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| **Project 47** | **The Regents of the University of California, Cooperative Extension** | **$166,000** |

**PROJECT TITLE**

Understanding and Controlling Ice Nucleating Bacteria to Prevent Frost in Vineyards

**PROJECT DURATION**

**Start Date:** October 1, 2016

**End Date:** March 31, 2019

**SUMMARY**

Grapes are damaged when tissues freeze in spring frosts. Frost damage is triggered by certain bacteria that catalyze ice formation near 32 degrees Fahrenheit. Without those bacteria, grapes can supercool as low as 25 degrees Fahrenheit and avoid damaging ice formation. Overhead sprinklers can prevent damage to vines, but it takes considerable water, which is not always available. The use of wind machines for frost control is expensive, energy demanding, and noisy, requiring air inversions to work. Understanding the microbiology of grape vines will enable farming practices that avoid damaging ice formation without the need to warm plants by unsustainable methods, reducing water and energy demand. The project will research strategies to enable grapes to tolerate cold temperatures by reducing ice nucleating bacteria on leaves and by selecting and managing cover crops that do not support ice nucleating bacteria that migrate to grapes.

**PROJECT PURPOSE**

**Issue, Problem or Need**

Grape vines need to be protected from freezing as temperatures below 32 degrees Fahrenheit will damage green tissue resulting in serious crop loss. Sprinkler frost protection requires significant water which may not be available during drought. Wind machines use large amounts of fuel, and are noisy. This research proposes controlling ice nucleating bacteria with copper sprays and competitive bacteria. Without ice nucleating bacteria, wine grapes can drop to about 28 degrees Fahrenheit without damaging tissue.

Losses from frost are a potential problem every year, and affect nearly 250,000 acres of wine grapes in California. Frost protection with sprinklers requires copious water, which is difficult in drought years and interferes with endangered species habitat if surface water is used. Frost fans are expensive, noisy and use considerable expensive fuel. Additionally, they only work when atmospheric inversions are present. Controlling ice nucleating bacteria is a low input frost prevention method.

This project will: 1) Determine how ice nucleating bacteria are growing and moving onto emerging green grapevine tissue in the spring; 2) Determine if small amounts of copper sprayed on vines can prevent ice nucleating bacteria from causing frost; 3) Investigate if competitive bacteria can be used to prevent ice nucleating bacteria from growing on treated grape tissue; and 4) Investigate how cover crops and vineyard floor management affect vineyard microbial ecology to minimize ice nucleating bacteria populations.

Copper sprays to control ice nucleating bacteria are applied precisely to emerging vine tissue, less than 0.75 pounds per acre per application and give protection for about five days. In most years, four applications will give adequate frost protection (three pounds total per year, meets National Organic Program restrictions). Sprinkler frost protection requires copious water, and can dewater surface water sources during drought years stranding endangered salmonids. Frost protection without water would be very desirable. Wind machines use nine gallons of propane per hour, are noisy, and will not work unless temperature inversions are present. Cover crops will be tested and selected for those that host the least amount of ice nucleating bacteria. Initially, wine grape tissue is sterile and is contaminated by migrating bacteria from cover crops. Cover crops fix nitrogen, increase soil organic matter, create habitat, biodiversity, and improve soil health.

**Project Objectives**

1. Determine if copper sprays can protect grape vines from freezing, and how vineyard floor management interacts with frost risk.
   * Field: This trial will be conducted in 2017 and 2018. With cooperating growers, the project team will establish replicated plots in vineyards prone to freezing that are not sprinkler frost protected by other means, using a split plot analysis of variance design. Two main treatments will be used: 1) Copper (Kocide) applied at the rate of 0.75 pounds AI per acre in 25 gallons of water targeted to emerging shoots for five days of protection during the frost season, which would usually require four applications (three pounds AI per acre of copper total per season is the target, but more applications may be needed in extremely cold years) and 2) An untreated control, with no sprays. Frost season is variable, but normally it is expected that bud break starts around March 21 and ends the first week of May, which is historically when 90 percent of frost episodes are anticipated. Frost risk conditions are not continuous, so copper sprays are applied when weather forecasting indicates that a period of freezing weather is likely. The project team would begin treating when the first shoots are out, and on average, try to cover shoots every six inches of growth. Spraying will be done with a light all-terrain vehicle (ATV) with a low volume sprayer that targets only the vine cordon and emerging canopy; this allows spraying when the vineyard floor may be wet, and larger and heavier equipment may not be able to operate. Sub plot treatments will be different vineyard floor management, including: a. mowing native vegetation shortly; and b. removing all vegetation by disking. Replicated plots will be at least half acre in size, as ice nucleating bacteria can migrate by wind from outside of the individual plot areas. There will be a minimum of three replications for each treatment, for a total of 12 plots, covering six acres. 20 individual shoots or leaves will be assessed for each replicate of each treatment at a given sample time, usually once a week. Twelve treatment replications x 20 samples per replication x four weeks = 960 samples per season. Data loggers will be placed in the canopy and above the vineyard floor at the rate of four per plot for a total of 48. Additionally, a weather station will be placed in the plot to measure temperatures at one, five, and 35 feet (to assess inversions), wind, rainfall, humidity, dew points, and surface reradiation (black globe temperature). This can be viewed in real time and will also be logged for analysis. This allows an in depth record of any freezing events that may occur.
   * Additionally, aerial bacteria deposition samples will be taken weekly during the frost period each season at vine canopy height. Dishes will be placed in wire holders, their lids opened for three hours, and then closed and collected during the day. Each sub plot will have four samples, for a total of 48 plates, sampled five times per season, for a total of 960 samples. Each plate will be cultured and evaluated for total bacteria deposited, and the percentage of ice nucleating bacteria of the total population, which will be determined by sub sampling and freezing samples on specially designed glass slides.
   * If a frost event occurs in the plots, 100 random shoots total selected from at least 10 individual vines in the center of each replicated plot will be evaluated for incidence and severity of frost damage, for a total of 1,200 shoots.
   * Note on copper: While high rates of copper can cause environmental problems over time, these rates are targeted to be consistent with the National Organic Program rules that only three pounds of copper may be applied annually. Each application is a relatively small amount applied very precisely to emerging shoots in a low volume of water (25 gallons per acre). Once applied to tissue, the formulation the project proposes to use is rain fast on sprayed tissue and not likely to cause run off into water ways, or leach into the soil. Over time, the project team hopes to find a competitive bacteria (antagonist) that will fill the same ecological niche that ice nucleating bacteria utilize, and the need for copper sprays to control ice nucleating bacteria will be unnecessary. In the short run, applying small amounts of copper involves far less energy and water than using sprinklers or wind machines for frost protection, and represents a far lower potential environmental impact. The project team views this as a more sustainable approach than is presently being used for frost protection in the region. Copper sprays applied to walnut orchards for walnut blight control are permitted to use up to four pounds of copper per application, and in some years, almost 16 pounds of copper are applied per acre for the season.
   * Laboratory: Shoots (early in the season) or small emerging leaves (later in the season) will be sampled to determine the freezing point of the leaves and bacterial populations. Samples will be sent immediately to the laboratory. The frost susceptibility of the plants will be measured by determining the supercooling point of detached leaves and shoots. Tissue samples will be immersed in 15 mm diameter test tubes containing 20 mm of 10 mM phosphate buffer. Tubes containing grape shoots or leaves will then be introduced into a circulating ethanol bath held at -2°C for 30 minutes. The number of leaves which freeze will then be assessed. At 30 minute intervals, the temperature of the bath will be reduced by 0.5°C and the cumulative number of leaves which freeze at a given assay temperature will be determined until all the leaves have frozen, usually by -6.5°C. Total bacterial population size on individual shoots or leaves will be assessed by dilution plating of bacteria removed from the plant material in sterile buffer solution. Plant material will be placed in 2 cm diameter test tubes containing 20 mL of 10 mM phosphate buffer containing 0.1 percent peptone and sonicated for 7 minutes and then agitated for one minute by vortexing to remove bacteria from leaf surfaces. Appropriate dilutions of these leaf washings will then be plated on to 10 percent TSA media, and total colonies enumerated after five days. Bacterial population size will be normalized per gram of tissue. Statistical analysis will be conducted and graphic presentation of data developed.
2. Investigate how ice nucleating bacteria are acquired onto cover crops and develop during the winter growing season, and how the cover crops differ in ice nucleating bacterial population numbers.
   * Field: This trial will be conducted in 2016, 2017, and 2018. The project team will establish plots of 10 cover crop species replicated three times that are reported to have a wide range of ice nucleating bacterial populations, for a total of 30 plots. General groups include legumes, grasses, and forbs. Individual plots will be approximately seven feet by 20 feet and will be planted in a vineyard, covering 4,200 square feet. The plots will be established by seeding in mid-October and sprinkler irrigated to help ensure cover crop establishment. Following germination, plots will be sampled every two weeks through vineyard bud break (late October to early April) to determine ice nucleating bacterial populations by sampling vegetation from ten plants per plot, and consolidating them into one sample. This will be approximately 12 sampling events x 30 samples per event = 360 samples. Additionally, each cover crop species will be followed for phenology (seed emergence, bolting, and flowering), biomass, and percent ground cover. This will help to assess carbon biomass contribution to the vineyard soil.
   * The project will change cover crop species each year to allow for the opportunity to evaluate 30 species. These plots will be located adjacent to an existing weather station at the University of California Hopland Research and Extension Center (UC HREC).
   * Note on cover crops: While eliminating all vegetation beneath grape vines is a proven method to reduce the risk of frost, the potential for erosion during rainfall is undesirable and therefore cover cropping the vineyard floor is a logical way to prevent this. Previous studies by the investigators have shown that there can be a 10,000 fold difference between bacterial populations on different cover crop species. Consequently selecting the right species to give all of the positive benefits of cover crops (carbon sequestration, biodiversity both above and below the soil, habitat for beneficial insects and mites) without increasing the risk of frost for the vineyard.
   * Laboratory: Samples will be processed similar to grape vine shoots and leaves noted in Objective 1.
3. Determine if antagonistic bacteria can be successfully established on cover crops and vines, and prevent vines from freezing
   * Field: This trial will occur starting in 2016, 2017, and 2018. In year one, four cover crop species known to support ice nucleating bacteria will be established in small replicated plots (planted in seven x 20 feet plots, replicated three times, for a total of 12 plots, 1,620 square feet total area) and sprayed with Pseudomonas fluorescens A506, a bacterium known as an antagonist to ice nucleating bacteria that will compete for the same ecological niche. Sprays will be applied monthly following germination, and vegetation will be sampled monthly similar to Objective 3 sampling to determine if the bacterium is establishing. A total of five sampling events will occur x 12 plots = 60 samples.
   * If the trial conducted in 2017 shows that A506 bacterium can be successfully established on cover crops with populations dominating the microflora on leaf surfaces, a larger field trial with a cooperating grower will be conducted in 2018 in a vineyard with no other frost protection system. A randomized complete block design will be used which will include two treatments: 1) Cover crops and vines sprayed with A506 monthly before bud break, and every two weeks after bud break; and 2) A control treatment planted similarly but with no A506 applications. There will be four replications of each treatment covering 0.5 acres for a total of eight replications, covering a total of four treated acres. Instrumentation will be the same as used in Objective 1 including a weather station and temperature data loggers in each replication. Sampling would also be similar, two treatments x four replications x 20 shoot or small leaf samples following bud break x four sampling events = 640 samples for the experiment.
   * Laboratory: Samples will be processed as noted in Objective 1.
4. Investigate if different wine grape cultivars support similar ice nucleating bacterial populations.
   * This experiment will be conducted in 2017 and 2018. It is unknown if wine grape cultivars differ in their ability to support ice nucleating bacteria. There are known differences in leaf morphology (presence or absence of leaf hairs, thickness of cuticles, etc.). An existing replicated wine grape cultivar trial at UC HREC consisting of 12 different wine grapes will be sampled following bud break in two sampling events and evaluated in the laboratory to determine ice nucleating bacterial populations. Samples will consist of 20 shoot or small leaf samples following bud break x 12 cultivars x two sampling events = 480 samples. Samples will be processed in the laboratory as in Objective 1. As noted in Objective 2, there is an existing weather station at the UC HREC.
5. Compare cost and energy of frost protection with copper sprays to sprinkler and wind machine frost protection to validate this as a more sustainable approach than is presently being use in the region.
   * This activity will be conducted in 2017 and 2018. Existing data from UC Cost Studies for vineyard frost protection will be compared for the different frost protection systems, which accurately describe the cost to install and operate both sprinkler and wind machine frost prevention systems in vineyards. Metrics include machinery operating costs and depreciation, fuel, labor, and water. The project team will keep accurate records of materials applied, fuel used, operator time, and machinery costs during copper and antagonistic bacteria applications in the experiment to make direct comparisons to the present methods used for frost protection. The results will be presented at grower meetings, on the UC Cooperative Extension (UCCE) Mendocino website, and articles in the popular press.
6. Outreach.
   * These activities will occur during 2017, 2018 and 2019.
   * Research results will be presented at the annual UCCE Mendocino Lake Integrated Pest Management (IPM) Seminar which is held in the fall and attended by over 100 growers and crop consultants from the two county area.
   * Presentations will be made at annual professional society meetings during or at the conclusion of the research, such as American Society of Enology and Viticulture, and the American Phytopathology Society.
   * Articles will be written for trade journals during and at the conclusion of the experiments (McGourty).
   * Articles will be written for peer reviewed journals (Lindow and McGourty).
   * Talks will be presented on the research outcomes to other regional winegrowing organizations in California and other states (McGourty).
   * Online summaries will also be developed and posted on the UCCE Mendocino website, as well as the UC IPM Online website, and the UC Integrated Viticulture Online website.

**Project Beneficiaries**

Estimated number of project beneficiaries: 5,900

Does this project directly benefit socially disadvantaged farmers as defined in the RFA? Yes  No

Does this project directly benefit beginning farmers as defined in the RFA? Yes  No

In California, there are 5,900 wine grape growers farming over 650,000 acres of vineyard, producing about four million tons of fruit per year. All are at risk of frost at some point. In 230,000 coast vineyard acres, some frost is likely almost every year. Vineyards have a farm gate value of $3 billion, supply 4,285 wineries employing 333,000 people, and sell $24.6 billion of wine annually. State and federal alcohol excise taxes bring in nearly $800 million to their treasuries. Nationally, there are 25,000 farms with nearly one million acres of vineyard, producing seven million tons of fruit with a farm gate value of $5 billion. Frost damage occurs annually somewhere in many of these districts. When frost occurs, serious yield reductions occur ranging from 25 to 100 percent, depending on severity. It is entirely possible that this same technology can be used to protect other specialty crops prone to freezing.

Controlling ice nucleating bacteria will be relatively inexpensive, easy to accomplish, require less energy, and precious water than any technique presently being used to control frost damage. Naturally occurring, competitive, antagonistic bacteria will be very environmentally friendly if shown to work, and safer than copper based on registrations and experience for existing crop use (citrus and pears). Since this research project will investigate the relationship of different cover crop species and ice nucleating bacteria, cover crop planting, management, and materials to apply to grape vines, the results can be a model system for other specialty crops concerned with spring frost damage, including fruit trees, berry vines, warm season vegetables, herbs, and nursery stock. At this time, the ecology of ice nucleating bacteria and its sources, growth on frost susceptible crops and ways to minimize the risk of frost are not well studied. This project may provide new techniques for frost control.

**Statement of Solely Enhancing Specialty Crops**

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| By checking the box to the right, the recipient confirms that this project solely enhances the competitiveness of specialty crops in accordance with and defined by [7 U.S.C. 1621](http://uscode.house.gov/view.xhtml?req=(title:7%20section:1621%20edition:prelim)%20OR%20(granuleid:USC-prelim-title7-section1621)&f=treesort&edition=prelim&num=0&jumpTo=true). |  |

**Continuation of Project Information**

This project does not build upon a previously funded Specialty Crop Block Grant Program project.

***Likelihood of the project becoming self-sustaining:***

The technology to plant and manage cover crops, spray emerging vine shoots, and safely apply pesticides in low volumes to specific parts of a vineyard are already well understood and practiced by many growers. The programs proposed in this project will require about four sprays per year, cost less than $50 per acre, and if effective, will most likely be readily adopted. Three pounds of copper per acre accurately applied to the canopy poses a small environmental risk. The use of naturally occurring competitive bacteria (it takes the ecological niche that ice nucleating bacteria occupies) is already registered for use on other fruit crops, and has been shown to be very safe to the environment. Frost protection water use will be minimized, potentially saving almost 0.5 acre feet per acre, half of total vineyard water use in the region. This helps critical water flow during dry years in surface water sources for endangered salmonid fish.

**Support from Other Federal or State Grant Programs**

Was this project submitted to a Federal or State grant program other than the SCBGP for funding and/or is a Federal or State grant program other than the SCBGP funding the project currently? Yes  No

**EXTERNAL PROJECT SUPPORT**

In Mendocino and Lake Counties where UCCE Farm Advisor McGourty works, there are approximately 700 growers farming over 28,000 vineyard acres. Organizations that are supportive of this project include the Mendocino County Winegrowers, Inc.; Lake County Wine Grape Commission; Anderson Valley Winegrowers Association; and the Mendocino County Farm Bureau. All are concerned with the reduced availability of water for frost protection during drought years, regulations by state agencies for sprinkler frost protection, noise from wind machines that can cause conflicts with neighbors, and public perceptions that protecting vines with sprinklers is not a "beneficial use" of a public trust resource. Having more options to protect vines from frost, while conserving valuable water would greatly improve risk management options, provide more water for improving endangered species habitat, and also potentially improve crop yields during dry years.

**EXPECTED MEASURABLE OUTCOMES**

**Outcome 4:** Enhance the competitiveness of specialty crops though greater capacity of sustainable practices of specialty crop production resulting in increased yield, reduced inputs, increased efficiency, increased economic return, and/or conservation of resources.

* **Indicator 2:** Adoption of best practices and technologies resulting in increased yields, reduced inputs, increased efficiency, increased economic return, and conservation of resources.
  + **a.** 700 growers/producers indicating adoption of recommended practices.
  + **b.** 700 producers reporting increased dollar returns per acre or reduced costs per acre.
  + **c.** 28,000 acres in conservation tillage or other best management practice.
* **Description:** 
  + **a.** 700 growers are in the area of this study, and they have regular contact with UCCE Mendocino and Lake County offices and grower organizations. Nearly all receive newsletters, emails, and other communications, and attend seminars and meetings on production practices.
  + **b.** Frost reduces yields for the region by as much as 25 percent during years when multiple frost incidents occur. These new approaches will help to reduce risk from frost damage, and increase income to producers. Drought years are especially problematic because there is reliance on surface water sources for frost protection with sprinklers; dew points and air temperatures are low in dry years, and frost risk is higher than in humid years. In some years (2014, 2015) state regulators have curtailed water diversions to ensure that there is adequate stream flow for endangered salmonid fish. By having alternatives to sprinkler frost protection, growers reduce risk of frost damage and could potentially increase surface water flows for fisheries and urban use, which are priorities in water right categories. When sprinkler frost protection is used in the upper Russian River watershed and Navarro watershed, approximately 450 acre feet of water are used per event. If frost protection by reducing ice nucleating bacteria can be used for five events per season, 2,250 acre feet of water would be saved, which is about 25 percent of the water used most years by vineyards in both watersheds. Additionally, diesel and electric pumps which use considerable energy would not be needed, nor would wind machines, which create noise and use about eight gallons of propane per hour to operate. These costs are potentially much greater than applying low amounts of copper sprays by light weight ATVs and small sprayers.
  + **c.** Cover cropping is widely practiced in this region. Previous limited research indicates that cover crops greatly affect ice nucleating bacteria populations, and the project team hopes to show growers how to minimize frost risk with different vineyard floor management techniques, and by also selecting cover crops that do not support large populations of ice nucleating bacteria. In turn, erosion prevention, carbon sequestration, habitat for beneficial insects and mites, improved water infiltration, water retention, and increased biodiversity in cover crop canopies and beneath the soil are benefits that have been demonstrated by previous studies in the region and other areas using cover crops. Finally, copper applications may be potentially harmful if used in large amounts that could end up in waterways, but the project proposes to use small amounts to reduce the risk of freezing to emerging vine tissue. The project will very precisely apply 0.75 pounds per acre in 25 gallons of water per application to just the emerging leaf shoots. Four applications five days apart will give protection most years for the entire frost season (which usually lasts about 20 days) for a total of three pounds per acre, which is allowable under the National Organic Program and viewed as being environmentally benign. The project will also evaluate antagonist bacteria that multiply on both cover crops and grape vine tissue, eliminating the ecological niche on plant tissue for ice nucleating bacteria, reducing frost risk. This strategy has been effective on pears and citrus, but has not been demonstrated on grape vines. An antagonist bacteria known as Blight Ban (Pseudomonas fluoresscens a506) is already registered for use in California for pears and citrus, and is presently registered for use on grapes for bunch rot control. If effective, this could reduce the need for copper applications, and offer yet another very environmentally friendly alternative for frost protection.

**Outcome 5:** Enhance the competitiveness of specialty crops through more sustainable, diverse, and resilient specialty crop systems.

* **Indicator 1:** Two new or improved innovations models (biological, economic, business, management, etc.), technologies, networks, products, processes, etc. developed for specialty crop entities including producers, processors, distributors, etc.
* **Indicator 2:** Two innovations adopted.
* **Indicator 3:** 700 specialty crop growers/producers (and other members of the specialty crop supply chain) that have increased revenue expressed in dollars.
* **Indicator 4:** Two new diagnostic systems analyzing specialty crop pests and diseases (Diagnostic systems refer to, among other things: labs, networks, procedures, access points.).
* **Indicator 5:** One new diagnostic technologies available for detecting plant pests and diseases (The intent here is not to count individual pieces of equipment or devices, but to enumerate technologies that add to the diagnostic capacity.).
* **Indicator 7:** Two viable technologies/processes developed or modified that will increase specialty crop distribution and/or production.
* **Indicator 8:** 700 growers/producers that gained knowledge about science-based tools through outreach and education programs.
* **Description:** 
  + **Indicators 1 and 2:** The use of (1) copper and (2) competitive antagonistic bacteria can reduce the populations of ice nucleating bacteria including Pseudomonas syringae as demonstrated in other crops, including pears and citrus, but not wine grapes so far. Initial testing indicates in the laboratory that vineyards treated with copper have reduced ice nucleating bacterial counts and allows vine tissue to super cool and freeze at lower temperatures (at least two degrees below 32 degrees Fahrenheit, even lower under ideal conditions). Additionally, vineyard floor management practices will be assessed to determine best management practices to protect the soil and improve soil health, since cover crops are the most likely source of ice nucleating bacteria. Limited past testing indicated that cover crop species vary greatly in the size of ice nucleating populations that they support. Mowing cover crops just before bud break will likely reduce frost risk significantly by reducing the source of ice nucleating bacteria.
  + **Indicator 3:** In field testing during the past two years there have been no frosts, but copper treatments were demonstrated to reduce the freezing point of green grape vine tissue in the lab and greatly reduce ice nucleating bacteria populations. Growers are very interested in using technologies that are less expensive than present approaches that are energy and water intensive, including sprinkler frost protection and wind machines, and are expensive to operate.
  + **Indicator 4 and 5:** Lab tests to assess ice nucleating bacteria on the surface of cover crops and grape vine leaves are already available but not used by wine grape growers, but could be adapted by private labs as they are relatively simple tests and do not require expensive equipment. This includes plating bacteria and identification and assessment of populations and species, and determining populations of the bacteria by air deposition samples.
  + **Indicator 7:** Less damage to vineyards due to frost will result in increased yields and reduced risk by growers. In turn, this will also reduce crop insurance payments to growers if their vineyards are not injured.
  + **Indicator 8:** There at approximately 700 growers in the immediate area who quite likely will implement a program to reduce water, energy use, and money to protect vines from freezing if the techniques to be studied are effective.

**COST SHARING SUMMARY**

This project will not utilize cost sharing.

**BUDGET NARRATIVE**

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| **Budget Summary** | |
| **Expense Category** | **Funds Requested** |
| **A. Personnel** | $0 |
| **B. Fringe Benefits** | $0 |
| **C. Travel** | $4,500 |
| **D. Equipment** | $15,000 |
| **E. Supplies** | $4,800 |
| **F. Contractual** | $141,700 |
| **G. Other** | $0 |
| **Direct Costs Subtotal** | **$166,000** |
| **H. Indirect Costs** | $0 |
| **Total Budget** | **$166,000** |

**A. Personnel**

No costs requested.

**B. Fringe Benefits**

No costs requested.

**C. Travel**

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| **#** | **Trip Destination** | **Type of Expense** | **Unit of  Measure** | **Number  of Units** | **Cost per Unit** | **Number  Claiming  Expense** | **Funds  Requested** |
| 1 | Mendocino Country | Mileage | Miles | 8,333.33 | $0.54 | 1 | $4,500.00 |
| **Travel Subtotal** | | | | | | | **$4,500** |

**Trip 1 (10/2016-03/2019):** In each year of the study, an estimated 50 trips will be made by UCCE Principal Investigator and/or Agriculture Technician between the office at Ukiah and the field sites in Mendocino County to apply treatments to plants, collect plant material for analysis, maintain monitoring equipment etc. These trips will vary in length, but mileage is estimated at 55.56 miles/trip x 50 trips/year = 2,777.78 miles x 3 years = 8,333.33 miles x $0.54/mile = $4,500.

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| **CONFORMING WITH YOUR TRAVEL POLICY**  By checking the box to the right, the recipient confirms that the organization’s established travel policies will be adhered to when completing the above-mentioned trips in accordance with [2 CFR 200.474](http://www.ecfr.gov/cgi-bin/retrieveECFR?gp=&SID=988467ba214fbb07298599affd94f30a&n=pt2.1.200&r=PART&ty=HTML#se2.1.200_1474) or [48 CFR subpart 31.2](http://www.ecfr.gov/cgi-bin/text-idx?SID=3f25ca1f21583e03b13f595d0d9c518d&node=pt48.1.31&rgn=div5#sp48.1.31.31_12) as applicable. |  |

**D. Equipment**

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| **#** | **Item Description** | **Rental or  Purchase** | **Acquire  When?** | **Funds  Requested** |
| 1 | Two weather stations with tower | Purchase | Year 1 | $15,000.00 |
| **Equipment Subtotal** | | | | **$15,000** |

**Equipment 1:** Detailed weather data will be required at the field sites. Funds are requested for two weather stations based on a Campbell Scientific data logger and cell phone modem capable of monitoring air temperature at three different heights (one, five, and 35 feet above the vineyard floor), humidity, wind speed, solar irradiance, rainfall, canopy temperature, and black globe (leaf surface reradiation). Multiple sensors will be arrayed on a tower so that vertical temperature gradients can be measured (inversion layer formation). This allows for very exacting monitoring of environmental conditions during any frost events, and sensors are recommended by bioclimatologists who work on frost research. The weather station will also be equipped with communications capability to enable remote access of temperature and other weather parameters so that the sites can be monitored and treatment decisions made in real time. The cost of each weather station and associated tower is estimated as $7,500 based on the cost of a third existing station that is in use near one of the proposed plots. These stations will be purchased at the beginning of the experiment as soon as funds are available, and installed by February 2017.

**E. Supplies**

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| **#** | **Item Description** | **Cost Per  Unit** | **Number  of Units** | **Acquire  When?** | **Funds  Requested** |
| 1 | Hobo data loggers 64k pendant temperature measurement | $60.00 | 80 | Year 1 | $4,800.00 |
| **Supplies Subtotal** | | | | | **$4,800** |

**Supply 1:** Data loggers will go into plots at different locations to determine temperatures near the ground and in the canopy of the experiment. In addition to the weather stations, this allows for the measurement of actual vineyard microclimates at near the ground and canopy levels, giving the project team replicated temperature data that can be correlated to any damage that might occur in individual sub plots in the experiments. These devices can be reused in the different plots throughout the time frame of the investigation.

**F. Contractual**

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| **#** | **Name/Organization** | **Hourly Rate /**  **Flat Rate** | **Funds**  **Requested** |
| 1 | University of California, Berkeley | Flat Rate | $141,700 |
| **Contractual Subtotal** | | | **$141,700** |

**Contractor 1: University of California, Berkeley.**

Throughout the entire duration of this investigation, Co-Principal Investigator Dr. Lindow will be helping to coordinate application of treatments and sampling the trials. In the laboratory, he and his staff will run all microbiological investigations including determining the presence of bacteria and population numbers on foliage of grape vines and cover crops, determining freezing points of sampled shoots and leaves, and doing statistical analysis, interpretation, writing project summaries, and make presentations on data during and at the conclusion of the study. These activities will closely parallel the work of Glenn McGourty in managing the field component of these studies.

***Contractor 1: A. Personnel***

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| **#** | **Name/Title** | **Level of  Effort** | **Funds  Requested** |
| 1 | Renee Koutsoukis, Staff Research Associate II | 60% FTE | $81,451.00 |
| **Personnel Subtotal** | | | **$81,451** |

***Employee 1:*** Ms. Koutsoukis will perform the extensive laboratory-based experimentation related to the project including enumerating bacterial numbers on grape and cover crop species in the research plots. She will also perform measurements of the supercooling temperatures of treated grape plants by cooling large numbers of grape buds/leaves in tubes of water and determining the temperature to which they can be cooled before they nucleate and freeze. These activities are very intensive and require the preparation of large amounts of culture media and other supplies such as sterile dilution tubes for the dilution plating of leaf washings to enumerate bacteria and preparation of large numbers of test tubes with ice nucleus-free water. She also must coordinate receiving, storing, and processing promptly a large number of perishable samples. She will prepare samples for enumeration of bacteria by weighing tissues and introducing them into sterile buffer in flasks where bacteria will be removed from tissues by sonication. After enumerating bacteria recovered on various selective and non-selective culture media she will quantify ice nucleating bacteria recovered on non-selective media by a replica freezing procedure. These studies will be intense and will be concentrated in the late winter to early summer period of December through May in 2017, and the same period in 2018.

***Contractor 1: B. Fringe Benefits***

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| **#** | **Name/Title** | **Fringe Benefit  Rate** | **Funds  Requested** |
| 1 | Renee Koutsoukis, Staff Research Associate II | 47.33% | $38,549.00 |
| **Fringe Benefits Subtotal** | | | **$38,549** |

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| **CONFORMING WITH YOUR FRINGE BENEFITS POLICY**  By checking the box to the right, the recipient confirms that the organization’s established fringe benefits policy was used in determining the fringe benefits costs listed above. |  |

***Contractor 1: C. Travel***

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| --- | --- | --- | --- | --- | --- | --- | --- |
| **#** | **Trip Destination** | **Type of Expense** | **Unit of  Measure** | **Number  of Units** | **Cost per Unit** | **Number  Claiming  Expense** | **Funds  Requested** |
| 1 | Mendocino Country | Mileage | Miles | 8,333.33 | $0.54 | 1 | $4,500.00 |
| **Travel Subtotal** | | | | | | | **$4,500** |

***Trip 1 (10/2016-03/2019):*** In each year of the study, 15 round trips are anticipated for the Staff Research Associate and/or UC Berkeley PI between UC Berkeley to the field sites in Mendocino county to collect plant material and to evaluate frost injury to field sites. Mileage is estimated at 185.19 miles/trip x 15 trips/year = 2,777.78 miles/year x 3 years = 8,333.33 miles x $0.54/mile = $4,500.

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| **CONFORMING WITH YOUR TRAVEL POLICY**  By checking the box to the right, the recipient confirms that the organization’s established travel policies will be adhered to when completing the above-mentioned trips in accordance with [2 CFR 200.474](http://www.ecfr.gov/cgi-bin/retrieveECFR?gp=&SID=988467ba214fbb07298599affd94f30a&n=pt2.1.200&r=PART&ty=HTML#se2.1.200_1474) or [48 CFR subpart 31.2](http://www.ecfr.gov/cgi-bin/text-idx?SID=3f25ca1f21583e03b13f595d0d9c518d&node=pt48.1.31&rgn=div5#sp48.1.31.31_12) as applicable. |  |

***Contractor 1: D. Equipment***

No costs requested.

***Contractor 1: E. Supplies***

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| **#** | **Item Description** | **Cost Per  Unit** | **Number  of Units** | **Acquire  When?** | **Funds  Requested** |
| 1 | Bacteriological culture media | $0.20 | 30000 | Year 1-Year 3 | $6,000.00 |
| 2 | Expendable glassware (freezing tubes) | $0.50 | 2000 | Year 1-Year 3 | $1,000.00 |
|  | Expendable glassware (flasks) | $5.00 | 200 | Year 1-Year 3 | $1,000.00 |
| 3 | Expendable plasticware (tips for pipets) | $500.00 | 1 | Year 1-Year 3 | $500.00 |
|  | Expendable plasticware (petri dishes) | $0.05 | 30000 | Year 1-Year 3 | $1,500.00 |
| **Supplies Subtotal** | | | | | **$10,000** |

***Supply 1:*** Large amounts of bacteriological culture media ingredients such as nutrients, buffer compounds, agar, antibiotics, and other media ingredients such as fungicides that are added to the culture media will be needed in each year of the study. It is estimated that in each year of the study about 10,000 petri dishes containing culture media will need to be prepared and thus $2000 is requested yearly for such supplies.

***Supply 2:*** Large numbers of glass test tubes and flasks will be required in each year of the study. The test tubes are used to contain ice nucleus-free water into which grape leaves or buds are added and then cooled to determine their nucleation point. Flasks are required to contain leaf samples for sonication to remove bacteria from the plant surfaces. At least 200 flasks will be required at a cost of $5 per flask. While test tubes can be re-used for a limited time at least 2000 test tubes costing 50 cents apiece will be required.

***Supply 3:*** Considerable amount of disposable plasticware will be needed, such as tips for pipets. $500 will be required for the purchase of such materials over the course of this study. The largest costs will be for plastic petri dishes. A total of 30,000 petri dishes (costing 5 cents per petri dish) will be required over the course of this study ($1,500).

***Contractor 1: F. Contractual***

No costs requested.

***Contractor 1: G. Other***

No costs requested.

***Contractor 1: H. Indirect Costs***

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| **Total Personnel and Fringe Benefits** | **Indirect Cost Rate** | **Funds Requested** |
| $120,000 | 6% | **$7,200** |

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| **CONFORMING WITH YOUR PROCUREMENT STANDARDS**  By checking the box to the right, the recipient confirms that the organization followed the same policies and procedures used for procurements from non-federal sources, which reflect applicable State and local laws and regulations and conform to the Federal laws and standards identified in [2 CFR Part 200.317 through.326](http://www.ecfr.gov/cgi-bin/retrieveECFR?gp=&SID=988467ba214fbb07298599affd94f30a&n=pt2.1.200&r=PART&ty=HTML#sg2.1.200_1316.sg3), as applicable. If the contractor(s)/consultant(s) are not already selected, the organization will follow the same requirements. |  |

**G. Other**

No costs requested.

**H. Indirect Costs**

No costs requested.