CSU-OMRC Viticulture Research
Annual Report
July 1995 - June 1996

June 1996

Submitted to:

The Colorado Wine Industry Development Board

by
Rick Hamman
Research Viticulturist
Colorado State University
Orchard Mesa Research Center
Grand Junction, CO 81503

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Introduction:

This report is a summary of the activities, accomplishments and results of the viticulture program outlined in the Memorandum of Understanding (MOU) between the Colorado Wine Industry Development Board (CWIDB), the Colorado Department of Agriculture (CDA), and Colorado State University (CSU) for the period of July 1995 through June 1996. The CSU viticulture program has now successfully completed six years of contractual arrangements (MOU's) with the CWIDB. Technical support and research objectives are being accomplished. Long term research projects, cooperative research projects, demonstrations, and technical assistance aspects are continuing and have developed a well rounded successful program.

Various technical papers, graphs, summaries and Colorado wine industry statistics have been included in the Appendix. These accomplishments are presented below.

Objectives:

The overall objectives of the CSU Viticulture program are to provide growers, future growers and vintners with technical assistance and to plan, implement and evaluate a research/extension/development program for the needs of the developing wine industry.

Colorado offers distinct challenges to its grape growers and winemakers. Its disease and insect pressure, soils, irrigation, micro and macro-climates are unique from other states and countries and they require a different set of solutions than those

available to growers in other viticultural regions.

Colorado growers and vintners need a better understanding of the specific limits to grape production, methods to address those limits and an understanding of what impact they have on grape composition, wine making objectives and ultimately wine quality.

Colorado State University is currently addressing many of these industry related concerns with its yearly contractual arrangement with the CWIDB. The research activities provided by CSU during this period are a continuation and expansion of the current and long term mission, goals and objectives of the CWIDB.

Viticulture Research Beyond the Year 2000:

A long range perspective of Colorado viticulture research that closely follows the overall mission and goals of the CWIDB is provided below.

Cold Hardiness -- Ongoing Research: The capability to produce very high quality wines from *Vitis vinifera* cultivars grown in the Grand Valley region of Colorado has been well demonstrated. Most years, climatic elements during the growing season are ideally suited to the production of several high quality cultivars. Winter injury does pose a major potential constraint that can interrupt stable production with unpredictable frequency. We need stable production and the use of any new tools or methods that can favor production is important for industry growth and vitality.

Disease and Pest Management: Every season is different and new diseases and pests can flare up without notice. As options shrink (EPA registrations canceled), the need for alternative biocontrol methods becomes greater. Examples; Grape Leafhopper and Grape Mealy Bug and Powdery Mildew epidemics.

Grape Phylloxera is a 40 million dollar nightmare in California. Phylloxera is a devastating insect that can destroy vineyards and reduce wine

production. Colorado is not prepared for an epidemic of Phylloxera. The industry is 98% self-rooted, with very few vineyards planted on phylloxera resistant rootstocks. Phylloxera Survey and monitoring programs need implemented before the insect becomes established in Colorado.

Technical Support and Resource Needs: Ongoing and mandatory for future growth and enhancement. Training and educational workshops, field site visits/assessments. The industry is not self-supporting and private consultants do not exist.

Nutritional Disorders: Vine deficiency and fertility requirements in Colorado have not been determined and the need for establishing base lines is necessary. Nutritionally deficient vines are unproductive vines.

Nematodes and Replant Problems: The Dagger nematode can injure by feeding alone but more seriously transmits viruses such as GFLV (Grape Fan Leaf Virus). Vineyards can become unproductive or even destroyed.

Variety and Rootstock Trials -- Long term ongoing research:

-New clones that may be more winter hardy
-Disease resistant clones
-Phylloxera resistant rootstocks
-Clones with high quality enological characteristics.

Irrigation System Efficiency: When and how much water do I apply? Site and system variabilities are tremendous in western Colorado. Assistance with this area is vital to increase yields.

Production Enhancement: Average current yields are 2.9 T/A, we need 7 T/A. Increasing the grape supply directly impacts wine sales. Vineyards poorly managed are unproductive and produce inferior quality.

Vineyard Floor Management: New strategies, cover crops, what's new and will it work in Colorado? Weed control, chemical or mechanical? Inferior floor management can directly affect yields.

Vineyard Mechanization: As labor costs increase, total vineyard mechanization becomes possible, currently well implemented in New Zealand, Australia and New York. Mechanical harvesting and pruning reduces labor and lowers production costs. Cooperative mechanization is very near to the Colorado industry. Problems associated with mechanization need to be addressed, (wine quality, oxidation, vine hardiness).

Enology: Winery technical support and Enology research make a difference in wine quality. New developments, varieties, techniques and resources continually change and experiments addressing these issues will help the overall integrity of wine production in Colorado.

Unforseen Problems: New pests, diseases, water quality issues are constantly changing and cannot be ignored when a crisis arises.

Urbanization: Its impact on wine production.

The above list of long range viticulture research for Colorado is currently being addresed by the CSU Viticulture program. Research and technical assistance are the two principal areas of the Viticulture program. These areas are designed to provide answers regarding vine management, irrigation scheduling, variety testing, vineyard development, hardiness, vine growth and physiology, crop load, fruit composition, wine quality, area soils and public relations.

Climatic Overview and Year End Summary:

1995 was a record year for the Colorado wine industry despite the unusually cool, moist spring and slow start. The record wine grape harvest (563 tons) was a 37% increase over 1994 tonnage. The mild winter led to above average high temperatures in February and March encouraging an early bud break. Bud break for most vinifera varieties occurred by April 15 however, the cool wet months of April, May and June delayed bloom until June 15. Spring frost damage was minimal. Moderate hail damage was observed in several vineyards from unusual May storms. Rainfall was extremely high during the months of April, May, June and July (5.64 inches) and represented a 95% increase over the 31 year average and a 250% increase over 1994. The increased moisture created prime conditions for powdery mildew infections and growers were heavily engaged with its control. Insect pressure was mainly limited to isolated out breaks of leafhoppers. Phylloxera presence has not been identified in

commercial vineyards in Colorado to this date.

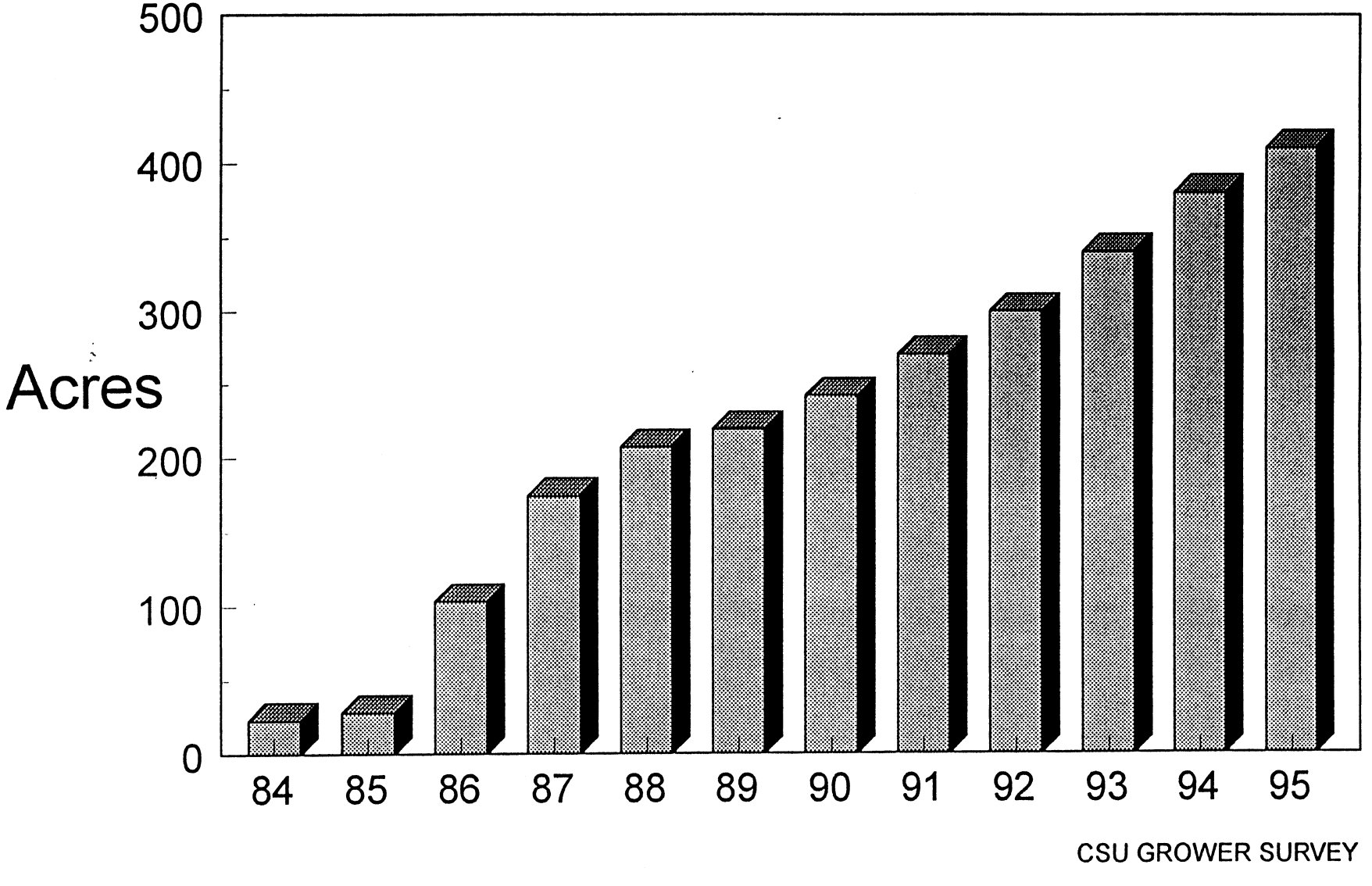
The most dramatic event of the 1995 growing season was the late harvest caused by the cool spring. An Indian Summer blessed most growers with a long sunny fall that completely matured the fruit. Harvest was 3 to 4 weeks later than last year and some growers were concerned if the fruit would ever ripen.

Chardonnay represents 30% of the Colorado industry followed by 18% Merlot, 10% Cabernet sauvignon, 8% Pinot noir with the remainder consisting of Cabernet franc, Riesling, Gewurztraminer, Sauvignon blanc, Viognier and other varieties. Thirteen percent of the 563 ton record harvest was sold out of state. The state average yields were 2.92 T/A. Growers averaged $1050 return per ton with a range of $600 - 1260/ton. Hand picking costs averaged $124 per ton.

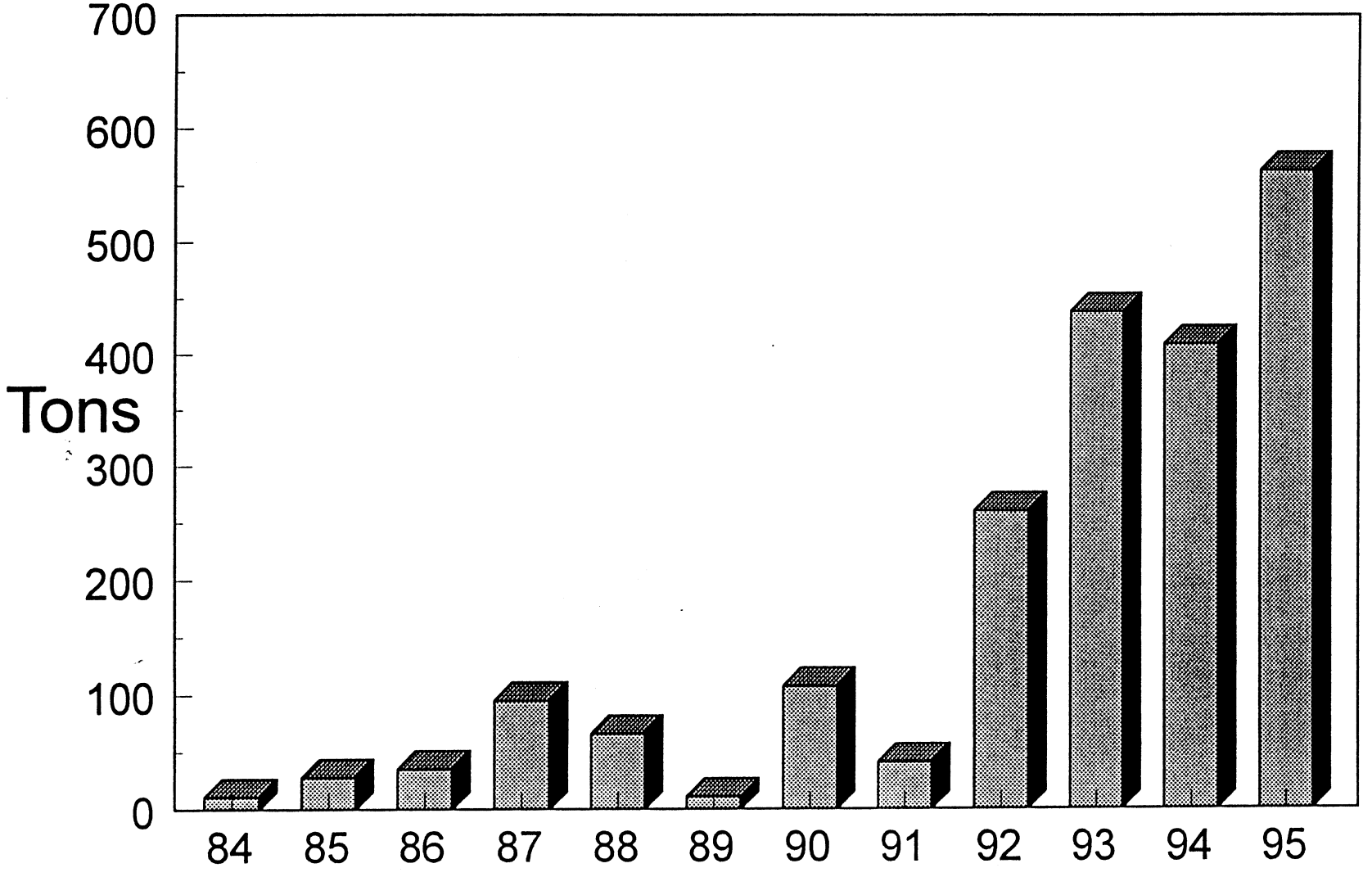
This year, Colorado has thirty more acres planted to vines, bringing the state wide wine grape average to 410 acres. Wineries have expanded their productive capacity by 30% and one more winery became licensed in 1995 for a total of 15.

The following graphs and charts clearly describe the growth and development of the Colorado wine industry.

Colorado Wine Grape Acreage



Colorado Wine Grape Production

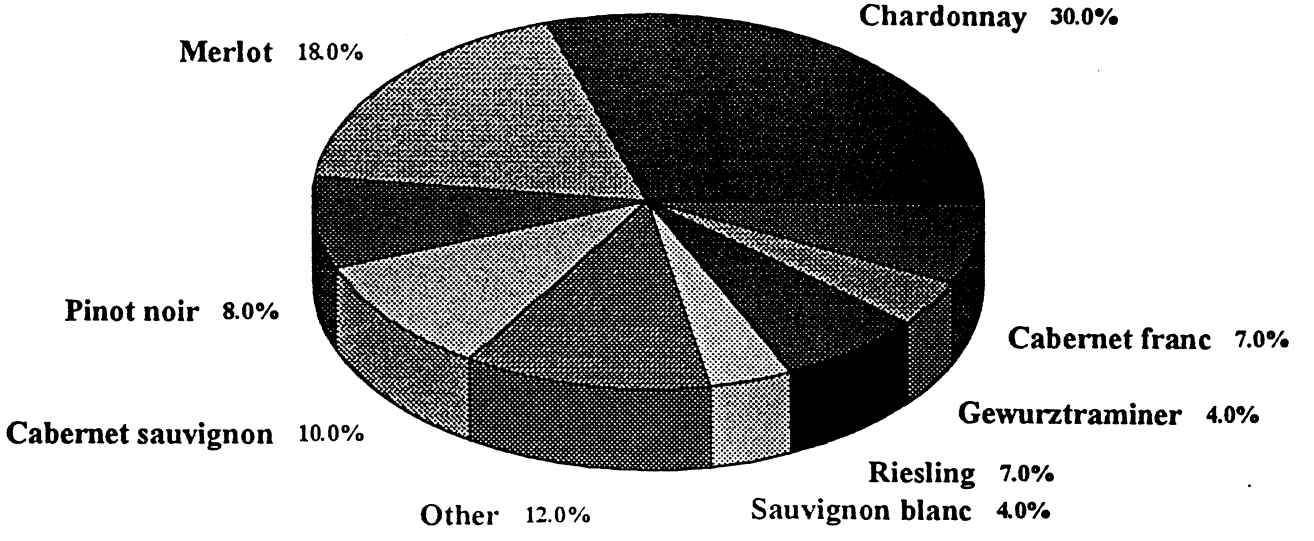


CSU GROWER SURVEY

Colorado

Variety by Acreage

1995



CSU GROWER SURVEY 95

Research Program:

The CSU Viticulture research program is providing a very solid base of research activities upon which the Colorado industry uses and relies upon. The research performed is of extremely high quality (published papers) and is a combination of experience and collaborative interdepartmental cooperation that follows the overall mission and goals of the CWIDB.

The following is a list of collaborative research projects that have been implemented as part of the CSU Viticulture program for the 1995-96 period.

A. **Vine Hardiness** - Cold Hardiness Enhancement and Bud Break Delay of Chardonnay.

B. **Varietal Testing** - Hardiness, growth, canopy, yield and fruit composition of research variety.

C. **Powdery Mildew Trials** - Bio-control Alternatives.

D. **Spring Deaclimation Studies**

A. Cold Hardiness Enhancement and Bud Break Delay of Chardonnay

This cooperative research project is continuing and involves faculty/staff in the Department of Horticulture/Biochemistry on main campus.

The objectives of this project were to; field test selected sugar treatments that improved the cold hardiness of grape tissues, to determine the optimum treatment from the lab and apply to the field and to evaluate gel-encapsulation techniques, equipment

and feasibility under field conditions.

The following 3 page paper summarizes this project and was presented to over 100 growers at the Rocky Mountain Association of Vintners and Viticulturists (RMAVV) March meeting and at the New Mexico Wine and Vine Society meeting this April held in Albuquerque, New Mexico.

Results of Bud Break Delay experiments under field conditions this April-May have shown substantial differences with various treatments and are displayed in the Bud Break Delay graphs. Overall, bud break delay treatments were effective. Our results indicate that treated chardonnay buds were delayed from bursting by as much as 7 to 10 days over control buds. The results of this research may lead to alternative methods for protecting vines from spring frost.

Cold Hardiness Enhancement and Bud Break Delay of Chardonnay

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Winter injury of dormant grapevine tissues and developing grape shoots are serious problems in temperate climate regions such as the Grand Valley in Colorado or the high desert regions of New Mexico. Nationally, the Federal Crop Insurance Agency (FCIA) reports over 43% of all grape production loss is due to freeze/frost conditions. In 1989 and 1991 Colorado experienced wine grape losses of 93% and 80% respectively from extreme freezes. These losses are of concern to any grape grower growing vines under these climatic conditions. Various methods to protect dormant grape tissues from winter injury such as burying canes, insulating vines with household insulation material, wind machines and evaporative cooling techniques have been used with limited success and are quite expensive. For this reason we began to study some low-cost alternative approaches to enhance hardiness of grapevine tissues.

Our studies of dormant wine grape tissues have identified several endogenous compounds that are associated with hardiness. These cryoprotective-like compounds are expressed exclusively during the winter season and showed significant relationships to the freezing resistance of *Vitis vinifera* cultivars such as "Chardonnay" and "Riesling" (Hamman et al. 1996). The endogenous cryoprotectants consist of soluble sugars of different molecular weights. Based on these studies and other cryopreservation studies, we attempted to increase the freezing resistance of dormant buds and cane tissues by exogenously applying several sugars to dormant grape tissues. Application of the soluble sugars was made with a tractor-driven air sprayer and/or hand brushing. Encapsulation with sugars was used to facilitate incorporation into grape tissues. In our lab studies we attempted to simulate field conditions in a temperature- and humidity-controlled incubation chamber. In this controlled environment samples were exposed to temperatures close to field conditions for two weeks.

Table 1."Chardonnay" Lab Testing - Killing Temperatures

Collection Date	Treatment	T50 (C) Bud	T50 (C) Cane
4 January 95	Control Treated	-25.3 -28.4*	-26.2 <-30*
12 February 95	Control Treated	-23.1 -26.6*	-24.6 -28.9*
22 February 95	Control Treated	-17.8 -24.5*	-21.3 -24.9*

T50 based on Spearman-Karber method and means were compared using an unpaired T-test, * significance at p = 0.05.

Lab samples were then frozen in a computer controlled freezing chamber and evaluated via the tissue browning method according to Stergios and Howell (1973). These laboratory studies indicated that we were able to significantly increase freezing resistance of Chardonnay buds and canes (Table 1).

The significant results of the laboratory work gave us enough confidence to begin applying treatments on grapevines growing under field conditions. The field testing consisted of three separate experiments.

Table 2. "Chardonnay" Field Testing - Killing Temperatures

Collection Date	Treatment	T50 (C) Bud	T50 (C) Cane
24 February 95	Control Treated	-16.6 -21.7*	-17.7 -24.2*
22 March 95	Control Treated	-6.6 -9.1*	-13.3 <-15*
10 April 95	Control Treated	-6.2 -11.2*	-11.2 -15.6*

T50 based on Spearman-Karber method and means were compared using an unpaired T-test, * significance at $p = 0.05$.

Treatments were applied in February and March. Cane and bud tissues were collected two weeks after treatment application and frozen in the computer-controlled freezer and tissue browning evaluations were made. Treated buds and canes showed significant differences from controls (Table 2). This preliminary field study gave us good results however, we did experience some difficulties with degradation of the encapsulation materials under field conditions. Exposure to moisture and fluctuating temperatures may be the cause of this degradation. It was concluded that the treated tissues under laboratory and field conditions appeared to slow bud and cane deacclimation and thus enhance hardiness levels.

The effect of the treatment on bud break was also attempted. A delay in bud break of grapevines by even a few days is very important and could be the difference between a full crop and no crop at all.

The bud break study also consisted of three separate experiments. Dormant treated and control one-nodal cuttings were placed in moist peatmoss and forced to grow. Our results indicated that treated samples delayed growth as much as 5 phenological stages later than controls (Table 3).

Table 3. "Chardonnay" Lab Testing - Bud Break

Collection Date	Treatment	Bud Stage[z]
14 December 94	Control Treated	12 2*
4 January 95	Control Treated	9 5*
8 March 95	Control Treated	9 3*

[z]Bud stage is based on the Eichorn-Lorenz (1977) method. * significance at p = 0.05.

All of these studies enhancing hardiness and delaying bud break are promising but are preliminary. Further research, evaluations and field trials are needed. Our current research involves several cultivars and different formulas. Data collected this spring appear to support last years research however, evaluations and analyses are not complete.

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Eichorn, K. W. and D. H. Lorenz. Phanologische Entwicklungsstadien der Rebe. Nachrichtenbl. Dtsch. Pflanzen-schtzdienstes (Braunschweig) 29:119-120 (1977).

Hamman Jr, A. R., I. Dami, T. M. Walsh, and C. Stushnoff. Seasonal carbohydrate changes and cold hardiness of Chardonnay and Riesling grapevines. Am. J. Enol. Vitic. 47:31-36 (1996).

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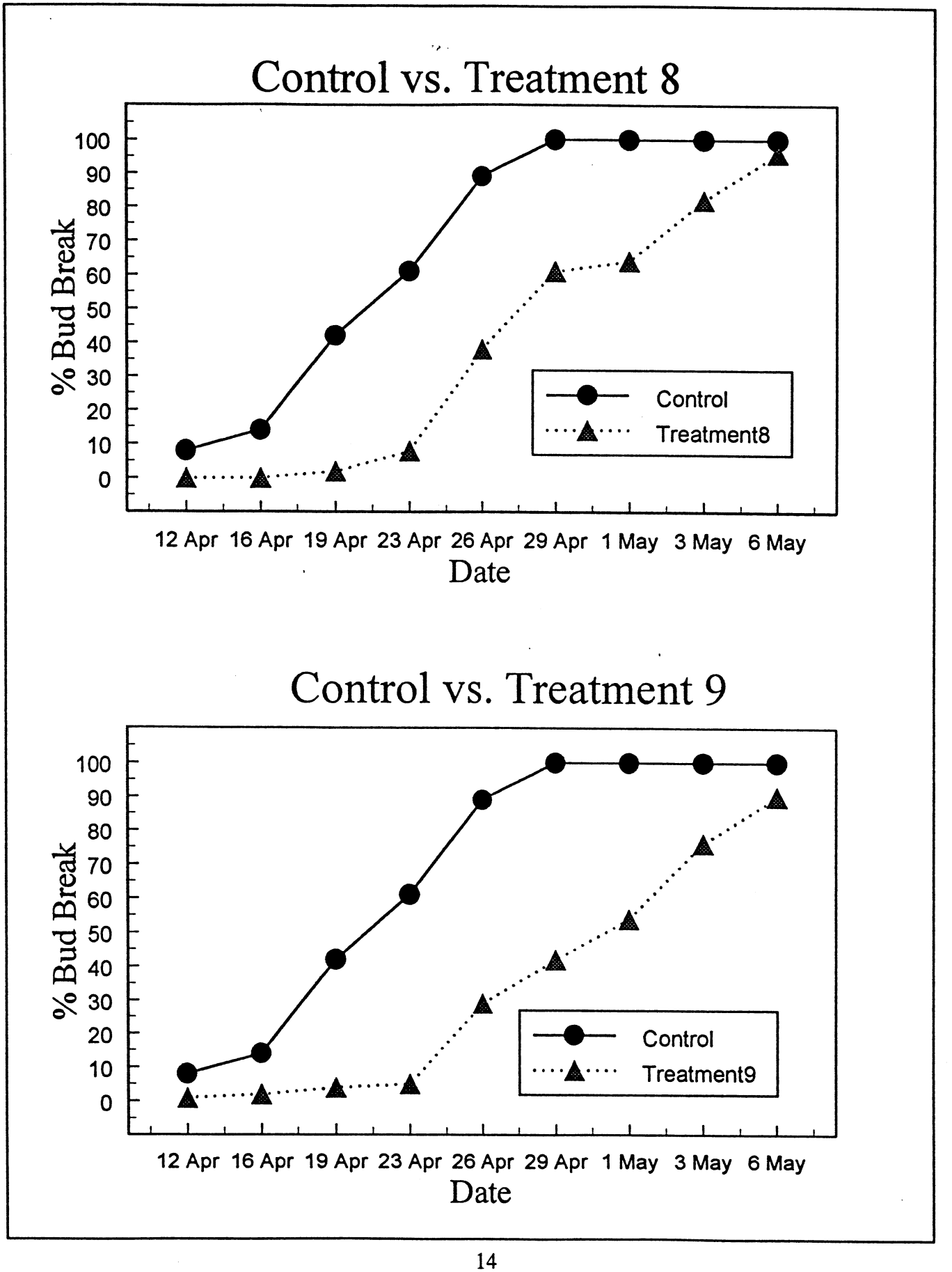
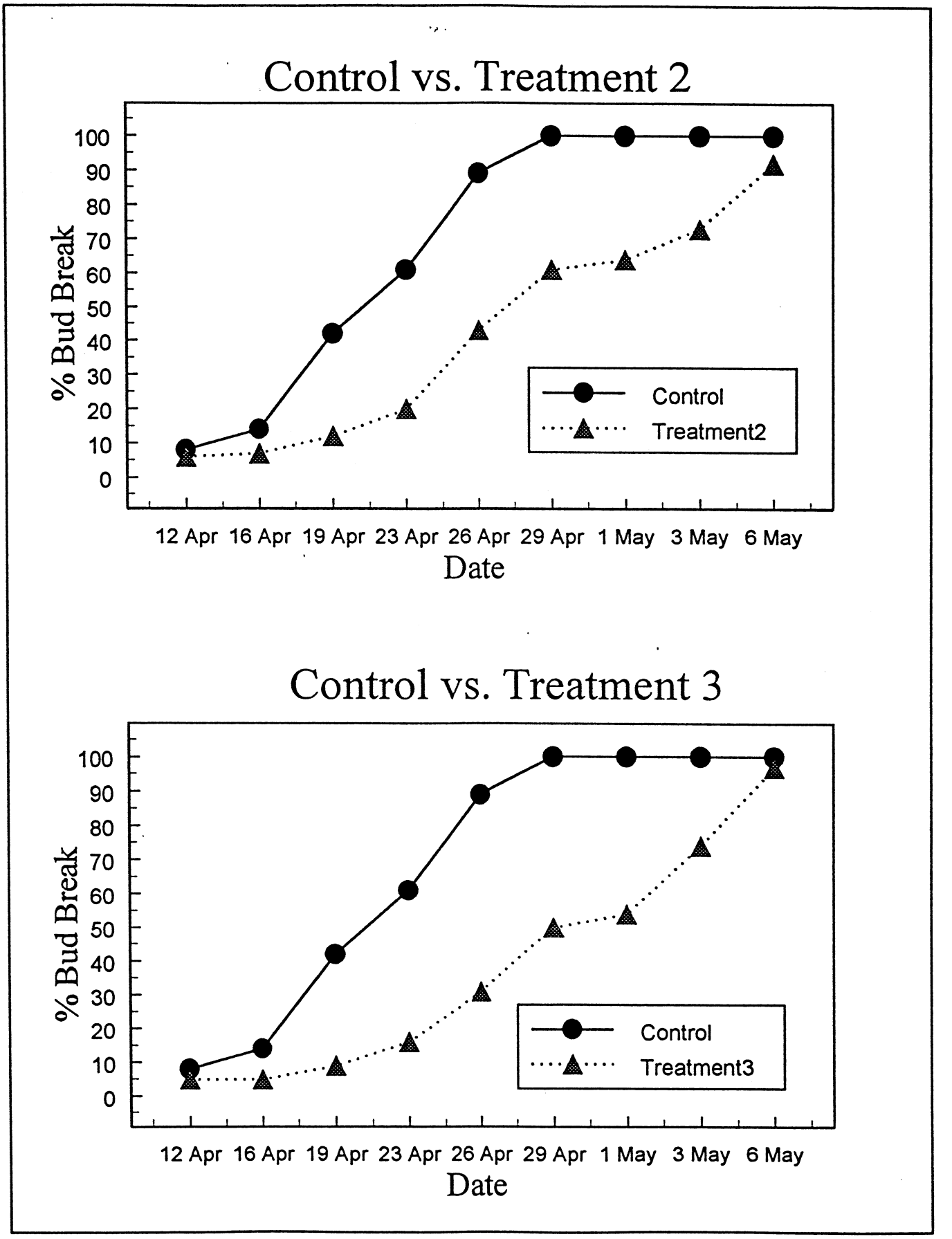
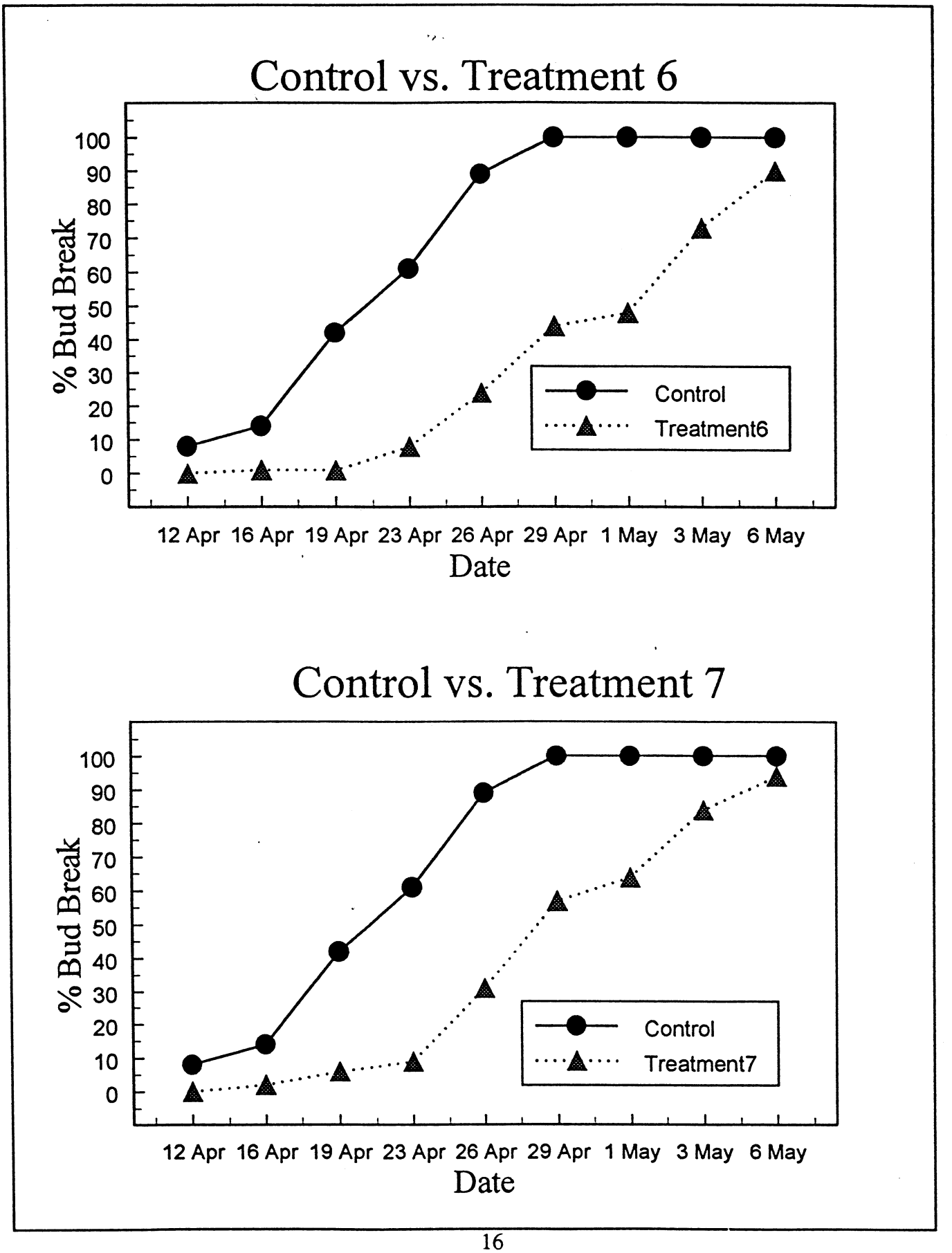
Bud Break Delay in Chardonnay



Bud Break Delay in Chardonnay



Bud Break Delay in Chardonnay



B. Variety Trials

The two acre wine grape variety trial established at OMRC in 1990 is now going into its fifth season. The vineyard has been invaluable for research purposes and public demonstrations. The vineyard contains 20 different varieties, 4 phylloxera resistent rootstocks grafted to Chardonnay 108 and self-rooted Chardonnay 108. (See Appendix - Variety block).

This year research data was collected throughout the vineyard and included the following categories: Growth, canopy, yield, fruit composition and cold hardiness of several varieties. This data is presented below. The data also includes cultivars growing in the furrow irrigated research block established in 1985 at OMRC.

The 1995 season was the first year of data collection in the OMRC rootstock trial. The results are presented below. Unfortunately a May 13 hail storm reduced yields by an estimated 40% of normal.

Cold Hardiness evaluations of several cultivars were performed on various dates (listed below). Cold hardiness evaluations require a dedicated time commitment of around 30-40 hours to complete a successful run. Due to equipment limitation, only four cultivars can be tested at once. A complete hardiness test includes; sample collection, sample preparation, controlled freeze test, evaluation of samples after thaw for both buds and canes, calculation of the T_{50} (T_{50} = low temperature that kills 50% of plant tissue) and statistical data analysis. Our evaluations this season indicate the American species Cynthiana/Norton to be more resistant to the cold than many other cultivars.

1995
Growth, Canopy, Yield and Fruit Composition
Variety Block 10

Variety	Age	Harvest Date	Shoots /Vine	Yield #/vine	Sugar °Brix	Acid	pH	Yield* Ton/Acre
Andover	4	9/13	24	2.6	21	14.7	3.24	1.1
Aurora	5	9/13	---	2.2	21.6	11.1	3.75	1.0
Baco Noir	4	10/12	36	7.8	25.5	13.6	3.34	3.4
Cabernet franc	4	10/12	---	7.6	25.2	7.6	3.35	2.3
Cabernet Sauvignon	4	10/13	---	6.1	25	7.5	3.4	2.0
Carmine	9	10/13	---	19.5	24.2	9.7	3.17	5.8
Cayuga White	4	9/20	32	6.9	21.4	9.6	3.4	3.0
Chardonnel	4	9/20	28	7.8	25.2	13.3	3.29	3.4
Chardonnay #108	4	9/25	33	4.8	23.6	11.3	3.32	2.1
Cynthiana	4	10/10	28	1.2	23.8	16.7	3.45	0.5
Dolcetto	3	10/6	30	11.3	21.3	7.5	3.65	4.9
Gewurztraminer #457	4	9/20	40	9.8	23.4	8.4	3.45	4.3
Malbec	9	10/13	---	12.5	25	9.3	3.24	3.8
Merlot #3	4	10/6	32	11.5	24.5	7.2	3.27	5.0
Muller thurgau	3	9/21	16	4.7	22.0	6.9	3.47	2.0
Muscat blanc	9	10/23	---	22.4	23.4	8.5	3.19	6.7
Norton	4	10/10	30	1.5	24.2	16.7	3.48	0.7
Pinot gris	3	9/21	17	4.5	22.1	7.8	3.39	2.0
Pinot Noir #374	4	10/6	34	10.7	21.8	8.8	3.31	4.7
Pinot noir Dijon	4	10/10	22	8.4	20.7	7.9	3.24	3.7
Pinot blanc #159	4	10/9	25	11.5	21.7	7.4	3.32	5.0
Riesling #9	4	10/25	28	11.2	23.6	9.9	3.02	4.9
Rkatsetelli	4	10/25	---	6.5	24.6	8.0	3.09	2.0
Sangiovese	4	10/24	32	11.8	20.2	9.2	3.07	5.1
Semillon	9	10/19	---	16.8	23.5	7.65	3.19	5.1
Seyval blanc	4	9/14	38	10.1	22.4	10.0	3.39	4.4
Shiraz	4	10/9	29	8.46	22.4	7.8	3.40	3.7
Siegerrege	4	9/13	---	3	24.0	7.3	3.67	1.3
Vignoles	4	9/18	33	4.9	24.6	12.7	3.59	2.1
Viognier	3	10/25	27	3.5	24.0	9.0	3.12	1.5

*Yields were reduced from a May 13 hail storm and were estimated to be 40% less than normal.

Rootstock Trials
OMRC 1995
Chardonnay

Growth Canopy and Yield

Stock	Vine Age	Harvest Date	Yield* T/A	Average Pruining Weight (ounces)	Average Cane length (inches)	Clusters/ Vine
Self rooted	4	9/25	2.1	------	-----	14.0
5-C	3	9/25	2.8	13.4	30.0	11.6
3309	3	9/25	2.5	10.5	28.0	15.7
101-14	3	9/25	2.2	14.3	28.0	12.6
420-A	3	9/25	2.5	11.0	28.5	13.1

*Yields were reduced from a May 13 hail storm and were estimated to be 40% less than normal.

Fruit Composition

	Sugar °Brix	Acid TA g/l	pH
5-C	20.8	14.0	3.46
3309	20.2	13.2	3.35
420-A	21.0	10.4	3.42
101-14	21.4	10.5	3.36
Self rooted	23.6	11.3	3.32

Cold Hardiness of Grape Cultivars

Tested in Block 10 at OMRC

Winter-Spring 1996

Collection Date	Cultivar	T_{50} Bud °C (F)*	T_{50} Cane °C (F)*
1/19	Chardonnay	-22.9 (-9.2)	-23.6 (-10.5)
2/5	Sangiovese	-21.8 (-7.2)	-27.0 (-16.6)
	Cayuga	-25.7 (-14.3)	-28.1 (-18.6)
	Chardonel	-25.9 (-18.4)	<-28 (-18.4)
	Cynthiana/Norton	<-28 (-18.4)	<-28 (-18.4)
2/12	Chardonnay	-24.0 (-11.2)	-24.2 (-11.6)
2/19	Rkatsiteli	-22.9 (-9.2)	-25.5 (-13.9)
	Gewurztraminer	-24.0 (-11.2)	-21.63 (-6.3)
	Dolcetto	-25.5 (-13.9)	-27.0 (-16.6)
	Vignoles	-24.6 (-12.3)	-29.2 (-20.6)
2/29	Gewurztraminer	-24.2 (-11.6)	-22.3 (-8.1)
	Pinot Noir	-25.1 (-13.2)	-23.9 (-11.0)
	Dolcetto	-25.5 (-13.9)	-27.3 (-17.1)
	Pinot Blanc	-25.6 (-14.1)	-25.0 (-13.0)
	Cynthiana/Norton	-25.7 (-14.3)	-30.2 (-22.4)
3/19	Chardonnay	-24.9 (-12.8)	-24.7 (-12.5)
4/9	Riesling	-13.7 (7.3)	-18.7 (-1.7)
	Merlot	-11.3 (11.7)	-14.3 (6.3)
4/10	Chardonnay	-11.6 (11.1)	-15.4 (4.3)
4/15	Dolcetto	-10.8 (12.6)	-15.9 (3.4)
	Riesling	-11.2 (11.8)	-15.4 (4.3)
	Chardonel	-11.8 (10.8)	-18.0 (-0.4)

*T_{50}: low temperature that kills 50% of plant tissue.

C. Powdery Mildew Trials - Bio-control Alternatives

Powdery mildew is the most important disease facing Colorado growers. The CSU Viticulture research program began evaluating natural alternative methods for control in 1994 and continued in 1995 with the addition of several vegetable oils.

During the 1994 season we tested several bio-control products supplied by Mycogen Corporation. We found M-Pede, a fatty-acid and registered pesticide, to be effective against powdery mildew however, 1994 was not a good mildew year, too dry and hot.

In 1995 we tested several oils (rape seed, sunflower, mustard seed and linseed) and a new fatty acid from Mycogen. Other vegetable oils have been tested in Canada and California with limited success. Oils and fatty acids (soaps) kill mildew spores and mycleium upon direct contact as a liquid spray. Therefore they are used primarily as a "curative" agent rather than the traditional preventative mode.

The mildew study consisted of six treatments, four of which were replicated. Treatments were applied to *Vitis vinifera* CV. Chardonnay. The materials were applied via recycling tunnel sprayer that was specially fabricated at OMRC. A brief summary of the treatments is described below.

Treatments:

1. MYX - 403, 1.0% V/V @ 10-14 day intervals
2. Rape seed oil, 1.0% V/V @ 10-14 day intervals
3. Sunflower oil, 1.0% V/V @ 10-14 day intervals
4. Standard Program, Thiolux @ 5#/100gal @ 10 day intervals until bloom then Rubigan @ 3oz/100gal @ 21 day intervals
5. Linseed oil + Joy soap, 1.0% V/V @ 10-14 day intervals
6. Mustard seed oil + Joy soap, 1.0% V/V @ 10-14 day intervals

Each treatment (except 5 and 6) was 3 full row replicates and each row had 3 control vines (no spray).

1995 was an excellent powdery mildew year, high relative humidity, moisture and cool temperatures. Powdery mildew ratings were taken in Mid-August. Fruit clusters were rated clean to 100% infection.

The results of the study are presented in the following graph.

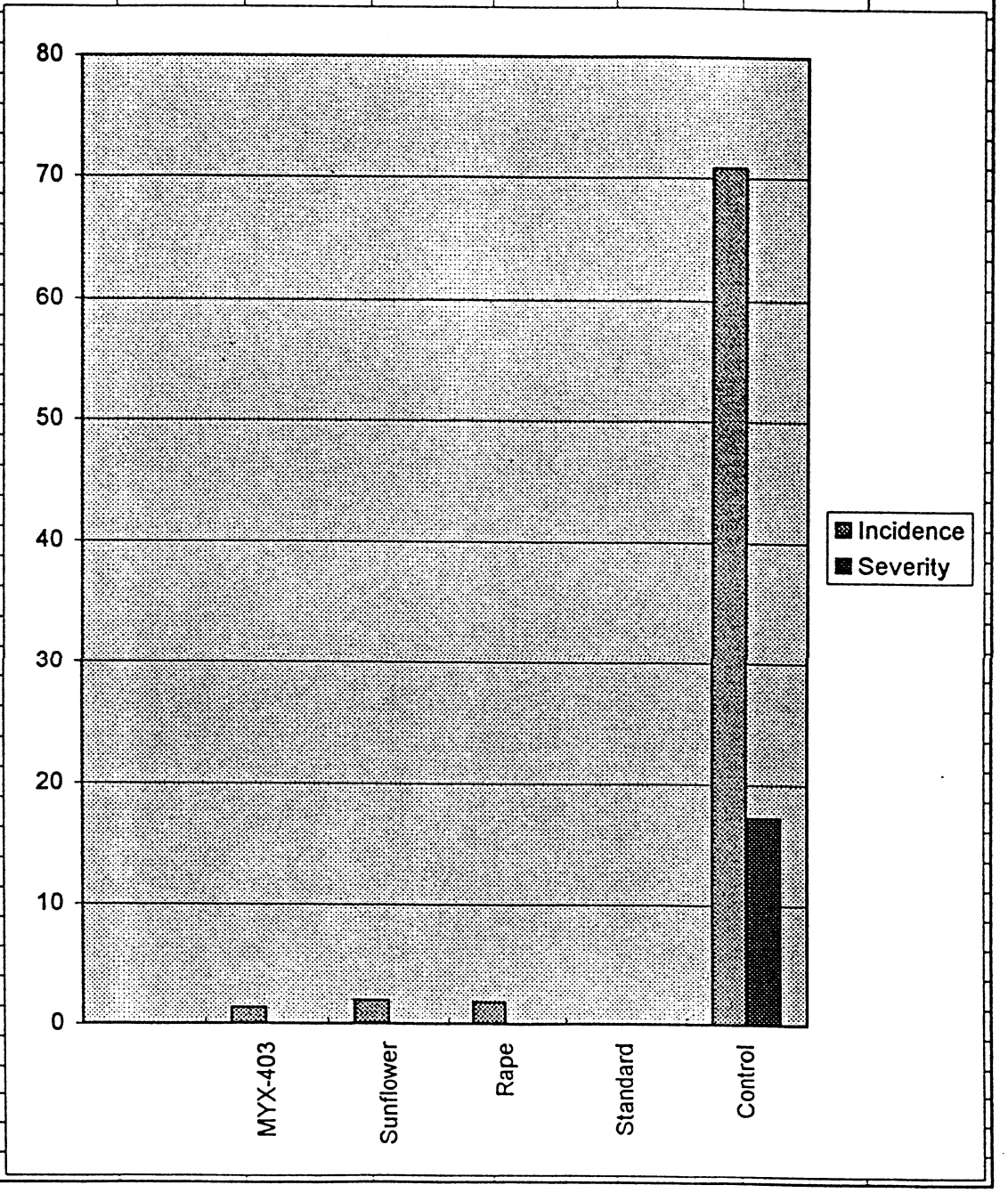
All replicated treatments (1-4) showed significant differences from control. Percent mildew incidence and percent mildew severity are methods to determine how bad the infection really was. Percent severity is the estimated coverage of the cluster surface by mildew based on the weighted average of ratings of 30 to 100 clusters per replicate.

Overall, the results of the study indicate very good control with oils and or fatty acids. Problems that we encountered were; sprayer cleaning, oils do not clean off the inside of the tank and lines easily, high foaming of M-Pede and MYX-403, fruit and leaves had a high glossy appearance (not natural looking) and the oils and MYX-403 required more repeat applications than the standard treatment. The 1996 powdery mildew trials consist of 3 treatments, sunflower oil, M-Pede and standard program. Data analysis is not complete, however the results of 1994 trials, this study and 1996 trials appear promising for alternative bio-control methods of grape powdery mildew control.

Grape PM Trials 1995

CSU-OMRC

	Incidence	Severity
	% Infected	%Infection
MYX-403	1.334	0.013
Sunflower	1.979616	0.019796
Rape	1.821167	0.018212
Standard	0	0
Control	70.84716	17.26469



D. Spring Deacclimation Studies

Objectives and goals:

This experiment was conducted during the spring of 1996 to study the relationship between hardiness of buds and canes as they progress through the dehardening period. The focus was to detect whether there is a soil temperature threshold at which vines lose hardiness.

Three cultivars were used. Chardonnay was used to represent *V. vinifera* species known for being cold tender. We also used Chardonnay grafted on rootstock '5C' to evaluate whether rootstock influences cold hardiness of the scion. Seyval blanc was also used to represent a hybrid with better cold hardiness than *vinifera.*

The results are summarized in the following table:

Collection Date	Cultivar	T50 (C)		Water Content (%)		Soil Temp (F)	
		Bud	Cane	Bud	Cane	6 "	15"
28 Mar	Chardonnay	-19.5	-22.6	42.4	46.4	49.4	47.9
	Chardonnay / 5C	-19.2	-22.7	45.0	46.6	47.6	46.6
	Seyval blanc	-22.3	<-28	43.9	46.9	47.7	46.9
2 Apr	Chardonnay	-12.7	-17.9	44.9	46.5	57.3	51.7
	Chardonnay / 5C	-11.6	-17.4	51.7	47.6	56.6	51.2
	Seyval blanc	-17.9	-23.0	44.9	47.3	55.9	50.9
9 Apr	Chardonnay	-10.0	-16.1	51.9	47.4	55.6	52.7
	Chardonnay / 5C	-9.6	-15.6	58.9	48.7	53.6	52.0
	Seyval blanc	-12.8	-18.0	48.4	46.4	58.0	54.2
15 Apr	Chardonnay	-12.2	-15.6	53.0	48.5	56.6	52.7
	Chardonnay / 5C	-10.9	-15.5	67.7	49.6	56.2	54.0
	Seyval blanc	-13.9	-17.3	53.6	47.8	54.6	51.7

Discussion of Results:

- On each date, significant differences in cold hardiness of buds and canes between Seyval blanc and self and grafted Chardonnay. This confirms previous published results. However, no difference was detected between Chardonnay cultivars either self- or grafted.

- First week of April: buds and canes began losing hardiness for all cultivars. In 4 days, there was a drop in freezing resistance of buds between 4 and 8C. This also corresponded with an increase in water content levels in buds and canes.

- When soil temperature reached 50F at 15 inch-depth, there was dehardening of buds and canes in all cultivars observed on 4 April 1996. This could be used as an indicator of loss of hardiness.

- Another indicator of loss of hardiness is bleeding of excised canes. Once bleeding began, cold hardiness of buds dropped to about -10 to -12.8C in all cultivars.

- In this experiment, the first observed bleeding occurred 3 to 4 weeks before bud break of the 3 cultivars. Data on first bleeding and bud break of the 3 cultivars is indicated in the following table.

Cultivars	First observed bleeding date	Bud break date
Chardonnay	9 April 1996	28 April 1996
Chardonnay / 5C	2 April 1996	22 April 1996
Seyval blanc	9 April 1996	10 May 1996

Summary of results:

Dehardening of vines is associated with the following:
- Rise in water content in buds and canes
- Bleeding of sap from excised canes
- Temperature of soil at 15 inch-depth is 50F or above

Pre-pruner Fabrication:

A low-cost prototype mechanical pre-pruner was fabricated in February. The pre-pruner was fabricated to address high labor costs common to all vineyards in Colorado. The pre-pruner components were purchased from AIM Inc. and the attachments for the tractor mounting were fabricated at the Fruita Research Center. This cooperative project was very successful and a demonstration was made in March to several growers. A study was developed in March to investigate canopy development and fruit composition of vines that were pre-pruned only. Data will be collected at harvest and at pruning.

Cover Crops:

Three drought tolerant grasses were planted in the test vineyard at OMRC. The grasses were planted without supplemental irrigation and germination was dependent upon rainfall. Two of the grasses, "Ephriam" and "thick spike" are crested wheat grasses and were chosen because of their good performance the previous year. The third grass, "sheep fescue" was also chosen for its drought tolerant abilities. The grasses were planted November 6, 1995. All three grasses were planted at 20# per acre with a T.G. Schmeiser 6' vineyard drill. Germination occurred during March and April. Sheep fescue failed to become established. Ephriam and thick spike did germinate but rainfall was not abundant this spring and establishment was 30% at best. 1994 plantings are maintaining a very good cover for these two cultivars. Ephriam crested wheatgrass to date is the most successful cultivar in our trials. Planting time appears to be crucial for these grasses and based on 2 years of data, late September or early October allows for better establishment.

Technical Assistance:

Technical advice on site selection, vineyard development, pruning, training, planting, fertilization, irrigation and pest management was provided throughout the year upon request. During this 12 month contractual period, viticulture and enology related phone calls and personal visits became more demanding. There were over 492 phone calls answered and more than 174 personal grower contacts via vineyard site visits or grower research center visits.

The Colorado Grape Growers Guide first printed in 1991 and reprinted in 1993 has been revised this year and is available at the CSU Cooperative Extension Resource Center (970-491-6198). The cost of the guide is $12 plus shipping. The Colorado Grape Growers Guide has been an extremely reliable resource for prospective and experienced growers. The 82 page grape guide contains excellent information on Viticulture in Colorado; including vineyard establishment, site preparation, trellising/training, planting, irrigation management, pruning, pest management, varieties, vine sources, useful books and publications and vineyard establishment and production cost estimates.

The Colorado Grapevine, a newsletter column published bi-monthly in the CSU Cooperative Extension Fruit Growers Newsletter is now distributed to over 700 fruit growers and industry related people. The Colorado Grapevine contains various articles of technical value for grape growers, vintners and the public at large. Articles published for this period July 1995 - June 1996 include the following:

Powdery Mildew and standard fungicide programs, Pruning, 1995 CSU wine grape

production survey, Crop thinning and hedging/topping, Grow tubes, Grape Powdery Mildew, and Nitrogen deficiency and correction. The Fruit Growers Newsletter is a free publication and always contains a calendar of events section for upcoming seminars, meetings and conventions.

The Rootstock Review, a newsletter published by the Rocky Mountain Association of Vintners and Viticulturists, is distributed to over 70 grape growers. I submitted meeting announcements and demonstration activities for all viticulture related events for this July 1995 - June 1996 period.

1995 - 1996 Publications:

- 1995 Colorado Harvest Summary. February 1996. R.A. Hamman Jr. Wine and Vines. Volume 77 Number 2, page 28-29.
- Colorado Grape Growers Guide. Colorado State University Cooperative Extension Publication 550A. 1996 Revision. R.A. Hamman Jr., Steven D. Savage and Harold J. Larsen. 82 pages.
- The Colorado Grapevine. Technical article title "Crop Thinning and Hedging/Topping". R.A. Hamman Jr. 1996 Colorado State University Cooperative Extension Fruit Growers Newsletter. Vol. 16 Number 2, page 3.
- The Colorado Grapevine. Technical article title "CSU Wine Grape Production Survey". R.A. Hamman Jr. 1996 Colorado State University Cooperative Extension Fruit Growers Newsletter. Volume 15 Number 1, page 7.

- The Colorado Grapevine. Technical article title "Grape Powdery Mildew". R.A. Hamman Jr. 1996 Colorado State University Cooperative Extension Fruit Growers Newsletter. Volume 15 Number 5, page 5.
- The Colorado Grapevine. Technical article title "Grow Tubes". R.A. Hamman Jr. 1996 Colorado State University Cooperative Extension Fruit Growers Newsletter. Volume 15 Number 6.
- The Colorado Grapevine. Technical article title "Nitrogen Deficiency and Correction". R.A. Hamman Jr. 1996 Colorado State University Cooperative Extension Fruit Growers Newsletter. Volume 15 Number 4, page 2-3.
- The Colorado Grapevine. Technical article title "Powdery Mildew and Standard Fungicide Programs". R.A. Hamman Jr. 1996 Colorado State University Cooperative Extension Fruit Growers Newsletter. Volume 16 Number 3, page 7.
- The Colorado Grapevine. Technical article title "Pruning". R.A. Hamman Jr. 1996 Colorado State University Cooperative Extension Fruit Growers Newsletter. Volume 16 Number 2, page 5.
- Field Response of *Vitis vinifera* Cultivars to Methanol 1996. Imed-Eddine Dami, C. Stushnoff and R.A. Hamman Jr. Am. J. Enol. Vitic. Volume 47 Number 3. In press.
- Seasonal Carbohydrate Changes and Cold Hardiness of Chardonnay and Riesling Grapevines. 1996. R.A. Hamman Jr., I.E. Dami, T.M. Walsh and C. Stushnoff. Am. J. Enol. Vitic. Volume 47 Number 1, pages 31-36.

Colorado Production:

Production records for Colorado were recorded via the 1995 CSU Wine Grower Survey. Results of the survey indicate that Colorado increased production by 37% over last year (409 tons) with a 1995 total of 563 tons. The increase is primarily due to better vineyard management and techniques, more vineyards becoming mature and good mid-winter and spring weather conditions. Vineyard yields varied from .1 to 9.4 tons/acre. The survey included picking costs per ton which averaged $124/ton. The 9 variety average dollars per ton received to a grower was $957 with a high of $1260 per ton for Cabernet sauvignon and a low of $600 for Seyval blanc. The results of this survey are included in the appendix under "Colorado Wine Grape Production."

Monitoring Vineyard Irrigation Management Practices:

The CSU Viticulture technical support program has offered irrigation scheduling assistance to western Colorado growers. The vineyard irrigation monitoring program was initiated 4 years ago and continues to be very well received. The program involves the use of a soil moisture measuring gauge (neutron probe) and cooperating vineyards in western Colorado. For the participating vineyards, soil moisture measurements are taken at 3 depths on a 2 week interval through the growing season. The information is condensed to a graph format and made available to growers for assessment. The benefits of this assistance are more efficient irrigation practices, more confidence with individual irrigation systems, better quality, higher yields, improved vine vigor, and hardiness.

Technical Seminars:

The following technical seminars were sponsored, coordinated and presented by the CSU Viticulture program for Colorado grape growers and the public during the period of July 1995 - June 1996.

- July 1995 - Field Day at OMRC. Viticulture field and laboratory research activities were demonstrated. Vineyard equipment was displayed and tours of vineyard research blocks were provided. Laboratory research wines individual sensory evaluation were made available.
- September 1995 - Presented a wine industry seminar and displayed a research and technical assistance demonstration booth at the 4th Annual Colorado Mountain Winefest.
- October 1995 - Presented a technical briefing on current research at OMRC to the Rocky Mountain Association of Vintners and Viticulturists fall meeting, Holiday Inn Grand Junction.
- October 1995 - Coordinated and sponsored a technical seminar on Viticultural practices and techniques of California, presented by Dr. Kieth Strieger for the Rocky Mountain Association of Vintners and Viticulturists.
- January 1996 - Coordinated and implemented a seminar on grapevine diseases presented by Dr. Bill Brown (CSU IPM specialist) at the 53rd Western Colorado Horticultural Society Annual Convention.
- March 1996 - Presented a field pruning demonstration to Master Gardeners and growers, workshop demo at OMRC.

- March 1996 - Presented a technical seminar on Colorado Viticulture and provided tasting of research wines to East Slope growers and wineries in Loveland at Trail Ridge Winery.
- March 1996 - Provided a summary of grape grower survey and a pruning demonstration at OMRC, varietal discussion and a tasting of research lab wines to growers and others attending the Spring Rocky Mountain Association of Vintners and Viticulturists meeting.
- March 1996 - Coordinated a seminar on Cold Hardiness Enhancement and Bud Break Delay of *Vitis vinifera*, presented by Graduate Student Imed Dami to the Rocky Mountain Association of Vintners and Viticulturists.
- April 1996 - Presented a seminar on Cold Hardiness Enhancement of *Vitis vinifera* to the New Mexico Wine and Vine Society.
- June 1996 - Presented a technical briefing and field tour of vineyard research trials to Utah extension personnel and a group of students from the Netherlands.

APPENDIXES

A. Colorado Wine Grape Production

B. OMRC Map - Block 10

C. Publications:

1. Seasonal Carbohydrate Changes and Cold Hardiness of 'Chardonnay' and 'Riesling' Grapes
2. Field Response of *Vitis vinifera* Cultivars to Methanol
3. The Colorado Grapevine Vol 15 (1, 4-6) Vol 16 (2,3)
4. The Colorado Grape Growers Guide

A

1995

Colorado Wine Grape Production

- Total 1995 Production (tons) = 563
- Producing Acreage = 193
- Average Ton/Acre = 2.92
- Range of Production ton/acre = 0.1 - 9.4
- New Acreage planted in 1995 = 30
- Total Colorado Acreage* = 410

Source: CSU Wine Grower Survey '95

*Colorado Department of Agriculture

Colorado

Wine Grower Survey '95

$/ton Received

Chardonnay	$1236
Cabernet sauvignon	1260
Cabernet franc	1183
Merlot	1120
Pinot noir	990
Lemberger	800
Gewurztraminer	725
Riesling	700
Seyval blanc	600

Total 9 variety average = $957/ton

Data are average of 3 or more.

Source: CSU Grower Survey '95

1995

Picking Costs/Ton

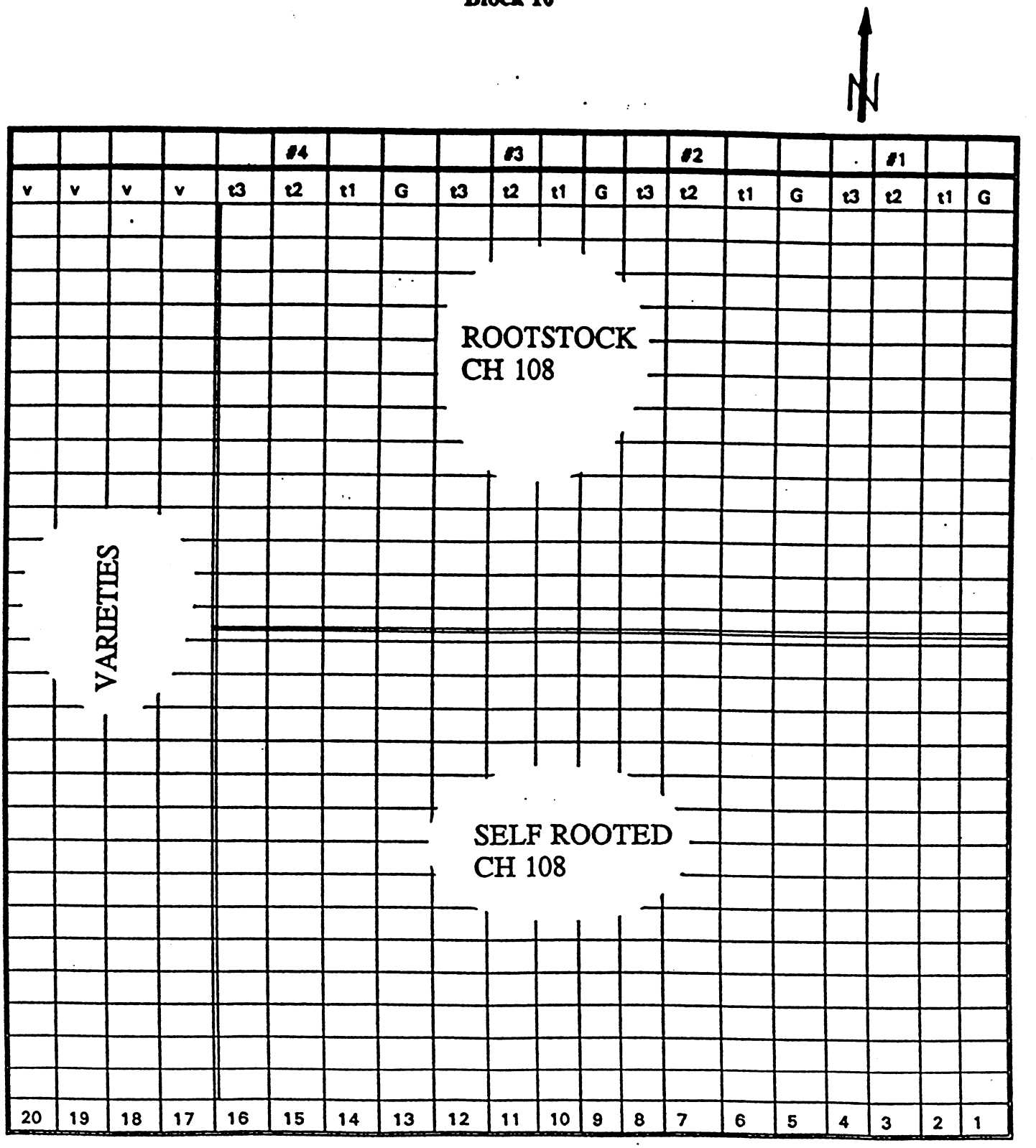
Average Cost = $124.00/ton

Range = $60 - $223/ton

Source: CSU Wine Grower Survey '95

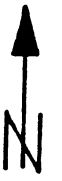
B

CSU Orchard Mesa Research Center
Vineyard Irrigation - Rootstock-Variety Trial
Block 10



Chardonnay 108 5 x 10' spacing, 80 vines/row, drip (8L/hr)

CSU Orchard Mesa Research Center
Wine Grape Varieties
Within Block 10



Key

Plot	Variety
1	Muller thurgau
2	Viognier
3	Sangiovese
4	Pinot Gris
5	Merlot #3
6	Chardonnel
7	Baco Noir
8	Vignoles
9	Cynthiana
10	Pinot Noir - Dijon 115 x 3309
11	Cayuga White
12	Norton
13	Andover
14	Riesling #9
15	Gewurztraminer #457
16	Shiraz
17	Pinot Blanc #159
18	Pinot Noir #374
19	Seyval Blanc
20	Dolcetto x 1616

Row 20	Row 19	Row 18	Row 17
7	4	8	9
14	15	13	20
10	14	9	11
11	5	1	8
15	11	11	3
4	20	2	7
6	3	7	16
13	8	6	2
16	19	3	19
17	16	17	14
18	7	20	6
8	18	14	13
19	17	12	1
2	13	10	5
5	1	4	10
12	10	19	4
9	2	15	18
1	12	18	15
3	6	16	17
20	9	5	12

Grape Varieties
CSU-Orchard Mesa Research
Block 10

Andover - New variety bred at CSU, not released.
A cross between Valient (American species) and Fr. Columbard.

Baco noir - Fr. hybrid - Maurice Baco 1900 - also known as Baco 1, very vigorous, winter hardy.

Cayuga White - white hybrid developed at Geneva, 1st planted in the 1970's, large berries, medium vigor.

Chardonnel - Seyval x Chardonnay developed at Geneva, NY, medium vigor, winter hardy.

Dolcetto - Italian red - Piedmont region. big cluster/berries, early maturing.

Gewurztraminer - #457, widely grown in Alsace France, Germany, Austria, northern Italy, small tight clusters, winter hardy.

Merlot #3 - France, Bordeaux 1/2 the acreage (North Italy (friuli region) 10-15 million cases/year.) medium winter hardiness, excellent red varietal and blending grape.

Muller thurgau - Riesling x Riesling from Germany and is winter hardy.
Riesling x sylvaner?

Norton - Cythiana - American Species *V. aestivalis*, deep red wine, small cluster, red cane color, medium to low vigor.

Pinot blanc - a true Pinot - from Alsace, Germany, Austria, early maturing, low vigor.

Pinot gris - evolved from Pinot Noir - Gray rose colored grape - also known as Tokay d Alsace - France or Rulander in Germany.

Pinot noir - great red burgundies of France,
Clones - Dijon 115, #374, low-medium vigor, fairly hardy, wine color is often light.

Riesling #9 - Germany, winter hardy white, smaller clusters, medium vigor.

Sangiovese - Italian Red - Tuscany, Italy. Dominant grape in Chianti wines, late maturing (1st years data) light colored wine.

Seyval blanc - Fr. Am. Hybrid. - Fr. breeder Seyve-Villard, very vigorous, winter hardy.

Shiraz - Australian name for Syrah - Northern Rhone Valley, France, most widely planted red variety of Australia, medium hardiness.

Vignoles - also known as Ravat 51 - Fr. Am. Hybrid, J. F. Ravat planted NY, medium vigor.

Viognier - Northern Rhone Valley. France, white aromatic.

CSU-OMRC Rootstock Trials

Chardonnay 108

10 Vines/Rep.

16	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
3309	5-C	101-14	3309	5-C	420-A	420-A	101-14	420-A	420-A	3309	420-A	3309	3309	3309	420-A
5-C	3309	5-C	101-14	101-14	3309	5-C	5-C	3309	5-C	420-A	5-C	101-14	420-A	420-A	3309
101-14	420-A	420-A	420-A	420-A	5-C	101-14	3309	101-14	3309	5-C	101-14	420-A	101-14	5-C	5-C
420-A	101-14	3309	5-C	3309	101-14	3309	420-A	5-C	101-14	101-14	3309	5-C	5-C	101-14	101-14

Rootstock	Parentage	Breeder
420-A	berlandieri x riperia	Milardet 1887
3309	riperia x rupestris	Couderc 1881
101-14	riperia x rupestris	Millardet 1882
5-C	berlandieri x riperia	A. Teleki 1922 from 5A

C

Publications:

1. Seasonal Carbohydrate Changes and Cold Hardiness of 'Chardonnay' and 'Riesling' Grapes
2. Field Response of *Vitis vinifera* Cultivars to Methanol
3. The Colorado Grapevine Vol 15 (1, 4-6) Vol 16 (2,3)
4. The Colorado Grape Growers Guide

Seasonal Carbohydrate Changes and Cold Hardiness of Chardonnay and Riesling Grapevines

R. A. HAMMAN, Jr.[1], I.-E. DAMI[2], T. M. WALSH[3], and C. STUSHNOFF[4]*

Cold hardiness and endogenous levels of soluble sugars were monitored during the dormant season for Chardonnay and Riesling (*Vitis vinifera* L.) dormant buds and stem cortical tissues. Endogenous levels of glucose, fructose, raffinose, and stachyose were strongly associated with cold hardening, increasing from the onset of cold acclimation in August to maximum cold hardiness in December and January. During dehardening in March and April, endogenous levels of these sugars dropped as temperature increased. A high ratio of glucose and fructose to sucrose coincided with maximum cold hardiness, and a low ratio was associated with the dehardened condition in fall and spring. Sucrose levels, however, were not associated with cold hardiness in either cultivar. Neither cold hardiness nor soluble sugars of grape tissues were influenced by a late harvest compared to harvest at normal fruit maturity.

KEY WORDS: *Vitis vinifera,* soluble sugars, HPLC, late harvest

Grand Junction, Colorado, has an ideal growing-season climate for the production of high quality wine grape cultivars. Although abundant sunshine, low relative humidity, cool night and hot day temperatures, and a reasonably long frost-free season are ideal for must and wine quality, high-elevation sites can experience damaging winter temperatures with unpredictable frequency (3). Accordingly, physiological and biochemical information, providing insight into mechanisms associated with resistance to freezing injury, is essential for the development and implementation of management practices which may minimize injury. This study was designed to characterize the cold hardiness response of Chardonnay and Riesling grapevines relative to seasonal environmental changes and to determine if the metabolism of one or more soluble sugars may be associated with cold hardiness.

Soluble sugars are generally known to increase, and starch to decrease, at the onset of cold acclimation in a number of woody plant species (7,10). Decreases in starch and/or increases in soluble sugars of cane tissues have also been associated with acclimation and cold hardiness in grapes (2,8,9,13,17). Recently, Wample and Bary (16) demonstrated that fructose, glucose, and sucrose were highest during the dormant, cold hardy state in buds of Cabernet Sauvignon grown in Washington state. Contrary to prevailing opinion, total soluble carbohydrates have not been found to indicate the state of cold hardiness in many plant species; *i.e.,* high levels of total soluble sugars do not necessarily coincide with increases in cold hardiness (15). On the other hand, some specific sugars, especially the galactosides of sucrose such as raffinose and stachyose, have been observed to change in accordance with acclimation to cold temperatures (5,11,12,15). Stushnoff *et al.* (15) found that raffinose and stachyose were the only specific soluble sugars which were statistically associated with season-long cold hardiness status in the cold hardy cv. Valiant (*Vitis labrusca* × *riparia)* and that total soluble sugars were not related to cold hardiness.

Raffinose family oligosaccharides (RFO) have been shown to be strongly associated with cold hardiness of several woody species; wherein, endogenous concentrations of raffinose and stachyose, but not sucrose, increase during cold acclimation and decrease with loss of hardiness (15). However, the plant species studied (*Amelanchier alnifolia* Nutt., *Cornus sericea* L., *Malus baccata* × *domestica, Prunus besseyi* Bailey, *Prunus virginiana* L., *Ribes rubrum* L., and *Vitis riparia* × *labrusca*) are extremely cold hardy (< -40°C), and it is possible that cold-tender plants such as European wine grapes have different cryoprotectant mechanisms or lack regulation of biosynthetic pathways necessary for optimal development of cryoprotection.

In this investigation, we evaluated cold hardiness of two relatively cold-tender European grape cultivars from August 1992 to April 1993. The soluble sugars were monitored individually during this period to examine the relationship between cold hardiness and the most predominant soluble carbohydrates. Another objective was to determine the influence of crop load on cold hardiness and sugar production by comparing tissues from vines harvested during late September, with fruit at 22° Brix, with those from vines with fruit at 28° Brix harvested after the first frost of 1 November 1992. It was hypothesized that late-harvested fruit might accumulate sugar at the expense of the cane and bud tissues, thereby, altering cold hardiness.

Materials and Methods

Plant materials: Dormant buds and cortical tis-

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This research was conducted at the Orchard Mesa Research Center and the Department of Horticulture, Colorado State University.

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sues were collected from canes of healthy six-year-old vines of *V. vinifera* cv. Chardonnay and Riesling grown at Orchard Mesa Research Center, Grand Junction, Colorado. The cordon-pruned vines were trained to a six-wire vertical, shoot positional trellis system, grown under clean cultivation with a fall cover crop of winter wheat sown 20 September 1992, and irrigated to maintain 75% field capacity as determined by a neutron probe. All normal production practices were followed. In this experiment, the normal harvest date was based on fruit sugars attaining about 22° Brix. The normal harvest date for Chardonnay and Riesling was 11 September 1992 and 7 October 1992, respectively. The late harvest date was 3 November 1992, following the first killing frost (-3°C) of 1 November 1992. Late harvested fruits of Chardonnay and Riesling attained 28° and 26° Brix, respectively.

Sample collection of plant material and fruit was from treatments assigned at random to four replications of vines grown in a randomized block design. Samples were collected at approximately two-week intervals, from 1 September to 6 October 1992, and thereafter at three- to four-week intervals until 18 April 1993. Bud break was considered to be 21 April 1993 for Chardonnay and 23 April 1993 for Riesling.

Cold hardiness. ***Lowest survival temperature by visual examination (LST_{100}):*** Dormant buds were harvested with a 20-mm long section of stem on each harvest date. These buds were placed in plastic bags to prevent moisture loss and subjected to gradual freezing by lowering the temperature 2°C/hr. Four samples were removed at 2.5°C intervals, at each of four stress temperatures chosen to span the probable lethal temperature at a particular sampling date. The test buds were thawed and maintained at room temperature in sealed plastic bags for seven days, at which time they were sectioned freehand and examined for oxidative browning, an indicator of lethal freeze injury (14). The temperature at which no injury (100% survival) was detected, was recorded as the lowest survival temperature (LST_{100}) for the harvest date.

Low temperature exotherm (LTE): Cold hardiness was also determined by monitoring the low temperature exotherms (LTE) detected at the ice nucleation temperature for overwintering buds. Copper constantan, 36 gauge, thermocouples were attached to the outside surface of each bud with parafilm. Seven test buds and a dried reference bud of approximately equal weight were subjected to controlled-rate freezing at 2°C/hr in an aluminum block placed in a Tenny Jr. programmable freezer (Tenney Inc., South Brunswick, NJ). Temperature data were recorded every second from 0°C to -30°C using an Omega WB-AAIB high resolution interface card (Omega, Stamford, CT). Differential thermal analysis freezing curves were plotted from stored data using Axum graphics software (Trimetrix Inc., Seattle, WA). LTEs for the overwintering buds were recorded as the killing temperatures.

Quantitative determination of soluble sugars: On each sample date (eleven), mid-cane internode segments, approximately 10 cm long, were frozen and shipped by overnight delivery with dry ice in insulated styrofoam containers from Grand Junction to Fort Collins. Unexpended dry ice was always present upon arrival. The samples were immediately plunged into liquid nitrogen, freeze dried and stored at -20°C in a desiccated environment until analysis.

Three to five internodal stem segments (with the bark removed) were combined in each replicate and ground with a Wiley Mill using a 40-mesh screen, further pulverized with a mortar and pestle and passed through a 100-mesh stainless steel screen. About 1 mg of screened sample was solubilized in 1 mL, 100 m*M* NaOH, centrifuged for five minutes at 4°C, at 10 000 rpm, and filtered through a 0.22 µ*M* Whatman Nylon 66 syringe filter. The filtrate was analyzed using a Dionex DX-300 series HPLC system (Dionex Co., Sunnyvale, CA), equipped with a 25-µL injection loop. Oligosaccharides were separated on a Carbopac PA-100 column (4.6 × 250 mm) using a flow rate of 1 mL/min at ambient temperature, equipped with a Dionex guard column (3 × 25 mm). A pulsed electrochemical detector was used for detection of oligosaccharides. An eluant gradient was used to optimize the oligosaccharides separation. NaOH concentration was linearly increased from 70 m*M* to 120 m*M* in 18 minutes, combined with sodium acetate concentration held at 3 m*M* for 10 minutes, then increased to 35 m*M* for the last 8 minutes. Sugar concentrations were calculated on a moles per gram of dry weight basis. Two to three extractions were analyzed for each of the four replicates.

Statistical analyses: Correlation analyses were conducted to determine the association among the following factors: injury estimated by assessment of visual browning for LST_{100} and LTE for overwintering buds; mean outdoor minimum temperature for 2, 4, 7, 10, and 15 days preceding sampling date and LST_{100} of buds and endogenous levels of fructose, glucose, sucrose, raffinose and stachyose in internodes. An unpaired t-test was used to compare differences in bud cold hardiness based on LST_{100} and on LTE for canes taken from vines on the normal and late harvest dates.

Results and Discussion

Cold hardiness and the environment: Cold hardiness of Chardonnay and Riesling dormant buds, for both normal- and late- harvested vines, was closely associated with the mean minimum temperature preceding determination of cold hardiness (Table 1). The lowest temperature experienced was -17°C, 26 December 1992, and according to results of the controlled freezing tests, at no time in 1992-1993 did outdoor minimum temperatures drop low enough to injure the buds of either cultivar (Fig. 1). Bud injury, evaluated by tissue browning (LST_{100}), produced results similar to evaluation of injury by monitoring LTEs for the buds in both cultivars (Fig. 1, 2).

For both normal- and late-harvested vines, endogenous levels of fructose, glucose, raffinose, and stachyose, but not sucrose, were directly related to low tempera-

Table 1. Linear correlation coefficients (r) for soluble sugars (moles/g dry wt) in internodes and bud cold hardiness (determined by visual browning, lowest survival temperature with no injury [LST_{100}]), and the mean minimum temperature (°C) for 2, 4, 7, 10, and 15 days preceding sampling for Chardonnay and Riesling grapevines. Data are based on means of four replicates for each collection date.

Cultivars	Days preceding sampling	Glucose	Fructose	Sucrose	Raffinose	Stachyose	LST_{100}
Chardonnay							
	2	-0.76*	-0.81**	-0.47 ns	-0.77**	-0.88***	0.93***
	4	-0.82**	-0.88***	-0.58 ns	-0.84**	-0.92***	0.96***
	7	-0.84**	-0.91***	-0.60 ns	-0.83**	-0.92***	0.97***
	10	-0.83**	-0.90***	-0.60 ns	-0.84**	-0.92***	0.97***
	15	-0.82**	-0.89***	-0.59 ns	-0.82**	-0.90***	0.97***
Riesling							
	2	-0.71*	-0.73*	-0.51 ns	-0.81**	-0.82**	0.93***
	4	-0.80**	-0.83**	-0.62 ns	-0.88***	-0.88***	0.95***
	7	-0.83**	-0.85**	-0.64*	-0.88***	-0.89***	0.96***
	10	-0.83**	-0.86**	-0.64*	-0.89***	-0.90***	0.96***
	15	-0.80**	-0.84**	-0.61 ns	-0.87**	-0.87***	0.96***

*$p = 0.05$; **$p = 0.01$; ***$p = 0.001$.

tures from 2 to 15 days preceding analyses (Table 1) and to cold hardiness status 25 August 1992 through 30 March 1993 (Table 2). We cannot imply from this study that increasing accumulation of any one of these sugars in internodes causes an increase in bud cold hardiness; however, low temperature and endogenous levels of fructose, glucose, raffinose and stachyose in internodes are very strongly associated with bud cold hardiness in these two cold-tender cultivars.

Soluble sugars: At the end of the growing season (August, September, October), sucrose was the most predominant reserve sugar for Chardonnay and Riesling internodes (Fig. 3, 4). Glucose and fructose were also found in lesser amounts. RFO were present in even lower quantities in the stems of both cultivars. As the mature shoots began to cold acclimate, RFO levels increased slowly but steadily. Sucrose, glucose and fructose, however, remained at almost the same levels until the first frost.

About one month after the first frost (1 November 1992), there was a major change in the sugar content of internodes (Fig. 3, 4). Glucose and fructose increased markedly and peaked in late December. The rise in monosaccharides was accompanied by an increase in RFO. These sugars reached their maximum levels during mid-winter, then started to decline with the other sugars as the spring season approached. Furthermore, despite the presence of the same sugars throughout this period, their proportions relative to total soluble sugars changed substantially, especially the monosaccharides and disaccharide. In mid-winter (December, January, February), the monosaccharides (glucose +

Table 2. Linear correlation of bud cold hardiness (lowest survival temperature with no injury [LST_{100}]), 25 August 1992 to 30 March 1993, with internode soluble sugars (moles/g dry wt) for Chardonnay and Riesling grapevines. Data are based on means of four replicates for each collection date.

Cultivars	Coefficients and probability	Glucose	Fructose	Sucrose	Raffinose	Stachyose
Chardonnay	Correlation (r)	-0.76	-0.88	-0.55	-0.83	-0.92
	Probability (p)	0.01	0.0007	0.09	0.003	0.0001
Riesling	Correlation (r)	-0.71	-0.75	-0.50	-0.84	-0.82
	Probability (p)	0.02	0.01	0.1	0.002	0.003

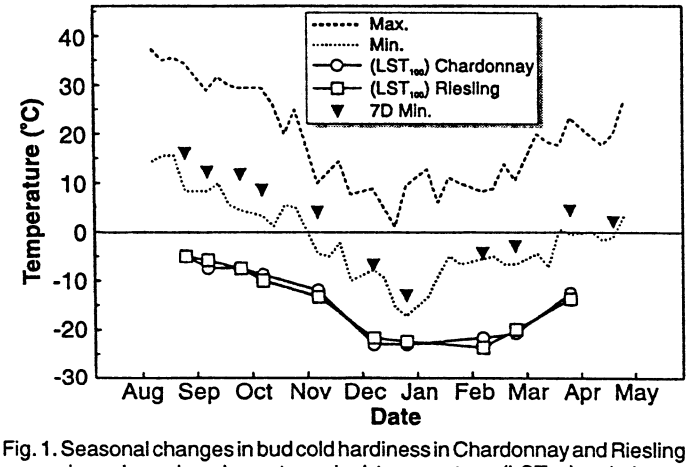

Fig. 1. Seasonal changes in bud cold hardiness in Chardonnay and Riesling grapevines, based on lowest survival temperature (LST_{100}), relative to weekly maximum and minimum temperatures (°C). ▼ denotes the average minimum temperature for seven days prior to the sample date of cold hardiness tests and biochemical analysis.

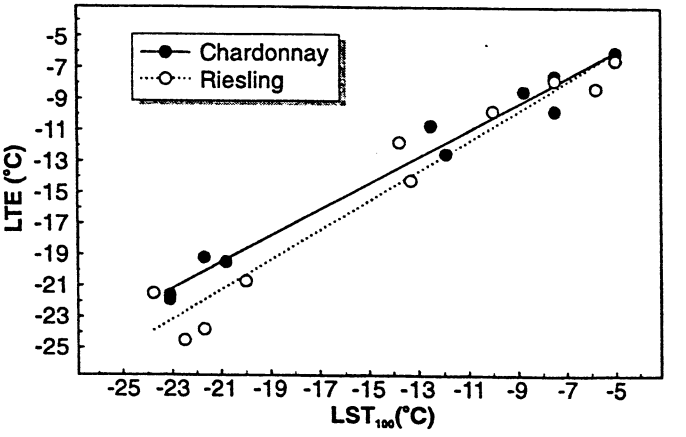

Fig. 2. Relationship of bud cold hardiness in Chardonnay and Riesling grapevines, based on lowest survival temperature (LST_{100}), and low temperature exotherms (LTE), determined by regression analysis. Data are based on means of ten dates from August through March.

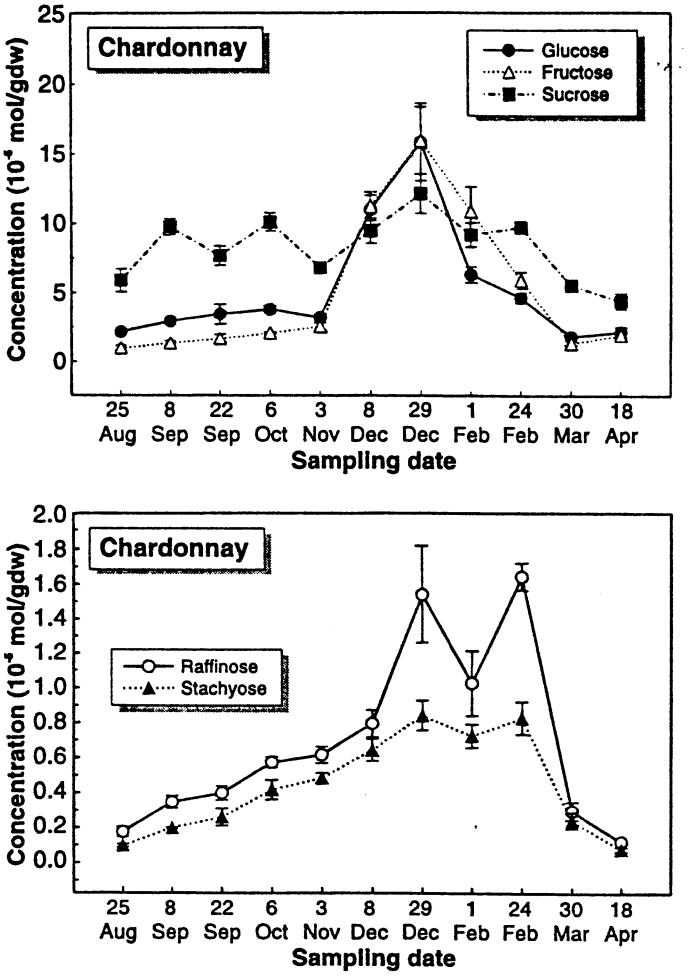

Fig. 3. Seasonal changes in soluble carbohydrates derived from cane internode tissues of Chardonnay grapevines, 25 August 1992 to 18 April 1993 at Grand Junction, Colorado. Data are means ± SE from four replicates.

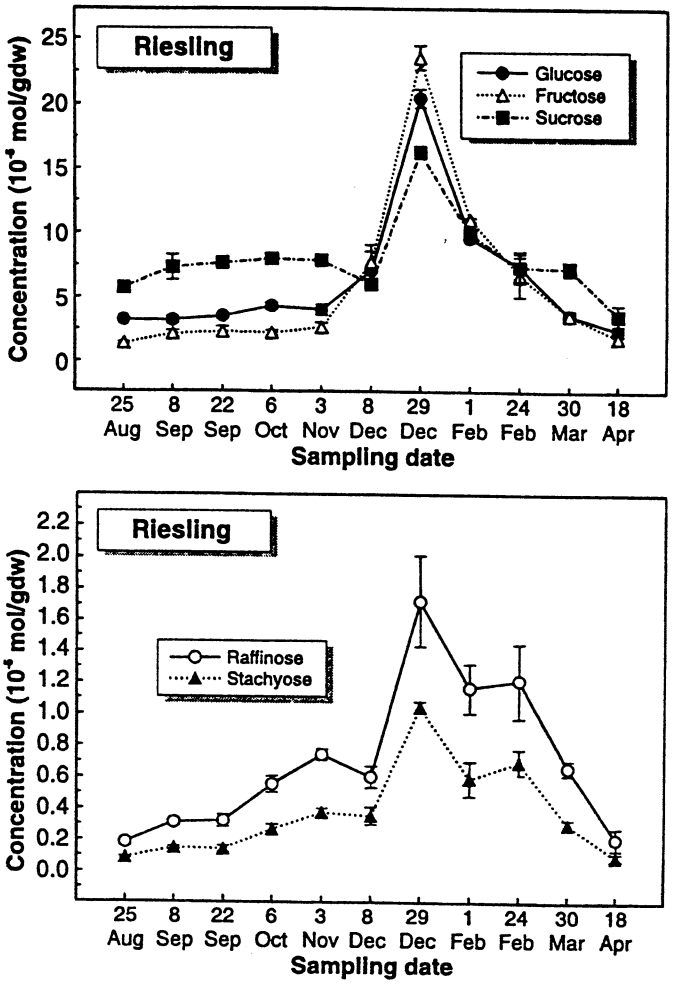

Fig. 4. Seasonal changes in soluble carbohydrates derived from cane internode tissues of Riesling grapevines, 25 August 1992 to 18 April 1993 at Grand Junction, Colorado. Data are means ± SE from four replicates.

fructose) and sucrose represented 60% to 70% and 25% to 33% of the total soluble sugars, respectively (Table 3). These proportions were reversed during the deacclimation season (late spring and summer), when monosaccharides ranged between 27% and 47% of the total soluble sugars, and disaccharides between 50% and 70%. The ratio of the monosaccharides to disaccharide [(glucose + fructose)/sucrose] indicates, in a striking manner, the sugar changes during the season (Table 3). For example, the ratio in Chardonnay was lowest in late August (0.53), early September (0.38) and again in March (0.57), when the buds and stems were most cold sensitive. This ratio reached its maximum (2.63) in late December when the buds were the hardiest.

Extremely cold hardy species such as currant, dogwood, and chokecherry (LT_{50} from -60°C to -80°C) produce substantial amounts of RFO, but undetectable amounts of monosaccharides during winter (15). Amounts of RFO correlate strongly with cold hardiness in cold hardy taxa as measured by LT_{50}(15). In addition, the cold hardy cultivar, Valiant, accumulated raffinose, stachyose, sucrose, and glucose but not fructose (15); however, only raffinose and stachyose correlated significantly with cold hardiness, whereas sucrose and glucose did not.

Although other studies have implicated RFO as cryoprotectants (4,6,15), it is not clear whether glucose and fructose play a significant role as cryoprotectants. This study suggests they may play such a role because of their abundance and association with hardiness. Another interpretation is that their accumulation pattern may interfere with RFO synthesis and thus limit cold hardiness. This may explain the differences in cold hardiness potential observed between European grapes, Chardonnay and Riesling (about -25°C), compared to American grapes such as Valiant (about -40°C). The monosaccharide synthesis pathway may utilize sucrose and interfere with the RFO pathway, which uses sucrose as a substrate for the synthesis of raffinose and stachyose (1). This shift may result in reduced synthesis of RFO, providing only limited cryoprotection, such as found in *V. vinifera* dormant buds. This hypothesis also assumes that the monosaccharides do play a limited role in freeze protection.

Normal versus late harvest: No consistent or insightful trends were detected in vines subjected to a late harvest after the first killing frost on 1 November

Table 3. Proportion of monosaccharides and sucrose to total soluble sugars in internode tissues of Chardonnay and Riesling grapevines from August 1992 through March 1993.

	Chardonnay				Riesling			
Date of collection	Glucose (%)	Fructose (%)	Sucrose (%)	(Glu + Fru) /Sucrose	Glucose (%)	Fructose (%)	Sucrose (%)	(Glu + Fru) /Sucrose
25 Aug	23.1	10.5	63.4	0.53	30.4	13.2	53.9	0.81
8 Sept	18.4	8.4	69.7	0.38	24.4	16.2	55.9	0.72
22 Sept	25.5	12.3	57.2	0.66	25.3	16.6	54.8	0.76
6 Oct	22.3	12.2	59.7	0.58	28.0	147.5	52.1	0.81
3 Nov	23.4	18.6	49.9	0.84	24.5	15.7	52.6	0.76
8 Dec	33.1	34.0	28.6	2.34	32.4	35.6	27.6	2.46
29 Dec	34.3	34.5	26.1	2.63	32.4	37.4	25.8	2.70
1 Feb	22.4	38.7	32.7	1.86	29.8	34.1	30.7	2.08
24 Feb	20.3	26.0	42.8	1.08	31.4	28.9	31.5	1.91
30 Mar	19.6	14.4	60.0	0.57	23.5	23.4	46.8	1.00

Table 4. Endogenous sugars (10^{-5} mol/g dry wt) and cold hardiness (LTE temperature) of Chardonnay and Riesling dormant buds from vines harvested at 22°Brix compared to vines late-harvested at 28° Brix. Non-significant data for other sugars is not shown.

Sample date	Glucose		Sucrose		LTE (°C)	
	N[x]	L[y]	N	L	N	L
Chardonnay						
3 Nov	3.18	3.27	6.79	8.08	-12.5	-17.8**
8 Dec	10.95	11.41	9.44	10.57	-21.9	-23.5
29 Dec	15.85	20.15	12.08	14.72	-21.6	-18.7
1 Feb	6.28	7.07	9.12	9.15	-18.8	-21.9
24 Feb	4.60	5.66	9.58	9.68	-19.5	-18.7
30 Mar	1.80	1.91	5.51	7.11*	-10.7	-9.9
Riesling						
3 Nov	4.03	4.22	7.87	7.14	-14.2	-16.7**
8 Dec	-	-	-	-	-23.8	-24.8
29 Dec	20.48	24.22*	16.29	14.94	-24.5	-23.3
1 Feb	9.64	8.07	9.93	11.01	-21.5	-19.5
24 Feb	7.37	7.31	7.39	9.44	-20.7	-25.5**
30 Mar	3.65	4.07	7.27	7.35	-11.7	-9.3

*$p = 0.05$; **$p = 0.01$

[x] = Normal harvest (Chardonnay: 11 Sept 1992; Riesling: 7 Oct 1992)

[y] = Late harvest (Chardonnay and Riesling: 4 Nov 1992)

1992. Comparisons for each sampling date and cultivar, using an unpaired t-test, revealed only a few significant differences (Table 4). There were no meaningful trends relating soluble carbohydrate levels to cold hardiness of either cultivar for normal versus delayed harvest. LTEs for both Chardonnay and Riesling were significantly lower from buds of late-harvested vines tested 3 November 1992, and for Riesling tested 24 February 1993, but there were no corresponding differences using LST_{100} to evaluate injury. Wample and Bary (16) concluded that neither delaying harvest nor failing to harvest Cabernet Sauvignon vines adversely influenced cold hardiness of vines grown at Prosser, Washington from 1985 to 1988.

Grand Junction, Colorado, is a very suitable high-elevation site for high quality wine grape production. It has high light intensity, high daytime temperatures and cool night temperatures. Unfortunately, the high elevation is also accompanied by a significant risk of low temperature injury to *V. vinifera* during the dormant season. Growers could benefit significantly if a single production practice, such as avoiding a late harvest, would influence winter hardiness, as suggested by observations in several other growing areas. On the basis of these data and three years of data from Prosser, Washington (16), we conclude that with normally high photosynthetic conditions and no other apparent limiting growth factors, there is no adverse influence of a delayed fruit harvest on bud hardiness nor on endogenous accumulation of soluble carbohydrates in cane internodes.

Conclusions

A strong association between cold hardiness and endogenous content of several sugars was found. This may be a good starting point to search for biochemical mechanisms that may be responsible for stabilization of grape tissues during freezing stress. Because the monosaccharide synthesis pathway is stimulated during dormancy as cold hardiness increases, it probably interferes with the RFO pathway, which uses sucrose as a substrate for the synthesis of raffinose and stachyose. This 'competition' may lead to restricted synthesis of RFO, limiting protection to the extent of that found in *V. vinifera* dormant buds, in contrast to much higher levels of RFO found in cold hardy Valiant cultivar and other woody plant species. With the cold-tender cultivars such as Chardonnay and Riesling, a high monosaccharides to disaccharide ratio is a better indicator of cold hardiness than RFO levels *per se*.

If good conditions exist for growth, such as optimal photosynthesis and no apparent abiotic or biotic stress, there is no adverse influence of a delayed fruit harvest on bud hardiness nor on endogenous accumulation of soluble carbohydrates.

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Field Response of *Vitis vinifera* Cultivars to Methanol

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The response of *Vitis vinifera* cultivars to methanol was investigated at the Orchard Mesa Research Center in Grand Junction, Colorado. Sublethal methanol doses, based on visual observations, were 90% for leaves and 100% for trunks. The accumulation of total soluble solids (TSS) in berries was also monitored weekly until harvest. There was no significant difference between the treated and control vines during the period of study, with the exception of Muscat blanc, which showed a slightly higher TSS content in methanol-sprayed vines than in controls. This difference, however, disappeared during ripening. There was no significant effect of methanol on photosynthesis, transpiration, nor stomatal resistance. At harvest, data on yields estimated by cluster weight, berry weight and berry size indicated no differences between the two treatments. Methanol did not alter cold hardiness of dormant buds, measured by differential thermal analysis. It was concluded that in this study methanol had little or no physiological, nor practical effect on grapes.

KEYWORDS: cold hardiness, photosynthesis, total soluble solids, yield components

Improving quality and yield of cultivated plants have been major goals of growers and scientists during past decades. Manipulation of the environment and the genome have resulted in substantial progress toward attaining these goals. Application of methanol recently produced impressively high yields, surpassing expectations with several crops of economic importance (12,13). Because of the ease and low cost of application, methanol was considered by some to be a breakthrough with potential to revolutionize agricultural production of several C_3 crops, but the concept needed further research investigation with untested crops.

Capacity to utilize methanol by bacteria, fungi, algae, animals, and plants was reported many decades ago. Large *et al.* (7) were the first to document that bacteria could grow on methanol as a sole source of carbon and energy in an aerobic environment. Other reports indicated that methanol is rapidly metabolized and converted to amino acids under dark conditions by plants (2). A Russian group led by Doman and Romanova (3) demonstrated that plant leaves are capable of photosynthetic assimilation of methanol under light conditions. They added that bean and barley leaves assimilated vapors of formic acid, formaldehyde and methanol with the formation of products of about the same qualitative composition and about the same sequence of appearance as observed in CO_2 assimilation.

Nonomura and Benson's conclusions (12,13) were not limited to the assimilation of methanol *per se*, but went one step beyond. They discovered that, if properly applied to C_3 crops grown in areas of intense sunlight, methanol can substantially boost crop production and shorten the maturity cycle, hence potentially conserving water by reducing irrigation requirements. Since C_4 plants did not respond to methanol, Nonomura and Benson (12) suggested that methanol may affect the photo-respiratory pathway, which is functional only in C_3 plants. They proposed that methanol may inhibit photorespiration in C_3 plants, thus rendering the latter to act like the more efficient C_4 plants (1, 12). Another possible mechanism is the incorporation of methanol into the photo-respiratory pathway, leading to the production of more amino acids such as serine, and ultimately doubling carbohydrate (*i.e.*, sucrose) synthesis (13).

The objective of this study was to evaluate whether the application of methanol on grapevines is beneficial under conditions of high temperature and high light intensity relevant to the Grand Valley in the Western slope of Colorado. These conditions are presumably prerequisites for the most effective methanol treatment.

Materials and Methods

The experiments were conducted at the Orchard Mesa Research Center, Grand Junction, Colorado (trickle irrigated), and two other private vineyards at Palisade, Colorado, seven kilometers from the research center, both furrow irrigated. *Vitis vinifera* L. mature vines (5 - 12 years old), both white and red cultivars, were used in this study. They were all own-rooted, trained to bilateral cordons, spur-pruned, and shoots were vertically positioned with a wire trellis system. The three vineyard sites were regularly irrigated and all normal production practices were followed. The experimental design was a randomized complete block, replicated four or five times at each site, with a single vine per replicate.

The first part of the study consisted of determining the sublethal dose of methanol for leaves and trunks as described by Nonomura and Benson (12). Assessment

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of methanol-toxicity symptoms was based on visual observations of necrosis, chlorosis or any other form of methanol-related injury on different parts of the sprayed vine. Solutions of methanol, ranging from 10 to 100% (v/v) in 10% increments, were each mixed with a surfactant, 0.1% (v/v) Triton X100 (Sigma, St. Louis). Hand- held (7.5 liters, Optimum Model 1402 RL Co., Lowell, Michigan) and backpack (15 L, Solo Model 425, Solo Inc., Newport News, Virginia) sprayers were used for all applications. The spray was applied on clear-sky days between 1300 hr and 1600 hr, when temperatures ranged between 27°C and 35°C. The developmental stages at which vines were sprayed ranged between full bloom and berry set. Cultivars sprayed to runoff included Chardonnay, Semillon, Riesling, Muscat blanc, Pinot blanc, Sauvignon blanc, Gewürztraminer, Aligote, Sylvaner, French Colombard, Gamay Beaujolais, Pinot noir, Merlot, Cabernet Sauvignon, Malbec, and Carmine.

Once sublethal doses were determined, cultivars were selected to evaluate the effect of further applications of methanol on physiological and biochemical characteristics of grapevines. The treated vines consisted of multiple applications (2 - 3) of methanol. Sprays included foliage (90% methanol + 0.1% Triton X100) or trunk (100% methanol + 0.1 Triton X100) application. Control vines were sprayed with water + 0.1% Triton X100. The volume of methanol solution sprayed to runoff was estimated to range between 0.2 L and 0.7 L per vine, depending on the vigor and the part of the grapevine sprayed (trunk or foliage). Treatments were applied under the same conditions mentioned above. Since the results among several cultivars between foliage and trunk treatments were similar, only the former are discussed with the following cultivars: Muscat blanc, Chardonnay and Merlot. Dates of application (A), veraison (V) and harvest (H) for each cultivar were as follows: *(1)* Muscat blanc: 27 July, 3 August, 23 August (A); 13 August (V); 21 September (H); *(2)* Chardonnay: 29 July, 22 August (A); 14 August (V); 13 September (H); *(3)* Merlot: 29 July, 22 August (A); 18 August (V); 1 October (H).

Total soluble solids (TSS) were monitored following the first application until harvest. Ten to twenty berries on a replicate vine were taken on each sampling date at about seven-day intervals, and a laboratory refractometer (Abbe-3L Milton Roy, Rochester, NY) was used to measure TSS (°Brix) from the squeezed juice. At harvest, the clusters from each vine were counted, the weight per vine recorded and the average cluster weight calculated. Also, berries were randomly collected from clusters and the average weight of ten berries recorded, and their size measured with a caliper (Max-Cal Jensen Tools Inc., Phoenix, AZ).

In an attempt to confirm the results of Nonomura and Benson regarding the effect of methanol on photosynthesis, measurements of CO_2 uptake, transpiration and stomatal resistance were taken in an open system configuration. The photosynthesis rate was determined by measuring the rate of CO_2 concentration assimilated by a known leaf area in a given time. Transpiration rate is based on measuring the water vapor flux per one-sided leaf area. Stomatal resistance was obtained by measuring transpiration and leaf surface temperature and applying the appropriate equation for calculation. These parameters were simultaneously taken on a single leaf clamped inside a leaf chamber connected to a CO_2 gas analyzer Model CI-301 (CID Inc., Vancouver, WA). Readings were from fully expanded, but not senescent leaves in the middle part of the vertically-trained shoots. The leaves were also fully exposed and in almost a perpendicular position to the sunlight. Data were collected in the morning between 0900 h and 1100 h under saturating light > 1000 $\mu molm^{-2}s^{-1}$ PPF. Measurements on Muscat blanc were taken 24 hours after the first and the second spray. Measurements on Chardonnay and Merlot were taken 24 hours and one week after the first spray. No further measurements were taken because of technical problems encountered with the equipment. Three to five leaves were measured per vine, and four to five vine replicates were used in this experiment.

In the winter of the same year, buds were collected from dormant vines of Muscat blanc which were sprayed with methanol in the summer of 1993. Bud cold hardiness was monitored from 2 December 1993 until 5 April 1994, using differential thermal analysis described elsewhere (15). The low temperature exotherms (LTE) for the overwintering buds were recorded as the killing temperatures. It was hypothesized that if methanol altered sugar metabolism and translocation, it might also alter bud cold hardiness, since the latter has been shown to be strongly correlated with endogenous sugar metabolism (5).

All data were subjected to analysis of variance where applicable and were presented as treatment means. Means were separated using T-tests.

Results and Discussion

Sublethal dose: None of the concentrations between 10% and 90% for leaves and 60% to 100% for trunks showed any toxicity symptoms during the six-week visual observation period. The only damage observed was on vines sprayed on foliage with 100% methanol. The effect was immediate; within a few hours after spray application, young leaves and shoot tips curled and turned necrotic. Also, the small green berries discolored and turned purple at the surface of contact with methanol. The effect of absolute methanol on berries was irreversible since the latter never recovered and remained small in size and cracked by the time of ripening. The shoot tips, however, resumed growth normally. It was, therefore, concluded that the sublethal doses for foliage and trunk application were 90 and 100%, respectively. These concentrations were used for subsequent experiments.

Effect of methanol on TSS: The TSS in the berries, monitored every week until harvest, showed significant differences in Muscat blanc during veraison. The methanol-treated vines had about 1° Brix more

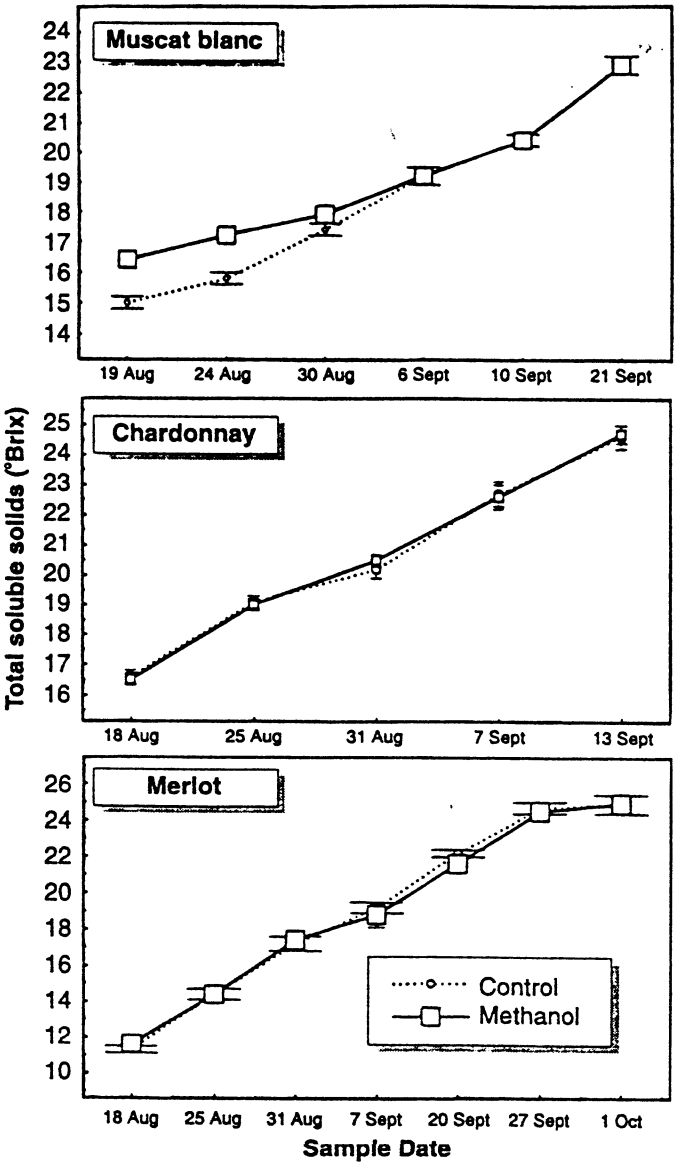

Fig. 1. Changes in berry total soluble sugars following methanol applications on Muscat blanc, Chardonnay, and Merlot. Data points are means ± SEM. Control treatment: water + 0.1 % Triton X100. Methanol treatment: 90% methanol + 0.1% Triton X100.

than the control on 19, 24, and 30 August (Fig. 1a). This difference, however, disappeared as fruit maturity approached, and at harvest the TSS for both treatments were almost the same. On the other hand, Chardonnay and Merlot showed no significant differences in TSS between the two treatments throughout the fruit development period (Fig. 1b, 1c). A late ripening cultivar, Riesling, exhibited a rather negative effect of methanol where TSS was even lower in treated vines than that of control (data not shown). Similar conclusions were drawn by Wutscher (17), where no significant differences in juice content or soluble solids in orange fruits were observed between control and methanol-treated trees. These results, however, do not support the positive effect of methanol on sugar accumulation reported for tomato plants (12).

Effect of methanol on photosynthesis: Methanol stimulation of growth was potentially attributed to reduced photorespiration by Nonomura and Benson (12,13). They did not, however, test the direct effect of methanol on photosynthesis. In this experiment, readings of photosynthesis, transpiration and stomatal resistance were taken in three cultivars. We tested a short-term response (readings taken 24 hours after spray) and relatively long-term response (readings taken 1 week after spray) of grapevines to methanol. In all cases, no statistically significant differences between control and methanol-treated vines were observed, indicating no effect of methanol on photosynthesis, at least in the cultivars studied. Furthermore, the results in this study contradict the findings by Nishio *et al.* (11). These authors reported that 20% methanol treatment of spinach plants stimulated photosynthetic CO_2 gas exchange as quickly as three hours after treatment, with a continued positive response, compared to the control, for two weeks. Nishio *et al.* (11), however, could not explain the perplexing long-term stimulatory effect of methanol on photosynthesis.

Effect of methanol on yield components: The effect of methanol on yields, as reported by Nonomura and Benson (12), was the most interesting response, attracting researchers throughout the world to test the hypothesis. Unfortunately, in this study methanol had no effect whatsoever on any component of yield in all three cultivars (Table 1). Numerous studies had similar

Table 1. Effect of methanol on yield components of Muscat blanc, Chardonnay, and Merlot. Data are means ± SEM[x].

Cultivars	Treatments (g)	Cluster weight (g)	Berry weight[y] (mm)	Berry size
Muscat blanc	Control	155.3 ± 10.7	20.4 ± 1.0	15.4 ± 0.2
	90% methanol	152.5 ± 14.1	18.4 ± 0.4	15.4 ± 0.1
Chardonnay	Control	73.1 ± 6.3	12.3 ± 0.3	12.1 ± 0.1
	90% methanol	72.6 ± 11.5	11.9 ± 0.4	12.3 ± 0.2
Merlot	Control	108.6 ± 12.3	13.3 ± 0.2	13.1 ± 0.1
	90% methanol	104.1 ± 12.5	13.2 ± 0.2	

[x] = Treatments are foliar applications as follows: control = water + 0.1% Triton X100; methanol = 90% methanol + 0.1% Triton X100.
[y] = average weight of 10 berries.

Table 2. Effect of methanol on bud cold hardiness of Muscat blanc determined by differential thermal analysis and expressed as low temperature exotherm (LTE, °C) at different dates of collection. Data are means ± SEM[x].

Sample date	LTE (°C)	
	Control	Methanol
2 Dec	-23.7 ± 0.3	-21.8 ± 1.6
13 Jan	-22.2 ± 0.9	-21.9 ± 1.0
8 Feb	-23.0 ± 0.8	-23.2 ± 0.5
2 Mar	-21.9 ± 0.3	-21.5 ± 0.6
16 Mar	-12.6 ± 1.0	-13.5 ± 0.6
22 Mar	-13.9 ± 1.2	-12.9 ± 0.5
5 Apr	-14.2 ± 1.0	-12.3 ± 0.8

[x] = Treatments are foliar applications as follows: control = water + 0.1% Triton X100; methanol = 90% methanol + 0.1% Triton X100.

disappointing results with a wide range of crop species such as orange (17), okra, chili pepper, and eggplant (16), creeping bentgrass, lemon, carrot, romaine lettuce, radish, wheat, corn and pea (14), tomato and melon (6), peppermint (10), cotton (9), snapbean (8), and potato (4).

Effect of methanol on cold hardiness: LTEs of buds collected from treated and control Muscat blanc vines (Table 2) revealed no significant differences for any sample dates. Data comparing specific soluble sugars in cane tissues between the two treatments also showed no differences (data not shown).

Conclusions

Even though we attempted to follow a similar protocol to that used by Nonomura and Benson (12), there were no responses to methanol in any of the parameters measured in grapevines. Other experiments comparing single *vs.* multiple applications showed no influence of methanol (data not shown). The methanol formulation used in this study was modified from that reported by Nonomura and Benson (12) since we did not include any nutrients, amino acids or nitrogen fertilizer. The reason was to exclude any extra- or side-effect(s) that may interfere or override the methanol action *per se*. Nevertheless, we can conclude that methanol had no physiological or practical effect on the C_3 species, *Vitis vinifera*, and we do not recommend its use in a commercial production scale. Most of the published studies agree that there is no measurable response of methanol to enhance crop quality or quantity, and our study is another to support that claim.

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Fruit Growers Newsletter

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Good Neighbor Practices

by Harold Larsen

Diseases, insect pests, weeds, pesticides, and water do not recognize property boundary lines. Growers are occasionally confronted with problems that are not of their own making, but rather the result of actions (or inactions, as the case may be) of other property owners/managers nearby. A grape or pear grower suddenly notices in early summer that the new growth on his plants is severely distorted, with strong symptoms of 2,4-D herbicide injury. A nearby neighbor has applied a 2,4-D herbicide even though temperatures reached into the 90's at the time. Another grower finds one of his peach blocks has been flooded for a week when an uphill neighbor "forgot" to clean out a waste ditch at the bottom of property being irrigated above.

In both of the above examples, the damage inflicted can be traced fairly readily back to the responsible party who is liable for any damages caused by his/her actions. Any individual who applies any pesticide is required by law to be certain that the application complies with all provisions/restrictions on the label and that drift (whether as fine mist or volatile vapors) off of the property does not occur. Similarly, by law, one is responsible for what happens to the wastewater from any irrigation application.

Some insect pests and weeds may be subjects of control where pest districts have been established or where noxious weeds are present (under state law requiring control of certain noxious weeds). However, the number of weeds and insect pests falling under these regulations are quite few, and no plant diseases are included. This means that most weeds, insect pests, and all fruit diseases are not under regulation. Thus, each grower and property owner needs to manage their properties in a conscientious manner. Application of this Golden Rule approach ("Do unto others as you would have them do unto you.") will both help minimize adverse impacts on neighbors and encourage a cooperative spirit between neighbors. One never knows when these aspects may become critical for one's own situation in the future.

IN THIS ISSUE:

	page
Good Neighbor Practices	-- 1
Beginner's Branch	-- 2
CSU Implements A Few Changes	-- 2
Leaf Analysis Time	-- 2
The Colorado Grapevine	-- 3
WCHS Transcripts Available	-- 4
Calendar	-- 4

Colorado State University, U.S. Department of Agriculture and Colorado counties cooperating.
Cooperative Extension programs are available to all without discrimination.

The Colorado Grapevine

by Rick Hamman

Crop Thinning:

Crop thinning is a final adjustment technique of crop regulation that results in the most significant fruit quality improvement. The demand for quality fruit is extremely important for premium wine making and crop thinning is a technique that can improve fruit/wine quality. Crop thinning adjustments may be necessary to mature the fruit earlier or change wine style. Vigorous varieties such as Seyval blanc and Dechuanac have high initial fruit set and continue to produce abundant flowers late in the season which must be removed for proper fruit composition and maturation. Many other varieties such as Chardonnay, Merlot, Pinot noir, Gewurztraminer, Cabernet savignon, Semillon and Cabernet franc may also need some crop adjustment. The earlier the thinning, the greater the benefit to the remaining inflorescence. Early inflorescence thinning, i.e., before flowering, is an operation that is rapid and easily visible. Bunch thinning or cluster thinning is done after flowering and set. This operation involves removal of undersized, poorly-set or immature clusters. Researchers in Italy have demonstrated the best time to cluster thin red colored grapes was at the veraison stage. Veraison is a French term describing the ripening stage when the grapes begin to change color. They found at that time, quality is not impaired, clusters are more visible, thinning is faster and more accurate.

Hedging/Topping:

Hedging or topping consists of cutting off shoot tips during the early to mid summer. Late season trimming should be avoided since lateral regrowth may be stimulated and cause a delay in fruit maturation. Trimming to less than 10-12 nodes may impair fruit ripening. If trimming is not performed previous to a late season topping, healthy leaves could be removed from the canopy exterior exposing previously shaded older unproductive senescing leaves and thus lowering fruit ripening potential. Non-positioned canopies are very prone to this practice.

If vine trimming is needed for increased light exposure, spray penetration, harvesting or to ease the application of bird netting, etc., alternative practices should be considered. Controlling vine vigor through proper irrigation management, fertilization, use of an improved trellis system, or matching variety to site are better viticultural strategies than vine trimming. Vine trimming is a temporary "band-aid" solution to vineyard canopy problems.

Grow Tubes

Several growers have asked about grow tubes -- should they use them or not? The following information was collected from personal contacts in Colorado, Washington and California.

The grow tubes or plastic shelters have become a very popular method for training newly planted vines in California and other grape growing regions. Colorado has also joined the trend with several growers beginning to use the tubes in 1995. The grow tubes are plastic shelters available in various colors (white, blue, green, etc.) and sizes. Typical stock sizes are from 24 to 44 inches high with diameters of 2-3/4 inches to 4-1/2 inches. The grow tubes have been reported to enhance growth, reduce training costs and protect vines from chemical sprays and rodents. Suppliers recommend the grow tube to be placed over the young vine at planting. They also recommend the grow tube be pressed into the soil 2 inches and tied to the grape stake or wire for stability and microclimate maintenance.

Growers in Washington state find the plastic shelter useful, especially with young interplants that are struggling. Recent unpublished work at UC Fresno, California suggest the narrow tubes (less than 3-1/2") increase temperatures to extreme levels causing foliar burning. Colorado growers have had good and bad experiences with the plastic shelters. Some Colorado growers excessively burnt their vines with grow tubes and they claim late planting to be the major cause. Other growers had positive experiences, claiming first year growth was better than they had ever seen.

I personally visited several vineyards with grow tubes this summer and noticed that vineyards planted late experienced more heat stress damage than vineyards planted in early spring. Removal of the tubes was a topic of confusion. Some growers removed the tubes when burning was evident while others waited until early fall. I noticed that growers who used the tubes were planning on single trunk, not double trunk training. Double trunk training may be more challenging with grow tubes. Most growers felt the grow tubes were beneficial but expensive. Grow tubes normally range in price from $.40 to $1.40 each plus the cost of installation and removal. It appears that grow tubes will be used by some growers in Colorado regardless of the pros and cons. I feel the tubes are expensive and more experience under Colorado conditions is needed. Correct grow tube use may be a cultural practice that can favor early vine development and enhance vine establishment; however, incorrect use could be detrimental. (Hamman)

Fruit Grower Information Via the Code-A-Phone

It's hard to believe that fruit production information has been available to area farmers by way of the telephone for over 16 years now. If the number of times the recorded information is accessed each growing season (almost 8,000 times in 1995), we then surmise that the information is both accurate and timely. Here's how it works.

Each week (approximately March through September) the Colorado State University Research and Extension personnel pool their respective information that seems appropriate for the season for Delta, Mesa and Montrose Counties and records it in the automatic information delivery system. The weekly messages are customized primarily for Mesa and Delta counties.

When we have surveyed local Code-A-Phone users regarding timeliness and content, the response has generally been quite favorable; however, we are always open to ideas for improvement. If you have suggestions, please send us a note or give us a call on the phone. (Gene Nelson)

The Colorado Grapevine

Results of the 1995 CSU Colorado Wine Grape Production Survey:

On October 11, fifty-seven surveys were mailed and by December 11, thirty-two growers had responded. The following is a summary of those responses:

Total 1995 Production	563 Tons
Producing Acreage	193 Acres
Average Production	2.92 Tons / Acre
Range of Production	0.1 - 9.4 Tons / Acre
New Acreage (Planted 1995)	30 Acres
Total Colorado Acreage*	410 Acres

*Colorado Department of Agriculture

Variety	% of Acreage	Price Received / Ton*
Chardonnay	30%	$1,236
Merlot	18%	1,120
Cabernet sauvignon	10%	1,260
Pinot noir	8%	990
Cabernet franc	7%	1,183
Riesling	7%	700
Gewurztraminer	4%	725
Sauvignon blanc	4%	950
Other	12%	950

*Data are averages of 3 or more survey reports.

Hand Picking Costs / Ton	
Average	$124
Range	$60 - 223

Wine grape production for the 1995 season set a record and was up 27% (154 Tons) from 1994. Overall, harvest was a month behind previous years due to the cool months of May and June. The late harvest resulted in a longer "hang time" for most varieties which was needed to satisfy grower/winery contracts. Several wineries have reported the 1995 grape quality to be excellent. (Hamman)

The Colorado Grapevine
by Rick Hamman

Pruning:

Pruning is the removal of living shoots, canes, leaves, and other vegetative parts of the vines. It does not include removal of flowers or fruit. The purpose of dormant pruning is to balance the leaf surface area with the capacity of the vine to produce a mature crop.

Pruning in Colorado should begin in March. The threat of severe cold temperatures (below 0°F) is minimal by then, and any winter damage can be assessed and adjustments made.

Pruning intentions:

1. To establish and maintain the vine in a form that will facilitate vineyard management.
2. To produce fruit of a desired quality.
3. To select nodes that are fruitful.
4. To regulate the number of shoots/clusters.
5. To regulate the vegetative growth of the vine.

Pruning should be completed before bud swell, which typically occurs the third week in April in Mesa County. If pruning occurs during bud swell, crop loss can occur by physically rubbing off the tender swelling buds when removing unwanted canes. The actual removal of the "unwanted" pruned canes can be quite abrasive to the "wanted" remaining buds. Extra care should be used if pruning during bud swell.

Bleeding may also be noticeable when vines are pruned at bud swell or later. Bleeding is defined as the exudation of sap from cut canes but has no reported effects on the vine. The sap exudate comes from the cut ends of the vessels of the xylem tissues and consists mainly of water with small concentrations of sugars, mineral nutrients, solutes and plant hormones. (Hamman)

Code-A-Phone To Be Reactivated in March

The commercial fruit Code-A-Phone message system will be reactivated in March. It will provide weekly updates on crop development, pest and disease observations or things to watch for, pesticide registration announcements or notices, and announcement of meetings, workshops, etc.

Growers are asked to help provide input for this information service by sharing observations on crops, pests, diseases in their orchard areas. It's impossible for us to cover all areas with weekly personal visits. Your information will be shared on an area basis, without naming orchards. (Larsen)

Colorado Grape Guide Revised:

The revised Colorado Grape Growers Guide (publication 550A) is now available through the CSU Cooperative Extension Resource Center, CSU, Fort Collins, CO 80523, 970/491-6198. The 82 page guide contains excellent information on Viticulture in Colorado including; vineyard establishment, site preparation, trellising/training, planting, irrigation management, pruning, pest management, varieties, vine sources, useful books and publications and vineyard establishment and production cost estimates. Cost is $12.00 + $4.00 S&H + $0.48 state sales tax.

Powdery Mildew:

Powdery mildew (PM) is the most important grape disease challenging Colorado grape growers. Preventing PM infection is the best offense for clean, healthy production. The PM season is now upon us and implementing a good fungicide program is essential. The following fungicide programs may be useful when designing your grape PM prevention program:

Standard Fungicide Programs

The most highly recommended fungicide programs for grape powdery mildew control are as follows:

Standard DMI[2] program:

1. Wettable or dusting sulfur starting at budbreak and continuing on a 10-14 day pattern (7 days for dust) until bloom or 10" shoot growth.
2. DMI applications every 18 days from bloom or 10" shoot growth until the total, annual dosage/acre has been applied (usually around 3 sprays).
3. Sulfur applications on a 10-14 day interval (7 day interval for dust) until veraison.

Standard Sulfur Program:

1. Wettable sulfur starting at bud break.
2. Dusting or wettable sulfur on a 7-14 day cycle until veraison.

Older recommendations (first written in 1905) began the program at 6 or 12 inches of shoot growth, but recent research indicates that much better control is usually achieved by starting the spray program earlier.

These programs are commonly modified by grape growers for various reasons.

DMI program modified for resistance management:

- Alternation: sulfur sprays inserted between the DMI applications.
- Tank Mixing: sulfur added with the DMI applications.
- Substitutions: the total number of DMI applications reduced to 2 or 1 with sulfur used to cover the interval.

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[2]DMI's are Demethylase inhibitor fungicides such as Nova, Rubigan, and Bayleton.

The Colorado Grapevine

Rick Hamman

Grape Powdery Mildew

Grape powdery mildew (*Uncinula necator*) is native to the eastern U.S. on various *Vitis* species which occur there. In the mid-1800's it spread to Europe through England where it began to cause serious economic problems on the European grape, *Vitis vinifera*. It is now found in all grape growing areas of the world and annual control expenditures are approximately $250 million world wide.

The organisms which cause what are called "powdery mildew" diseases belong to an unusual group of fungi called the Erysiphales. They are distinguished by several features. They are obligate parasites which means that they can only grow and reproduce on a living plant host. They are much more independent of moisture for their growth and particularly for the process of spore germination. Nearly all fungal pathogens require at least a film of free water in order to germinate and infect a plant, but powdery mildew fungi do not. They are favored by higher humidities, but can function at remarkably low humidities in many cases. Powdery mildews tend to be very host specific. For instance, the powdery mildew which infects grape (*U. necator*) only infects a few other plants while the powdery mildew which infects apples cannot infect grape.

The most common errors and problems in mildew management:

It would be rare to find someone who has grown grapes for several years who has not at some point been "caught" by mildew. The epidemic is explosive and it is easy to have flaws in a mildew program which allows the disease to break through. Also, mildew pressure varies from season to season. A program which is completely adequate one year may not stop the disease in a year which is particularly conducive for disease development (e.g. mild winter, early spring rains, relatively cool summer conditions). Powdery mildew management mistakes usually fall into the following categories:

1. **starting too late:** new recommendations for mildew control begin at budbreak; this is particularly important in some areas where there is significant over-wintering inoculum. This can happen to even the best intentioned grower since some vineyards can be too wet to allow entry of spraying equipment at the beginning of the season.

2. **severe over-wintering infection:** in certain areas, over-wintering by cleistothecia is so common that a serious battle with the disease starts at budbreak. This is not common except in some coastal areas of California.

3. **long spray intervals:** it is very easy to let mechanical failures, bad weather (rain, wind) or holidays combine to interfere with fungicide applications in the early season. A planned 10-day interval can easily slip to 16 or 18 days. With the rapid growth of the vine in the early summer, this allows the disease epidemic free reign and makes subsequent control difficult.

4. **wrong fungicide:** this is usually only a problem with inexperienced growers. Copper, mancozeb, Ronilan or Rovral do NOT provide significant control of powdery mildew.

5. **tolerant strains:** in some areas, mildew populations are now very difficult to control with DMI (Demethylase Inhibitor) fungicides because of previous selection for resistance.

Harvest:

The 1995 growing season started off cool and wet with several isolated sites damaged by hail. It has turned out to be a good powdery mildew year. Anticipated harvest dates for most varieties appear 1-2 weeks later than last season. I predict the 1995 Colorado wine grape production to be over 500 tons.

on nitrogen stored in the vine from the previous season.

Nitrogen fertilizer should be applied only during periods of active uptake to minimize loss through soil leaching. This includes the period from bud-break to veraison. Recommendations for application of actual nitrogen must be translated into rates based on commercial formulations. For example, a recommendation of 40 pounds of actual nitrogen per acre would require 190 pounds of ammonium sulfate per acre. Calculation: 40/21% = 190.

Noninsured Crop Disaster Assistance Program Signup Deadline is July 15

Secretary of Agriculture Dan Glickman recently announced the publication of interim regulations to implement the Noninsured Crop Disaster Assistance Program (NAP), a key component of the Clinton Administration's Federal Crop Insurance Reform legislation enacted by Congress last year.

The NAP applies to crops for which federal crop insurance is currently unavailable. Eligible commodities include commercially produced crops grown for food or fiber, floricultural and ornamental nursery crops, Christmas trees, turfgrass sod, industrial crops, and aquacultural species.

To be eligible for NAP protection, farmers must report their acreage to their local Consolidated Farms Service Agency (CFSA) office shortly (within 15 days) after planting. Farmers will not be eligible for NAP protection if they do not report their acreage to CFSA offices.

"Farmers across America need to be aware of this new reporting requirement and should contact their local CFSA office about reporting deadlines in their area," Glickman said. [editor's note: Colorado Deadline is July 15, 1995]. USDA's FCIC has started to communicate information out to various farm groups so that they can be aware of these dates and the reporting requirements for farmers.

The NAP regulations are effective immediately to enable the Department to assist farmers whose crops have already been impacted by natural disasters. The NAP regulations were published in the Federal Register on Thursday, May 18, 1995, with a 60 day comment period. Comments on the regulations can be sent to: Regulatory and Procedural Development Staff, FCIC/USDA, Rm. 500, 2101 L Street, N.W., Washington, DC 20037. (S. Phipps, Federal Crop Insurance Corp.)

Beginner's Branch

Go to the OMRC field day on July 13; attend one of the workshops with Dr. Marini (see calendar); water young trees every 7 - 10 days; consider taking a leaf analysis (see related article); thin fruit; line up all harvesting labor; check ladders; and in early July, prune out sucker growth in peaches. Listen to the code-a-phone each week, watch out for fruit rots that start near harvest, especially late season coryneum infections. Watch out for mite and leafhopper buildup in hot, dry weather. Cherry growers should watch out for pear slugs after harvest. Finally, always, always remember safety first in everything you do. (Gaus)

The Colorado Grapevine
Nitrogen Deficiency and Correction

by Rick Hamman

Vines use nitrogen to build many essential compounds for growth and development; among these are proteins, including all enzymes, amino acids, nucleic acids and pigments (chlorophyll and anthocyanins).

Symptoms of nitrogen deficiency:

The classical symptom is a light green color of leaves, as opposed to dark green leaves of vines receiving adequate nitrogen. If the leaves show this uniform light green color, it will be most noticeable on basal to mid-shoot leaves. Newly developing leaves often have a pale green color and should not be confused with nitrogen deficient leaves.

Some growers confuse nitrogen deficiency with iron deficiency. In western Colorado, soil pH is typically in the range of 7.3 to 8.4. High pH soils like these make iron unavailable to the vine. Symptoms of iron deficient vines are easy to detect. The classical symptom is a pronounced intervenal leaf yellowing. The veins of a moderately iron deficient grape leaf remain green. On the other hand, nitrogen deficiency results in a complete fading of green color including the veins. Other symptoms that point to nitrogen deficiency are a slow rate of shoot growth, short internodal length, and small leaves. Insufficient nitrogen can also reduce crop through a reduction in clusters, berries and berry set.

Excess nitrogen:

Nitrogen stimulates vegetative growth. If excess nitrogen is available to the vine, a tremendous stimulation of vine growth may occur. Shoots of such vines can grow late into the fall and may attain 8 to 10 feet in length. The percentage of shoot nodes that mature can also be decreased when excessive nitrogen results in late season continuation of growth. The belief that any added nitrogen will reduce cold hardiness of a vine is an unfortunate misconception. The addition of moderate amounts of nitrogen (20-40 pounds of actual nitrogen per acre) will not reduce the vines cold hardiness level and will likely improve their overall performance.

Causes of nitrogen deficiency:

Nitrogen is the essential element most often requiring addition on a routine basis. Once absorbed by the vine, nitrogen can be lost through fruit harvest and the annual pruning of vegetation. Considering that grape berries contain about 0.18 percent nitrogen, a 5 ton crop will remove approximately 18 pounds of nitrogen per acre from the vineyard. Yearly crop removal exports nitrogen; with no fertilizer input most soils will eventually be exhausted of readily available nitrogen. The nitrogen depletion will occur most rapidly with soils low in organic matter content. Soils higher in organic matter content can more easily convert organic nitrogen to forms (ammonical and nitrate nitrogen) capable of being absorbed by the vines.

Correcting nitrogen deficiency:

Maintaining an appropriate nitrogen status is based on past experience, vine performance and supplemental use of bloom-time petiole analysis for nitrogen concentration. Adequate nitrate-nitrogen levels at bloom time range between 350-1200 ppm for most grape varieties. Site, variety and year to year differences may exist. This data is based on leaf petioles taken from opposite clusters at full bloom. If nitrogen fertilization is needed, application of 20-40 pounds of actual nitrogen per acre on sandy loam soils is a good starting point for mature vineyards. Young vineyards (first and second growing season) in need of nitrogen fertilization typically require no more than 30 pounds of actual nitrogen per acre. It occasionally takes two years for added nitrogen to have an impact on vine performance. This is because much of a vines early-season nitrogen needs depend

THE COLORADO GRAPE GROWERS' GUIDE

1996 Edition

by

Richard A. Hamman, Jr[1]., Steven D. Savage, and Harold J. Larsen

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TABLE OF CONTENTS

Introduction

Introduction 1
Anatomy of the Grapevine 2
Grapevine Structures 3

Establishing a Vineyard

Site Selection 4
Common Mistakes in Vineyard Development
Plant Selection 6
Planning a Commercial Vineyard in Colorado 8
Nursery Stock and Standards 9
Phylloxera/Rootstocks 10
Site Preparation 13
Vineyard Development Checklist 14
Trellising/Training 15
Planting -- The First Year 20
Grow tubes 22
The Training Year (Year 2) 23
The Third Year 27

Maintaining a Vineyard

Water Management 29
Protection Against Winter Injury 30
Frost Protection and Damage Treatment
Pruning 32
Bleeding 36
Re-training Winter Damaged Vines
Shoot Tying and Suckering 39
Shoot Thinning
Leaf Removal 40
Crop Thinning
Hedging 41
Nutrition Analysis and Foliar Sprays
Zinc, Manganese, & Iron
Crop Estimation 44
Harvest Timing 45
Sugar content, acidity, pH, & sampling

Pest Management / Control

Disease Management 47
Powdery mildew, crown gall, Botrytis bunch rot, sour bunch rot, & virus diseases.
Powdery Mildew Fungicide Program 50
Insect & Mite Pest Management 55
Nematodes 60
Weed Management 61
Bird Control 62

Wine Grape Varieties

White Varieties -- *Vinifera* Cultivars 63
White Varieties -- Hybrid Cultivars 65
Red Varieties -- *Vinifera* Cultivars 66
Red Varieties -- Hybrid Cultivars 67
Relative Cold Hardiness of Grapevines 68

Table Grape Varieties

Himrod, Interlaken, Lakemont, etc. 69

Vine Sources

Nurseries 72
Vineyard Supply Sources 73

Cost Estimates

Vineyard Establishment Costs 76
Vineyard Production Costs 77

Useful Books and Publications

Books and Publications 78
Viticulture Trade Magazines 81
Resources on the Internet 82

Supplemental Materials (Appended)

Cornell University, Grape IPM Sheets
Michigan St. University, (Common Diseases of Grapevines)
Univerity of Illinois, Grape Diseases I
Washington St. University, (Grape Phylloxera)