A RESEARCH PROPOSAL TO CONDUCT PIERCE'S DISEASE SYMPTOM FIELD EVALUATION AT THE SOLANO COUNTY RESEARCH BLOCK

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Reporting Period: The results reported here are from work conducted September 1, 2014 through June 30, 2016.

ABSTRACT
In September, 2014, September, 2015 and May, 2016, the PI and a team of grapevine pathologists scored Pierce's disease (PD) symptom severity in a Solano County research block planted with transgenic grapevines mechanically injected with *Xylella fastidiosa* (*Xf*; Pierce's disease strain). Analysis of the variation in the data overall and among individuals indicated that, regardless of when vines were scored, all scores agreed for greater than 50% of the vines and the majority of scores agreed for at least 96.5% of the vines. This indicates that the rating system was well understood by team members and provided a relatively uniform measure of PD symptoms. Score variation was highest in May, 2016, suggesting that PD symptoms might be more variable in the spring, resulting in a less uniform measure of disease. In addition, the percent agreement of scores for vines with less severe symptoms was lower suggesting that raters have more difficulty scoring these vines.

LAYPERSON SUMMARY
In September, 2014, September, 2015 and May, 2016, the PI and a team of grapevine pathologists scored Pierce's disease (PD) symptom severity in a Solano County research block planted with transgenic grapevines mechanically injected with *Xylella fastidiosa* (*Xf*; Pierce's disease strain). Analysis of the variation in the data overall and among individuals indicated that, regardless of when vines were scored, all scores agreed for greater than 50% of the vines and the majority of scores agreed for at least 96.5% of the vines. This indicates that the rating system was well understood by team members and provided a relatively uniform measure of PD symptoms. Score variation was highest in May, 2016, suggesting that PD symptoms might be more variable in the spring, resulting in a less uniform measure of disease. In addition, the percent agreement of scores for vines with less severe symptoms was lower suggesting that raters have more difficulty scoring these vines.

INTRODUCTION
The Product Development Committee (PDC) of the California Department of Food and Agriculture (CDFA) Pierce's Disease and Glassy-winged Sharpshooter Board requested research into uniform evaluation of Pierce's Disease symptoms exhibited by grapevines developed by four PIs as part of the Board's research portfolio. These vines are planted in a single research block in Solano County.

PI Golino and FPS Plant Pathologists with multiple years of grape disease experience made up the core evaluation team. Several Plant Pathology PhD graduate students with grape pathology thesis research were also invited to participate. Each individual participated in training in evaluating PD symptoms according to the scoring system below. That training included 'calibration' by examining a subset of vines including healthy and PD-inoculated controls to insure that ratings are as uniform as possible. Vines were evaluated twice in mid-September and once in mid-May.

Scoring Technique:
A visual rating system on a scale of 1-5 was used by each member of the team to rate every vine individually. All vines were labeled by row and vine number. Data was collected by row and vine number without any information about the particular treatment that the vine received. This is a slightly modified version of the rating system used by the Kirkpatrick lab.

GOLINO/ GILCHRIST SIMPLIFIED RATING SYSTEM –

0 – Healthy vine. All leaves green with no scorching, good cane growth, no cordon dieback or failure to push canes at bud positions. Dry or yellowing leaves may be present but do not show characteristic Xyllela symptoms.

1 – Leaves on one or two canes showing characteristic Xyllela scorched leaf symptoms. No evidence of physical damage to leaf petiole(s) or cane(s). On cane in question at least TWO leaves are symptomatic, 1 single leaf is NOT enough to warrant a rating of #1.

2 – More than 2 canes possess multiple scorched leaves. HOWEVER canes with symptomatic leaves are still confined to just one area of the vine.

3 – Canes with clearly scorched leaves are found on several canes including canes which have not been inoculated.

4 – Ends of cane(s) begin dying back; some canes failed to push in the spring. Vine is clearly symptomatic on all or nearly all surviving canes. Main point is that the vine is NOT yet dead but is clearly facing a terminal fate.

5 –Dead vine or a vine that had a few canes weakly push in the spring but those canes later died with onset of hot temps in July or August. There are NO visible signs of other potential problems such as gophers, crown gall, phytophthora, or Eutypa/Bot dieback of cordons.

If a vine appeared to have died for reasons other than PD that was entered in the comments field for that vine and no score was entered in the rating field.

OBJECTIVES

The objectives were to:

1. Train individuals to evaluate PD symptoms according to the above scoring system.
2. Score the grapevines during the fall and spring.
3. Evaluate the extent to which the scores for any given vine agreed.

RESULTS AND DISCUSSION

In September 2014, nine members of the evaluation team scored 616 vines and the data was analyzed with the purpose of determining the extent to which the scores for any given vine agreed. Scores for a vine were counted as "in agreement" if they equaled one of the integers above or below the mean. Although mode and frequency are typically used for analyzing ordinal data, the scores in the rating system are quantitative in the sense that they follow a logical sense of order and the difference between the scores is roughly equivalent. Therefore, we felt that using the mean as a measure of central tendency was justified. The purpose of the interval was to accommodate integer data and in practice, allows scores to vary by one integer and still be counted as "in agreement".

The percent agreement of scores for individual vines is shown in Figure 1. Cells of varying shades of green represent vines where at least five out of nine scores agreed.

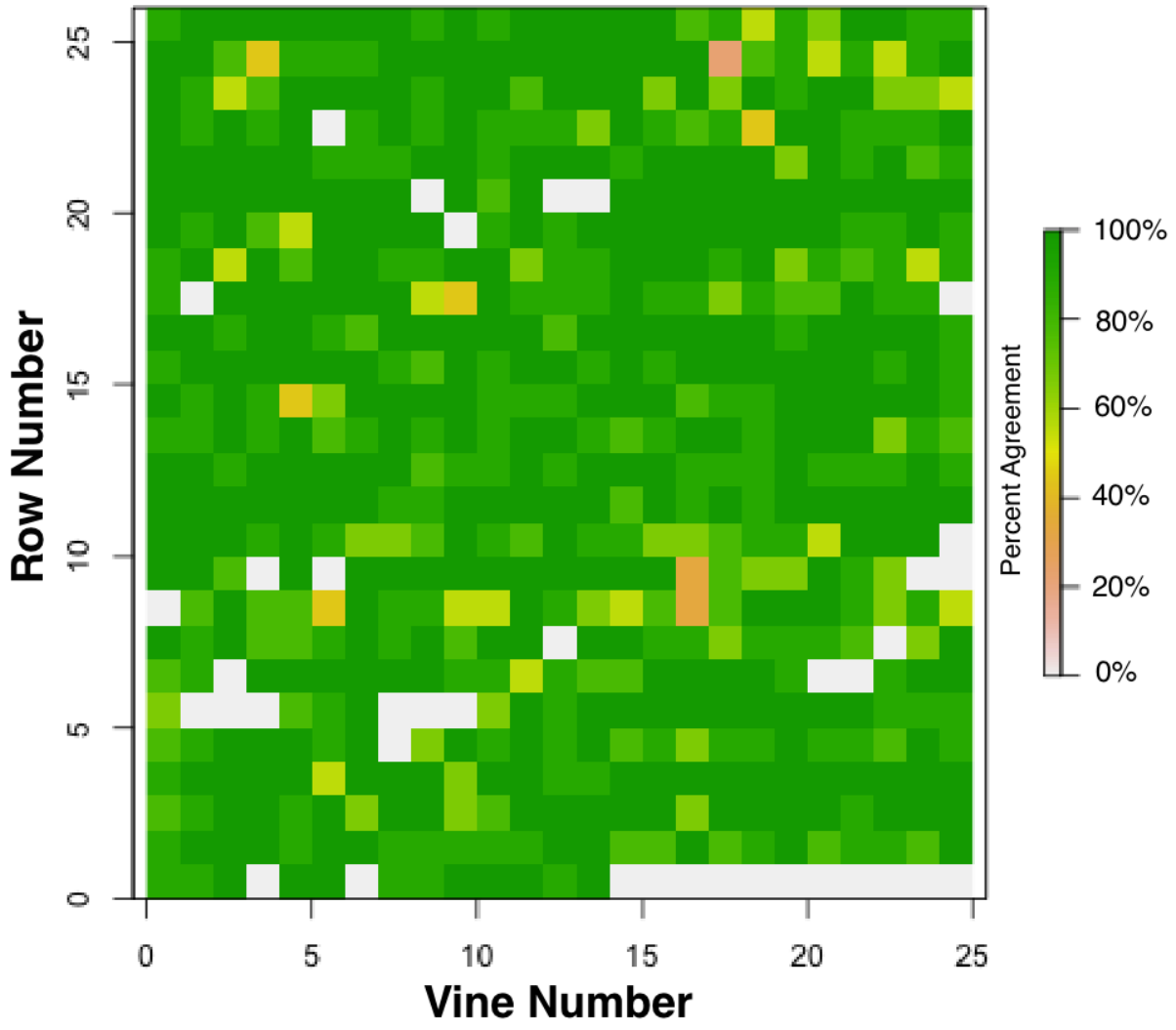

Figure 1. Cell plot of the 616 vines that were rated in September 2014. The colors indicate the percent agreement among scores of individual raters. Scores are counted as "in agreement" if they equal the integer above or below the mean for any given vine. Grey areas indicate missing vines.

The number and percent of vines in each agreement category for the first scoring in September, 2014 is shown in Table 1. Adding columns "56%" through "100%" indicates that for 97.4% of the vines, at least five of the nine scores agreed. For 51.0% of the vines, all nine scores agreed i.e. were within one integer above or below the mean.

Percent Agreement Sept. 2014	0% (0/9)	11% (1/9)	22% (2/9)	33% (3/9)	44% (4/9)	56% (5/9)	67% (6/9)	78% (7/9)	89% (8/9)	100% (9/9)
Number of Vines	4	0	1	3	8	19	41	62	164	314
Percent of vines	0.60	0.00	0.16	0.49	1.30	3.08	6.66	10.1	26.6	51.0

Table 1. The number and percent of vines in each of the ten agreement categories in September, 2014.

The vines were scored again in September 2015 by ten people; the percent agreement of scores for individual vines is shown in Figure 2.

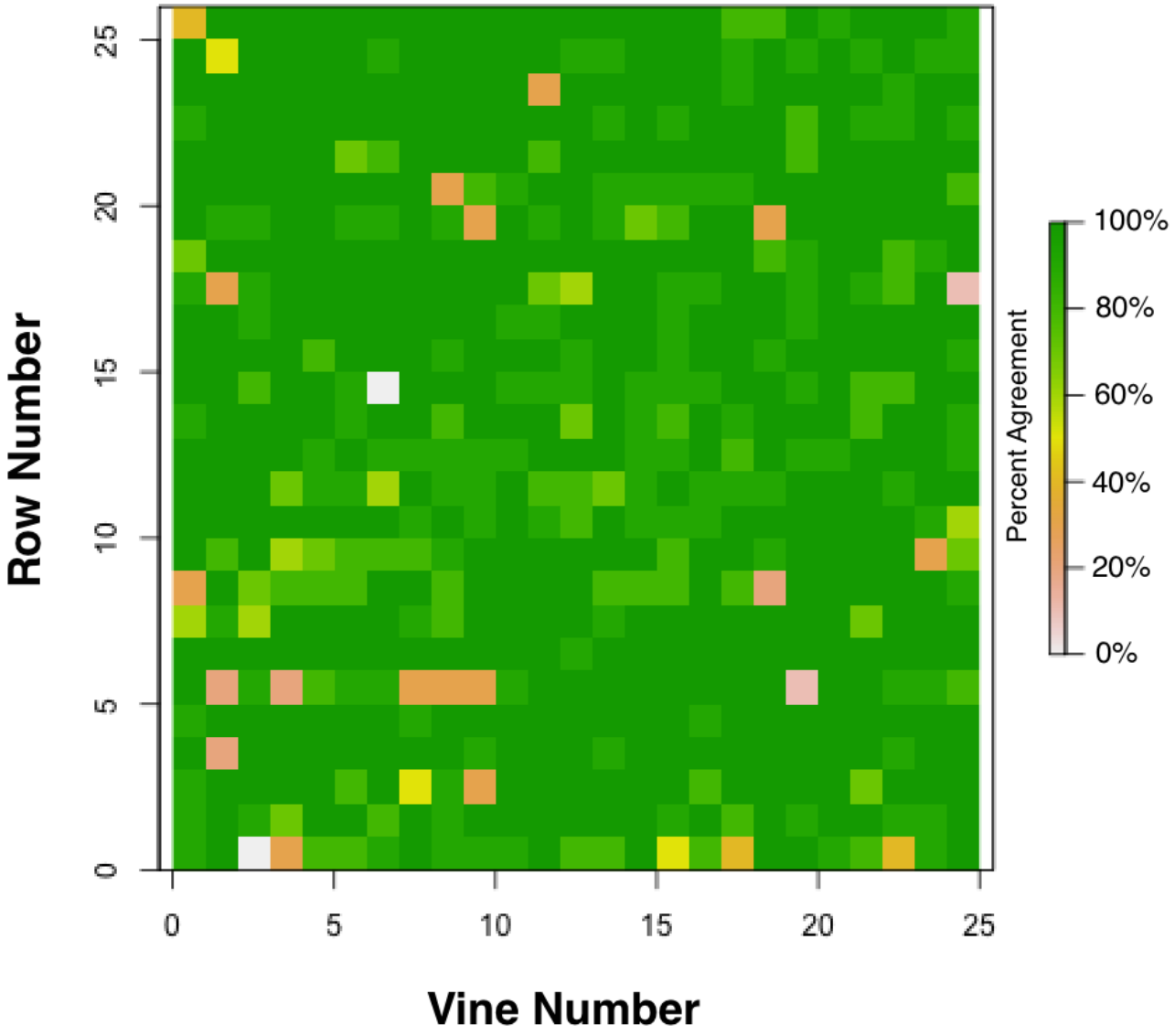

Figure 2. Cell plot of the 650 vines that were rated in September, 2015. The colors indicate the percent agreement among scores of individual raters. Scores are counted as "in agreement" if they equal the integer above or below the mean for any given vine. Grey areas indicate missing vines.

The number and percent of vines in each agreement category is shown in Table 2. Adding columns "50%" through "100%" indicates that for 96.5% of the vines, at least five of the ten scores agreed. For 66.5% of the vines, all ten scores agreed i.e. were within one integer above or below the mean.

Percent Agreement Sept. 2015	0% (0/10)	10% (1/10)	20% (2/10)	30% (3/10)	40% (4/10)	50% (5/10)	60% (6/10)	70% (7/10)	80% (8/10)	90% (9/10)	100% (10/10)
Number of Vines	2	2	4	12	3	3	6	13	49	124	432
Percent of vines	0.31	0.31	0.62	1.85	0.46	0.46	0.92	2.00	7.54	19.08	66.46

Table 2. The number and percent of vines in each of the eleven agreement categories in September, 2015.

The per vine change in percent agreement between 2014 and 2015 is illustrated in Figure 3. For 290 and 251 vines respectively, the percent agreement increased or stayed the same. For 109 vines, the percent agreement decreased in 2015. In some cases, these latter vines appear to be clustered, indicating that some treatments were possibly more difficult to rate. However, percent agreement for most of these vines was still greater than 50% (data not shown). Vines with less than 50% agreement were scattered throughout the plot, indicating problems with individual vines and not entire treatments.

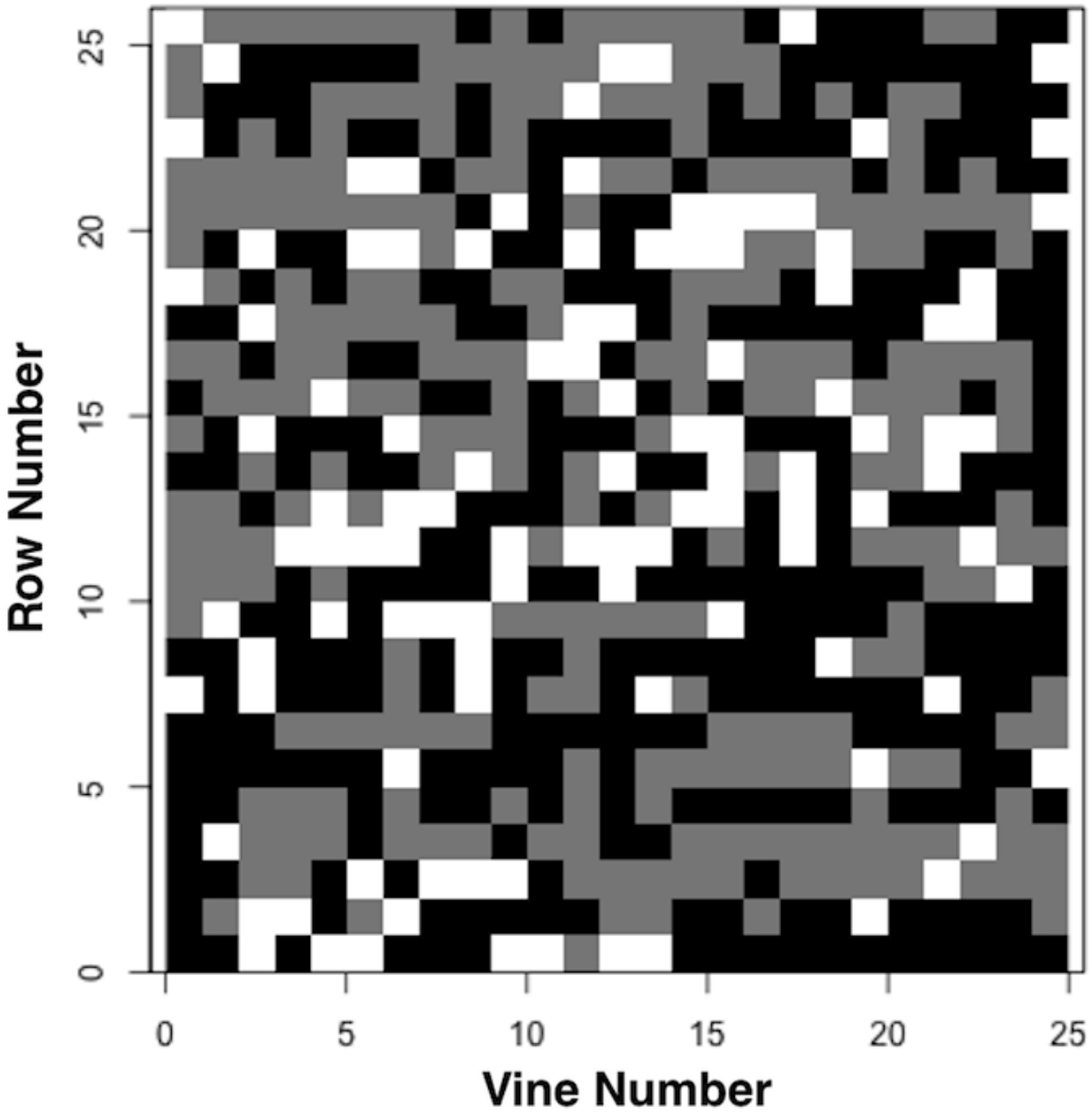

Figure 3. Cell plot representing individual vines and the change in percent agreement between September, 2014 and 2015. Colors indicate the level of change: Black = increase in percent agreement; grey = no change; white = decrease.

In May, 2016, eleven members of the evaluation team scored 622 vines and the data was analyzed. The percent agreement of scores for individual vines is shown in Figure 4. Cells of varying shades of green represent vines where at least six out eleven scores agreed.

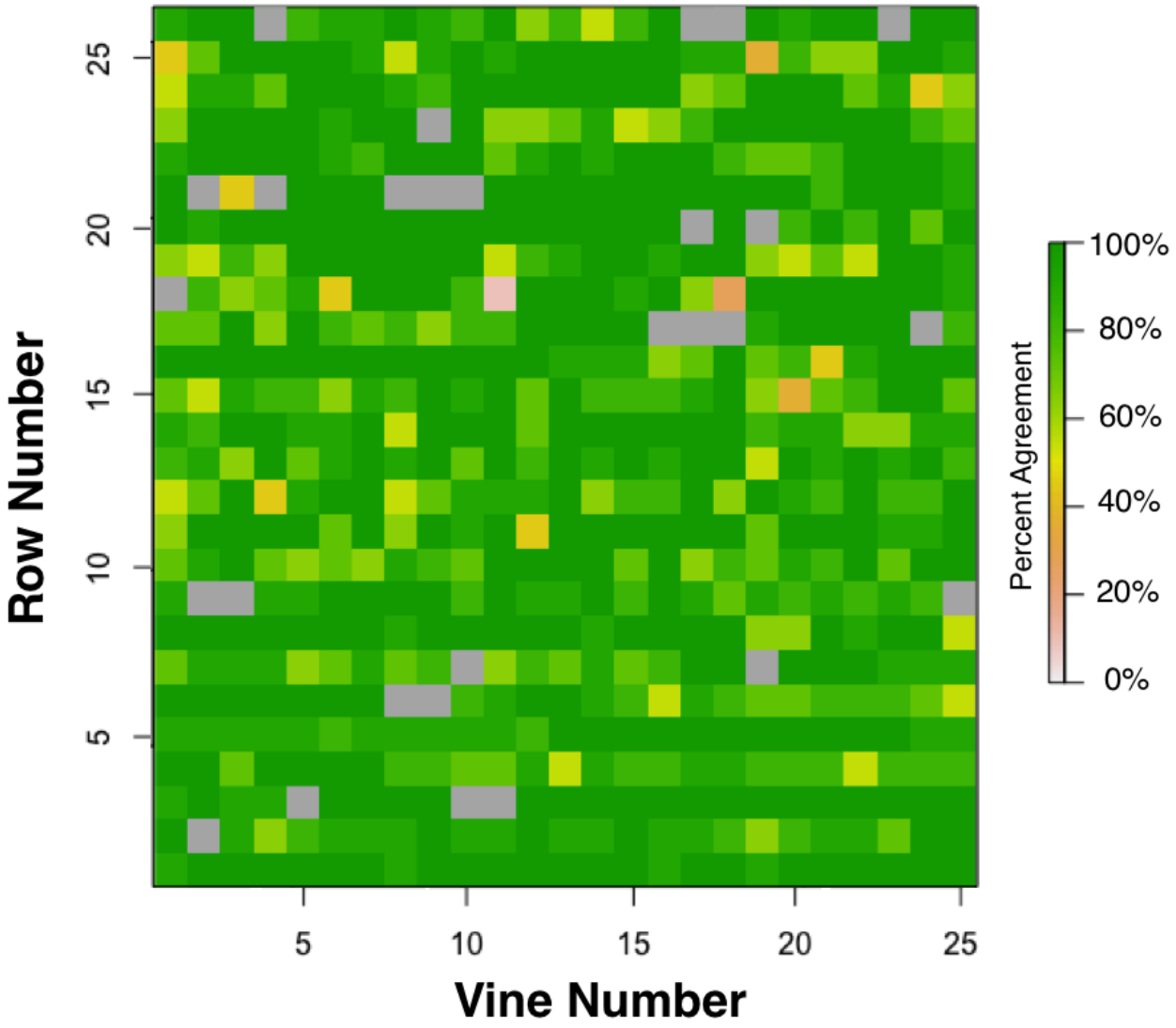

Figure 4. Cell plot of the 622 vines that were rated in May, 2016. The colors indicate the percent agreement among scores of individual raters. Scores are counted as "in agreement" if they equal the integer above or below the mean for any given vine. Grey areas indicate the 28 vines that had fewer than eleven scores and were not included in the analyses. Usually this was because a vine was missing and so was not scored.

The number and percent of vines in each agreement category is shown in Table 3. Adding columns "55%" through "100%" indicates that for 98.2% of the vines, at least six of the eleven scores agreed. For 50.6% of the vines, all eleven scores agreed, i.e. were within one integer above or below the mean.

Percent Agreement May 2016	0% (0/11)	9% (1/11)	18% (2/11)	27% (3/11)	36% (4/11)	45% (5/11)	55% (6/11)	64% (7/11)	73% (8/11)	82% (9/11)	91% (10/11)	100% (11/11)
Number of Vines	0	1	0	1	2	7	18	35	48	73	122	315
Percent of vines	0.00	0.16	0.00	0.16	0.32	1.13	2.89	5.63	7.72	11.74	19.61	50.64

Table 3. The number and percent of 622 vines in each of the twelve agreement categories from May, 2016.

The May, 2016 scores were compared with those from September, 2015 to determine if there was a significant difference in score agreement when vines were rated at a different time of the year. The September, 2015 scores are shown again below.

Percent Agreement Sept., 2015	0% (0/10)	10% (1/10)	20% (2/10)	30% (3/10)	40% (4/10)	50% (5/10)	60% (6/10)	70% (7/10)	80% (8/10)	90% (9/10)	100% (10/10)
Number of Vines	2	2	4	12	3	3	6	13	49	124	432
Percent of vines	0.31	0.31	0.62	1.85	0.46	0.46	0.92	2.00	7.54	19.08	66.46

Table 2. The number and percent of vines in each of the eleven agreement categories from September, 2015.

There are two notable differences in the level of score agreement between September, 2015 and May, 2016. First, the percentage of vines where the majority of scores, i.e. at least 50% of the scores, agree increases from 96.5% in September, 2015 to 98.2% in May, 2016. Second, the percentage of vines where 100% of the scores agree decreases from 66.5% in September, 2015 to 50.6% in May, 2016. Therefore, while the percentage of vines where the majority of scores agree increases slightly in May, 2016, the "strength" of the agreement decreases.

The per vine change in percent agreement between September, 2015 and May, 2016 is illustrated in Figure 5. In May, 2016, the percent agreement increased or stayed the same for 383 and 97 vines, respectively. The percent agreement decreased for 142 vines in May, 2016. In some cases, these latter vines are clustered, indicating that some treatments were possibly more difficult to rate. However, percent agreement for most of these vines was still greater than 50% (data not shown).

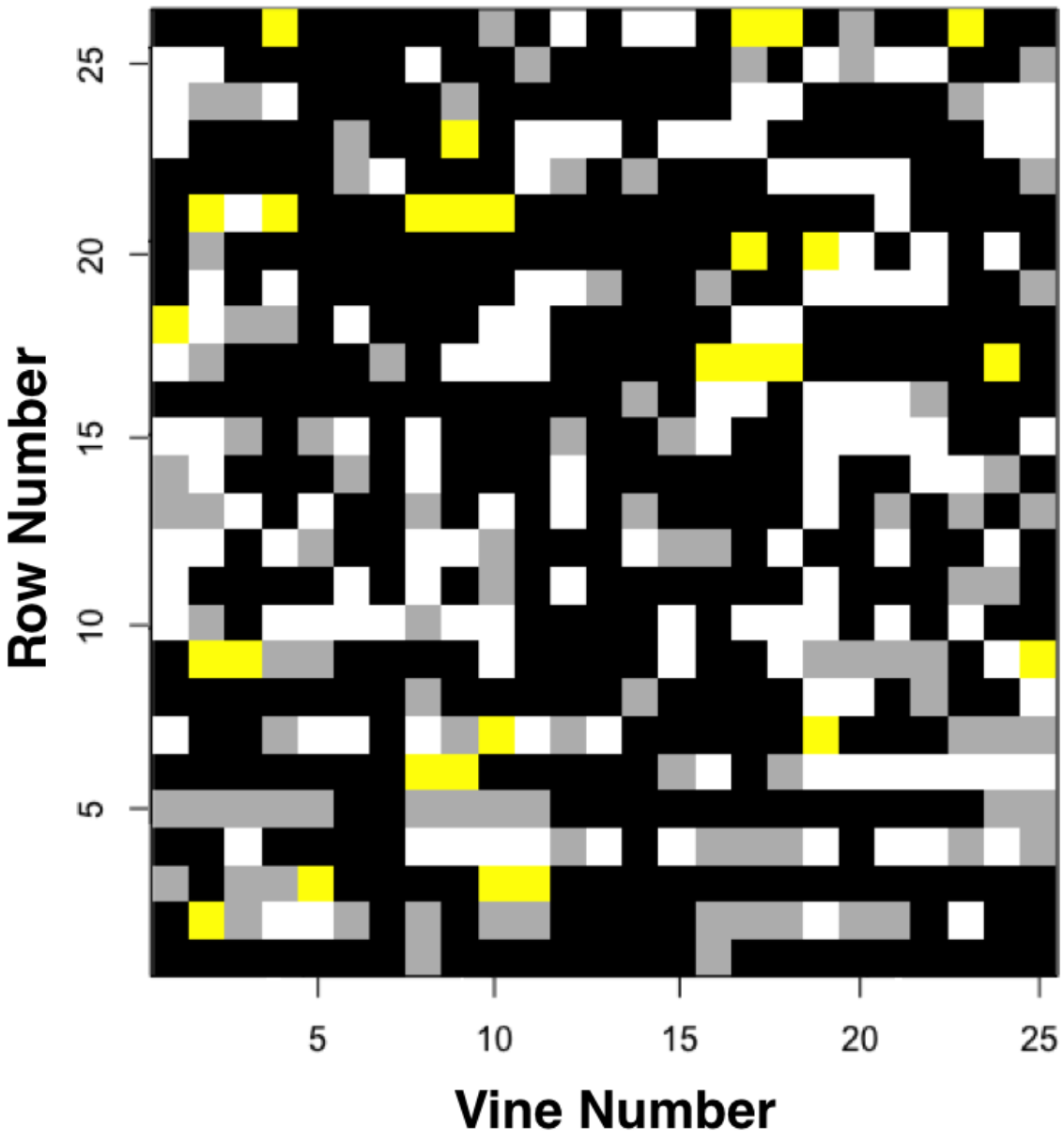

Figure 5. Cell plot representing individual vines and the change in percent agreement between September, 2015 and May, 2016. Colors indicate the level of change: Black = increase in percent agreement; grey = no change; white = decrease. Yellow = vines eliminated from analyses due to fewer than eleven scores.

To determine if the percent agreement for the May, 2016 data varied by score, we mapped mean score against percent agreement for individual vines (Figure 6). The "V" shaped scatterplot indicates that there is an agreement bias for low and high scores, i.e. vines that are not very symptomatic or are showing severe symptoms have scores that are in higher agreement. This is especially true for severely symptomatic vines. Of the 315 vines that had scores 100% in agreement (Table 3), 193 or 61.3% had a mean score of 5.

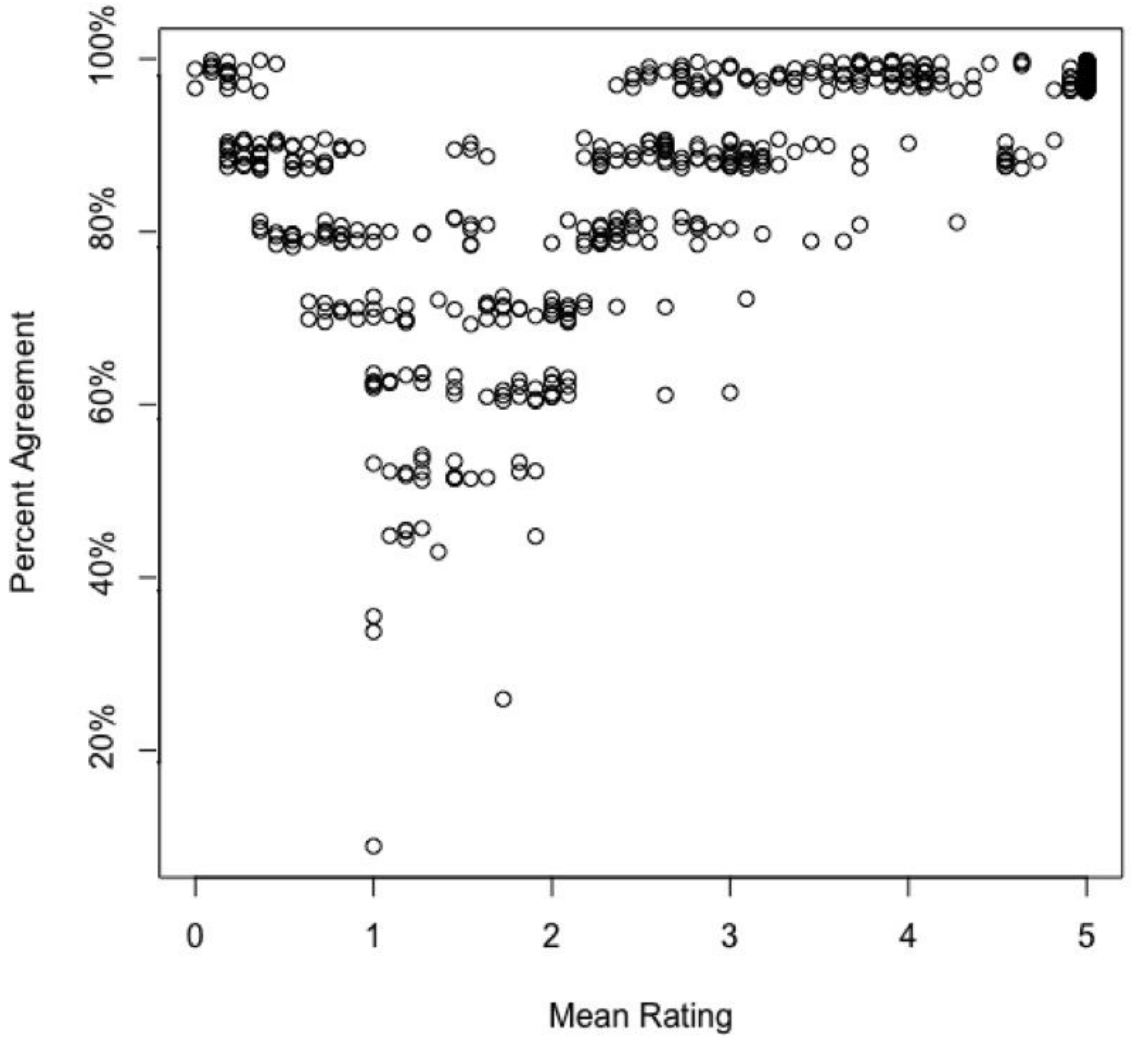

Figure 6. Scatterplot showing mean score plotted against percent agreement. Each circle represents one vine.

CONCLUSIONS

In conclusion, review of the data from the September, 2014 and 2015 ratings indicates that for approximately 97% of the vines, the majority of team members scored the vines within one integer above or below the mean. In 2015, the percentage of vines where all scores agreed increased from 51.0% to 66.5%. Overall, this demonstrates that the rating system was well understood by team members and provides a relatively uniform measure of PD disease symptoms that can be used to describe the vines in this experiment.

The percentage of vines where the majority of scores agreed increased by 1.7% in May, 2016 compared to September, 2015. However, there was a change in the extent to which scores agreed, with a 16% decrease in the percentage of vines where all scores agreed. This suggests that PD symptoms may be more variable in the spring and that rating vines at this time results in a less uniform measure of disease. In addition, the percent agreement of scores for vines with less severe symptoms is lower suggesting that raters have more difficulty scoring these vines.

FUNDING AGENCIES

Funding for this project was provided by the CDFA Pierce's Disease and Glassy-winged Sharpshooter Board.