Agriculture and Agri-Food Canada | Agriculture et Agroalimentaire Canada

Prediction of canola field establishment by ethanol-based seed deterioration assays

Final Project Report (CARP 2005-04-07)

Nov. 12, 2009

Prepared for

The Canola Council of Canada
Canola Agronomic Research Programs

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Abstract

The relationship between results of new, ethanol-based, 24-h seed deterioration/vigor assays and subsequent field performance of canola was studied. Results of color (Vigorcheck) and instrumental (Vigorscore) versions of the new assays were compared to seedling emergence, leaf area expansion, seedling biomass, number of plants at maturity and oilseed yield for numerous pesticide-treated and untreated seed lots of open-pollinated, hybrid and synthetic genotypes. Seedlings emerged 1-2 days earlier and to a higher percentage from seeds that tested high-vigor with the Vigorscore and Vigorcheck assays compared to those that tested low-vigor. Leaf area growth from seed testing high-vigor was greater than that from seed testing low-vigor. However, leaf growth of hybrid types showed less variation than open-pollinated types because hybrid types overcame the effects of seed deterioration more rapidly than open-pollinated types. Oilseed yield was significantly correlated with Vigorscore results in two out of four yield trials. Twelve seed quality tests were compared for their ability to predict seedling performance in the field. Vigorscore, the cool vigor stress test, 2-d germination and the prechill vigor test were the best predictors, while the vigor index and seed weight tests were the worst. The official germination test was in the middle of the group. It is recommended that official germination analysis be supplemented with Vigorscore analysis in order to better predict seedling emergence and growth in the field.

Background and Objectives

Canola seed deterioration can reduce seedling emergence, crop establishment and crop yield. Deterioration, or vigor loss, is caused by aging, which may be accelerated by agronomic history, poor storage conditions and pesticide seed treatments. Two new 24-hour assays detect deterioration of canola seed. The assays are based on the amount of ethanol (ethyl alcohol) emitted by partially imbibed seed. The assays identify seed that is not likely to perform as well as high quality seed of the same genotype. The first assay to be developed, called Vigorcheck, is a simple color test that can be run on the farm (Figure 1). The second assay is a quantitative instrumental test called Vigorscore (Figure 2).

Seed deterioration is the primary cause of low vigor in canola. It is readily recognized when seedlings from high quality and deteriorated seed are grown side by side when grown in hydroponics (Figure 3). Weak seedlings from deteriorated seed (right side of Figure 3) may struggle to overcome soil resistance or fail to emerge. In practice, vigor is recognized primarily as the ability of seedlings to emerge rapidly and uniformly in the field (ISTA, 1995).

The current popularity of canola hybrids and the frequent use of the term “hybrid vigor” inevitably leads to some confusion when discussing seed vigor. Differences in vigor among canola seed samples can be due to genetic effects (genetically determined differences among varieties or hybrids, e.g., hybrid vigor) or metabolic effects, mainly seed deterioration. It is important to distinguish between genetic and metabolic effects. In the present study, we are concerned only with changes in vigor arising from changes to seed metabolic status due to deterioration.

Many seed qualtiy tests measure deterioration. The official, or standard, germination test is the best known example. Biochemical-type deterioration tests include the electroconductivity and tetrazolium procedures. The new 24-h ethanol-based assays fall into the later group in that these assays detect biochemical changes that occur in deteriorated seed.

Deteriorated seed releases substantial quantities of ethanol that can be readily detected. Gaseous ethanol is emitted from canola seed when it accumulates water (imbibition). The ethanol emission indicates that the seed is undergoing anaerobic metabolism, which is inefficient compared to normal, aerobic seed metabolism. Our research has shown that the ratio of aerobic to anaerobic metabolism is lower in deteriorated compared to non-deteriorated canola seed. We found previously that the quantity of ethanol gas emitted by moist canola seed is an indicator of seed deterioration as measured by seedling growth in the laboratory (Buckley and Buckley, 2009; Buckley *et al.*, 2006; Buckley *et al.*, 2003). Study of a

large canola seed sample set, consisting of 151 seed lots (most of which were from the commercial seed trade) and including more than 50 non-hybrid and hybrid genotypes and 19 seed treatment formulations, revealed a high correlation (r = 0.81) between seed vigor (measured as seedling growth in hydroponics) and ethanol emissions (Buckley *et al.*, 2003). Results showed that seed treatment, hybridization, genetic modifications and mutagenic modifications had minimal effects on the correlation between seedling growth and ethanol emissions (Buckley *et al.*, 2003).

To our knowledge, there are no ethanol-based vigor assays currently in use for any crop; however the technique has been suggested several times in the scientific literature. Gorecki et al. (1985; 1992) found that the quantities of ethanol and acetaldehyde produced by imbibing or germinating pea and cocklebur seeds were proportional to the age of the seed. Artificially aged muskmelon seeds reduced their ability to germinate and increased the production of ethanol and acetaldehyde during imbibition (Pesis and Ng, 1986). Naturally or artificially aged soybean seed had higher ethanol and acetaldehyde concentrations in seed tissue than did un-aged seed (Woodstock and Taylorson, 1981).

The **objective** of the present project was "To provide field data that will establish the relationship between seed ethanol emissions and emergence/establishment of canola plants and, thereby, establish the reliability of newly developed ethanol-based vigor assays". During the course of the work, we also were able to investigate the relationship between ethanol-based assay results and canola oilseed yield. In addition, a comparison of the new Vigorscore assay with 11 other seed quality tests was also untaken. Preliminary results of the project were presented at a conference (Buckley and Irvine, 2006).

This report is presented in two parts: Part I addresses the capability of the ethanol-based canola seed deterioration assays, Vigorcheck and Vigorscore to predict seedling performance and oilseed yield. Results from nine field trials are included. Part II is a comparison of Vigorscore with 11 other laboratory measures of canola seed quality. Results from four of the nine field trials are used in the comparison.

Experimental Methods

***Field trials*.**
Field trials were conducted over three years (2004-2006) in and near Brandon, MB. Two types of trials were performed--seedling growth trials and conventional yield trials. All trials were a randomized block design. Conventional yield trials had four repetitions, while seedling trials had 6 repetitions. All seed was treated with commercial pesticides consisting of Gaucho, Gaucho CS, Helix, Helix Xtra, Prosper or Prosper 400. There were 97 unique seed samples with varying storage history in the seedling growth trials and 62 in the yield trials, with some overlap between the two. All trials included a check seed sample from a single high-quality lot of AC Excel. The check and all other seed samples were stored at -15 to -20 °C, which resulted in insignificant changes in seed quality over the three years of the trial. Almost all of the seeds tested were *Brassica napus*, however, 6 samples of *Brassica rapa* were included. Seedling growth trials were conducted on sandy loam at the Brandon Research Centre main complex. Yield trials were conducted on clay loam at a Brandon Research Centre remote site (section 21, township 12, range 18, WPM) and also at the main complex. Ten seedling growth trials were planted but three were lost to gopher damage or flooding. Three conventional yield trails were planted and one was lost to flooding. Two of the seedling growth trials were converted to yield trials in order to supplement the yield data.

In seedling growth trials, 60 seeds were placed with the aid of a vacuum planter on a 6 x 10 grid in 0.5-m^2 plots and covered with 13 mm of sieved soil. The plots were irrigated to prevent crust formation and provide uniform moisture among the trials. Emergence was counted daily, digital photographs were taken twice weekly for leaf area measurements and seedlings were harvested for biomass determination when the check reached the 3-4 leaf stage. In the two seedling growth trials that were converted to yield trials, seedlings were not harvested and the plots were taken to maturity. In the conventional yield trials, seed was sown into barley stubble at 100 seeds m^{-2} and 10-15 mm deep with a cone seeder and the plots were not irrigated. Fertilizer was applied in both seedling growth and yield trials to meet the requirements of the growth of the crop (seedling or mature

plant). Flea beetles were controlled in the seedling growth and yield trials with malathione, cypermethring, L cyhalothrin-lambda or carbaryl when needed as a foliar spray. A plot-size combine was use to obtain the oilseed yield from both the converted seedling growth trials and conventional yield trials. Numbers of plants per square meter in yield trials were counted in fall (after harvest). Multiple seed lots showing varying quality within a genotype were evaluated side-by-side in the same trial. Results were expressed as a percentage of the highest (or lowest) value within each genotype. Average daily temperature was recorded for the seedling growth and yield trials. Rainfall was recorded for the non-irrigated sites (conventional yield trials).

Field trials yielded data for the following variables: days to 50 % emergence, days to maximum emergence, maximum percentage emergence, maximum emergence rate (MER), leaf area per plot (i.e., leaf area per square meter), leaf area per plant, leaf growth rate, above-ground seedling fresh and dry matter per plot, and above-ground seedling fresh and dry matter per plant. Data for leaf area per plot, leaf area per plant and the weight variables was obtained when the check was at the 3-4 leaf stage.

Daily emergence data was fitted to a modified Gompertz growth function from which the three emergence variables were calculated. The Gompertz function used was as follows:

$$y = a \cdot \exp(-\exp(-(t - t_i) / b))$$

and, from the first derivative,

$$MER = a / (e \cdot b)$$

where y = cumulative emergence at time t; a = maximum achieved emergence; t_i = time corresponding to the inflection point of the Gompertz growth curve; b = constant with units d^{-1}; MER = maximum emergence rate (slope of the Gompertz growth curve at the inflection point); e = Euler's number; exp(argument) = e raised to the power supplied by the argument.

A variation of the Weibull function used for modeling fennel seed germination by Damata *et al.* (1994) and the conventional Gompertz function used for modeling wheat seedling emergence by Gan *et al.* (1996) were also evaluated, but neither function was satisfactory for modeling the seedling emergence from our field studies.

A composite variable derived from six seedling emergence and growth variables from the field trials was calculated to facilitate interpretation of results in Part II. The composite, called "Seedling Establishment", was derived from days to 50 % emergence, maximum emergence rate, maximum percentage emergence, leaf area per plot, leaf area per plant and rate of leaf area growth. Since the scale of days to 50 % emergence was reversed compared to the other variables, its inverse × 10^4 was calculated, which converted percentage of low in genotype to the equivalent of percentage of high in genotype. The results for each component variable were standardized to mean = 100 and SD = 20 using the Standard Procedure of SAS (SAS Institute Inc, 1999). Finally the unweighted average of the six variables was calculated to yield the composite variable.

***Seed analyses at the Brandon Research Centre*.**

Seed samples were tested with the Vigorcheck and Vigorscore seed deterioration assays; a hydroponics seedling growth assay; and 2-, 7-, 10- and 11-d germination procedures. In addition, seed weight was recorded and vigor index calculated. A description of each procedure follows.

Vigorcheck--Color seed deterioration assay. Two grams of seed and 0.5 ml of water were added to 2-oz glass bottles. The bottles, which contained proprietary chemicals dried on the inside surface, were sealed with caps that incorporated an indicating color disc. The bottles were shaken to mix the seed and water and dissolve the chemicals, then set aside at 23 °C (room temperature) for 24 h. If sufficient gaseous ethanol was produced by the seed over 24 h, it reacted with the indicator disc causing a color change from yellow to blue (Figure 1). Blue was interpreted as deteriorated seed, whereas yellow and all intermediate color stages were interpreted as non-deteriorated seed. In the graphs that follow, all the intermediate colors are shown as green.

Vigorscore--Instrumental seed deterioration assay. The instrumental procedure was the same as the color procedure except that bottles

were sealed with a cap containing a neoprene-Teflon septum and, after 24 h, the gaseous ethanol in the bottle was determined quantitatively with a modified hand-held breathalyser (courtesy of Draeger Safety, Inc.) (Figure 2). The septum in the bottle cap was pierced with a needle on the breathalyser and a small gas sample extracted by the instrument for analysis.

Hydroponics seedling growth assay. Four replicates of 100 seeds were placed on stainless steel screens suspended about 1 cm above an aerated, buffered, complete nutrient solution in a controlled-environment chamber (16 h at 6.6-8.3 µmol m^{-2} s^{-1} light, 23 °C and 70-83 % RH; 8 h dark at 16 °C and 77-88 % RH). The mist from the solution provided moisture for germination and early growth of seedlings and also raised the humidity surrounding the seedlings. After five days, fresh weight of combined roots and shoots were determined. Results were expressed as a percentage of the check that was included in each batch of samples. The check was the same high-quality lot of AC Excel that was used as a check in the field trials. Hydroponics results were expressed as fresh weight, rather than dry weight, because there is little increase in dry matter above that found in the original seed in the hydroponics growth period.

7-d germination. The germination procedure described by the Canadian Food Inspection Agency (CFIA, 1997) was followed except that a total of 168 seeds were tested instead of the minimum 200 seeds described by CFIA. Seeds were placed on 7 × 8-grids on saturated germination (blotting) paper in 17.1 × 12.0 × 8.6-cm (L × W × H) germination boxes (three boxes per seed sample) in a room controlled at 25 ºC for 24 h per d. Light was supplied from compact fluorescent bulbs at 9-15 µmol m^{-2} s^{-1} (750-1200 lux) inside the germination boxes (with lids in place) throughout the room for 8 h per d. Humidity was not controlled. Triplicate boxes were prepared for each seed sample and randomly arranged throughout the room. The boxes were arranged in a single layer in order to ensure uniform light for all seedlings. Control of light intensity within the range specified by CFIA (1997) was considered to be particularly important because hypocotyl elongation is sensitive to light intensity and our early observations indicated that short hypocotyls were a characteristic of seedlings grown from deteriorated seed. One end of the germination boxes was raised 19 mm so that excess water was drained to the low end. There was sufficient water so that the paper remained saturated throughout the test. Normal and abnormal seedlings were counted at 7 d. Several statements in the description of abnormal canola seedlings are somewhat ambiguous in the Canadian Methods and Procedures for Testing Seed (CFIA, 1997). Of particular concern is the lack of specificity about hypocotyl length. The manual states that seedlings with “markedly shortened hypocotyls” are abnormal, but does not define “markedly shortened”. In order to ensure consistency in assessing abnormal seedlings, we defined “markedly shortened” as less than 7 mm. The hypocotyl length of healthy seedlings frequently was more than 40 mm. Percentage germination was calculated from the number of normal seedlings. Statistical tolerances for differences among replications specified by CFIA and the International Seed Testing Association (Miles, 1963) were met.

2-, 10 and 11-d germination procedures. Forty-eight hours after the start of the 7-d germination procedure, the percentage of seeds with observable axis emergence was obtained to yield the 2-d germination result. The Canadian Methods and Procedures for Testing Seed (CFIA, 1997) allow seed analysts to extend germination measurements for canola to 10 or 11 days, if it appears that germination is not complete at 7 d. Consequently, we continued all germination analyses to 11 d and counted normal seedlings on d 10 and d 11 in addition to day 7.

Vigor index. Canola seed vigor index was calculated as described by Elliot et al. (2005) from seed weight and the official germination results (20/20 Seed Labs). Vigor index is the arithmetic product of germination percentage and seed weight.

***Seed analysis by commercial seed laboratories*.**

Ten-gram subsamples of each seed sample were sent to 20/20 Seed Labs (Nisku, AB) and Biovision Seed labs (Edmonton, AB) for vigor and official germination analyses. 20/20 Labs tested the seed with their pre-chill vigor test and the official germination test for canola. The 20/20 germination procedure followed CFIA (1997) rules including the option to extend

testing to 10 or 11 d at the analyst's discretion and the option of reducing the temperature to 15 ºC for 16 hours. Biovision Labs performed their cool vigor stress test and a 7-d germination for canola. The Biovision germination procedure also followed the CFIA (1997) rules, but was terminated at 7 days, without the option of extending the test to 10 or 11 d, even though it is their standard procedure to do so for official germination analysis. A significant number of samples were extended beyond 7 d by 20/20 Labs. Thus, although we had planned for official germination analyses from two commercial laboratories, official germination results were obtained only from 20/20 Seed Labs. Germination analyses at the commercial laboratories, but not at BRC, were performed by, or under the direction of, certified seed analysts.

Pre-chill vigor test. Two hundred seeds were distributed on the surface of a 4:1 mixture of potting soil and silica sand in 10- × 10-cm plastic boxes and loosely covered with 0.6 cm of soil. The soil was lightly watered and the boxes, without lids, were incubated at 5 ºC without light for 7 d then at 15 ºC for 16 h (dark) and 25 ºC for 8 h (light) per d for 5 d. Normal seedlings with a total root plus hypocotyl length of 2.5 cm or more were counted as vigorous and expressed as a percentage of the number of seeds planted.

Cool vigor stress test. Fifty seeds were distributed on the surface of a sand and soil mixture in 10- × 10-cm plastic boxes. The soil was lightly watered and the boxes, with lids in place, were incubated at 5 ºC for 6-7 d, then at 15 ºC for 16 h (dark) and 25 ºC for 8 h (light) per d for 5 d. Normal seedlings with uniform, healthy growth were counted as vigorous and expressed as a percentage of the number of seeds planted.

Statistical analysis

Part I. The ability of Vigorscore to predict field seedling emergence and growth was investigated with correlation analysis (CORR procedure of SAS) (SAS Institute Inc, 1999). Data were transformed (logit or square root) if necessary to improve distribution normality. In all but three data sets the absolute values for skewness and kurtosis were less than 1.0 after any transformations. The exceptions were a kurtosis value of -1.18 for the square root of Vigorscore for open-pollinated varieties, a skewness value of -1.14 for logit transformation of percentage emergence for hybrid plus synthetic genotypes and a kurtosis value of -1.45 for the logit transformation of leaf area growth rate for hybrid and synthetic genotypes. Although the square root of Vigorscore had a kurtosos value of -1.18, the data set passed the Kolmogorov-Smirnov goodness-of-fit test for normality (CORR procedure of SAS) (SAS Institute Inc, 1999). Transformations employed are indicated in the figures. Data for open-pollinated varieties and hybrid plus synthetic genotypes were fitted separately to a linear model.

Part II. The ability of the 12 laboratory assays to predict field seedling performance was investigated with correlation analysis using the CORR procedure of SAS (SAS Institute Inc, 1999). To perform the analysis, laboratory and field data were transformed if necessary to improve distribution normality. In all but one case (leaf area per plant, untransformed), one or more of the normality or goodness-of-fit tests in the Univariate procedure of SAS (SAS Institute Inc, 1999) were satisfied before or after transformation. Original datasets that met one or more of the tests were not transformed. In all but two of the data sets the absolute values for skewness and kurtosis were less than 1.0 after any transformations. The exceptions were the power transformation of Vigorscore (coefficient of kurtosis = -1.08) and vigor index (untransformed; coefficient of kurtosis = 1.18) The composite field variable, seedling establishment, was calculated with untransformed component variables. The composite variable did not require transformation, even though most of its component variables did require transformation prior to correlation analysis of the individual variables. Transformations employed are described in Table 1. Correlation analysis was performed using results of seed samples in three official germination ranges: 34-99, 75-99 and 90-99 %. Multiple comparisons among correlation coefficients (r) were performed in a manner analogous to Newman-Keuls-type multiple range testing as described by Zar (1984).

Results

***Field conditions*.**

Temperature and precipitation during the field trials is shown in Tables 2 to 4. Temperatures

for the six seedling establishment trials planted in May 2005 and 2006 was within 1 SD of the 30-yr average for the location. One seedling establishment trial was planted in June, 2004, at higher temperatures. Precipitation is not shown for the seedling establishment trials because the plots were irrigated nightly during emergence to prevent soil crusting.

Part I—Prediction of seedling performance by Vigorscore and Vigorcheck

Seedling emergence. Correlation coefficients between head space ethanol (Vigorscore) and three measures of emergence--percentage emergence, days to 50 % emergence and maximum emergence rate--for open-pollinated and hybrid (including synthetic) genotypes varied from -0.65 to -0.86 (Table 5). The highest absolute value was obtained for days to 50 % emergence for open-pollinated genotypes. Color results obtained with Vigorcheck were consistent with ethanol concentrations obtained with Vigorscore (Fig. 4).

Seedling leaf area. Measurements of leaf area correlated nearly as well as emergence with Vigorscore results for open-pollinated varieties, but less so for hybrid genotypes (Table 5). The correlation values varied from -0.19 to -0.78. The highest absolute value was obtained for leaf area/plot for open-pollinated genotypes. Correlations on a per plant basis were less than those on a per plot basis and values for hybrid and synthetic genotypes were less than those for open-pollinated varieties. Graphs of the data, including with Vigorcheck (color) results, are shown in Fig. 5.

Seedling weight. The correlation coefficients between seedling weight measurements and Vigorscore results varied from -0.44 to -0.74. (Table 5). The highest absolute value was obtained for fresh seedling weight/plot. As with leaf data, correlations on a per plant basis were less than those on a per plot basis. Graphs of the data, including Vigorcheck (color) results, are shown in Fig. 6.

Seedling emergence and leaf growth profiles. Typical S-shaped patterns, commonly found in emergence studies, were observed when accumulated daily emergence was plotted (Fig. 7). Good quality seed, as determined by a yellow result in the color assay, emerged more rapidly and to a higher percentage than deteriorated seed for both open-pollinated and hybrid plus synthetic genotypes, which is consistent with results shown in Fig. 4. Open-pollinated seed lots yielding a yellow color emerged more rapidly than those yielding a green color, which, in turn, emerged more rapidly than those yielding a blue color. Hybrid and synthetic genotypes yielding yellow and green colors emerged at about the same rate and more rapidly than those yielding a blue color.

Leaf area expansion followed a typical exponential growth function (Fig. 7). Leaf area from open-pollinated seed lots yielding a yellow color grew more rapidly than leaf area from seed yielding green or blue colors. On the other hand, leaf area expansion of hybrid and synthetic seedlings tended to be clustered together and not separated by vigor, which is consistent with the lower correlations found for leaf growth measurements with hybrid and synthetic genotypes (Table 5,).

Yield trials. Oilseed yield per plot, expressed as a percentage of the highest value found within each genotype, was significantly correlated with the results of the Vigorscore assay for open-pollinated varieties in one out of two trials and for hybrid genotypes in one out of three trials ($P =< 0.05$) (Table 6). Color results (Vigorcheck) were consistent with Vigorscore results in all trials (Fig. 8). Plant counts at oilseed harvest were significantly correlated with Vigorscore results in the same trials in which significant yield correlations were found (Table 6). Graphs of the plant count data, including Viogorcheck (color) results, are shown in Fig. 9.

Part II—Evaluation of 12 seed deterioration tests for predicting canola seedling performance in the field

Results of the Vigorscore assay and those of 11 other laboratory seed tests were evaluated for their ability to predict field performance of canola. Data are presented in three germination ranges determined with the official procedure (CFIA, 1997): 34-99, 75-99 and 90-99 % germination.

Seedling establishment. Seedling establishment is a composite variable calculated from the results of three emergence and three leaf growth variables. Results of seedling establishment were correlated with the results of the 12

laboratory seed quality methods. Absolute values of r for 34-99 % germination varied from 0.18 to 0.88 (Table 7, Fig. 10). A similar range of r values was found for the 75-99 % and 90-99% germination ranges, but the number of methods yielding higher r values decreased as the germination range was restricted (Tables 8 and 9, Fig 11 and 12). The r for seed mass and the vigor index were significantly lower than the r for 10 laboratory methods in the 34-99 and 75-99 % germination ranges and 1 laboratory method in the 90-99 % germination range (P <= 0.05). Eight methods yielded r => 0.8 in the 34-99 % range, four methods in the 75-99 % range and one method in the 90-99 % range. The r for the official germination procedure decreased from 0.83 to 0.59 as the germination range decreased.

Although the ranking of the laboratory methods varied slightly among the three germination ranges, correlation coefficients between seedling establishment and Vigorscore, cool vigor stress test, 2-d germination and prechill vigor test were consistently among the highest values. For the two highest germination ranges (75-99 % and 90-99 %), Vigorscore yielded the highest correlation coefficients. Although there was relatively little variation among the top laboratory procedures in the 34-99 % and 75-99 % ranges, the difference in correlation coefficients between the highest-ranked method (Vigorscore, r = 0.84) and the second method (2-d germination, r = 0.73) in the 90-99 % range was substantial.

Seedling emergence. In general, seedling emergence variables were more highly correlated with the laboratory variables than leaf area and seedling weight variables. Eight laboratory tests – 2-d, 7-d (BRC), 7-d (Bio),and official germination as well as Vigorscore, seedling growth, the pre-chill vigor test and the cool vigor stress test – yielded one or more correlation coefficients with emergence measurements that were equal to or greater than 0.8 in the 34-99 % germination range (Table 7). Six laboratory variables – 2-d, 7-d (Bio) and official germination, as well as Vigorscore, seedling growth, and the pre-chill vigor test – yielded correlation coefficients equal to or greater than 0.8 in the 75-99 % range (Table 8), while only Vigorscore yielded a correlation coefficient greater than 0.8 in the 90-99 % range (Table 9). Correlation coefficients for seed weight and vigor index were significantly less than that for the highest ranking laboratory test (P =< 0.05). Across all germination ranges the seedling emergence variable most highly correlated with all of the laboratory variables was d to 50 % emergence (average r = 0.64). Days to 50 % emergence also was the most highly correlated of all the field variables. The emergence variable least correlated with the laboratory variables was d to maximum emergence (average r = 0.43).

Seedling leaf area. Nine laboratory tests – 2-d, 7-d (BRC), 7-d (Bio), 10-d and official germination as well as Vigorscore, seedling growth, the pre-chill vigor test and the cool vigor stress test – yielded one or more correlation coefficients with respect to leaf area variables that were equal to or greater than 0.7 in the 34-99 % germination range (Table 7). Three laboratory variables – 2-d germination, Vigorscore and the cool vigor stress test – yielded correlation coefficients equal to or greater than 0.7 in the 75-99 % range (Table 8), while only Vigorscore yielded a correlation coefficient equal to or greater than 0.7 in the 90-99 % range (Table 9). Correlation coefficients for seed weight and vigor index were significantly less than that for the highest ranking laboratory test in both the 34-99 and 75-99 % germination ranges, while only seed weight differed in the 90-99 % range (P =< 0.05). Across all germination ranges the leaf area variable most highly correlated with the lab variables was leaf area per plot (average r = 0.54) while the one least correlated with the lab variables was leaf area per plant (average r = 0.45).

Seedling weight. Seven laboratory tests – 2-d, 7-d (Bio) and official germination as well as Vigorscore, seedling growth, the pre-chill vigor test and the cool vigor stress test – yielded one or more correlation coefficients with respect to seedling weight variables that were equal to or greater than 0.7 in the 34-99 % germination range (Table 7). Two laboratory variables – 2-d germination and the cool vigor stress test – yielded correlation coefficients equal to or greater than 0.7 in the 75-99 % germination range (Table 8), while no laboratory variables had a correlation coefficient equal to or greater than 0.7 in the 90-99 % range (Table 9). Correlation coefficients for seed weight and vigor index frequently differed from that for the highest ranking laboratory test in the 34-99 and 75-99 % germination ranges (P =< 0.05), but not

in the 90-99 % range. Across all germination ranges the seedling weight variable most highly correlated with all laboratory variables was dry weight per plot (average r = 0.56) while the least correlated was dry weight per plant (average r = 0.47).

Part II summary. Results of Part II, sorted by correlation coefficients over three germination ranges, are summarized in Table 10.

Discussion

Field conditions

It is a generally accepted principle of vigor testing that high-vigor seed will have a performance advantage over low-vigor seed under adverse (usually cold) field conditions, and that the advantage will be less under good field conditions (ISTA, 1995). Indeed, it has sometimes been stated that high vigor provides an advantage only in adverse environments (Hamman *et al.*, 2002). This concept of a vigor x soil temperature interaction, though, was developed with large-seeded crops, particularly maize, soybeans and peas (Egli and TeKony, 1996; Johnson and Wax, 1978). In contrast, a vigor x soil temperature interaction has not been found with small-seeded crops (Perry, 1982). In studies with flax, sugar beet and onion, no interaction between seed vigor and field conditions was observed in trials with seeding dates varying from very early to very late (Bekendam *et al.*, 1987). Bekendam et al. (1987) found that high vigor lots of the small-seeded crops showed the same percentage improvement in emergence with improving field conditions as did low-vigor lots. Similar results were reported by Perry (1978) for carrot and beet seeds. Hegarty (1974) reported no interaction between sowing date and seed lot quality with respect to emergence of calabrese (broccoli) even though soil temperature varied from 6.4 to 13.4 ºC over four sowing dates. Elliott et al. (2005) concluded that the standard germination test and a cold germination test (pre-chill test) "provided an equally good indication of establishment in warm dry soil, cool moist soil or cool dry soil." Such a result (Elliott *et al.*, 2005) is consistent with the absence of a vigor x soil temperature interaction. Perry (1982) speculated on the reasons for the different vigor x soil temperature response of small and large seeds. He pointed out that, although small seeds may be subject to the same environmental stresses as large seeds, the affects of soil impedance are much more important for small seedlings. It appears that soil impedance may be the predominant or limiting environmental factor for small seedlings. Consequently, the vigor x soil temperature interaction observed with larger seeds/seedlings may be masked with small seeds/seedlings. It is well known that canola is very sensitive to planting depth and crust formation, which is consistent with the soil impedance hypothesis. Thus, an important exception to the vigor x soil temperature interaction is found with small-seeded crops, including the brassicacae.

Since we did not expect to observe a vigor x soil temperature interaction with canola in our field trials, we decided to test soil temperatures that would apply to the greatest majority of canola growers in most years. Consequently, six of the seven seedling trials were sown in mid May. The 2004 trial was sown in June after a trial planted in mid-May was lost to ground squirrel damage. Comparison of the long-term average temperature for May with temperatures in May 2005 and 2006 showed that those experiment years were, indeed, average. Furthermore, the present results show that the effects of canola seed deterioration on seedling establishment under average temperature conditions are substantial and that those effects can be predicted by a number of seed quality tests. Even though one would expect the emergence of both high- and low-vigor canola to be lower under adverse field conditions, the proportional difference in performance between high- and low-vigor seed should remain relatively constant. Since canola seed is not expected to show a vigor x soil temperature interaction, the impact of low vigor seed on canola production in Western Canada is being felt by all growers in all years, not just by those growers experiencing adverse field conditions in some years.

Insect damage was minimized in the trials by an aggressive spraying program. Thus, the ability of Vigorscore and other tests to predict resistance of canola seedling to insect damage was not evaluated. Nevertheless, damage by insect pests is a very important factor affecting canola establishment. Elliott et al. (2008; 2007b) found that large canola seedlings derived from large seeds were more resistant to flea beetle damage than small seedlings derived from small seeds. Thus, one might expect also that large seedlings from non-deteriorated seed

would have greater flea beetle resistance than small seedlings from deteriorated seed.

Isolating seed deterioration effects.
In order to study the efficacy of seed quality tests in this study, vigor effects derived from seed deterioration were isolated from vigor effects derived from genetic variation by expressing field seedling results as a percentage of the highest or lowest value within each genotype. Expression of results in this manner also permitted comparison of results across trials. When results are expressed as percentage of high or low in genotype, differences in emergence and growth are due to seed deterioration.

Vigorscore
When evaluating analytical methods, linear correlation coefficients are a measure of accuracy, with values of 1.0 or -1.0 indicating perfect accuracy and zero indicating no analytical reliability whatsoever. Coefficients for Vigorscore and seedling emergence variables were -0.86 for days to 50 % emergence for open-pollinated types and -0.84 for final percentage emergence for hybrid types. The high correlation coefficients are consistent with emergence being the primary field vigor characteristic (ISTA, 1995). We conclude that Vigorscore is a good predictor of canola field emergence, and therefore vigor, for both open-pollinated and hybrid seed types.

Early in our studies, we observed that vigor could affect seedling growth (for example, see Figure 3). Thus, we included measurements of leaf area and seedling mass in the present study. For open-pollinated varieties, Vigorscore correlated with leaf growth and seedling mass nearly as well as with emergence. Thus, Vigorscore accurately predicted leaf expansion and dry matter accumulation for open-pollinated seed types. Results, however, were not as good for hybrid and synthetic genotypes (Table 5). Hybrid leaf area growth rate was relatively high and showed little variation among seed lots (Figure 7). Once emerged, seedlings from deteriorated hybrid seed were able to grow at a rate similar to that of high quality seed. The reason for this likely is related to the physiological cause of poor vigor. The fundamental cause of poor vigor arising from seed deterioration is believed to be damage to cell membranes (Powell, 1988). However, once water has been imbibed by deteriorated seed, repair of the membrane damage begins and, if damage has not been too severe, the seed and seedlings eventually recover. Our results suggest that hybrid types are able to recover from the damaging effects of deterioration more rapidly than open-pollinated types. Hybrid seedlings that manage to emerge appear to have largely recovered from the effects of deterioration. This is consistent with the relatively low correlation coefficients obtained for hybrid types when results were expressed on a per plant basis compared to a per plot basis (Table 5).

Vigorcheck
We did not attempt correlation analysis with the Vigorcheck results. Nevertheless, the results shown in Figures 4-7 demonstrate that color results from the Vigorcheck assay were consistent with Vigorscore results and with seedling performance. We view Vigorcheck as a screening procedure that could be used by growers prior to planting canola seed. It is ideal as an on-farm screening procedure because if does not require laboratory facilities or training. Nor does it require close control of incubation temperature and may be run at normal room temperature. The Vigorcheck assay was designed to have very high reliability for identifying high quality seed. An examination of figures 4-6 shows that the potential of a false positive result is very low. In no case was there a disagreement between Vigorcheck and Vigorscore results.

A survey conducted by our laboratory in 2005 showed that 92 % of 129 seed lots planted by 42 growers that spring produced a yellow color (positive result) in the assay. Eight percent yielded a blue color, which indicated that some deterioration had occurred in the seed. However, the color assay does not distinguish between minor and severe deterioration of seed and further testing is desirable when a negative result is obtained.

Seedling growth metabolism
Leaf area and seedling weight data expressed on a per plot basis include variation in number of plants/plot due to differences in emergence plus variation in growth rate per plant due to differences in seedling metabolism. The data expressed on a per plant basis, though, eliminates the effect due to differences in emergence and leaves only the effect of seed vigor on seedling growth metabolism. Three

correlation coefficients for leaf growth and seedling mass for open-pollinated types and one for hybrid and synthetic types from data expressed on a per plant basis were statistically significant (P =< 0.05) (Table 5). Thus, seedling growth metabolism after emergence was noticeably reduced by seed deterioration for open-pollinated types and less so for hybrid and synthetic types.

Comparison of Vigorscore with other seed quality tests

The seedling establishment variable. Comparisons among the laboratory methods were complicated by the fact that, for each of 12 laboratory variables, there were observations on 9 field variables. To simplify the data interpretation a composite field variable, called seedling establishment, was calculated. Since it can be argued that the most important aspects of seedling establishment are rapid and uniform emergence followed by canopy closure, the six most important emergence and leaf growth variables were included in the composite variable.

Statistical analysis and seed germination ranges. In method comparison studies of this sort, it is important to test the data ranges of interest, because results of correlation analysis vary with the range of data analyzed. We have compared the ability of laboratory results to predict seedling field responses over three data ranges: 1) all available data, which includes results from seeds with 34-99 % official germination, 2) data from seed samples with 75-99 % official germination and 3) data from seed samples with 90-99 % official germination. We expect that 75 % germination represents the practical lower limit for the germination of canola seed lots in the Western Canadian seed trade. However, since virtually all canola seed on the market is certified #1 and must have achieved => 90 % germination at some time, the 90-% threshold also is important. There is interest in being able to identify seed lots with superior field performance within the 90-100 % germination range. Seed with < 90 % germination will be rejected by the seed trade and not be tested further. However, seed may deteriorate in storage after official germination analysis and decline to less than 90 % germination prior to planting.

When using correlation analysis in method comparison studies, it is usually not possible to make definitive conclusions about differences among methods. The number of samples required to detect significant (P =< 0.05) differences among all but extreme r values is prohibitive for field studies. Another approach, determining if r values are significantly greater than zero, is often employed. However, most r values usually are greater than zero, and the results still do not indicate whether or not there are any differences among most r values, themselves. Most authors of method comparison studies simply tabulate correlation coefficients (or coefficients of determination) and discuss the resultant ranking of the methods. This is the approach we have adopted, although we also performed multiple range tests of r values. Except for differences between the high-ranking methods and seed weight or vigor index (P =< 0.05, Tables 3-5), the variations among correlation coefficients in the present study should be considered to be trends that may or may not be verified if the study could be repeated with a much larger number of samples.

Ranking the seed tests. The results in Tables 7-9 and particularly the seedling establishment summary in Table 10 serve to rank the seed tests evaluated in the present study. Seed tests with correlation coefficients => 0.8 can be considered to be good measures of field performance. Interestingly, the official germination procedure achieved r => 0.8 only in the broadest germination range studied, 34-99 % (Table 10). In the 90-99 % range official germination was a relatively poor predictor of field performance (r = 0.59, Table 10). Vigorscore, the cold-type vigor tests and the 2-d germination (axis emergence) tests were the top-ranked tests across all germination ranges. Only Vigorscore had an r value => 0.8 in the 90-99 % germination range.

Ruggedness of seed quality tests is an important characteristic if tests are to be routinely used in certified seed laboratories. Vigorscore likely is the most rugged of the four top-ranked procedures, mainly because of its relative insensitivity to variations in incubation temperature and incubation time. The 2-d germination procedure is probably the least rugged because it is very sensitive to temperature and time variations (measurements must be made nearly exactly 48 h after starting incubation).

Germination analysis. The 7-d germination procedure conducted at BRC was consistently higher-ranked than the official germination procedure for predicting seedling establishment. Although the BRC procedure mostly followed the CFIA rules there were four differences between the BRC protocol and the official germination procedure performed at 20/20 Seed Labs. Firstly, the analysis was terminated after 7 d at BRC, whereas the CFIA rules include an option for extending the test beyond 7 d. The option was exercised during official germination analysis at 20/20 Seed Labs. Secondly, one of the indicators of an abnormal seedling in the CFIA rules is a "substantially shortened hypocotyl". At BRC, a "substantially shortened hypocotyl" was interpreted as a hypocotyl less than 7 mm, whereas seed analysts performing the official germination protocol used the subjective interpretation specified by the rules. Thirdly, the temperature regime adopted at BRC was a constant 25 ºC for 24 h/d, whereas the regime adopted at 20/20 Seed Labs was 15 ºC for 16 h (dark) and 25 ºC for 8 h (light). Both regimes are permitted in the CFIA protocol. Fourthly, 168 seeds were analyzed in the BRC procedure instead of the minimum 200 seeds specified by CFIA (1997). The first three differences might be expected to contribute to the apparently enhanced predictability of the BRC germination method.

Seed weight. Results showed that canola seed weight within a genotype is not a good predictor of seedling field establishment. Seed weight and vigor index, which is derived from seed weight, were substantially poorer predictors of seedling establishment than the highest-ranked laboratory procedures evaluated in the present study and are not recommended for predicting field establishment when making comparisons within genotypes. There is, however, little doubt that differences in canola seed weight or size over broad ranges affect seedling performance (Elliott *et al.*, 2008; Elliott *et al.*, 2007a; Elliott *et al.*, 2007b). Nevertheless, within the relatively small range of seed weights found among seed lots of the same genotypes in the Western Canadian canola seed trade, the effect is insignificant.

Supplemental vigor analysis. Although germination analysis is required for certification of canola seed in Canada, it is unlikely that the official germination procedure is the best predictor of canola seedling establishment in the field. Thus, supplemental analysis by one of the high-ranking procedures in the present study appears to be justified. In the 34-99 and 75-99 % germination ranges, it is unlikely that there is any difference among the four top-ranked methods: Vigorscore, the cool vigor stress test, 2-d germination and the pre-chill vigor test. In the range of 90-99 % germination, the Vigorscore assay is likely to provide the most useful supplemental information because of its substantially higher correlation coefficient compared to all other methods evaluated. Since nearly all canola seed with < 90 % germination is excluded from the seed trade, the supplemental vigor method of choice for the Canadian canola seed trade is the Vigorscore assay.

ISTA validation

Presently, there is no ISTA-validated procedure for determination of canola seed vigor. It seems likely that the Vigorscore assay could meet the ISTA requirements for validation (ISTA, 2006). The present project provides data evaluating the reliability of the new assays for predicting field performance, which is one of the ISTA requirements. Another requirement for ISTA validation, a successful inter-laboratory evaluation of the assay, has not been undertaken. Now that the development of the new assay and three years of field evaluation have been completed, an inter-laboratory comparison with the object of achieving ISTA validation could be undertaken.

Conclusions

Part I

- Vigorscore is a suitable assay for evaluating the quality of canola seed with respect to seedling emergence and seedling growth in the field.
- Vigorcheck is a suitable screening procedure for canola seed deterioration.
- Seedlings from deteriorated open-pollinated seed grew significantly slower after emergence than non-deteriorated seed. The effect was less noticeable in hybrid and synthetic genotypes apparently due to rapid recovery from the effects of seed deterioration.

Part II

- The laboratory tests most suitable for predicting canola seedling establishment in

the field were Vigorscore, the cool vigor stress test, 2-d germination (axis emergence) and the pre-chill vigor test.

- The only tests that were significantly inferior to others in statistical evaluation were seed weight and vigor index ($P < 0.05$).
- The correlation of official germination results with field establishment of canola seedlings was intermediate with respect to that of the 11 other laboratory tests. Consequently, supplemental analysis by more highly ranked tests may provide a superior estimate of seedling establishment than official germination alone.
- On average, the emergence variable most highly correlated with all laboratory variables was d to 50 % emergence, while the most highly correlated leaf area variable was leaf area per plot (check at the 3-4 leaf stage) and the most highly correlated seedling weight variable was dry weight per plot (check at the 3-4 leaf stage).
- Vigorscore was the most highly ranked test for prediction of seedling establishment in the 75-99 and 90-99 % germination ranges.
- Vigorscore appeared to be substantially better than all other tests with respect to Its ability to predict seedling establishment in the 90-99 % germination range.
- The cool vigor stress test was the most highly ranked test for prediction of seedling establishment in the 34-99 % germination range.
- The use of relatively good field conditions for comparing the prediction capabilities of vigor/deterioration tests in the present study was justified because emergence of brassicacae and other small seeded crops do not demonstrate the vigor x soil temperature interaction characteristic of large-seeded crops.

Acknowledgement

Data analysis by Jianzhong (John) Huang and advice on data transformations from Alan P. Moulin are gratefully acknowledged.

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Table 1. Transformations employed during correlation analysis of field and laboratory variables in Part II.

Variable	Units	Abbrev-iation	Transformation
Laboratory variables (Brandon Research Centre)			
2-d axis emergence	%	2dgerm	logit(0.99 × 2dgerm / 100)
7-d germination	%	7dgermBRC	logit(0.99 × 7dgermBRC / 100)
10-d germination	%	10dgerm	logit(0.99 × 10dgerm / 100)
11-d germination	%	11dgerm	logit(0.99 × 11dgerm / 100)
Vigorscore	µl/l	EtOH	$-1 \times EtOH^{0.4}$
seed weight	g / 100 seeds	seedwt	no transformation required
seedling growth in hydroponics	% of check	hydro	no transformation required
vigor index	(% × g / 100 seeds) / 10	vigdex	no transformation required
Laboratory variables (20/20 Seed Labs)			
official germination	%	ISTAgerm	logit(0.99 × ISTAgerm / 100)
pre-chill vigor test	%	vig2020	logit(0.99 × vig2020 / 100)
Laboratory variables (Biovision seed labs)			
cool vigor stress test	%	vigBio	logit(0.99 × vigBio / 100)[a]
7-d germination	%	7dgermBio	logit(0.99 × 7dgermBio / 100)
Field variables			
days to 50 % emergence	% of low in genotype	d50emrg	$\log(stdz_{0.5,0.1}(-10{,}000 / d50emrg))$[b]
days to max emergence	% of low in genotype	dmaxemrg	10,000 / dmaxemrg
dry weight per plant	% of high in genotype	dwplant	no transformation required
dry weight per plot	% of high in genotype	dwplot	no transformation required
leaf area per plant	% of high in genotype	lfplant	no transformation required
leaf area per plot	% of high in genotype	lfplot	no transformation required
leaf area growth rate	% of high in genotype	lfgrw	$\log(stdz_{0.5,0.1}(-1*lfgrw))$[b]
max emergence rate	% of high in genotype	MER	$MER^{0.5}$
max % emergence	% of high in genotype	%emrg	logit(0.99 × emrg / 100)[a]
seedling establishment[c]	% of high in genotype	estblmt	no transformation required

[a] $logit(j) = \ln(j / (1-j))$, $0.01 =< j =< 0.99$

[b] $stdz_{0.5,0.1}$ = standardized to mean = 0.5 and SD = 0.1

[c] Composite of six seedling emergence and leaf expansion variables (untransformed) as described in the text: d50emrg, MER, %emrg, lfplot, lfplnt and lfgrw.

Table 2. Average daily temperatures (ºC) for the months of the seedling establishment field trials. One 2004 trial was planted on June 22 and completed on July 13. Six 2005 and 2006 trials were planted on May 11-15 and completed on June 8-13. Trials were concluded when the check reached the 3-4 leaf stage. Temperature data was collected at the AAFC Brandon Research Centre main complex (Brandon CDA, Environment Canada, National Climate Data and Information Archive) where the trials were conducted.

Month	Average daily Temperature (ºC)			
	30-yr average	2004	2005	2006
May	11.8 ± 2.2	-	9.9	12.2
June	16.6 ± 1.8	14.2	17.1	17.8
July	18.9 ± 1.4	18.0	-	-

Table 3. Average daily temperatures and monthly precipitation for the two conventional yield trials. Weather data is from the Brandon air port (Brandon A, Environment Canada, National Climate Data and Information Archive), which is 23 km from the research site (Brandon Research Centre remote site). The 2004 trial was planted on June 4 while the 2006 trial was planted on May 9. Plants were pushed (stems kinked and laid down in the plots) at about 30 % seed color change on September 10 and August 16, respectively. Oilseed yield was obtained by combining when the pods were ripe.

	30-yr average (1971-2000)		2004 yield trial		2006 yield trial	
Month	Temperature (ºC, average ± SD)	Precip-itation (mm)	Average temperature (ºC)	Precip-itation (mm)	Average temperature (ºC)	Precip-itation (mm)
May	11.4 ± 2.1	52.7	6.3	145	11.6	41
June	16.1 ± 1.7	74.4	13.7	52	17.2	82
July	18.4 ± 1.5	75.8	17.4	80	19.9	8
August	17.5 ± 1.8	69.2	13.6	95	18.9	76
September	11.4 ± 1.4	50.1	13.1	25	12.1	75

Table 4. Average daily temperatures and monthly precipitation for the two yield trials derived from seedling establishment trials. Weather data is from the Brandon Research Centre main complex (Brandon CDA, Environment Canada, National Climate Data and Information Archive) where the trials were conducted. The trials were planted on May 12. Plants were cut at the ground and bagged July 21 and August 2 at about 30 % seed color change.

	30-yr average (1971-2000)		2006 yield trials	
Month	Temperature (ºC, average ± SD)	Precipitation (mm)	Average temperature (ºC)	Precipitation (mm)
May	11.8 ± 2.2	53	12.2	40
June	16.6 ± 1.8	76	17.8	84
July	18.9 ± 1.4	73	20.5	16
August	18.0 ± 1.8	70	19.4	75
September	11.9 ± 1.5	48	12.5	68

Table 5. Pearson correlation coefficients (r) between results of canola seed deterioration assay (Vigorscore; µl ethanol/l, transformed by square root) and 10 field measurements of seedling performance. Emergence results were obtained by fitting data to a modified Gompertz function. Leaf area growth rates were obtained by fitting data to a three-parameter exponential growth function. Results were expressed as a percentage of the highest or lowest value within each genotype (% hi gtype, % lo gtype). Data sets, except for two, were transformed to minimize skewness and kurtosis and thereby approach a normal distribution. n = number of seedlots tested.

Field variable group	Field variable	Units	Data transform-ation	Open-pollinated varieties		Hybrid and synthetic genotypes	
				r	n	r	n
Emergence	Percentage emergence	% hi gtype	logit	-0.65*	56	-0.84*	29
	D to 50 % emergence	% lo gtype	logit	-0.86*	56	-0.79*	29
	Max emergence rate	% hi gtype	square root	-0.76*	56	-0.80*	29
Leaf growth	Leaf area/plot	% hi gtype	none	-0.78*	56	-0.42*	29
	Leaf area/plant	% hi gtype	none	-0.70*	56	-0.19	29
	Leaf area growth rate	% hi gtype	logit	-0.77*	56	-0.43*	29
Seedling weight	Fresh weight/plot	% hi gtype	none	-0.74*	43	-0.65*	16
	Fresh weight/plant	% hi gtype	none	-0.68*	43	-0.44	16
	Dry weight/plot	% hi gtype	none	-0.73*	43	-0.70*	16
	Dry weight/plant	% hi gtype	logit	-0.66*	43	-0.50*	16

*Correlation coefficients are statistically significant (P <= 0.05).

Table 6. Pearson correlation coefficients (r) between results of canola seed deterioration assay (Vigorscore; µl ethanol/l, transformed by square root) and harvest measurements (untransformed) for 59 seedlots of 10 open-pollinated and hybrid *Brassica napus* genotypes in four trials in 2004 and 2006. Trials 2004 and 2006c were conventional yield trials, while trials 2006a and 2006b were converted seedling establishment trials (see trial descriptions in the text).

Trial	Oilseed yield		Plants/m^2 at oilseed harvest	
	Open-pollinated varieties	Hybrid genotypes	Open-pollinated varieties	Hybrid genotypes
2004	-	-0.55*	-	-0.78*
2006a	-0.80*	-	-0.86*	-
2006b	-	0.38	-	-0.41
2006c	-0.037	-0.28	0.049	-0.22

*Correlation coefficients are statistically significant (P <= 0.05).

Table 7. Pearson correlation coefficients (r) between field and laboratory variables for seed samples with 34-99 % germination.

Lab variables[d] / *Field variables*[d] *(seed samples, n)*		Brandon Research Centre								20/20 Labs		Biovision Labs	
		2d-germ	**7dgerm-BRC**	**10d-germ**	**11d-germ**	**EtOH**	**hydro**	**se100m**	**vigdex**	**ISTA-germ**	**vig-2020**	**7dgerm-Bio**	**vigBio**
Emergence	**%emrg[e] (51)**	0.74[a]	0.84[a]	0.75[a]	0.73[a]	0.71[a]	0.66[a]	-0.10[b]	0.52[a]	0.75[a]	0.77[a]	0.76[a]	0.72[a]
	d50emrg[e] (51)	0.83[a]	0.83[a]	0.75[ab]	0.73[ab]	0.89[a]	0.83[a]	-0.08[c]	0.51[b]	0.81[ab]	0.86[a]	0.84[a]	0.83[a]
	dmaxemrg (51)	0.53[a]	0.59[a]	0.53[a]	0.5[a]	0.65[a]	0.57[a]	-0.03[b]	0.33[ab]	0.59[a]	0.58[a]	0.59[a]	0.59[a]
	MER[e] (51)	0.75[a]	0.78[a]	0.79[a]	0.78[a]	0.88[a]	0.75[a]	-0.17[c]	0.46[b]	0.81[a]	0.83[a]	0.81[a]	0.81[a]
Leaf area	**Lfgrw[e] (51)**	0.80[ab]	0.79[ab]	0.73[ab]	0.69[ab]	0.74[ab]	0.72[ab]	-0.26[c]	0.45[b]	0.73[ab]	0.77[ab]	0.73[ab]	0.82[a]
	Lfplant[e] (51)	0.62[a]	0.57[a]	0.48[a]	0.48[a]	0.64[a]	0.55[a]	-0.10[b]	0.28[ab]	0.60[a]	0.63[a]	0.59[a]	0.74[a]
	Lfplot[e] (51)	0.73[ab]	0.73[ab]	0.66[ab]	0.64[ab]	0.75[ab]	0.68[ab]	-0.11[c]	0.45[b]	0.75[ab]	0.76[ab]	0.74[ab]	0.82[a]
Weight	**dwplant (39)**	0.60ab	0.51[ab]	0.39[ab]	0.37[ab]	0.58[ab]	0.59[ab]	0.04[b]	0.33[ab]	0.56[ab]	0.61[ab]	0.57[ab]	0.68[a]
	dwplot (39)	0.72[a]	0.69[a]	0.61[a]	0.57[a]	0.71[a]	0.73[a]	0.08[b]	0.58[a]	0.74[a]	0.75[a]	0.75[a]	0.79[a]
estblmt (51)		0.85[a]	0.85[a]	0.80[a]	0.78[a]	0.86[a]	0.78[a]	-0.18[b]	0.52[b]	0.83[a]	0.87[a]	0.85[a]	0.88[a]

[abc]Correlation coefficients within rows that do not have a common letter in the superscript are different (P =< 0.05).

[d]Data was transformed as described in Table 1. Also see Table 1 for abbreviations and units.

[e]Components of the composite variable, estblmt.

Table 8. Pearson correlation coefficients (r) between field and laboratory variables for seed samples with => 75 % germination.

Lab variables[d] / *Field variables*[d] *(seed samples, n)*		Brandon Research Centre								20/20 Labs		Biovision Labs	
		2d-germ	**7dgerm-BRC**	**10d-germ**	**11d-germ**	**EtOH**	**hydro**	**seedwt**	**vigdex**	**ISTA-germ**	**vig-2020**	**7dgerm-Bio**	**vigBio**
Emergence	**%emrg[e] (45)**	0.65^{ab}	0.77^{a}	0.58^{ab}	0.56^{ab}	0.59^{ab}	0.54^{ab}	-0.08^{c}	0.18^{bc}	0.70^{ab}	0.68^{ab}	0.65^{ab}	0.60^{ab}
	d50emrg[e] (45)	0.80^{a}	0.78^{a}	0.64^{ab}	0.62^{ab}	0.85^{a}	0.80^{a}	-0.05^{c}	0.26^{bc}	0.8^{a}	0.82^{a}	0.82^{a}	0.77^{a}
	dmaxemrg (45)	0.47^{ab}	0.57^{ab}	0.46^{ab}	0.43^{ab}	0.64^{a}	0.51^{ab}	-0.04^{b}	0.14^{ab}	0.55^{ab}	0.51^{ab}	0.52^{ab}	0.55^{ab}
	MER[e] (45)	0.69^{a}	0.70^{a}	0.69^{a}	0.69^{a}	0.86^{a}	0.68^{a}	-0.21^{b}	0.11^{b}	0.76^{a}	0.75^{a}	0.71^{a}	0.72^{a}
Leaf area	**Lfgrw[e] (45)**	0.73^{a}	0.68^{a}	0.53^{a}	0.48^{a}	0.67^{a}	0.66^{a}	-0.19^{b}	0.06^{b}	0.62^{a}	0.68^{a}	0.58^{a}	0.76^{a}
	Lfplant[e] (45)	0.65^{a}	0.56^{ab}	0.4^{abc}	0.3^{8abc}	0.61^{ab}	0.53^{ab}	-0.11^{c}	0.07^{bc}	0.50^{ab}	0.57^{ab}	0.51^{ab}	0.70^{a}
	Lfplot[e] (45)	0.71^{a}	0.67^{a}	0.52^{ab}	0.48^{ab}	0.70^{a}	0.62^{ab}	-0.09^{c}	0.14^{bc}	0.60^{ab}	0.66^{a}	0.60^{ab}	0.73^{a}
Weight	**dwplant (34)**	0.68^{a}	0.59^{a}	0.41^{a}	0.36^{a}	0.64^{a}	0.64^{a}	0.11^{a}	0.28^{a}	0.61^{a}	0.66^{a}	0.63^{a}	0.71^{a}
	dwplot (34)	0.71^{a}	0.66^{a}	0.47^{a}	0.41^{a}	0.66^{a}	0.69^{a}	0.19^{a}	0.39^{a}	0.67^{a}	0.69^{a}	0.68^{a}	0.71^{a}
	estblmt (45)	0.81^{a}	0.79^{a}	0.66^{a}	0.63^{a}	0.83^{a}	0.74^{a}	-0.14^{b}	0.16^{b}	0.77^{a}	0.81^{a}	0.78^{a}	0.82^{a}

[abc]Correlation coefficients within rows that do not have a common letter in the superscript are different (P =< 0.05).
[d]Data was transformed as described in Table 1. Also see Table 1 for abbreviations and units.
[e]Components of the composite variable, estblmt.

Table 9. Pearson correlation coefficients (r) between field and laboratory variables for 31 seed samples with =>90 % germination.

Lab variables[d] / *Field variables*[d] *(seed samples, n)*		Brandon Research Centre								20/20 Labs		Biovision Labs	
		2d-germ	7dgerm-BRC	10d-germ	11d-germ	EtOH	hydro	se100m	vigdex	ISTA-germ	vig-2020	7dgerm-Bio	vigBio
Emergence	%emrg[e] (31)	0.51^{abc}	0.70^{a}	0.49^{abc}	0.47^{abc}	0.60^{ab}	0.43^{abc}	-0.24^{c}	-0.04^{bc}	0.61^{ab}	0.54^{ab}	0.50^{abc}	0.5^{abc}
	d50emrg[e] (31)	0.71^{ab}	0.64^{ab}	0.43^{ab}	0.40^{ab}	0.77^{a}	0.69^{ab}	0.08^{b}	0.24^{ab}	0.56^{ab}	0.68^{ab}	0.60^{ab}	0.59^{ab}
	dmaxemrg (31)	0.43^{ab}	0.55^{ab}	0.36^{ab}	0.32^{ab}	0.71^{a}	0.45^{ab}	-0.22^{b}	-0.09^{b}	0.43^{ab}	0.43^{ab}	0.36^{ab}	0.51^{ab}
	MER[e] (31)	0.66^{ab}	0.68^{ab}	0.57^{ab}	0.58^{ab}	0.86^{a}	0.58^{ab}	-0.23^{c}	0.00^{bc}	0.61^{ab}	0.70^{a}	0.54^{ab}	0.59^{ab}
Leaf area	Lfgrw[e] (31)	0.63^{a}	0.59^{ab}	0.33^{ab}	0.26^{ab}	0.62^{a}	0.61^{a}	-0.14^{b}	-0.01^{ab}	0.38^{ab}	0.47^{ab}	0.28^{ab}	0.60^{a}
	Lfplant[e] (31)	0.66^{ab}	0.53^{ab}	0.28^{ab}	0.24^{ab}	0.64^{ab}	0.56^{ab}	-0.05^{b}	0.06^{ab}	0.46^{ab}	0.52^{ab}	0.44^{ab}	0.69^{a}
	Lfplot[e] (31)	0.66^{ab}	0.61^{ab}	0.38^{ab}	0.32^{ab}	0.71^{a}	0.59^{ab}	-0.07^{b}	0.08^{ab}	0.48^{ab}	0.54^{ab}	0.44^{ab}	0.65^{ab}
Weight	dwplant (24)	0.59^{a}	0.46^{a}	0.15^{a}	0.09^{a}	0.56^{a}	0.55^{a}	0.20^{a}	0.22^{a}	0.40^{a}	0.50^{a}	0.42^{a}	0.58^{a}
	dwplot (24)	0.59^{a}	0.53^{a}	0.21^{a}	0.13^{a}	0.60^{a}	0.60^{a}	0.26^{a}	0.30^{a}	0.41^{a}	0.49^{a}	0.43^{a}	0.54^{a}
estblmt (31)		0.73^{ab}	0.70^{abc}	0.50^{abc}	0.46^{abc}	0.84^{a}	0.65^{abc}	-0.14^{bc}	0.05^{c}	0.59^{abc}	0.68^{abc}	0.57^{abc}	0.70^{abc}

[abc]Correlation coefficients within rows that do not have a common letter in the superscript are different (P =< 0.05).

[d]Data was transformed as described in Table 1. Also see Table 1 for abbreviations and units.

[e]A component of the composite variable, estblmt.

Table 10. Summary of correlations between seedling establishment of canola (various genetic types) and results of seed quality tests over three germination ranges (Pearson correlation coefficients, r; values => 0.8 are highlighted). Seedling establishment was calculated from three emergence and three leaf growth variables in field studies. BRC = Brandon Research Centre. See text for descriptions of the seed tests.

Germination range					
34-99 % (51 seed samples)		**75-99 % (45 seed samples)**		**90-99 % (31 seed samples)**	
Seed quality test	**r**	**Seed quality test**	**r**	**Seed quality test**	**r**
Cool Vigor Stress Test	0.88	Vigorscore	0.83	Vigorscore	0.84
Prechill Vigor Test	0.87	Cool Vigor Stress Test	0.82	2-d germination	0.73
Vigorscore	0.86	2-d germination	0.81	Cool Vigor Stress Test	0.70
2-d germination	0.85	Prechill Vigor Test	0.81	7-d germination (BRC)	0.70
7-d germination (seed lab)	0.85	7-d germination (BRC)	0.79	Prechill Vigor Test	0.68
7-d germination (BRC)	0.85	7-d germination (seed lab)	0.78	growth in hydroponics	0.65
official germination	0.83	official germination	0.77	official germination	0.59
10-d germination	0.80	growth in hydroponics	0.74	7-d germination (seed lab)	0.57
growth in hydroponics	0.78	10-d germination	0.66	10-d germination	0.50
11-d germination	0.78	11-d germination	0.63	11-d germination	0.46
vigor index	0.52	vigor index	0.16	seed weight	-0.14
seed weight	-0.18	seed weight	-0.14	vigor index	0.05

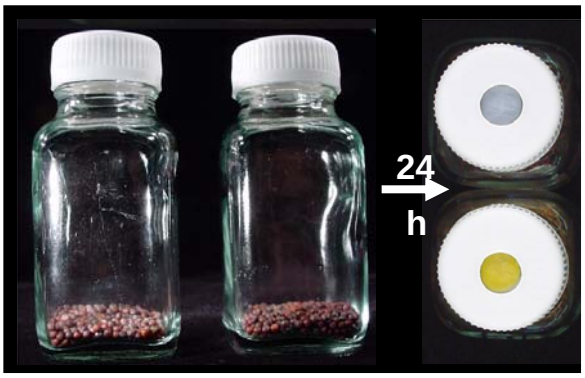

Fig. 1. Canola seed deterioration color assay. Seed and water are added to bottles, shaken and set aside at room temperature for 24 hours. Yellow indicates good quality; blue indicates deterioration. Intermediate colours, shown as green in figures, are also interpreted as good quality.

Fig. 2. Canola seed deterioration instrumental assay. The procedure is the same as for the color assay except that results are measured electronically.

Fig. 3. Seedlings from good (left) and deteriorated canola seed of the same variety and germination percentage grown hydroponically.

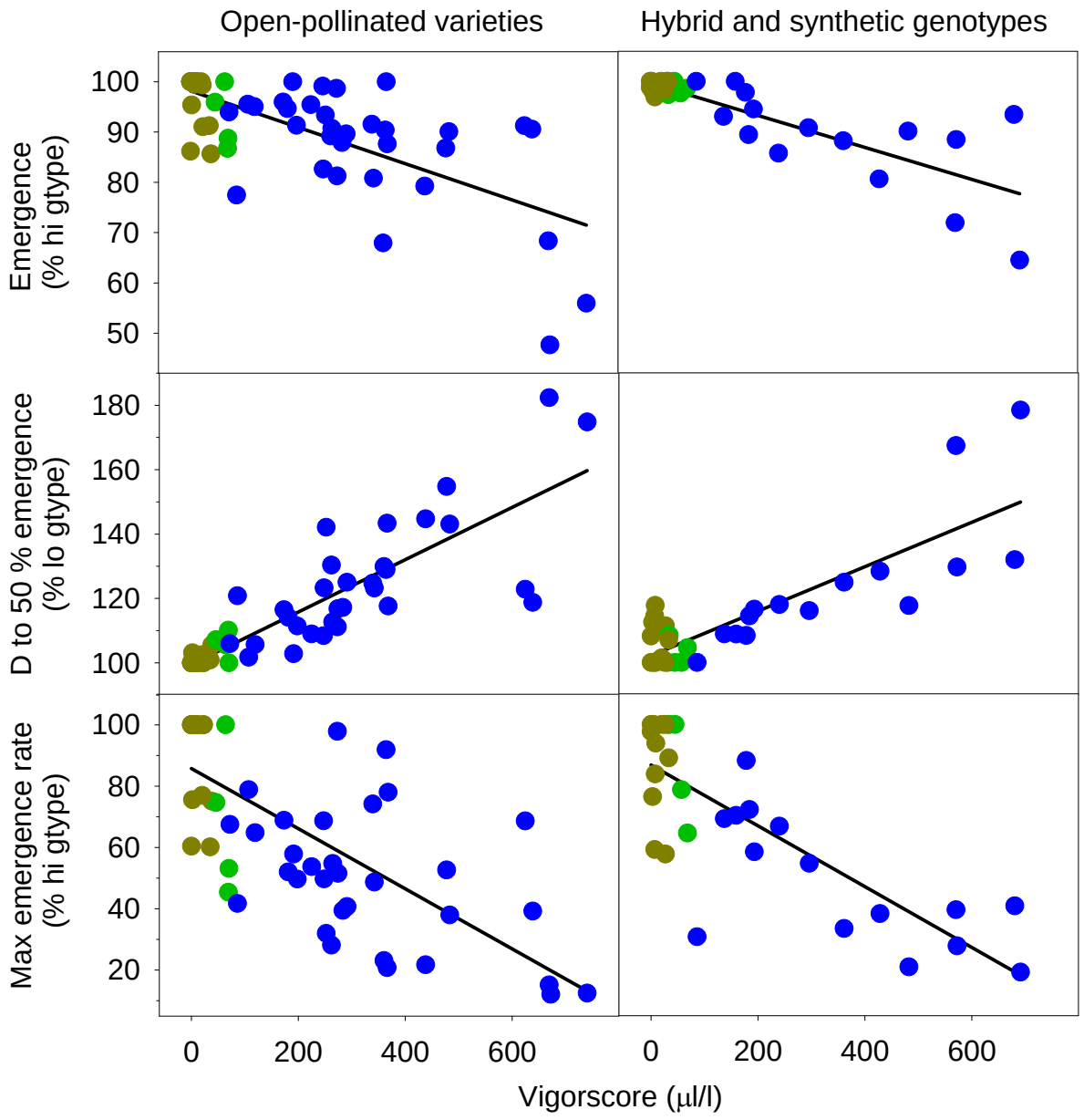

Figure 4. Prediction of seedling emergence in field plots by instrumental (Vigorscore) and color (Vigorcheck) ethanol-based canola seed deterioration assays. Emergence variables were obtained by fitting data to a modified Gompertz function. All results were expressed as a percentage of the highest (or lowest) value within each genotype (% hi gtype, % lo gtype). There were 56 seed lots of 13 open-pollinated varieties and 29 seed lots of 5 hybrid and synthetic genotypes.

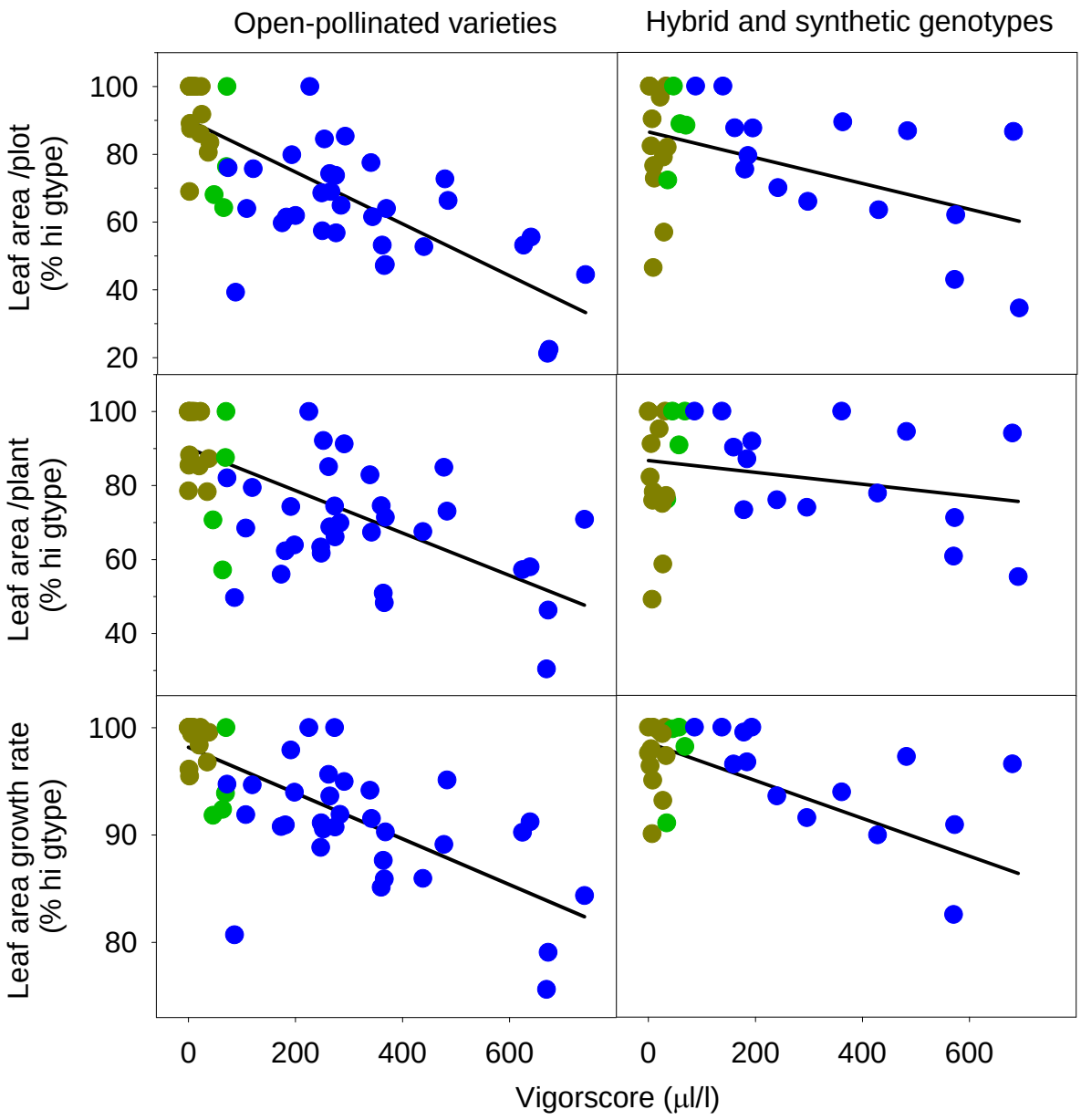

Figure 5. Prediction of seedling leaf growth in field plots by instrumental (Vigorscore) and color (Vigorcheck) ethanol-based canola seed deterioration assays. Data was collected when the check was at the 3-4 leaf stage. Growth rate was obtained by fitting data to an exponential growth function. All results were expressed as a percentage of the highest value within each genotype (% hi gtype). There were 56 seed lots of 13 open-pollinated varieties and 29 seed lots of 5 hybrid and synthetic genotypes.

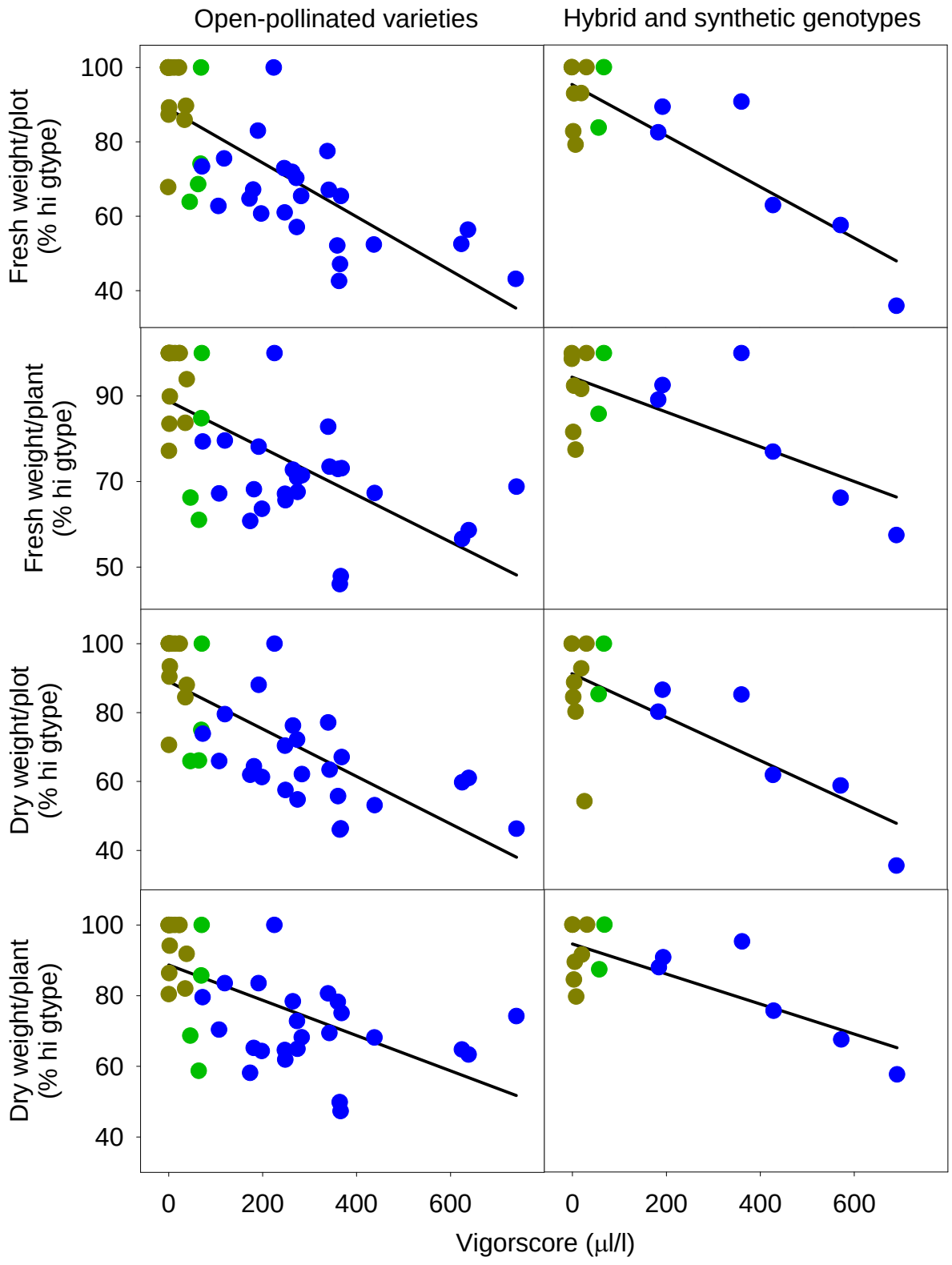

Figure 6. Prediction of seedling weight (check at the 3-4 leaf stage) in field plots by instrumental (Vigorscore) and color (Vigorcheck) ethanol-based canola seed deterioration assays. All results were expressed as a percentage of the highest value within each genotype (% hi gtype). There were 43 seed lots of 8 open-pollinated varieties and 16 seed lots of 3 hybrid and synthetic genotypes.

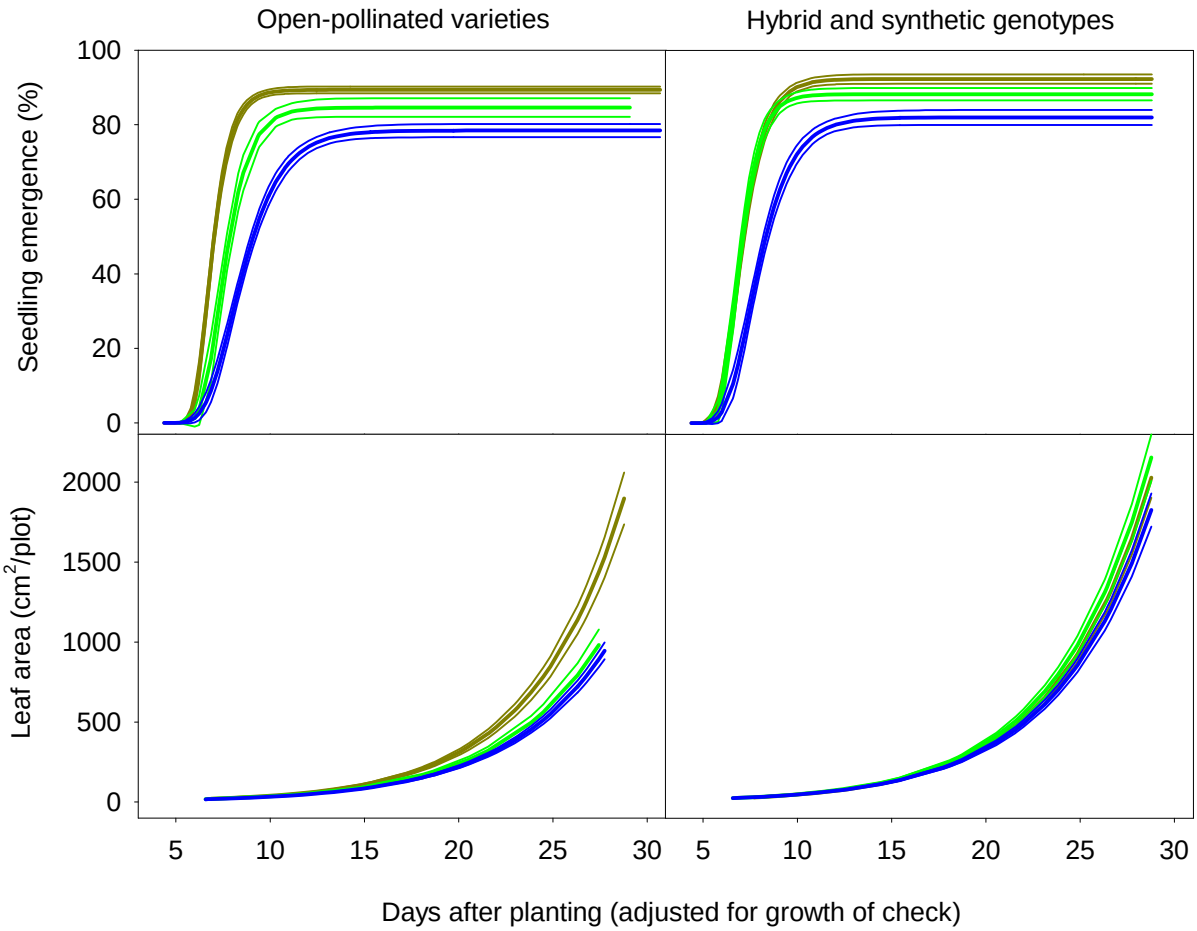

Figure 7.--Canola seedling emergence and leaf growth profiles observed in field plots over three years. Percentage emergence curves were obtained by fitting data to a modified Gompertz function for which there were 56 to 506 observations per curve (one observation is the mean of 6 repetitions, i.e., 6 plots). Leaf area curves were obtained by fitting data to an exponential growth function for which there were 28 to 234 observations per curve. In both cases, the green curves had the fewest observations because most seed lots yield a yellow or blue color. There were 55 open-pollinated seed lots of 13 varieties and 29 hybrid and synthetic seed lots of 5 genotypes. Line color indicates the Vigorcheck result. Heavy lines are the modified Gompertz function, while the light lines are 95 % confidence intervals.

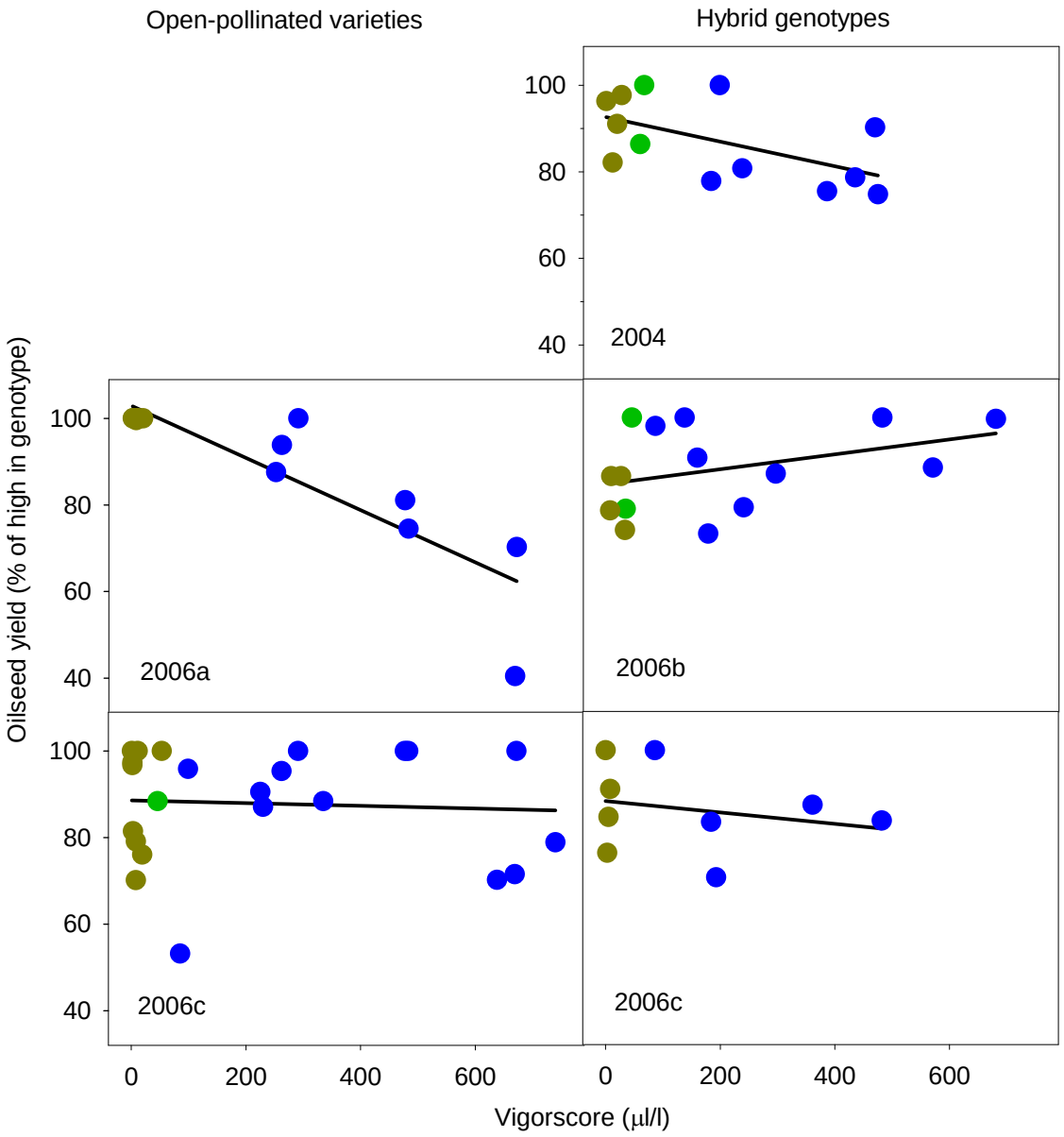

Figure 8. Prediction of air-dried oilseed yield from Vigorscore assay results for 59 seedlots of 10 open-pollinated and hybrid canola genotypes in four trials (symbol color = color assay result; 12 seed lots were repeated in two trials). Trials 2004 and 2006c were conventional yield trials (n = 4), while trials 2006a and 2006b were seedling establishment trials (n = 6) (see trial descriptions in the text).

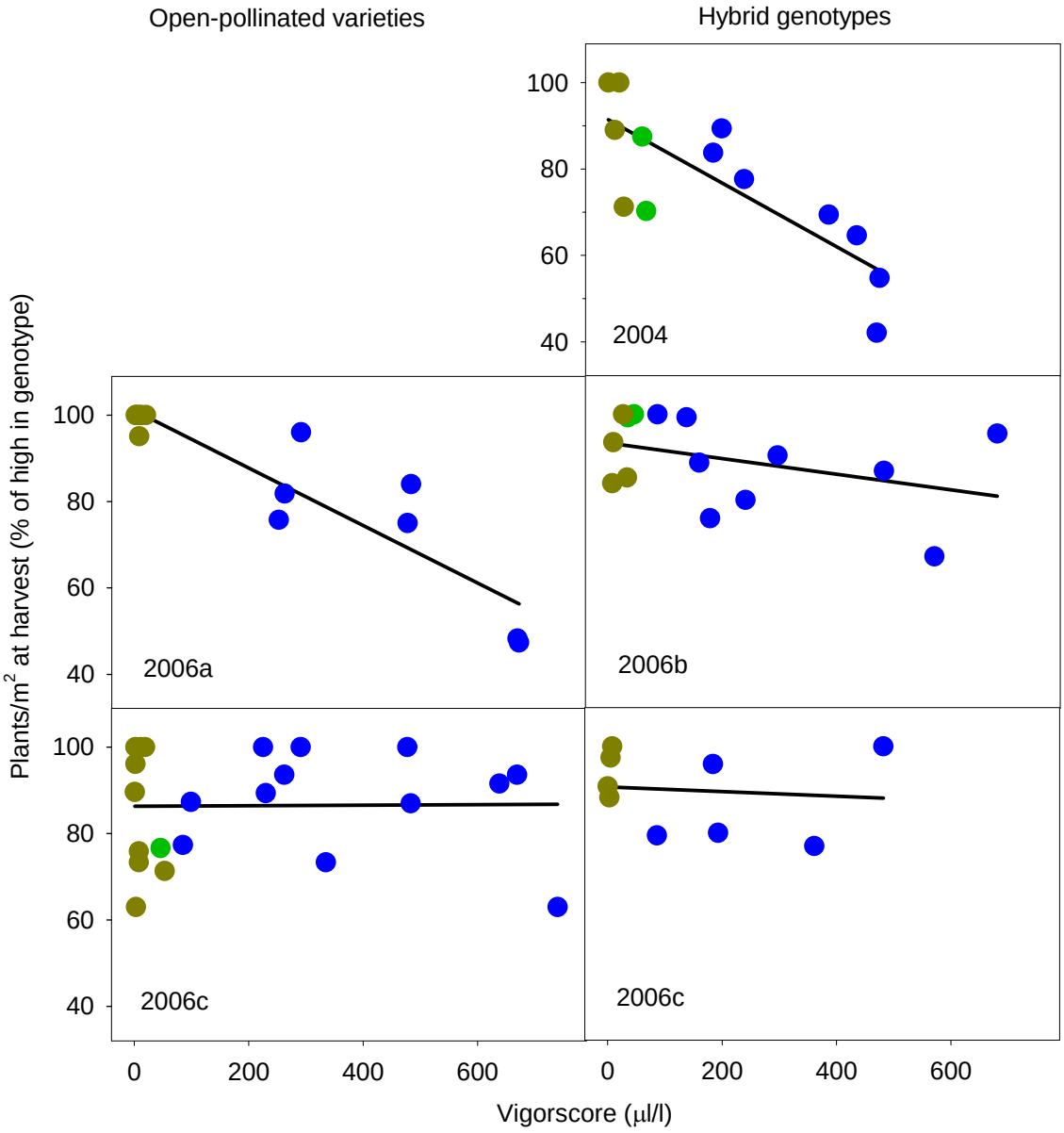

Figure 9. Prediction of plant/m^2 at harvest from Vigorscore assay results for 59 seedlots of 10 open-pollinated and hybrid canola genotypes in four trials (symbol color = color assay result; 12 seed lots were repeated in two trials). Trials 2004 and 2006c were conventional yield trials (n = 4), while trials 2006a and 2006b were seedling establishment trials (n = 6) (see trial descriptions in the text).

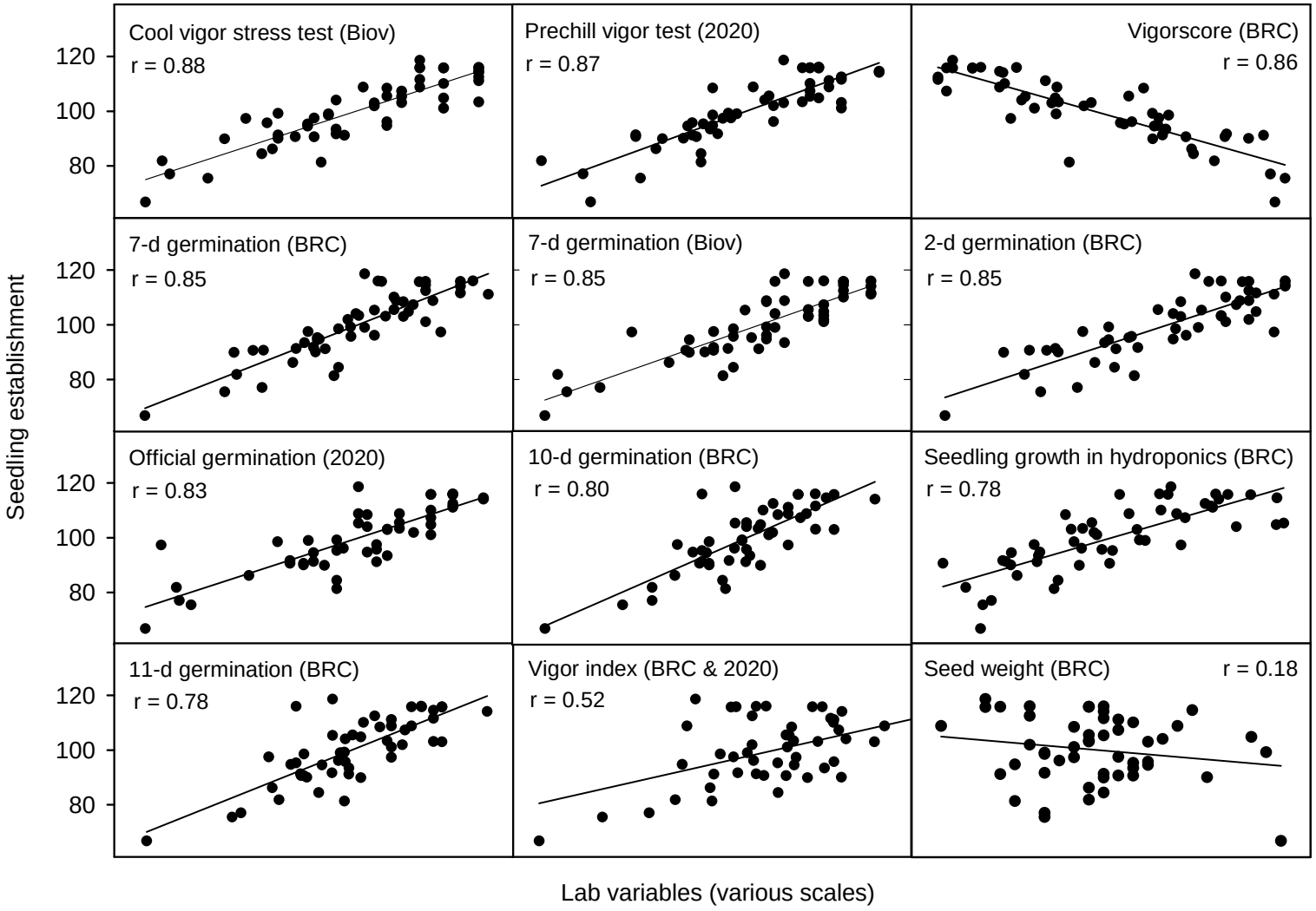

Fig. 10--Ability of laboratory seed vigor/deterioration tests to predict canola seedling field establishment for 51 seed samples with a germination range of 34-99 %. There were 44 samples of 11 open-pollinated varieties, 5 of 1 hybrid type and 2 of 1 synthetic type. Laboratory data was transformed as described in the text. Seedling establishment is an average of six field measurements. BRC = Brandon Research Centre; Biov = Biovision Seed Lab, Edmonton, AB; 2020 = 20/20 Seed Labs, Nisku, AB; vigor index = seed weight x official germination; r = Pearson correlation coefficient.

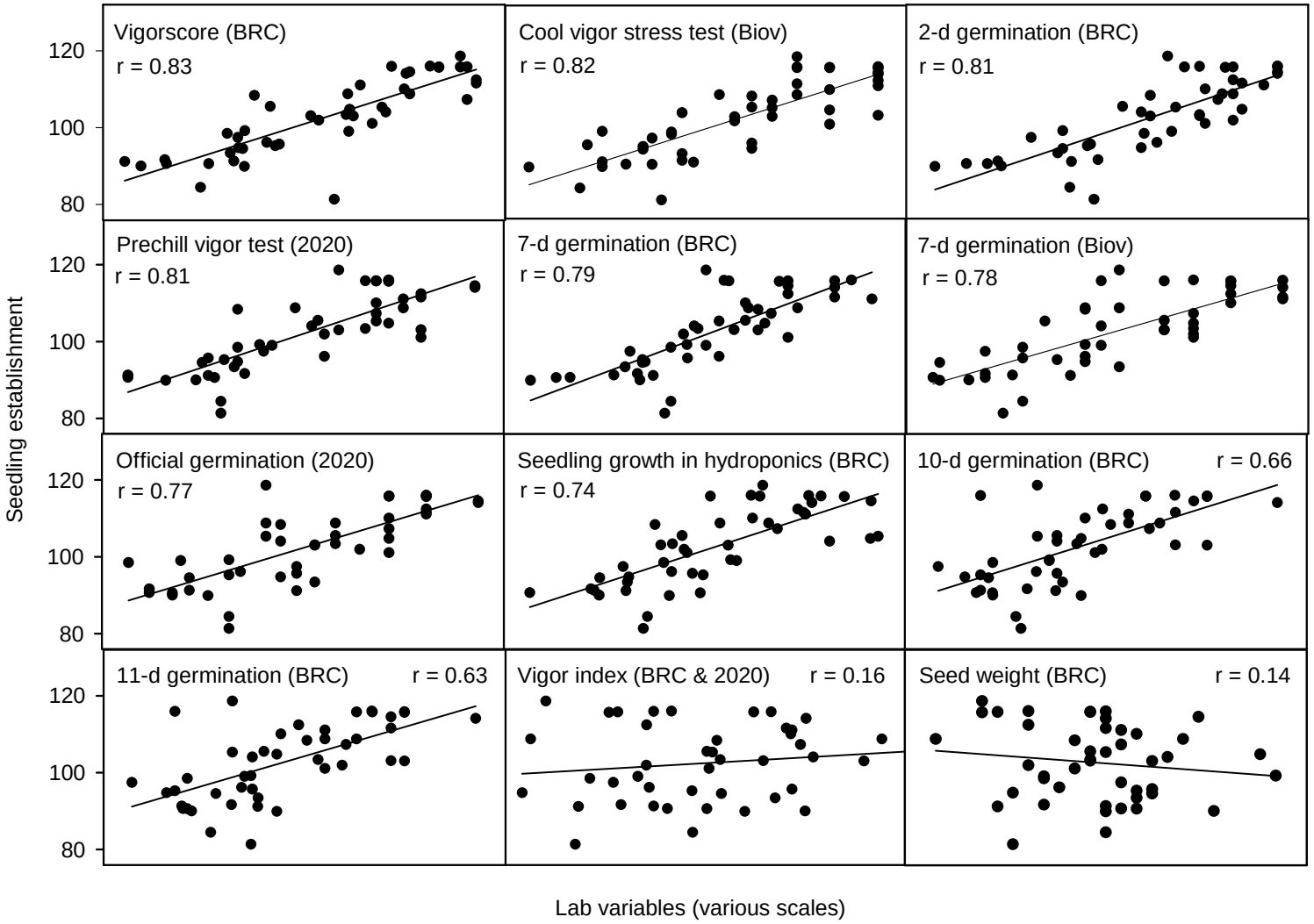

Fig. 11--Ability of laboratory seed vigor/deterioration tests to predict canola seedling field establishment for 45 seed samples with a germination range of 75-99 %. There were 40 samples of 11 open-pollinated varieties, 4 of 1 hybrid type and 1 of 1 synthetic type. Laboratory data was transformed as described in the text. Seedling establishment is an average of six field measurements. BRC = Brandon Research Centre; Biov = Biovision Seed Lab, Edmonton, AB; 2020 = 20/20 Seed Labs, Nisku, AB; vigor index = seed weight x official germination; r = correlation coefficient.

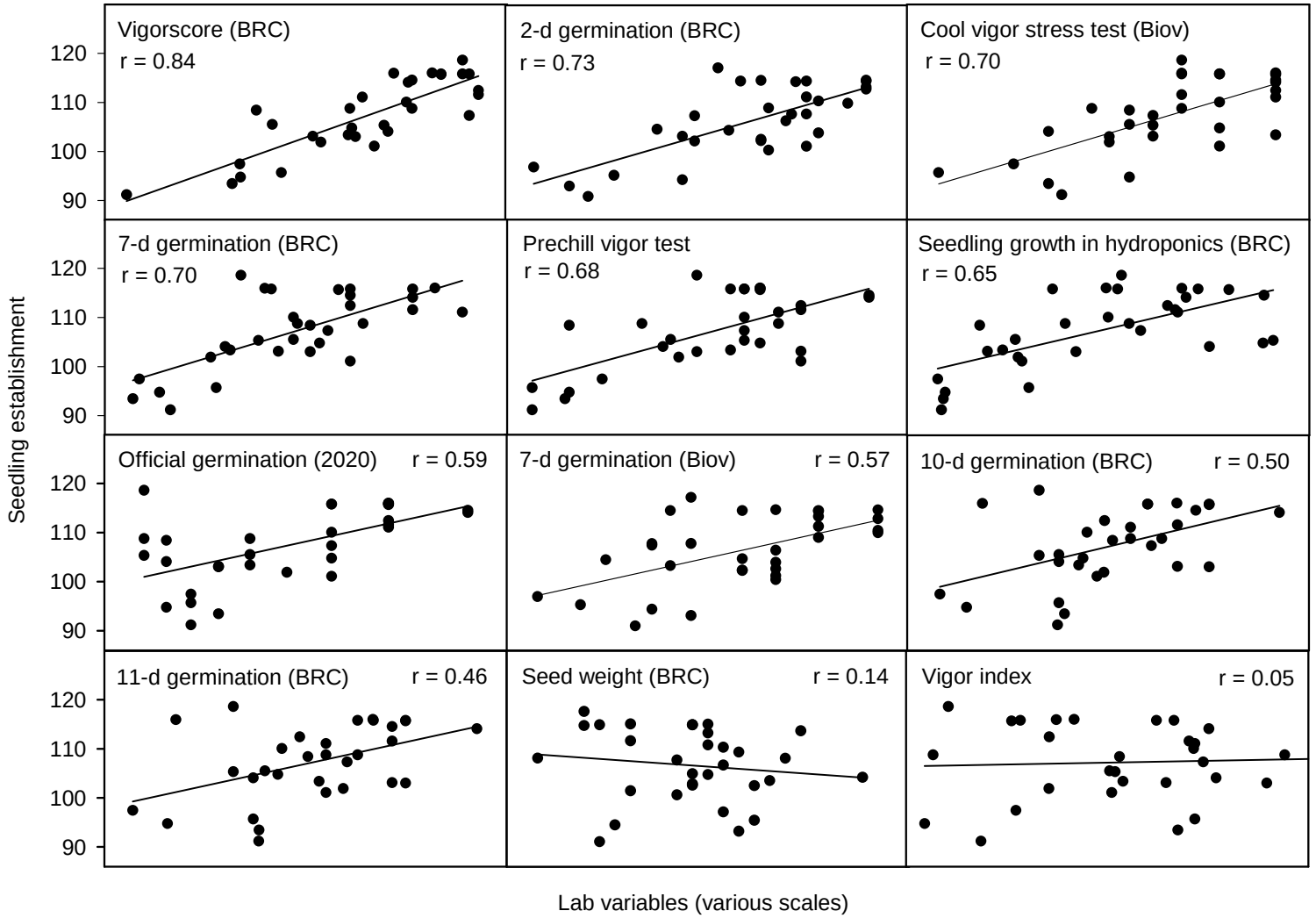

Fig. 12--Ability of laboratory seed vigor/deterioration tests to predict canola seedling field establishment for 31 seed samples with a germination range of 90-99 %. There were 27 samples of 11 open-pollinated varieties, 3 of 1 hybrid type and 1 of 1 synthetic type. Laboratory data was transformed as described in the text. Seedling establishment is an average of six field measurements. BRC = Brandon Research Centre; Biov = Biovision Seed Lab, Edmonton, AB; 2020 = 20/20 Seed Labs, Nisku, AB; vigor index = seed weight x official germination; r = correlation coefficient.