Final Report Submitted to:
The Pennsylvania Wine Marketing and Research Board
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Title: Monitoring fungicide residue retention on grapes in Pennsylvania

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INTRODUCTION:

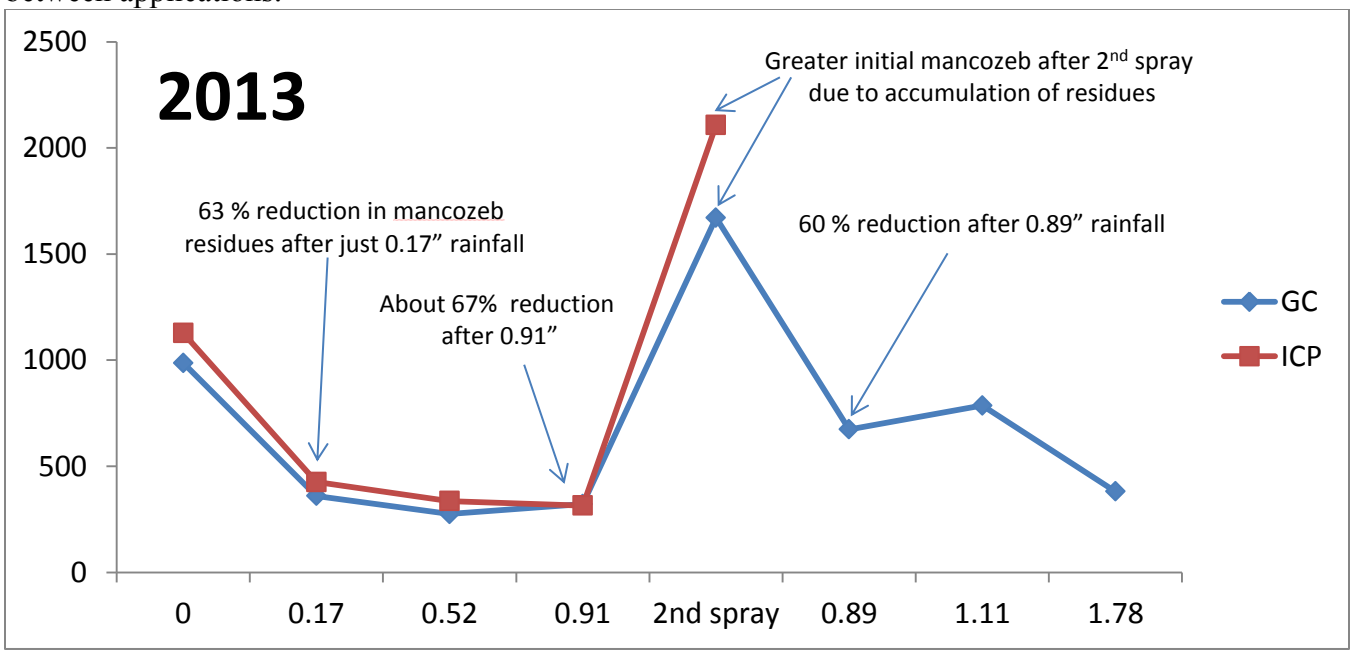
Protectant fungicides like mancozeb are extremely important to the Eastern wine grape industry in the control of black rot and downy mildew of grapes. The development of these diseases is dependent on rainfall events, and the retention of mancozeb on plant surfaces during infection periods is critical to disease control efficacy especially for production of highly susceptible premium wine varieties like Chardonnay and Riesling. A better understanding of how rainfall events affect the retention and efficacy of mancozeb will help create more informed grape disease management recommendations and help growers make more accurate decisions about pesticide applications under varying weather conditions.

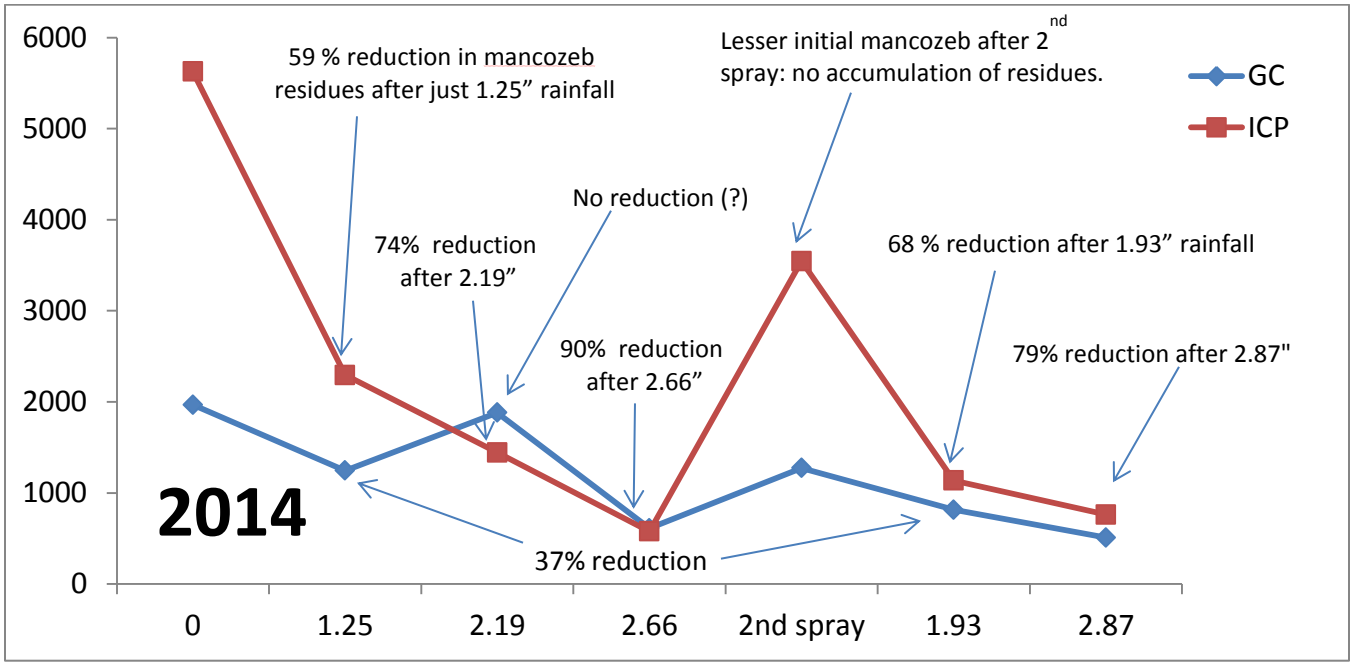
In 2013 and 2014, funding by the Pennsylvania Wine Marketing and Research Board enabled us to conduct monitoring of mancozeb residues on mature Chardonnay (2013) and Riesling (2014) leaves under various patterns of rainfall and to compare the two methods of analyzing residues on those samples. The data was used to establish the relationship between natural rainfall amounts and the decline of mancozeb residue on grape tissue. Our basic objectives were accomplished through experiments at the Lake Erie Regional Grape Research and Extension Center, a Penn State facility in North East PA. In 2013, Manzate (mancozeb) was applied at full label rate (4 lb/A) to *Vitis vinifera* 'Chardonnay' vines with corresponding unsprayed check plots. To determine the effects of rainfall on fungicide residue decline we sampled and analyzed residues on well exposed, fully expanded leaves immediately following application (to establish the baseline residue concentration) and after each of 3 rainfall periods. Residues on leaves were quantified using gas chromatography (GC) and inductively coupled plasma mass spectrometry (ICP) and the results were analyzed and compared. The experiment was conducted twice to assemble a variety of initial and post-rainfall residue concentrations. In 2014, we had planned to expand the design to include the monitoring of residues of 0, 0.5, 1, 2, and 4 lbs/acre of Manzate in the same vineyard (Chardonnay) and to examine clusters in addition to leaves. Unfortunately the loss of our Chardonnay vineyard to winter cold, forced us to downsize our field work and basically repeat our work from 2013 (0 and 4 lbs/A Manzate) on a single, partially surviving row of Riesling at our location. To compensate for this loss, we designed and conducted some simple bioassays on greenhouse grown vines (Riesling) and used the data to determine the efficacy of varying amounts of mancozeb residue on diseases like black rot and downy mildew. Our hypothesis is that this would allow us to relate fungicide application rates, rainfall, and residue accumulation or depletion to the need for future applications for protection from these diseases. The data will be used to establish the relationship between weathering, fungicide rates, and the degree of protection remaining on grape tissue.

Observations with regard to mancozeb residue versus rainfall over two seasons: Figures 1 and 2. What a difference a year makes; there were many contrasts between the results of 2013 and 2014 (See figure 1). First, initial mancozeb deposits from spray applications (vertical y-axis) were much greater in 2014 than in 2013. There is also a large difference in amount of initial deposit detected by GC and ICP in 2014. This is in strong contrast to results of 2013 where initial concentrations were roughly the same with both analyses. The larger initial deposit in 2014 is thought to be due to improvements made to our sprayer after the 2013 season; the upgraded sprayer deposited two (GC) to five (ICP) times more mancozeb in 2014 than in 2013. But, which analysis is more accurate? The GC analysis relies on samples that are immediately frozen after sampling and shipped frozen, overnight to the lab at Michigan State University. Samples are then stored in methanol where fungicide breakdown can occur over time. During the analysis, mancozeb residue values are calculated by converting the molecular mass of carbon disulfide detected from the sample and converting to mancozeb molecular mass. On the other hand, ICP relies on the detection of manganese in the sample. A single manganese atom is part of the mancozeb molecule. Being an element, manganese does not break down into anything else; ICP samples can be dried and stored for long periods of time and shipped without concerns about manganese break down. While it is possible that the mancozeb molecule could break down on the leaf surface over time, releasing the manganese atom, it is unlikely this occurred within 24 hours of the initial sampling.

Nevertheless, the higher initial deposit in 2014 resulted in higher levels of residual mancozeb remaining after subsequent rainfall events, even though rainfall amounts in 2014 were twice those of 2013. **So, lesson number 1: better spray efficiency = higher initial deposit = higher residual after subsequent rainfall events, even though rainfall amounts may be higher and the same 4 lb/A rate was applied.** In both years, the ICP analysis consistently shows that initial residue is quickly reduced after first rain (by about 60 %) and remaining residue is more tightly stuck. On the other hand, the GC results show only a 36 % decrease after about 2 inches of rain (inconsistent with 2013). In this regard, the GC analysis is less consistent than the ICP analysis. Also in 2014, the GC analysis shows that the initial residues were no higher than after 2.19 inches of rain. Years also contrasted with respect to mancozeb accumulation after successive sprays: **2013 results indicated that there is mancozeb accumulation on leaves whereas results of 2014 show that there is *not* an accumulation. This may be due, in part, to the much higher rainfall levels recorded in 2014, compared with 2013.**

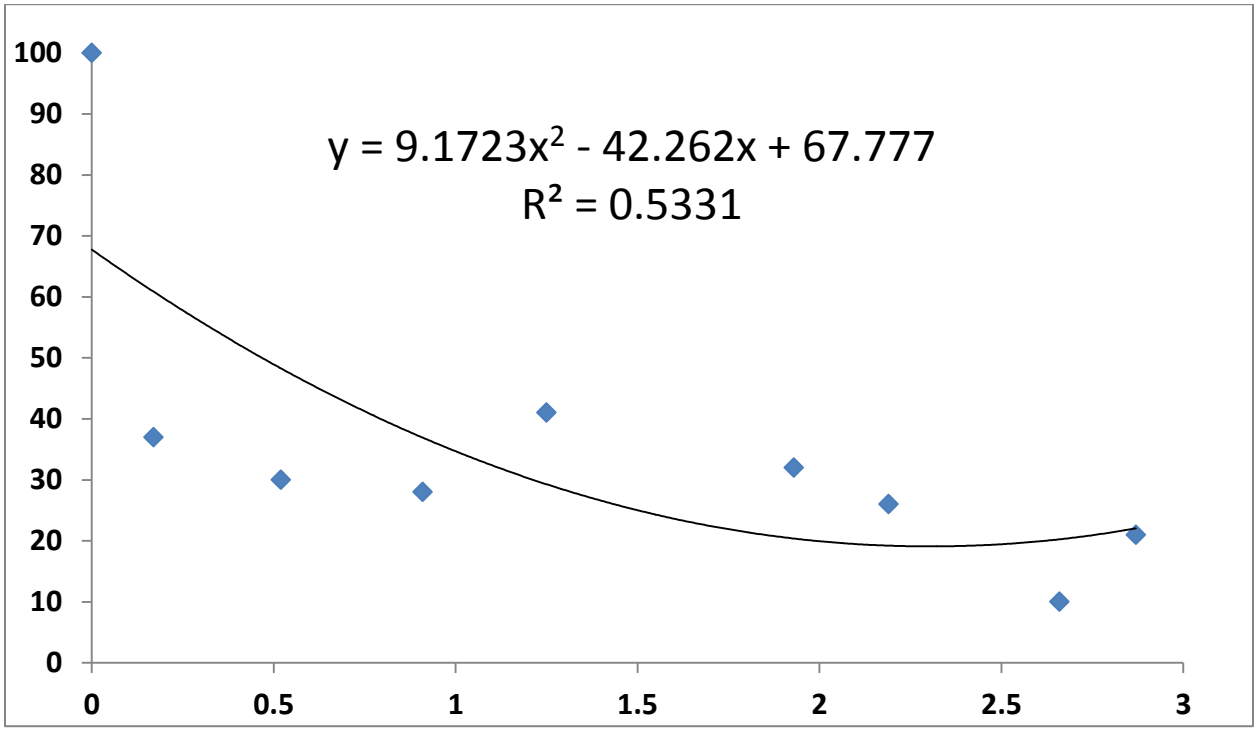
Figure 1. Graphs representing changes in the concentration of mancozeb on Chardonnay (2013) and Riesling (2014) leaf samples collected after various rainfall events and analyzed by two different methods; GC and ICP. Y/vertical axis: ppm (0-2500) of mancozeb on leaves from GC versus ICP analysis. X/horizontal axis: 1st and 2nd applications of Manzate and rainfall amounts between applications.





<u>In summary</u> and based on the graph below (Figure 2), the first inch of rain (or perhaps just the first rainfall event) appears to remove about 60-70% of the initial deposit, two inches of rain removes about 70-80% of the deposit, and three inches removes about 80-90% of the initial deposit. This suggests that the majority of the initial deposit is highly soluble.

Figure 2: Relationship between rainfall (in inches; x-axis) and % of mancozeb residue remaining after rainfall (y-axis). Graph is based on combined ICP results from 2013 and 2014. The equation for the trendline is based on the polynomial equation where % remaining residue = $9.17x^2$ – 42.26x + 67.78.

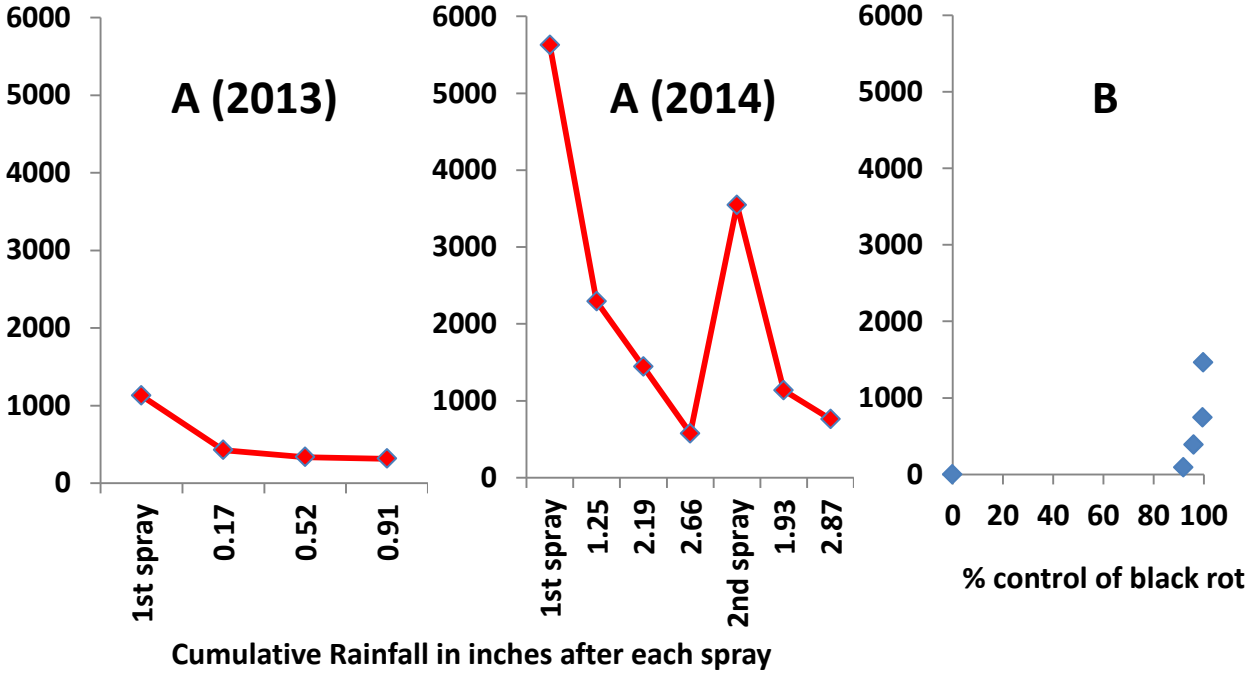


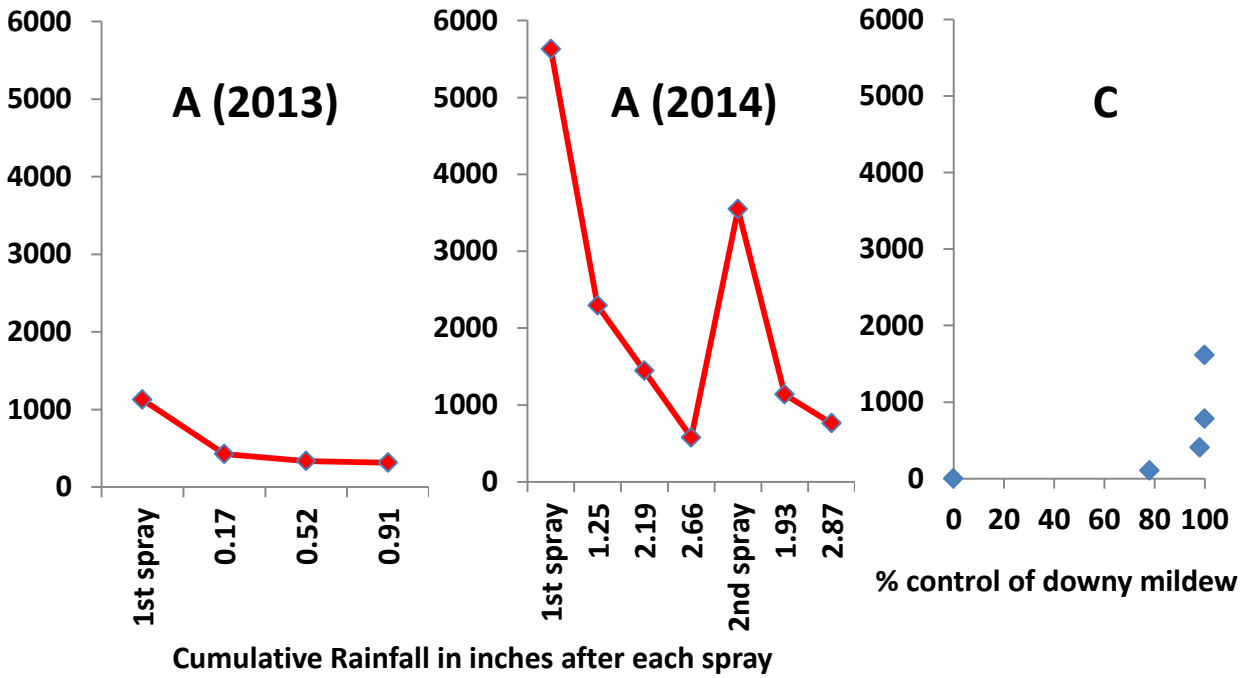
<u>Observation with regard to mancozeb residue versus disease control: Figure 3.</u> After the loss of our Chardonnay vineyard, and heavy damage to our Reisling from winter cold, and no opportunity to gather data as originally planned, we shifted our focus to examine the relationship between mancozeb residues and disease control using potted, greenhouse grown Riesling vines. Potted vines were sprayed with various rates of Manzate Prostick (0, 0.1, 0.25, 0.5 and 1 lb/100 gal). Deposits on potted vines were allowed to dry for about 3-4 hours and leaves were then inoculated with a spore suspension of either the black rot or the downy mildew pathogen. A separate sample of potted vines was also sprayed with the same concentrations (0, 0.1, 0.25, 0.5 and 1 lb/100 gal) and the leaves were harvested for ICP analysis to determine the relationship between Manzate rate and mancozeb concentration. The severity of disease that resulted with each rate was recorded and used to determine the relationship between mancozeb concentration on the leaf and disease control. The results show that we achieved good to excellent control of black rot and downy mildew with as little as 100 ppm of mancozeb per gram of leaf tissue (µg/g). According to the charts below (Figure 2) mancozeb deposits in the field in 2013 and 2014 never fell below 300 and 500 ppm, respectively, which would have been adequate to provide 92-100 % (excellent) control of black rot and downy mildew on Riesling leaves according to our greenhouse bioassays. In our experience in the field, this seems hard to believe.

A possible explanation (hypothesis?) for this high level of control at very low rates, may relate to the differences in the nature of the deposits in each experiment (greenhouse versus field), and raises some interesting questions. In the field trial, the deposits left after rainfall may differ in solubility from the deposits in the bioassays in the greenhouse; the fresh deposits on leaves in the greenhouse bioassay would be expected to be more soluble (remember that 60-70%

of initial deposits are easily washed off by first rainfall) and hence more toxic to the pathogens they target than weathered deposits of similar concentrations remaining on leaves after rainfall in the field. For example, a fresh deposit applied to a leaf at 1 lb/100 gal in the greenhouse with no coverage limitations (about 1500 µg/gram leaf tissue), may have more available (soluble) active ingredient than a 4 lb/100 gal application applied in the field, against wind and sprayer limitations, that has been weathered to the equivalent of a 1 lb/100gal concentration or 1500 µg/g of leaf surface. Hence the bioassay results may only apply to initial deposits made in the field or at least deposits unaltered by rainfall and may not accurately reflect disease control levels by weathered deposits of similar concentration. Thus there may be a loss in efficacy by a rain challenged deposit that relates to both the reduction in initial deposit concentration AND the reduction in solubility of that deposit. Experiments to begin to test/examine this phenomenon are described next.

Figure 3. ICP analysis of mancozeb residues (vertical axis) on leaves of field grown Riesling vines versus rainfall (A) and % control of black rot (B) and downy mildew (C).





Efficacy of rain weathered mancozeb residues for controlling downy mildew: In spring of 2015, field tests were conducted with Niagara grapevines (after the total loss of our Riesling due to winter cold damage in February 2015) to determine how well rain-weathered mancozeb residues control downy mildew on leaves. Mancozeb was applied (Manzate prostick at 4 lbs/100 gallons) to the tops and undersides of fully expanded Niagara leaves in 3 replications with a small, handheld, aerosol charged sprayer. This was matched by 3 replications of unsprayed leaves for comparison (check plots). Samples were collected from each treatment after residues were allowed to dry (full baseline residue) and again after each of 3 subsequent heavy rainfall periods (0.41", 1.64", and 1.46"). Leaf samples were inoculated by applying a spore suspension of the downy mildew pathogen (10^5 sporangia/ml) to the undersides of leaves and then symptom development (percent area of leaf covered with downy mildew sporulation) was recorded a week later. We applied the spore suspension to the undersides of leaves because experience has shown us that far more infection occurs through the undersides than through the top sides of leaves. Interestingly, rainfall amounts had no effect on the efficacy of the manzate application for controlling downy mildew, despite the fact that residues were subjected to a total of 3.51 inches of natural rainfall, and by our earlier experiments, should have been reduced by 90%. Levels of infection on unsprayed leaves averaged about 42 % leaf area covered with sporulation, whereas manzate sprayed leaves averaged about 0.4 % leaf area covered with sporulation. Inoculations at initial mancozeb application and after each subsequent rainfall period did not alter disease control on sprayed leaves; control was always at 98-99% (nearly complete) after each rain period/inoculation! A possible explanation for this lies in the fact that coverage of leaves with manzate (in this experiment) was nearly perfect and may not accurately reflect the results we would have had with less than perfect coverage using an airblast sprayer. This raises more questions and funds from other sources will be used to continue to examine this phenomenon and provide more answers.

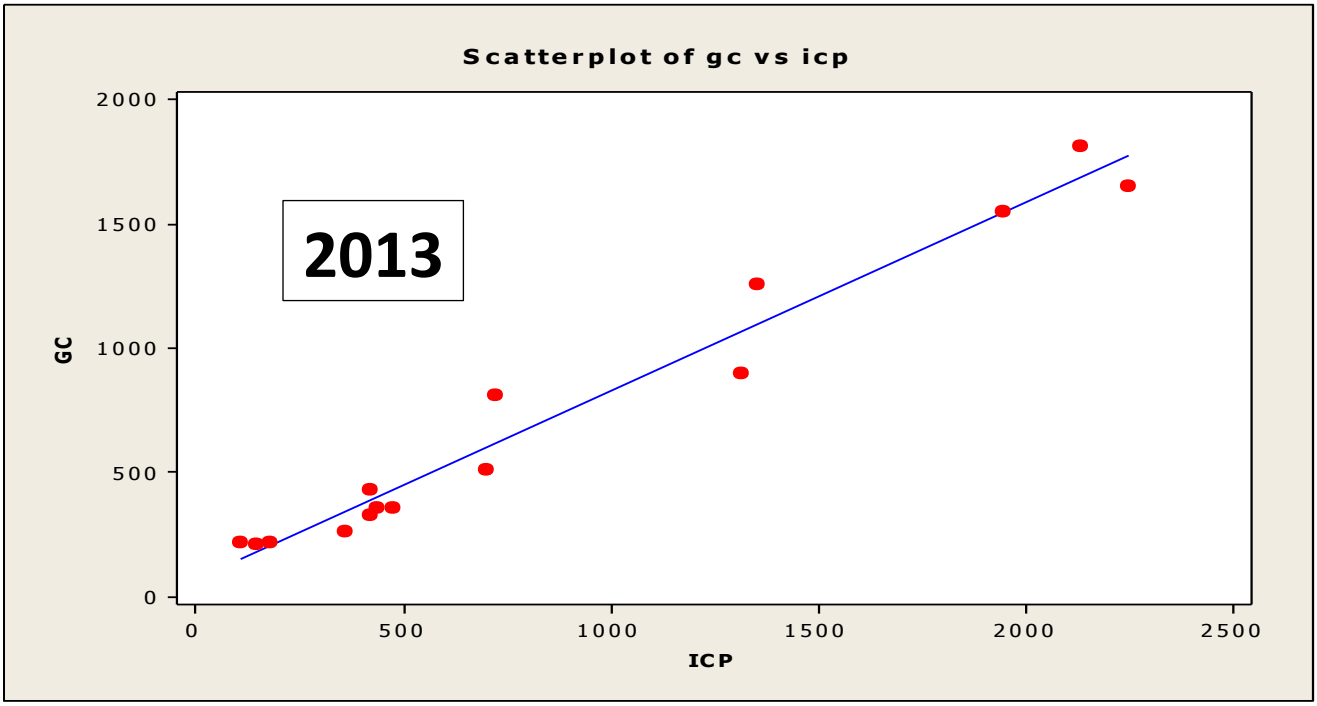
Comparison of GC versus ICP for mancozeb residue concentration: Figure 4.

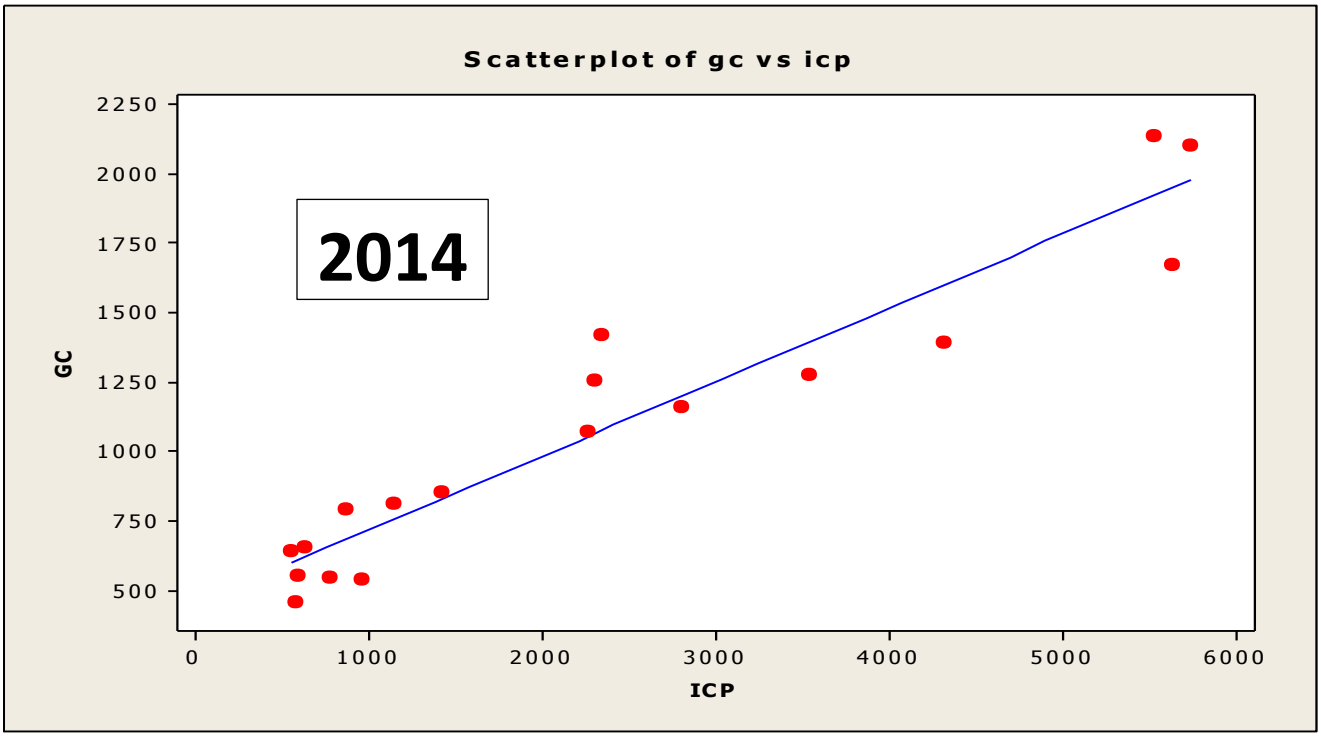
Gas Chromatography (GC) is used to monitor and analyze residues of mancozeb on plant surfaces by measuring the carbon disulfide formed after hydrolysis of the active ingredient. However, this method can be expensive, costing $100 or more per sample. Samples harvested for GC must be kept frozen until processing for analysis, and there can be loss of active ingredient during storage. On the other hand, Inductively Coupled Plasma mass spectrometry (ICP), may be a suitable substitute for detecting and enumerating mancozeb residues by focusing on the presence of manganese in the mancozeb active ingredient. This method is much less expensive, representing a potential cost savings of 75-80 % for mancozeb residue analysis in future experiments. Samples for ICP can be dried and stored at room temperature for long periods of time with little concern over loss of manganese from the sample, as manganese is an element and will not change.

Linear regression analysis was used to compare the data drawn from the two methods (GC and ICP) and create a model that shows how closely correlated the matching samples are (how interchangeable the methods are at each measurement/sampling). Ideally, we had hoped for at least a 95 % degree of correlation as shown by the R^2 value. Our results of 2013 surpass that goal with $R^2 = 96.65$ %. The model was also highly significant with $P < 0.000001$, and our linear regression formula was gc = 68.21 + 0.76 icp (where the gc and icp units are both in parts per million of mancozeb). Our results from 2014 were somewhat lower, especially when data from the third sampling is included, where mancozeb concentrations after 2.19 inches of rain were no different than what was applied initially. After removing this outlier data, the resulting $R^2 = 90.5$ %, the model was highly significant with $P < 0.001$, and our linear regression formula was gc = 452 + 0.27 icp.

As a result of these findings, we were reasonably confident that ICP analysis was at least as good as HPLC and could be used for the rest of the sample analysis to draw reasonably accurate conclusions about mancozeb residue amounts, at a much lower cost.

Figure 4. Linear regression models illustrating the relationship between matching data points (mancozeb residue concentration in ppm) from gc (vertical axis) and icp analysis (horizontal axis) used in the detection of mancozeb residues on Chardonnay (2013) and Riesling (2014) leaves. The line for 2014 was generated after removing the outlier data points from the third sampling date where initial mancozeb concentrations with gc were essentially the same as concentration after 2.19 inches of rainfall.





Annual budget

Funds were requested for funding of summer student labor for sampling, postage (for mailing samples), laboratory fees for fungicide residue analysis, and travel for presentation of information. Below is a summary of how the various expenditures were made, to the nearest dollar amount.

	Amount
Salary and wages: Seasonal student labor; Category III	$ 500
+ Fringe Benefits (7.9%)	$ 39
Postage	$ 111
Travel	$ 200
Sample analysis	$ 4,050
Total	**$ 4,900**