CENTER for PRODUCE SAFETY

CPS 2011 RFP
FINAL PROJECT REPORT

Project Title
Identifying causative factors contributing to positive leafy green samples

Project Period
August 1, 2011 – December 31, 2013

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Objectives

This proposal consists of six primary objectives:

1.) prepare a data management plan
2.) formalize the agreement with individual company participants
3.) obtain company data and populate the database
4.) analyze the data for correlations between pathogen occurrences and individual practices/practice failures
5.) review the findings with participants
6.) complete a final report.

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CPS Campaign for Research

FINAL REPORT

Abstract

While there have been numerous studies and programs designed to understand the occurrence of microbial pathogens in leafy greens, few of the studies have focused on occurrences when pathogen levels are present but at levels that may not result in illness if the produce is consumed. Without root cause analysis, it is unclear if best practices are contributing to reductions in disease-causing microbial pathogen levels and if so, the significance of the contribution. Our goal was to identify factors that may impact the occurrence of human pathogens in leafy greens. For this project, we worked with several California leafy green companies to collect and evaluate retrospective case-control field level data. The results were compiled in a database that over time can become a reference point for improving best practices. This work was intended as a pilot program. Future work will build on this database and may result in a data-intensive leafy green risk assessment.

Background

When a foodborne illness outbreak occurs, an epidemiological investigation, laboratory testing and an environmental assessment are conducted to determine the cause and source of the outbreak. Case-control survey instruments are used in the epidemiological investigations (CDC, 2014) where the food vehicle has not been identified. For the investigation, individuals exposed to and ill from the foodborne illness answer a series of survey questions about what they ate, where they ate, etc. (case surveys); individuals not having a foodborne illness complete the same survey (control surveys). Results from both surveys are compared to identify patterns and to determine the food vehicle and ultimate source of the outbreak (WHO, 2008). In cases where human pathogens are present in food but not at a level causing human illness, producers or buyers would like to understand the cause(s) of contamination. Even when the contaminated food is known, often the variables leading to the contamination are not. Case-control survey methods can be employed in this type of root cause investigation. Findings from the analysis may lead to a better understanding of preventive control points and eventually to improvements in managing contamination risk factors.

Research Methods and Results

This joint Western Growers and Intertox Decision Sciences (IDS) project identified potential contributing factors to several California occurrences of human pathogens in leafy green products. With Western Growers support, six of the leading California leafy green companies (processors, handlers and growers) referred to as the leafy green sample group (LGSG) agreed to participate in the study. Using case-control methodology, a leafy green survey instrument was developed with input from the LGSG. Participating companies completed case-control field surveys and submitted them for analysis.

The project scope included leafy green crops currently covered by the California Leafy Green Marketing Agreement (CLGMA) primarily grown in the Salinas Valley. The current CLGMA crops are: arugula, baby leaf lettuce, butter lettuce, cabbage, chard, endive, escarole, green leaf, iceberg, kale, red leaf, romaine, spinach and spring mix (CLGMA, 2014). Study group participants were asked to collect surveys for any of the CLGMA crops produced during the summer through fall months of the 2011-13 growing seasons, particularly in the Salinas Valley, once the product was no longer in commerce. Whenever a pre-production test yielded a positive result for *Salmonella* species, Shiga toxin-producing *E. coli* (STEC), enterohemorrhagic *E. coli* (EHEC) or verocytotoxin-producing *E. coli* (VTEC), participants completed a case survey. In addition, participants were asked to complete one or more control survey(s) for either the same field at a later data or a similar field at the same timeframe as the positive test result. Information from both survey types was used to populate a Microsoft® Office Excel database and then analyzed using Stata® statistical software.

There were six research objectives: prepare a data management plan; solicit and formalize agreement with LGSG participants; obtain LGSG data and populate the database; analyze the data for correlations between pathogen occurrences and individual practices/practice failures; review the findings with the LGSG; and complete a final report.

The data management plan describing the intended use of the data, a draft survey instrument and a plan for working with LGSG companies on data collection was completed in the first few months of the project. Western Growers and IDS prepared a working document for the LGSG detailing confidentiality terms and mutual roles and responsibilities. The confidentiality terms included a discussion of IDS standards for collecting data and protecting confidential company details. The roles and responsibility section of the document provided a process and timing for data transmittal. During the pilot program, six companies agreed to complete case-control surveys.

Several conference calls and one in person meeting with the LGSG were held to finalize the survey instrument ensuring it addressed industry practices and study objectives. The face-to-face meeting was held in early 2012 and served as a project kick-off event.

After finalizing the data management plan and formalizing an agreement with LGSG members, participants worked to complete and submit surveys. When a positive crop test occurred, LGSG members completed a questionnaire for that particular field/processing period (case) as well as a questionnaire for comparable fields/processing period with negative crop/product test results (control). The data collected from both survey types was used to populate a Microsoft® Office Excel database. In total 5 case and 20 control studies were collected. One case study out of the five was not included in the following analysis since it was from an Arizona location and not the Salinas Valley.

Obtaining completed surveys took longer than anticipated for two reasons. First, the LGSG companies did not always have direct access to the data, especially control data. Second, there were few positive test results. Investigating the cause of positive leafy green test results required the use of retrospective analysis using small sample sizes. Since case survey data collection was limited to actual occurrences and occurrences were infrequent, the opportunity for collecting case surveys was limited.

Questionnaire data for positive crop test occurrences (case) were compared to data from questionnaires for negative crop tests results (control) in order to identify differences. Contextual details such as food safety practices, geographical, and meteorological data were collected and considered in the analysis. Limitations in collecting the data and data quality were also considered. Data was analyzed using Stata® statistical software. Final survey results were reviewed with LGSG members prior to submittal of this document.

The case-control survey covers pre-planting through product shipment. Questions were organized by production stage - pre-plant, production/pre-harvest and harvest to finished product shipment and were based on the CLGMA audit checklist (CLGMA, 2011). Within each production stage, questions were arranged by topic. Subject areas included environmental influences, water source and use, fertilizers and soil amendments, and weather. In total there were 141 possible questions in the survey. Case studies yielded positive test results for *E coli* O157:H7 (3), EHEC (2), STEC (1) and *Salmonella* spp. (2). In two of the case studies, positives were detected during pre-harvest testing and the product was not harvested. For the third case, the positive test occurred at receipt of product. The fourth positive was detected as part of USDA random sampling on finished products. Because of the small number of case studies (2) where the affected product was harvested prior to a positive detection, this report does not include findings on harvest through finished product shipment. Results by subject area are as follows:

Environmental influences

In the environmental section of the survey, questions were asked about animal activity and flooding for both pre-planting and pre-harvest timeframes. Pre-planting questions asked about the proximity to feedlots and grazing lands and evidence of urban encroachment.

A pre-plant risk assessment was performed on 100% of the fields/farms in all of the case and control studies. Having crops previously grown on the farm testing positive was not an indicator of a positive test result. None of the four case study farms and only one of the control study farms had crops previously grown that tested positive.

Pre-plant flooding was noted in 30% of the control studies but not in any of the case studies. There was no evidence of flooding in any of the case and control studies in the production/pre-harvest time period.

Urban encroachment was not a factor in any of the case or control surveys. None of the case studies or control studies was located near an animal feedlot. Grazing in proximity to production was significant; however, the results were counterintuitive as shown in Table 1 below. Since a large number of control studies (95%) and only half of the case studies were for fields proximate to grazing land, there appears to be an inverse correlation between fields approximate to grazing land and positive test results. The odds ratio is 0.05, implying that a field located adjacent to a grazing field is 95% less likely to test positive then one that is not adjacent to a grazing field. Based on previous research demonstrating correlations between the proximity to grazing lands and positive test results (Berry, 2013, Hoar, 2012, Suslow, 2011), this result warrants further study before definitive conclusions can be reached.

There was evidence of animal activity present in 3 of the four positive results. Still, the odds ratio of 7 is not significant, most likely because 6 of the 20 control cases also reported animal activity. There was also one case study where a pre-harvest risk assessment was performed and identified evidence of animal intrusion. To address the intrusion, buffer zones were employed to isolate and remove the affected crop area from production. There was no evidence of any excessive bird, insect, amphibian or reptile activity in any of the survey results.

Table 1. Environmental Impacts

	Cases (n=4)		Controls (n=20)		Odds ratio
	Yes	No	Yes	No	
Field approximate to grazing land	2	2	19	1	0.05
Evidence of animal activity	3	1	6	14	7

Water source and uses

Questions about pre-planting water use were asked to determine if the field was irrigated prior to planting, whether the irrigation water was tested and if testing met the LGMA irrigation water standards. All of the case study fields were irrigated before planted, were tested and met LGMA standards. Results were similar for control fields except one field was not irrigated prior to planting. All case and control survey water test results also met the LGMA standards throughout production.

Water sources were evaluated in the surveys to determine the impact of canals, reservoirs, and wells on test results. Of the case study farms, 75% use well water and 25% use canal water. Fifty percent of control farms also use on-farm reservoirs. Control survey farms use wells (95%) and canal water (5%). None of the control farms use on-farm reservoirs. Based on the odds ratio, canal users are 2.5 times more likely than farms without canals to have a positive test result. There were no issues or observations of debris among canal users.

Table 2. Irrigation Types

	Cases (n=4)		Controls (n=20)		Odds ratio
	Yes	No	Yes	No	
Furrow	3	1	1	19	57
Drip	2	2	1	19	19
Sprinkler	3	1	19	1	0.15

Irrigation methods used are displayed in Table 2 above. Ninety-five percent of control farms use sprinklers; 5% use furrows and 5% use drip irrigation methods. Case farms use furrows (75%), drip (50%) and sprinkler irrigation (75%). Furrow irrigation users are 57 times more likely to have a positive test result than farms that do not use furrow irrigation. The odds ratios for furrow and drip irrigation are significant; however, the confidence interval ranges are quite large indicating either model instability or a strong correlation between furrow irrigation and to a lesser extent between drip irrigation and positive test results. Further data collection and analysis is needed to ensure the correlations are accurate.

Fertilizers and soil amendments

Pre-planting fertilizer questions covered the types of fertilizers used and the timing of the application. All case and control companies applied fertilizers or soil amendments to the crop after planting. Only one case company applied organic fertilizers and application occurred 45 days or more prior to planting (Table 3). The organic soil amendment, chicken pellets, was tested for pathogens. Neither of the soil amendment odds ratios was significant – most likely as a result of the small number of case studies.

Table 3. Soil Amendments (Post-planting)

	Cases (n=4)		**Controls (n=20)**		**Odds ratio**
	Yes	No	Yes	No	
SA applied to soil or foliage	3	1	19	1	0.16
Chicken pellets applied pre-planting	1	3	0	20	1

Companies were asked if they apply their soil amendments to the soil or directly on the foliage. Based on the wording of this question, it is unclear where respondents are applying soil amendments. Confusion regarding the question could explain the resulting low odds ratio. For future surveys, this question will need to be revised.

Weather

Even with the limited number of case studies and issues relating to accuracy of the statistical analysis given the small sample size, weather does appears to be the most significant of the four subject research areas. The odds of a positive test result for rainfall occurrence at any time during production was 57 times greater than if no rainfall occurred during production (the p-value is significant; however, the confidence interval was quite large indicating either model instability or a strong correlation.). To analyze weather-related variables, data on rainfall, humidity, wind and temperatures corresponding to the production periods of the case-control studies was obtained from the Weather Underground - a service that collects data on current weather conditions from more than 42,000 weather stations across the country (Wunderground, 2014). Weather Underground includes data from:

- Nearly 2,000 Automated Surface Observation System (ASOS) stations located at airports throughout the country. These are maintained by the Federal Aviation Administration and observations are updated hourly or more frequently when adverse weather affecting aviation occurs (low visibility, precipitation, etc.).
- Over 16,000 Personal Weather Stations (PWS's) that are part of Weather Underground's ever-expanding PWS network. Stations are put through strict quality controls and observations are updated as often as every 2.5 seconds.
- Over 26,000 weather stations that are part of the Meteorological Assimilation Data Ingest System (MADIS) which is managed by the National Oceanic and Atmospheric Administration (NOAA)[1].

[1] For further information, see http://www-sdd.fsl.noaa.gov/MADIS.

Using the Weather Underground service, farm locations were entered into the online database and the geographically closest station was displayed.

The weather data obtained for each case study is presented below in Table 4 and in the appendices.

Table 4. Weather conditions

	Case Number			
Wind Gust Speed (MPH)	**1**	**2**	**3**	**4**
4 Days before harvest	23	33	24	0
3 Days before harvest	16	21	19	26
2 Days before harvest	21	24	15	0
1 Days before harvest	102	21	12	14
Day of harvest	16	25	0	0
Minimum Humidity (%)				
4 Days before harvest	53	55	37	55
3 Days before harvest	64	34	37	47
2 Days before harvest	68	56	33	32
1 Days before harvest	34	60	31	29
Day of harvest	16	43	23	36
Maximum Temperature (°F)				
4 Days before harvest	74	63	65	58
3 Days before harvest	74	68	68	60
2 Days before harvest	75	65	69	65
1 Days before harvest	87	65	72	68
Day of harvest	92	65	78	62
Other Weather Variables				
Total Dust Score	668	288	271	175
Maximum Precipitation event (inches per day)	1	0.97	0.009	0.39
Reported Rainfall during production (reported by grower); Y = yes; N = no	Y	Y	N	Y

Each weather variable was analyzed using logistic regression to establish confidence intervals for the odds ratios. Significant factors identified are included in Table 5 below. If the farm reported any rain during production, a positive test result was 57 times more likely than when no rainfall was reported (odds ratio of 57, 95% confidence interval 2.76 to 1117.76). The maximum precipitation (in inches) per day was found to correlate positively with contamination (odds ratio of 16.17, 95% confidence interval of 1.10 to 238.46). However, for both of these variables, the width of the confidence interval can either indicate a strong relationship or model instability because of the small sample size. Humidity, temperature, and wind gust speed were also found to be significant (see Table 5). Rainfall within 3-5 days of harvest and the difference between observed and historical average conditions were not found to be significant.

Table 5. Weather factors

	Odds Ratio	p-value	95% Confidence Interval	
Rainfall occurrence	57.00	0.01	2.76	1176.76*
Maximum precipitation per day (inches)	16.17	0.04	1.10	238.46*
Minimum humidity, 2 days before harvest	0.90	0.04	0.81	1.00
Mean humidity, during production	0.85	0.03	0.74	0.99
Minimum humidity, day of harvest	0.84	0.03	0.72	0.98
Wind gust speed (MPH), day of harvest	0.82	0.04	0.68	0.99
Minimum humidity, during production	0.81	0.05	0.65	1.00
Minimum temperature, during production (°F)	0.64	0.04	0.42	0.97
Average minimum temperature, during production (°F)	0.61	0.01	0.42	0.90

*See note in text about confidence interval size.

For further weather analysis, a dust score was created for each sample by coding the maximum temperature (F), minimum humidity, and wind gust speed (in miles per hour (MPH)) from the day of harvest to five days prior to harvest. Temperature was scored on a scale from 1 to 7 with 1 given to temperatures from 0 - 40°F and seven given to temperatures greater than 96°F. Wind gust speed was given a score from 1-10, with an increase of gust speed resulting in an additional code score (i.e. 0-10 MPH = 1, 11-20 MPH = 2). Humidity was given an inverse coding as a lower humidity would increase the likelihood of dust. Humidity from 0-10% was given a score of 10 versus humidity of 90-100% was given a score of 1. This was completed for each day prior to harvest. In addition, a total dust score was created using the sum of each of the daily scores. The dust score, using the current dataset, does not appear to be a good metric given the limited number of samples.

Additional analysis was performed using mean humidity levels up to ten days prior to harvest. Results from this analysis indicate a significant correlation between mean humidity levels on the day of harvest (Table 6 below).

Table 6. Humidity

Days prior to harvest	Odds Ratio	p-value	95% Confidence Interval	
10	0.92	0.08	0.83	1.01
9	0.91	0.06	0.82	1.00
8	0.92	0.05	0.83	1.03
7	0.96	0.59	0.84	1.11
6	0.94	0.29	0.83	1.05
5	1.00	0.95	0.87	1.15
4	0.99	0.84	0.87	1.12
3	0.91	0.24	0.78	1.06
2	0.79	0.05	0.62	1.00
1 (day of harvest)	0.72	0.03	0.53	0.97

Outcomes and Accomplishments

All six of the project objectives were successfully completed. With strong industry support from leading leafy green companies and Western Growers, we were able to assemble a leafy green study group to review and agree on an industry survey and collect industry data (5 case and 20 control surveys). Although there are few case studies, we were able to analyze the data and identify areas for further research and analysis when additional case studies are available. Having assembled the LGSG and established a process for collecting and analyzing case-control surveys, we recommend continuing this research in 2014.

Summary of Findings and Recommendations

<u>Findings</u>

An analysis of the data reveals potential correlations between positive product tests and several variables: field proximity to grazing land (although the correlation is counter intuitive), irrigation type (furrow irrigation), and weather, particularly rainfall and humidity. Given the small number of positive samples, the data should be considered preliminary. However, the findings are useful and warrant further data collection for confirmation.

Critical to the success of the project is the ability to collect sufficient numbers of case and control surveys. During the 2011- fall 2013 growing season, there were few “positives”, limiting our ability to collect additional surveys responses. Therefore, a program collecting this type of data needs to run for several years to obtain enough survey responses for statistical validity.

As for the pilot program, the importance of the research was not only to determine if the data could be collected and then to collect it and analyze it, but also to determine the program limitations in order to make improvements in any future study. Based on the observations during the pilot program, several improvements can be made going forward. First, a consistent methodology for the selection of case and control sites needs to be adopted by all companies for statistical purposes. Consistent sampling patterns and protocols need to be used by all companies. Finally, the program would benefit from one individual completing all surveys for all companies to ensure consistency.

Recommendations

We recommend CPS continue its support of the case-control program as a means of collecting sufficient data to make a determination of the causative factors contributing to positive leafy green samples.

Acknowledgement

We would like to thank the companies and their employees supporting this project.

References

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APPENDICES

Publications and Presentations (required)

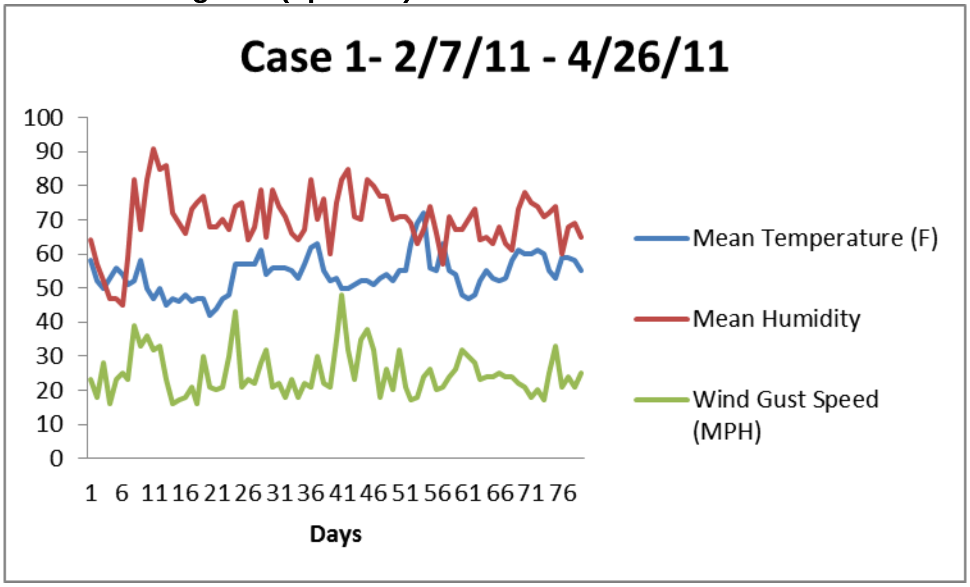
No publications or presentations have been made to date. Project participants received a copy of the study and reviewed the findings prior to submittal of this report.

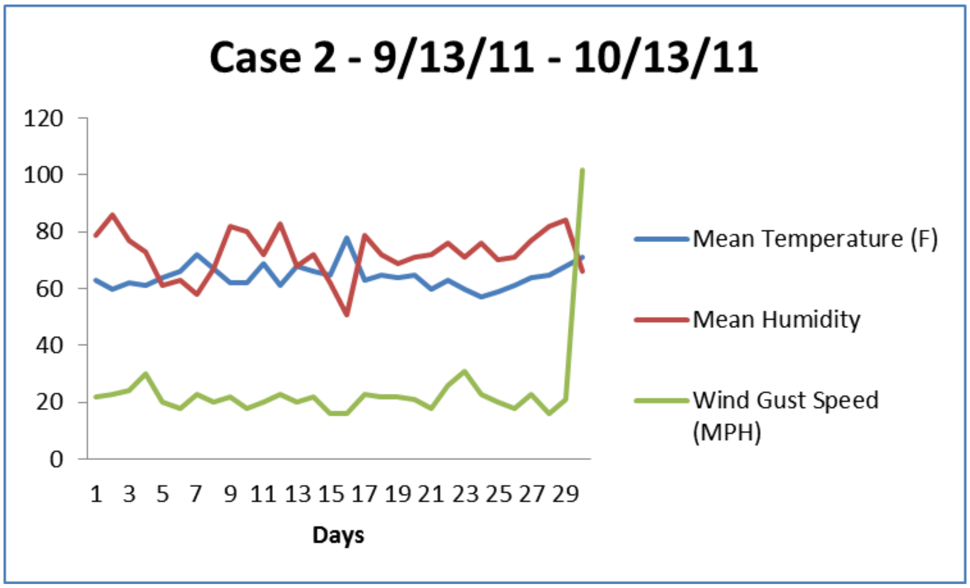
Budget Summary (required)

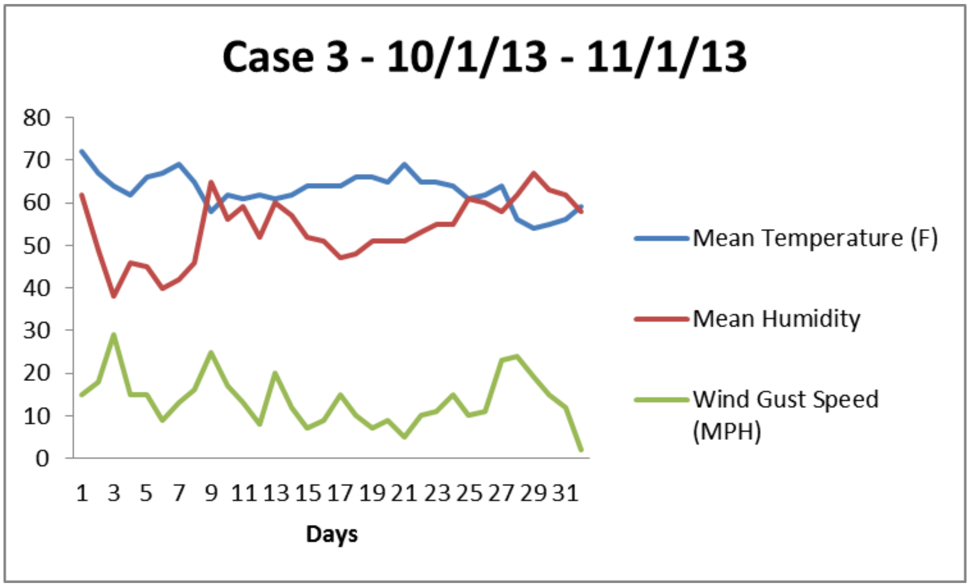
Category	Budgeted	Expended	Remaining
Salaries	$17,830.35	$17,830.35	$0
Benefits	$3,387.77	$3,387.77	$0
Travel	$1,410.00	$1,410.00	$0
Supplies and Materials	$100.00	$100.00	$0
Indirect Costs	$2,271.88	$2,271.88	$0
Total Costs	**$25,000.00**	**$25,000.00**	**$0**

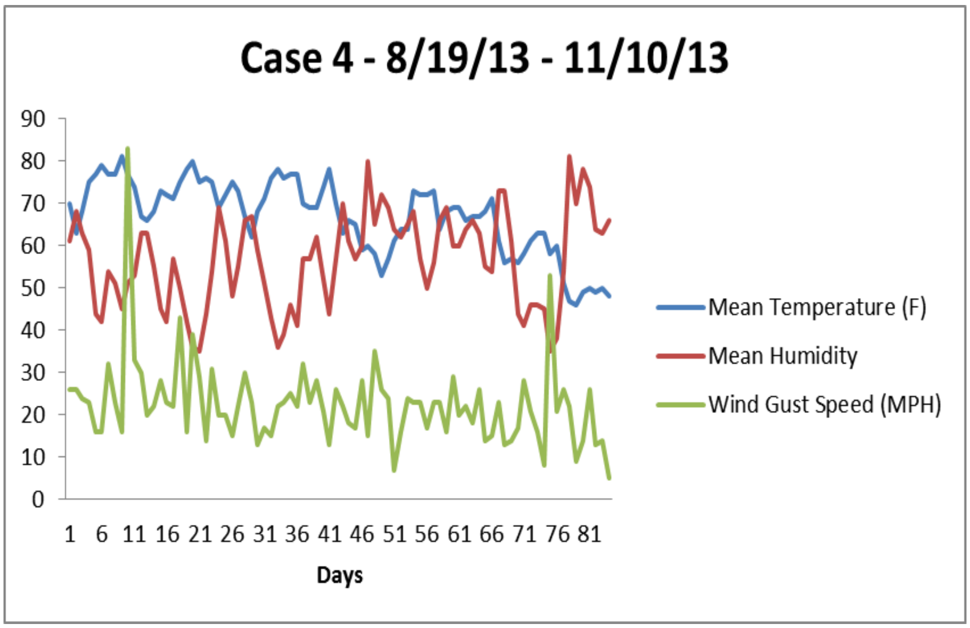
Budgetary money was spent on salaries and benefits for researchers who compiled and analyzed the data for the project (85%), travel (6%), supplies and materials (0.4%), and indirect costs (9%). Travel included five meetings to Salinas to meet with participating companies. Other than travel costs, with the budget allotted and the in-kind contributions, we had adequate funds based on the number of participants and surveys.

Tables and Figures (optional)






Case 3 - 10/1/13 - 11/1/13
80
70
60
50
40
30
20
10
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31
Days
Mean Temperature (F)
Mean Humidity
Wind Gust Speed (MPH)


Case 4 - 8/19/13 - 11/10/13
90
80
70
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50
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30
20
10
0
1 6 11 16 21 26 31 36 41 46 51 56 61 66 71 76 81
Days
Mean Temperature (F)
Mean Humidity
Wind Gust Speed (MPH)