

MANUAL AND MECHANICAL LEAF REMOVAL IN THE BUNCH ZONE (*VITIS VINIFERA* L., cv BARBERA): EFFECTS ON BERRY COMPOSITION, HEALTH, YIELD AND WINE QUALITY, IN A WARM TEMPERATE AREA

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Abstract

Aims: The main objective of the fruit zone leaf removal is the microclimate improvement around fruits in order to enhance grape ripening and fruit health. The aim of this work was to investigate the consequences of manual or mechanical leaf removal according to the phenological stage (fruit set or véraison) on spraying efficiency, yield composition, berry colour, must and wine quality, and grape health.

Methods and results: A three-year experiment (2001-2003) on the cv. Barbera was carried out in northwest Italy. Weather conditions during the three experimental seasons were very different. Leaf removal improved the efficiency of the fungicide application, reducing fungal diseases, but increased grape sensitivity to sunburn due to greater sunlight exposure. Some significant decrease on yield components was observed but not because of the same treatment. In leaf-removed vines, berry quality increased in the year least favourable for ripening (2002); in the warmer years, no quality improvement in must and wine was observed.

Conclusion: Leaf removal effects are not strictly tied to intervention time; the main effects were due to the vintage weather conditions: in unfavourable ripening conditions, leaf removal can improve grape health and quality. Due to the higher level of bunch temperature induced by this technique, in warmer conditions its usefulness needs to be accurately evaluated. Although a faster process, mechanical leaf removal did not provide any substantially different results when compared to manual intervention.

Significance and impact of study: The study showed potential and limits of leaf removal, suggesting that its application may vary, depending on climate and weather conditions of vineyards and vintage.

Key words: phenological stages, spraying efficiency, *Botrytis cinerea*, grape quality, wine sensory analysis

Résumé

Objectif : Les objectifs principaux de l'effeuillage sont l'amélioration du microclimat des fruits de manière à favoriser un bon état sanitaire de la vendange et la maturation des baies. L'objectif de cette étude a été de comparer les effets des techniques d'effeuillage (manuelle et mécanique) et de l'époque d'intervention (nouaison ou véraison) sur l'efficacité de la pulvérisation de produits phytosanitaires, le rendement des ceps, la couleur des baies, la qualité des moûts et des vins et l'état sanitaire des grappes.

Méthodes et résultats : L'expérimentation a été menée pendant trois ans sur le cépage Barbera en Italie du Nord Ouest, dans une région à climat tempéré (Piedmont). Une amélioration de l'efficacité des traitements fongicides et de l'état sanitaire des baies a été observée sur les plantes effeuillées. En revanche, le risque de brûlure des baies a été augmenté du fait d'une surexposition des grappes à la radiation solaire. Quelques variations des composantes du rendement ont été observées, mais pas toujours suite au même traitement. L'effeuillage a permis l'amélioration du millésime 2002, jugé peu qualitatif en terme de maturation du raisin. Par contre, pour les millésimes 2001 et 2003, jugés plus favorables, on n'a pas observé de changement qualitatif de l'état de la vendange.

Conclusion : L'effet de l'effeuillage n'est pas strictement lié à l'époque d'intervention, mais plusieurs effets sont dus aux conditions climatiques de l'année. L'effeuillage mécanique n'a pas entraîné de résultats particulièrement différents par rapport à l'effeuillage manuel, sauf en terme de main d'œuvre. Dans les millésimes défavorables, l'effeuillage peut améliorer l'état sanitaire et la qualité de la vendange. Par contre, lors de saisons plus chaudes, cette technique peut être inefficace à cause des influences négatives.

Signification et impact de l'étude : L'étude a montré les potentialités et les limites de l'effeuillage manuel et mécanique. On suggère que le choix soit fait chaque année en fonction du climat de la saison.

Mots-clés : stades phénologiques, efficacité de la pulvérisation, *Botrytis cinerea*, qualité du raisin, analyse sensorielle des vins

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INTRODUCTION

The cv. Barbera is one of the most important varieties grown in northwest Italy. Its ripening is favoured, especially in the latter stages, by a warm and dry climate, inasmuch as this variety has a highly acidic must and shows high vulnerability to *Botrytis cinerea*. In such a situation, canopy managements that regulate the microclimate of fruit zone may control the evolution of ripening.

Fruit-zone leaf removal is one of the most common summer practices aimed at an improvement of cluster microclimates, thus favouring grape ripening and reducing fungal diseases (GUBLER *et al.*, 1991; PERCIVAL *et al.*, 1994; STAFF *et al.*, 1997). Many studies have been carried out in order to assess the effects of this technique on plant development, grape ripening, grape health and wine quality. The results depend on the phenological stage and intensity of intervention, as well as on the climatic conditions of the season (HUNTER *et al.*, 1991, 1995; PRICE *et al.*, 1995, PETRIE *et al.*, 2003). Climatic parameters, such as temperature, atmospheric humidity, solar radiation, water availability (rain or irrigation), in fact, have an important impact on vegetative growth and physiology, berry composition and bunch health.

In cool, non-arid areas, the shaded grape berries can ripen in unfavourable microclimatic conditions, thus presenting lower soluble solid content, higher acidity value (CRIPPEN and MORRISON, 1986a; REYNOLDS *et al.*, 1986; SMART *et al.*, 1990), lower quantity of anthocyanins and of aromatic compound (MORRISON and NOBLE, 1990; BUREAU *et al.*, 2000). Moreover, these grapes are more sensitive to fungal diseases, grey mould in particular (*Botrytis cinerea* Pers.). Leaf removal can improve cluster exposure and ventilation and the efficiency of pesticides, resulting not only in a considerable reduction in fungal diseases (ENGLISH *et al.*, 1989; PERCIVAL *et al.*, 1994; STAPLETON and GRANT, 1992; ZOECKLEIN *et al.*, 1992), but also in better grape ripening and sensory properties of wines (KLIEWER and LIDER, 1968; KLIEWER, 1970; HALE and BUTTROSE, 1974; SMART *et al.*, 1985; CRIPPEN and MORRISON, 1986 a, b; ILAND, 1988; HUNTER *et al.*, 1991; ZOECKLEIN *et al.*, 1992; DOKOOZLIAN and

KLIEWER, 1996, SERRANO *et al.*, 2002; MAIN and MORRIS, 2004; MURISIER and FERRETTI, 2004). However, as the direct sunlight exposure of the grape may cause, specially in the hottest seasons, an increase in berry temperature (BERGQVIST *et al.*, 2001; SPAYD *et al.*, 2002; DELOIRE and HUNTER, 2005) and possible sunburn damages, the technique may not always improve quality (KLIEWER, 1977; HASELGROVE *et al.*, 2000; VASCONCELOS and CASTAGNOLI, 2000, BERGQVIST *et al.*, 2001; SPAYD *et al.*, 2002). In addition, even if single-leaf photosynthesis may increase when the vine leaf area is reduced (Hunter and Visser, 1988), it has been shown that the fruit-zone leaf removal can reduce the whole-vine photosynthesis and thus the level of fruit maturity (PETRIE *et al.*, 2003).

The above remarks, the necessity to reduce vineyard management costs, as well as a larger availability of leaf-removing machines, gave reason to study the influence of fruit-zone leaf removal on harvest quality and quantity (grape health, must and wine composition) of cv. Barbera. We studied the leaf removal method (manual or mechanical) and the phenological stage for the intervention (berry set or *véraison*), evaluating the efficiency of pesticide distribution as well as cluster health in leaf-removed vines without specific fungicide applications

MATERIALS AND METHODS

The experiment was performed from 2001 to 2003 on *Vitis vinifera* vines cv Barbera, R4/Kober5BB clone, in Carpeneto (northwest Italy). Average climatic conditions in the area of the experiment (350 m above sea level) report 882 mm average rainfall and a 2134 Huglin Index. The vineyard, planted in 1987, on a hillside with a 20 ° slope with south-southeast exposure, and vine rows oriented north-south. Vine spacing was 1.0 x 2.5 m, training system was an espalier with Guyot pruning system (8-10 buds per vine). Vineyard was managed according to the best agronomical practices for the cultivar and the region.

Climatic conditions during the three experimental seasons differed significantly (table 1).

Table 1 - Growing degree-day (base 10 °C) (GDD) and rainfall at Carpeneto (North-West Italy).

	2001		2002		2003		average 1990-2003
	annual	seasonal ^a	annual	seasonal ^a	annual	seasonal ^a	seasonal ^a
GDD (°C)	1847	1799	1822	1752	2311	2262	1904
rainfall (mm)	746	396	1349	637	784	329	512

^a from 1 April to 31 October.

Table 2 - Treatments and abbreviations employed in tables and text.

Early = leaf removal at berry pea size (75 BBCH-identification keys); late = leaf removal at *véraison* (83 BBCH-identification keys).

Treatments	Abbreviations
No fungicide applications - no leaf removal	untr. test
No fungicide applications - early mechanical leaf removal	untr. EM
No fungicide applications - late mechanical leaf removal	untr. LM
Fungicide applications against grey mould (table 3) without leaf removal	test
Fungicide applications against grey mould - early mechanical leaf removal	EM
Fungicide applications against grey mould - early manual leaf removal	EH
Fungicide applications against grey mould - late mechanical leaf removal	LM
Fungicide applications against grey mould - late manual leaf removal	LH

Table 3 - Main technical parameters of leaf stripping machine and sprayer used in the experiment.

Leaf stripping machine	Sprayer
Type: helicoidal blades	Type: airblast sprayer
Working: intake and cut	Type of fan: axial (600 mm diameter)
Blades positioning: in front of the tractor	Fan output: 14000 m ³ /h
Controls: hydraulic	Type and nozzles number ATR violet (3+3)
Regulations: head inclination, working height	Working pressure: 0.7 MPa
Working height: 0.4 m	Forward speed: 3.3 km/h
Forward speed: 3.6 km/h	Volume rate: 200 L/ha

Each agronomical treatment (table 2) consisted of three replicates of twenty-five contiguous vines with a completely randomized design.

1. Vineyard and grape evaluations

Manual leaf removal was carried out by eliminating both leaves of primary and secondary shoots in the fruit zone (at a height of 70-80 cm). Mechanical leaf removal was carried out with a helicoidal blade cutter leaf-stripping machine, whose helicoidal rotor-cutting device performs a double function of cutting and in-taking of leaves. Fungicides were applied using an airblast sprayer via a traditional axial fan distribution system (table 3).

Spraying efficiency on clusters was assessed at fruit set and at *véraison* by spraying a mixture of water and a 10 % tracer (E102 Tartrazine). After the application, two groups of 10 clusters each were picked from each treatment (10 clusters on the side of sprayer passage and 10 on the other side). The settled mixture on the picked clusters was assessed through a colorimetric method (BALSARI and TAMAGNONE, 1994). Product penetration was assessed by relating values of the measured deposits on the two groups of picked clusters. Product distribution evenness was assessed for each treatment by calculating the coefficient of variability (CV %) of the product quantity found on samples.

Two fungicide applications (500 L/ha of fungicide solution against *Botrytis cinerea* were applied with a

knapsack sprayer. The applications were done at the bunch closure stage (77 BBCH-identification keys) and at *véraison* (87 BBCH-identification keys) (table 4).

Based on a published procedure (CRAVERO *et al.*, 2002), mechanical damages to the clusters due to the leaf stripping machine (8-10 days after intervention), berry burns linked to sun exposure (3-4 weeks before harvest), grey mould and sour rot (at harvest) were also evaluated.

For plants treated with fungicides only, the following measurements were done at harvest: yield per vine, number of clusters per vine, cluster weight and berry weight. A sample of 200 berries was randomly collected from each treatment. Thirty berries were randomly chosen from this sample, and further divided into three groups of 10 berries, which were used as triplicates. Skins were separated from the pulp, extracted in pH 3.2 buffer (DI STEFANO and CRAVERO, 1991), homogenised and then analyzed for anthocyanin and polyphenol concentration by spectrophotometry. Remaining berries were pressed and the juice was analysed for soluble solids content (SSC) and titratable acidity.

2. Winemaking, chemical and sensory wine analysis

Grapes from the eight treatments were separately vinified without replications; they were destemmed and crushed, SO₂ was added (4 g/100 kg of grapes), and they were transferred for maceration and alcoholic fermentation into 100-liter tanks. All musts were inoculated with

Table 4 - Date of fungicide applications against grey mould, active ingredient and doses.

Date of fungicide applications	active ingredient	(g/ha)
July 2, 2001; September 6, 2002; September 1, 2003	cyprodinil+fludioxonil	300+200
September 4, 2001; July 2, 2002; June 25, 2003	pyrimethanil	1000

Table 5 - Treatment influence on spraying efficiency. Synthesis of results obtained after spraying at fruit set and at *véraison*.

For the same year and the same column, means within each column followed by the same letter are not statistically different at $p \leq 0.05$.

		fruit set			véraison		
		product on the grape ($\mu\text{l/g}$)	penetration (%)	evenness (CV*) (%)	product on the grape ($\mu\text{l/g}$)	penetration (%)	evenness (CV) (%)
2001	test	2.61 a	35 a	55	0.31 b	90 b	32
	mechanical	2.53 a	37 a	57	0.42 a	95 a	30
2002	test	1.95 a	42 a	60	0.56 a	78 b	33
	mechanical	1.89 a	47 a	54	0.58 a	64 c	28
	manual	2.18 a	37 a	49	0.72 a	90 a	38
2003	test	1.59 a	32 b	60	0.40 a	61 b	23
	mechanical	1.82 a	52 a	37	0.50 a	59 b	20
	manual	1.71 a	38 ab	51	0.41 a	79 a	33

* = coefficient of variability

Saccharomyces cerevisiae yeasts and with a fermentation activator later on. During the 12 maceration days the cap was punched down twice a day and, at the end of this period, the wines were drawn off, only detaining free-run wine. The malolactic fermentation (MLF) took place in an air-conditioned room at 22-24 °C, after inoculation with selected bacteria *Leuconostoc oenos*. At the end of the MLF, SO₂ was added to the 8 wines (40 mg/L), which were decanted twice during winter. The stabilization process took place only in a cold room, so that wines would be sufficiently limpid. At the end of the process, wines were bottled using crown caps.

In June and July following the harvest, the wines were subject to physical-chemical analyses and to a sensory analysis, carried out by a panel of 15 wine experts.

Physical-chemical parameters (alcohol percentage, dry matter, titratable acidity, volatile acidity, pH, free and total SO₂ ash and ash alkalinity) were analysed according to the official EU system (COMMISSION REGULATION n° 2676/90). Fixed acids were analyzed by HPLC (CANE, 1990), total flavonoids, free and total anthocyanins, total phenolics (Folin index), proanthocyanidines, and vanillin assay by spectrophotometry (DI STEFANO *et al.*, 1989). In addition, colour intensity (E420 nm + E520 nm) and colour hue (E420 nm/E520 nm) were measured.

Wine sensory analysis was carried out using ranking tests, scoring wines according to the following parameters: red colour intensity and acceptability, odour intensity and acceptability, taste balance and acceptability, and the wine

total acceptability. The three wines from *Botrytis cinerea* non-treated vines were tasted separately from the 5 from fungicide-treated vines.

3. Statistical data processing

Results obtained from vineyards and grape assessments were submitted to the ANOVA statistical analysis system, applying the average discrimination through either Tukey's test or Duncan's test. Where necessary, percentage values were converted into the corresponding angular values. The significance of the differences between frequency distributions and cytological observations were evaluated by the χ^2 test. Since only one repetition was done, wine data were not submitted to statistical analysis. Ranking test sensory results were processed with the non-parametric Quade test, combined with the multiple comparisons procedure (CONOVER, 1980).

RESULTS AND DISCUSSION

1. Leaf-removal effects on the efficiency of pesticide distribution

A significant improvement of spraying efficiency was observed for 3 years in leaf-removed vines (table 5). In the second and third years, when distribution was applied at fruit setting, leaf removal improved deposit uniformity on clusters (lower coefficient of variability values). In the first year, when the application was applied at *véraison*, a significant product deposit improvement was seen on the clusters in leaf-removed vines (table 5). In 2002 and

Table 6 - Effects of mechanical and manual leaf removal on yield components, at harvest. Annual value and 2001-2003 mean value.

Means within each column followed by the same letter are not statistically different at $p \leq 0.05$.

	yield/vine (kg)				cluster number/vine			
	2001	2002	2003	mean value	2001	2002	2003	mean value
test	3.33 ab	4.90 a	3.01 a	3.75 a	10.0 ab	16.2 a	15.1 a	13.8 a
EM	4.31 a	3.64 b	2.05 a	3.33 a	12.8 a	14.8 ab	12.2 b	13.2 a
EH	4.40 a	4.06 ab	1.96 a	3.47 a	11.9 ab	13.6 b	12.5 ab	12.6 a
LM	3.18 b	3.94 ab	2.66 a	3.26 a	9.2 b	13.9 ab	14.0 ab	12.4 a
LH	3.31 ab	3.29 b	2.55 a	3.05 a	11.3 ab	13.1 b	13.2 ab	12.5 a

	cluster weight (g)				berry weight (g)			
	2001	2002	2003	mean value	2001	2002	2003	mean value
test	334 ab	302 a	200 a	279 a	3.00 a	3.27 a	2.52 a	2.95 a
EM	339 ab	246 a	168 a	251 a	2.96 ab	3.21 a	2.19 ab	2.77 ab
EH	371 a	298 a	157 a	275 a	3.01 a	3.32 a	1.91 b	2.72 bc
LM	344 ab	283 a	190 a	272 a	2.82 ab	3.37 a	2.21 ab	2.88 ab
LH	296 b	249 a	193 a	246 a	2.74 b	2.87 b	1.96 b	2.57 c

2003, in terms of deposit on clusters, no difference was observed according to mechanical or manual leaf removal, whereas manual leaf removal improved product penetration in the canopy. The manual process, however, required 10 times longer in comparison with the mechanical intervention (BIASI *et al.*, 1993).

2. Leaf-removal effect on yield

The decrease in yield and in the cluster number observed in some cases in Barbera (table 6) was probably caused by some small damage caused by the leaf-stripping machine or by an increase in undeveloped buds and/or by a decrease in shoot fertility in the year after leaf removal (HOWELL *et al.*, 1994; KOBLET *et al.*, 1994). Berry-weight differences were also observed, mostly related, however, to the season, in agreement with results obtained by other authors (HUNTER *et al.*, 1991). In 2002, the high mean berry-weight was partially due, on the one hand, to a lower number of berries (balancing effect), and on the other to the rainy summer, which increased berry size because of greater water absorption. Plant water status was not measured, but it seems reasonable to think that, in the particularly hot and dry vegetative season of 2003, low berry weight could have been caused either by the reduced enlargement of pericarp cells due to the reduced spring water availability (OJEDA *et al.*, 2001) (table 1), or to the berry dehydration during the final phase of ripening.

In 2001, cluster and berry weights were significantly lower than the test only in the late manual leaf-removed vines (LH) (table 6). In the following two years, only berry weight was significantly different from test: in the LH

vines, in 2002, and in early manual leaf-removed vines (EH) and LH vines, in 2003. Two elements may have influenced the mentioned result: the leaf area reduction (KOBLET *et al.*, 1994) and the increased cluster temperature due to greater sunlight exposure, resulting in increased berry transpiration and dehydration (HALE and BUTTROSE, 1974; CRIPPEN and MORRISON, 1986 b; BERGQVIST *et al.*, 2001; DELOIRE and HUNTER, 2005). In 2003, when the warmest weather was registered, in fact, the gap was higher, whereas in 2001 and 2002 it was lower. In accord with these observations, previous studies showed that sun-exposed clusters could have an inferior berry mass in comparison to shaded clusters (HASELGROVE *et al.*, 2000; SPAYD *et al.*, 2002).

3. Leaf-removal effects on must and berry skin composition

In 2001, a significant soluble solids content increase was observed only for LH vines; in 2002 it increased only in the manual leaf-removed plants (table 7); no significant differences were observed in 2003. The highest soluble solids amount was assessed during the warmest years, but for different reasons: in 2001, the climatic conditions favoured a better ripening, whereas in 2003 the higher soluble solid levels were better attributed to their concentration due to berry dehydration, which occurred during ripening. In 2001, leaf removal induced an increase in titratable acidity; in 2002, it caused a decrease in it, in accordance with an improved microclimate in the fruit zone. In 2003, the acidity showed a relevant and widespread decrease in all treatments due to the high air temperature registered during ripening that, by increasing

Table 7 - Soluble solid content and titratable acidity of must at harvest as influenced by leaf removal treatments.Means within each column followed by the same letter are not statistically different at $p \leq 0.05$.

	soluble solid content (%)			titratable acidity (g/L tartaric acid)		
	2001	2002	2003	2001	2002	2003
test	22.8 b	20.6 c	26.9 a	9.5 b	13.7 a	5.27 a
EM	23.2 b	21.2 bc	27.4 a	11.6 a	12.4 ab	5.33 a
EH	23.3 ab	23.6 a	26.5 a	10.6 a	11.0 b	5.85 a
LM	23.1 b	20.2 c	26.8 a	11.1 a	14.0 a	5.93 a
LH	24.3 a	22.0 b	26.8 a	10.7 a	11.4 b	4.65 a

Table 8 - Effect of leaf removal treatments and seasons on polyphenol (g/kg f.w.) and anthocyanin (g/kg of f.w. and mg/berry) amount at harvest. Data are the mean for the three replicates.Means within each column followed by the same letter are not statistically different at $p \leq 0.05$.

	total polyphenols (g/kg)				total anthocyanins (g/kg)				total anthocyanins (mg/berry)			
	2001	2002	2003	mean*	2001	2002	2003	mean*	2001	2002	2003	mean*
test	1.54 a	1.86 d	3.05 a	2.15 b	0.93 a	0.77 b	1.24 a	0.98 b	2.79 ab	2.53 b	3.09 ab	2.80 b
EM	1.67 a	2.06 ab	3.71 a	2.66 a	0.90 a	1.04 b	1.31 a	1.08 b	2.68 ab	3.31 b	2.87 b	2.95 ab
EH	1.60 a	2.83 a	3.66 a	2.70 a	0.99 a	1.56 a	1.35 a	1.30 a	2.98 a	4.47 a	2.58 c	3.34 a
LM	1.49 a	2.10 cd	3.54 a	2.37 bc	0.93 a	0.99 b	1.48 a	1.13 b	2.62 ab	3.33 b	3.27 a	3.07 ab
LH	1.55 a	2.29 bc	3.66 a	2.50 ab	0.91 a	0.89 b	1.45 a	1.08 a	2.48 b	2.94 b	2.83 bc	2.75 b

* Combined data over three seasons

berry temperature (BERGQVIST *et al.*, 2001), probably also increased malic acid degradation (LAKSO and KLIEWER, 1978).

The average amount of polyphenols in berry skin (expressed as g/kg of fresh weight - f.w.) varied from 1.6 g/kg in 2001 to 2.3 g/kg in 2002, to 3.5 g/kg of f.w. in 2003 (table 8). The amount was higher in the warmest and driest year (2003), very low in the warm but less dry year (2001), whereas in 2002, with wet and cool weather, it reached intermediate absolute values. As already observed for the soluble solid content, the polyphenol concentration improved, compared to the test vines, only in 2002 in the early leaf removal vines and in the LH vines. Similar observations may be made as those concerning total anthocyanins (table 8). Many differences were assessed over the years: in 2001 an average of 0.93 g/kg of f.w., 1.05 g/kg in 2002 and 1.37 g/kg in 2003. In 2002, leaf-removal caused a general increase in the concentration of anthocyanins, although significant differences were only obtained with EH vines. No difference was observed in 2001 and 2003 anthocyanin concentrations, expressed as g/kg f.w. Considering the results expressed as mg/berry (table 8) however, a tendency of decreasing concentration due to leaf removal may be noted in 2001 and 2003. This is possible tanks to small berry sizes at harvest, caused by water deficiency

during ripening (OJEDA *et al.*, 2002), and the warmer microclimate in the leaf-removed fruit zone which partially compromised anthocyanin synthesis (KLIEWER, 1970; BERGQVIST *et al.*, 2001).

On the contrary, in 2002, higher concentration values were reached by all the treated vines (as mg/berry), both in comparison with the test vines and with the other experimental years. These observations confirmed that the modifications of microclimatic conditions due to leaf removal might promote anthocyanin synthesis in rainy years more than in warm and dry years.

4. Leaf-removal effects on grape health

No important damage to the grape linked to the mechanical process was observed. In 2001, with strong epidemic pressure due to *Botrytis cinerea*, leaf removal had a positive influence in curbing overall damage, without resort to specific fungicide treatments (table 9), in accordance with previous works (GUBLER *et al.*, 1994; PERCIVAL *et al.*, 1994; STAFF *et al.*, 1997). In 2002, from the end of July, cluster impairment related to sour rot was widely manifested; the leaf removal process, as well as the specific fungicide applications against *Botrytis cinerea*, did not reduce spread of the disease. The appraisal of cluster sunburn damage proved difficult because of an unclear symptomatology (necrosis and

Table 9 - Estimation of the disease diffusion in the different years (untr. = no fungicide applications against grey mould). Values correspond of disease diffusion (%) on cluster.Means within columns followed by different letters differ significantly at $p < 0.05$ by Tukey's test.

	2001*		grey mould	2002		2003**
	grey mould	sunburn		sour rot	sunburn	sunburn
untr. test	34.9 a	0.5 d	4.3 a	7.0 a	0.1 d	13.2 a
untr. EM	19.3 ab	1.9 bcd	0.7 ab	2.7 a	0.2 d	6.4 a
untr. LM	11.7 b	8.1 a	2.5 ab	7.3 a	2.8 ab	10.1 a
test	0.6 c	0.5 d	0.5 ab	3.5 a	0.1 d	5.3 a
EM	0.6 c	1.9 bcd	0.1 b	2.5 a	0.5 cd	10.2 a
EH	0.3 c	1.5 bcd	0.8 ab	2.7 a	0.5 cd	12.0 a
LM	0.3 c	6.0 ab	2.0 ab	7.0 a	2.4 ab	15.9 a
LH	0.2 c	3.3 abcd	1.2 ab	3.7 a	0.7 bcd	8.5 a

* In 2001 no sour rot was observed

**In 2003 no grey mould and sour rot were observed

Table 10 - Phenolic parameters of the wines obtained by microvinification (untr. = no fungicide applications against grey mould).

	total phenols (mg/L)			proanthocyanidins (mg/L)			anthocyanins (mg/L)		
	2001	2002	2003	2001	2002	2003	2001	2002	2003
untr. test	1872	768	1673	866	300	1427	327	128	397
untr. EM	1257	957	1748	477	633	1474	159	169	318
untr. LM	1243	952	1671	522	502	1349	204	141	377
test	1526	775	1478	802	367	1241	352	129	341
EM	1667	851	1816	889	488	1611	285	150	332
EH	1179	1093	2083	401	803	1904	201	262	444
LM	1447	919	1817	866	431	1534	327	175	456
LH	1764	1233	1728	954	828	1470	355	265	387

withering), thus determining highly variable data and less significant differences. Except for 2003, whose extraordinary climatic conditions also determined sunburn phenomena on test vines, early intervention showed a positive effect against sunburn, compared to late leaf removal (table 9).

5. Leaf-removal effects on wine composition and sensory characteristics

Leaf removal, whether combined with specific applications against *Botrytis cinerea* or not, did not, in our experience, determine physical-chemical variations in wines (data not showed). Major influence was observed on the wine phenol content (table 10). In 2001, leaf removal that was not combined with specific anti-*Botrytis* applications brought about lower values in the all analyzed phenolic parameters, whereas when combined with fungicide applications, it caused a negative variation in EH vines only. In 2002, a clear improvement of the wines' phenol composition was observed, regardless of the fungicide application, in accordance with the must observations (table 8). The absolute value of each

parameter was, however, much lower than in the other years, probably because the 2002 was colder and wetter than the other experimental seasons (table 1). In 2003, total phenols increased in sprayed and leaf-removed vines when compared with the treated test; anthocyanins decreased in untreated leaf-removed vines when compared with untreated test vines (table 10); in this season the higher anthocyanin amount is attributable to the solute concentration caused by berry dehydration (table 6).

In 2001, no positive effects on colour intensity was observed, whereas in 2002 only the wine from vines with leaves removed manually attained a higher value than the test; in 2003, every wine from leaf-removed vines were more intensely coloured than the test (table 11). In 2002, hue values were highest, though values did not increase following leaf removal. In the other two years, there were increases only after early leaf removal (table 11).

In 2002, the leaf removal improved taste characteristics of the wines, which were the most appreciated by the tasting panel (figure 1). The best wines

Table 11 - Wine colour intensity (E420 nm + E520 nm) and hue (E420 nm/E520 nm) of the wines obtained by microvinification (untr. = no fungicide applications against grey mould).

	2001*		2002		2003**	
	grey mould	sunburn	grey mould	sour rot	sunburn	sunburn
untr. test	34.9 a	0.5 d	4.3 a	7.0 a	0.1 d	13.2 a
untr. EM	19.3 ab	1.9 bcd	0.7 ab	2.7 a	0.2 d	6.4 a
untr. LM	11.7 b	8.1 a	2.5 ab	7.3 a	2.8 ab	10.1 a
test	0.6 c	0.5 d	0.5 ab	3.5 a	0.1 d	5.3 a
EM	0.6 c	1.9 bcd	0.1 b	2.5 a	0.5 cd	10.2 a
EH	0.3 c	1.5 bcd	0.8 ab	2.7 a	0.5 cd	12.0 a
LM	0.3 c	6.0 ab	2.0 ab	7.0 a	2.4 ab	15.9 a
LH	0.2 c	3.3 abcd	1.2 ab	3.7 a	0.7 bcd	8.5 a

* In 2001 no sour rot was observed

**In 2003 no grey mould and sour rot were observed

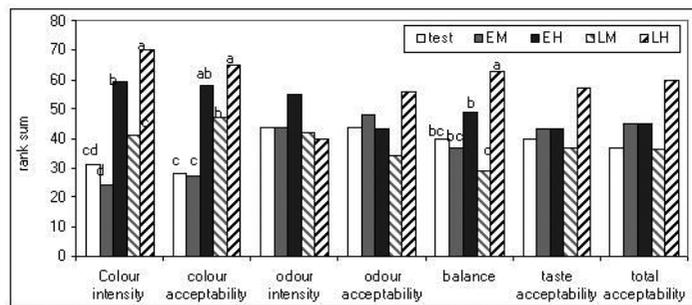


Figure 1 - Ranking test on 2002 wines: the higher is rank sum and the higher are intensity and acceptability of considered parameter. Different letters indicate significant differences at $p \leq 0.05$ by Quade's test.

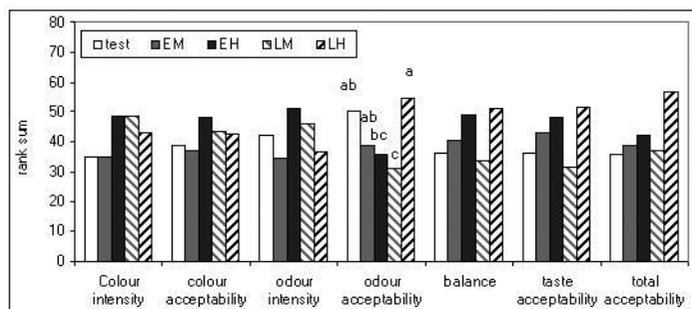


Figure 2 - Ranking test on 2003 wines: the higher is the rank sum the higher are intensity and acceptability of considered parameter. Different letters indicate significant differences at $p < 0.05$ by Quade's test.

in terms of sensory characteristic and acceptability (especially as for the colour) were the ones obtained by hand-treated (EH and LH) vines. In 2003, the differences were not so relevant. In terms of total acceptability, the most appreciated wines, however, came from LH vines, confirming the results of the previous year (figure 2).

CONCLUSION

Leaf removal in the fruit zone modifies the Barbera cluster microclimate in a way that may be considered an important weapon against *Botrytis cinerea*, which is

especially suitable for vineyards with thick cluster foliage. Leaf removal also improved product penetration in the canopy, and fungicide efficiency, with a subsequent reduction in pesticide dosage. This result is particularly interesting for organic viticulture, as well as for integrated pest management programs. In this case, a practical advantage is represented by the integration of early leaf removal and fungicide application at *véraison*. Leaf removal had, however, no impact on sour rot, a severe disease against which no effective protection product is currently available.

Over the three-year period, in terms of quantity, no decrease in crop level, cluster number or weight was observed with this technique, whereas some berry weight decreases were observed, due to manual leaf removal. The phenological intervention stage did not clearly affect qualitative and quantitative parameters, depending on the experimental season. Only in the coolest and rainiest year (2002), the least suitable weather for Barbera ripening, was there an improvement in the pulp soluble solid content, skin anthocyanin and polyphenol, as well as in quality parameters of the wines following leaf removal, which were registered regardless of treatment modality (manual or mechanical). Mechanical leaf removal did not show any substantial differences when compared with the manual process, except in the time it takes to complete the operation, which is substantially shorter. In two experimental seasons out of three, the favoured wines tasted came from grapes from vines with leaves removed at *véraison*.

An inconvenience linked to leaf removal is, however, represented by the cluster direct exposure to sunlight, which increases cluster temperature, frequently causing berry withering or sunburn, and modifying the accumulation of some berry quality components. In microclimatic conditions unfavourable for ripening, or in the less favourably exposed vineyards, leaf removal may improve grape health and quality with little occurrence of the mentioned inconvenience. In warmer areas and years, or in south- or southwest-exposed vineyards, the real usefulness of this process needs to be accurately evaluated. Removal of leaves on the less exposed sides of vines in order to avoid excessive direct sunlight exposure to clusters may be considered. From this point of view, randomized leaf-removal performed by the mechanical process may prove to be of greater advantage than a manual intervention that removes leaves around clusters.

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