Evaluation of Northern Grape Hybrid Cultivars for Their Susceptibility to Anthracnose Caused by *Elsinoe ampelina*

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Abstract

Use of winter-hardy grape cultivars has enabled expansion of wine grape production in the province of Quebec, Canada, but some of the cultivars have been reported as susceptible to grape anthracnose, a serious disease. The disease causes grape leaves and berries to dry up and drop prematurely, resulting in poor or no yield and, in cold climates, reduced winter survival. On susceptible cultivars, anthracnose management is costly, making grape production unprofitable. Therefore, cold climate grape cultivars were evaluated for their susceptibility to leaf infection by *E. ampelina*. Plants were grown from dormant cuttings and on each plant the youngest three leaves were tagged and inoculated with a conidial suspension of *E. ampelina* (1×10^6 spores per ml). Immediately after inoculation, plants were maintained under high humidity conditions at $24 \pm 2°C$ for 72 h. The number of lesions per leaf was determined 14 days after inoculation. Cluster analysis was used to group the cultivars based on their susceptibility. The cultivars were classified as: (i) resistant or slightly susceptible − DM 8521-1, ES 10-18-30, St-Pépin, Sabrevois, Vidal banc, Baltica, Frontenac gris, Ste-Croix, Somerset, Frontenac, Seyval blanc; (ii) susceptible − Muscat de Swenson, Geisenheim, La Crescent, Frontenac blanc, Louise Swanson, Delisle; and (iii) highly susceptible − Swenson White, Vandal Cliche, Traminette, and Marquette. Knowledge of the susceptibility of grape cultivars to anthracnose will help growers to make prudent cultivar choices when new vineyards are established.

Introduction

Cold climate wine grape production is an emerging business in the province of Quebec, Canada. Vineyards are being established either to replace apple orchards or as a new crop and appear to stimulate agro-tourism, especially in the south-eastern part of the province in an area called the "wine road." It is generally not recommended to grow tender (non-winter-hardy) grape cultivars in cold climates because they tend not to withstand the lowest winter temperatures (13). Cold-climate or winter-hardy cultivars are mainly French-American hybrids also known as inter-specific hybrids that were developed mainly in Minnesota, but also by breeders in Quebec (13). These cultivars can survive winter temperatures of -30 to -37°C and hence represent a good option for the growers in cold climate regions. However, some of these cultivars were reported to be highly susceptible to anthracnose caused by the fungus *Sphaceloma ampelinum* de Bary (teleomorph *Elsinoë ampelina* Shear) (15).

Fig. 1. Lesions caused by *Elsinoe ampelina* on a grape leaf. showing typical shot-hole appearance (cv. Vandal Cliche).

Grape anthracnose was first reported in Europe (2), and the exact history of the disease in North America is not known. It is likely that the pathogen was introduced into North America through plant material imported from Europe in the mid-1800s. The pathogen infects leaves, tendrils, shoots, petioles, and immature berries (11). On the leaves, the fungus causes small, circular, black or brown spots. As lesions mature, the center of the lesion drops out, creating a shot-hole appearance (Fig. 1). On the petioles and green shoots, *E. ampelina* causes deep, elongated cankers, with a greyish centre and black edges (Fig. 2). Under favorable conditions for disease development, numerous lesions develop on the shoots, predisposing them to breakage in windy weather (Fig. 3). Following severe infections, the leaves shrivel and fall off (Fig. 4). Infected berries show sunken spots with a greyish center and black edges (Fig. 5). Severely infected berries dry up and drop prematurely. The disease is difficult to control once it becomes established in a vineyard.

Fig. 2. Anthracnose cankers on a shoot of grape (cv. Vandal Cliche).

Fig. 3. Vine shoot weakened by cankers caused by *Elsinoe ampelina* (cv. Vandal Cliche).

Fig. 4. Anthracnose lesions on green shoots (cv. Vandal Cliche).

Fig. 5. Anthracnose lesions on berries (cv. Vandal Cliche).

The literature on grape anthracnose is scarce, and epidemiology of the disease is not fully understood, especially under northern climates. Suhag and Grover (17) reported that in northern India, *E. ampelina* remained viable through winter on diseased shoots on the vine and on prunings debris on the ground or buried under 3 to 5 cm of soil. In northern climates, however, *E. ampelina* most probably overwinters as sclerotia (5), which may remain viable for up to 5 years as reported in temperate climates such as New Zealand's (3). In the spring, the sclerotia produce numerous conidia when a wet period of at least 24 h and temperatures above 2°C occur (1,11). Conidia are spread by free water or rain. Conidia germinate and cause primary infections if free water is present on leaf surfaces for at least 12 h and the temperature is between 2 and 32°C (10). It has been shown that the higher the temperature is, the faster infections take place (18,19). Symptoms of grape anthracnose develop within 14 days at 2°C and within 4 days at 30 to 32°C (2,18). New conidia are produced within one to five days after lesions become visible and infected leaves may produce conidia until early autumn (2).

Because *E. ampelina* can produce initial inoculum at relatively cool temperatures, reducing viability of survival structures with liquid lime sulphur before bud break is critical, especially in vineyards with a history of anthracnose (10). During the growing season, using pruning and training systems that improve air circulation, promote rapid leaf drying, and allow for full spray coverage and canopy penetration are helpful for managing anthracnose. Avoiding susceptible varieties such as those of *Vitis vinifera* and some related hybrids is also important in reducing the impact of anthracnose.

Anthracnose has the potential to cause severe crop losses on susceptible cultivars when frequent periods of wet weather occur during spring and summer (11). In such situations, anthracnose may become a serious limiting factor to grape production. In years with frequent rainfall, anthracnose can be very destructive and practically impossible to control on susceptible cultivars, even with fungicide applications every 7 to 10 days (8,9). In the province of Quebec, springs are generally rainy. Consequently, anthracnose reduces yield on susceptible cultivars and increased fungicide costs making grape production unprofitable. However, in northern areas such as the province of Quebec, the most important agronomic criterion for selecting cultivars is their ability to survive winter temperatures. For most of the winter-hardy cultivars, however, the susceptibility to anthracnose is not known.

Therefore, this study was conducted to evaluate the susceptibility of winter-hardy grape cultivars suitable for northern climates to anthracnose. It was expected that the research would facilitate informed choices among cultivars as new vineyards are established in the province of Quebec.

Evaluating Cold Climate Grape for Susceptibility to *E. ampelina*

Preparation of the inoculum. *E. ampelina* grows slowly on artificial media forming compact colonies. However, it rarely sporulates under artificial conditions (12). Hence, the inoculum suspension was prepared from grape leaves of the cultivar 'Vandal Cliche' collected in a vineyard naturally infected with *E. ampelina*. The inoculum was prepared as follows: leaves with typical symptoms of grape anthracnose disease were collected and placed in plastic trays with wetted brown paper at the bottom to maintain high relative humidity. The trays were sealed with a plastic cover and incubated at room temperature (20 to 24°C) for 2 days. The leaves were then placed in an Erlenmeyer flask containing 2 liters of distilled water and were agitated for five minutes. The suspension was filtered twice through two layers of cheese cloth and the concentration of *E. ampelina* spores determined with a hamacytometer. The final inoculum concentration was adjusted to 1×10^6 *E. ampelina* spores per ml by dilution in distilled water. Conidia of *E. ampelina* are small (3-6 × 2-8 μm), ovoid, hyaline with mucilaginous walls and one or two refringent spots (11,15).

Plant material. Cultivars currently grown or promising for cold climate regions were selected for this study (Table 1). Plant material used in this work consisted of cuttings obtained from a commercial nursery. The cuttings were

produced from canes collected in the fall in vineyards free of anthracnose. The cuttings were transplanted in 15-cm diameter pots filled with a mixture of mineral soil and peat moss (1:1, vol/vol), and kept in an unheated plastic greenhouse (22 ± 3°C). The plants were inoculated at the seven to nine true-leaf stage. Prior to inoculation, the three youngest fully expanded leaves on each plant were tagged. The tagged leaves were inoculated by misting them with the suspension containing *E. ampelina* conidia. Additional plants were sprayed with water alone and served as negative controls. Immediately after inoculation, the plants were covered with a plastic sheet to favor high humidity and were kept shaded for 48 h to avoid an excessive increase of temperature under the plastic. The number of lesions per inoculated leaf was determined 14 days after inoculation. A sub-sample of one leaf per cultivar was kept aside and used to confirm that the lesions were caused by *E. ampelina*. These leaves were first washed with tap water and isolations were performed according to standard methods for pathogenic fungus isolation. Mycelium growing out of the lesion was transferred onto potato dextrose agar medium containing antibiotics (tetracycline at 15 mg/liter and novobiocin at 100 mg/liter). Cultures were maintained at room temperature (20 ± 2°C) for one month. Total DNA was then extracted from mycelium using a commercial kit (Fast DNA spin Kit, MP Biomedicals LLC, Solon, OH, USA) following manufacturer recommendations. PCR amplification was done using ITS primers (ITS1F and ITS4) resulting of an approximately 1000-pb amplicon. For each isolate, sequence of the amplicon was compared with sequences available in GenBank (accession numbers AY826762, AY826763, and AY826764) (16).

The experiment was completed four times, twice in 2008 and twice in 2009. In each experiment, three leaves were inoculated with the pathogen on each of three replicate plants for each of 21 cultivars. In each experiment, an additional three plants per cultivar were sprayed with sterile water to serve as controls; the control plants were otherwise treated in the same manner as the pathogen-inoculated plants.

Data analysis. All statistical analyses were done with the statistical analysis system SAS (version 9.2, SAS Institute Inc., Cary, NC). The numbers of lesions on the three inoculated leaves per plant were averaged. An analysis of variance was conducted to determine if there was an interaction between experiments and cultivars and to determine the data could be combined across experiments.

The effect of cultivars on the mean number of lesions per plant was tested using analysis of variance and the Tukey means separation test (PROC GLM). The mean number of lesions per cultivar (mean of 4 experiments × 3 replicates × 3 leaves per plant) was subjected to cluster analysis in order to classify the cultivars based on their susceptibility to *E. ampelina.* The cluster analysis was done using the linked average method, allowing for a determination of the relatedness of the cultivars in their reaction to the mean number of lesions per cultivar. The classes were obtained by selecting the point, in the Ward's minimum-variance cluster analysis, where $R2$ began to decline significantly (PROC CLUSTER).

Table 1. Cultivars evaluated for susceptibility to grape anthracnose caused by *Elsinoe ampelina*.

Cold tolerance category[x]	Cultivar[yz]	Origin
Cold-climate cultivars	Baltica (Hasansky Slaki)	Danyvostochyni#60 × *V. amurensis*
	Delisle E.S. 7-5-41	E.S. 2-2-22 × Esprit
	DM 8521-1	[E.S. 283 × (*V.riparia* × Merlot)] × (*V.riparia* x Chambourcin)
	ES 10-18-30	E.S. 5-6-64 (E.S.634 × Chardonnay) × E.S. 3-16-21(E.S.643 × Cabernet Sauvignon
	Frontenac blanc	White selection of Frontenac
	Frontenac gris	Gray selection of Frontenac
	Frontenac	*Vitis riparia* 89 × Landot (L.4511)
	La Crescent	St-Pépin × (*V.riparia* × Muscat Hambourg)
	Louise Swenson (E.S.4-8-33)	E.S.2-3-17 × Kay Gray
	Marquette	Ravat noir (S8365 × Pinot noir) × MN 1094
	Muscat de Swenson (E.S. 8-2-43)	(E.S.56 × E.S.56) × SV 23-657
	Sabrevois	E.S. 283 (E.S. 114 × Seyval) × E.S. 193 (Mn 78 × Golden Muscat)
	Somerset (E.S. 12-7-98)	Unknown
	Ste-Croix	E.S.283 (E.S 114 × Seyval blanc) × E.S.193
	St-Pépin,	E.S. 114 (Mn 78 × Rosette) × Seyval blanc
	Swenson White	Edelweiss × E.S. 442 (Mn 78 × Rubilande)
	Traminette	JS 23-416 × Gewürztraminer
	Vandal Cliche	Vandal 63 (Prince of Wales × *V.riparia*) × Vandal 163 (Aurore × Chancellor)
	Vidal blanc (256)	Rayon d'or (S 4986) × Ugni blanc
Cool-climate cultivars	Hibernal (Geisenheim GM322-58)	Chancellor × Riesling
	Seyval blanc	Rayon d'or (S.4986) × S. 4995

[x] Cold-climate cultivars can withstand minimum winter temperatures of -29°C, cool-climate cultivars can withstand minimum winter temperatures of -23°C.

[y] University of Minnesota. 2008. Cold hardy grapes. www.grapes.umn.edu.

[z] University of Minnesota. 2010. Excellent wines from cold hardy grapes. www.grapes.umn.edu/wine.html.

Anthracnose and Northern Grape Cultivar Selection

Regardless of the cultivars and experiments, for all fungal isolated from lesions on leaves inoculated with the inoculum used to evaluate cultivar susceptibility, the sequence of the amplicon matches all three available sequences indicating that the lesions were caused by *E. ampelina*. No symptoms developed on plants inoculated with water alone. The interaction between experiments and cultivars was not significant ($P = 0.2764$), therefore the data from the four experiments were pooled.

There was a significant effect of cultivar on the mean number of lesions per leaf ($P < 0.0001$). Based on the Tukey test at the 0.05 level of confidence, there were significantly fewer lesions on the cultivars DM 8521-1, ES 10-18-30, and St-Pépin than on Delisle ES 7-5-41, Swenson White, Vandal Cliche, Traminette, and Marquette (Fig. 6).
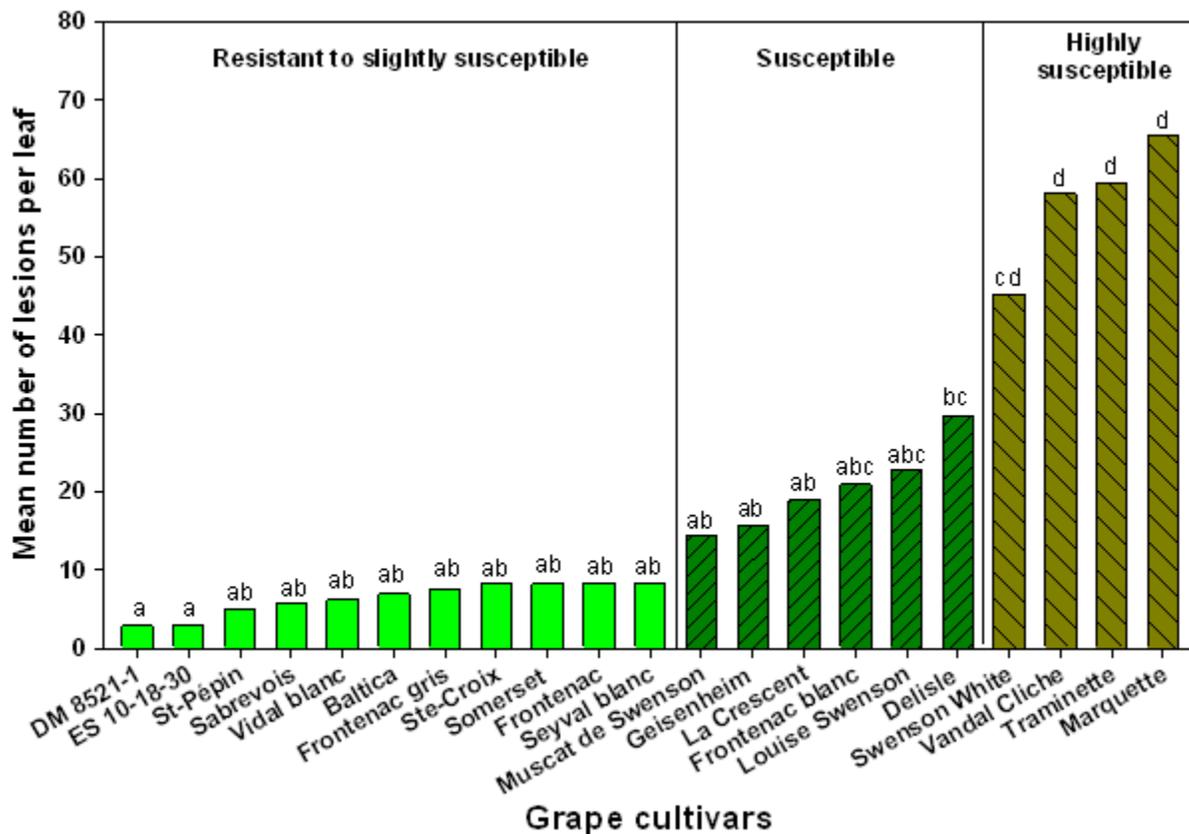
Fig. 6. Relative resistance of grape cultivars to *Elsionoe ampelina* as assessed in potted plant assays. The value of each bar represents the average number of lesions on 36 inoculated leaves. The bars with the same letters are not significantly different according to the Tukey test at the 0.05 level of confidence. The categories of susceptibility were based on the cluster analysis.

Based on cluster analysis, the cultivars were divided into three categories. Resistant to slightly susceptible cultivars included DM 8521-1, ES 10-18-30, St-Pépin, Sabrevois, Vidal blanc, Baltica, Frontenac gris, Ste-Croix, Somerset, Frontenac, and Seyval blanc; susceptible cultivars included Muscat de Swenson, Geisenheim, La Crescent, Frontenac blanc, Louise Swanson, and Delisle; highly susceptible cultivars included Swenson White, Vandal Cliche, Traminette, and Marquette. The resistant to slightly susceptible cultivars had means of 0 to 10 lesions per leaf, while the susceptible cultivars had means of 11 to 30 lesions per leaf. The highly susceptible cultivars had means of more than 30 lesions per leaf (Fig. 6). Most of the cultivars evaluated fell into the resistant to slightly susceptible category.

Little is know about resistance of grape to anthracnose. It was reported that resistance to anthracnose is controlled by three independently inherited genes; two dominant genes for susceptibility (An_1 and An_2) and one dominant gene for resistance (An_3) (6,12). The results of our evaluations of resistance are in accordance with previous reports of severe outbreaks of anthracnose in vineyards planted with the cultivar Vandal Cliche (4). Hopkins and Harris (8) evaluated grape seedlings for their susceptibility to anthracnose and concluded that cultivars inoculated with 1×10^6 conidia per ml which developed less than 10 lesions per leaf were resistant and those with more than 20 lesions were considered as susceptible.

The work reported here will contribute valuably to cultivar selection criteria for production of wine grapes in northern regions such as the province of Quebec. Although anthracnose resistance has been one of the most important criteria for grape breeding programs in Florida, China, and Korea (7,8,12,14,20), resistance to the disease has not been a selection criterion in most other grape breeding programs, including those for northern climates. In

northern climates, the choice of grape cultivars is mostly based on winter survival and on criteria that are related to the type and quality of wine (red, rose, white, late harvest, and ice wine). As a consequence, susceptibility to anthracnose has not generally been considered for northern grape cultivars. However, major outbreaks of anthracnose in the province of Quebec and the lack of fungicides registered for its control indicated that resistance to the disease should be considered when selecting cultivars for the region. Our results address grape growers' concerns about anthracnose susceptibility in cultivars recently developed for northern climates. It is anticipated that our assay for resistance can be optimized further and used more widely for continuing evaluations of susceptibility to anthracnose in northern grape cultivars.

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