

Canola Seed Vigour Ethanol Test

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Abstract

Seed germination, seedling emergence and crop establishment are important aspects of canola production, and are the main components of seed/seedling vigour. A major concern of seed businesses and canola growers alike is loss of seed vigour that may go undetected before planting. Two vigour assays, based on off-gassing of ethyl alcohol from poor vigour seed, are being developed. They are a simple colour test that has been designed for on-farm use, and a technique using a hand-held gas monitor that will be suitable for various applications. Neither technique requires laboratory facilities or training. Assays with both techniques accurately distinguished between high- and low-vigour untreated seed. Preliminary work with treated seed is promising. The assays, which normally can be completed in 24 h, can be run at ambient temperatures varying from 19 to 27 °C (66 to 81 °F) and can be used to test seed that is 4.5 to 10.5 % moisture. Hybrid, non-hybrid, genetically modified and mutagenically modified genotypes appear to be equally suitable.

Introduction

Seed germination, seedling emergence, and crop establishment are important aspects of agricultural and horticultural production, and are important components of seed/seedling vigour. These factors are related to early growth of the crop, and may be related to resistance to early-season stresses and final yield. A major concern of growers is that deterioration of some seed lots, leading to loss of vigour, may be undetected before planting. Current vigour tests vary in reliability and in their applicability to individual crops. Canola is one crop for which a reliable vigour test is not available.

Canola seed/seedling vigour can be readily demonstrated by differences in fresh seedling biomass (**Figure 1**). Relatively little has been published about vigour tests for canola. Germination percentage of seeds in a standard test correlated poorly with field performance, whereas germination after controlled deterioration correlated significantly with plant growth and final yield (Larsen et al. 1998). Thompson, Elliott and Gusta (1999-2000) reported recently that 1) 24-h germination correlated positively with field emergence; 2) smooth spherical dark seed performed better in vigour tests than misshapened, damaged or light-coloured seed; and 3) water uptake by canola seed was correlated with seedling growth. Last year at this conference the need for and interpretation of canola seed vigour tests were discussed (Button 2002)

During imbibition, seeds undergo a period of anaerobic metabolism during which ethanol and acetaldehyde are produced. Fairly soon, most seeds shift to aerobic metabolism and the production of ethanol and acetaldehyde drops dramatically or ceases. However, it appears that seeds

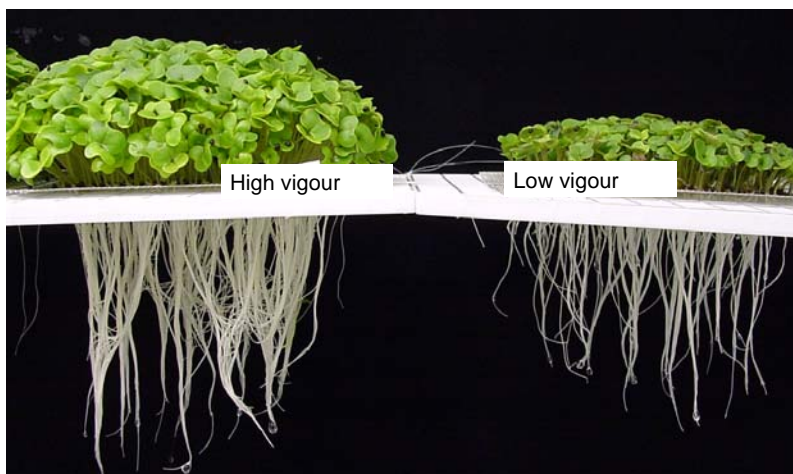


Figure 1. High- and low-vigour lots of AC Excel grown hydroponically for 7 d after beginning of seed water uptake (imbibition).

that have deteriorated are less successful at making a rapid conversion to aerobic metabolism. Ethanol and acetaldehyde production by moist soybean (Woodstock and Taylorson 1981), pea (Gorecki et al. 1985), muskmelon (Pesis and Ng 1986) and cocklebur (Gorecki et al. 1992) seeds were found to increase following natural or artificial aging, or were associated with loss of germination percentage. We have investigated the significance of ethanol (and to a lesser extent, acetaldehyde) production by moist high- and low-vigour canola seed and found that ethanol production is highly correlated with measures of vigour. Here, we report progress in the development of canola seed vigour assays based on ethanol production.

Methods

In order to evaluate the efficacy of experimental vigour tests, it is necessary to compare the results to a reference vigour assay. Reference seed vigour was determined by germinating seed and growing seedlings in hydroponics. The hydroponics bioassay consisted of placing 100 canola seeds on a stainless steel mesh suspended 1-2 cm above an aerated complete nutrient solution. Bubbling in the nutrient solution created a mist that wetted the seeds, yielding suitable conditions for germination and growth. The seeds and seedlings were incubated for 5 d at 22 °C during daily light periods (16 h) and 17 °C during dark periods. Total fresh weight of seedlings (roots and shoots), expressed as a percentage of that of a high-vigour check (a high-vigour sample of AC Excel), was taken as the reference vigour determination.

Volatile compounds found in the head space gas above moist seed were analysed by capillary gas chromatography (GC). Seed was weighed into vials and water was added to make the seed up to desired moisture concentrations. The vials were sealed and incubated at room temperature. After 24 h, volatile compounds in the head space gas were pre-concentrated by means of automated solid phase micro-extraction and then introduced onto the GC column. Results for ethanol determinations were expressed as peak areas.

Two experimental vigour assay techniques were investigated. The first was a colour test utilizing the yellow-to-blue colour reaction of a potassium dichromate/sulphuric acid solution when exposed to alcohol. The second was an instrumental test utilizing a hand-held gas monitor (Pac III single gas monitor fitted with an XS EC Organic Vapors A sensor, part no. 6809115, courtesy of Draeger Canada Ltd., Mississauga, ON). A minor modification was made to the gas monitor by manufacturing and installing a sharpened flanged tube over the ethanol sensor.

Results

Gas chromatography

Duplicate 0.5-g subsamples of 151 seed lots were made up to 20 % moisture in sealed vials and, after 24 ± 2.5 h at room temperature, were subjected to head space gas analysis by GC. Results (**Figure 2**) show that

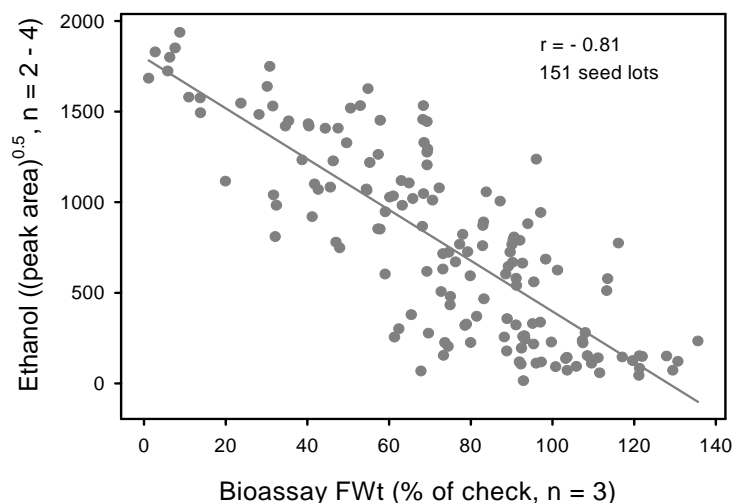


Figure 2. Ethanol in head space gas of high- and low-vigour canola seed lots. Fifty-one varieties and hybrids are represented. About half of the seed lots were treated with insecticide and/or fungicide. Bioassay fresh weight (FWt) determined by hydroponics was expressed as a percentage of a high-vigour lot of AC Excel.

ethanol emission by moist seed is a good indicator of seed vigour in a large diverse collection of canola seed samples.

The sample set can be subdivided into several categories including untreated (bare) and treated lots; treatment with fungicide and treatment with

fungicide plus insecticide; hybrid and non-hybrid varieties; and conventional, genetically modified and mutagenically derived varieties (**Table 1**). When the 151 seed lots were divided into untreated (75 lots) and treated (76 lots) categories, correlation coefficients were -0.85 and -0.73, respectively. Although the difference between the two coefficients was not statistically significant ($P > 0.05$), there may be a trend to poorer correlations with treated seed compared to untreated seed. When the treated seed lots were further divided into those treated with fungicides only (44 lots) and those treated with fungicides and insecticides (22 lots), correlation coefficients of -0.76 and 0.71 were obtained. The insecticide in most of the 22 lots was lindane. Since lindane is no longer in use, it will be necessary to test insecticides that have recently replaced it. It is possible that chemicals used as carriers or adjuvants in seed treatment also might influence correlation coefficients and should be further investigated. Division of the sample set into hybrid (23 lots) and non-hybrid (123 lots) varieties yielded correlation coefficients of -0.84 and -0.80, respectively. Conventional varieties (37 lots) yielded a correlation coefficient of -0.86, whereas all genetically modified varieties (54 lots) yielded a correlation coefficient of -0.78 (**Table 1**).

Laboratory vigour assays must be consistent with field vigour determinations. Seventeen samples from a field vigour study lead by one of the co-investigators, R.H. Elliott, were investigated and the results of various tests compared (**Table 2**). Good correlations were found between the results of field vigour

Table 2. Correlations between various vigour tests and hydroponic bioassay results as well as ethanol emissions for a set of 17 canola seed samples.

Vigour test	Pearson correlation coefficients*	
	Hydroponics assay	Ethanol emission
Field fresh weight 14 d after planting	0.79	-0.78
Field fresh weight 21 d after planting	0.80	-0.77
Field fresh weight 28 d after planting	0.83	-0.71
Pre-chill test [^]	0.77	-0.77
Special cold test [§]	0.84	-0.76
Germination after 4 d (blotting paper)	0.89	-0.81
Germination after 5 d (blotting paper)	0.74	-0.79
Germination after 6 d (blotting paper)	0.68	-0.72
Germination after 7 d (blotting paper)	0.64	-0.68

*All coefficients in this table are statistically significant ($P < 0.05$).

[^]Seed was planted in a potting soil, chilled at 5 °C for 7 d, then warmed to 25 °C during 16-h light periods and 15 °C during 8-h dark periods for 5 d, after which the number of emerged seedlings was counted.

[§]Seed was planted in potting soil and sand mix (1:1), and maintained at 8 °C during 8-h light periods and 16-h dark periods for 14 d, after which the number of emerged seedlings was counted.

Table 1. Comparisons of Pearson correlation coefficients (r) within several seed categories.*

Category name	r	n	Category name	r	n	Difference between r
Untreated	-0.85	75	treated	-0.73	76	NS
			F	-0.76	44	NS
			F + I	-0.71	22	NS
Non-hybrid	-0.80	128	hybrid	-0.84	23	NS
Conventional genetics	-0.86	37	GM -- all	-0.78	54	NS
			GM -- LL	-0.85	12	NS
			GM -- RR	-0.70	32	NS
			MD -- SM	-0.77	19	NS

*F = fungicide; F + I = fungicide plus insecticide; GM = genetically modified; LL = Liberty Link; RR = Roundup Ready; MD = mutagenically derived; SM = Smart System; NS = not significant ($P > 0.05$).

determinations and ethanol emissions. The correlations, though, are based on only 17 samples, and further studies should be performed to confirm the results.

Correlations also were quite good with two cold tests and a 4-d germination test, but they were weakest with the standard 7-d germination test. Although a 7-d germination test is a measure of total germination

percentage, the 4-d test is at least in part a measure of rate of germination. We have shown previously that vigour as determined by 5-d hydroponics biomass is well correlated with rate of germination, as one might expect.

In summary, gas chromatographic analysis of volatile emissions from 151 canola seed samples indicated that ethanol emission from moist seed may serve as the basis for practical vigour assays.

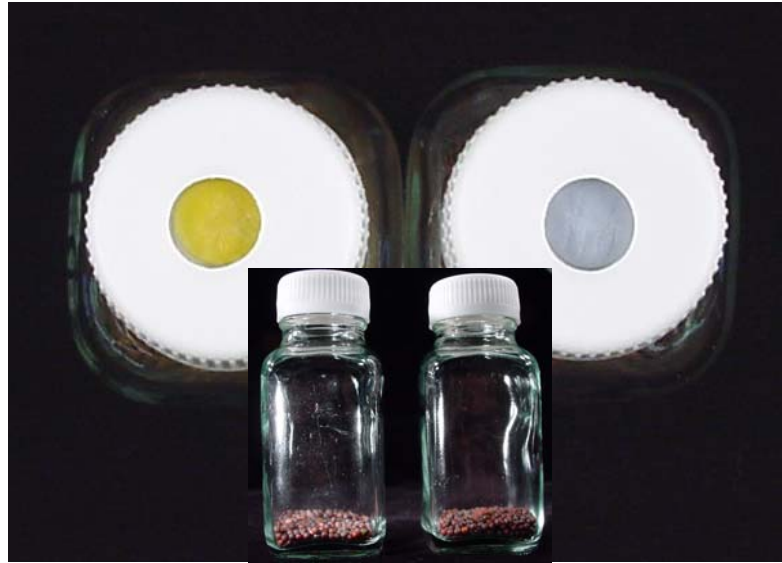


Figure 3. Colour development when high- and low-vigour seed samples were incubated for 24 h at room temperature in the on-farm assay kit.

On-farm (colour) canola seed vigour assay

The on-farm assay kit consists of a specially prepared 2-oz clear glass bottle and a cap that incorporates ethanol-sensitive colour reagents. The assay is run by adding 2 g of canola seed (measured by volume in a small test tube) to the bottle, followed by 0.5 ml of water (measured with a small syringe). After capping, the bottle must be shaken for a few seconds to distribute the moisture throughout the seed, after which it is set aside at room temperature for 24 h. Low vigour seed is indicated by the development of a blue colour in the colour disc; high vigour seed by a yellow colour (**Figure 3**).

A major advantage of the ethanol-based vigour assay is that it is relatively insensitive to variations in ambient temperature and seed moisture. Tests at 19, 23 and 27 °C and 4.4, 7.4 and 10. % seed moisture

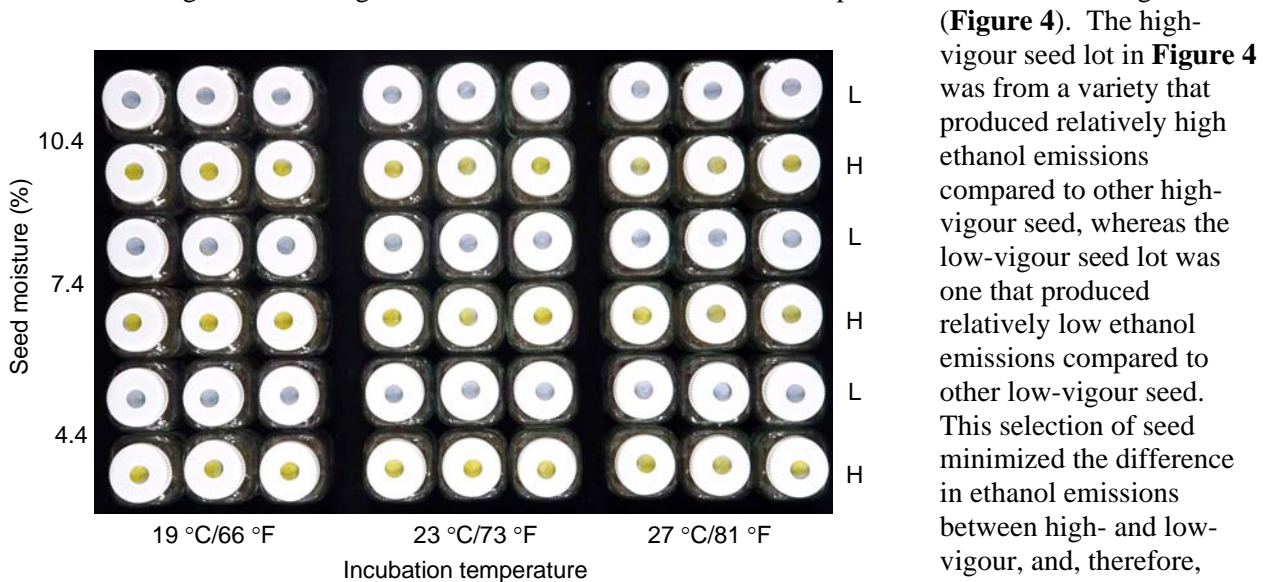


Figure 4. Effect of starting seed moisture and ambient (incubation) temperature on performance of the on-farm assay. “L” and “H” indicate rows of low- and high- vigour seed.

(**Figure 4**). The high-vigour seed lot in **Figure 4** was from a variety that produced relatively high ethanol emissions compared to other high-vigour seed, whereas the low-vigour seed lot was one that produced relatively low ethanol emissions compared to other low-vigour seed. This selection of seed minimized the difference in ethanol emissions between high- and low-vigour, and, therefore, challenged the capability of the assay to resolve the seed types. Although

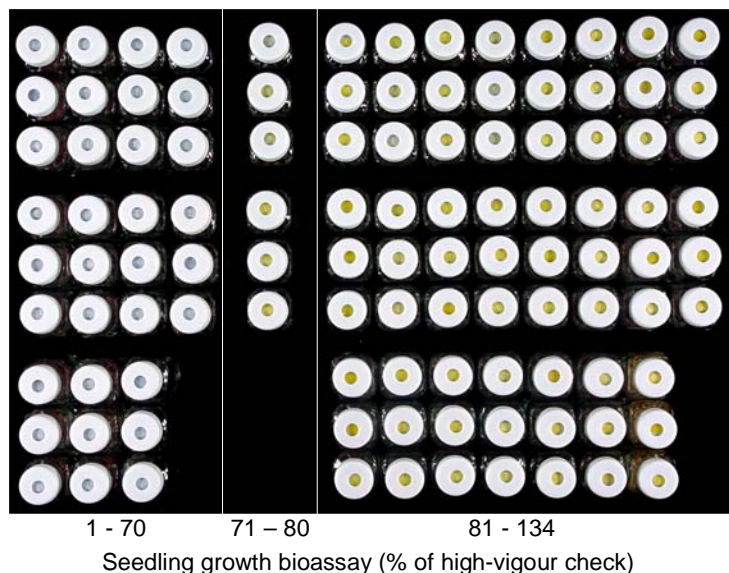


Figure 5. On-farm canola seed vigour assay performed with 36 seed lots including 23 varieties (triplicate determinations). The assay bottles are grouped by results of the hydroponics bioassay.

marginal range of 71-80 % of the high-vigour check. One lot was yellow, the other was dark yellow at 24 h. During the following 24 h, one of the samples in the marginal range continued to darken, however, none of the other samples showed any further colour change.

Several trials with treated seed have shown promising results. However, the occasional treated, high-vigour seed lot has yielded a blue colour. Also, the vigour threshold for the change from yellow to blue seems to be slightly lower for treated seed compared to untreated seed. The tests with treated seed were performed with seed treatments no longer in use. At the time of writing this report, we are gathering samples of newly registered seed treatments and expect to investigate them shortly.

Instrumental canola seed vigour assay (Figure 6)

Although a simple colour test will be convenient for producers or others who may want to test two or three samples, seed processors, seed merchants, seed laboratories and others associated with the canola industry may find a simple instrumental assay more suitable. An instrumental assay would provide quantitative data that may be more accurate and precise than colour change information. The main advantage of an



Figure 6. Hand-held gas monitor in use for determining vigour of canola seed.

starting seed moisture has little effect on the assay results, there was a trend for high moisture seed (10.4 %) to develop colour slightly faster and slightly more intensely than low moisture seed (4.4 %).

Thirty-six untreated, non-hybrid seed lots, including 23 varieties, were successfully separated into high- and low-vigour by the on-farm assay (**Figure 5**). All seed lots with hydroponics biomasses in the range of 1-70 % of the high-vigour check yielded blue colour. Twenty-three lots with a biomass in the range of 81-134 % of the high-vigour check yielded a yellow or dark yellow colour.

There were two seed lots in a

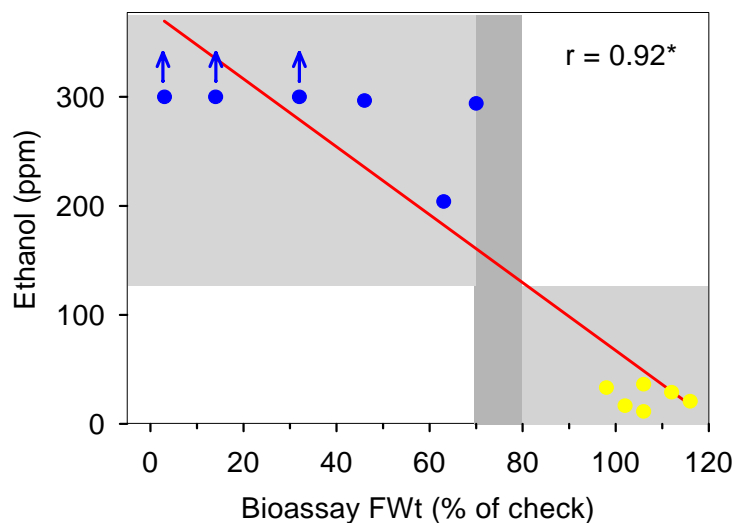


Figure 7. Ethanol concentration in the headspace of canola seed determined by the instrumental assay after 22.5-24 h incubation. The colour of dots is the colour of the on-farm assay for the same samples. Arrows indicate results greater than the upper limit of the instrument (300 ppm).

Teflon membrane and sealing the bottle over the ethanol sensor. The ethanol concentration in the head space can then be read directly on the instrument display.

Figures 7 & 8 show ethanol analysis of the head space of a selection of high and low-vigour, untreated seed after 22.5-24 and 4.5-6 h incubation. At both incubation times high- and low-vigour seed was accurately identified based on ethanol concentration. It appears that a short incubation time permitting assays to be run in one working day will be feasible.

instrumental assay, though, may be a shorter incubation time.

The use of a hand-held gas monitor (**Figure 6**) is being investigated. The instrumental vigour assay is similar to the on-farm (colour) assay in that seed and water are added to a 2-oz bottle and the bottle is capped with a special cap. For the instrumental assay, the cap incorporates a neoprene seal and a Teflon membrane. As in the on-farm assay, the bottle is then shaken and set aside. Once the incubation is complete, the bottle can be pushed over the flanged tube of the instrument piercing the

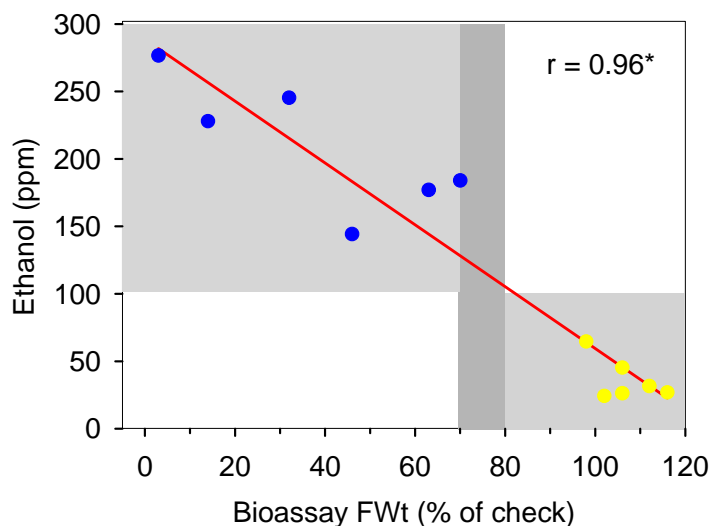


Figure 8. Ethanol concentration in the headspace of canola seed determined by the instrumental assay after 4.5-7 h incubation. The colour of dots is the colour of the on-farm assay (24-h incubation) for the same samples.

Conclusions

- Ethanol emission by moist canola seed is correlated with seed/seedling vigour.
- Correlations between ethanol emissions and vigour are significant for treated and untreated seed, for hybrid and non-hybrid seed and for conventional and genetically modified or mutagenetically derived genotypes.
- Simple and rapid canola seed vigour assays, requiring no special facilities, are possible based on colourimetric or instrumental detection of seed ethanol emissions.
- Twenty-four-hour colour assays and 24- and 5-h instrumental assays with untreated seed accurately distinguished between high and low vigour canola seed.
- Further work is required on 1) the relationship between assay results and field performance and 2) the assay procedure for treated seed.

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