Identifying Essential Test Locations for Oat Breeding in Eastern Canada

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ABSTRACT

The oat (Avena sativa L.) breeding program at the Eastern Cereal and Oilseed Research Centre of Agriculture & Agri-Food Canada has the responsibility to breed new oat cultivars for producers in eastern Canada, which includes Ontario, Quebec, and the Atlantic provinces. A 3-yr multilocation test was conducted to understand the genotype × location interaction patterns and the relationships among test locations in eastern Canada. A genotype + genotype × environment interaction biplot analysis of yield data revealed three distinct oat mega-environments in eastern Canada: (i) northern Ontario, (ii) southern and eastern Ontario, and (iii) Quebec and Atlantic Canada. To breed for all mega-environments, initial yield screening must be conducted at locations representing each of these mega-environments. Based on the relationships among test locations, six essential test locations were identified: three in Ontario, two in Quebec, and one in Atlantic Canada. Testing at all six locations appeared to provide a good coverage of the whole oat-growing area in eastern Canada. Based on these findings, a breeding and test strategy was developed. This includes conducting initial yield screening at three locations in Ontario, Quebec, and Atlantic Canada, followed by a formal yield test at all six essential test locations. Specifically adapted genotypes selected from this test will then be tested in the Registration Tests in their respectively adapted subregions.

Oat (Avena sativa L.) is an important cereal crop in eastern Canada, which is traditionally divided into three subregions: Ontario, Quebec, and Atlantic Canada (New Brunswick, Nova Scotia, and Prince Edward Island). The current hec tareage of oats in the three subregions is roughly 35,000, 105,000, and 18,000 ha, respectively (Statistics Canada, 2009). Previously, there were two
oat-breeding programs for eastern Canada within Agriculture & Agri-Food Canada (AAFC), located in Ottawa, ON, and Quebec City, QC. The Ottawa program was responsible for breeding for Ontario, whereas the Quebec City program, cooperating with an AAFC cereal breeder in Prince Edward Island, was responsible for Quebec and Atlantic Canada. Both programs were successful in breeding oat cultivars for their respective service areas. The Quebec program was terminated in 1996 on retirement of the oat breeder. It is now the responsibility of the Ottawa program to serve all regions of eastern Canada.

Historically, oat cultivars that yielded well in Quebec usually yielded poorly in eastern and southern Ontario, and vice versa. For example, while cultivar Bia was among the highest yielding cultivars in the 2006 Quebec Oat Registration and Recommendation Trials, it was the lowest yielding in the 2006 Ontario Oat Performance Trials. Similarly, the high-yielding Ontario cultivar Sherwood never yielded above average in the Quebec trials. Quebec cultivars tended to perform well in northern Ontario locations, however. No systematic study has been conducted so far to investigate oat mega-environments in eastern Canada.

For the Ottawa program to breed oat cultivars efficiently for all subregions, it is imperative to know (i) the magnitude and pattern of genotype × location interactions across all subregions, (ii) the extent to which the test locations in Ontario (ON) represent those in Quebec (QC) and Atlantic Canada (AC), and (iii) the minimum number of locations at which initial genotype evaluations need to be conducted. In this paper, we report on a 3-yr multilocation study conducted across eastern Canada, aimed at addressing these issues.

MATERIALS AND METHODS

Multilocation Trials

The Ontario oat-registration trials have traditionally been conducted at four or more test locations covering southern, eastern, and northern Ontario (Fig. 1). This was extended, for this study, to include four locations in Quebec, covering central and northern Quebec, one in New Brunswick, and one in Prince Edward Island, in 2006 (Table 1 and Fig. 1). There were 30 entries in the trial, including 27 breeding lines and three check cultivars. Two of the three checks were different in different subregions, and checks were not included at two locations (ON8 and AC1). Consequently, orthogonal data were available only for the 27 breeding lines. There were four replicates at the Ontario locations and three replicates at all other locations except AC1, where the trial was not replicated. A randomized complete block design was used at each location.

The same experimental design was used in 2007 and 2008, with four replications at Ontario locations and three replications at other locations. In 2007, 30 entries were tested orthogonally at the same 10 locations as in 2006 (Table 1 and Fig. 1). In 2007, entries included 22 breeding lines and eight official checks from Ontario, Quebec, and Atlantic Canada. In 2008, 30 entries, including 23 breeding lines and seven official checks, were tested orthogonally at 12 locations (Table 1 and Fig. 1). The 2007 and 2008 tests were also grown at three locations in western Canada (Lacome, AB; Glenlea, MB; and Portage la Prairie, MB), but only data from eastern Canada are reported here.

The breeding lines tested in each year were different because of the usual practice of discarding inferior genotypes and adding new breeding lines every year. The location sets were also different because of the change of locations in southern Ontario (Table 1). Nevertheless, the locations used each year were assumed to adequately represent the oat production areas of eastern Canada.

Local production management practices were followed at each test location in all years. The experimental unit was a four- or five-row plot, 3 m long with 15 cm between rows. The plots were 15 or 30 cm apart, depending on the location. Upon maturity, the whole plot was combine-harvested and yield, test weight, kernel weight, and other agronomic and quality traits were determined. However, in this study only the yield data are considered. The genotypes tested in each of the 3 yr are listed in Table 2.

Data Analysis

Because different sets of genotypes were tested in different years, the analysis was conducted by year, followed by a summary across years. In addition to conventional analysis of variance to quantify the relative importance of genotype main effect vs. genotype × location interaction, genotype + genotype × environment interaction (GGE) biplot analyses (Yan et al., 2000, 2007) were conducted to visualize the genotype × location patterns and the interrelationships between test locations.

The standard deviation (SD)–scaled GGE biplot was used, although many other data transformation methods are possible (Yan et al., 2007). This is the same model as used in standard principal component analysis (DeLacy et al., 1996; Cooper et al., 1997) or genotype × trait biplot analysis (Yan and Frégeau-Reid, 2008). The genotype × location two-way table was first centered with the respective location means and then divided by the SD of the respective location. The resulting standardized two-way table had a 0 mean and unit variance, and thus the test locations were given the same weights (equal importance) on genotype evaluation. Consequently, when this standardized two-way table is displayed in a biplot, the location vectors, which are the distances between the biplot origin and the positions of the locations, should be of equal or similar length if the biplot adequately displays the patterns in the data.

This principle can be used to judge whether the biplot is an adequate display of the patterns in the data. If all location vectors are of similar length, then the biplot is adequate; in such cases, the relationships between locations can be visualized by the angles between their vectors based on the cosine-correlation equality property, that is, the cosine of the angle between the vectors of two locations approximates the correlation between them. If the vector of a location is considerably shorter than other location vectors, it indicates that the biplot does not adequately display patterns involving this location and its relationships with other locations cannot be reliably visualized from its angles with other locations. A shorter vector is an indication that the relevant location is not associated with
In a standard error– (or related statistics) scaled GGE biplot, and under the same provision of adequacy, the vector length of a location is associated with the heritability of the trait under investigation in the location. The SD-scaled GGE biplot, which treats all locations with equal weights and is more effective in displaying the similarities among test locations, does not have an interpretation about the discriminating power of the locations. Analysis of variance and GGE biplot analysis were conducted using the GGEbiplot software (Yan, 2001).

RESULTS

Relative Magnitude of Genotype vs. Genotype × Environment Interaction

Analysis of variance indicated that location main effect (L), genotype main effect (G), genotype × location interaction (GL), and blocks-within-locations were all highly significant (Yan et al., 2007). In a standard error– (or related statistics) scaled GGE biplot, and under the same provision of adequacy, the vector length of a location is associated with the heritability of the trait under investigation in the location. The SD-scaled GGE biplot, which treats all locations with equal weights and is more effective in displaying the similarities among test locations, does not have an interpretation about the discriminating power of the locations. Analysis of variance and GGE biplot analysis were conducted using the GGEbiplot software (Yan, 2001).
As is typical in most multilocation trials, L was much greater than other variation sources. Of greater interest was the ratio of G over (G + GL), which was 13% in 2006 and 2008, and 39% in 2007. Thus, GL varied from greater to much greater than G, suggesting the existence of multiple oat mega-environments in eastern Canada.

Differentiation between Ontario Locations vs. Quebec and Atlantic Canada Locations

In 2006, the Ontario (ON) locations were clearly distinct from the Quebec (QC) and Atlantic Canada (AC) locations (Fig. 2A). Each of the Ontario locations had a right or obtuse angle with each of the Quebec and Atlantic Canada locations, suggesting that the two groups of locations were not associated or were negatively associated. This means that selection at an Ontario location was irrelevant or even counterproductive to selection at a Quebec or Atlantic Canada location.

The distinctiveness of the Ontario locations from the Quebec plus Atlantic Canada (QC + AC) locations was also apparent in 2007, although there were fewer negative correlations between the Ontario locations and the QC + AC locations (Fig. 2B). This pattern was strongest in 2008, where most Ontario locations, with the exception of ON8 (representing northern Ontario), were negatively associated with most QC + AC locations. ON8 was positively correlated with the QC + AC locations, and negatively associated with the other Ontario locations.

Thus, GGE biplots, for all 3 yr, indicated that the Ontario locations, with the exception of ON8, were distinctly different from the Quebec and Atlantic Canada locations in ranking genotypes. This confirmed and

Table 3. Analysis of variance and basic statistics.

<table>
<thead>
<tr>
<th>Source</th>
<th>2006</th>
<th>2007</th>
<th>2008</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>df  MS  F  P</td>
<td>df  MS  F  P</td>
<td>df  MS  F  P</td>
</tr>
<tr>
<td>Genotype (G)</td>
<td>26 1,238,251 9 0.00001</td>
<td>29 6,127,656 33 0.00001</td>
<td>29 2,043,834 11 0.00001</td>
</tr>
<tr>
<td>Location (L)</td>
<td>9 152,497,500 1070 0.00001</td>
<td>9 138,488,000 737 0.00001</td>
<td>12 194,386,800 1059 0.00001</td>
</tr>
<tr>
<td>G × L</td>
<td>234 897,100 6 0.00001</td>
<td>261 1,078,644 6 0.00001</td>
<td>348 1,169,938 6 0.00001</td>
</tr>
<tr>
<td>Block (Location)</td>
<td>22 762,671 5 0.00001</td>
<td>22 2,242,129 12 0.00001</td>
<td>33 1,699,777 9 0.00001</td>
</tr>
<tr>
<td>Error</td>
<td>572 142,487 637 187,898</td>
<td>957 183,558</td>
<td></td>
</tr>
<tr>
<td>Grand mean (kg ha⁻¹)</td>
<td>4268 3817 4035</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SE (kg ha⁻¹)</td>
<td>377 433 428</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LSD₉₅₃ (kg ha⁻¹)</td>
<td>535 614 607</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CV (%)</td>
<td>8.9 11.4 10.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>G/(G + GL) (%)</td>
<td>13.3 38.7 12.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
explained the observation that oat cultivars selected in Ontario were generally unadapted to Quebec and Atlantic Canada, and vice versa. For the Ottawa oat-breeding program to also serve the oat growers in Quebec and Atlantic Canada, selection at Ontario sites alone is inadequate; initial selections must also be conducted at test sites in Quebec and/or Atlantic Canada.

**Test Locations in Ontario**

In the biplots in Fig. 2, some locations had shorter vectors than others; for example, ON8 in Fig. 2A, and QC2 in Fig. 2C. This indicates that information related to these locations was not well displayed by the biplot. To better understand the relationships among the Ontario test locations, biplots containing only Ontario locations were constructed (Fig. 3). In 2006, the four locations appeared to fall in two groups: ON8 vs. ON2 + ON3 + ON6 (Fig. 3A). The latter three locations were positively correlated with one another ($P < 0.01$) but were not correlated with ON8. In 2007, ON6 and ON2 were independent of each other (Fig. 3B), and ON6 and ON8 were only weakly associated (Fig. 2B). Therefore, ON2, ON6, and ON8 each represented an independent environment. This relation is more obviously visualized using a three-dimensional biplot (not shown). The location ON3 can be grouped with either ON2 or ON8 (Fig. 3B); it had a correlation of 0.62 ($P < 0.01$) with both locations. Here we chose to group ON3 with ON2, considering that it was geographically much closer to ON2 than to ON8 (Fig. 1). ON3 is a southern Ontario location but had low crown rust (*Puccinia coronata* var. *avenae* W.P. Fraser & Ledingham) pressures; it therefore had some similarities to both ON2 (southern Ontario, with high rust pressure) and ON8 (northern Ontario, with little crown rust pressure). In 2008, the Ontario locations fell into three independent groups: ON3, ON8, and ON1 + ON4 + ON5 + ON6 (Fig. 3C). This latter group had a high level of crown rust pressure and locations within it were all strongly correlated ($P < 0.01$). The location groupings are summarized in Table 4.

Northern Ontario (ON8) always differed from southern and eastern Ontario locations in cultivar ranking. Eastern Ontario (ON6) tended to be independent of southern Ontario locations when crown rust was less severe (2007) but tended to group with the southern Ontario locations when crown rust was heavy (2006 and 2008). The south-

![Figure 2. (right) Genotype + genotype × environment interaction biplots including all locations for the (A) 2006, (B) 2007, and (C) 2008 data. See Table 1 for full location names and Fig. 1 for geographical positions of the locations. “Scaling = 1” means that data were scaled by the standard deviation of the location; “Centering = 2” means the data were centered by the mean of the location; “SVP = 2” means the biplot was constructed using location-focused singular value partitioning and is therefore appropriate for visualizing correlations between locations. Note that AC2 and QC1 overlap in Fig. 2C. PC = principal component.](image)
ern Ontario location ON3 differed from other southern Ontario locations in that it had lower crown rust pressure.

**Test Locations in Quebec and Atlantic Canada**

GGE biplots involving only the Quebec and Atlantic Canada locations were prepared to investigate the relationships among these test locations (Fig. 4). In 2006, the six locations were more or less evenly distributed with a right angle between QC1 and QC3, meaning that genotype rankings at these two locations were not correlated (Fig. 4A). Using these two locations as anchors, the six locations can be divided into two groups: QC3 + AC2 + QC4 and QC1 + AC1 + QC2. The members within each group were significantly correlated, but there were no significant correlations between groups, with the exception of QC2 which was located between the two major groups and was positively correlated \((P < 0.05)\) with all locations.

In 2007, all locations were positively correlated \((P < 0.01)\) but fell into what appears to be two groups, QC1 + QC3 + QC4 and AC1 + AC2; with QC2 falling approximately midway between the two groups (Fig. 4B). In 2008, the six locations could be divided into two apparent groups: QC3 + QC2 + QC4 and AC1 + AC2 + QC1. These locations were more strongly correlated within groups and less so between groups (Fig. 4C).

In all 3 yr, the Atlantic Canada locations could not be clearly separated from the Quebec locations, confirming that Quebec and Atlantic Canada belonged to the same mega-environment. The grouping results are summarized in Table 4.

**Northern Ontario as a Separate Mega-environment**

Comparing the biplots for the Ontario locations (Fig. 3) and those for the Quebec and Atlantic Canada locations (Fig. 4), it was evident that the environments in Ontario were more variable even though QC + AC covers a much wider geographical region and oat hectareage. This was mainly because of the distinctness of ON8, representing northern Ontario, from the other Ontario locations (Fig. 2 and 3).

ON8 was not significantly correlated with any other test location in 2006 (Fig. 2A). In 2007, ON8 was positively correlated with all Quebec and Atlantic Canada locations \((P < 0.05 \sim 0.01)\), but only positively correlated...
with the Ontario site ON3 ($P < 0.01$). In 2008, ON8 was positively correlated with most Quebec and Atlantic Canada locations ($P < 0.05$–$0.01$), but was negatively correlated ($P < 0.01$) or uncorrelated with other Ontario locations. Thus, northern Ontario represented by ON8 was more similar to Quebec and Atlantic Canada locations than to ON locations, but it could not be represented by the Quebec and Atlantic Canada locations in years such as 2006. As an important oat-growing region in eastern Canada, northern Ontario should be regarded as a different oat mega-environment. Retrospectively, it is probable that current oat cultivars grown in northern Ontario were not the optimum for this region because the initial yield screening, either from the Ottawa program or from the Quebec City program, had never been conducted there.

### Identifying Essential Test Locations

The test locations within each group, identified above, were positively correlated in genotype ranking (Fig. 2 and 3; numerical correlation coefficients not presented). Assuming that each grouping can be effectively represented by a single test location, a set of essential locations can be identified (Table 4). For example, in 2006, ON2, ON3, and ON6 formed a group. ON6 could be chosen to represent the group because it is the home of the Ottawa oat-breeding program and is, therefore, most accessible. Similarly, the

<table>
<thead>
<tr>
<th>Year</th>
<th>Location groups</th>
<th>Essential or most accessible location</th>
<th>Essential test locations</th>
</tr>
</thead>
<tbody>
<tr>
<td>2006</td>
<td>ON2, ON3, ON6</td>
<td>ON6</td>
<td>AC2 (3)†</td>
</tr>
<tr>
<td></td>
<td>ON8</td>
<td>ON8</td>
<td>ON3 (2)</td>
</tr>
<tr>
<td></td>
<td>AC1, QC1, QC2</td>
<td>QC1</td>
<td>ON6 (3), ON8 (3)</td>
</tr>
<tr>
<td></td>
<td>AC2, QC3, QC4</td>
<td>AC2 or QC3</td>
<td>QC1 (1)</td>
</tr>
<tr>
<td>2007</td>
<td>ON2, ON3</td>
<td>ON3</td>
<td>QC3 (3)</td>
</tr>
<tr>
<td></td>
<td>ON6</td>
<td>ON6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ON8</td>
<td>ON8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>QC1, QC2, QC3, QC4</td>
<td>QC3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AC1, AC2</td>
<td>AC2</td>
<td></td>
</tr>
<tr>
<td>2008</td>
<td>ON3</td>
<td>ON3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ON4, ON5, ON6</td>
<td>ON6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ON8</td>
<td>ON8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>QC2, QC3, QC4</td>
<td>QC3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>AC1, AC2, QC1</td>
<td>AC2</td>
<td></td>
</tr>
</tbody>
</table>

†Number of years the location was selected as essential or most accessible.

Figure 4. (right) Genotype + genotype × environment biplots including all Quebec (QC) and Atlantic Canada (AC) locations for the (A) 2006, (B) 2007, and (C) 2008 data. See Table 1 for full location names and Fig. 1 for geographical positions of the locations. “Scaling = 1” means that data were scaled by the standard deviation of the location; “Centering = 2” means the data were centered by the mean of the location; “SVP = 2” means the biplot was constructed using location-focused singular value partitioning and is therefore appropriate for visualizing correlations between locations. PC = principal component.
group of AC1, QC1, and QC2 can be represented by QC1, where the Ottawa breeding program often does reciprocal tests with a private breeding company. When an essential or highly accessible location was selected for each grouping, essential locations could be identified for each year. A list of six test locations was developed, which represented the minimum set of locations for early breeding-line testing in eastern Canada. These were locations AC2, ON3, ON6, ON8, QC1, and QC3 (Table 4).

GGE biplots involving only these six locations were constructed (Fig. 5) to compare with those based on data from all test locations (Fig. 2). The general GL interaction patterns and genotypes to be selected for different subregions remained largely the same based on data from the selected six test locations. For 2006, genotypes ‘1175–11’ and ‘1168–3’ would be selected for southern Ontario (represented by ON3), and ‘1068–3’ and ‘1130–1’ would be selected for the Quebec and Atlantic Canada subregions, whether the selection was based on the full data (Fig. 2A) or the subset of test locations (Fig. 5A). The high-yielding genotypes in an environment are visually identified as those that have longer projections onto the vector of the environment, which starts from the biplot origin and points to the marker of the environment. Note, however, that ON6 (eastern Ontario) and ON8 (northern Ontario) had short vectors in both Fig. 2A and Fig. 5A, reflecting their independence of, or distinctness from, other test locations. The highest yielding genotypes for these two locations are thus not obvious from these biplots.

For 2007, the GL patterns remained almost the same because of smaller GL interactions in that year. The genotypes that could be selected for Ontario included ‘Sherwood’, ‘1189–1’, ‘1180–4’, and ‘1180–5’, and those selected for Quebec and Atlantic Canada included ‘Sylva’, ‘Rigodon’, and ‘Triple Crown’, based on both the full location data set (Fig. 2B) and the minimum location subset (Fig. 5B).

For 2008, the same genotypes (Sylva, ‘Sutton’, Rigodon, ‘1234–1’, ‘1234–2’) were selected for the Quebec and Atlantic subregions (plus ON8), whether based on the full data set (Fig. 2C) or the minimum subset (Fig. 5C). For the rust areas of Ontario, the same genotypes would be selected for eastern and southern Ontario using the subset of locations: ‘1232–5’, ‘1231–2’, ‘1225–2’, ‘1174–3’, ‘1180–4’, and ‘1180–5’. An obvious change is that while ‘1228–1’ stood out as a high-yielding genotype in most test environments in the full data set (Fig. 2C), it was much less outstanding in the subset (Fig. 5C). This is a caution that some superior genotypes might fail to be identified if tested only in this subset of locations. Nevertheless, the six test locations (Fig. 5 sites) appeared to represent well the whole of eastern Canada oat-growing regions. Instead of testing at 10 or 12 locations, six locations appeared to be sufficient in any given year.

We must point out that Fig. 2 and 5 are not most appropriate for accurate genotype evaluation because they include environments from different mega-environments and because the biplot is based on location-focused singular-value partitioning (SVP = 2). Once mega-environments are defined, genotype evaluation as well as test-environment evaluation should be conducted within individual mega-environments (Yan et al., 2007; Yan and Holland, 2010). Genotype-focused singular-value partitioning (SVP = 1) is needed for accurate visual genotype evaluation.

There are still some close correlations among the “essential” locations (Fig. 5), for example, QC1 and QC3 in 2007, and QC1, AC2, and ON8 in 2008. However, none of these correlations was repeatable across years and, therefore, no locations can be further removed from the list.

**DISCUSSION**

**Oat Mega-environments in Eastern Canada**

This study leads to the conclusion that the growing regions in eastern Canada are composed of three different mega-environments: (i) eastern and southern Ontario, (ii) northern Ontario, and (iii) Quebec plus Atlantic Canada. The eastern and southern Ontario region was distinctly different from the QC + AC subregion. The northern Ontario region was in between but was more similar to the QC + AC region than to eastern and southern Ontario.

The formation of different mega-environments in eastern Canada may be attributed to several causes. A major cause can be differences in crown rust pressure. Crown rust is a major yield-limiting factor in most locations in eastern and southern Ontario, and resistance to this disease often explains 50 to 60% of the yield variation. On the contrary, crown rust has not been an issue for oat production in Quebec, Atlantic Canada, or northern Ontario. A second cause may be related to the differences in latitude and therefore daylength. Southern and eastern Ontario regions have lower latitudes and shorter daylength in the oat-growing season than Quebec, Atlantic Canada, or northern Ontario. Consequently, oat cultivars adapted to southern and eastern Ontario tend to be earlier, shorter, and have lower yield potentials than those adapted to the other regions. A third cause may be the difference in soil properties. It is known that some Quebec regions have severe boron (Tran et al., 2003) and magnesium deficiencies (Andre Comeau, personal communication, 2009), which may require genotypes that are more efficient in uptake and utilization of these minor elements.

Because of administrative reasons, eastern Canada has three independent cereal crops committees (Ontario, Quebec, and Atlantic Canada or Maritimes) that govern the registration and recommendation tests of cereal crop cultivars in their respective regions. The finding that QC sites and AC sites are similar in oat genotypic responses
suggests that greater test efficiency and better recommendations can be achieved if a single, coordinated oat registration and recommendation test can be conducted across Quebec and Atlantic Canada.

**Strategies of Breeding-Line Screening**

Based on the relationships among the 13 test locations, six were identified as essential locations and appeared to provide a good coverage of the whole eastern Canada oat-growing region: ON8—New Liskeard (representing northern Ontario), ON6—Ottawa (eastern Ontario), ON3—Nairn (southern Ontario), QC1—Princeville (southern Quebec), QC3—Normandin (northern Quebec), and AC2—Harrington (Prince Edward Island, representing Atlantic Canada).

Because of the strong and persistent GL interactions, initial yield screening should be ideally conducted at all of the six essential test locations. This is in contrast to the test strategy used in the past, where initial yield tests were conducted only at Ottawa (ON6) in the first year (“Home Test”), selected lines tested at multiple locations in Ontario in the second year (“Preliminary Test”), and surviving lines further tested at several locations in Ontario in the third year (“Registration Test”). Testing at locations in Quebec and Atlantic Canada might or might not be conducted in the third year, depending on the availability of resources. Under this test system, it is highly probable that breeding lines potentially adapted to Quebec and Atlantic Canada might have been discarded during the testing in Ontario and never had a chance to be tested in the sub-regions to which they were most adapted. It is, therefore, not surprising that cultivars selected in Ontario are generally unadapted to Quebec and Atlantic Canada.

Testing a large number of lines, say 300, at all six locations, even with only two replicates, is often unrealistic, because of limited seed and other resources. As a compromise, starting in 2007, a three-step strategy was adopted. In Step 1, the initial yield screening was conducted at three locations with one or two replications: Ottawa, ON (ON6); Normandin, QC (QC3); and Harrington, PE (AC2). These locations are all AAFC research facilities and, therefore, more accessible than others. The initial screening would reduce the 300 lines to about 50. In Step 2, lines that survived the initial screening were further evaluated at five of the six essential locations (Princeville [QC1], Normandin...
ally and specifically adapted genotypes. Frégeau-Reid (2008), can be used to identify both genotypic and specific adaptation to each mega-environment should be the main mode of selection, although genotypes adapted to a specific subregion, are placed into one or more of the Ontario, Quebec, and Atlantic Canada regional registration tests. In the light of our findings, it would be ideal to include ON8 (New Liskeard) at the Step 1 test, or at least at Step 2, so that northern Ontario is covered in the initial screening.

Strategies of Selection within and across Mega-environments

There are several potential genotype–selection strategies when multiple environments are involved. One is to select for wide adaptation, which is based on the genotype’s mean performance across all environments, with some consideration for stability across environments. This strategy is appropriate only when the environments belong to a single mega-environment. Another strategy is to select for specific adaptation, where selection is based on a genotype’s mean performance across a subset of environments that belong to a mega-environment, while ignoring data from other test environments. These two strategies largely correspond to the “index selection” and “independent selection” strategies, as used in selecting for multiple traits (Yan and Frégeau-Reid, 2008). In practice, a mixture of the two strategies is probably more appropriate, with different weights given for different test environments according to the relative magnitude of G vs. GL or GE (genotype × environment interaction). Because of the substantial rank changes between mega-environments (subregions) in eastern Canada, selection for specific adaptation to each mega-environment should be the main mode of selection, although genotypes adapted to all mega-environments (e.g., ‘1228−1’ in Fig. 2C) should not be neglected. A procedure combining both independent selection and index selection, as described in Yan and Frégeau-Reid (2008), can be used to identify both generally and specifically adapted genotypes.

Orthogonal vs. Nonorthogonal Tests

Orthogonal testing, in which all genotypes are tested at all locations, is always more desirable, given sufficient resources, as the information from such tests is much richer than from other testing methods. However, orthogonal testing is also something the breeder tries to avoid, as it is much more expensive. From the perspective of breeding, it makes little sense to test breeding lines in a region where they are highly likely to be unadapted. Orthogonal test is essential only when there is no knowledge about which lines are likely to be specifically adapted to which subregion(s). Once such knowledge is available, either through an initial orthogonal test or through a prior knowledge of the parents used to develop the selection population, breeding lines should be tested only in subregions where they are likely to be adapted. If some lines appear to be adapted to two mega-environments, of course, they can and should be tested in both.

To avoid unnecessary orthogonal tests in the future, it is essential that some orthogonal studies still be conducted to understand the causes of genotype × mega-environment interaction. Such tests can be used to search for associations of yield performance (or other important breeding objectives) in off-sites (such as in northern Ontario, Quebec, or Atlantic Canada) with a trait that is less subjected to GE (genetic markers being an extreme example) or a phenotype that can be reliably determined at the primary site (Ottawa). Such associations will allow a greater selection/culling intensity at the primary site, thereby minimizing the need for initial screening at off-sites.

Some Methodology Issues

Biplot Analysis Facilitates Mega-environment Investigation

Dividing a target region into meaningful subregions (mega-environments) has long been an interest of plant breeders. For example, Horner and Frey (1957) investigated the possibility of dividing Iowa oat-growing region into subregions by grouping nine test locations covering the state into groups of locations. Based on the principle that GL between groups should be maximized and that within groups minimized, they calculated the within-group GL variance for all possible combinations of 1, 2, 3, …, or 9 sites, hoping to find a site combination that gives the smallest within-group variance. A related study is that of Atlin et al. (2000) on barley (Hordeum vulgare L.)-growing regions in Canada, applying the theory of indirect selection. They concluded that dividing the Canadian barley-growing region into western Canada (including sites in Alberta, Saskatchewan, and Manitoba) and eastern Canada (including sites in Ontario, Quebec, and Prince Edward Island) was not justified. This was because increase in heritability of barley yield within subregions was offset by reduced genetic correlation between the divided subregions and the undivided whole region. However, based on GGE biplot analysis of the same barley data set, Yan and Tinker (2005) revealed a clear differentiation between western vs. eastern Canadian sites when the Manitoba (central Canada) sites were grouped into the eastern rather than the western subregion. The current study corroborates Yan and Tinker (2005) regarding the usefulness of GGE biplot analysis in facilitating investigation of crop mega-environments or subregions.

GGE Biplot Analysis Should Be Supported by Conventional Statistical Analysis

GGE biplots are a visual tool for identifying patterns among test locations or genotypes. However, its use is justified only when either G or GE in the data are real, that
is, statistically significant. Therefore, analysis of variance should be conducted to see if GGE biplot analysis is worthwhile to conduct. A related issue is the error heterogeneity among test environments. When the environments are heterogeneous, patterns observed from a GGE biplot can be from experimental errors rather than being real. Methods to address this include using a random-effect-model GGE biplot (Smith et al., 2002), a within-environment standard error–scaled GGE biplot (Blanche and Myers, 2006), and a heritability-adjusted GGE biplot (Yan and Holland, 2010). However, the environment-standard GGE biplot used here appears to be most effective in studying relationships among test environments and mega-environments. The problem that it takes no consideration of environmental heterogeneity can be overcome by using data from multiple years.

**Interpretation of Test Environments with Short Vectors in the Biplot**

Compared with other types of GGE biplots, the SD-scaled GGE biplot used in this study has the property that the environmental vectors tended to be of equal length if the biplot adequately approximates the environment-standardized data. Shorter environmental vectors are indicative of the fact that the specific environments were not strongly correlated with environments with longer vectors and that they were probably not strongly correlated with one another either. So, these short-vector environments can be regarded as independent test environments. This can be validated by generating a biplot that contains only these short-vector environments, or more decisively, by examining the correlation matrix among environments. When many independent test environments are suggested, the researcher has to decide if it is realistic and meaningful to treat each environment as a separate mega-environment. It is probably more appropriate to treat them as unique (therefore essential) test environments of a single but complex mega-environment (Yan et al., 2007).

**Using a Subset of Genotypes in Grouping Test Environments**

A GGE biplot displays the G and GE of a genotype × environment two-way table, whereby it allows visual mega-environment delineation, test-environment evaluation, and genotype evaluation. The shape of the biplot

Figure 6. (right) Genotype + genotype × environment biplots including genotypes that performed well in one or more environments, for (A) 2006, (B) 2007, and (C) 2008. See Table 1 for full location names and Fig. 1 for geographical positions of the locations. “Scaling = 1” means that data were scaled by the standard deviation of the location; “Centering = 2” means the data were centered by the mean of the location; “SVP = 2” means the biplot was constructed using location-focused singular value partitioning and is therefore appropriate for visualizing correlations between locations. PC = principal component.
and the patterns shown in the biplot, including the genetic correlations among test environments, all depend on the relative magnitude of G vs. GE. The G arises from similar relative performance, whereas GE from differential relative performance of genotypes in different environments. Inclusion of genotypes that are “universally” superior or inferior will lead to a larger G. While universally superior genotypes are what breeders look for, universally poor genotypes have no place in mega-environment investigation and test-environment evaluation, because it is all too easy to add such genotypes in the test, which will artificially inflate G, G:GE ratio, and genetic correlations among test environments. Therefore, it is advisable to exclude universally poor genotypes in biplot analysis so that the genetic correlations among test environments shown in the biplot are more objective. Some researchers may prefer to visualize a GGE biplot that contains only genotypes that are among the best performers in one or more test environments, such as those presented in Fig. 6. This is probably an extreme approach to exclude universally poor genotypes, but this will exclude genotypes that performed reasonably well in one or more environments. Because the number of genotypes is dramatically reduced, the genetic correlations among test environments, and hence test environment grouping, may become less credible. Nevertheless, it is interesting to note that the main conclusions from this study are largely reflected in the GGE biplots based on the much reduced subset of possible winners, despite the clear differences between the two sets of biplots (Fig. 6 vs. Fig. 2).

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