Impacts of Standard Wine-Making Process on the Survival of *Lobesia botrana* Larvae (Lepidoptera: Tortricidae) in Infested Grape Clusters

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ABSTRACT To determine the risk winery waste poses for the spread of *Lobesia botrana* (Denis & Schiffermüller) (Lepidoptera: Tortricidae) in California, we evaluated the survival of larvae in artificially infested grape clusters (*Vitis vinifera* L.) processed for wine making. The trial consisted of five treatments: whole cluster pressing to 1 bar (100,000 Pa); whole cluster pressing to 2 bars (200,000 Pa); destemming and berry pressing to 1 bar; destemming and berry pressing to 2 bars; and control. Each treatment was replicated with the following five winegrape varieties: Chardonnay, Sauvignon Blanc, Gewürztraminer, Yellow Muscat, and Cabernet Sauvignon. All winery waste was inspected for larval survival. No live larvae were recovered from any of the treatments in all five varieties; therefore, the hypothesis that green winery waste contributes to the spread of *L. botrana* was rejected.

KEY WORDS invasive carpophagous species, wine grape, mortality, winery waste

Lobesia botrana (Denis & Schiffermüller) (Lepidoptera: Tortricidae) is a significant pest in the grape-growing regions of Europe, the Middle East, northern and western Africa, southern Russia, and central Asia (Bovey 1966, Centre for Agricultural Bioscience International [CABI] 2012). Larvae feed on grape inflorescences and on green and ripe berries (Marchal 1912, Ioriatti et al. 2011). Injury to berries allows for infection by various fungi that frequently results in bunch rots (Fermaud and Le Menn 1989). In May 2008, *L. botrana* was reported for the first time in the Western Hemisphere, in Chile, and subsequently in Argentina in March 2010 (Gonzalez 2010).

In September 2009, *L. botrana* was reported in North America from Napa County, CA (Mastro et al. 2010, Varela et al. 2010). In pheromone trap surveys conducted in vineyards at densities of 6–10 traps/km^2, male moths were found in nine California counties in 2010 plus one additional county in 2011 (Varela et al. 2013). Napa County had the highest populations with >100,000 moths caught in 2010 in an area of ≈10,000 vineyard ha (Cooper et al. 2011). Infestations in all other counties were comparatively low. The three counties neighboring Napa had 4–26 infested sites with the total number of moths caught in 2010 ranging from 11 to 59. The remaining seven counties had one or two infested sites per county with total moth counts ranging from 1 to 19.

How the moths arrived in California was investigated by the U.S. Department of Agriculture, but no conclusions were reached regarding the entry pathway into California. Assuming that the county with the highest moth populations, Napa, was where the original infestation began, the spread within California may be associated with the movement of infested wine grapes to wineries, movement of infested machinery, and, in one case, movement of wooden vineyard stakes.

The detection of *L. botrana* in California triggered regulatory action by the U.S. Department of Agriculture and the California Department of Food and Agriculture to prevent the further spread of *L. botrana* (U.S. Department of Agriculture–Animal and Plant Health Inspection Service [USDA–APHIS] 2010). In commercial vineyards, insecticide applications and mating disruption are being used in an ongoing eradication program. To prevent the spread of this moth, regulations were implemented on the movement from quarantined areas of fruit, plant material, machinery, and winery waste. Green winery waste that results from processing fruit originating from a *L. botrana* quarantine area must be composted on site, transported to an approved green waste or composting facility, or returned to the original vineyard by an approved hauler.

Red wines receive most of their flavor from the berry skins, whereas white wines do so from the juice contained in the berry pulp. In red varieties, berries are fermented, and after fermentation, seeds and pieces of skins are pressed to separate wine from these solids. In white varieties, berries are pressed to release

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juice that is then fermented (Henderson and Rex 2007).

Grape clusters of red varieties are destemmed and then the berries are often crushed. The crusher is a set of rollers designed to break open the berries and release the juice. The gap between the rollers can be adjusted to provide a greater or lesser degree of crushing. The resulting mixture of juice, skins, and seeds, called must, is transferred to the fermentation tank. After fermentation, the wine is drained and the remaining solids are pressed. Processing red grapes into wine produces green winery waste (stems) and fermented winery waste (solids); the latter is referred to as pomace or marc. If grapes are mechanically harvested, the stems remain on the vines; thus, only berries are processed and green winery waste is not produced.

In white wine production, extracting the juice can be accomplished in different ways, two of which are: 1) grapes clusters are destemmed, berries are sometimes crushed, and the resulting must or berries are pressed; 2) whole clusters (berries attached to stems) are loaded directly into the press ("whole cluster press"). The process of pressing can have several cycles, each with a different duration and pressure. The number of cycles and the pressure reached depends on the different wine styles. Green winery waste produced after processing a white variety is either separated stems, seeds, and skins or the intact pressed clusters. When fruit is mechanically harvested, only berry skins and seeds are produced as green waste.

The risk of dispersal of *L. botrana* posed by winery waste has not been previously studied in countries where this insect is established. Thus, the objectives of this study were to evaluate the potential for survival of *L. botrana* life stages after processing infested grapes and determine the risks of spreading this insect through the transport and deposition of winery waste.

Materials and Methods

We evaluated the survival of *L. botrana* larvae in clusters that were artificially infested and processed for wine making. Five treatments were conducted: 1) whole cluster pressing to 1 bar (100,000 Pa); 2) whole cluster pressing to 2 bars (200,000 Pa); 3) destemming and berry pressing to 1 bar; 4) destemming and berry pressing to 2 bars; and 5) no pressing for the control. Each treatment was replicated with the following five winegrape (*Vitis vinifera* L.) varieties: Chardonnay, Sauvignon Blanc, Gewürztraminer, Yellow Muscat, and Cabernet Sauvignon. These varieties were chosen from the most representative international cultivars based on potentially different mechanical characteristics of the berries, irrespective of the color.

The experiment was conducted at Fondazione Edmund Mach, San Michele all'Adige (TN), Italy, as the varieties reached harvest maturity on the following dates: Chardonnay on 1 September 2011, Sauvignon Blanc on 2 September 2011, Gewürztraminer on 15 September 2011, Yellow Muscat on 29 September 2011, and Cabernet Sauvignon on 30 September 2011. For each variety, the berry mechanical properties were measured on the day of the experiment using a Universal Testing Machine TAxT2i Texture Analyzer (Stable Micro System, Godalming, Surrey, United Kingdom) according to the methodology of Letaief et al. (2008a,b). A 20-berry sample was used for each mechanical parameter. Skin break force was determined by needle probe and berry firmness by a 35-mm diameter flat probe, the latter being defined as the force needed to reach a berry deformation of 50%. The acquisitions were made at 400 Hz using Texture Expert Exceed software version 2.54 (Stable Micro Systems, Godalming, Surrey, United Kingdom) working in a Windows environment.

Five kilograms of fruit was used for each treatment and for the control. Twenty-five kilograms of fruit per variety was infested with *L. botrana* third instar larvae. Larvae were obtained and selected from the colony maintained at the Fondazione Edmund Mach. The 25 kg was divided, and each kilogram of grapes was placed inside a plastic square container and 10 larvae were added, placing approximately two larvae per cluster. Containers were covered with organdy and fastened with elastic bands. All containers were placed in a rearing room and maintained for 7 d at 23 ± 2°C and a photoperiod of 16:8 (L:D) h.

For whole cluster press treatments to 1 or 2 bars, 5 kg of infested clusters was placed in an experimental press (20 liters Hydropress, Speidel Tank-und Behälterbau GmbH, Ofterdingen, Germany). For the 1-bar treatment, the pressure was ramped up to 1 bar and maintained for 5 min. After decompression, the fruit was mixed by hand and the procedure was repeated for a total of four cycles of 1 bar each. For the two-bar treatment, the procedure was similar, except that it comprised a total of seven cycles that were divided into the first four cycles, in which pressure reached 1 bar, and the remaining three cycles, in which pressure reached 2 bars. Pomace, stems, and any other possible residual material was carefully removed manually and placed on trays for examination. The press was washed after each treatment.

For the crushing–destemming and press treatments, 5 kg of infested clusters was processed with a crusher–destemmer (Ares 15 inox, OMAC, Corridonia, MC, Italy). The stems were placed in plastic bags for later examination. The berries were placed in the press and processed to 1 and 2 bars as mentioned above for the whole cluster press.

On the day treatments were conducted, for each variety, five boxes were randomly selected and inspected under dissecting scopes for larval survival as the control. One hundred control berries were weighed. After each treatment, all winery waste (pressed whole clusters, stems, and pressed berries) was transported to the laboratory and examined individually under a dissecting stereo microscope for the presence of dead and live larvae. Because all larvae were not recovered during examination, the winery waste was placed in small rearing cages (BugDorm, Megaview Corp., Taichung, Taiwan) for possible adult emergence.

Table 1. Number of larvae alive, dead, or missing in the control and after processing wine grapes by pressing whole clusters to 1 or 2 bars or by destemming and pressing the berries to 1 or 2 bars

Treatment	Mean percent larvae		
	Alive	Dead	Missing
Whole cluster press 1 bar	0.0a	10.4 ± 1.9a	89.6 ± 1.0a
Whole cluster press 2 bars	0.0a	3.2 ± 1.3b	96.8 ± 1.3b
Berry press 1 bar	0.0a	1.2 ± 1.2b	98.8 ± 1.2b
Berry press 2 bars	0.0a	0.8 ± 0.5b	99.2 ± 0.5b
Control	92.8 ± 2.7b	0.0b	7.2c

Within each column, means followed by a different letter are significantly different (Tukey pairwise comparison, $P < 0.05$).

Table 2. Compression, force to crack berry, and skin thickness by grape variety

Date	Variety	Compression (G)	Force to crack berry (*N*)	Skin thickness (μm)
1 Sept. 2011	Chardonnay	661.9	0.583	178.1
2 Sept. 2011	Sauvignon Blanc	644.6	0.441	196.8
15 Sept. 2011	Gewrztraminer	518.1	0.899	208.3
29 Sept. 2011	Yellow Muscat	953.7	0.553	199.5
30 Sept. 2011	Cabernet sauvignon	442.9	0.630	209.7

To recover larvae that might have moved with the juice, the juice was sieved through 1-mm mesh for all treatments. The sieve was examined for dead or live larvae. To evaluate whether the different grape-pressing processes affected product quality, the amount of juice extracted was measured and analyzed for its chemical basic composition (percentage of total soluble solids as °Brix, pH, titratable acidity, malic and tartaric acids, potassium, and yeast assimilable nitrogen) using a Fourier transform infrared spectrometer (FT-IR Grapescan 2000; FOSS, Hillerød, Denmark). The yield was measured for each pressing cycle and variety.

Analysis of variance (ANOVA) was used to determine the treatment effect. To accommodate sampling and spread data collection over different harvest dates, each replicate was a different variety, and for this reason the "replicate" was analyzed to be treated as a covariate. If the replicate was not significant, the covariant was dropped and Tukey's pairwise comparison was used to separate treatment means.

Results

There was no treatment effect of replicate (variety) on the number of live ($F = 0.004$; df = 4, 20; $P = 1.000$), dead ($F = 0.257$; df = 4,20; $P = 0.902$), or missing ($F = 0.010$; df = 4, 20; $P = 1.000$) larvae; for this reason, variety was not a factor in the analysis and was not included as a covariate. Of the 50 larvae inoculated per variety, the number of live larvae recovered from the clusters randomly selected as controls ranged from 42 in Cabernet Sauvignon to 50 in Yellow Muscat. No live larvae were recovered from any of the four treatments, whereas 92.8 ± 2.7 live larvae were recovered from the control ($F = 1{,}157$; df = 4,20; $P < 0.001$; Table 1). There were treatment differences for the number of recovered dead ($F = 12.330$; df = 4,20; $P < 0.001$) and missing ($F = 541.9$; df = 4,20; $P < 0.001$) larvae (Table 1). The percentage of dead larvae recovered ranged from 0 to 16% of the number of larvae inoculated on each sample of 5 kg of fruit. All dead larvae were found on the berries; none were found on the stems or in the juice. We assumed the remaining larvae were destroyed and were not recognizable in the waste material. Slightly higher percentages of dead larvae were recovered from whole cluster press treatments (10.4 and 3.2% for one- and two-bar whole cluster press treatments, respectively) than from the crushing–stemming and berry press treatments (1.2 and 0.8% for one- and two-bar berry press treatments, respectively). No moths emerged from the rearing cages.

Berry mechanical properties (Table 2) and the yields obtained with the experimental press (Figs. 1 and 2) are consistent with the values measured for other samples of the same varieties in the region and achievable using industrial wine-making equipment. The targeted percentage yield was reached by both pressing whole clusters and crushed–destemmed berries, but higher percentages were achieved when whole clusters were pressed to 2 bars. The juice composition for all five grape varieties (Table 3) was considered normal based on the 2011 vintage in Trentino (Italy), a year characterized by high temperatures in September. The high pH values may be attributed to the time elapsed between harvest and pressing when the *L. botrana* larvae were allowed to web inside the clusters.

Discussion

Under the conditions of these trials, winery waste was not a source of live larvae. The only larvae found were inside or between pressed berries and they were dead. Whole cluster pressing to 1 bar is gentler than

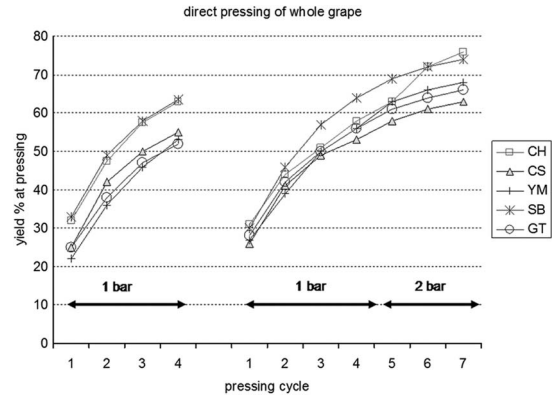

Fig. 1. Percent yield by grape variety of whole cluster press to 1 and 2 bars. (CH, Chardonnay; CS, Cabernet Sauvignon; YM, Yellow Muscat; SB, Sauvignon Blanc; GT, Gewürztraminer).

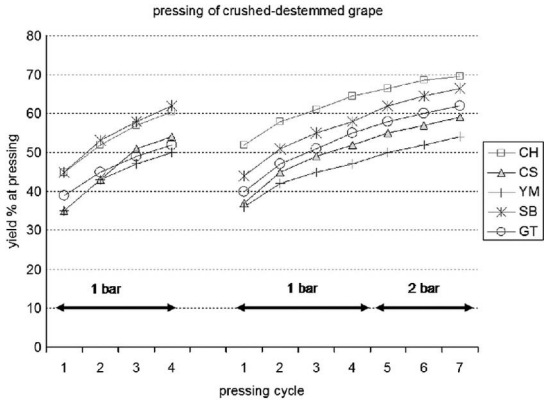

Fig. 2. Percent yield by grape variety of pressed destemmed berries to 1 and 2 bar. (CH, Chardonnay; CS, Cabernet Sauvignon; YM, Yellow Muscat; SB, Sauvignon Blanc; GT, Gewürztraminer).

the other treatments, which may explain why fewer larvae were missing. In California, winery waste is composted on site, taken to a composting facility, or collected into piles that remain unmanaged through the winter. If larvae had survived, to be a source of adult moths the following year, they must successfully pupate inside the piles, survive the winter, and be able to emerge from the pile. In California, the practice of spreading green winery waste directly into the vineyards' row middles has been discouraged because it was shown that vine mealybug, *Planococcus ficus* (Signoret), can be spread with untreated winery waste (Smith and Varela 2008).

The varieties tested, having highly different berry and cluster characteristics, can be considered representative of the mechanical properties of a wider range of varieties. Further studies are needed to determine how small-scale trials compare with commercial operations, in particular when white whole clusters are pressed to low pressures. In this case, in *Lobesia*-infested regions, an extra-high pressure cycle may be warranted, discarding the extra juice produced if necessary. The incidence of larval survival during the process of wine making may be low, but with large volumes of grapes and higher infestation rates, a low survival rate could still represent a significant risk. In light of the results of the present work, fermented pomace obtained from red wine making cannot be considered a risk, deriving from crushing–destemming, punching down the cap, pumping, followed by additional pressing to obtain the press wine.

Infestation level is also a factor in the risk posed by the movement of infested fruit and subsequently the risk posed by winery waste. Given that flight populations in the spring of 2010 were very high in the core infested area of Napa Valley, it is very likely that the spread in California may have taken place from infested loads in prior years. Presently, infestation levels are substantially reduced. Regulations require economic resources from the government to enforce and from the growers to comply. Determining which pathways of spread have the highest risk allows for better use of the limited resources available. Based on our results, the movement of infested fruit to the winery is a substantially higher risk than that posed by winery waste. Because we only studied the survival of larvae on green winery waste, further studies are needed to determine the risk of larval survival in the process of unloading the fruit or during the cleaning of containers and machinery. Nevertheless, the most rigorous crushing and destemming methodologies are advisable in quarantine situations where extra insurance is needed against the potential spread of *Lobesia* via winery waste, especially if there is no negative impact on juice quality.

Table 3. Juice composition for five grape varieties, whole cluster press or destemmed, and berries pressed to 1 or 2 bar

Var	Grape pressing	Maximum pressure applied (bar)	°Brix	pH	Titratable acidity (g/liter)	Tartaric acid (g/liter)	Malic acid (g/liter)	Potassium (mg/liter)	Yeast assimilable nitrogen (mg/liter)
CS	Whole cluster press	1	22.6	3.49	5.2	6.1	2.2	1,755	36
	Whole cluster press	2	21.4	3.46	5.1	6.0	2.1	1,637	32
	Crushed–destemmed	1	22.4	3.43	4.8	5.6	2.0	1,673	44
	Crushed–destemmed	2	21.7	3.45	4.7	5.6	2.0	1,684	28
CH	Whole cluster press	1	19.9	3.18	8.4	7.4	4.6	1,731	186
	Whole cluster press	2	19.3	3.20	7.4	6.8	4.0	1,677	127
	Crushed–destemmed	1	20.1	3.19	7.6	7.1	4.1	1,688	148
	Crushed–destemmed	2	20.3	3.26	7.0	6.6	4.2	1,814	175
SB	Whole cluster press	1	19.6	3.22	6.6	6.9	2.9	1,556	127
	Whole cluster press	2	20.1	3.34	6.0	6.7	2.7	1,752	135
	Crushed–destemmed	1	19.5	3.34	5.9	6.5	2.8	1,668	133
	Crushed–destemmed	2	18.9	3.28	6.3	6.8	3.0	1,739	124
GT	Whole cluster press	1	21.2	3.52	5.7	5.9	2.2	2,047	84
	Whole cluster press	2	21.4	3.49	5.0	6.2	1.9	1,905	86
	Crushed–destemmed	1	21.0	3.50	4.7	5.7	2.1	2,027	76
	Crushed–destemmed	2	21.8	3.53	4.8	5.6	2.4	2,017	89
YM	Whole cluster press	1	20.2	3.52	3.4	4.4	1.7	1,426	80
	Whole cluster press	2	21.4	3.72	3.0	4.2	1.9	1,793	100
	Crushed–destemmed	1	20.9	3.57	3.2	4.2	2.1	1,617	80
	Crushed–destemmed	2	21.2	3.75	2.9	4.2	2.0	1,863	85

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