HETEROSIS IN HYBRID ALFALFA

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Yield History

- Alfalfa forage yield improvement over the last 20 years has been stagnant.
- Similarly, open pollinated corn varieties experienced very little yield improvement in the early 1900’s.
Hybrid Technology

- From 1860 to 1930, corn yield improved only 2 bushels/acre in a period of 70 years.
- When hybrid corn technology entered the marketplace in 1930, yield improvement increased to 1.0 bushel/acre/year.
- Today, corn yield improvement continues to gain 1.8 bushels/acre/year using hybrid technology.
Average U.S. Corn Yields & Kinds of Corn, Civil War to 2000; periods dominated by open pollinated, by four-parent crosses, and by two-parent crosses are shown, "b" values (regressions) indicate average gain per year; data compiled by USDA.

Heterosis

- Hybrid alfalfa technology has the potential for yield improvement similar to hybrid corn.
- The success of hybrid technology in alfalfa, however, will depend on the ability to capture heterosis.
Purpose of the Study:

- Determine if heterosis could be found in elite, hardy alfalfa germplasm adapted to the Midwest.
The effect of plant spacing on heterosis:

- An observation from previous work is that yield differences are more dramatic when plants are spaced further apart:
  
  hills > rows > multiple-row plots

- As a result, the amount of heterosis observed in hills and rows will generally be greater than the heterosis observed in multiple-row plots.

- Heterosis values from multiple-row plots should better reflect what can happen in a farmer’s field.
The effect of replication on heterosis:

- The purpose of this study was to determine the average level of heterosis.
- The number of reps, cuts, years, and locations of forage yield testing should not effect the average level of heterosis observed.
- More replication, cuts, years, and locations will, however, improve the accuracy of the individual heterosis values and, as a result, could effect the minimum and maximum heterosis observed.
Materials and Methods:

- 6 separate experiments were conducted
- 4 female (male sterile) lines were used to produce alfalfa hybrids.
- 167 male (male fertile) lines were used as pollinizers to produce alfalfa hybrids.
- A total of 326 alfalfa hybrids were produced and tested for forage yield.
Seed Production

- Hybrid seed was produced in 4-row plots (40inch spacing), 25 feet long, in Sloughhouse, CA in 1999, 2000, and 2001.
- Hybrid seed was produced using male sterile, hybrid alfalfa technology.
Forage Yield Testing

- Forage yield plots were conducted using NAAIC standards.
- Forage yield plots were established in Clinton, WI in 2000, 2001, and 2002.
- Plots were planted in 6-row plots, 5 feet wide by 20 feet long.
- Plots were harvested 3 times in the year of establishment, and 5 times/year thereafter.
- Plot designs were either a 6x6 or 8x8, 2 or 3 rep lattice.
## Details of the 6 Experiments

<table>
<thead>
<tr>
<th>EXPERIMENT</th>
<th>PLANTING YEAR</th>
<th>LATTICE</th>
<th>REPS</th>
<th>HYBRIDS</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>2000</td>
<td>8X8</td>
<td>2</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
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<td>8X8</td>
<td>2</td>
<td>40</td>
</tr>
<tr>
<td>3</td>
<td>2001</td>
<td>6X6</td>
<td>2</td>
<td>148</td>
</tr>
<tr>
<td>4</td>
<td>2001</td>
<td>6X6</td>
<td>2</td>
<td>29</td>
</tr>
<tr>
<td>5</td>
<td>2001</td>
<td>8X8</td>
<td>3</td>
<td>39</td>
</tr>
<tr>
<td>6</td>
<td>2002</td>
<td>8X8</td>
<td>3</td>
<td>20</td>
</tr>
</tbody>
</table>
Heterosis Calculation

- **Mid-parent Heterosis (%) =**
  \[
  \frac{(\text{Hybrid} - \frac{\text{Female} + \text{Male}}{2})}{\frac{\text{Female} + \text{Male}}{2}} \times 100
  \]

- **High-parent Heterosis (%) =**
  \[
  \frac{(\text{Hybrid} - \frac{\text{High Parent}}{2})}{\frac{\text{High Parent}}{2}} \times 100
  \]

  - Hybrid = total forage yield of the hybrid expressed as a % of mean.
  - Male = total forage yield of the male expressed as a % of mean.
  - Female = total forage yield of the female expressed as a % of mean.
  - High Parent = total forage yield of the female or male, the higher value, expressed as a % of mean.
Calculation of Significance

- Heterosis values were calculated for each hybrid.
- An average heterosis value was determined for each of the six experiments.
- The heterosis values in each experiment were compared against a normal distribution using the Z-test\(^1\) to test whether the heterosis in that experiment differed from 0%.

# Heterosis by Experiment

## Mid-parent Heterosis

<table>
<thead>
<tr>
<th>EXPERIMENT</th>
<th>Average</th>
<th>p-VALUE</th>
<th>Maximum</th>
<th>Minimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.2%</td>
<td>&lt; 0.001</td>
<td>11.4%</td>
<td>-5.9%</td>
</tr>
<tr>
<td>2</td>
<td>2.5%</td>
<td>&lt; 0.001</td>
<td>7.2%</td>
<td>-4.4%</td>
</tr>
<tr>
<td>3</td>
<td>3.7%</td>
<td>&lt; 0.001</td>
<td>11.9%</td>
<td>-3.4%</td>
</tr>
<tr>
<td>4</td>
<td>4.4%</td>
<td>&lt; 0.001</td>
<td>13.1%</td>
<td>-3.2%</td>
</tr>
<tr>
<td>5</td>
<td>2.3%</td>
<td>&lt; 0.001</td>
<td>6.8%</td>
<td>-1.5%</td>
</tr>
<tr>
<td>6</td>
<td>4.3%</td>
<td>&lt; 0.001</td>
<td>11.0%</td>
<td>-3.8%</td>
</tr>
<tr>
<td>Average</td>
<td>3.4%</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

## High-parent Heterosis

<table>
<thead>
<tr>
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<th>Average</th>
<th>p-VALUE</th>
<th>Maximum</th>
<th>Minimum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.4%</td>
<td>&lt; 0.001</td>
<td>9.0%</td>
<td>-6.8%</td>
</tr>
<tr>
<td>2</td>
<td>1.1%</td>
<td>&lt; 0.010</td>
<td>6.4%</td>
<td>-5.4%</td>
</tr>
<tr>
<td>3</td>
<td>1.9%</td>
<td>&lt; 0.001</td>
<td>9.3%</td>
<td>-7.8%</td>
</tr>
<tr>
<td>4</td>
<td>2.1%</td>
<td>&lt; 0.001</td>
<td>7.4%</td>
<td>-4.2%</td>
</tr>
<tr>
<td>5</td>
<td>1.4%</td>
<td>&lt; 0.001</td>
<td>6.6%</td>
<td>-2.7%</td>
</tr>
<tr>
<td>6</td>
<td>1.0%</td>
<td>n.s.</td>
<td>7.4%</td>
<td>-7.2%</td>
</tr>
<tr>
<td>Average</td>
<td>1.6%</td>
<td>&lt; 0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
The Distribution of Mid-parent Heterosis

Mid-parent Heterosis

Frequency

-12% -10% -8% -6% -4% -2% 0% 2% 4% 6% 8% 10% 12%
Mid-parent Heterosis Summary

- More than 87% of the hybrids exhibited positive mid-parent heterosis.
- Average mid-parent heterosis was 3.4%.
- Values for mid-parent heterosis were as high as 13.1%.
The Distribution of High-parent Heterosis

Frequency

High-parent Heterosis

-10%  -8%  -6%  -4%  -2%  0%  2%  4%  6%  8%

28%  72%

72%  28%
High-parent Heterosis Summary

- More than 72% of the hybrids exhibited positive high-parent heterosis yielding more than both of the parents of the hybrid.
- Average high-parent heterosis was 1.6%.
- Values for high-parent heterosis were as high as 9.3%.
- The 326 hybrids averaged 0.3% better than the Magnum V check.
- The best hybrid was 9.9% better than Magnum V.
Summary

- Significant heterosis exists in elite, hardy germplasm adapted to the Midwest.
- Mid-parent heterosis averaged 3.4%, but was as high as an 13.1%.
- Positive high-parent heterosis was exhibited in 72% of the hybrids, and was as high as 9.3%.
- Heterosis in elite, hardy germplasm is obtainable and can be utilized to make superior yielding, hybrid alfalfa varieties.