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Methods of Cold Stabilization

By Murli Dharmadhikari

Precipitation of Potassium bitartrate (KHT) in bottled wine is considered undesirable and therefore, vintners employ various methods to prevent this problem. The common stabilizing methods include chillproofing or cold stabilizing, contact processes, and ion exchange. In some cases addition of certain compounds such as metatartaric acid and carboxymethyl cellulose (CMC) has been successfully tried to prevent KHT precipitation. Metatartaric acid is not very stable and its inhibitory effect is temporary.

Cold Stabilization by Chilling

In a conventional method of cold stabilizing a wine is chilled to a temperature just above its freezing point and is held at that temperature for

two to three weeks. Chilling the wine lowers the solubility of KHT and facilitates its crystallization and precipitation. During cold storage the rate of KHT precipitation is rapid in the initial stage and slows down with time. This is attributed to the reduction in KHT saturation level. The low temperature and the storage time to stabilize wine depend on the kind of wine. For example, wines containing sugar and/or higher amounts of alcohol would require a lower storage temperature than the dry table wines with 11 to 12% alcohol. Other factors such as the concentration of acids, cations, anions, pH, and various complexing agents will also affect the tartrate stability and precipitation. To minimize the influence of complexing agents and colloidal substances on KHT precipitation, the wine should be clarified by fining and filtration before chilling and cold storage. As stated earlier, storage of wine close to freezing for two to three weeks is considered sufficient to remove excess KHT. However, the wine should be tested for bitartrate stability to determine the length of storage time. To separate the crystallized deposit of KHT following cold storage, the wine should be cold filtered. This is necessary to avoid the redissolving of KHT. It should be noted that since oxygen is more soluble in cold wine (than at

cellar temperature), great care should be taken to minimize oxygen pickup. Nitrogen or CO₂ blanketing and/or sparging is advisable.

A wine can be chilled by using refrigeration. Using insulated tanks can save the energy and cost of refrigeration. Many wineries take advantage of low winter temperatures to chill the wine. Installing fans to pull in the cold air can be helpful in lowering the cellar temperature and chilling the wine. This method of relying on mother nature to obtain cooling can be economical but it may not be convenient and effective. For example, one may not be able to lower the temperature to the desired point or may not be able to hold it for the desired length of time. Sometimes cooling the entire cellar can interfere with other cellar operations.

Wine stabilization by chilling is widely practiced in the wine industry. The process is time consuming and can also be costly. Although there are other methods that are fast and in some cases economical, many winemakers prefer stabilizing high quality wines by chilling.

Cold Stabilization by Contact Process

Grape juice and/or wine can be considered as a supersaturated solution of KHT. Under certain conditions such as low temperature storage, the dissolved KHT becomes insoluble and small crystals settle to the bottom in the form of sediment. In a supersaturated solution (e.g.,

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wine) the crystallization process occurs in two stages. In the first stage, also referred to as nucleation phase, nuclei or seed crystals are formed. It is important to note that for nucleation to occur in a supersaturated solution the concentration of KHT must reach a critical level. Below this level (such as slightly supersaturated wine) the nucleation will be slow. In the second stage the nuclei grow into crystals. Crystal growth occurs when KHT ions migrate to active sites on the surface of the seed crystals and are incorporated into a crystal lattice. If for some reason the active sites on the crystal surface are blocked (due to adsorption of colloids) crystal growth is impeded. Certain phenolic compounds and polysaccharides present in wine have been shown to impede crystal growth.

It has been shown that in a supersaturated solution the rate of nucleation and crystal growth depends on the degree of supersaturation. In a contact process of stabilization, powdered KHT crystals are added. These crystals serve as seed crystals and crystal growth begins immediately. Thus the addition of KHT eliminates the nuclei induction phase and this speeds up the time required to stabilize the wine. Many trials have been conducted to determine the optimum amount of KHT needed to stabilize wine. The research has shown that the addition of 4 g/L of powdered KHT is sufficient to render a wine stable. Adding 4 g/L of powdered KHT gives 17 million KHT fragments/ml of wine (Rhein & Neradt, 1979). Adding KHT in amounts less than 4 g/L takes a longer time to stabilize the wine. Using higher amounts than 4 g/L of KHT is not recommended because it

is uneconomical, rather difficult to remove, and the improvement in KHT reduction is marginal. Table 1 contains the data showing the effect of KHT additions (in increasing amounts) on K⁺ and tartaric acid levels in wine.

| Table 1. Decrease of potassium and tartaric acid levels in wine as a function of KHT additions (8). | | | | |
|--|---------------------------|----------------------------|---------------------------|----------------------------|
| KHT additions | K⁺ mg/L | DK⁺ mg/L | H₂T g/L | DH₂T g/L |
| Control | 1150 | - | 1.82 | - |
| Chilled, none | 1150 | - | 1.82 | - |
| 1 g/L | 1020 | 130 | 1.34 | 0.48 |
| 2 g/L | 1020 | 130 | 1.34 | 0.48 |
| 4 g/L | 970 | 180 | 1.15 | 0.67 |
| 6 g/L | 970 | 180 | 1.12 | 0.70 |
| 9 g/L | 960 | 190 | 1.10 | 0.72 |
| 12 g/L | 960 | 190 | 1.08 | 0.74 |

Source: Neradt (1980).

As the results indicate, the addition of 4 g/L of KHT decreased the tartaric acid level by .67 g/L i.e., 36.8%. Increasing the rate of KHT to 12 g/L (3 times) reduced the tartaric acid level by .74 g/L or 40.6%. Thus increasing the KHT addition three times the suggested level (4 g/L) gave marginal improvement in tartaric acid reduction. A similar trend is observed with reference to K levels.

Cost of KHT

At 4 g/L, 33 1/3 lbs of KHT will be needed to treat 1000 gallons of wine. At a price of \$2.11/lb, (the current cost could be higher) the cost of KHT powder to treat 1000 gallons of wine will be \$70.26. In practice the KHT crystals can be recovered from the wine and used again.

Generally, the KHT powder can be reused between 5 to 8 times. For red wines the number of times that the KHT can be reused is much less. If the KHT can be reused 5 times, then the cost of treating wine would be reduced to about \$14.00/1000 gal or 1.4¢/gal, which is a small expense to achieve wine stability.

With repeated use the KHT crystals grow and become larger in size, thus increasing the amount of contact time required to stabilize a wine. For this reason wet grinding of the crystals is recommended. Neradt (1980) reported that in a commercial KHT powder; about 40% of the particles were less than 40 µm in size. After five uses this fraction was reduced to less than 4% (due to crystal growth). However after grinding, the fraction (i.e., particle size less than 40 µm) increased to about 50% (Table 2). In practice one may be able to reuse KHT crystals about five to ten times before grinding. For more frequent uses, i.e., over five to ten times, wet grinding would be necessary to reuse the crystals.

Fouling of Crystals

Fouling of crystals occur when, (during crystallization processes), certain colloidal substances occupy the active sites on the crystal surface and, consequently, halt crystal growth. The fouling substances include certain pigments, polyphenols, proteins, and polysaccharides. The extent of crystal fouling depends on the amount and types of colloidal substances present in the wine. Pre-treatment of wine, such as clarification, fining, and filtration, will reduce the level of colloidal material and thus minimize the fouling of the KHT crystals with repeated use. The fouled or blinded crystals

Table 2. Particle size distribution of KHT in %.

| Particle size | Commercial KHT from German source, unused | Commercial KHT from Spanish source | | |
|---------------|---|------------------------------------|--------------|---------------------|
| | | Unused | After 5 uses | Ground after 8 uses |
| < 5 µm | 5.0 | 0.5 | 0.2 | 2.5 |
| 5 - 15 µm | 10.0 | 4.0 | 0.3 | 2.5 |
| 15 - 40 µm | 28.0 | 34.0 | 3.5 | 45.5 |
| > 40 µm | 57.0 | 61.5 | 96.0 | 49.5 |

Source: Neradt (1980).

can be washed to remove the contaminants. The washing or rinsing will cause a loss of KHT depending on the temperature and the amount of water used in washing. Generally, the loss of 3 to 6% of KHT has been noted in the washing of the crystals.

Contact Process Compared to Conventional Cold Stabilization and Ion Exchange

In 1979 Rhein and Neradt reported the results of an experiment in which the contact process was compared with chilling and the ion exchange methods. Their data is given in Table 3.

Table 3. Tartaric acid and potassium content of a wine before and after tartrate stabilization.

| Stabilizing method | Tartaric acid g/L | K+ mg/L |
|--------------------|-------------------|---------|
| A | untreated | 720 |
| | contact | 565 |
| | chilling | 715 |
| | ion exchange | 360 |
| B | untreated | 735 |
| | contact | 575 |
| | chilling | 655 |
| | ion exchange | 340 |

Source: Adapted Rhein and Neradt (1979).

From the results shown in Table 3, it is clear that the contact process yielded significant reduction in tartaric acid and potassium levels; whereas, conventional cold stabilization gave inconsistent results.

Contact Process Procedure

1. Clarify, fine and filter the wine to remove colloidal material. (Remember this causes fouling of crystals.)

2. Chill the wine to the desired stability temperature. For table wine the stabilizing temperature is generally 28 - 32 °F

3. Chilling the wine may cause precipitation of unstable material (especially in red wines). In such a case, polish filtration of chilled wine may be needed. Filtration before seeding makes later removal of KHT crystals easy.

4. Add KHT powder crystals @ 4 g/L.

5. Provide a CO₂ or nitrogen blanket to minimize oxygen pickup. It is important to remember that a larger amount of oxygen is dissolved at the lower temperature, but the oxidation effect becomes evident later when the wine is warmed up.

6. Agitate the wine thoroughly. Agitation promotes crystal growth.

7. Allow 1 1/2 to 2 hours of contact time.

8. Take the conductivity reading of the filtered wine sample and compare it with the reading obtained during the laboratory trial. This assumes that a lab trial for a given batch of wine was conducted earlier and the conductivity reading of the wine stabilized to the desired temperature is known. In place of a conductivity reading, changes in the tartaric acid level can be determined analytically and compared with the tabulated value to determine wine stability.

9. After the treatment the KHT can be allowed to settle and the wine can be racked and filtered. If time is too short to allow settling, then the wine can be filtered to remove KHT. (The wine should be filtered while cold.)

10. To lower the cost of processing the wine, the KHT crystal should be ground.

11. Repeated use of KHT powder can cause microbial contamination of the wine. To prevent contamination, the KHT slurry should be stored with 500 ppm SO₂.

Wine stabilization with contact process has many benefits. The results are reliable and consistent. It is rapid as compared to the conventional cold stabilizing method, and with repeated use of the KHT seed crystals, the process can be very economical.

Stabilization by Ion Exchange

Wine stabilization by an ion exchange treatment is practiced by many large wineries. The process is suitable for producing bulk wines. When properly applied the wine can be stabilized without significantly affecting the quality. However, some winemakers believe that it adversely affects the quality of a high quality wine, especially one with a delicate flavor.

The ion exchange treatment consists of passing the wine through a column containing resin in cationic or anionic form. In cation form the resin may be charged with sodium (Na^+) or hydrogen (H^+), or a mixture of Na^+ and H^+ . When the wine is treated with cationic resin in sodium form, the Na^+ of the resin is exchanged with K^+ (and other cations such as Ca^{++} and Mg^{++}) from the wine. This results in the formation of sodium bitartrate, which is more soluble. There is a slight reduction in acidity. The increase in the sodium content of the wine could be undesirable. In such a situation a mixed resin in Na^+ and H^+ form could be used. This would limit the amount of Na^+ in the wine; however, due to the exchange between the H^+ ion (from resin) and the K^+ ion (from wine), the acidity of the wine would increase. This may be suitable for treating low acid wine, which would benefit from increased acidity while being stabilized.

When a wine is treated with anionic resin in hydroxyl (OH^-) form, the OH^- ion is exchanged for the tartrate anions (and other anions). This lowers the tartrate content of the wine. By passing the wine through both cation (H^+ form) and anion (OH^- form) exchange resins, one exchanges

H^+ and OH^- ions for potassium and tartrate ions. Thus the net result is the exchange of bitartrate for water.

Procedure

In the ion exchange procedure, usually a cation exchanger resin in Na^+ form is commonly used. The resin is prepared by treating it with 10% sodium chloride (common salt). The column is washed with ion exchanged water to remove chloride. Wine is then introduced from the bottom until the column is full and the air removed. Then the wine is introduced from the top and allowed to flow downward. As the wine flows through the column it picks up Na^+ and loses K^+ to the resin.

There comes a point when the resin is exhausted of its Na^+ ions. This is referred to as the "break point". It is important to detect the break point by monitoring K^+ levels in effluent wine at various intervals. After the resin is exhausted (as indicated by the presence of potassium in out-coming wine), it is washed with water and can be regenerated again.

After repeated use the resin loses its exchange capacity. When this occurs the resin is soaked in hydrogen peroxide solution to remove contaminants.

Ion-exchange treatment has been reported to remove certain vitamins and growth factors from wine. This causes difficulty in secondary fermentation and, therefore, many winemakers do not prefer to use this method for sparkling wine production.

The ion exchange method of stabilizing wine is rapid and economical. It is specially suited for stabilizing very large quantities of wine.

References

1. Rhein, O. H., and F. Neradt. 1979. "Tartrate stabilization by Contact process." *Am. J. Enol. Vitic.* 30:265-271.

American Society for Enology and Viticulture

Eastern Section

July 8-11, 2003
Corning, New York

Please join us for the 28th Conference of the ASEV Eastern Section. This year's event will be located in Corning, New York, in the heart of the **Finger Lakes** wine region. The Finger Lakes AVA covers **8000 acres** and is home to **73 wineries** producing 35 million gallons of wine. Over 2.5 million tourists visit the region each year. You will have the opportunity to do the same either on your own – most wineries are within a short driving distance from Corning - or by joining us on an attractive **pre-conference bus tour** on July 8.

The **symposium program** will answer all the questions you ever had about wine bottle closures. An historic look back at wine storage and closures will lay the groundwork for today's options: natural, technical and synthetic corks, crown and screw caps. National and international experts from academia and industry will address expectations, production techniques and performance criteria for each type.

The efforts to eliminate TCA from natural corks, the research and performance of screw caps since 1974, the current status of technical and synthetic corks, quality control protocols, cost comparisons and practical "How to" issues will be

addressed. Participants will have the opportunity to evaluate TCA and other musty aromas and taste samples stored with different closures. Industry and consumer perspectives will be discussed, and finally a **Crystal Ball – The Perfect Wine Closure in 2020** – will be offered.

Confirmed speakers:

Terry Acree, Professor
 Cornell University
 Mark Bassel, APM
 Bob Fithian, Cork Quality Council
 Leigh Francis, Senior Research
 Chemist, The Australian Wine
 Research Institute
 Ken Fugelsang, Professor,
 Fresno State University
 Jürg Gafner, Professor, Swiss
 Federal Research Station for
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 Wolf-Rüdiger Sponholz, Professor,
 Geisenheim Research Institute
 Chris Stamp, Lakewood Vineyards

Registrants will have the opportunity to visit a first class **trade show**, taste many award winning wines from the region, and be treated to the famous local cuisine through **special receptions, tours, and meals**. The ASEV Eastern Section is a venue for the knowledge and enjoyment of grapes and wine – a great opportunity



to showcase *your* fine wines! Plan to come early and/or stay late to join us on the bus tour and/or visit the local wineries. There is easy access to Corning by car or from the Rochester, Ithaca or Corning airports.

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 Or contact Ellen Harkness:
harkness@foodsci.purdue.edu,
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Buzzwords: Genomics, Functional Genomics, Proteomics and Metabolomics

By Wenping Qiu

Jargons denoted by “omics” begin to appear more frequently in newspapers and journals. These buzzwords sound quite intimidating at first glance. The aim of this short article is to briefly discuss definitions of genomics, functional genomics, proteomics, and metabolomics that were recently coined in the biology field. Researches in these emerging biology fields are transforming our view and perception of living organisms. Understanding their meanings will help us embrace the new biological concepts and technologies.

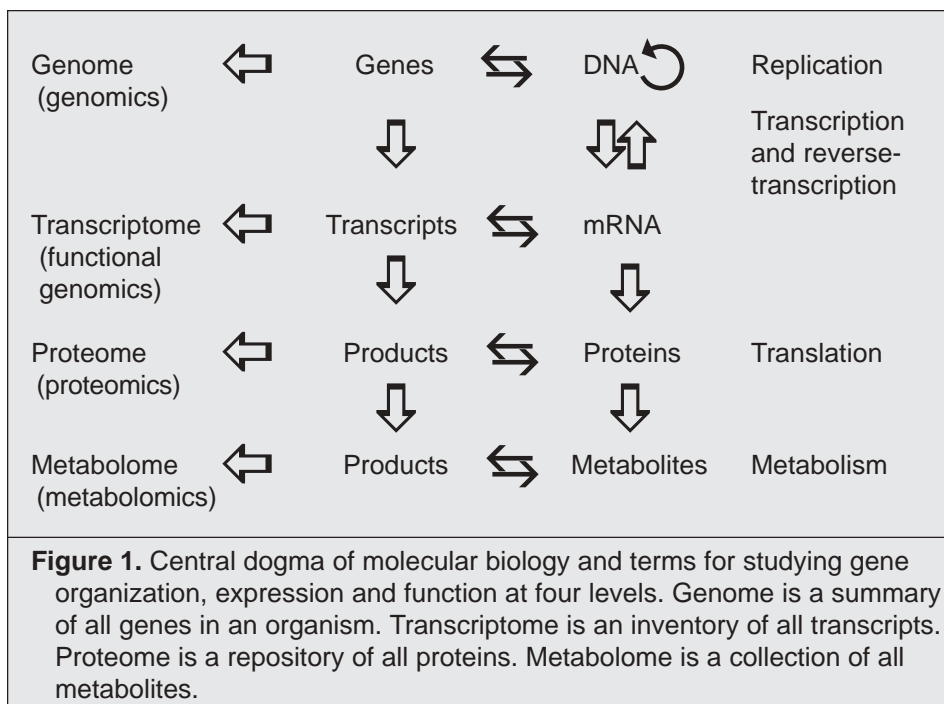
According to the central dogma of molecular biology, a specific trait of an organism is determined by the expression of a group of genes in response to physiological and environmental changes at a given time (Figure 1). Genes are stored in

DNA molecules, the genetic makeup of an organism. Genes *per se* are inert unless they are “expressed”. The gene of a living organism is analogous to the text of a speech, expressing a gene is akin to delivering a speech. Therefore, the abstract genetic information programmed by a gene has to be transferred to the effectors to exert its function and dictate other events. The first effectors are the messenger RNAs (mRNAs), which are produced by a process of transcription. The mRNAs receive the genetic information from genes and serves as template for the synthesis of the second effectors, proteins. Proteins perform structural, regulatory and catalytic roles that keep cells of an organism alive. The third effectors are chemical compounds, the end products of complex

metabolism that is catalyzed by a group of catalytic proteins (enzymes). The type and level of mRNAs, proteins and metabolites can be regarded as the ultimate response of an organism to genetic, physiological or environmental changes.

Consequently, molecular genetic and biological information of an organism can be explored at four levels. Genomics is to surf the genome and identify all the genes an organism possesses, and also to search physical features of genes on the genome. Functional genomics is to reveal the function of a gene by systematically analyzing its expression pattern and its interaction with other genes. Proteomics is to catalogue all the proteins expressed in a specific tissue responding to regulatory and external factors. Metabolomics is to archive all the chemical compounds. To put it simply, genomics is to study **what genes are** while functional genomics, proteomics, and metabolomics are to investigate **what genes do**. Figure 1 illustrates objectives and interrelationships of the four “omics”. Genes, mRNAs, proteins and metabolites form a hierarchical network in a cell. The molecular genetic and biological information thus is getting more and more complicated as it flows from genes to metabolites.

Functional genomics is specially mentioned here since it is emerging as a new biotechnology recently in viticulture. Its goal is to identify the gene expression pattern that underlies growth and development of an organism. It is becoming a major discipline in molecular biology in the post-genomic era¹. We also plan to employ the functional genomics approach



in the newly initiated *Vitis* Gene Discovery Program at the Missouri State Fruit Experiment Station. In contrast to the previous one-gene-at-a-time strategy, a high throughput technology has been developed in functional genomics for searching, screening and sorting groups of genes. Instead of discovering a few of genes, functional genomics approach can identify thousands of genes in a short time. The technology also can analyze the expression pattern of multiple genes that determine a special trait simultaneously and enable linking a group of genes to a special trait. Automatic gene-hunting instruments and sophisticated softwares are now industrializing the gene discovery scheme and allow us to harvest novel genes at an unprecedented pace.

The second buzzword specially mentioned is metabolomics. Based on the same principle as functional genomics, the newly emerging metabolomics is to sort out and archive massive sets of metabolites. The methods to be used are automation of solvent extraction and sample clean-up procedures that are tailored for particular classes of compound, followed by automated gas chromatography (GC)-mass spectrometry (MS) and liquid chromatography (LC)-MS. As aroma, flavor and color is a complex composition of thousands of metabolites, metabolomics is very suitable and useful to characterize the unique metabolite spectrum of each grape and wine. For instance, a library of metabolites can be constructed for each grape and wine and forms a composite reference for evaluating a unique wine.

Traditionally, the metabolite characterization of grape and wine

restricts to the selected classes of major compounds while minor compounds are frequently ignored. But it is well known that the minor substances can influence the nuance and uniqueness of a special wine. Advanced from the conventional methods, metabolomics enables more in-depth analysis of berry and wine characteristics by enlarging the spectrum of grape and wine metabolites. Based on the same principle, we might entertain coining new terms: aromaome (aromaomics), flavorome (flavoromics), and the like. It is not doubtful that characterization of massive sets of metabolites through metabolomics will become a new trend in viticulture and enology.

The new buzzwords not only reflect new trends in science, but also reshape our perception of a living organism. They transform our previous views of studying an organism at molecular biological level from the isolated case-by-case strategy to the overall holistic revelation of mechanisms for a biological system. Functions of an organism are conventionally revealed by dissecting genes by genes, proteins by proteins and compounds by compounds. Now we can understand their functions and characteristics by integrating all information and forming a comprehensive biological information system². As a result, the complexity of an organism can now be unveiled in a more nature-like manner. The automated instruments revolutionize the biotechnology just like automobile and personal computer dramatically change our daily lives. The “omics” currently are generating information of an organism at an industrial scale¹. This lead to amass-

ing titanic set of data. As currently we are in the information era, biology will enter a new millennium of bio-information. Like computer information system changes our daily lives, biological information system (bioinformatics) is renovating our way of thinking of an organism profoundly. The ultimate goal of these “omics” is to achieve a true and comprehensive understanding of biological functions of an organism. The accumulation of biological data ultimately will convert biology from an observation science into an information science.

The new technologies considerably increase the efficiency of identifying genes and analyzing their functions. To embrace the new technologies and to seize the opportunity of discovering more novel genes that are closely related with special traits of grapevines, we are looking forward with excitement to applying these new technologies to the viticulture.

References

1. Brent, R. Genomic Biology. *Cell* **100**, 169-183 (2000).
2. Fiehn, O., Kloska, S. & Altmann, T. Integrated studies on plant biology using multiparallel techniques. *Curr. Opin. Biotech.* **12**, 82-86 (2001).

Coming Events

1. **17th Annual Midwest Regional Grape and Wine Conference.** February 8 - 10, 2003. Marriott's Tan-Tar-A Resort, Osage Beach, Missouri. For information contact: Denise Kottwitz, Grape and Wine Program, Division of Market Development, Missouri Department of Agriculture, PO Box 630, Jefferson City, Missouri 65102
Denise_Kottwitz@mail.mda.state.mo.us 800/392-WINE
2. **23rd Missouri Small Fruit Conference.** February 17 - 19 2003. Clarion Inn and Conference Center, Springfield, Missouri. For information contact: Patrick Byers Fruit Grower Advisor plb711t@smsu.edu
 SMSU Department of Fruit Science
 9740 Red Spring Road
 Mountain Grove, MO 65711-2999
 417.926.4105 <http://mtngrv.smsu.edu>
3. **ASEV Eastern Section 28th Annual Conference "Wine Closures: Put a Cork In It?"** July 8-11, 2003, Corning, New York Conference Hotel: Radisson Hotel Corning, 125 Denison Parkway East, Corning, New York, 14830, USA 607-962-5000, www.radisson.com/corningny
 For more information visit the ASEV-Eastern Section website:
<http://www.nysaes.cornell.edu/fst/asev> Or contact Ellen Harkness:
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