Project Report
Arizona Iceberg Lettuce Research Council

For period
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Breeding high quality and disease resistant iceberg lettuce cultivars for Arizona

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Growers and shippers throughout the region

SUMMARY

Our objectives are to incorporate disease resistance into crisphead lettuce cultivars and breeding lines adapted to low desert production conditions. Major breeding efforts targeted resistance to lettuce big vein, Fusarium root rot, lettuce drop (*Sclerotinia sclerotiorum*), tipburn, and powdery mildew. In 2006, we confirmed resistance of previously identified germplasm to lettuce drop, and we obtained preliminary data on inheritance of resistance to Fusarium wilt. Advanced breeding lines were evaluated and selections were made for resistance to tipburn, big vein, and powdery mildew. Seed was increased for advanced breeding lines with resistance to bolting.

INTRODUCTION

Production of iceberg lettuce from December through April is centered in the low desert region of Arizona and California, and is critical to maintaining a year round supply of high quality lettuce. Lettuce production is continually faced with preexisting and new challenges; therefore, development of improved cultivars with superior adaptation and pest resistance is important for sustaining this industry. Our research emphasizes the development of landmark cultivars adapted to early, middle, or late season conditions with resistance to pests, physiological disorders and environmental stresses.

The USDA lettuce breeding project has an ongoing desert program, both in Arizona and in the Imperial Valley. Currently, the USDA is the only publicly funded lettuce breeding program operating in the low desert. In recent years, most of the desert production has been in the Yuma area. Therefore nearly all of our on-site work has been done in Yuma and has focused on the production concerns of this region. The objectives are to develop iceberg lettuce breeding lines possessing 1) bolting resistance for early fall production, 2) big vein and powdery mildew resistance for mid-winter production, 3) tipburn resistance for late spring production, 4) lettuce drop resistance for fall, winter, and spring planting, and 5) Fusarium wilt resistance for fall, winter, and spring planting.

REPORTS ON SPECIFIC PROJECTS

Big Vein Resistance

Big vein is a serious viral disease in the low desert region, and the development of resistant cultivars is needed to reduce economic damage resulting from big vein. Partially resistant cultivars adapted for coastal California production environments have been released which have a reduced frequency of symptomatic plants and/or symptom expression that is delayed until plants reach market maturity. Partially resistant cultivars have not been developed for Yuma production conditions. Furthermore, progress in increasing the level of partial resistance through breeding has been slow.

Big vein is caused by Mirafiori Lettuce Big Vein Virus (MLBVV) which is vectored by the soil fungus *Olpidium brassicae*. Complete resistance to MLBVV has only been identified in accessions of *Lactuca virosa* L., a distant relative of lettuce. No cultivars with big vein resistance from *L. virosa* have been developed. *Lactuca virosa – L. sativa* hybrid families developed by B. Maisonneuve, INRA, Montfavet, France were provided to the USDA for big vein resistance breeding. Through greenhouse based selection for big vein resistance, hybrid breeding lines were identified with a reduced percentage of symptomatic plants. This is a type of resistance similar to what is observed in cultivated lettuce. The genes responsible for resistance in these *L. virosa – L. sativa* hybrids are most likely distinct from those in currently available resistant cultivars (Hayes and Ryder, 2007, HortScience 42:35-39). Introgression of resistance from *L. virosa* into cultivated lettuce may lead to higher levels of resistance than what is currently available.

The objectives of big vein research for Arizona are to:

1) Introgress partial resistance from cultivated lettuce into low desert adapted cultivars.
2) Determine the level of resistance in commercially available low desert adapted iceberg cultivars.
3) Identify new sources of resistance.
4) Introgress resistance from *L. virosa* into cultivated lettuce.

Field experiments; Objective 1 and 2

Procedures

A mid-winter field trial with 3 replications was conducted in Yuma, AZ to identify breeding lines and cultivars with big vein resistance. The experiment was planted on October 15; the incidence of big vein symptomatic plants was recorded on January 15 for 10 $F_{2:3}$ families with resistance from Pavane, 13 $F_{3:4}$ families with resistance from Margarita, four $F_{4:5}$ and $F_{5:6}$ breeding lines from Winterhaven x Pacific, and 18 cultivars. Additionally, 11 F_2 families generated from backcrossing Winterhaven x Pacific breeding lines to adapted cultivars were planted for selection of head type and resistance.

Results and Discussion

Variation was observed among cultivars for the percentage of big vein symptomatic plants, with the mean percent symptomatic plants ranging from 0% (Pacific and Pavane) – 41% (Vanguard 75). Two Winterhaven x Pacific breeding lines had less than 10% disease, and were selected for further breeding. Nine families ($F_{2:3}$ and $F_{3:4}$) with resistance from Margarita and Pavane had less disease than Winterselect (11% symptomatic plants). These lines will be continued for further breeding. Over 1800 F_2 plants were evaluated for head type and symptoms expression. Eighty-four non – symptomatic plants were selected with horticulturally acceptable head characteristics.

Two years of testing have been conducted on mid-winter adapted iceberg breeding lines. The goal of this research was to document the level of resistance in this material in order to make grower recommendations. Variation in the percentage of big vein symptomatic plants was observed in 2005 and 2006, although the differences were not significant in 2006 due to lower disease pressure (Figure 1). The resistant controls Pavane and Pacific were the first and third most resistant cultivars in 2005 respectively, and both had 0% symptomatic plants in 2006. Among desert adapted cultivars, Winterselect had the lowest percentage of symptomatic plants, followed by Coyote, Honcho, and Winterhaven. The remaining cultivars appear to be highly susceptible.

Greenhouse experiments, Objectives 3 and 4

Procedures, Results and Discussion

Hybrid materials between *Lactuca virosa* accession IVT280 and several European cultivars provided by B. Maisonneuve, INRA, Montfavet, France have demonstrated a high level of partial resistance to big vein disease. Two related lines, 03-366-3M and 03-366-9M were selected for breeding, and we subsequently demonstrated the high likelihood that these breeding lines contain novel genes from *L. virosa* for big vein resistance (Hayes and Ryder, 2007, HortScience 42:35-39). We are using these parents to develop big vein resistant breeding lines that are genetically independent of Pacific. In 2006, we tested 492 $F_{4:5}$ breeding lines from 03-366-3 x Salinas, 03-366-3 x Salinas 88, 03-366-9 x Clemente, 03-366-9 x Salinas, and 03-366-9 x Salinas 88 in two replicated greenhouse experiments. Sixty-eight percent and 41% of families were as or more resistant than Pacific in experiments 1 and 2 respectively. Seventy-seven families combined high levels of resistance with romaine or crisphead characteristics, and 408 symptomless plants were selected from these families for further breeding.

We will continue to screen PI accessions for big vein resistance. Over the life of this project, we have reported at least 65 iceberg, leaf, romaine, and butterhead accessions with partial resistance to big vein. Based on a series of greenhouse and field experiments in 2004 and 2005, 15 accessions were selected as putative sources of partial resistance. We will continue to characterize this material for its value for big vein resistance breeding. Starting in 2006, we began to focus this research on complete resistance to big vein. For this reason, over 80 accessions were dropped from the program based on data from greenhouse experiments.

Powdery Mildew Resistance

Powdery mildew caused by the fungus *Erysiphe chicoacearum* can damage lettuce during mid-winter production conditions. Resistance can be an effective control of powdery mildew, although no resistant iceberg lettuce cultivars are known. While several sources of resistance have been reported, a few butterhead cultivars consistently have the highest level of resistance in low desert field experiments.

Our research objectives are to:

1) Introgress resistance from butterhead cultivars Big Boston and Soraya into new iceberg cultivars and breeding lines.

Procedures

A field experiment was planted at the University of Arizona, Yuma Valley Agricultural Research Station in Yuma, AZ on November 7. The field was allowed to become naturally infested with *Erysiphe chicoacearum*, and then evaluated for resistance and horticultural characteristics on March 8. The germplasm evaluated including 40 $F_{4:5}$ breeding lines from Salinas x Soraya and 9 F_2 families derived from crosses between 4 resistant $F_{4:5}$ Winterhaven x Big Boston lines to low desert adapted iceberg cultivars.

Results and Discussion

Sufficient levels of disease occurred prior to market maturity to allow selection of horticultural characteristics and resistance. Thirteen plants from 5 $F_{4:5}$ Salinas x Soraya breeding lines were selected for resistance and head type. These selections will be backcrossed to low desert adapted iceberg cultivars for further breeding. Over 1000 F_2 plants with resistance from Big Boston were evaluated and 18 progeny were selected with improved head type and resistance. These lines will be evaluated in 2007/08 field experiments for further selection.

Bolting Resistance

Procedures, Results, and Discussion

High temperatures during fall plantings in the Yuma production region can result in premature bolting. Development of cultivars adapted to high temperature conditions are necessary to expand the production season in Yuma. Our breeding objective is to identify and select iceberg breeding lines with resistance to bolting and appropriate head characteristics for fall planting in Yuma, AZ. Previous research developed 9 F_6 87-714-8 x Autumn Gold breeding lines, and we reported in 2006 on their yield and horticultural performance in two commercial trials. In 2007, seed production was conducted with this material to generate sufficient seeds quantities for further field experiments.

There has been increased interest in bolting resistance in romaine cultivars for fall plantings. An unreplicated experiment with 24 romaine cultivars was planted in Yuma, AZ on September 11. Valmaine, Medallion, Sunbelt, Siskyou, PIC714, and Tall Guzmaine had fewer bolted plants and or shorter cores than 16 other cultivars. These lines will be retested next year to confirm these results.

Tipburn Resistance

Lettuce planted for harvest in March and April is exposed to high temperatures and increasing day length near market maturity, making the crop vulnerable to tipburn. Tipburn resistance is needed in cultivars adapted to late spring production to insure reliable quality for spring harvested lettuce. Additionally, romaine and leaf cultivars can be extremely susceptible to tipburn, and less breeding has occurred to improve leaf and romaine types for tipburn resistance. No report exists on the tipburn resistance of leaf and romaine cultivars.

The objectives of this research are to:

1) Develop iceberg cultivars with a high level of tipburn resistance.
2) Determine the variation for tipburn resistance in romaine and leaf cultivars.
3) Identify romaine and leaf cultivars with tipburn resistance for use as parents in breeding.

Objective 1:
Procedure
We are developing iceberg breeding lines from the cross Salinas x Vanguard 75 with resistance to tipburn for late spring harvests in the low desert. The procedure for this project has been to evaluate tipburn resistance in a December planted trial at the Yuma Valley Agricultural Center. Late season plantings in Yuma work well for tipburn evaluations. However, they are not conducive for selection of head type due to high heat and advanced stages of plant deterioration. Consequently, a second experiment is planted each year in the production regions east of Yuma (Wellton, Tacna, or Texas Hill) to select for yield, head type, and horticultural quality. In previous years, this approach had identified breeding lines with low tipburn incidence, high yield, and good horticultural performance

For 2006, the first experiment was planted on December 20 at the Yuma Valley Agricultural Center to evaluated tipburn resistance. This trial contained 47 F_5 breeding lines and the check cultivars Gabilan, Calicel, Diamond, Head Master, Salinas, Silverado, Tiber, and Vanguard 75.

Five heads per plot were evaluated for tipburn, and data was analyzed as percent tipburn. The second field experiment was planted on January 17 near Wellton, AZ to evaluate horticultural characteristics and tipburn resistance. Fifty foot plots were established for 42 F_5 breeding lines and the check cultivars Gabilan, Navajo, Diamond, Head Master, Dominquez 67, and Green Lightning. On April 18, five heads per plot were selected for collection of data on head weight (g) and core height (cm).

Results and Discussion

Variation for tipburn resistance was observed at the Yuma Valley Agricultural Center experiment. Percent tipburn ranged from 0 – 100%; ten breeding lines and the cultivars Gabilan and Silverado had 0% tipburn. The susceptible check Calicel and one breeding line had 100% tipburn. Resistant breeding lines were retained for further breeding. Tipburn was also observed at the Wellton experiment, which ranged from 0 – 100 %. Gabilan, Head Master, Navajo, plus 13 breeding lines all had 0% tipburn. However, the correlation between the Wellton experiment and the YVAC experiment was low ($r = 0.13$, $p = 0.39$), and only three breeding lines had 0% tipburn at both locations. Head weight ranged between 1038 grams to 1622 grams, and all of commercially cultivars had competitive head weights: Diamond (1622 g), Head Master (1582 g), Gabilan (1528 g), Navajo (1497 g), Dominques67 (1489 g), and Green Lightning (1426 g). Head weights of eight breeding lines fell within this range, but none of these breeding lines had adequate levels of tipburn resistant. Core height ranged from 3.1 to 7.7 cm. Among check cultivars, Diamond had the tallest cores with a mean of 6.3 cm. Positive correlations were observed between percent tipburn and head weight ($r = 0.36$, $p = 0.01$) and core height ($r = 0.42$, $p = 0.002$), indicating that tipburn resistant lines generally had lighter heads with shorter cores. To the extent that small cores and light head weight are indicative of late maturity, it's possible that strong selection pressure for tipburn resistance has indirectly selected for slower development. We have not previously observed this trend in these breeding lines. These experiments will be repeated to see if these results are reproducible.

Objective 2 and 3:

Procedure

A late spring tipburn trial with 3 replications was planting on December 20 at the Yuma Valley Agricultural Center to identify leaf and romaine cultivars with high tipburn resistance for use as parents in breeding. Five plants per plot were evaluated for tipburn symptoms on April 5, and data was analyzed as percent tipburn. The same experiment was conducted in 2004, and the data from both years was combined into a single analysis for this report. The proportion of plants per plot with tipburn was transformed to arcsine values for analysis in Proc Mixed of SAS to determine if the variation between cultivars was significant. Least square means and 95% confidence intervals were calculated, and then back transformed to the original scale and reported as tipburn rating. Crisphead, green leaf, red leaf, and romaine were pooled into a single analysis, but reported separately.

Results and Discussion

Genetic variation for tipburn resistance was observed between lettuce cultivars in 2 years of testing in Yuma, AZ. The rank correlation between the two years was statistically significant, and of moderate magnitude ($r = 0.46$, $p < 0.001$). Gabilan had the highest level of tipburn resistance among crisphead cultivars, although several modern cultivars were competitive (Figure 2). Cochise 47, Block Buster, Desert Spring, and a handful of heirloom cultivars were highly susceptible to tipburn (Figure 2). Among leaf cultivars, Xena and Red Fox appear to be the most resistant (Figure 3). Among romaine cultivars, King Henry had consistently low levels of tipburn. Medallion, Siskyou, and Clemente also performed well, while many other modern cultivars had high levels of tipburn (Figure 4). Crosses among resistant cultivars will be conducted to generate breeding populations.

Lettuce Drop (*Sclerotinia sclerotiorum*) resistance (Collaborative with B. Pryor)

Lettuce drop is a destructive disease of lettuce caused by *Sclerotinia sclerotiorum* and *Sclerotinia minor* that occurs worldwide. Breeding for *S. minor* resistance in the Salinas Valley of California has been ongoing, and has identified sources of resistance in cultivated lettuce and developed breeding lines. *S. minor*, which predominates in the Salinas Valley, differs from *S. sclerotiorum* in that it infects lettuce only through eruptive germination. *S. sclerotiorum* predominates in the Yuma Valley, and can infect lettuce through eruptive germination of sclerotia or through airborne ascospores that infect lettuce heads. Breeding for resistance to *S. sclerotiorum* in Yuma has not previously been conducted.

The objectives of this research are to:

1) Determine if genotypes resistant to *S. minor* in the Salinas Valley are additionally resistant to *S. sclerotiorum* in Yuma, AZ.
2) Identify sources of resistance to *S. sclerotiorum.*

Procedure

Field trials were conducted in 2004, 2005, and 2006 at the University of Arizona, Yuma Valley Agricultural Center in collaboration with Dr. Barry Pryor (U of AZ). The results from the 2004 and 2005 experiments were previously reported to the AILRC. Three replications of 22 lines were planted in mid-November in each year. The field was infested by incorporating Sclerotia of *S. sclerotiorum* into the beds prior to planting. Non-inoculated plots of the *S. minor* susceptible check Batavia Reine des Glaces was included as a negative control. Disease incidence was collected on a weekly basis over 6 (2004) or 7 weeks (2005), and the sum mortality over this period was used to calculate the disease incidence as the proportion of plants that died. Some disease was observed in non-inoculated plots of Batavia Reine des Glaces. However, the amount of disease was always significantly less than the inoculated plots, and indicates that the field infestation method was successful at increasing the amount of lettuce drop disease. The data from the non-inoculated plots was removed from the data set prior to final analysis. Disease incidence was transformed to the arcsine scale and analyzed with Proc Mixed in SAS. LSmeans for each line was calculated with 95% confidence intervals, and back transformed to the original scale to be reported as disease rating.

Results and Discussion

Genetic variation for resistance was observed, with the percent mortality totaled across each year ranging from 10% to 55%. The line x year interaction was significant. This interaction was due to differences in disease pressure between years and rank changes between lines. Regardless, several sources of resistance that we are using for breeding performed well in all three years (Table 1). These include PI251246, Eruption, Little Gem and Pavane. Great Lakes 54 was an exception. This cultivar has been widely used in our breeding program for resistance to *S. minor* in the Salinas Valley, but performed poorly in these experiments. The romaine cultivar Medallion had not been previously reported as resistant, but performed well in these experiments.

We previously reported (2005 AILRC report) on the effect of using breeding lines bred for *S. minor* resistance in the Salinas Valley for developing *S. sclerotiorum* resistant breeding lines adapted to Yuma. The results indicated that germplasm with previous selection for *S. minor* resistance are better populations for selection of *S. sclerotiorum* resistance than a randomly selected group of iceberg cultivars. Furthermore, this advantage appeared to result from previous selection for resistance to *S. minor*, and from the use of parents with moderate levels of resistance to *S. minor*.

Fusarium wilt resistance (a.k.a. Fusarium root rot)

Procedure, Results, and Discussion:

Fusarium wilt is a potential threat in California and is spreading in Arizona. Three physiological races of Fusarium are known in Japan. Our greenhouse and field data indicate that race 1 is present in California and Arizona. Limited greenhouse data from F_1 individuals and three F_2 families indicate that resistance to race 1 in ‘Costa Rica No.4’ and ‘Salinas’ is controlled by recessive genes.

The level of resistance in ‘Salinas’ and Coast Rica No. 4’ is not sufficient for the early plantings. The field tests have been highly variable; the data from the 2005 field inheritance study did not provide any useful information. Greenhouse test have proven better than the field tests but could be improved. To that end, several new greenhouse inoculation procedures were evaluated. A new and potentially higher source of resistance was obtained for comparison with ‘Salinas’ and several romaine cultivars.

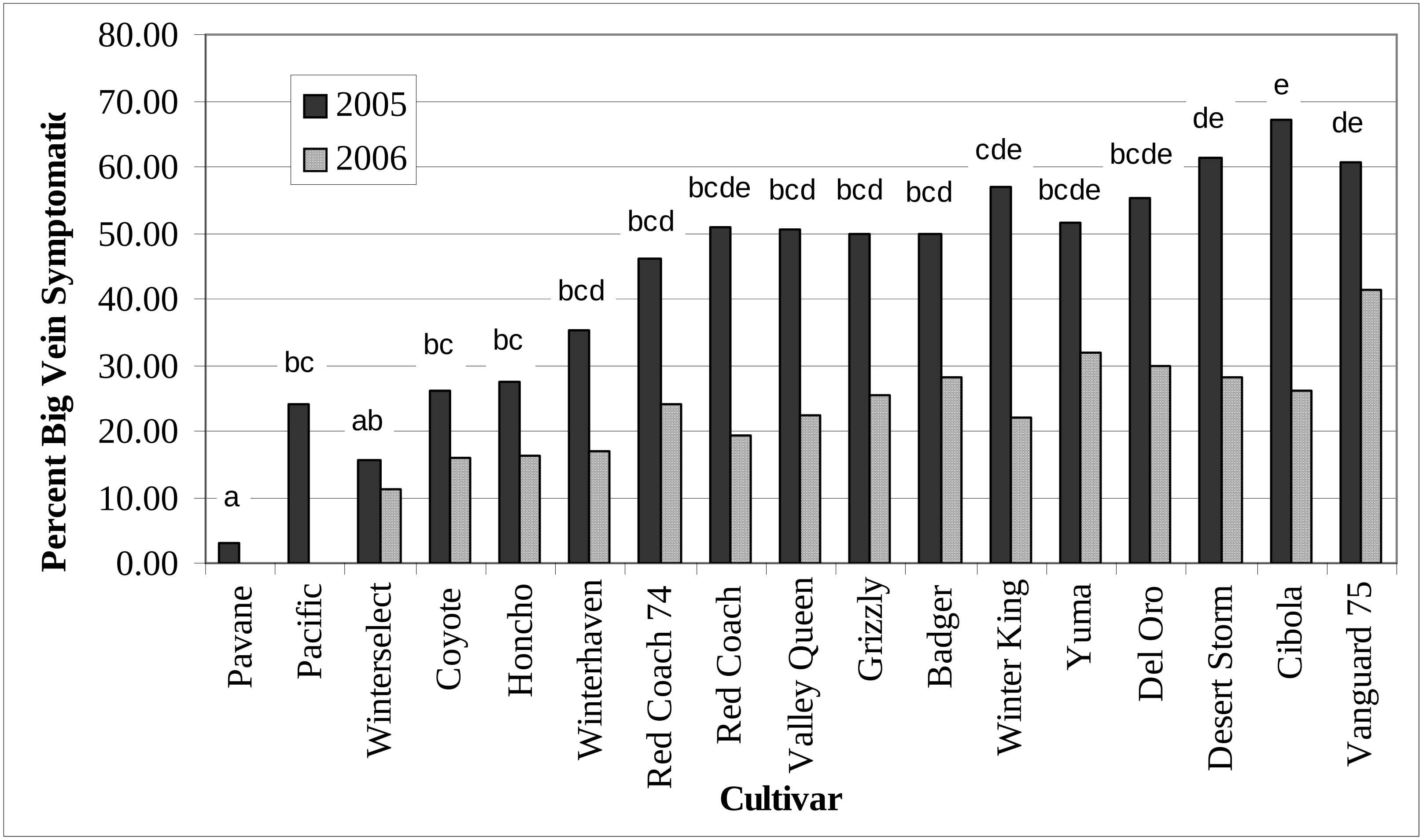

Figure 1. Variation for percent big vein symptomatic plants in Pavane, Pacific, Great Lakes 65 and 15 low desert adapted iceberg cultivars evaluated in 2005 and 2006 in Yuma, AZ. Bars followed by different letter are significantly different ($p < 0.05$); the results from 2006 were not significantly different.

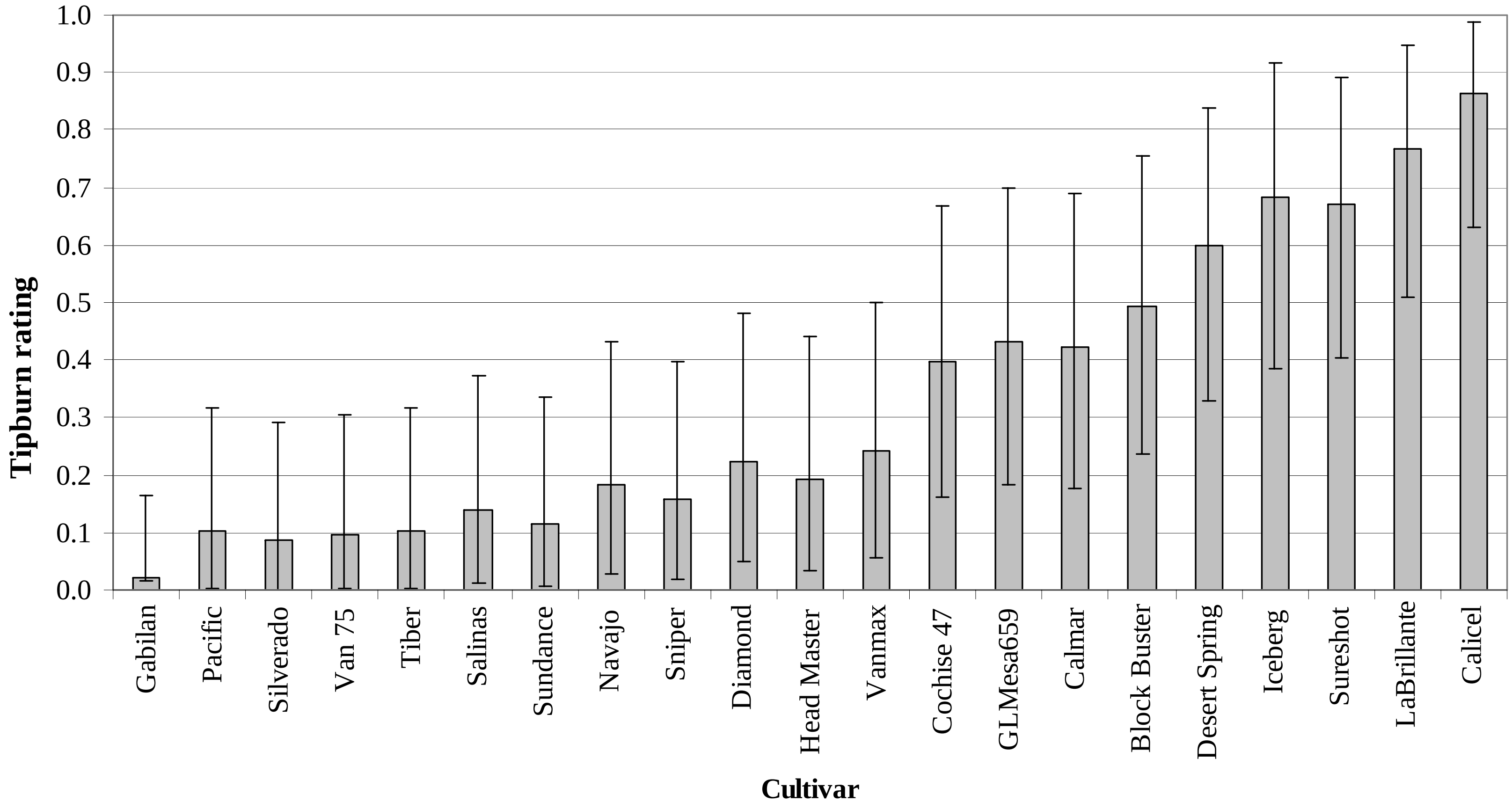

Figure 2. Variation for tipburn resistance in 21 crisphead lettuce cultivars evaluated in 2004 and 2006 in Yuma, AZ. Tipburn rating is the back transformed value from the arcsine analysis of the proportion of plants per plot with tipburn. Lower tipburn rating indicates a lower proportion of tipburn affected plants. Error bars are 95% confidence intervals.

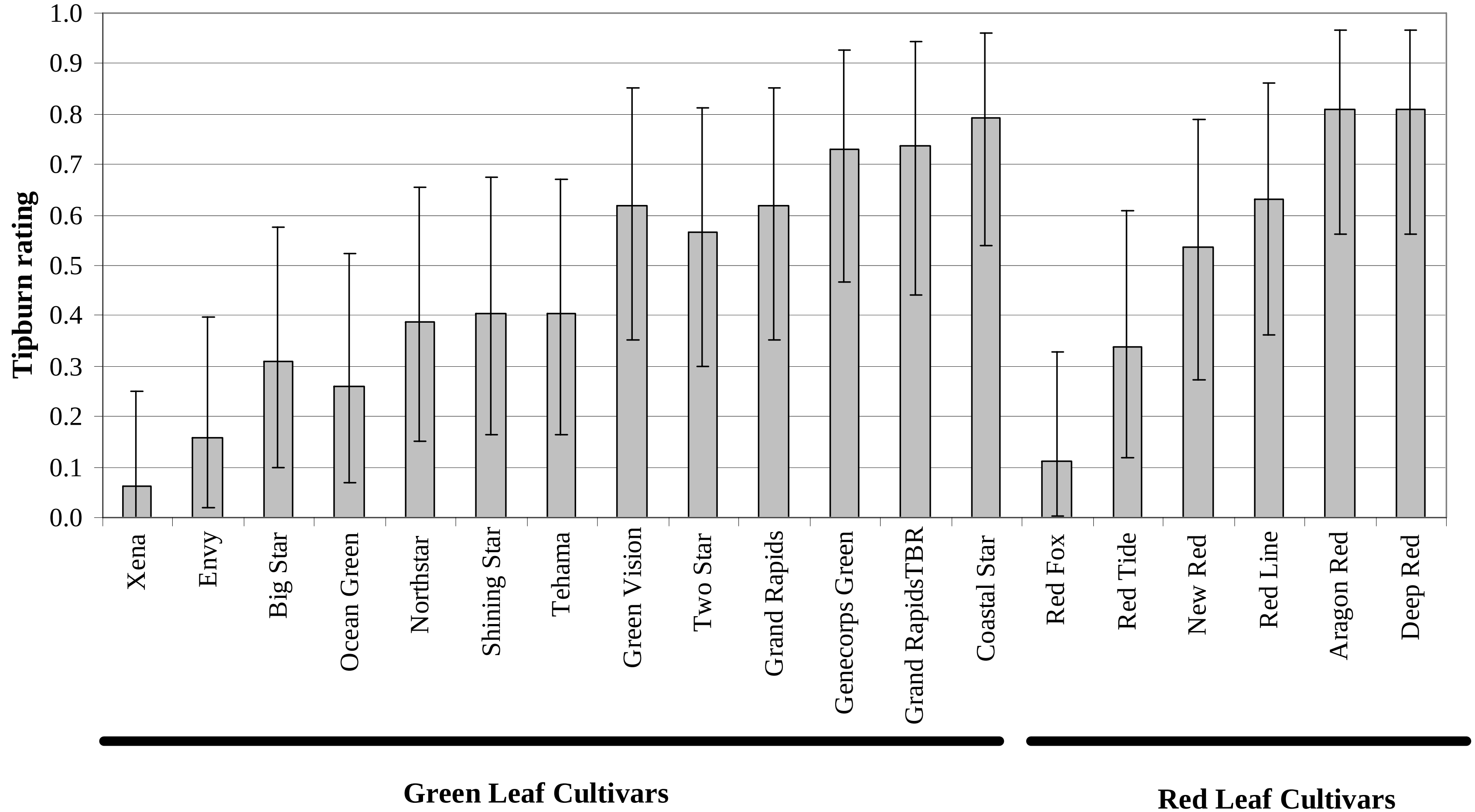

Figure 4. Variation for tipburn resistance in 13 green leaf and 6 red leaf lettuce cultivars evaluated in 2004 and 2006 in Yuma, AZ Tipburn rating is the back transformed value from the arcsine analysis of the proportion of plants per plot with tipburn. Lower tipburn rating indicates a lower proportion of tipburn affected plants. Error bars are 95% confidence intervals.

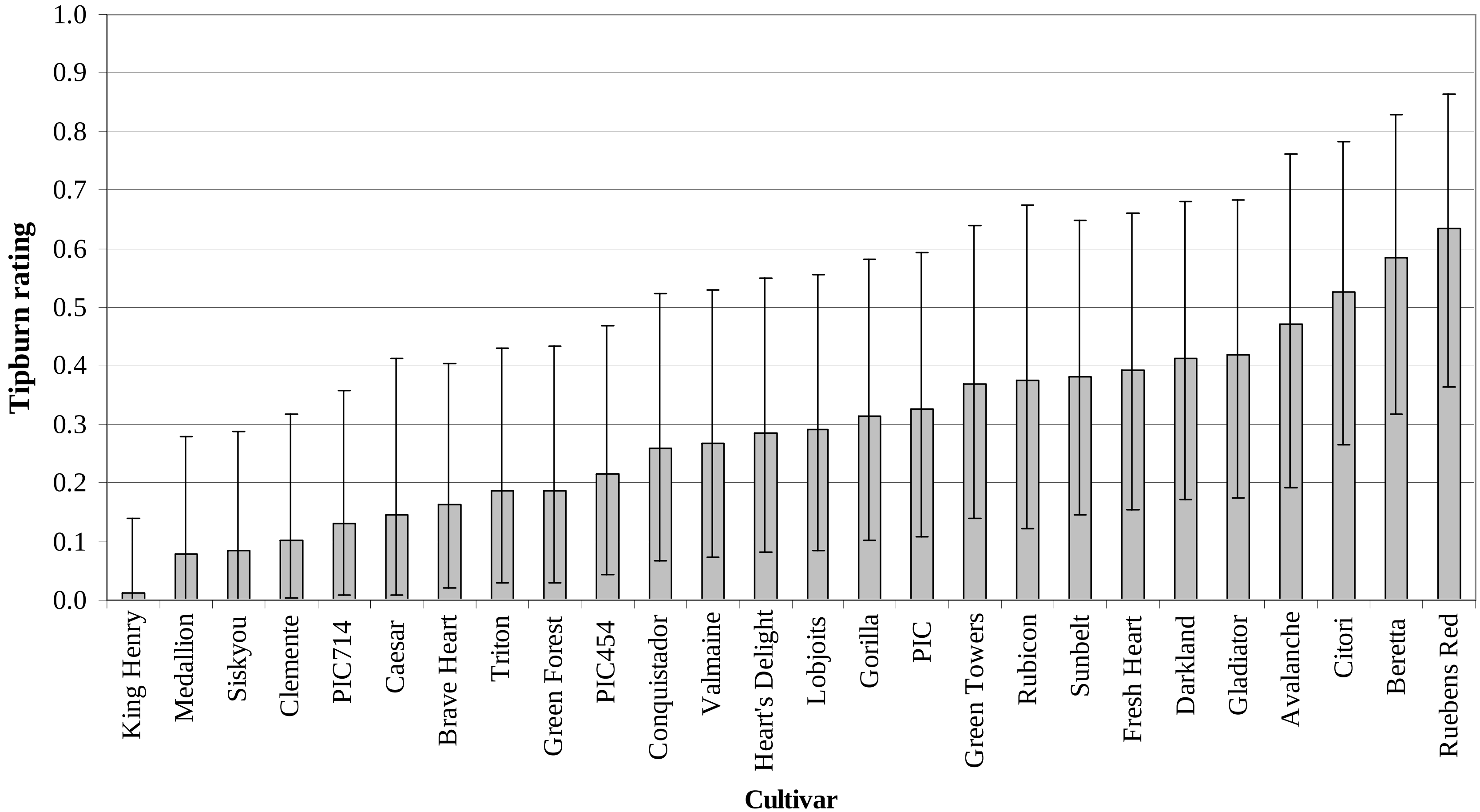

Figure 5. Variation for tipburn resistance in 26 romaine lettuce cultivars evaluated in 2004 and 2006 in Yuma, AZ. Tipburn rating is the back transformed value from the arcsine analysis of the proportion of plants per plot with tipburn. Lower tipburn rating indicates a lower proportion of tipburn affected plants. Error bars are 95% confidence intervals.

Table 1. Genetic variation for percent mortality in 22 *Lactuca sativa* accession evaluated in a *Sclerotinia sclerotiorum* infested field in Yuma, AZ in 2004, 2005, and 2006.

		Disease Rating[1]		
	Percent		95% confidence Interval	
Line	Mortality	Mean	Lower	Upper
PI251246	10	0.09	0.03	0.16
Little Gem	18	0.17	0.09	0.26
Eruption	22	0.21	0.13	0.31
Madellion	26	0.24	0.15	0.34
Pavane	27	0.25	0.16	0.36
Salinas 88	25	0.26	0.14	0.39
Winterhaven	28	0.28	0.18	0.39
Vangaurd 75	30	0.28	0.18	0.39
Holborn Standard	28	0.29	0.21	0.37
Iceberg	33	0.31	0.18	0.45
Australian	32	0.33	0.23	0.44
Tehama	35	0.33	0.23	0.44
Red Tide	31	0.35	0.24	0.47
Grizzly	40	0.39	0.28	0.50
Syn352	42	0.40	0.29	0.51
Coyote	40	0.40	0.29	0.51
Desert Storm	40	0.41	0.30	0.52
Red Fox	43	0.41	0.30	0.52
Two Star	46	0.42	0.31	0.54
Imperial 850	45	0.47	0.35	0.58
Great Lakes 54	40	0.47	0.38	0.57
Batavia Reine de Glacies	55	0.57	0.49	0.65

1/ Back transformed values from analysis of arcsine transformed proportion of dead plants.