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INFLUENCE OF GREENHOUSE MAINTENACE TREATMENTS ON GROWTH OF SEEDLING GRAPEVINES (*Vitis* spp.)

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Abstract

Vegetative clonal propagation of scions and rootstocks represent the commercial method of grapevine production; currently, techniques such as hardwood rooting, grafting, and tissue culture are readily exploited by the nursery and grapevine industries. Seedlings of highly heterozygous grapevines are not true to type, as a result they are not utilized in the mass production of planting stock for commercial grape vineyards. However, seedling growth and quality is key for plant breeding programs initiating thousands of unique seedlings each year. To investigate treatments for their impact on diverse interspecific cold-hardy grapevines, seedlings were grown under greenhouse conditions. Four weeks after transplant, seedlings were cut back to one true node (CUT), leaf thinned (LT), or left as untreated control vines (Control). Eight weeks after treatment, plants were analyzed for total node number, periderm encompassed nodes, lateral shoot number, and total number of base stems. Control and LT vines had more periderm encompassed nodes and total nodes than CUT vines, while CUT vines had the highest number of shoots arising from the base nodes of the vine.

***Key words**: grapevine breeding, hybrid grapevines, seedling nursery.*

INTRODUCTION

Commercial production of grapevines (*Vitis* spp.) in North Dakota is limited by short growing seasons and extreme winter freeze events (Hatterman-Valenti et al., 2014; Svyantek et al., 2020). For these reasons, the North Dakota State University Grape Germplasm Enhancement Project (NDSU-GGEP) generates seeds each year from novel crosses across wide genetic backgrounds to combine desirable traits contributing to yield components, fruit composition, and abiotic and biotic stress resistances. The timeline for crossing and seedling production is a multi-year process for generational progress from seed to seed due to the extended period of grapevine juvenility. The annual schedule of activities is summarized in Figure 1.

Using gibberellic acid treatments to reduce stratification requirements, the earliest harvested field-crossed seeds can begin germination in late October-to-early November most years. Using greenhouse conditions to extend the growing season and enhance seedling growth, seedlings are grown in a controlled environment for as many as seven months before hardening-off and field transplant. Due to the limited growing season of North Dakota's climate, seedling field transplant is delayed until after the threat of spring frost has subsided, and typically occurs from June to July depending on the number of seedlings to be field planted.

Ideal seedlings for transplant have excellent periderm development, with lignified wood protecting vines from immediate desiccation and increasing the potential height of trunks surviving winter. A single, strong trunk enhances the likelihood of fruit production in the following season. For these reasons, early seedling germination may be considered as part of a breeding scheme to develop larger plants for field planting.

Efforts have been made to characterize seedling treatments for the purposes of disease resistance screening and induction of precociousness (Srinivasan & Mullins, 1978;

Srinivasan & Mullins, 1981; Hopkins & Harris, 2000). Likewise, seedling production has been explored and emphasized in numerous, field-planted, seed propagated woody plants (Mexal & Landis, 1990; Fraysse & Crémière, 1998). Wholesale nurseries frequently utilize seedling production to produce rootstocks for a variety of plants because the method is both low cost and convenient for producing massive amounts of rootstock for grafting purposes (Beckman & Lang, 2002) However, despite its importance to breeding vineyard establishment, grapevine seedling production is rarely investigated due to the vegetative nature of commercial grapevine cultivar propagation.

Early germination may generate larger, more vigorously establishing seedlings for field planting, thus decreasing time until fruit production, evaluation, and selection in the context of a breeding program. This may conversely lead to increased labor and chemical requirements within the greenhouse stemming from the extended duration of intensive seedling care within greenhouses.

The aim of this research was to screen grapevine seedlings with a diverse genotypic background for their response to treatments that improve ease of maintenance throughout extended durations under greenhouse conditions.

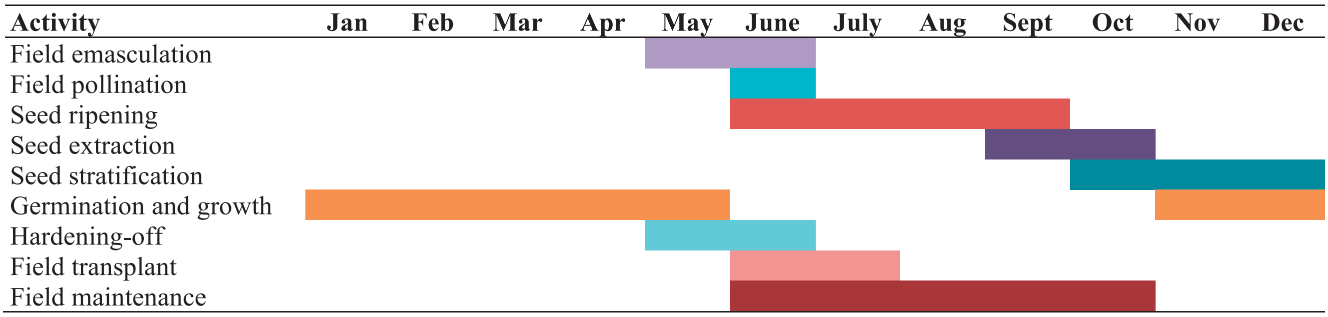

Figure 1. General schedule of seedling production for the North Dakota State University Grape Germplasm Enhancement Project

MATERIALS AND METHODS

Growth conditions

Seedlings were germinated and grown in a greenhouse under a 16:8 light:dark photoperiod with temperatures maintained at 30°C. Seeds were sown in Pro-Mix BX General Purpose potting media (Premier Tech Horticulture, Quakertown, Pennsylvania, USA) placed into 1 L pots. Individual seedlings were transplanted into individual D40H Deepots (Stuewe and Sons, Inc., Tangent, Oregon, USA) at the two-to-four leaf stage. Fertilizer was applied throughout the experimental period as a biweekly drench of 20-20-20 fertilizer adjusted to 400 ppm of nitrogen. Irrigation was provided daily. Four weeks after D40H transplanting, management treatments were applied.

Experimental treatments

A 4x3 factorial was employed in a completely randomized design in the spring of 2019. The first factor (genotype) was a result of specific crosses conducted in the spring of 2018. Parents used in seedling cross development were representative of the interspecific hybrid, cold-hardy grapevines used in the NDSU GGEP. The second factor (management treatment) was conducted at three levels, manually applied to actively growing seedlings. Leaf thinning (Leaf) was conducted for the first six nodes of leaf thinned vines; leaf removal was conducted using a manual pruner. Cutting back of vines (Cut) was conducted to the first true bud above the cotyledon using a manual pruner. Control vines were not treated.

A total of six populations were constructed using E.S. 10-18-14 (ES), ND.1.35.35 (ND), 'Marquette' (MQ), 'Prairie Star' (PS), and TP 2-2-50 (TP) as parents in a NC Design II factorial design with E.S. 10-18-14 and ND.1.35.35 serving as seed parents. Bagged pistillate clusters of ND.1.35.35 were exposed to pollen collected from 'Prairie Star', 'Marquette', and T.P. 2-2-50. Emasculated clusters of E.S. 10-18-14 were bagged and pollinated by 'Prairie Star', 'Marquette', and T.P. 2-2-50. However, due to poor seed set, only seedlings derived from the E.S. 10-18-14 cross with 'Marquette' were utilized in this study.

Parent description

E.S. 10-18-14 (E.S.5-6-64 × E.S. 3-16-21) is a slip-skin, tight clustered, perfect flowered white wine breeding line with both 'Chardonnay' and 'Cabernet Sauvignon' as *V. vinifera* grandparents (Chateau Stripmine, 2022). It yields fruit with acceptably low levels of titratable acidity for the region.

A pistillate breeding line, ND.1.35.35 came from a 2015 cross between E.S. 5-8-17 and 'Louise Swenson' (syn. E.S. 4-8-33). ND.1.35.35 was first selected in the program in 2017; it has small, early ripening berries with unique fruity aroma and pubescent backed leaves.

'Marquette' (MN 1094 × 'Ravat Noir' [syn. Ravat 262]) is a 2006 commercial release from the University of Minnesota grapevine breeding program (Hemstad and Luby, 2008). 'Marquette' is grown widely throughout the Upper Midwest region of the United States.

'Prairie Star' (E.S. 2-7-13 × 'Alpenglow' [syn. E.S. 2-8-1]) is a slip-skin, conical clustered, white wine grape from the late Elmer Swenson's breeding program (Clark, 2019; Chateau Stripmine, 2022).

T.P. 2-2-50 ('Montreal Blue' syn. E.S. 5-3-16 × 'Jukka' [syn. E.S. 6-4-47]) is the result of a 2000 cross conducted by private breeder Tom Plocher (Chateau Stripmine, 2020). It has large, slip-skin berries and long, loose clusters when grown in Fargo, ND.

Data collection and analysis

Eight weeks after application of management treatments, data were collected. Focusing on the health of above ground plant matter, seedlings were evaluated for number of nodes on the main stem, periderm encompassed nodes, number of lateral shoots, and number of stems branching from the base nodes (true buds and cotyledon buds).

Statistical analysis was conducted in JMP Pro 15.0.0. The experiment was evaluated as a mixed model with genotype, treatment, and genotype-treatment interaction set as fixed effects, and replicate set as a random effect. Significantly different Least Squares means estimates were separated using all pairwise comparisons according to Tukey's honestly significant difference (HSD) test. Graphics were generated with R software using the ggplot2 package (Wickham et al., 2021).

RESULTS AND DISCUSSIONS

The genotype main effect of ES×MQ seedlings produced the greatest number of periderm-encompassed nodes, though not statistically different from ND×MQ. Within the treatment main effect, Cut vines produced the fewest number of periderm encompassed nodes (0.7), while Control (4.6) and Leaf (3.9) produced the most. A significant genotype-treatment interaction was detected for the number of periderm-encompassed nodes; ES×MQ - Control (6.5), ES×MQ - Leaf (5.1), and ND×MQ - Control (4.7) had the most, contrastingly, ND×TP - Cut (0.1), ND×MQ - Cut (0.8), and ES×MQ - Cut (0.5) had the fewest.

Table 1. Plant growth eight weeks after treatment application to grapevine seedlings under greenhouse conditions

Treatment	Periderm nodes (no.)	Total nodes (no.)	Lateral shoots (no.)
Genotype (G)[z]			
ND×MQ	3.2 ab[y]	19.2 ns	4.8 ns
ND×PS	2.8 B	17.1	5.2
ND×TP	2.3 B	17.9	4.3
ES×MQ	4.0 A	18.3	3.9
Treatment (T)			
Control	4.6 A	19.6 a	5.3 a
Cut	0.7 B	15.9 b	2.0 b
Leaf	3.9 A	18.9 a	6.4 a
G×T			
ND×MQ - Control	4.7 ab	20.1 ns	5.3 ns
ND×PS - Control	3.6 bc	18.7	7.2
ND×TP - Control	3.7 bc	19.3	3.8
ES×MQ - Control	6.5 A	20.5	5.0
ND×MQ - Cut	0.8 E	16.5	2.4
ND×PS - Cut	1.5 cde	15.4	2.4
ND×TP - Cut	0.1 E	16.2	2.2
ES×MQ - Cut	0.5 de	15.5	0.8
ND×MQ - Leaf	4.2 B	21.0	6.7
ND×PS - Leaf	3.2 bc	17.2	6.0
ND×TP - Leaf	3.1 bcd	18.3	6.8
ES×MQ - Leaf	5.1 ab	19.0	5.9
P value			
G	0.0017	0.0708	0.3827
T	<.0001	<.0001	<.0001
G × T	0.0407	0.6804	0.4907

[z]Seedlings of crosses between specified parents: MQ= 'Marquette'; ND= ND.1.35.35; PS= 'Prairie Star'; TP= T.P. 2-2-50.

[y]Means separated within effects by Tukey's HSD at α=0.05; ns= Not significant.

No genotype main effects or genotype-treatment interaction effects were detected for total node number or lateral shoot number. The treatment main effect was significant in both cases. Control and Leaf vines had more nodes and more laterals than Cut vines.

Cut vines had a higher percentage of stems sprouting from the base cotyledon and true buds (Figure 2). Leaf and Control vines rarely had more than one base stem, this likely contributed to their greater number of total nodes for a single main stem.

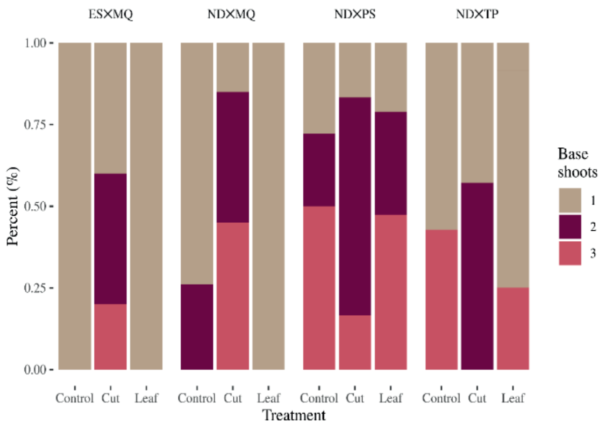

Figure 2. Percent of grapevines with one, two, or three total base shoots stemming from the first true node and cotyledon nodes, separated by genotype and manual vine treatments

In dissecting the impact of total number of base shoots on the growth of the longest shoot, no significant linear relation was found (data not shown). However, base shoot number was negatively correlated (R= -0.45, p=0.0009) with the number of periderm-encompassed nodes for plants within the control treatment (Figure 3). The regression equation indicated that for each stem, nearly two fewer nodes were encompassed by periderm.

Examining the relationship between total nodes and periderm-encompassed nodes revealed control vines with a greater number of total nodes had an increase in periderm encompassed nodes (Figure 4). This relationship, however, was not significant for either Cut vines or Leaf vines. Possible causes for these discrepancies include the reduced length of time for plant growth between the time of treatment (cutting or leaf removal) and the time of monitoring. While eight weeks eclipsed between treatment and measurement, there is a lag time between the cutting back of grapevines and the bursting of latent buds. This period before shoot regrowth may have given control vines effectively three to four extra weeks of growth compared to cut vines, this is reflected in the differences in total node number between the two treatments (average number of nodes: Control=19.6, Cut= 15.9).

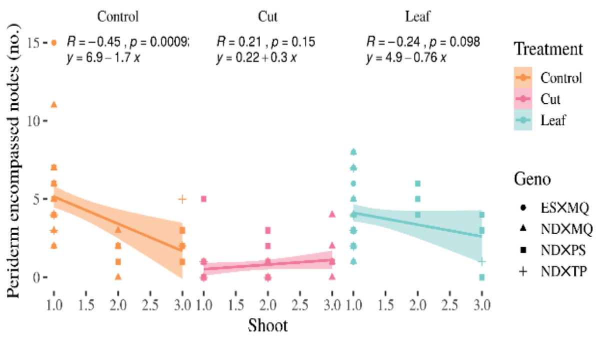

Figure 3. Relationship between total number of base shoots (x) and periderm encompassed nodes (y) for grapevine seedling populations following three management treatments

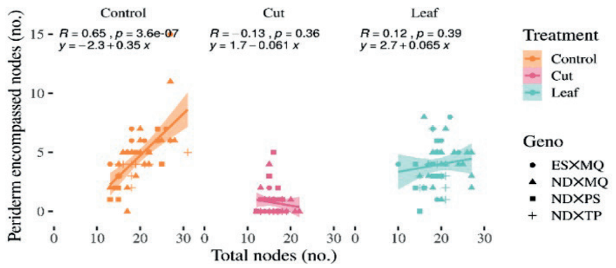

Figure 4. Relationship between total number of nodes (x) and periderm encompassed nodes (y) for grapevine seedling populations following three management treatments

Further work may strive to identify ideal container dimension and time in given container sizes to maximize root proliferation, water use efficiency, and transplant survival. Additional evaluations investigating treatments on seedling growth conditions for their impact on grapevine physiology, root distribution, and subsequent seedling establishment will aid in refining efficiency of grapevine seedling production for breeding programs. Fertilizer, irrigation, lighting, container size, and staking are also necessary topics for investigation by researchers hoping to isolate ideal grapevine seedling production conditions.

From the results of our study, the Cut treatments, which reduced the total vine size, should not be recommended for seedling production in their current format. From a practical standpoint, periodic rejuvenation and reduction of leaf mass and shading via cutting back is implemented in our grapevine improvement program to reduce labor of training seedlings under greenhouse conditions. Certain techniques, such as shoot thinning to a single stem shortly after initiation of latent buds may prove indispensable in reshaping seedling vines to continue healthy, vigorous growth during prolonged durations within a greenhouse environment proceeding field planting.

CONCLUSIONS

Both crosses containing MQ as a pollen parent (ES×MQ and ND×MQ) produced a high rate of periderm encompassed nodes at the time of analysis. MQ may be a useful parent when selecting for early periderm development under greenhouse conditions; however, the consequences on field grown vines and hardiness of vines was not determined.

Cut vines had fewer periderm encompassed nodes, total nodes, and lateral shoots, but more base stems. More than one base stem is an undesirable trait and the proliferation of new shoots from lowers nodes is likely derived from the removal of dominant growing point. This practice should not be recommended for maintaining seedling stock in a greenhouse for field transplanting. However, under certain circumstances this technique triggers subsequent regrowth, and may be useful. Directly prior to field transplanting new seedlings without acclimation, this technique should be evaluated under minimal irrigation conditions, as it would allow the vine to start anew with fresh shoots in the field, rather than acclimating greenhouse grown tissue to field conditions.

Control and Leaf vines produced the most periderm-encompassed nodes. Leaf-thinning may be implemented, along with thorough training of grapevines to produce trunks rapidly under greenhouse conditions. This gives breeders the greatest opportunity to screen for cold hardiness under field conditions in the first dormant season of a grapevine’s life. It also increases the potential for the grapevine to transition to sexual maturity, set fruitful buds, and have fruit for phenotyping under field conditions in the grapevines second growing season. These two advantages would allow for faster selection and genetic advancement. The physiological implications of establishing seedling vine trunks under greenhouse conditions for selection in challenging environmental conditions is unknown.

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