HDC

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Research Report

FV/10

Seed Vigour in species of Field Vegetable

Produced by means of machine vision

SEED VIGOUR IN SPECIES OF FIELD VEGETABLES: PREDICTION BY MEANS OF MACHINE VISION

(final report for the Horticultural Development Council, A.C. McCormac, December, 1989).

FV/10

Project coordinator	: S.R. Draper
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The fundamental aim of this project was the prediction of vegetable seed vigour through laboratory testing, and incorporating the use of machine vision as a means to record and analyse such test data.

The concept of seed vigour and its importance to crop production has already been discussed in depth in a literature review ("Seed Bed Ecology, Seed Physiology: Predicting the Interaction") presented to the Horticultural Development Council as a preliminary to this project. Briefly, vigour is a multi-faceted quality, its applied definition being dependent on the desirable features of the particular crop and the environmental conditions which the seed is required to tolerate. The establishment of a predetermined level of plant population is particularly critical in vegetable crops as, in the case of direct-drilling, vegetables cannot yield compensate as readily as crops with a tillering habit and, in the glasshouse raising of transplants a high percentage of module fill is important to justify the high expenditure. Therefore for the purposes of this project, seedling emergence from a soil environment was taken as the definitive expression of vigour. This parameter also had the advantage of being relatively quick to score in comparison to, say, harvestable yield.

Machine vision is a means by which the manual recording of data can be replaced by a camera/ computer set-up and hence has potential for the automation of testing, saving in terms of labour both at the actual measurement stage and by removing the need to key-in data for statistical analysis. The high resolution of measurements also enables rapid and accurate measurement where manual techniques are impractical. Both of these assets are illustrated by the applications used in the course of this project.

The results and conclusions of the work carried out during this project's tenure are reported here in five self-contained sections. Three of these sections (I, II & V) have been submitted to journals for publication as research papers and are presented here in the same format.

Section I demonstrates the potential of machine vision for automating the measurement of seedlings, here recording the root lengths of the slant board test, an already established vigour test of lettuce and carrot seed lots. Section II describes modifications to the standard protocol which enable the slant board test to be used routinely for all small seeded vegetables, including the rounded type of the brassica species. The results of this test are shown to be predictive of the emergence vigour of seed lots of cauliflower (*Brassica oleracea* L.) and this correlation is compared with that of the standard germination score and controlled deterioration vigour test. The problems of applying the machine vision routine developed for lettuce slant boards to measurement of seedlings with a non-linear root growth pattern, such as cauliflower, are discussed.

The attempt to express growth rate in terms of thermal units and thereby compensate for uncontrolled fluctuations of temperature during seedling growth tests is reported in section III. It was hoped that by use of a data-logger to monitor the temperature being delivered to

the seedlings during the incubation period it would become possible to set a numerical standard, at least for trials carried out within the same laboratory. This would allow comparison of results between trials conducted on different occasions, currently not possible due to inadequate control of incubator conditions. The obstacles to achieving this, both at the theoretical and practical level, are described.

Section IV demonstrates the ability of machine vision to accurately measure the dimensions of seeds; manual measurement using a microscope and graticule is highly time consuming and therefore only small numbers of seeds can be handled whereas employing machine vision allows large samples to be rapidly processed. This function was further exploited for the study described in section V.

In order to formulate a novel approach to testing for seed vigour it is important to establish the factors which limit seed performance and thereby provide a theoretical basis for the test procedure. Emergence failure of cauliflower seed was concluded to be largely at the pre-germination stage; seeds which were chitted in the laboratory and then transferred to peat-filled modules almost invariably survived to emerge. The imbibition process in dry seeds was selected as an early germination event to study with regards to growth vigour and the results of this investigation are reported in section V. Using the machine vision facility to record the increase in seed size with water-uptake, it proved possible to follow the time course of imbibition for individual seeds of the small seeded brassica species. Formerly such studies have been restricted to the legumes whose large seed size makes them amenable to weighing. It was seen that high rates of imbibition in dry cauliflower seeds are associated with poor seedling growth and the importance in relation to this of a physically intact seed coat was shown. Improved emergence through restriction of seed bed moisture content demonstrated the significance of imbibition damage to expression of seed quality in a soil environment. The practical implications of these findings to seed production and cultivation methods are discussed.

Prediction of emergence vigour of cauliflower seed lots from the measurement of imbibition rates did not prove reliable and it is considered that this indicates differential sensitivities of seed to damage during water-uptake. A laboratory test which accounted for the interactive effect of these two factors could prove valuable in the prediction of emergence and would identify those seed lots most susceptible to failure at the pre-germination stage. It is proposed that the electroconductivity of the soak water of seeds could provide a measure of the membrane disruption incurred as a result of imbibition damage whether due to the absolute rate of imbibition or sensitivity of the embryo to such damage. This test has, to date, been most successful for seed species with large cotyledons such as the legumes; for application with the small brassica seeds, the problems encountered in the OSTS, Cambridge, of a large background "noise" level of electrolytes relative to that leaching from the seeds, need to be over-come.

The observation that imbibition damage is an important influence on field emergence also has implications for the protocols of laboratory growth tests. Maximising the risk of such damage by pre-imbibing seed in water before transfer to the growth conditions could help to distinguish between susceptible lots. We believe that the effect on the correlation with field emergence of vigour tests conducted with such pre-imbibed seed, compared to with seed imbibed slowly on dampened paper or by humidification, deserves investigation.

Cold soil conditions are a major limitation to the direct drilling of a number of horticultural crops including the brassicas. The association of the cold sensitivity of ungerminated cauliflower seed with the imbibition period, as indicated in section V, could prove a fruitful line of investigation with regards to improving cold tolerance through seed selection or seed treatment.

Section I

AUTOMATED VIGOUR TESTING OF FIELD VEGETABLES USING IMAGE ANALYSIS

A.C. McCormac, P.D. Keefe and S.R. Draper

(accepted for publication by Seed Science and Technology, February, 1989)

ABSTRACT

The slant board test is a potentially useful predictor of seed vigour in small seeded field vegetables. However in its standard form the test is time consuming. Image analysis had already been used to characterise and measure seeds in the authors' laboratory but the resolution of the very fine roots produced in early seedling growth proved difficult. The paper describes modifications to the slant board test necessary to achieve automated measurements by image analysis. In particular a novel growth support medium is described consisting of a thick paper blotter overlaid with black fabric, to provide an optically favourable background. Problems of phytotoxicity, poor colour contrast, dye-leaching, root penetration and growth distortion are reported for many of the support media tested. The automated slant board test offers the prospect of more accurate and less expensive predictions of seed vigour in field vegetables.

INTRODUCTION

The slant board test developed by Jones and Cobb (1963) and modified by Smith, Welch and Little (1973), involves the measurement of seedling root lengths after a number of days growth in the dark. This test is widely used within the seed trade to assess the vigour of lettuce (*Lactuca sativa* L.), and to a lesser extent carrot (*Daucus carota* L.) seed lots. Its

value for the assessment of potential field performance has been demonstrated for several species. Smith, Welch and McCoy (1973) reported a correlation of lettuce seed vigour, as measured by the slant board test, with total emergence in field trials and head size at harvest, and these results were confirmed by Wurr and Fellows (1985). Results of the slant board tests have also proved to be of use in estimating the field emergence of carrot seed lots, being as accurate as the ISTA germination test. In addition, interpretation of the coefficient of variation (CV) of seedling root lengths can provide a prediction of size variation at harvest (Gray and Steckel, 1983). As well as the potential of the slant board test for other small seeded root crops such as radish (*Raphanus sativus* L.) and sugar beet (*Beta vulgaris* L.), noted by Perry (1981), Finch-Savage (1986) measured early seedling growth of cauliflower (*Brassica oleracea* L.), leek (*Allium porrum* L.) and onion (*Allium cepa* L.) seed lots by placing pre-germinated seed on slant boards and reported a positive relationship of the results with seedling vigour and subsequent field performance.

In common with many of the vigour tests methods in current use, measuring and recording the results of the slant board test is time consuming and therefore expensive. A system for the automatic recording of seedling lengths has been developed by interfacing a microcomputer with hand potentiometric calipers (Keys, Margapuram and Reusche, 1984) but although this eliminates the need to key-in data, it fails to significantly reduce the time spent on the actual measurement stage.

The purpose of the work reported in this paper was to investigate the potential of image analysis (machine vision) for the measurement and recording of data from vigour tests, taking the slant board as an example.

If automation through the use of machine vision is to enhance efficiency and economy in terms of labour, then the changes made in the test protocol, necessary for compatibility with the Image Analyser, need to avoid the use of expensive materials or the introduction

of additional procedures. The obstacles to achieving this which are reported here, identify a number of problems which are potentially common to other systems attempting the accurate measurement of seedling performance using imaging technology.

MATERIALS AND METHODS

Seed

Three samples of lettuce seed and one of carrot were taken from stocks maintained at the Official Seed Testing Station, Cambridge (table 1). Preliminary tests indicated that sample C was of lower vigour than A or B, probably due to its greater age.

Growth substrate

Coloured, absorbent papers were obtained from the manufacturers, and black fabrics from local retail outlets (table 2). These were cut to size but otherwise, in contrast to Smith, Welch and Little (1973) who leached blotters prior to use in order to remove toxic residues, were used as supplied.

Image Analysis (IA)

The equipment used for image analysis has been described elsewhere (Keefe and Draper, 1988). Essentially it consists of a quantimet Q10 image analyser with a vidicon scanner, the latter being movable in the x, y directions by a gantry system controlled by the Q10. Gantry movement and image analysis functions were executed according to programs written in-house in Modified Microsoft Basic. Image digitisation was into 512 x 480 pixels and 64 grey levels; on each occasion, before starting measurement, a single level of the latter was chosen as the dark/light segmentation threshold to give a binary image.

Hand measured slant board test

Substrate blocks (18.5 x 10.5 cm) were saturated in distilled water and placed on 3 mm thick perspex supports. Twenty dry seeds were positioned, radicle end downwards, in a horizontal line (the sowing line) 40 mm from the top of the block using a perspex ruler as a guide. The spacing pattern consisted of four 30 mm wide groups (each containing five seeds) separated by 15 mm gaps. Plates were placed in a plastic propagator tray and held at 70^{o} from the horizontal by a perspex rack. The tray contained sufficient water to submerge 1 cm of the lower edge of the blocks, and was covered with a plastic lid, the vents of which were closed to maximise humidity. Plates were incubated in the dark at $20^{o}C$ +/- $1^{o}C$ for four (lettuce) or six (carrot) days. Root lengths were measured using a plastic template marked at 2 mm intervals, the zero-line of which was aligned with the sowing line on the plate (Smith, Welch and Little, 1973).

Machine vision slant board test

The protocol for preparing slant boards was as described above. To measure root-lengths, the plates was placed beneath the camera such that the sowing line aligned with the zero axis seen on the live video monitor. By pressing a single key, automatic measurement of all 20 seedlings was initiated, the movable gantry allowing the camera to scan the length of the slant board assessing one group of five seedlings at a time. Root length was calculated as the vertical distance between the zero-line on the monitor and the root tip as detected by the image analyser. Acceptance or rejection of the set of 20 measurements could be made at the end of each replicate by a single key-stroke. Results were stored on floppy disc.

Presentation of results

The mean and CV values were calculated for a set of four slant boards for each seed lot. Ungerminated seedlings were included in this calculation and no attempt was made to distinguish between normal and abnormal seedlings. Results were analysed using the BBC Micro Statistics Package version 86 (International Software, UK).

RESULTS AND DISCUSSION

The work reported here can be considered in two phases: the development of a protocol for measurement of slant board results by image analysis and comparison of the results obtained from the automated and original hand methods.

Automated seedling measurement

To allow measurement of seedling root lengths by image analysis it was necessary to modify the materials and methods of the slant board test as practised at the OSTS. This involved changing the colour of the growth substrate by the addition of a second, fabric layer and introducing a more defined seed layout.

Preliminary observations of seedlings grown on the standard paper (No. 0, table 2) through a black and white TV camera, and attempts to detect seedling roots by IA with this background, showed that there was insufficient contrast between root and substrate. Therefore a range of alternative, coloured substrates, comprising both papers and fabrics, were assessed for suitability by using them in standard slant board tests of the three lettuce seed lots, to compare root growth with that on the standard and determine how reliably measurement by IA recorded true lengths. Initially none of the materials tested proved adequate for one or more of the reasons presented in table 2. However, the observation that growth retardation as seen on the polyester (no. 9) and acrylic (no. 10, table 2) fabrics was only approximately 20% and was not associated with morphological distortions suggested that in these cases it might be the result of a reduction in water availability caused by the thinness of the fabrics rather than a toxic effect. This possibility was investigated by repetition of the trials of these two fabrics with the addition of an underlying layer of standard paper which, it was considered, would alleviate the water stress. A comparison of root growth recorded over a four day period indicated that there was no significant difference between

the growth rate of seed lots on the fabric over standard paper and on the standard paper alone (data not presented). A double-layered substrate of black (for maximum colour contrast) polyester-lining fabric over standard paper was therefore adopted for subsequent investigations involving image analysis.

The arrangement of seeds on the slant board was changed from equal spacing to a series of groups because only a limited number of seedlings could be viewed at any one time in an approximately square video frame. The layout was such that a set of five seedlings could be accommodated within a single frame while leaving sufficient room between individuals to prevent the roots from touching one another. The groups of five were separated by larger gaps so that seedlings from one group did not impinge on the video frame of neighbouring groups. The same spacing pattern was used for all boards since this significantly simplified the camera movement control software.

Details of software and image analysis methods developed during this work are not presented here, however brief mention of some of the imaging difficulties which arose is considered relevant for those contemplating the use of digital imaging systems with seedlings.

Despite the apparently binary quality of the root-background contrast as seen by the human eye, problems in obtaining a binary image suitable for analysis were nevertheless encountered. The fundamental cause of these problems was the long thin shape of seedling roots, vigorous roots having a width to length ratio of 0.01. Given an image digitisation of 500 x 500 pixels, if the full length of a root was viewed in a single TV frame then its width might only occupy five pixels. As a result any factor which decreased the apparent width of the root such as lack of contrast could lead to a break in the binary representation of the root. Two independent factors gave problems in this respect. Firstly, when viewed and illumi-

nated from above it was noted that seedling roots were not opaque white but translucent which made them appear grey and second, because the growth substrate was wet, any unevenness in its surface tended to catch the light causing areas of reduced contrast and glinting (specularities) of sufficient brightness to be detected as areas of white, which were interpreted as additional roots by the image analyser. The first problem was overcome by the use of lateral rather than overhead illumination. The seedling roots then appeared brighter/less translucent, possibly due to internal refraction of the incident light. The second problem was minimised by ensuring that no air bubbles were trapped between the fabric and paper and by reducing the intensity of the lighting. Even so it as necessary to incorporate various exclusion criteria into the software, based on area, size and position with respect to the sowing line of detected objects to prevent the inclusion of the dimensions of artifacts in seedling data files. In the final arrangement a single 12v tungsten filament lamp was held 5 cm above and 30 cm in both horizontal directions away from the area of substrate being viewed. This gave reliable results except in the case of roots which deviated from a straight growth pattern. This was a rare occurrence and a brief visual examination of the boards prior to measurement allowed such roots to be straightened with a small paintbrush.

Comparison of hand and machine methods

To test the ability of image analysis to reliably measure seedling root lengths, slant boards using the double-layered substrate were measured by the machine and hand methods. No significant differences were detected between the mean values as derived by IA and the hand method for the root lengths of all four seed lots (table 3). Discrepancies between the absolute values of the means as measured by hand and machine vision can be accounted for by the accuracy of the template being limited to the nearest 1 mm. Also, alignment of the sowing line with the zero measuring line on the template and under the camera was unlikely to correspond exactly as it relied upon judgement by eye. Therefore, a more informative test of the reliability of IA for measuring slant board test results was to regress

a set of individual root lengths measured by IA onto the measurements of the same roots recorded by the hand method. Regression equations were calculated for each slant board separately. The reason for this was that any discrepancy in alignment of the zero-line under the camera and template, as mentioned above, may not have been consistent between plates. A regression line with a gradient of 1.0 and intercepting the y-axis at zero would be expected if the two sets of data were consistent. Exact correspondence of values is unlikely due to the differences in the resolution of the two systems; image analysis records data to 0.001 mm in contrast to the integer values recorded using the template. However, assuming that hand measurement to the nearest 1 mm was not biased to either under or over estimation, any such deviations from the model equation should be non-significant. The regression equation calculated from the data of one lettuce slant board:

$$y = 0.146(+/-0.753) + 0.993(+/-0.026)x$$

(where y = the values measured by IA and

x = those measured by hand).

is consistent with this requirement and figure 1 shows an even scatter of data points about the regression line. This result is representative of the other three slant boards. Similarly, a comparison of the measurement of the root lengths of 20 carrot seedlings gave a regression coefficient of 0.993 +/- 0.019 with a y-axis intercept point of 0.544 +/- 1.003. This shows that IA is capable of recording root lengths at least as accurately as the hand method.

The work reported in this paper demonstrated that the technique of IA can provide an accurate means of measuring the results of the slant board test for vigour without incurring significant extra costs or time requirements at the sowing stage. The introduction of an additional fabric layer and the more defined seed layout add only slightly to the cost of preparation since the fabric is cheap and reusable and the spacing pattern can be quickly achieved using a plastic template.

The advantages of using this technique include an increase in the speed and accuracy of measurement, and elimination of keying of data for statistical analysis, the latter being time-consuming and a potential source of error. In addition the automatic recording of data within a computer facilitates its statistical interpretation allowing both graphical display and the application of a number of alternative quality criteria to the results without extra operator effort (figure 2). The latter for instance might include calculation of the mean with or without incorporating data for ungerminated seeds, or the calculation of the CV of seedling root length for predicting crop uniformity (Currah, 1978). The computerised recording of measurement data also allows storage of results on floppy disc and offers the opportunity for integrating other automatic data capture activities, such as incubator temperature logging, on the same computer. This might permit the calculation of thermal time/growth parameters which could be used to compare seed lots grown in different incubators and at different times.

Although the image analysis equipment used proved adequate for the present application a number of limitations were met which suggest that alternative imaging configurations would be better suited for work with seeds and particularly seedlings. Several of these restrictions such as the low (64) grey level resolution and the use of a single grey-level threshold, which impose strict requirements on image contrasts and illumination, are largely a result of the age of the equipment and are discussed elsewhere (Keefe and Draper, 1988).

The most fundamental problem however is the aspect ratio of the image which is captured and analysed. Because roots are long and thin it was not possible to view the full length of a root in a single frame (ie without moving the camera) and at the same time have sufficient resolution in terms of analysable pixels to reliably interpret width related features of the root such as absence of root hairs or discolouration.

An approximately square image frame with a resolution of around 500 x 500 pixels is common to the majority of commercially available image analysers in the price range which might currently be considered acceptable for seed work, we would therefore conclude that such "off the shelf" general purpose systems are not at present well suited to applications involving seedlings. The measurement of root lengths as implemented in this work represents possibly the simplest useful analysis of the captured image, being a direct replacement for manual operation. More sophisticated analyses of the image were precluded largely by the problems detailed above. However in view of how rapidly advances are currently being made in computer and imaging technology, it seems likely that more detailed assessment of seedling quality by image analysis will be possible in the future. Desirable, features would include the measurement of shoot growth, the identification of damaged or diseased areas and the ability to deal with overlapping or non-linear growth patterns. Such improvements would yield a system close to being capable of full germination assessment. An alternative approach to seed quality, which does not require an advance in imaging technology and which is currently under investigation in this laboratory is the detailed study of changes occurring in the seed/seedling at the very earliest stages of germination.

Table 1. Seed stocks used for slant board vigour test.

Sample code	Species	Variety	Percentage germination	Year of production
A	Lettuce	Romaine	99	1987/88
B	Lettuce	Premier Great Lakes	97	1987/88
C	Lettuce	Saladin	95	1986/87
D	Carrot	Red Flame	84	1987/88

Table 2. Summary of the inadequacies of the alternative substrates. Trials were performed using two lettuce seed lots (A and B) with a replication of four plates each and repeated on two separate occasions. Comparisons of results were made on data from within a single trial.

			Substrate inadequacies				
Substrate code no.	Manufacturer's reference	Colour	Growth inhibition[1]	Poor colour contrast	Dye leach[3]	Root pen'n[4]	Growth pattern[5]
Paper							
0	T-10-D(tinted) T.D. Bridger Ltd.	light grey	-	+	-	-	-
1	Nr. 3236 Schleider and Schuell	grey	-	+	-	-	-
2	Fords Gold Medal Blotting; Wiggins Teape	blue	+	+	-	-	-
3	Fords Gold Medal Blotting; Wiggins Teape	red	+	+	+	-	-
4	Fords Gold Medal Blotting; Wiggins Teape	violet	-	+	-	-	-
5	Frieze Paper Fyne Paper, Manchester	grey	+	+	-	-	-
6	Frieze Paper Fyne Paper Manchester	black	+	-	+	-	-
Fabric							
7	Polyester Crepe	black	+	-	-	+	-
8	Polyester Twill	black	+	-	-	+	+
9	Polyester Lining	black	+	-	-	-	-
10	Acrylic	black	+	-	-	-	-
11	Denim-cotton	black	-	-	-	-	+
12	Dyed cotton (plain weave)	black	-	-	-	+	-

(cont.)

(table 2. cont.)

13	Felt	black	-	-	-	+	-
14	Wool and Nylon mixture	black	+	-	-	+	-

+ = observed; - = not observed.

[1] Seedling growth was inhibited compared to the standard paper.

[2] The colour did not contrast sufficiently with the roots to allow efficient camera detection.

[3] Dye-leaching caused root colouration thereby resulting in poor contrast.

[4] Root penetration into the fabric made them invisible to the camera.

[5] Textured surface encouraged meandering growth so making a straight measurement from zero-line to root-tip unrepresentative of true length and also creating a disjointed binary image due to unequal lighting down the root length.

Table 3. Mean and CV (in parenthesis) values of the slant board test of lettuce and carrot seed lots measured by the hand and IA methods.

		SEED LOT			
Measurement method	Trial number	A	B	C	D
Hand	1	36.7 (17.5)	40.8 (23.8)	25.7 (50.4)	17.7 (76.7)
IA	1	35.8 (17.5)	40.6 (22.7)	26.4 (50.3)	17.9 (72.9)
Hand	2	33.8 (20.9)	36.1 (32.2)	29.0 (47.2)	18.0 (75.9)
IA	2	34.8 (21.4)	37.1 (31.9)	28.1 (47.7)	17.3 (73.4)

Each value was calculated from the root lengths of 80 seeds (ie four replicate plates) which had been grown in a single trial on the double layered substrate.
T-tests ($p = 0.05$) show that there is no evidence of any differences between the means as measured by the hand and IA methods.
Seed lots: A, B and C - lettuce; D - carrot.

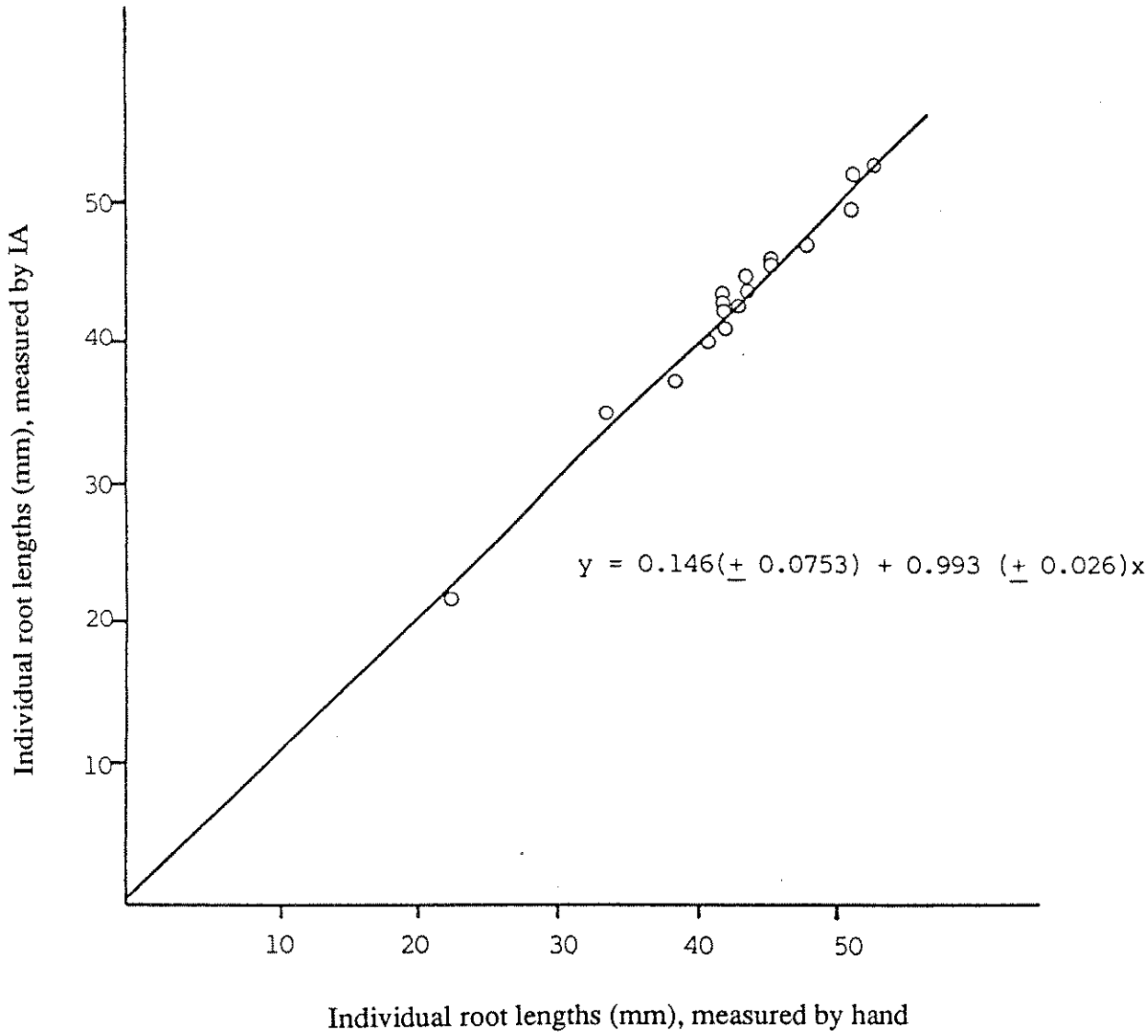
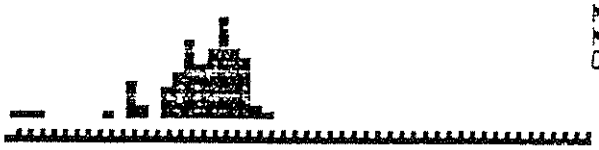


Figure 1. Comparison of measurement by image analysis and hand methods by regression analysis of 20 seedling root lengths (image analysis values regressed onto hand measured values.



SEEDLOT: PVZ62/CO.2

80 SEEDLINGS MEASURED
SEEDLINGS LONGER THAN 2 mm. = 79

MEAN LENGTH A = 35.4875 See note A.
MEAN LENGTH B = 35.9367 See note B.
COEFFICIENT OF VARIATION = .926621 See note C.

NOTES

A. This is the mean length calculated using all the seedlings.
B. This mean length refers only to those seedlings which were greater than 2 mm. in length
C. The C.V. is calculated for those seedlings which exceeded 2 mm.

Figure 2. Specimen computer printout for lettuce slant board tests. An operator defined length limit of 2 mm was used as the criterion for deciding which seeds had failed to germinate. A value greater than zero is used because some ungerminated seeds overlap the zero line slightly. The graph is a distribution histogram of "number of seedlings" (y-axis) against root length (x-axis).

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Section II

THE VIGOUR OF CAULIFLOWER (*BRASSICA OLERACEA* L.) SEED LOTS: NOVEL USE OF THE SLANT BOARD TEST.

A.C. McCormac and P.D. Keefe

(paper in the process of writing for submission to Plant Varieties and Seeds)

ABSTRACT

Modifications were made to the protocol for the slant board test of lettuce vigour, enabling its use with the seed of brassica species. These included the use of a colloid gel (Laponite) to secure the rounded seed. Mean root length after five days incubation and root extension between day four and day five were shown to be positively correlated with the vigour of soil emergence of thirteen cauliflower seed lots. The predictive value of these growth parameters compared favourably with those of the standard germination score and the controlled deterioration test of vigour.

INTRODUCTION

Despite the requirement for a minimum level of viability (HMSO 1975), there is considerable variation in the field emergence potentials of commercially sold seed lots (Perry 1972). Even under the protected environment of glasshouse raised modules, as widely used in the production of brassica transplants (Mathews & Powell 1986), high vigour of a seed lot is important in ensuring an economic level of module-fill. The rate (rapidity) of emergence is also related to seedling vigour (Finch-Savage, 1986) and lack of uniformity in seedling development can be reflected in a lower marketable yield of certain vegetable

crops (Benjamin, 1982).

A number of laboratory methods have been developed for assessing the relative vigour of seed lots (ISTA, 1976) and of these the controlled deterioration test has been reported to show good corelation with field emergence for a number of vegetable species including swede (*Brassica napus* L.) (Powell & Mathews 1981) and Brussel sprout (*Brassica oleracea* L.) (Powell & Mathews 1984). The CD test however has the disadvantage that it is expensive in comparison with certain other tests and, depending on the rapidity of germination of the species in question, it can be slow to get a result.

An alternative vigour test which has also been used with vegetable species is the measurement of root growth in the slope or slant board test. This has proved a valuable predictor of field vigour in lettuce (*Lactuca sativa* L.) (Smith *et al.* 1973b; Wurr & Fellows 1985) and carrot (*Daucus carota* L.) (Gray & Steckel 1983), and Finch-Savage (1986) reported its use for assessing the vigour of pre-chitted seed of cauliflower (*Brassica oleracea* L.), leek (*Allium porrum* L.) and onion (*Allium cepa* L.). Its use for dry brassica seed however has been precluded by the difficulties of keeping the near-spherical shaped seed in place on an almost vertical surface without affecting the subsequent growth of the seedling.

In this paper we describe a modification to the standard slant board test which allows it to be used routinely with dry brassica seed and report the results of an investigation which compared the value of the standard and CD germination tests with two parameters of seedling growth in the modified slant board test for predicting the emergence of cauliflower seed lots sown in a modular raising system.

MATERIALS AND METHODS

Seed material

Six seed lots of cauliflower cultivar Flora Blanca and seven of Winter St. George were obtained from commercial suppliers. Of these, three lots of Winter St. George (W1, W2, W3) and two lots of Flora Blanca (F1, F2) had been exposed to room conditions for 12 months prior to use. The remaining four lots of Flora Blanca (F3, F4, F5, F6) and four of Winter St. George (W4, W5, W6, W7) were used within three months of being received.

Emergence vigour

Seeds of each lot were sown (in three replicates of 56 seeds) directly into Hassy trays filled with "Shamrock" peat-based potting compost and kept in a hot-box at 20°C for two days or in a cold room at 7°C for two weeks before transfer to an unheated glasshouse. Seeds were also sown into Levington F1 (low nutrient) potting compost and these trays were placed immediately into the glasshouse where day and night temperatures did not fall below 20°C and 13°C respectively. All trials were carried out during June - August, 1989. The number of emerged seedlings was counted one and two weeks after sowing except for those trays initiated at 7°C which were scored two weeks after transfer to the glasshouse. A seedling was judged to be emerged when the cotyledons were 90° or more apart. The emergence vigour was assessed as the final percentage module-fill. Emergence after the first week (where scored), expressed as a percentage of the final score was taken as a measure of the emergence rate.

Germination test

The germination test of seed viability was conducted according to standard ISTA procedure (ISTA 1976). The percentage of seeds producing normal seedlings was scored 7 and 14 days after sowing.

Controlled deterioration test

The procedure for the controlled deterioration test (CD) has been previously described by Powell & Mathews (1981). The moisture content of all 13 seed lots, determined by the weight loss after 17 hours at 103°C (ISTA 1976) was between 6.0% and 7.5%. Seed moisture was raised to 20% before incubation at 45°C for 24 hours. Four replicates of 25 seeds were germinated in the dark at 20°C on moist paper. Seeds were scored for radicle emergence after 14 days.

Slant board test

The slant board test protocol was a modification of that devised by Smith *et al.* (1973a) for lettuce seed. The growth substrate consisted of water saturated blocks (10.5 x 18.5cm) of absorbent paper (T-10-D, T.D. Bridger Ltd.) over-layed with a black polyester fabric; this has previously been shown to be a suitable medium for seedling growth (McCormac *et al.* in press). A strip of 3% (w/v) Laponite gel (an inorganic colloid) (Laporte Industries Ltd.) was applied 40mm from the top of the block along the edge of a ruler, using a plastic pipette with a 2mm diameter aperture. The ruler was then removed by sliding it towards the top of the block, so leaving a thin layer of gel. Twenty dry seeds were placed in a horizontal line along the lower edge of this band of gel. In the absence of gel, seeds were held in place using a series of small (0.5 cm diameter) plastic cups, each of which had a 2 mm diameter hole at the base to allow the radicle to extend. These cups were removed when the root was > 2 mm in length. The blocks were put on 3mm thick perspex supports and placed in a covered propagator tray which contained water sufficient to submerge 0.5 cm of the lower edge of the block. For each seed lot, three replicate plates (ie 60 seeds) were sown and these plates were distributed in a split-plot design within the propagator tray.

The seeds were kept in the dark at 20 +/_ 1 °C (except during measurement) for the times stated. Seedlings were measured from the hypocotyl/root transition zone to the root-tip

using a plastic template marked at 2mm intervals. Measurement of individual roots was to an accuracy of 1mm. The vigour of a lot was expressed as (a) the mean root length of all germinated seeds after five days incubation (no attempt was made to differentiate between normal and abnormal seedlings, Wurr & Fellows 1984) (b) the extension rate (mm per 24 hours), calculated as the increase in root length between day four and day five of all seeds whose radicle had emerged by day four.

RESULTS

Slant board test

To test whether the results of the slant board test were influenced by the use of Laponite gel to hold the seeds in place, the root growth of cauliflower seeds germinated on the slant boards with a strip of 3% (w/v) Laponite was compared with that of seeds which were instead held in place by plastic cups until the radicle had emerged. There was no significant difference in the mean root-lengths measured after five days at 20°C (data not shown) and it was therefore concluded that Laponite, when applied so that the roots grew immediately away from the gel (as described in materials and methods), was a satisfactory agent for attaching brassica seed to a sloping surface. Root growth through the Laponite gel was avoided because in preliminary growth trials in petri dishes this was observed to retard root growth. Anaerobiosis of the gel environment was suspected as a cause since the magnitude of the retardation was not increased by increasing Laponite concentration, suggesting that toxicity or osmotic effects were not responsible.

Whereas for lettuce seedlings, measurement of the linear distance from the sowing line to the root-tip is equal to the root-length (Smith *et al.* 1973a), cauliflower roots tend to deviate slightly from a linear growth pattern and comparison of the values recorded from the boards "as-grown" and after manual straightening of the roots showed a difference between the linear measurement and the actual root length of the seedlings (table 1). This

difference, although small in magnitude, could be sufficient to affect the rank order of a seed lot. Changing the angle of the slope of the boards from 70° to the horizontal (as used for the lettuce test (Smith *et al.* 1973a)) to 90° (ie vertical) helped to minimise this effect, the difference between the "as-grown" and straightened measurements being significantly ($p = 0.001$) less for seedlings grown at 90° (table 1). Vertical slant boards were therefore adopted in the following tests and any obviously errant roots were straightened before measurement. Use of the fabric over-lay on the growth blocks greatly facilitated straightening of the roots; attempts to move roots grown directly on paper resulted in damage because the penetrative growth of the root hairs strongly attached the roots to the substrate.

Laboratory test results and soil emergence

Significant differences were found between the seed lots for the standard germination percentages, controlled deterioration germinations (CD) and seedling growth rates. Soil emergence percentages also differed significantly between the lots, ranging from a combined average of 28% (lot W1) to 91% (lot F6) (Table 2). Environment by seed lot interaction was also significant, indicating that the relative performance of each seed lot differed between environments. The results of each test were correlated with the seed lots' respective emergence vigours by calculating Spearman's coefficient of rank correlation (Steel & Torrie 1980). This statistic was used as the assumptions necessary for the more usual coefficient of product moment correlation were not thought to be valid. The results of the slant board test from each of three separate test runs were correlated with emergence individually.

The results of all variables measured in the three laboratory tests were significantly correlated with the final percentage seedling emergence and, to a lesser extent, with the rate of emergence (table 3). Both the CD and slant board tests of vigour gave higher correlation coefficients with emergence than did the standard germination test. The results of the slant

board test were best related to emergence from the unfavourable seed bed conditions of 7°C; the results of the CD test were significantly less well related for this environment. CD gave the highest correlation of the three tests with rate of seedling emergence. Of the two parameters of seedling growth measured in the slant board test, ie root length after 5 days and extension growth during day 5, the former was more highly correlated with emergence vigour (table 3).

Repeatability of results

The slant board test was repeated on three separate occasions, keeping the incubator growth environment as uniform as possible. The ranking of the thirteen seed lots within a single trial according to mean root length after five days incubation was reasonably consistent between the test runs (table 4); a slightly higher degree of fluctuation was apparent for extension growth as the vigour parameter.

The slant board test of cauliflower vigour.

Table 1. The tendency of brassica roots to wander when grown in the slant board test, makes linear measurements inaccurate, as seen by the discrepancy between the measurements taken of cauliflower seedling roots "as-grown" and following straightening. Growing the seedlings on slant boards angled at 90° reduces this discrepancy compared to that when grown at 70° ($p = 0.001$). Each value is the mean (SE) of all roots greater than 2 mm in length, from three slant boards (20 seeds per board) incubated at 20°C for five days

		root length (mm)		
seed lot	angle of slant board	as-grown	straightened	discrepancy
F2	70°	17.5 (1.400)	19.6 (2.192)	2.1 (0.120)
	90°	17.6 (0.350)	18.8 (0.910)	1.2 (0.555)
F4	70°	18.2 (1.200)	21.4 (2.052)	3.2 (0.830)
	90°	22.0 (0.810)	23.0 (0.505)	1.0 (0.310)

The slant board test of cauliflower vigour.

Table 2. Results of standard laboratory germination and field emergence percentages.

seed lot	standard germination % (14d)	emergence % field trial[1] A	B	C
W1	62	37	16	32
W2	76	65	39	52
W3	82	61	20	70
W4	85	57	19	68
W5	78	65	34	75
W6	68	60	27	50
W7	83	78	56	55
F1	81	68	55	77
F2	94	81	88	80
F3	84	55	40	41
F4	93	89	88	95
F5	95	85	87	84
F6	97	96	90	84

All values are presented to 2 significant figures.

[1] Field trials were conducted for three seed bed conditions: A - hot box (20°C) for 2 days, B - cold room (7°C) for 2 days, C - glasshouse, day time temperatures > 20°C. Each value is the mean of 3 reps of 56 seeds.

The slant board test of cauliflower vigour.

Table 3. The correlations of the field emergence of 13 cauliflower seed lots with the standard laboratory germination test of viability (scored after 7 and 14 days) and the controlled deterioration (CD) and slant board tests of vigour. The slant board test measured vigour as mean root length (measured from 3 replicates of 20 seeds) after 5 days incubation at 20°C or root extension between days 4 and 5; this was repeated on three separate trials occasions. Field vigour was assessed as the emergence percentage in each of three trial conditions (A = hot-box (20°C) for 2 days, B = cold room (7°C) for 2 days, C = glasshouse) and as the overall average[D]. Emergence rate = percentage of total emergence occurring within the first week.

	germination		CD	slant board test					
	7day	14day		mean root length			extension rate		
				trial n°.					
field variable				1	2	3	1	2	3
% emergence									
field trial:									
A	0.82***	0.71**	0.90***	0.87***	0.87***	0.83***	0.81***	0.78**	0.79**
B	0.70**	0.75**	0.79**	0.97***	0.90***	0.94***	0.91***	0.75**	0.88***
C	0.85***	0.74**	0.83***	0.74**	0.66*	0.69**	0.70**	0.58*	0.65*
combined[D]	0.78**	0.71**	0.84***	0.89***	0.83***	0.83***	0.86***	0.81***	0.79**
emergence rate									
	0.66*	0.60*	0.85***	0.79**	0.86***	0.80**	0.66*	0.60*	0.72**

{degrees of freedom = 11

significance of correlations: *, **, *** represents $p < 0.05$ $p < 0.01$, $p < 0.001$ respectively.}

The slant board test of cauliflower vigour.

Table 4. Rank order of the 13 cauliflower seed lots according to the two parameters of growth vigour (mean root length after 5 days, root extension between days 4 and 5) measured in three trials of the slant board test.

	mean root length			extension rate		
	trial no.					
seed lot	1	2	3	1	2	3
W1	1	1	1	1	1	1
W2	6	8	6	8	11	7
W3	4	2	3	4	5	4
W4	2	4	4	3	3	6
W5	3	3	2	6	9	2
W6	5	7	5	2	2	3
W7	8	9	9	7	10	9
F1	9	6	8	5	4	5
F2	11	10	12	10	8	11
F3	7	5	7	9	6	8
F4	13	13	13	13	13	13
F5	10	11	10	11	7	10
F6	12	12	11	12	12	12

DISCUSSION

Formerly, the slant board test has been restricted to use with seed of species such as lettuce and carrot which, by virtue of their having at least one flattened surface and a low seed weight, can be held on a sloping surface purely by hydrostatic forces. By using Laponite gel, an inorganic colloid, as a biologically inert, water supplying "glue" it has been possible to grow the near spherical dry seed of a brassica species in the slant board format allowing the evaluation of this type of test for predicting the field planting value of cauliflower seed lots.

The suitability, in theory, of the slant board test as a means to predict seed vigour is supported by Woodstock (1969) who stated that seedling growth rates are a sensitive indicator of seed quality, growth tending to decline with seed age well in advance of a reduction in germination. In addition, rapid germination has been observed to be advantageous in minimising the risks of soil crusting (Rathore *et al.* 1982) and water stress (Taylor *et al.* 1982). The standard slant board test (Smith *et al.* 1973a) effectively integrates assessment of the rapidity of germination and the rate of root extension growth in the early days of seedling development. In the work reported in this paper, the value of the extension growth in isolation from the rapidity of germination was also investigated. Ranking of the 13 cauliflower seed lots according to total root length (the standard slant board measurement) gave good correlation with emergence data (comparing favourably with the predictive value of the ISTA germination and CD tests) and good repeatability. The rank order according to root extension rate, however, was both less well correlated with emergence and less consistent between trial occasions than total root length. This may be related to the growth under the exhaustion conditions of the slant board test: following a lag period there is a phase of maximum root growth after which the rate declines as the seedling reserves are depleted. Measurement of rate within an isolated period may record vigorous, fast germinating seed lots in the exhaustion phase of growth while lots which

were slow to germinate are at their peak rate. The possibility of such non-synchrony of growth phases between seed lots in a trial questions the suitability of root extension as an independent parameter of vigour.

From the above results it is proposed that the slant board test could provide a routine method of assessing cauliflower seed lots with respect to their relative field potentials and it seems likely that the method could easily be extended to a range of other brassicas. The introduction of the Laponite "glue" may also permit use of the slant board growth format with a much wider range of species than has been investigated hitherto, particularly those with irregularly shaped or rounded seed and possibly somewhat larger types.

From a commercial viewpoint the slant board test has a number of advantages over CD as a vigour test. It requires less expenditure in terms of consumables and man-hours, and the test result is obtainable in less than half the time. It is also possible to automate the recording of seedling root lengths grown on slant boards using machine vision (McCormac *et al.* in press). Using this technology, with an image processing function to reduce the root-image to a skeleton profile, it would be possible to measure the length of meandering roots without requiring their prior straightening by hand.

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Section III

TEMPERATURE COMPENSATION FOR SEEDLING GROWTH IN THE LABORATORY

INTRODUCTION

An important aspect of any laboratory test is the repeatability of results. Before a vigour test method can be recommended for general use the reproducibility of the results between testing stations, as well as within, has to be investigated. The requirements of a collaborative test are that means from each test occasion agree within tolerable limits, the rank order of seed lots are similar and the results of separate trials correlate well with each other. In the event of variation in the mean values, as is the case for growth tests such as the slant board test, the vigour test may still be usefully applied providing it ranks seed lots consistently (Perry, 1984). However, such a test will only be comparative and numerical standards cannot be used. The performance of seed lots can therefore be compared only according to seedling growths conducted simultaneously. It is believed that discrepancies in temperature are a primary source of this variation.

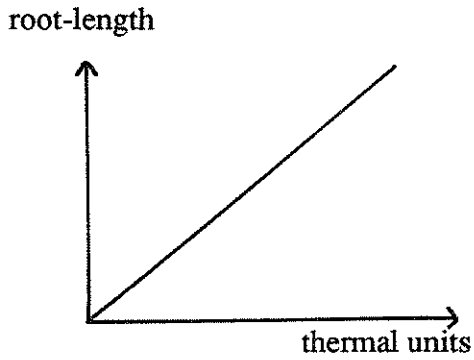
Perry (1984) considered that improvements in the level of reproducibility required the elimination of such variation by greater accuracy of control of the environmental conditions, however, the sensitivity of the growth rate/ temperature response makes the necessary level of control impractical using standard incubator facilities. As well as poor control of average levels between incubators, fluctuations in temperature during a trial can also occur depending on the speed of response of the thermostat. The alternative approach, to be investigated here, is to monitor the temperature delivered during the test period (by use of a data-logger) and compensate, by model fitting, for deviations of the test conditions from an agreed standard.

The times of development of a number of crops, under field conditions, have been successfully correlated with calculations of the thermal units delivered. Using Candolle's (1855) method of summing the mean ambient temperature for each day ("degree-days") the variability of harvest time for a wheat variety was much reduced compared to that based solely on number of days (Leopold and Kriedemann, 1975). A derivation of this incorporates knowledge of the minimum temperature required for growth and calculates for heat units exceeding this base-temperature (Arnold, 1959). This method has been used to predict the progress of crops of beans (Sully and Waines, 1988), and groundnut and millet (Mohamed *et al,* 1988). It was therefore investigated whether a similar approach, recording the temperature at hourly periods, could provide the required degree of accuracy for the prediction of seedling growth in a laboratory test.

Firstly, it was considered whether a model for temperature compensation could be formulated using theoretical growth rate/ temperature relationships. The practical application of such a model was then examined by assessing how well the actual growth of cauliflower seedlings corresponded to the theoretical assumptions made.

I. *Temperature Compensation Model*

The aim was to formulate a model such that seedling root length was proportional to the number of thermal units delivered during the growth period whatever the average temperature:



For these purposes it was taken that growth response to average temperature, at least over the limited range likely to be encountered under laboratory conditions, could be described by a linear[i] or quadratic[ii] equation:

[i] rate of growth = a + b(temperature)

[ii] rate of growth = a + b(temperature) + c(temperature)^2

{where a, b and c are real numbers}

This assumption was based on the data published for pea roots and maize coleoptiles (Leopold and Kriedemann, 1975), height of tomato plants (Went, 1945) and the leaf extension rate in millet (Mohamed *et al,* 1988).

Calculation of thermal units as the sum of the hourly temperature clearly does not provide the desired relationship; the gradient of the root-length (hourly growth rate x n^o. hours) versus thermal units (average temperature x n^o. hours) will continuously increase with the average temperature (figure 1a).

Calculation of thermal units as the sum of the squared temperature (average temperature^2 x hours) does not provide a linear relationship with root-length either but the gradient of the response does reach a maximum at the temperature defined by -2a/b (figure 1b):

$$\text{gradient of response (G)} = \frac{[a + b(\text{temperature})] \times \text{hours}}{\text{temperature}^2 \times \text{hours}} \quad \textit{(for [i])}$$

$$\Rightarrow \frac{d(G)}{d(\text{temperature})} = -2a(\text{temperature}^{-3}) - b(\text{temperature}^{-2}) = 0$$

$$\Rightarrow \text{temperature} = -2a/b \text{ at maximum value of (G).}$$

This also holds true for quadratic growth/ temperature relationships [ii].

Therefore, correction for root growth on a [temperature^2 x hours] basis will have least error for growth at temperatures around -2a/b where there is a plateau of the change in

gradient with average temperature. The error between the correct root-length (R_s) after the standard number of hours growth (H_s) at the standard temperature ($T_s = -2a/b$), and the root-length calculated (R_c) for the same number of thermal units ($T_s^2 \times H_s$) from the actual growth (R_a) at a temperature not equal to the standard ($T_a = T_s \times E$):

$$= T_s^2 \times H_s \times [\text{gradient}_{\text{standard}} - \text{gradient}_{\text{actual}}]$$

$$= (-2a/b)^2 \times H_s \times \{\frac{[a + b \times (-2a/b)]}{(-2a/b)^2} - \frac{[a + b \times (-2a/b) \times E]}{(-2a/b)^2 \times E^2}\}$$

$$= -a \times H_s \times [1 - T_s/T_a]^2$$

or, as a proportion of the correct standard root length, the error $(R_s - R_c)/R_s$

$$= (T_s/T_a - 1)^2$$

Similarly, for thermal units calculated as [average temperature x hours], error

$$= (T_s/T_a - 1)$$

Therefore for $T_s/T_a < 2$, the error of compensation based on the sum of the squared temperature will be less than for the sum of the temperature (figure 1c).

If the value of -2a/b is known accurately then the error will be known and the correct standard length can thus be calculated even for growth temperatures away from the standard and using [temperature x hours] as the thermal unit:

$$R_s = R_a \times \frac{(T_s \times H_s)}{(T_a \times H_a)} \times \frac{1}{(2 - T_s/T_a)}$$

{where $T_a \times H_a$ = actual thermal units delivered}

This holds true for linear growth rate/ temperature relationships [i] only.

Since the value of the standard temperature (T_s) has to be assumed, this is likely to be a source of error if the true value of -2a/b (T_t) is not identical for all seed lots being tested. The magnitude of this error, as a proportion of the correct standard root-length:

$$= \frac{2(T_t - T_s)(T_s - T_a)}{(T_t - 2T_s)(T_s - 2T_a)}$$

SUMMARY

method:

(1) approximation of a linear relationship (figure 1b, 1c) of root-length versus thermal units (TU = temperature^2 x hours) by growth around a standard temperature set by the estimated value of -2a/b.

{where: hourly growth rate = a + b(temperature), or

$$= a + b(\text{temperature}) + c(\text{temperature})^2\}$$

Standard root-length (R_s) is calculated by compensating the actual root-length (R_a) for the number of thermal units delivered (TU_a) compared to the standard (TU_s):

$$\Rightarrow \quad R_s = R_a \times TU_s / TU_a$$

The error of this estimation depends on the discrepancy between the standard (T_s) and the actual temperature (T_a) of growth:

$$\text{percentage error} = (T_s/T_a - 1)^2$$

This requires that the value of -2a/b is fairly consistent for all seed lots and that this corresponds to a temperature suitable for seedling growth, ie around 20^{o}C.

(2) calculation of the discrepancy of the gradient of root-length vs. thermal units (TU = temperature x hours) between standard (T_s) and actual (T_a) temperature to give accurate calculation of standard root-length:

{where: growth rate = a + b(temperature)}

$$R_s = R_a \times (TU_s/TU_a) \quad \times \quad \frac{1}{(2 - T_s/T_a)}$$

The error of this calculation depends on the accuracy of the estimation (T_s) of -2a/b compared to the true value (T_t):

$$\text{percentage error} = \frac{2(T_t - T_s)(T_s - T_a)}{(T_t - 2T_s)(T_s - 2T_a)}$$

This requires a high level of agreement of the value of -2a/b between the different seed lots of a given species.

II. ***Temperature response of seedling growth***

To test the practicality of applying these models, it was investigated how well actual seedling growth patterns agreed with the theoretical assumptions made for methods (1) and (2) ie:

a) is root growth versus average temperature adequately described by a linear (1, 2) or quadratic (1) equation, and if so,

b) can the value of -2a/b from this equation be considered the same (2), or similar (1) for all seed lots. Since -a/b = the x-axis intercept, this assumption states that the base temperature for minimum growth is constant within a species.

c) does the value of -2a/b correspond to a temperature suitable for growth, ie approximately 20^{o}C (1).

Materials and Methods

These assumptions were tested by measuring root growth at a range of temperatures. The seed lots used consisted of 2 lots each of 2 cauliflower varieties, Flora Blanca (F1, F2) and Winter St. George (W1, W2). Seeds were grown on slant boards as described in *Materials and Methods, Section II*, and root length was measured as the mean (from three replicate slant boards of 20 seeds each) of all germinated seeds after 5 days incubation at the temperature stated. The temperatures delivered during the growth period were recorded at hourly intervals using the Squirrel data logger (Grant Instruments (Cambridge) Ltd.). Measurement of temperature was taken from the water reservoir as this compared well with the temperature on the surface of the growth substrate, whereas that of the air-phase could differ by up to 1.5^{o}C. Growth at 35^{o}C was found to be markedly inhibited (figure 2) and therefore the growth/ temperature relationship was investigated for temperatures below 30^{o}C.

Results

The agreement of the actual seedling growth with the assumptions stated above was measured as follows:

a) The linear and quadratic regressions of root growth versus average temperature were assessed for the "goodness-of-fit" by the percentage variation of the data values accounted for by the regression equations (table 1a). From this it was concluded that when including the higher range of temperatures (ie up to 30°C) a quadratic expression was the best representation of the growth/ temperature relationship in cauliflower seedlings, but for levels not exceeding 25°C, a linear expression was adequate (figure 2).

b) Calculation of the value of -2a/b for the regression equations of each of the seed lots (table 1b) shows a high degree of variation so that calculation by method (2), assuming an universal constant, will have no advantage over estimation by (1) in terms of accuracy. However, there does appear to be some agreement of -2a/b between the 2 seed lots of the same cultivar perhaps indicating a genetic affect on the base temperature (-a/b). Whether or not this is a real effect would have to be determined by far more extensive trials.

c) In general, the values in table 1b are not unacceptably different from the 20°C standard usual for seedling growth experiments. Disagreement of the chosen standard from -2a/b does not make the calculations void but can increase the error margin.

The mean root lengths of 2 seed lots (F1 & F2) were recorded at various incubation times at average temperatures of 16°C, 19°C, 22°C. Plots of these values vs. thermal units (TU) = sum(hourly temperature) (figure 3a) and TU = sum(hourly temperature^2) (figure 3b) shows that the latter approaches a linear relationship significantly better. However, the regression line does not pass through the origin (y-axis intercept = -12.0 $^+/_-$1.29 & -23.1 $^+/_-$3.87 for lots F1 & F2 respectively) and this is thought to be due to the lag-period before radicle emergence occurs. Subtraction of the number of thermal units delivered before c. 50% radicle emergence is observed, (approximately 60 hours at 20°C for cauliflower seed) serves to rectify this: y-axis intercept = -0.62 $^+/_-$0.755 & -2.80 $^+/_-$2.26 for F1 & F2 respectively.

Figure 1. Theoretical assessment of estimation of a linear relationship of root-length vs. thermal units (TU) for TU = sum (hourly temperature) and sum (hourly temperature^2). Root length is calculated as growth rate x hours where:

growth rate (mm/ 24hour) = a + b(average temperature)

where a = -4.2, b = 0.4; -2a/b = 21°C.

This equation was based on the temperature response curve of seed lot F2 (Table 1b).

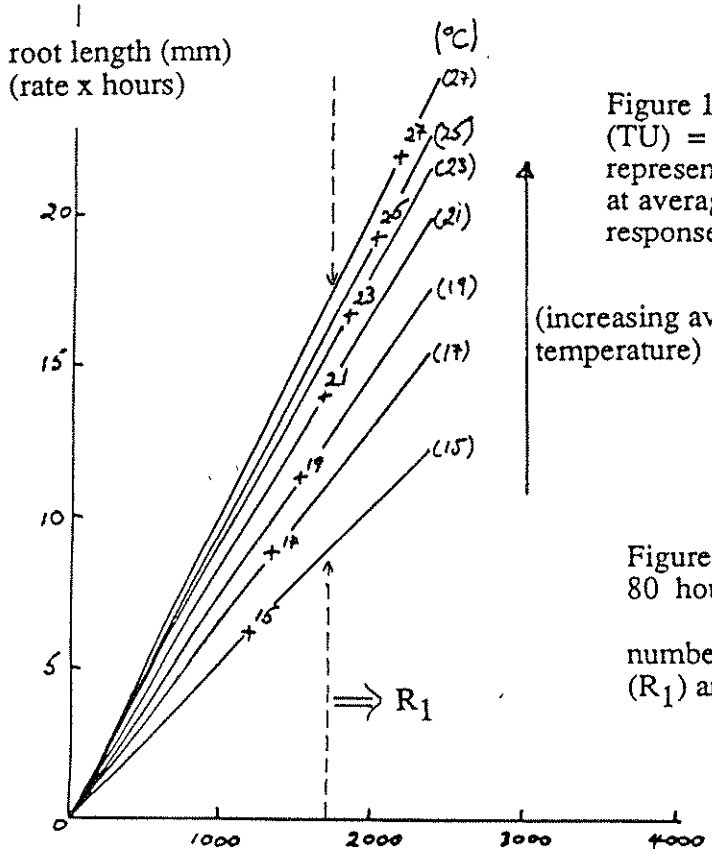
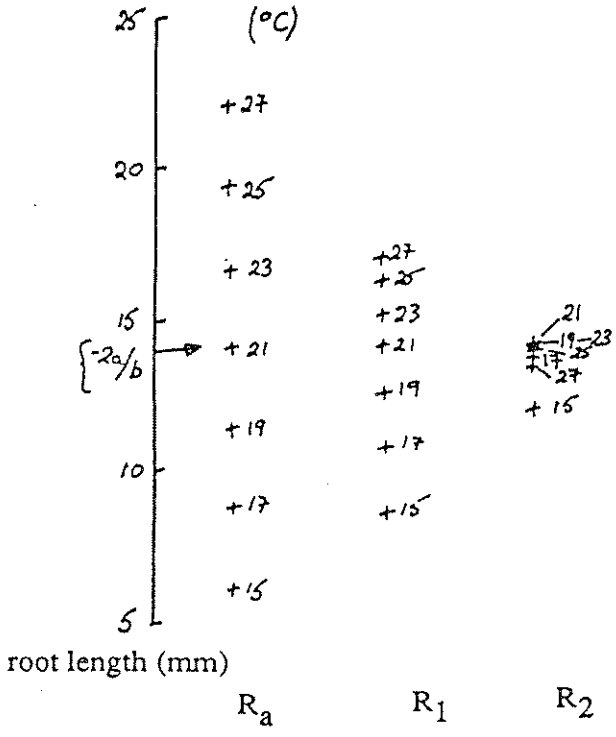


Figure 1a. Plot of root length vs. thermal units (TU) = sum of hourly temperature. Points plotted represent root length calculated for 80 hours growth at average temperature shown (°C). Gradient of response increases with average temperature.

Figure 1c. Comparison of actual root length (R_a) after 80 hours growth at the average temperatures (°C) with corrections for thermal units (where standard number = 80 hours at 21°C); TU = temperature x hours (R_1) and TU = temperature^2 x hours (R_2).



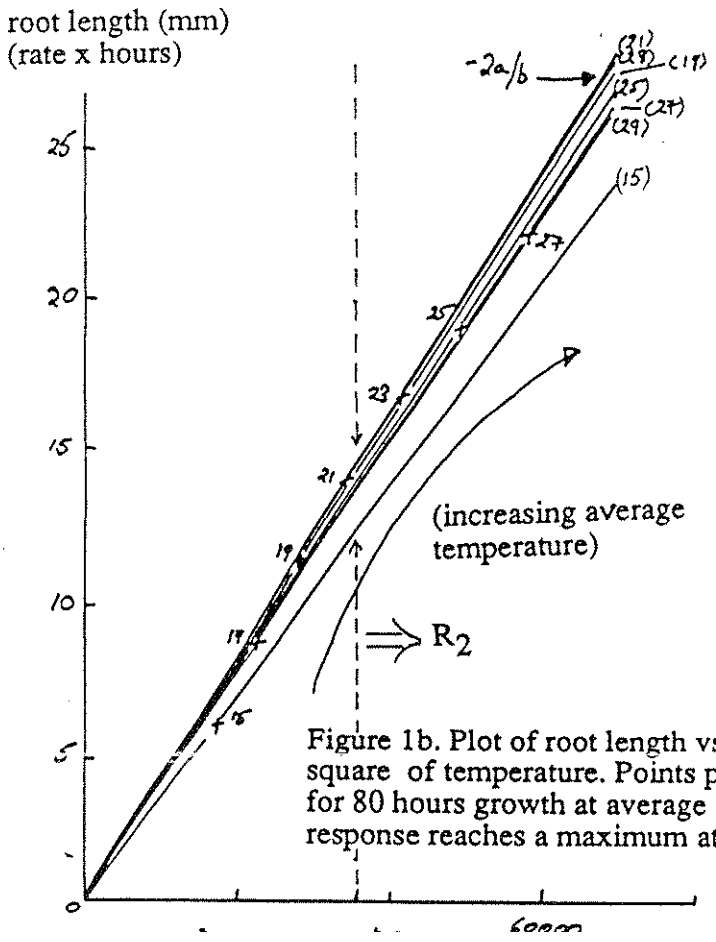


Figure 1b. Plot of root length vs. thermal units (TU) = sum of the square of temperature. Points plotted represent root length calculated for 80 hours growth at average temperature shown (°C). Gradient of response reaches a maximum at temperature = (-2a/b) = 21°C.

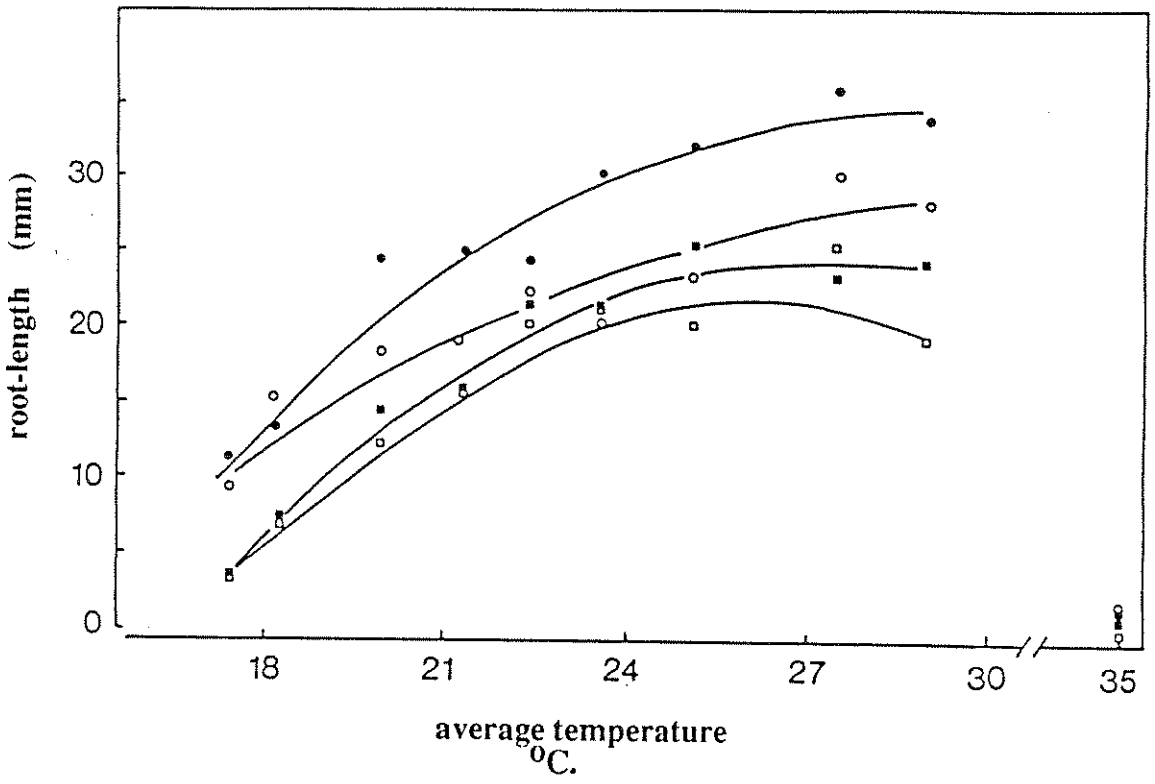


Figure 2. Root growth response to temperature. Points plotted are the mean root length values of all germinated seeds from 3 replicate plates of 20 seed each. Root length was measured after 5 days growth at the corresponding average temperature. Seed lots used were of 2 cauliflower varieties, Flora Blanca (F1,○; F2,●) and Winter St. George (W1,□; W2,■). Regression lines (—) show the quadratic equation which best fits the data for each seed lot.

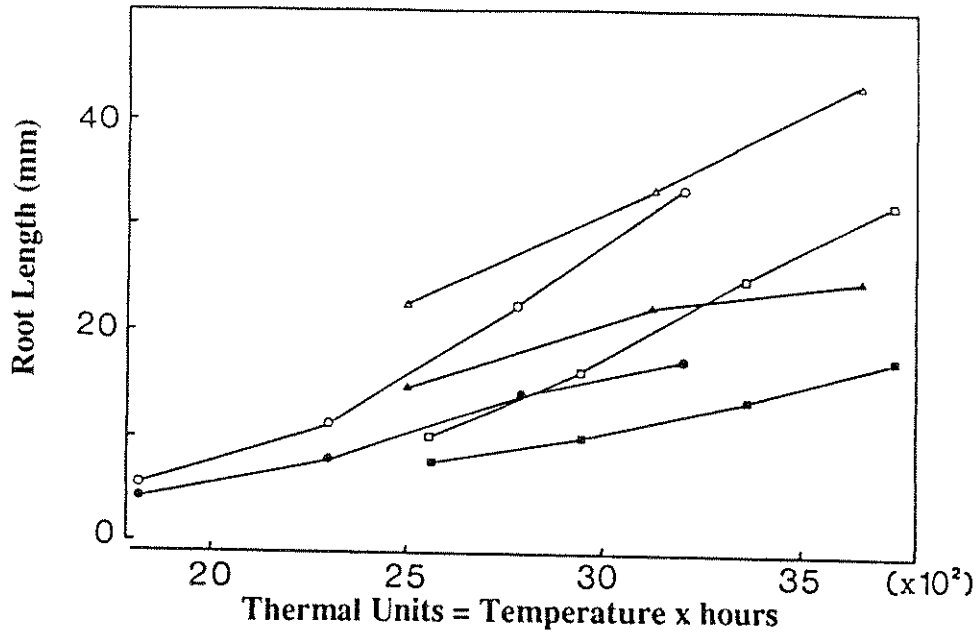


Figure 3a. Root length versus number of thermal units delivered. Thermal units = sum(hourly temperature). Each point is the mean of all germinated seeds from 3 replicate plates of 20 seeds each, of seed lots F1 (solid symbols) and F2 (open symbols). Seeds were incubated at average temperatures of 16°C (□,■), 19°C (○,●) and 22°C (△,▲). Measurements taken at different incubation times at the same temperature are joined (——).

Percentage variation in the data values accounted for by linear regression = 63% & 73% for F1 & F2 respectively.

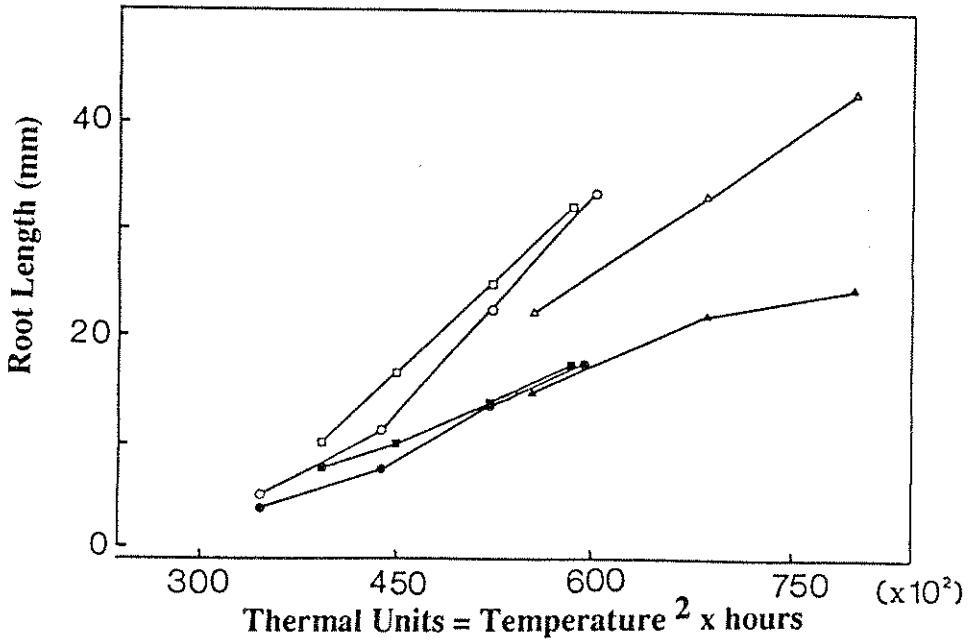


Figure 3b. Root length versus number of thermal units delivered. Thermal units = sum(hourly temperature ^{2}). For key to symbols see . figure 3a.

Percentage variation in the data values accounted for by linear regression = 98% & 93% for F1 & F2 respectively.

Temperature compensation for seedling growth.

Table 1a Percentage variation of data values of root-length (mean of 60 seeds) versus average temperature between 17-29°C & 17-25°C accounted for by linear or quadratic regression equations.

	17-29°C		17-25°C	
seed lot	quadratic	linear	quadratic	linear
W1	94	73	97	89
W2	96	78	94	92
F1	89	89	88	79
F2	93	87	89	89

Table 1b. Value of -2a/b calculated from the regression equations: root-length = a + b(average temperature) {c(average temperature)^2.

	17-29°C		17-25°C	
seed lot	quadratic	linear	quadratic	linear
W1	23.0	27.2	22.2	31.5
W2	23.1	27.3	23.1	31.7
F1	21.4	19.5	20.7	20.3
F2	22.2	21.5	21.9	25.6

DISCUSSION

Using the approach reported here, no reliable method was found of expressing thermal units such that a numerical standard could be accurately predicted from the seedling growth measured at non-standard temperatures. The fundamental reasons for this were:

i) no simple theoretical model for the temperature compensation of seedling growth was apparent.

ii) the growth responses to temperature did not readily conform to a linear relationship. This may be due to the sensitivity of root-length measurements to the interacting effects of both cell number and cell length. In a study using a wheat variety, the response of leaf length to average temperature was found to be influenced by cell length as well as cell number and each of these factors had a different temperature optimum (Friend and Pomeroy, 1970).

c) For a model to be of use in routine testing, it must be generally applicable to all seed lots of a given species. It was found necessary to make certain assumptions regarding the base temperature for growth which, from the data presented here, are not considered likely to hold valid for all seed lots included in a trial; cultivar-specific values are a possible solution to this.

Estimation of standard root growth using method (1) would not appear sufficiently accurate to allow comparison of numerical values between trials but could serve to reduce the treatment error term within a trial, especially where the number of seed lots involved necessitates a split-plot experimental design. Incubators which rely on air-flow for uniform heat distribution can have a temperature differential between shelves when the presence of growth trays impedes air-movement. Therefore replicate plates on different shelf-levels will show larger standard errors which may reduce the significance of the difference of the means between seed lots. Estimation of the correction for such non-homogeneities of temperature between replicate trays by method (1) could help improve the distinction between seed lots.

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Section IV

SEED PHYSICAL CHARACTERS: MEASUREMENT OF SEED SIZE BY MACHINE VISION AND RELATIONSHIP TO VIGOUR.

INTRODUCTION

Seed size is an important factor in determining the precision of sowing rates when using mechanical planters. The uniform grading of lettuce seed according to size and shape before coating is a common procedure (Asgrow International Corp., 1975) and has been recommended for other vegetable species including soybeans (Armstrong *et al*, 1988). Seed size can also be positively related to vigour as is the case for leek (Gray and Steckel, 1986), carrot (Gray and Steckel, 1983) and soybean (Fontes and Ohlrogge, 1972).

Existing measuring techniques are laborious, as when individual seed are measured under a microscope, or imprecise, for example the use of slotted screens to sort size fractions. The automation of such measurement through the use of image analysis would therefore be a valuable tool.

Automated measurement of seed size by machine vision

MATERIALS AND METHODS

The seed material used consisted of untreated samples of lettuce, carrot, onion and cauliflower lots. The seeds were placed in a petri dish and illuminated from below to present the camera with a 2 dimensional profile from which the area, perimeter, length (measured as the maximum diameter) and width (measured as the maximum diameter at an angle of 90^0 to the length) were recorded by the quantimet Q10 computer which was linked directly to the camera (see *Section I* for details of equipment). A shape factor, as a measure of the

"roundness" of the seed, could also be automatically calculated as:

$$\frac{4}{\text{pi}} \times \frac{\text{area}}{\text{length}^2}$$

ie having a value between zero and one where one corresponds to a perfect 2D circle/ 3D sphere. The seed positioned under the camera could be in any orientation, thereby allowing rapid placement of the sample.

Calibration of the camera into millimetre units was made by comparison with a small number of seed-length measurements taken under a microscope at X20 magnification and graticule resolution of 0.05 mm. This calibration needed to be accurate for comparison of the results with those measured by a different system.

RESULTS AND DISCUSSION

Comparison of the mean values of the length and width dimensions of 25 seed of each of the lettuce, carrot and onion seed lots, as measured under the microscope showed no significant differences compared to those recorded by machine vision (data not shown). Also, linear regression of the machine vision measurements of the individual lettuce seed lengths onto those taken by hand showed a relationship with a gradient not significantly different from 1.0 (figure 1), as expected if the two sets of data agreed.

The camera scanned each sample in groups of up to 10 seed at a time, this being the number that could readily be accommodated in the field of vision. The Q10 processor took approximately 15 seconds to record the group's data. The inclusion of several parameters in the measuring routine caused negligible increase in the time taken to process each sample by machine vision, in contrast to the manual technique.

Machine vision can therefore be seen to be capable of providing a rapid and accurate method of recording the physical dimensions of individual small vegetable seeds. By sig-

nificantly reducing the labour intensive nature of such work, automated measuring techniques enable far larger and therefore more representative samples to be handled at the testing stage. Such a system also has potential for use by the seed production industry for grading of seed on the basis of size or shape.

Seed size in relation to emergence vigour

MATERIALS AND METHODS

The emergence vigour of thirteen cauliflower seed lots comprising the two cultivars Winter St. George and Flora Blanca was assessed as described in *Materials and Methods, Section II*. Using the machine vision routine detailed above, the 2-dimensional areas of 100 seed from each sample were recorded and the mean values calculated.

RESULTS AND DISCUSSION

The correlation of emergence vigour with seed size for all 13 lots gave a positive, but statistically non significant, coefficient value of 0.52 ($p > 0.05$). Calculation of this coefficient (r) for the six lots of Flora Blanca only, greatly improved this relationship giving a value of $r = 0.984$ ($df = 4$; $p < 0.001$). However for the seven lots of Winter St. George there was no correlation between seed size and vigour ($r = -0.1475$; $df = 5$) indicating that, for these seed samples, embryo size was not the primary factor governing field performance.

This apparent anomaly between the two cultivars may be related to the susceptibility of the seed lots of Winter St. George to failure due to environmental factors, whereas for those of Flora Blanca, greater resistance to such influences allowed expression of the size advantage.

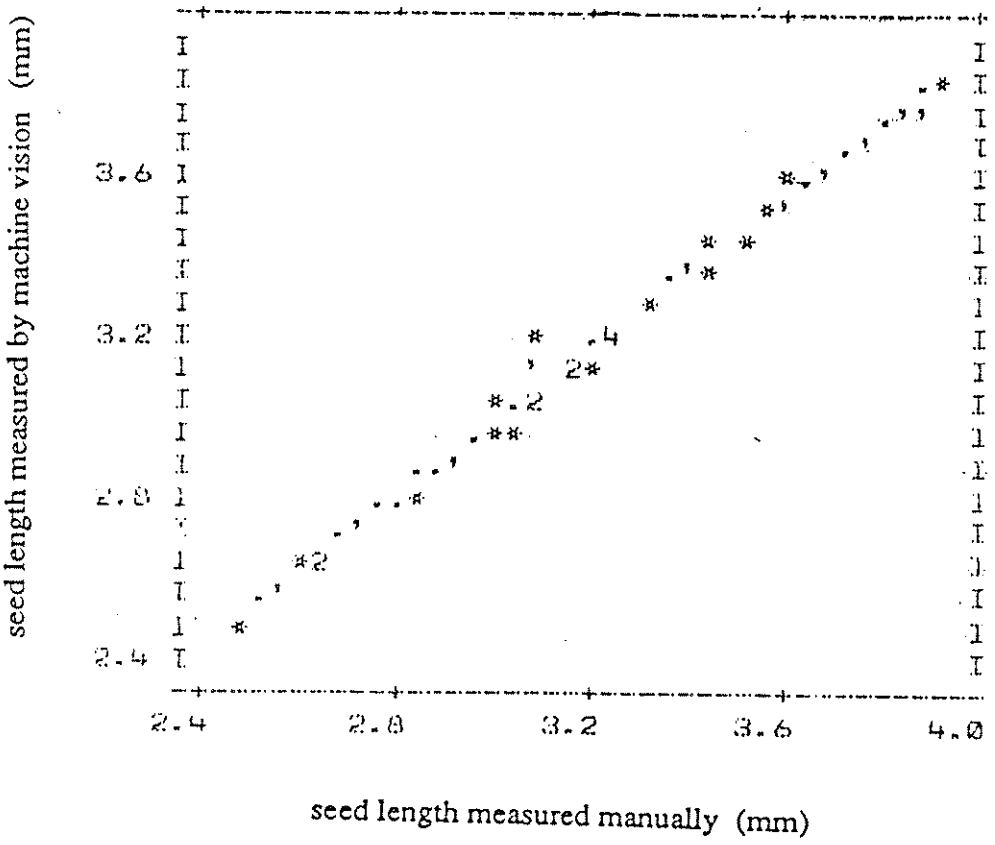

Figure 1. Comparison of measurements of seed size taken manually (using a microscope at X20 magnification, 0.05 mm resolution) with those recorded by the automated machine vision technique. Machine measurements of the lengths of 25 lettuce seeds were linearly regressed onto those by hand giving the regression equation :

$$y = 0.11\ (^{+}/_{-}\ 0.14) + 0.94\ (^{+}/_{-}\ 0.076)\ x$$

(machine) (hand)

(* = individual seed lengths; .,,, = regression line)

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Section V

CAULIFLOWER (*BRASSICA OLERACEA* L.) SEED VIGOUR: IMBIBITION EFFECTS

A.C. McCormac and P.D. Keefe

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ABSTRACT

By means of a machine vision facility, the process of water-imbibition in a small seeded brassica species was recorded as the visible increase in seed volume. Dry cauliflower seed (*Brassica oleracea*) showed an immediate rapid phase of imbibition upon the addition of water. This initial phase was associated with the reduction in seedling root growth resulting from the imbibition of cold water, and the rate of uptake of water at 20^{o}C was negatively correlated with subsequent seedling growth. Damage to the testa of dry seed resulted in an increased rate of imbibition and a corresponding decrease in seedling vigour measured according to the slant board test. This showed that the intact testa of cauliflower seed is capable of acting as a barrier to water influx and that a high rate of water uptake is damaging to the embryo. Testa damage reduced percentage soil emergence of seeds, as did raising the soil moisture content during the imbibition period by watering immediately after sowing. Both these treatments were believed to increase imbibition rate. Conditions which encouraged a low rate of water uptake also improved the rate and uniformity of emergence. Correlation of mean imbibition rates (measured in a laboratory test) with soil-emergence indicated that the sensitivity to imbibition damage varied between seed lots and interacted with the absolute rate of water influx to determine emergence vigour. This factor prevented reliable prediction of seed vigour from the ranked-order of measured imbibition rates. The significance of these findings to the seed production and modular transplant

raising industries is discussed.

INTRODUCTION

In current production of many vegetable crops, field stands are established using modular transplants as opposed to direct-drilling. The expenditure involved in such raising of seedlings under glass demands a high level of performance from seed lots to produce near 100 percent module-fill (Mathews and Powell, 1986). As stated by Hampton and Coolbear (1989), it is important to understand the variables which limit the various aspects of seed performance in order to evaluate vigour testing methods and to avoid vigour losses resulting from the treatment of seed during production. It is recognised that the events prior to radicle emergence, especially concerning water levels (Obroucheva, 1989), are critical to successful germination and the work presented in this paper focuses on the implications of the imbibition period to the vigour of brassica seed.

The imbibition of water into the dry seed is an essential process in the initiation of germination but has also been documented as a potentially hazardous period (Woodstock, 1988). Powell and Mathews (1978) showed that the rapid uptake of water by dry pea-embryos resulted in cell death (detected on the surface of the cotyledons by tetrazolium chloride staining techniques) and in seeds, impaired seedling growth (Powell and Mathews, 1980). Such imbibition damage has also been reported for several other legume species including soybean (Oliveira, Mathews and Powell, 1984) and dwarf French bean (Powell, Oliveira and Mathews, 1986). Powell and Mathews (1978) proposed that chilling injury of ungerminated seed is the result of a cold-enhanced sensitivity to imbibition damage, and in support of this Tully, Musgrave and Leopold (1981) reported a relationship between resistance to chilling injury in pea seed and the rate of uptake of cold water. Low soil temperatures have been cited (Herner, 1986) as a major limitation to the direct drilling of many horticultural crops; brassica seed shows a much reduced emergence below $10^{o}C$.

Imbibition is a physical process, water being absorbed by the protein and polysaccharide macromolecules of the cell wall and protoplasm which constitute the highly negative matric potential of the dry seed. This water potential gradient which exists between the seed and its surrounding medium determines the force of imbibition and may influence the rate (Woodstock, 1988). The rate of movement into the seed is also reported to be a function of the quality of the seed coat; in peas (Powell and Mathews, 1979) and soybeans (Mason, Vorst, Hankins and Holt, 1982; Oliveira *et al*, 1984) rapid rates were associated with a high incidence of testa damage.

The literature reports of imbibition patterns previously cited have been exclusively concerned with large seeded species, primarily the grain legumes. This restriction can be related to the use of weight increase as a measure of water-uptake. This report describes the use of machine vision to follow the imbibition process in a small seeded brassica species, namely cauliflower, by the visible swelling of the imbibing seed. As well as being more sensitive to small increments of increase, this technique avoids any inaccuracies due to surface held moisture and attenuates detection of the hydration of the seed coat or the filling of air-cavities which Herner (1986) noted may make measurement of initial imbibition on a weight basis unrepresentative of the hydration of the embryonic and cotyledonous tissues.

In this paper evidence is presented for the influence of imbibition damage on the vigour of cauliflower seed, both in laboratory tests and under glasshouse conditions, and the importance of an intact seed coat with respect to this is demonstrated. The use of imbibition rate as a predictor of seed performance is also discussed.

MATERIALS AND METHODS

Seed material

Ten seed lots of cauliflower seed comprising two varieties, Flora Blanca and English Winter St. George, were obtained from a number of commercial suppliers. Three lots of cultivar Winter St. George (W1, W2, W3) and two of Flora Blanca (F1, F2) were stored at room temperature and humidity for 12 months prior to their use, whilst the remaining two lots of Winter St. George (W4, W5) and three lots of Flora Blanca (F3, F4, F5) were used within two months of being received. All seed lots had a moisture level of 6.0 - 6.5% in the unimbibed state. Seed lots which are termed "cracked" had been subjected to rapid shaking for 30 minutes using a Griffin flask shaker at half maximum speed to cause repeated impact of the seeds with the sides of a glass flask. The testae of seeds referred to as "split" had been lightly scored with a scalpel.

Imbibition

During imbibition in the laboratory, the seeds were either submerged in standing water or were placed in contact with a flat, damp (55% (w/w) moisture content) pad of absorbent paper (T-10-D, T.D. Bridger Ltd.). Imbibition was at 20°C or 5°C in an incubator, or at room temperature (20°C - 23°C) (an incubator-controlled environment was not possible when using the machine vision device for measurement of the seed); the osmotic medium was left to equilibrate to the required temperature for 24 hours before contact with the seeds. All treatments within each trial were conducted simultaneously to nullify any effects of temperature fluctuations, and using the same water sample to avoid possible inconsistencies in pH levels.

The machine vision system used was the Quantimet Q_{10} described by Keefe and Draper (1988). Dry seeds were affixed to a petri-dish using a diluted solvent-based glue to prevent movement between measurement occasions. The seeds were illuminated from below to

present the camera, mounted directly above, with a two-dimensional profile of each seed. The area of this profile was recorded. Calibration of the measurements was automatically made at each measurement occasion; the calibration object was measured simultaneously with the seed to compensate for any optical effects of the water level.

In order to calculate seed volume from the two-dimensional measurements, it was necessary to assume that each seed approximated a sphere. The volume of water imbibed between measurement intervals was considered equal to the increase in seed volume. Measurements were taken when the seeds were unimbibed, and 30 minutes and 24 hours after the addition of water. Rate of imbibition was calculated as the increase in seed volume after the initial 30 minutes of imbibition expressed as a percentage of total increase after 24 hours.

Germination and vigour assessment

Vigour of seeds in the laboratory was assessed by the measurement of seedling root-growth. Seeds which had been previously imbibed for 24 hours were transferred to slant boards, as used for lettuce vigour tests (Smith, Welch and Little, 1973) and grown in the dark at 20°C. Root-lengths were measured after five days, non-germinating seeds were scored as zero. The growth vigour of a lot was calculated as the mean root-length of all germinating and non-germinating seeds.

The emergence vigour was measured as the percentage module-fill of seeds sown directly into Hassy-trays filled with "Shamrock" peat-based potting compost. For each seed sample, 180 seeds were sown in three replicates of 56 into each of two soil moisture levels ie 88% (w/w) (seeds were watered immediately after sowing) or 70% (w/w) (ie as taken from the bag, initial watering took place 2 days after sowing). Moisture content was determined by weight loss after five hours at 103°C. The soil and water were brought to

20°C before sowing and for the first two days following sowing the trays were kept at 20°C in a hot-box and covered with polythene sheets to prevent drying-out. The trays were then transferred to an unheated glasshouse and watered daily. All trials were conducted during June - July, 1989. The number of emerged seedlings was counted 1 and 2 weeks after sowing. A seedling was scored as emerged when the cotyledons were judged to be 90° apart.

RESULTS

The imbibition period and chilling damage

There is a rapid, roughly linear phase of water influx into dry brassica seed starting immediately upon the addition of water and lasting for approximately one hour, after which the rate becomes progressively slower (Fig. 1). Imbibition appears complete after approximately six hours.

Cauliflower seed which are exposed to sub-optimal temperatures (<10°C) during the early stages of germination, ie. prior to radicle emergence, produce less vigorous seedling growth upon transfer to 20°C compared to seeds maintained at 20°C throughout. To demonstrate that this cold sensitivity of the unchitted seed is specifically associated with the imbibition period, seeds from lots F5, W2 and W3 were imbibed initially in water at 20°C or 5°C for 2 hours and then half the seeds from each temperature were transferred to the opposite treatment for an additional 24 hours. The remaining half were also resuspended, in water at the same temperature. Comparison of the subsequent root growth (Fig. 2) shows that the damage incurred within the first 2 hours of imbibition can account for the total extent of chilling injury suffered after the full 26 hours exposure to 5°C. Partial imbibition at 20°C served to protect the seed from damage upon subsequent transfer to 5°C (Fig. 2), supporting the conclusion that chilling injury to ungerminated seed is a function of water uptake into the dry seed and is not due to exposure to sub-optimal tempera-

tures *per se*. From this it was concluded that the initial phase of rapid water uptake is the period of imbibition which is critical to the damage of cauliflower seed. Therefore the imbibition rates presented in the following results were calculated for the first thirty minutes of water uptake.

Rate of imbibition, vigour and the seed coat

For each of six of the seedlots, the rate of imbibition of individual seeds in standing water at 20°C was correlated with the vigour of subsequent seedling root growth (Table 1). The consistently negative values of the correlation coefficients indicate an inverse relationship between vigour and imbibition rate, and from point plots of seedling root-length versus seed imbibition rate (Fig. 3) it can be seen that seeds with imbibition rates at the higher end of the range were more likely to fail to germinate that those with slower uptake rates. However, the correlation coefficients, although statistically significant, were not large (Table 1) and this is related to the observation from Figure 3 that not all non-viable seeds are associated with rapid water uptake.

No correlation of seedling root growth with seed size (either when dry or fully imbibed), or final moisture content was apparent (data not shown).

In leguminous seed the variation in imbibition rate has been shown to be a property of the testa. To determine if the case for brassica seed is similar, the effect on imbibition rate of damaging the integrity of the seed coat was investigated. Scoring the testa of cauliflower seed with a scalpel (ie "split" seed) resulted in an approximately 100% increase ($p<0.001$) in the mean imbibition rate compared to seeds with intact seed coats (Fig. 4) demonstrating that the intact testa of brassica seed is capable of limiting the rate of water influx. The imbibition rate of "cracked" seeds (damaged by impact), when imbibed in standing water, also exceeded that of their intact counterparts by, on average, 50% ($P<0.001$) (Fig. 4). This

shows that impaction of dry seeds can damage the testa sufficiently to impair its function as a barrier to water movement.

Comparison of the average root growth for each seed coat treatment showed a reduction in vigour corresponding to increased imbibition rate (Fig. 4), indicating that the health of the embryo is adversely affected by a high rate of water influx into the dry seed. This therefore suggests a role for the seed coat in protecting the embryo from imbibition damage. Slowing the rate of water influx into cracked and intact seed by imbibing them on dampened paper resulted in an improved vigour score compared to those imbibed in standing water (Fig. 4) supporting the conclusion that the reduced root growth of the damaged seed samples was a direct result of the increased imbibition rate. The correlation coefficients of mean imbibition rate versus vigour for the five treatments were -0.9847 ($p<0.01$) and -0.9784 ($p<0.01$) for seed lots F2 and W2 respectively.

The final moisture content of the seed was unaffected by the condition of the testa or the imbibition medium (data not shown).

Imbibition rate and field emergence

There were significant differences in the emergence vigour of the ten seed lots as well as variation directly attributable to the sowing environment and treatment of the testa (Table 2). The final percentage module-fill of seed samples with damaged testae (ie "cracked") was consistently ($p<0.001$) lower compared with that of their intact counterparts sown under the same conditions. When the initial watering was delayed (soil moisture = 70%) both intact and cracked lots showed improved final emergence scores ($p<0.001$) compared to those watered immediately after sowing (soil moisture = 88%). Such restriction of the free water available to the seed would be expected to slow the rate of imbibition. These results therefore support the conclusion that imbibition damage, resulting from the rapid uptake of water into the dry seed, is a potentially important source of vigour loss in the

field and show that a low quality of testa, such as caused by physical injury, increases the susceptibility of a seed lot to such damage.

Following imbibition at low (70%) soil moisture, 76% of the final seedling emergence occurred within one week of sowing compared to 52% when the seeds had imbibed at high soil moisture ($p<0.001$) (Table 2). This emergence rate was also significantly different ($p<0.001$) between the intact and cracked seed samples which had been imbibed at high soil moisture (62% and 42% respectively) but not at low soil moisture (80% and 73%) ($p>0.05$). A slow rate of imbibition therefore encouraged faster, more uniform emergence times which, as well as being desirable for crop production (Mathews and Powell, 1986), has been shown to be associated with high performance of cauliflower seed (Finch-Savage, 1986).

Predicting emergence vigour

The vigour of twenty cauliflower seed lots (including ten with damaged testae, ie "cracked") was measured as the final emergence percentage under seed bed conditions of 70% moisture (Table 2). Significance of the correlation between emergence and imbibition data was calculated using Spearman's coefficient of rank correlation (Steel and Torrie, 1980) as this statistic does not assume a bivariate normal distribution as does the more usual coefficient of product moment correlation. Correlation with the mean imbibition rates (measured in the laboratory in standing water at room temperature) for all twenty lots, comprising the two varieties Winter st. George and Flora Blanca, gave a negative but non-significant coefficient of -0.1707 ($p>0.05$). Separate consideration of the data from the two cultivars improved the significance of the relationship for the ten lots of Winter St. George ($r = -0.8667$; $p<0.01$), but revealed poor correlation of imbibition rates with emergence for those of Flora Blanca ($r = -0.0909$; $p>0.05$) (Figure 5). This notable difference in the relationship of imbibition rate and vigour between the two cultivars suggests that there

is variation in the sensitivity of the seed to the effects of high imbibition rates, as has been reported for pea cultivars by Powell and Mathews (1980). In an attempt to compensate for any such variation, the seed-sensitivity was estimated as the difference in emergence percentage between intact and cracked samples of each lot expressed as a proportion of the difference in their laboratory measured imbibition rates (Figure 5). The correlation of the product of these two factors (imbibition rate x sensitivity) with emergence for all twenty samples is highly significant ($r = -0.6692$; $p=0.002$) and from this it is concluded that imbibition damage is a function of absolute rate of water uptake interacting with the susceptibility of the embryo to disruption by a rapid influx, and that this effect is important to the emergence vigour of module sown cauliflower seed.

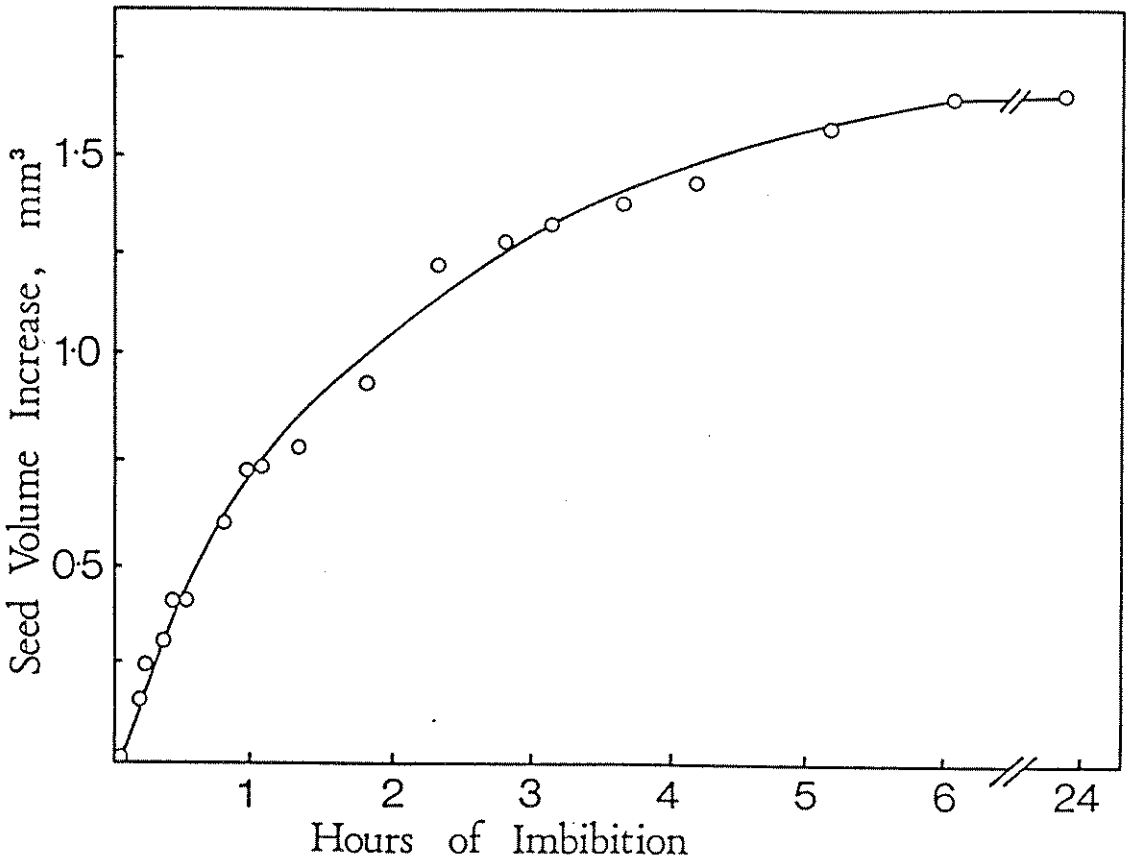

Figure 1. Time course of imbibition of cauliflower seeds (seed lot W2) in standing water at room temperature. The increase in seed volume from time zero is interpreted as equal to the volume of water imbibed. Each point is the mean of twenty seeds. Prior to imbibition, seed moisture content was in equilibrium with air (6.2% w/w).

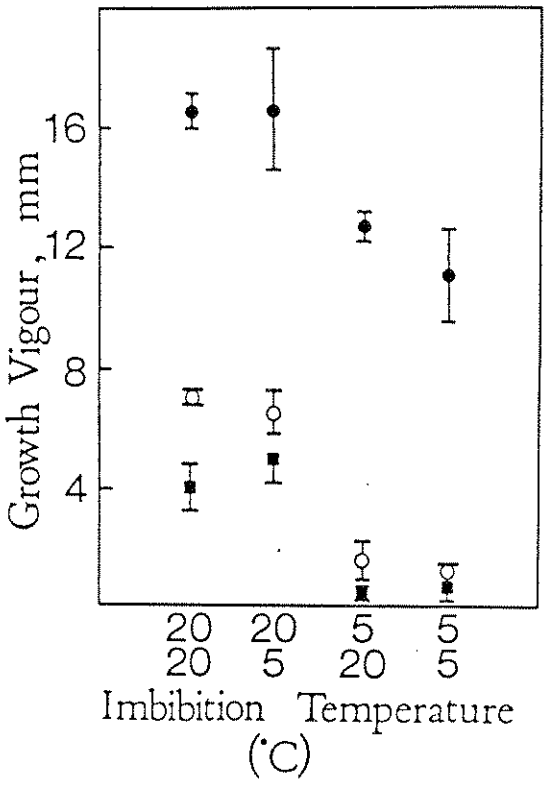

Figure 2. Effect on vigour of cold treatment during imbibition. Dry seeds of lots W2 (■), W3 (○) and F5 (●). were imbibed in standing water at 20°C or 5°C for 2 hours and then transferred to the opposite treatment or maintained as was, for a further 24 hours. Vigour was measured as mean (+/- SE) root growth of 40 seeds after 5 days at 20°C.

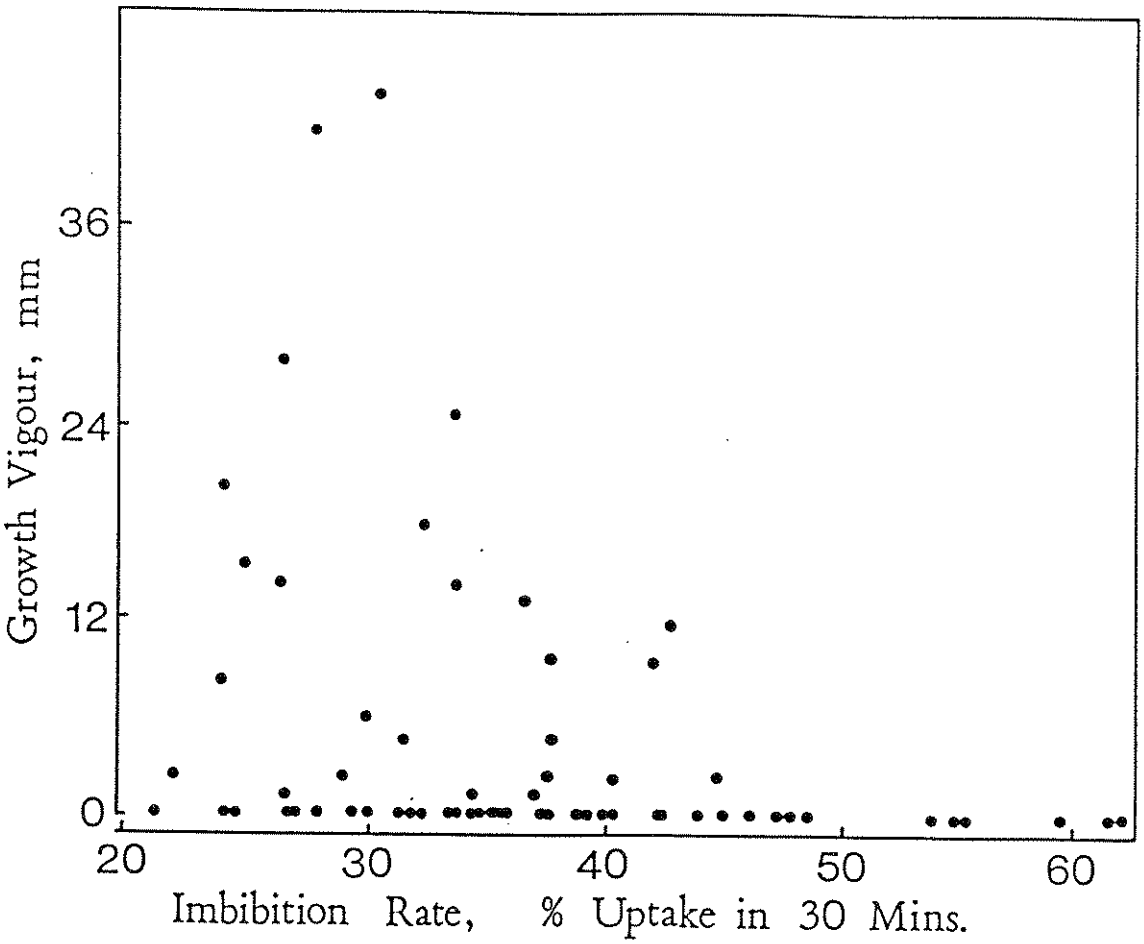

Figure 3. Distribution of root growth vigour with respect to imbibition rate of 64 individual seeds of seed lot W3. Seeds were imbibed in standing water at room temperature and the rate calculated as the percentage of total uptake achieved after the initial 30 minutes. Vigour was assessed as root-length, measured after the imbibed seed had been germinated at 20°C for 5 days.

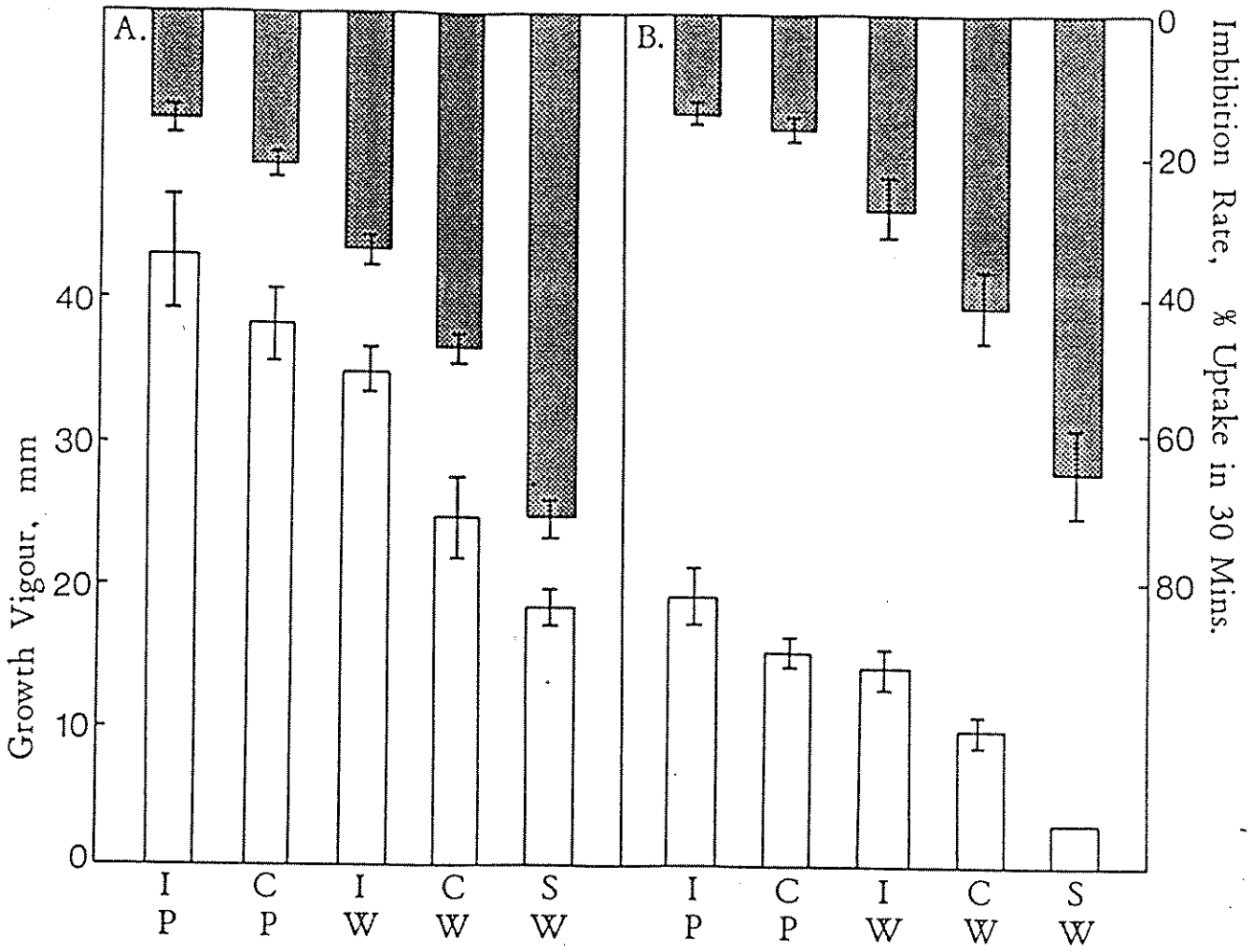

Figure 4. Relation of the mean imbibition rates (shaded bars) and corresponding growth vigour (open bars) of seeds with split (S), cracked (C) and intact (I) seed coats. Seeds of lots F2 (A) and W2 (B) were imbibed in standing water (W) or on damp paper (P) for 24 hours. Imbibition rate was calculated as the percentage of total water uptake (after 24 hours) which occurred within the initial 30 minutes. Imbibed seeds were germinated at 20°C for 5 days before measurement of root growth. Each bar is the mean value of 40 seeds (+/-SE).

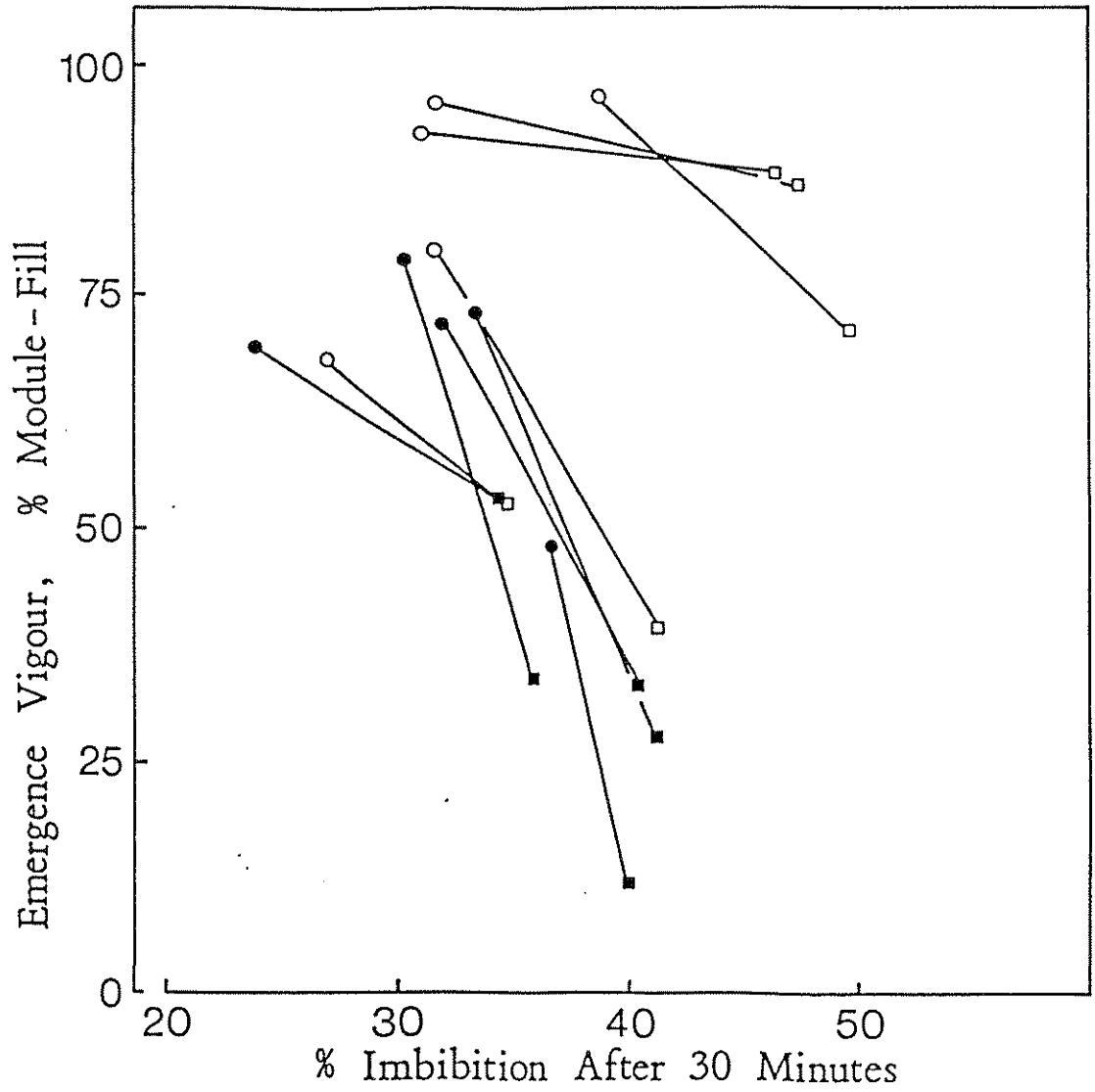

Figure 5. Negative correlation of imbibition rate (calculated as percentage imbibition after the initial 30 minutes) with emergence vigour of 20 cauliflower seed samples comprising the cultivars Flora Blanca (open symbols) and Winter St. George (solid symbols) with untreated ("intact") (●,○) and damaged ("cracked") (■,□) seed coats. Samples with intact and cracked seed coats taken from the same original lot are joined by a line (—). Imbibition rates (measured at room temperature in standing water) are the mean of 80 seeds. Vigour was assessed as percentage module-fill, of three replicates of 56 seeds, under seed-bed conditions of 70% soil moisture at 20°C.

Table 1. Correlation coefficients of imibition rates with the vigour of seedling root growth for 64 individual seeds of six cauliflower lots. Imbibition rates were calculated as percentage of final water uptake imbibed after the first 30 minutes for each seed individually. Vigour was assessed as the length of seedling roots after 5 days growth on slant boards at 20°C, non-germinating seed were scored as zero length.

Seed lot	W1	W2	W3	F1	F2	F5
Correlation coefficient	-0.2541	-0.2238	-0.3061	-0.5730	-0.3048	-0.4820
significance (df = 62)	$p<0.05$	$p>0.05$	$p<0.05$	$p<0.001$	$p<0.05$	$p<0.001$

Table 2. Percentage module-fill of cauliflower seed lots sown as supplied, ie "intact" (I) and with damaged seed coats, ie "cracked" (C). Emergence was scored 2 (1) weeks after sowing. Seed bed conditions were 20°C in a hot box and timing of the initial watering gave high (88%) or low (70%) soil moisture for the first 48 hours. Values shown are the mean of 3 replicates of 56 seeds each.

seed lot	W1		W2		W3		W4		W5		F1		F2		F3		F4		F5		mean of all lots	
seed coat	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C	I	C
soil moist%																						
high	26	10	57	16	53	11	44	25	50	16	56	13	70	52	41	28	81	28	74	50	55	25
	(10)	(2)	(34)	(5)	(25)	(5)	(18)	(7)	(32)	(7)	(36)	(3)	(54)	(24)	(14)	(10)	(71)	(16)	(58)	(24)	(35)	(10)
low	48	12	72	33	73	28	70	53	79	34	80	39	92	88	69	53	96	71	96	86	77	50
	(33)	(7)	(64)	(25)	(55)	(21)	(49)	(36)	(67)	(23)	(71)	(25)	(86)	(73)	(38)	(32)	(72)	(65)	(90)	(76)	(63)	(38)

DISCUSSION

The automated recording of the visible increase in seed size by means of a machine vision facility has been successfully used as an alternative to measurement of seed weight as a means to follow the course of imbibition. Using this technique, this paper presents evidence that imbibition damage resulting from the rapid uptake of water into dry seed, previously reported for the seeds of leguminous species eg pea (Powell and Mathews, 1979) and soybean (Oliveira *et al*, 1984), can also impair the vigour of brassica seed.

The reduced emergence in modules in which the seed was watered immediately after sowing, compared to when the initial watering was delayed to encourage a slower rate of imbibition, indicates that control of the soil moisture content during the imbibition period, as well as soil temperature, is an important aspect of glasshouse management if the full potential of high quality seed in terms of percentage emergence and uniformity of development is to be achieved. Low seedling emergence under conditions of high soil moisture has also been observed in peas (Baylis, Deshpande and Storey, 1943) and soybean (Ikeda, 1986).

The vigour rating of seeds as assessed in a laboratory test has also been shown to be affected by the degree of imbibition damage. In standard test procedure (ISTA, 1976) dry seeds are imbibed on damp paper pads which has been shown to encourage a slow rate of water uptake. Pre-imbibition of seeds in water before transfer to the germination conditions would serve to maximise imbibition stress and may therefore more closely represent the situation in the field. Conversely, if required, imbibition damage could be eliminated as a vigour component by humidification of the seeds before testing (Ellis, Hong and Roberts, 1989).

Differences in the imbibition rate between seed lots of leguminous cultivars have been shown to be a property of the testa (Mason *et al*, 1982; Oliveira *et al*, 1984; Powell *et al*,

1986) and the marked increase in imbibition rate as a result of damage to the testa of cauliflower seed demonstrates that the seed coat of brassicas can also act as a barrier to rapid water uptake. The observation that mechanical stress can damage the integrity of the testa sufficiently to impair this function and bring about a decline in emergence vigour has implications for the handling of seed. Seed coat damage resulting from mechanised harvesting and threshing processes has been reported for a number of species including soybean (Green, Cavannah and Pinnell, 1966), field bean (Wilson, 1987) and Amaranth (Krishna, Kasturi, Beeson, Berlage, Weber and Kauffman, 1987) and is a potential source of vigour loss in brassica seed.

The indistinctness of the division between the imbibition rates of individual dead and viable seeds within a lot, as seen in figure 3, and the variation in the threshold level due to differences in sensitivity between lots largely precludes the use of such results to predict the germination of a lot. Thornton, Powell and Mathews (1989) reached a similar conclusion for the seed leachate conductivity test performed on Brussel sprout cultivars. The use of group means to rank seed lots does appear to have a significant relationship with emergence potential. However, it has been demonstrated that variation in susceptibility to damage can also be influential. Imbibition damage therefore appears to be a function of embryo quality as well as absolute rate of water influx. This factor complicates interpretation of laboratory measured imbibition rates and thereby severely limits the value of such results as a predictor of field-vigour.

Osmoconditioning has been reported to increase germination rate and final germination percentage of cucumber and tomato seed at sub-optimal temperatures (Thanos and Georghiou, 1988) and the effectiveness of priming and pelleting treatments is currently being assessed by Hua, Chang and Tang (1989). Given that imbibition damage can be a significant factor limiting the performance of cauliflower seed, it may prove economical to pretreat brassica seed using such techniques.

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