The Pennsylvania State University

The Graduate School

Intercollege Graduate Degree Program in Integrative Biosciences

# THE INFLUENCE OF REPRODUCTIVE EFFORT ON ROOT DYNAMICS AND PHYSIOLOGY IN CONCORD GRAPE

A Thesis in

Integrative Biosciences

by

Huining Xu



Submitted in Partial Fulfillment
of the Requirements
for the Degree of

Master of Science

May 2008

The thesis of Huining Xu was reviewed and approved* by the following:

David Eissenstat
Professor of Woody Plant Physiology
Thesis Advisor

Kathy Brown
Professor of Post-harvest Physiology

Dawn Luthe
Professor of Plant Stress Biology

Peter Hudson
Willaman Professor of Biology
Director of Huck Institute of Life Sciences
Chair, IBIOS Graduate Education

*Signatures are on file in the Graduate School

## ABSTRACT

The effect of reproductive effort on root dynamics and physiology in *Vitis labruscana* Bailey cv. Concord grapevines was investigated in a vineyard in Fredonia, NY, USA. Grapevines were thinned to achieve target fruit crops of 100%, 75%, 50% and 25% of full cropping (~18.6 kg/vine) to establish different levels of reproductive effort. Actual three-year crop averages were 100%, 75%, 58% and 45% of full crop. The lowest level of reproductive effort resulted in a 30-56% greater aboveground vegetative growth compare to the other treatments. Arbuscular mycorrhizal colonization was found to be 36%-50% higher in the 25% treatment than in the other treatments, although colonization in all treatments was >50% root length colonized. Fine roots also tended to be higher in total nonstructural carbohydrate concentration in the 25% treatment than in the 100% treatment, although not significantly. In contrast, root production, lifespan, vertical and horizontal distribution and respiration were generally not decreased by an increase in reproductive effort. Thus, we conclude that Concord grape has remarkable ability to maintain root system functions under sustained high levels of reproduction.

*Keywords: crop load, roots, belowground carbon allocation, mycorrhiza, root lifespan, nonstructural carbohydrates.*

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## ACKNOWLEDGEMENTS

This work would not have been possible without the support and encouragement of my advisor, Dr. David Eissenstat. He not only helped me with my research, but also taught me how to think scientifically. Thanks to Dr. Dawn Luthe and Dr. Kathy Brown, my committee members for helpful suggestions for my thesis. Thanks to Kelly Link, Rick Dunst and staff of Vineyard Laboratory in Fredonia for vineyard maintenance and minirhizotron data gathering. Thanks to Tom Adams and Luis Valenzuela for general technical support. Thanks to Daniel Spade and Travis Haussener for assistance with image processing. Thanks to Daniel Spade and Jori Sharda for assistance with AM colonization assay. Thanks to Rick Marini for help on statistics. This research was supported by funds from the US Department of Agriculture Viticulture Consortium-East, Lake Erie Regional Grape Project, Grape Production Research Fund, the NY Wine & Grape Foundation and the State of New York to A.N.L and D.M.E., for which the author is grateful. I would also like to thank the IBIOS EMPP program and the program director, Dr. Teh-Hui Kao, for giving financial support to finish my work.

I would also like to thank all the members in my lab, for supporting me and sharing their experiences with me. Finally I would like to thank my parents for bringing me up and supporting me all the time

# Chapter 1

## Background

Concord grape (*Vitis labruscana* Bailey cv.*)*, a native of North America, is widely planted throughout the states of New York, Pennsylvania, Ohio, Michigan, and Washington for the juice and wine industries as well as some other industries (Gadoury et al., 2001). For example, in the Erie Region, each year about 800 growers produce more than 150,000 tons of grapes on 30,000 acres of vineyards, making a total economic impact of over $300 million dollars per year. Thus, Concord grape production is an important foundation to the economies of Chautauqua County, New York, and Erie County, Pennsylvania (Dufresne, 2007; Duncan, 2007).

Berry size, cluster size, sugar content and flavor components are important aspects for evaluating grape quality (reviewed by Williams *et al.*, 1996). These traits can be strongly influenced by reproductive effort (usually referred to as crop load or fruit level in agricultural and horticultural fields). Increasing reproductive effort tends to reduce quality of the fruit as it can reduce berry size and cluster weight as well as sugar content (reviewed by Williams *et al.,* 1996). Thus, understanding the ways reproductive effort affects whole vine dynamics and physiology is very important for the grape industries.

Multiple studies have focused on the effect of reproductive effort on grapevine morphological development and physiological response. Increasing reproductive effort has been shown to reduce shoot growth, leaf size, and total vine leaf area (Edison et al., 1993, 1995a and b). High reproductive effort can also delay wood and fruit maturity (reviewed by Williams *et al.*, 1996).

Various studies have shown leaf photosynthesis can be influenced by fruiting. Photosynthesis has been found positively correlated with reproductive effort in various species, including citrus (Iglesias et al., 2002), apple (Palmer 1992; Palmer et al., 1997; Wünsche et al., 2000), *Prunus cerasus* L (Layne and Flore, 1995), grape (Edison et al., 1995, Naoer et al., 1997), and mango (Urban et al., 2004). A possible explanation is that in low fruit producing plants, sink activity of the fruits is relatively low, which leads to more carbohydrate accumulation in the leaves. Carbohydrate accumulation has been shown to have an inhibitory effect on photosynthesis (Goldschmidt and Huber, 1992). Thus photosynthesis may be inhibited in plants with low reproductive effort.

Although there have been various studies on the effect of reproductive effort on the aboveground parts of plants, studies on the belowground functions are relatively limited. Grape root biomass has been found inversely correlated with reproductive effort at harvest time (Edison et al., 1995b; Marigoni et al., 2003). Root respiration was also shown to decrease with increasing reproductive effort in grapevines grown in large pots (Morinaga et al., 2003). However, because of the difficulty in accessing root systems and the complexity of field grown conditions, the effect of reproductive effort on the growth pattern and physiology of unconstrained root systems in the field has never been investigated.

In this study, we examined various aspects of root systems in Concord grapevines at four levels of reproductive effort over three years under field conditions, which will contribute to the understanding of the effect of reproductive effort on fruit crops at the whole-plant level.

## Chapter 2

## The influence of reproductive effort on root dynamics and physiology in Concord grape

### Introduction

Reproductive effort is defined as the proportion of total biomass allocated to reproductive structures such as flowers, fruits, and seeds, and is often expressed in terms of biomass, carbon or energy (Reekie and Bazzaz, 1986). Crop load is commonly used to indicate different levels of reproductive effort in agricultural and horticultural fields. In fruit crops, reproductive effort is widely recognized to affect fruit size, sugar content and other traits associated with fruit quality to consumers (e.g., Palmer, 1992; McFadyen *et al.,* 1996; Palmer *et al*., 1997; Naor, 1999 and 2001). Reproductive effort has also been found to affect many other aspects of plant physiology including above- and belowground vegetative growth, photosynthesis and respiration, and carbohydrate storage (Erf and Proctor, 1987; Edison *et al*. 1993, 1995a and b; Palmer *et al.,* 1997; Wünsche and Palmer, 2000; Inglese *et al*., 2002).

Most of the studies have focused on aboveground vegetative tissues as they are easy to access. Belowground parts have been much less studied. Among the relatively few studies that examined the effect of reproductive effort on roots, most have used container-grown plants (Buwalda and Lenz, 1992; Inglese *et al.,* 2002; Morinaga *et al*., 2003). Thus, there is a notable lack of studies on root responses to differential reproductive effort where roots are grown in unrestricted soil volumes under field conditions.

Root biomass partitioning is affected by both the relative demand for carbohydrates by roots compared to other competing organs and the total supply of carbohydrates from photosynthesis (Atkinson 1977). Increasing reproductive effort has been found to reduce total leaf biomass and root biomass in tomato (Heuvelink 1997), apple (Buwalda and Lenz 1992; Palmer 1992), peach (Inglese 2002) and grape (Edson *et al*., 1993, 1995b). In contrast, other studies in grape indicate reproductive effort does not affect total root mass per vine (Edison *et al.,* 1995a). While most of the research has focused on standing crop, which is the net result of roots produced over the season less those that have died, few have looked at root production and mortality separately. Root production is more directly related to carbon allocated belowground. If high reproductive effort tends to reduce production but increase root survivorship, this would minimize change in the standing crop. Thus direct estimates of both root production and survivorship gives a clearer understanding of how reproductive effort may affect belowground process.

Reproductive effort can also influence plant nonstructural carbohydrate storage. As expected, increasing reproductive effort increases total nonstructural carbohydrates partitioned to fruit (*e.g.* Edison *et al.,* 1993; Morinaga *et al.,* 2003). Leaf starch concentration often decreases with an increase in reproductive effort (Wünsche and Palmer, 2000). Root starch concentrations also may diminish as found in citrus (Duncan and Eissenstat 1993), French prunes (Ryugo *et al.* 1977) and peach (Inglese *et al.* 2002).

Respiration can be affected by carbohydrate (substrate) supply (Atkins and Tjoelker 2003). Thus, a decrease in starch and sugar content in tissues of plants with high reproductive effort may cause reduced respiration. For example, in a greenhouse study, Morinaga *et al*. (2003) found fine root respiration was higher in non-fruiting than high-cropping vines at all stages of fruit growth.

In this study, we examined various aspects of biomass production, storage and physiology of shoots and roots of potentially high-yielding grapevines at four levels of reproductive effort over three years under field conditions. We hypothesized that a linear increase in reproduction would lead to linear decreases in dry matter partitioning to roots, root longevity, root nonstructural carbohydrate storage, root respiration, and mycorrhizal colonization.

## Materials and methods

### Study site

The study site was located at the Fredonia Vineyard Lab in Fredonia, NY, USA. Plants were mature, 25-yr-old *Vitis labruscana* Bailey cv. Concord grapevines planted in 1978, with permanent arms 1.8 m aboveground and spaced at 2.4 m between vines and 2.7 m between rows. In the spring of 2004, these vines were pruned to 120 buds per vine to have a high crop potential among which 16 uniform 5-vine plots were identified and selected for our study.

The experimental design was a randomized complete block with four levels of reproductive effort (4 blocks x 4 levels of reproductive effort). Vines were blocked by initial levels of reproduction prior to treatment. In 2004, 2005, and 2006, vine crops were adjusted to four levels (from about 12 to 30 kg $m^{-3}$) by cluster thinning thirty days after bloom, with targets of 25, 50, 75 and 100% of full reproductive effort (crop). Each experimental unit had three adjacent vines within the same row, with a buffer vine on each end that received the same treatment. Drip irrigation was available for all vines to maintain adequate soil moisture.

**Root dynamics**

We used the minirhizotron technique to monitor seasonal root production and survival. Two tubes were installed for each experimental vine, 6 per experimental unit, and 96 in total. Acrylic minirhizotron tubes, 5.7 cm external diameter, and 91 cm in length, were installed 30° from vertical in the soil in late summer of 2003, approximately 0.5 m between adjacent experimental vines, and approximately 0.5 m from their trunks perpendicular to the rows on the south side of the vines with 10 cm of tube protruding above the soil surface. A hole in the top of the tube was used to fix the indexing handle. Tube sections that were not covered by soil were wrapped with black electrical tape, and capped with a rubber stopper to prevent moisture and light from getting into the tube. The portion of the minirhizotron above the soil surface was covered by white cans to minimize radiant heat exchange.

Images of roots visible in the windows were collected every two weeks with a miniature video camera system (Bartz Technology, Santa Barbara, CA, USA) beginning in April 2003 through the growing season until November. Dates that individual roots were produced and when they turned black or disappeared (dead) were recorded using specialized software (WinRhizoTron; Regent Instruments Inc, Quebec, Canada). Roots produced in one growing season were followed through the first imaging date of the following year. In Concord grape, approximately 90% of past season's $1^{st}$- and $2^{nd}$-order roots are dead by this time (Anderson *et al*. 2003). Root birth date was recorded as the midpoint between when a root was first observed and the previous date. Root death date was similarly recorded as the midpoint of the date a root was first observed to turn black or to have disappeared and the previous observation date. Root lifespan was calculated as the number of days between root birth and death. Roots that did not turn black or disappear were treated as censored (removed from the population). Number of roots appearing in each window on each video date was recorded and used for the calculation of root production. Soil depth of each window was calculated based on the location and size of the windows as well as the installation angle.

**Fine root distribution**

In August 2006, about 10 d before veraison (the time that berries turn from green to red and start to accumulate sugar), soil cores with a diameter of 4.5 cm and 60 cm in length were collected at 25, 50 and 75 cm from the trunk of the treatment vine and perpendicular to the row of the vines in each of three vines per experimental unit

(Figure **1** ). A second set of cores was taken 25 cm from the row middle and 40, 80 and 120cm from the vine trunk and parallel with the row of the vines. Soil cores were divided into 3 depth increments: 0-20 cm, 20-40 cm, and 40-60 cm and pooled over the tree vines for each depth x distance combination. Roots were washed from the core samples by hand in late August and early September 2006. In this root washing procedure, core samples were sifted through a fine screen (0.5mm x 0.5mm mesh) in running water and all living roots were collected and rinsed. Coarse roots were separated from fine roots, and all the roots were oven-dried at 70°C for 48 hours and weighed. The samples used for this study included only roots less than 1mm. In order to account for the influence of the soil residue on root mass, subsamples were ashed at 500°C for 10 hrs and weighed; data were expressed on an ash-free dry mass basis. Fine root spatial distribution was estimated from root mass density in the soil core taken from each distance and depth.

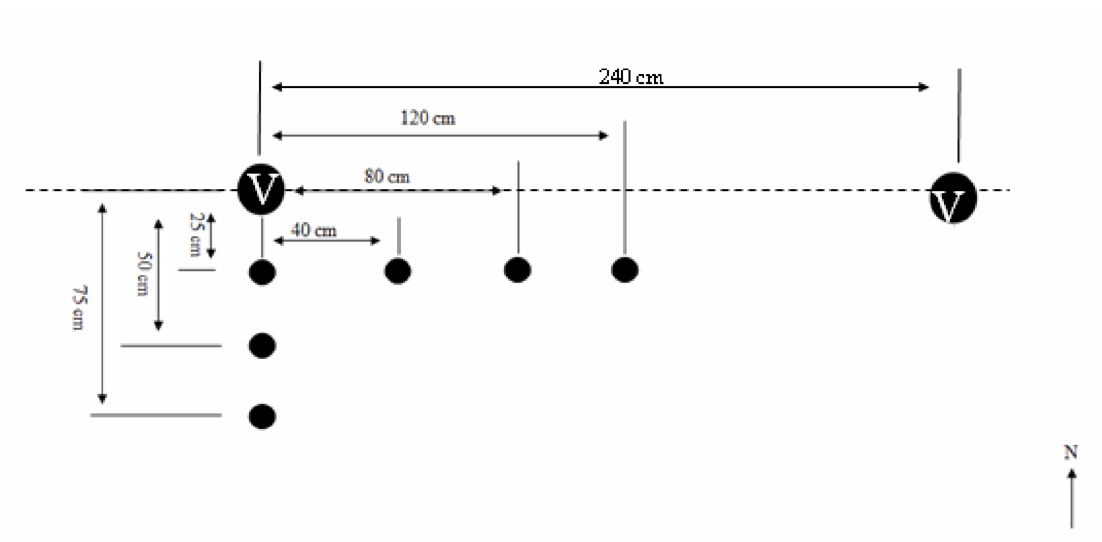


Figure **1**: Locations of soil coring (solid circles) for estimating root standing crop and root distribution. Rows of vines are oriented east to west (indicated by horizontal line through soil coring location). Soil cores with a diameter of 4.5 cm and 60cm in length were taken at 25, 50 and 75 cm from the trunk of the treatment vine (circle with V) and perpendicular to the row of the vines. A second set of cores was taken 25 cm from the row middle and 40, 80 and 120cm from the 25-cm-perpendicular core, parallel with the row of the vines.

To estimate total fine root mass per vine, root mass density was averaged over the cores sampled at different distances from the vine trunk for each depth interval, as no significant differences were found among cores at different distances from the vine for a particular depth interval. Total area occupied by a vine in the vineyard was estimated based on vine spacing. Total fine root biomass per vine was estimated by multiplying average root mass density by total vine area occupied, and summing over the different depth intervals.

## Mycorrhizal colonization

Root subsamples from the 0-20 cm soil cores sampled in August 2006, were examined for mycorrhizal colonization (Phillips and Hayman, 1970). Root samples were cleared in 10% KOH and stained with Trypan blue solution (1 L glycerol, 0.95 L $H_20$, 50 mL acetic acid, 0.2 g Trypan blue), using a modification of the method of Phillips and Hayman (1970). After staining, mycorrhizal colonization was assessed using a multiple-transect technique (McGonigle *et al*. 1990).

## Root respiration

Measurements were taken on only 25% and 100% treatment vines. On June 10th, woody roots about 5 mm in diameter, without fine laterals, located about 50 cm from the trunk of the vine and at a depth from 0-10 cm, were carefully excavated, placed into root bags (10 cm x 20 cm, nitrogen-free polyester with pore size of 50 microns, Ankom, NY, USA) and reburied. Four bags were buried to the northwest, northeast, southwest and southeast of each vine, of the three middle vines in each experimental unit. Twenty days later, new laterals were excised, rinsed, and placed in buffer (1 mM $CaSO_4$, 5 mM MES, adjusted to pH 5.5 with KOH) until measurements were taken. Roots were put into bag for 20 days for recovery from the disturbance and to insure that all the lateral roots were less than 20 day old to minimize the aging effect on respiration. Root respiration was determined with a Clark-type oxygen electrode system (Oxygraph, Hansatech, King's Lynn, UK) in August, 2007. Respiration was measured about 30 min after excision at a controlled temperature of 20°C. Only new roots (lightly pigmented) of 1$^{st}$ and 2$^{nd}$ order (where 1$^{st}$ order = the finest laterals with no branch roots = 1$^{st}$ order) from the vines were used for the measurement. After the measurements were completed, respiration was expressed on an ash-free dry mass basis.

## Root electrolyte leakage

In August 2007, electrolyte leakage was determined on new 1st and 2$^{nd}$-order lateral roots based on changes in electrical conductivity (Huang *et al.,* 2005).

Subsamples of roots separated from the sample intended for root respiration were used for root electrolyte leakage test. Roots were thoroughly rinsed of all soil particles and immersed in 40ml of deionized water. Percent electrolyte leakage of the sample was estimated by measuring the electrical conductivity (EC) of the water at immersion ($EC_{initial}$), after 30 min ($EC_{30}$), and after disrupting root cell membranes by boiling the sample for 5 min ($EC_{boil}$). Membrane leakage was estimated at a percent of total electrolytes in the roots:

$$\text{Electrolyte leakage (\%)} = 100 * (EC_{30} - EC_{initial}) / (EC_{boil} - EC_{initial}).$$

**Root carbohydrate analysis**

Roots less than 1 mm in diameter from the August 2006 collection were analyzed for nonstructural carbohydrates (Comas *et al.,* 2005). Samples were boiled for 30 min, and after cooling, two subsamples were digested with 0.5 M sodium acetate (pH 4.8), one subsample with 5 units of amyloglucosidase and 2.5 units of α-amylase and the other without. Concentration of reducing sugars was determined by colorimetric analysis of the supernatant extracted from each subsample with Nelson's Reagent (Nelson, 1944; Somogyi, 1952), and the two subsamples were compared to determine the amount of glucose-equivalents as soluble sugars and, by subtraction, that incorporated into starch. Nonstructural carbohydrates concentration was expressed as glucose-equivalents on an ash-free dry mass basis.

### Statistics

To examine the effect of different levels of reproductive effort on total root biomass per vine and on root spatial distribution, data were tested with PROC MIXED procedure in SAS (SAS Institute Inc., Cary, NC, USA) using a randomized complete block design. The effect of different levels of reproductive effort was considered a random effect in this study. Influence of different levels of reproductive effort on root survivorship was analyzed with Cox proportional hazards regression using PROC PHREG in SAS, which allow the influence of the "hazard" of an individual covariate is determined while all other covariates to be held constant (Cox 1972). Covariates tested included root diameter and depth of appearance. Differences at $P<0.05$ were considered significant. Proportional hazards regression was conducted separately on different cohorts of roots (bloom to 30 days after bloom, 30 days after bloom to veraison, and veraison to harvest) in each year.

## Results

### Environmental conditions and fruit production

This three-year study included a range in precipitation patterns (data not shown). Total precipitation was 39 cm, 32 cm and 42 cm, and the accumulated growing degree days was 2857, 3332, 2840 for the growing season from April to November from year 2004 to 2006 (http://lergp.cce.cornell.edu/weather.htm ).

Manipulation of reproductive effort was established by thinning clusters to different target levels 30 days after bloom each year (Table **1)** Grapes were generally high yielding throughout the study in the 100% treatment. In 2004, a clear gradient of levels of reproductive efforts was successfully established, although not exactly as targeted. However, in 2005 and 2006, the actual grape yields were quite off the target, causing a smaller gradient in reproduction than planned (Table 1). Averaged over three years, only the 25% treatment was substantially higher than targeted.

Table **1**: Cluster number and fruit biomass from each treatment at harvest for Concord grapevines in Fredonia, NY, USA. Grape biomass is expressed on a fresh weight basis.

| | Targeted reproductive effort | Cluster number ± SE (percentage) | Grape biomass (kg) ± SE (percentage) |
|---|---|---|---|
| 2004 | 25% | 111±8 (48%) | 7.0±1.1 (39%) |
| | 50% | 147±3 (64%) | 9.0±0.3 (51%) |
| | 75% | 208±7 (90%) | 15.0±0.8 (80%) |
| | 100% | 231±5 (100%) | 17.8±1.2 (100%) |
| 2005 | 25% | 147±6 (62%) | 11.4±0.4 (61%) |
| | 50% | 178±10 (75%) | 13 .3±1.4 (71%) |
| | 75% | 205±7 (86%) | 16.2±0.9 (86%) |
| | 100% | 238±4 (100%) | 18.7±0.3 (100%) |
| 2006 | 25% | 106±9 (41%) | 6.5±0.4 (34%) |
| | 50% | 151±5 (58%) | 9.8±0.3 (51%) |
| | 75% | 199±10 (77%) | 14.3±1.3 (59%) |
| | 100% | 259±3 (100%) | 19.2±1.2 (100%) |
| 3-Yr average | 25% | 121±13 (50%) | 8.3±1.6 (45%) |
| | 50% | 159±10 (65%) | 10.7±1.3 (58%) |
| | 75% | 204±2 (84%) | 15.2±0.6 (82%) |
| | 100% | 243±8 (100%) | 18.6±0.4 (100%) |

**Shoot vegetative production**

Variation in reproductive effort produced a non-linear response in shoot growth (Figure **2**). Shoot biomass in the 25% reproductive treatment was 26% to 60% higher than that in the other treatments. Vegetative biomass in the 50 and 75% treatment were very similar to that in the 100% treatment, especially in 2004 and 2006.

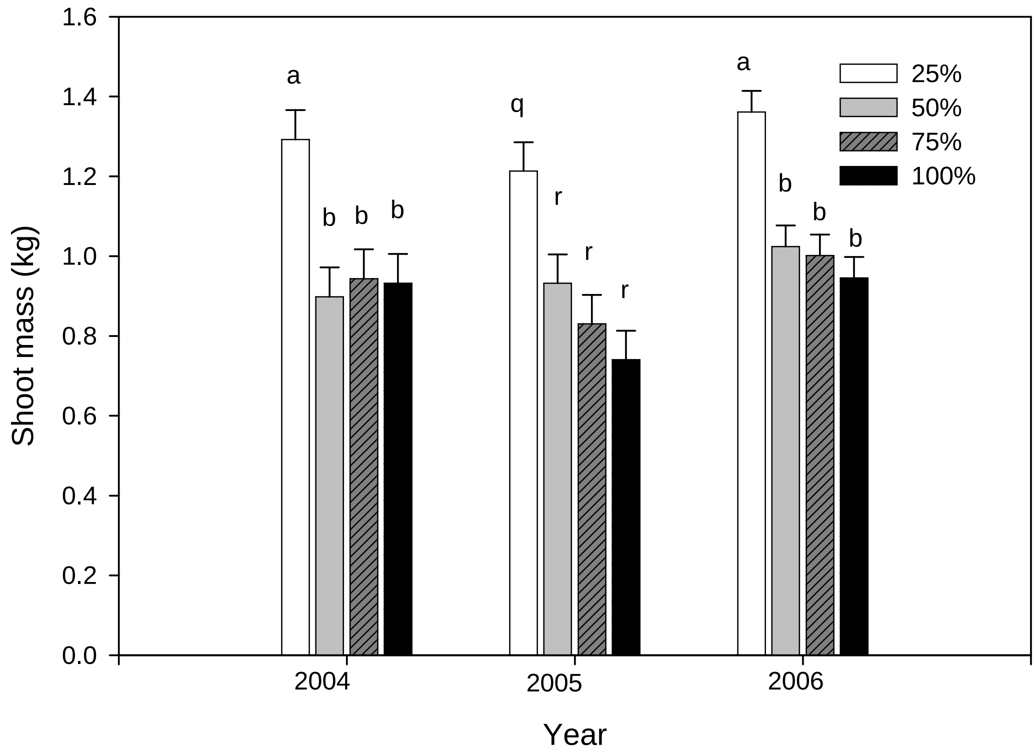


Figure **2**: Shoot mass (+SE, expressed on fresh weight basis) of winter canes of Concord grapevines in response to different levels of reproductive effort in Fredonia, NY, USA. See Table 1 for actual fruit biomass associated with targeted percentages. Within any year, a reproductive treatment followed by a different letter was significant (*P*<0.012).

**Root production**

Root production was monitored with minirhizotrons from 2004 to 2006 (Figure 3). In 2004, in order of increasing reproductive effort, the average root production was 47, 26, 87 and 49 roots $m^{-2}$ of observation area $yr^{-1}$ (pooled SE=19); no significant differences were found amongst the four treatments ($P$>0.3). In 2005, root production was lowest in the vines with the lowest reproductive effort (25% treatment, $P$=0.08), a result that was opposite our expectation. Thus, there was no evidence that a decrease in reproductive effort led to greater root production. In 2005, root production from 100%, 75% and 25% treatment all decreased from that observed in the previous year, while root production in the 50% treatment increased slightly. Interestingly, in 2006, root production was consistent with our hypothesis, exhibiting a non-significant decreasing trend as with an increase in reproductive effort ($P$>0.3). In this year, root production of 100% treatment recovered from the low level of 2005, while root production in the other treatments all dropped from the previous year. Root production was higher in 2004 than the other two years, which was probably due to the root growth stimulation following the disturbance associated with the minirhizotron installation in summer 2003 (Joslin and Wolfe, 1999) ( Figure **3** )

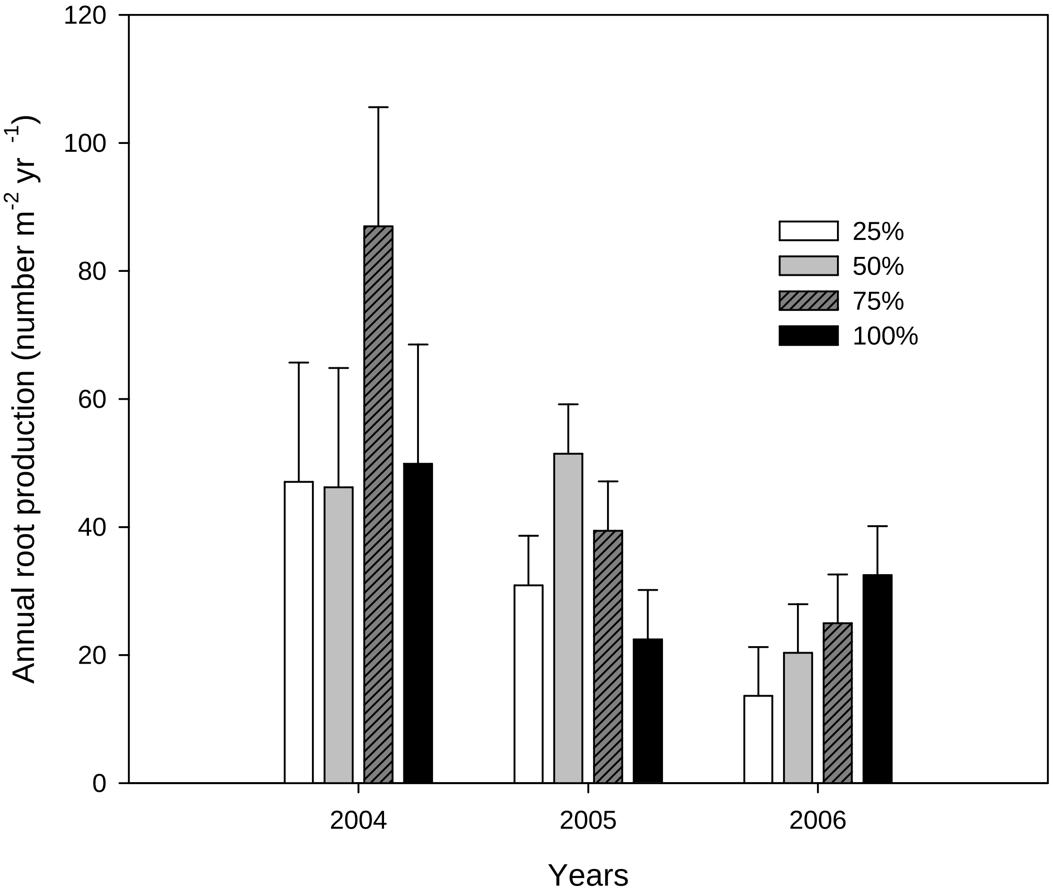


Figure **3**: The effects of reproductive effort on annual root production (number of roots $m^{-2}$ viewing area $yr^{-1}$; +SE) in Concord grapevines observed using minirhizotron tubes in Fredonia, NY, USA. (*P*>0.3 for 2004, *P*>0.08 for 2005 and *P*>0.3 for 2006). See Table 1 for actual fruit biomass associated with targeted percentages.

Temporal patterns of root production were also monitored. Reproductive effort had no consistent influence on root production in any particular month during the growing season (Figure **4** ). No significant differences were observed amongst the four different treatments in any year on any observation date (*P*>0.1). Root production began about two weeks after bloom, and reached its peak in early July. There was a rapid drop in root production beginning about two weeks before harvest, and after harvest, root production soon dropped to zero. In 2004, at peak production, the 75% treatment vines had the highest monthly root production while the 25% had the lowest. After veraison, the 75% dropped rapidly while others stayed about the same. In 2005, all the four treatments generally had similar root production patterns. In 2006, the 100% had a surprisingly higher root production at peak in mid June than the other treatments, which had very similar patterns. Unlike in other years, in 2006 some root production in all four treatments was observed in mid November.

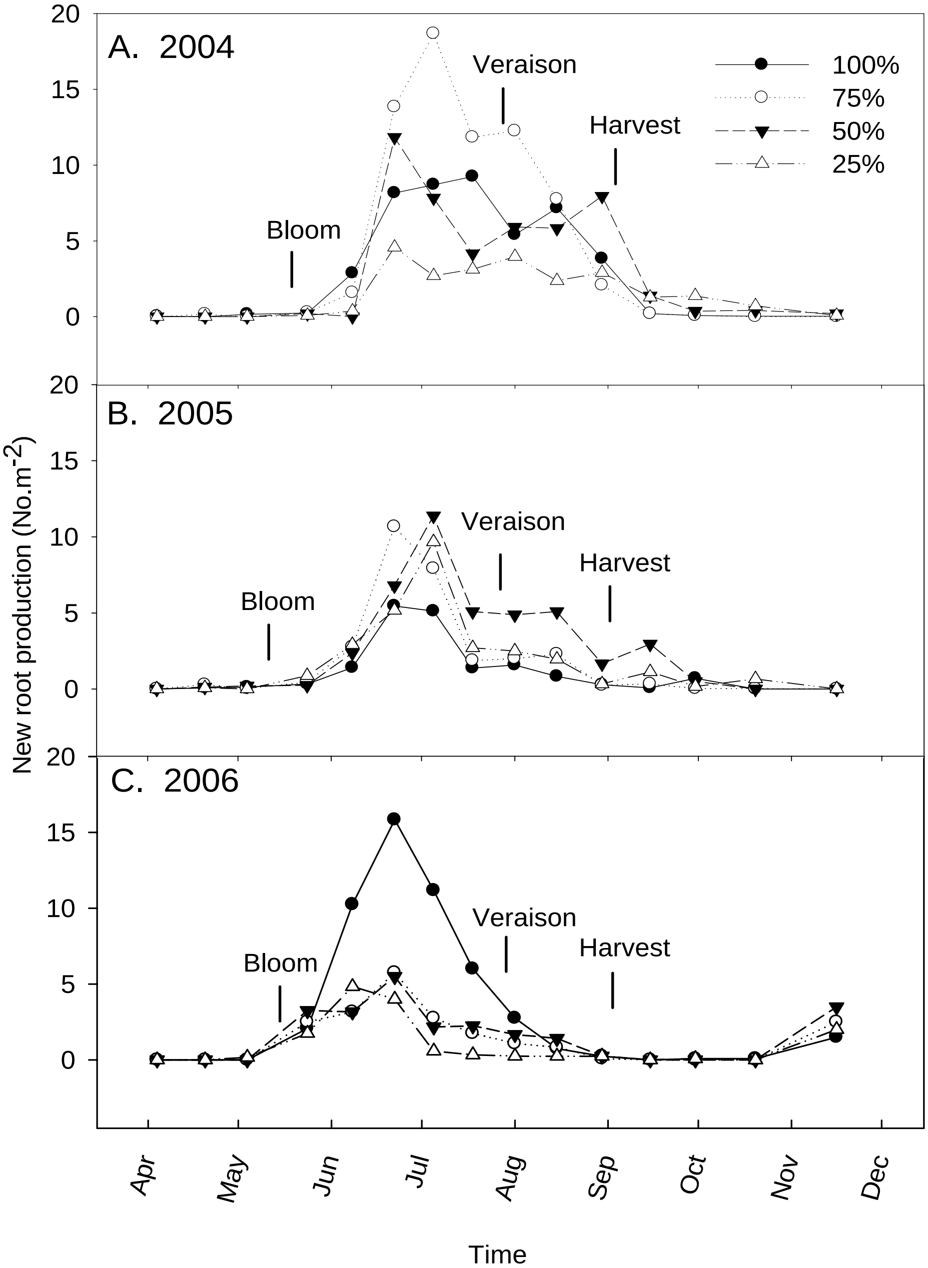


Figure **4**: The effect of different levels of reproductive effort on monthly fine root production in Concord grapevines from 2004-2006 in Fredonia, NY, USA. Shoot phonological stages for each year are indicated by vertical lines. See Table 1 for actual fruit biomass associated with targeted percentages.

**Fine root distribution and total root mass**

We expected more extensive roots for the vines with lower reproductive effort. However, this is not what we observed. For any depth layer, we found no significant differences in root density with distance for any of the treatments. We also did not observe more roots in deeper soil in vines of low reproductive effort (Figure **5** ). For the 0-20 cm depth interval, reproductive effort influenced root standing crop in a quadratic pattern: root density was about 377, 656, 890 and 457 g $m^{-3}$ with an increase in targeted reproductive effort of 25% to 100% (SE ± 141, $P$=0.03)(Figure 5), Within the 20-40 cm depth interval, treatment effect was also significant; root density for 25% treatment to 100% treatment was 139, 277, 135, and 168 respectively (SE± 33, $P$=0.04). Within the 40-60 cm depth interval, many soil cores did not contain any roots or only very few roots; no significant difference was found among different treatment ($P$>0.8). In any treatment, root density at 0-20 cm was over 200% higher than that at 20-40cm, or at 40-60cm. Roots at different distances from the vine trunk were not significantly different.

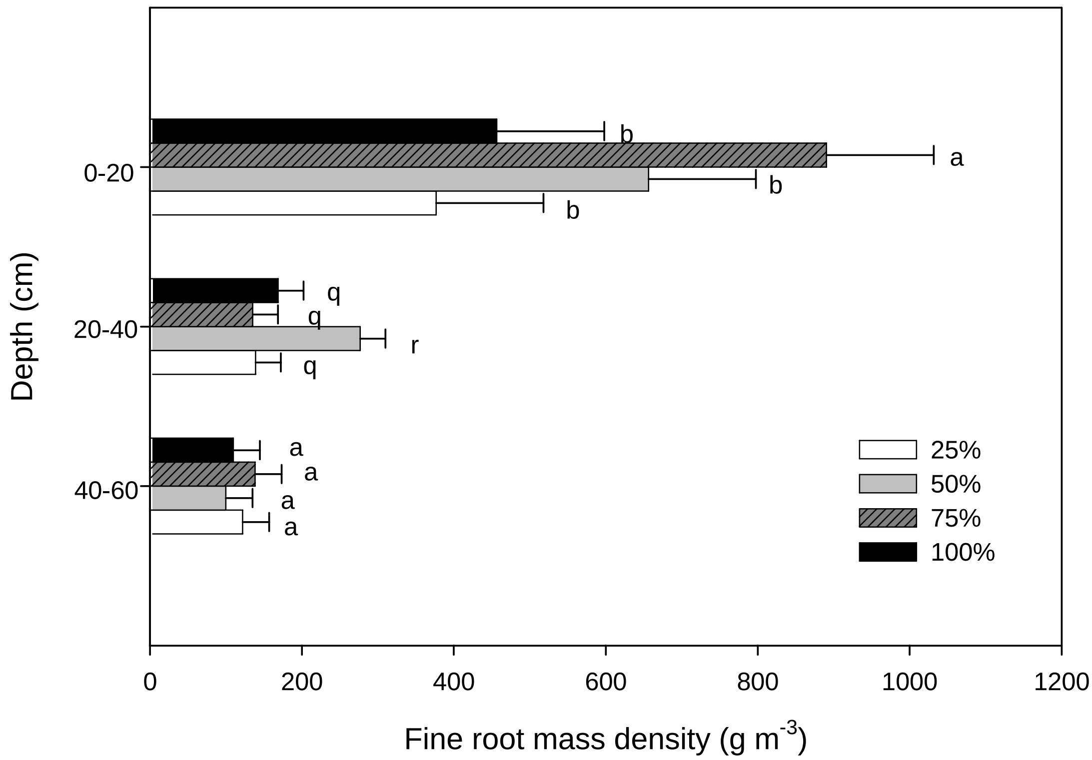


Figure **5**: The effects of three years of manipulation of reproductive effort on fine root mass density (+SE) in August 2006 in Fredonia, NY, USA. (*P*<0.04 at 0-20 cm and 20-40 cm; *P*>0.8 at 40-60 cm; mass is expressed on an ash-free dry mass basis). See Table 1 for actual fruit biomass associated with targeted percentages.

Effects of reproductive effort on total fine root biomass per vine were not in the direction hypothesized (Figure **6**). Fine root biomass per vine tended to be higher for vines of medium reproduction (50% and 75%) and lowest in the 25% and 100% reproduction treatments (*P*=0.057). Thus, our data provided little evidence to support the hypothesis that low reproductive effort allowed for greater root standing crop.

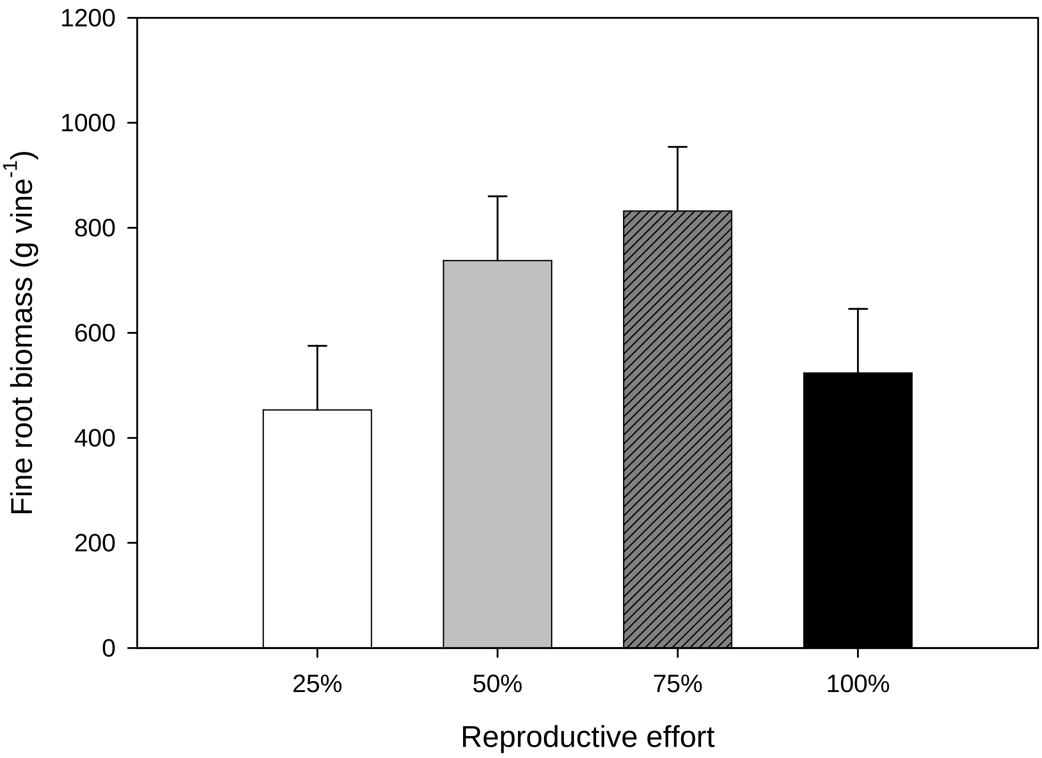


Figure **6**: The effects of three years of manipulation of reproductive effort (+SE) on total fine root biomass per vine in Concord grape in August 2006 (just prior to veraison) in Fredonia, NY, USA. (*P*=0.057, fine root biomass expressed on an ash-free dry weight basis). See Table 1 for actual fruit biomass associated with targeted percentages.

**Root respiration and carbohydrate analysis**

A decrease in reproductive effort was not associated with significant increases in respiration or in root nonstructural carbohydrate concentrations (Table **2**). The relationship of total carbohydrate concentration with increased reproductive effort was similar to that observed for shoot growth (Figure 2). There was a tendency for total non-structural carbohydrates to be highest in the 25% treatment, with plants of higher reproductive effort (50, 75 and 100%) exhibiting more similar total concentrations (Table **3** , $P$ = 0.48).

Table **2**: The effect of reproductive effort on fine root respiration in August (just prior to veraison) in 2006 in Concord grapevines Fredonia, NY, USA. Sample size 63 for the 25% treatment and 32 for the 100% treatment ($P$>0.4). See Table 1 for actual fruit biomass associated with targeted percentages.

| Reproductive Effort | Respiration Rate (SE) (μmol $O_2$ g $dw^{-1}$ $s^{-1}$) |
|---|---|
| 25% | 0.124 (0.032) |
| 100% | 0.168 (0.034) |

Table **3**: The effects of reproductive effort on fine root nonstructural carbohydrate concentrations of roots collected in August 2006 (just prior to veraison) in Fredonia, NY, USA. See Table 1 for actual fruit biomass associated with targeted percentages.

| Target reproductive effort | Soluble Sugars (g glucose equiv. $g^{-1}$ dw) ± SE | Total Carbohydrates (g glucose equiv. $g^{-1}$ dw) ± SE |
|---|---|---|
| 25% | 0.036±0.007 | 0.106±0.018 |
| 50% | 0.048±0.008 | 0.088±0.003 |
| 75% | 0.045±0.005 | 0.094±0.021 |
| 100% | 0.038±0.003 | 0.091±0.003 |

$P$>0.1 for both soluble sugar concentration and total carbohydrate concentration

**Mycorrhizal colonization**

Mycorrhizal colonization was similar among the three treatments with the higher levels of reproductive effort (50, 75, and 100%), all of which were between 50 to 54% root colonization (Figure **7**). However, AM colonization for the 25 % treatment was considerably higher than that in the other three treatments, at 73% root colonization ($P$<0.04), a pattern consistent with treatment differences observed for shoot growth and root total carbohydrates.

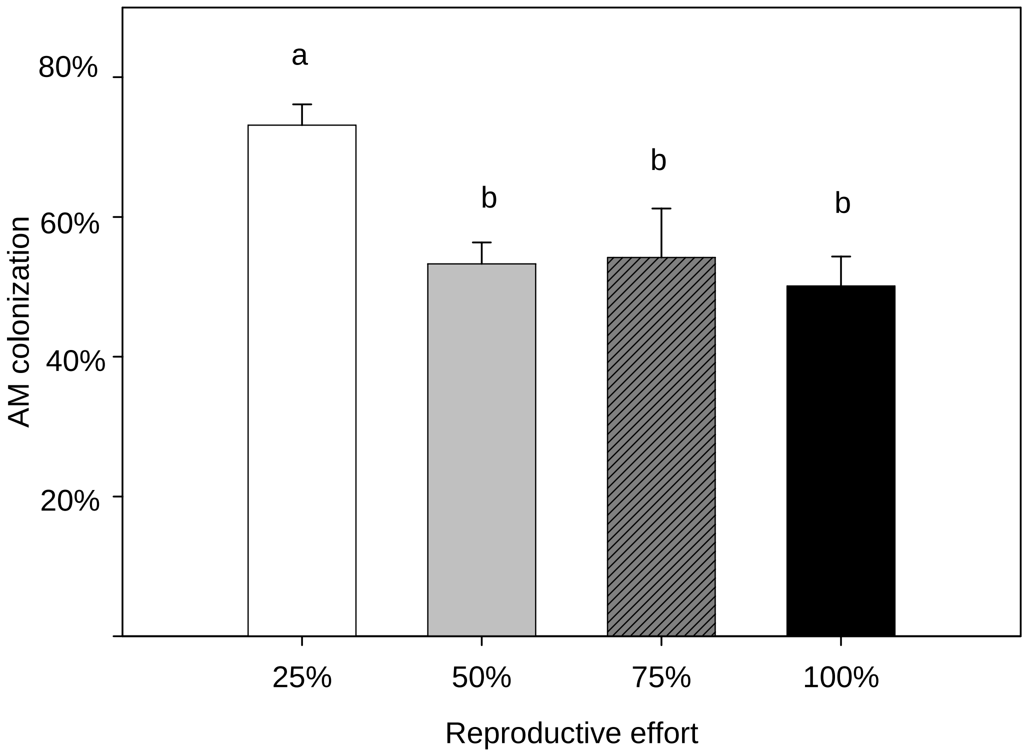


Figure **7**: The effects of different levels of reproductive effort on arbuscular mycorrhizal (AM) colonization in fine roots of Concord grapevines in Fredonia. NY, USA. Treatments followed by a different letter significantly different (*P*<0.04). See Table 1 for actual fruit biomass associated with targeted percentages.

**Root lifespan and electrolyte leakage**

Although an increased reproductive effort diminished root survivorship in 2004, this effect was not sustained in 2005 and 2006 (Figure **8**). In 2004, root survivorship decreased as reproductive effort increased in the root cohort produced between bloom and 30 days after bloom. The decrease in lifespan was fairly linear with an increase in reproductive effort ($P$<0.0001). However, in the other two root cohorts in 2004, although reproductive effort had a significant effect on root lifespan ($P$<0.0003), the response was not linearly related with level of reproductive effort. Generally, the vines of higher reproductive effort (75% and 100%) exhibited lower root survivorship than the vines of lower reproductive effort (Figure 8). In 2005, although the survivorship of the root cohort produced from bloom to 30 days after bloom and the cohort produced from veraison to harvest were significantly affected by reproductive effort ($P$<0.04), the effect was complex. Root survivorship was often higher in the 75 and 100% treatment than in the 25 and 50% treatments. In other cohorts of 2005 and 2006, we did not observe any significant effect of reproductive effort on root survivorship.

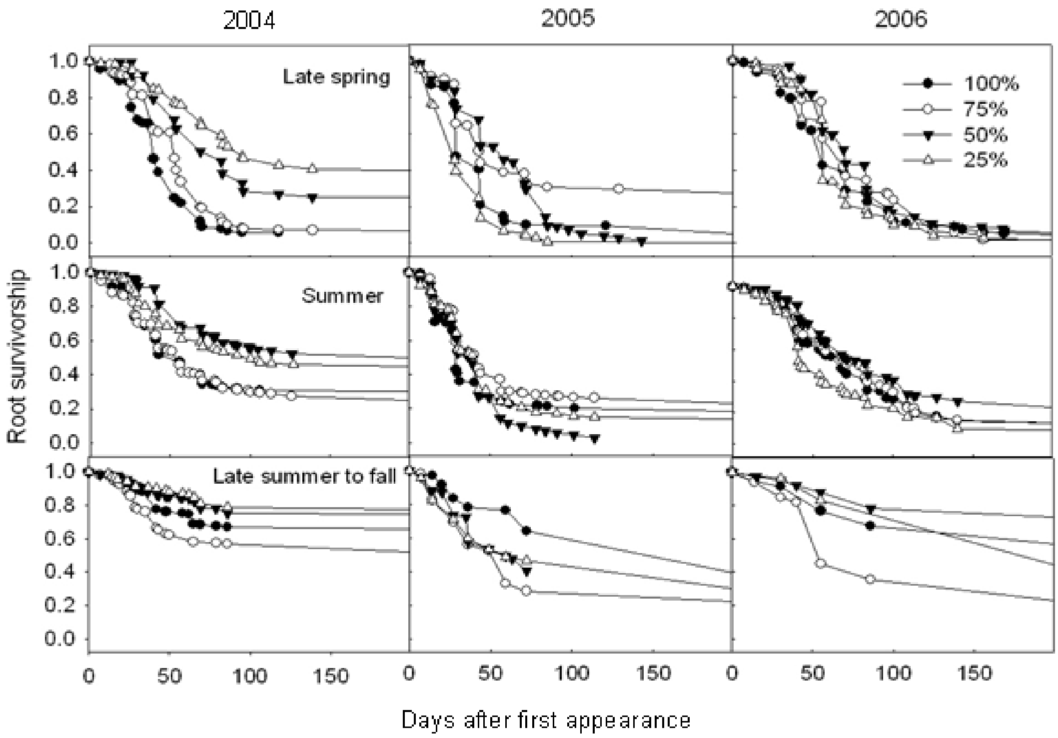


Figure **8**: Root survivorship in response to different levels of reproductive ability in Concord grapevines in Fredonia, NY, USA. Each row represents survivorship of a different root cohort: Late spring=cohort of roots born between bloom and 30 days post bloom; Summer=cohort born between 30 days post bloom and veraison; Late summer to fall = cohort born between veraison and harvest. See Table 1 for actual fruit biomass associated with targeted percentages.

In addition, electrolyte leakage of roots sampled in 2006 in the 25% and 100% treatments were similar (2.5%± 1.0% and 3.3%± 1.0% respectively). The low leakage indicated that the roots were very healthy.

Root diameter and depth have been shown to significantly affect root lifespan in Concord grape (Anderson, et al., 2003). However, in this study, only depth had a significant effect; diameter was not significant (data not shown).

## Discussion

The goal of this study was to quantify how increasing reproductive effort influences root system function. To address this question, we examined annual and seasonal patterns of root production, root distribution and total root mass per vines, carbohydrate storage, AM colonization, root respiration and root survival. Unlike the strong effects of reproductive effort on aboveground vegetative growth, effects belowground were more subtle than obvious.

In the few instances where effects were observed, the most consistent pattern was that the 25% treatment differed from the higher reproductive effort treatments. This was most evident with shoot vegetative growth (Figure 2), but also was observed with mycorrhizal colonization (Figure 7) and a similar non-significant pattern for total non-structural carbohydrates (Table 2). Surprisingly, root growth and total root standing crop were not affected consistently over the three years by manipulation of reproductive effort, despite the substantial differences in fruit production in these vines (Table 1).

While we were able to establish significant differences among the four treatments, the final reproductive biomass achieved in each treatment was not exactly as targeted (Table 1). This is probably because the reduction in individual grape clusters leads to reduced natural abortion and larger fruit size and single fruit mass (Naor 1999 and 2001, Palmer *et al*., 1997). Consequently, thinning clusters by number did not lead to a linear reduction in total fruit mass in the first year and adjustments only led to modest improvements in subsequent years.

Our study showed an increase in AM colonization in the vines with the lowest reproductive effort (Figure 7). It has been suggested that colonization can be increased by increasing carbon availability to the plant (Smith and Read 1997).

For example, in most groups of plants, increased atmospheric carbon often increases mycorrhizal colonization in roots (Tang *et al.,* 2006). Thus, this symbiosis seems relatively sensitive to changes in root carbohydrate status. However, even in the vines of high reproductive effort, a mycorrhizal colonization of 50% is generally considered adequate for nutrient uptake (Smith and Read 1997).

Although not significant, total non-structural carbohydrate concentration (Table 3) exhibited a similar pattern as that observed of mycorrhizal colonization (Figure 7) and shoot growth (Figure 2). These results are consistent with a previous study on Concord grape at the same site where reproduction was not manipulated, which found that heavy reproductive growth was generally associated with lower starch reserve in roots at the end of a season (Comas *et al*., 2005).

We hypothesized an inverse relationship of reproductive effort with root lifespan. This was observed in the first year, but was not evident in the second and third year. Lack of an effect of reproductive effort on root survivorship in 2005 and 2006 may have been the result of the vines acclimating to different reproductive demand. Annual variability in root survivorship associated with reproduction was also observed in a previous study in Concord grape examining the influence of irrigation and pruning (Anderson *et al*., 2003).

Annual root production was not related to reproductive effort (Figure 3). Vines from the 25% treatment exhibited relatively low root production in all three years. Indeed, in 2006 root production increased with an increase in reproductive effort, suggesting that vines may be producing roots to support the higher demand for nutrients associated with greater reproductive effort. Another possible explanation is that vines in the 25% treatment had greater aboveground vegetative growth (Figure 2), which should lead to greater leaf area and transpiration, causing drier soil during the summer. Dry soil can greatly restrict root growth in Concord grape (Comas *et al*. 2005).

We hypothesized that low reproductive effort should lead to a more extensive root system, with more roots further from the vine trunk and deeper in the soil compared with other treatments. Annual root production estimated from minirhizotron images at a fixed location may not be an adequate test of this hypothesis, thus, horizontal and vertical fine root biomass distribution was estimated by soil cores sampled from three different distances both in the vines rows and between the rows. However, we found no evidence that increased reproductive effort diminished the extent of the root system. In addition, total fine root biomass per vine in 2006 was reasonably consistent with total fine root production per vines estimated with minirhizotrons, except in the 100% treatment (Figure 5 and Figure 3).

Although no significant differences were found in monthly root production among treatments, the magnitude of differences among treatments was found to decrease annually over the three-year period (Figure 4). In 2004, the 25% and 50% treatment peaked in root production about 30 d after bloom, followed by the 75% treatment (about 45 d after bloom); the 100% treatment peaked latest, about 60 d after bloom. After veraison, when berries start to accumulate sugars at the highest rate, root production of both the 25% and 75% treated vines dropped rapidly. Interestingly, vines in the 50% treatment had an increase of root production over veraison and started to decrease again after harvest. Vines in the 100% treatment also had a small increase of root production after veraison but started to decline about 14 days after veraison. Differences were less significant during the following two years, suggesting that vines were becoming adjusted to their reproductive effort.

Although textbooks suggest that root production in grape can have two peaks during the year, one in spring and one in fall (Mullins *et al*., 1992), this was not observed in this location. Neither in this study (Figure 4) nor in an earlier study at this site (1997-2000; Comas *et al*. 2005) were two peaks in root production observed. Consistent with Comas *et al*. (2005), root production generally reached a peak before veraison and then decreased. Only in 2004 in the 50% and 100% treated vines was a secondary peak around harvest apparent.

We expected to see a higher root electrolyte leakage in the grapevines with higher reproductive effort as less energy was expected to be provided to the root system.

However, electrolyte leakage was not related to reproductive effort, indicating that reproductive effort did not affect the energy availability and cell integrity in roots. At the same time, root respiration did not vary between the two extreme treatments, indicating the grapevines were able to cope with different levels of reproductive effort and maintain their root vigor and cell integrity of roots under high reproductive demand.

A possible explanation of the result that reproductive effort had only subtle effects on the root system could be that the increased photosynthesis in plants with higher reproductive effort may compensate for the cost of reproductive tissues. Increasing photosynthesis in plants with higher reproductive effort has been found in apple (Palmer, 1992; Palmer et al., 1997, Wünsche and Palmer, 2000), grape (Edison et al., 1993, 1995a, b).

In conclusion, although different levels of cluster removal established large differences in reproduction of as much as 10 kg of fruits per vines in high-yielding Concord grapevines, I found little sustained impact on root respiration, root lifespan, fine root biomass production and distribution. Our data suggest that mature Concord grapevines on good sites cope effectively with the high carbon demand for reproduction.

## Chapter 3

## Synthesis

In conclusion, although different levels of cluster removal established large differences in reproduction, as much as 10 kg of fruits per vine, in high-yielding Concord grape vines, I found little sustained impact on root respiration, root lifespan, and fine root biomass production and distribution. Our data suggest that Concord grape on good sites copes effectively with the high carbon demand for reproduction.

Being so efficient, the Concord grape may have relatively “cheap” root systems that require less energy for nutrient uptake and maintenance; or it has high photosynthesis capacity that is able compensate the cost to high reproductive growth. So the finding of this study suggests future studies on how the vines are able to mitigate the carbon stress and maintain the root systems while reproductive demand is so high. For example, what is the energy requirement for nutrient uptake and maintenance of the root systems? Is there an increase in photosynthesis induced by high reproductive effort adequate to compensate for the high cost of reproductive growth? What is the photosynthetic capacity? What is the carbohydrate partitioning to the other tissues? Is there any cost expressed in other tissues, such as canes, shoots and leaves?

Concord grapes have also been known not to require extensive thinning, while various other species need substantial thinning to avoid high fruit abortion or decreased disease resistance. Thus it would also be interesting to study the differences in how different grape cultivars cope with variation in reproductive effort.

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