A survey of seed vigour in field and garden peas (*Pisum sativum* L.)

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Abstract

A survey of five seed lines of field peas and thirteen seed lines of garden peas was performed to obtain data on the relationship between a range of management factors (nitrogen application, windrowing and desiccation), nutrient concentration (macro and micro nutrients), seed vigour (assessed by accelerated ageing (AA), hollow heart, seedling weight, cold test, electrical conductivity) and field emergence (FE) in both March and June. Mechanical damage was also assessed. Mechanical damage was found to be an over-riding factor in seed performance. Examination of non-damaged seed indicated that zinc and calcium concentration were implicated in performance of both field ($R^2 = 0.95$) and garden peas ($R^2 = 0.67$) after AA. A critical concentration for garden peas of 47 µg/g zinc was identified for germination of >78% after AA, and 0.1% calcium for germination >65% after the cold test. Copper concentration was significantly and negatively related to FE in June in garden peas ($R^2 = 0.69$); for FE >90%, copper concentration should be below 7 µg/g. Incidence of hollow heart was significantly related ($R^2 = 0.82$) to concentration of zinc, calcium and nitrogen in the seed.

Additional key words: *accelerated ageing, cold test, electroconductivity, ferric chloride, field emergence, germination, hollow heart.*

Introduction

Seed vigour is defined as an index of the extent of the physiological deterioration and/or mechanical integrity of a high germinating seed lot which governs its ability to perform in a wide range of environments (Hampton, 1998). Several different methods are used to assess seed vigour (Hampton and TeKrony, 1995). For garden peas, electroconductivity is used routinely to predict field emergence (Hampton and TeKrony, 1995). In New Zealand no other species currently has vigour testing as a part of quality assessment.

For the pea industry, seed vigour is important at several stages. For the grower, canopy closure (thereby maximising radiation interception) is achieved more rapidly if seed lines are of even vigour than if patchy growth results from uneven vigour seed; in non-ideal environmental conditions, poor vigour seed may not germinate or emerge at all. For growers of garden peas, patchy emergence and growth can mean that their crop is unsuitable for processing. For consumers of pea sprouts, even height and weight are attractive; for sprout growers they are, therefore, a necessity. Thus pea seeds of high germination and vigour are required.

Unlike germination, which involves a series of enzyme reactions and mobilisation of stored carbohydrate and proteins, seed vigour is a concept encompassing several performance characteristics (Perry, 1981; Hampton and TeKrony, 1995). It can be considered as the reciprocal of the deterioration processes involved with seed ageing (Hampton, 1991). The nature of the concept of seed vigour has not yet been fully explained, but several factors such as loss of membrane integrity, maternal plant nutrition, poor seed storage, and environment (both macro and micro) have been proposed as factors affecting vigour (Hampton 1991). These factors have been reviewed extensively (Hampton and Coolbear, 1990; Hampton, 1991, Hampton and TeKrony, 1995). In the context of the current research, the major factors of importance are membrane integrity and maternal plant nutrition affecting seed nutrient status.

Loss of membrane integrity, whether mechanical or chemical, is a fundamental cause of differences in seed vigour (Powell, 1985, 1988). In peas, mechanical disruption readily occurs at harvest. Seed harvested moist (e.g., 40% seed moisture content (SMC)) is subject to bruising and internal damage; seed harvested at 15% SMC has a higher incidence of hollow heart than seed harvested at 25% SMC (Castillo *et al.*, 1992). Seed harvested at below 15% SMC is subject to cracking damage as the seeds become brittle (Gane *et al.*, 1984). Harvesting seed in the moist state has implications for drying requirements. The latter is, in itself, of importance: slow drying can increase the risk of fungal disease, which could affect seed vigour; fast drying could damage the embryo and affect seed vigour (Hill and Johnstone, 1985). Chemical deterioration of membranes could be due to inadequate nutrition (Powell, 1985), use of desiccants (Miller, 1988) or field deterioration (Matthews and Powell, 1986).

Maternal plant nutrition has been implicated in seed vigour, but research such as that reported by Ene and Bean (1975) confounded seed nitrogen concentration with the effect of increasing thousand seed weight; increased thousand seed weight is often associated with increased vigour (e.g., Hampton 1986, Charlton 1989), but the reverse has also been reported (Wang and Hampton 1991). In garden peas, research relating fertiliser treatment directly to seed vigour, has obviated the content/concentration problem by concentrating on the overall effect (Padrit *et al.*, 1996). Addition of nitrogen to the maternal plant increased germination after AA and decreased conductivity and hollow heart. Addition of phosphorus to the maternal plant increased germination after AA and reduced hollow heart. It is not clear from this work whether increased nutrition was having its main effect through a direct or indirect effect on seed physiology.

This research was established to compare methods of assessing seed vigour with field emergence, and to identify the effects of nutrient status, nitrogen application at flowering and harvesting practice on seed vigour in field and garden peas.

Materials and methods

Five lines of field pea seed and 13 lines of garden peas were obtained from Canterbury in 1996. Field peas had had one of the following practices: zero nitrogen (N) at flowering, plus N at flowering, windrow 5 weeks before harvest, windrow 3 weeks before harvest, diquat and direct heading. The garden peas represented eight different cultivars; management practicesfor the 13 lines were unknown. Germination tests were performed according to internationally agreed methodology (ISTA, 1996). Seed vigour was measured by electro-conductivity, seedling weight, cold germination and accelerated ageing (ISTA, 1995). Ferric chloride was used to assess mechanical damage (Duangpathra, 1986) and hollow heart was assessed in garden peas (Hampton and Scott, 1982). Field emergence was recorded in 1996 after sowing peas in a standard-prepared seed bed in March (average soil temperature at 100 mm soil depth at 9 am was 12°C) and June (100 mm soil temperature 6°C). Nutrient analyses were performed on all samples by Ruakura Analytical Services and were not replicated.

Statistical differences were analysed using Minitab version 10. ANOVA were performed and differences in seed vigour were related to seed nutrient status and management practice using correlation and regression analysis. Duncan's multiple range test at $P<0.05$ was used to establish significant differences. The Cate-Nelson separation technique (Cate and Nelson, 1965) was used to establish critical thresholds for responses where appropriate.

Results

Field peas

Seed moisture content of all lines was between 6.9 and 8.8% and thousand seed weight was between 338 and 372 g. The heaviest seed was associated with the treatment to which N had not been applied (Table 1). Mechanical damage was greatest in the windrowed 3-weeks and desiccated treatments, followed by the windrowed five-weeks treatment (Table 1).

Germination was significantly reduced by windrowing for five weeks; germination of all other seed lines was 89% or above (Table 1). There were no significant differences in seedling weight (20.8 mg/seedling),

Table 1. Effect of five management practices on seed moisture content (SMC; %), thousand seed weight (TSW; g) and mechanically-damaged seed (%) of field pea.

Management practice	SMC (%)	TSW (g)	Mechanically-damaged seed (%)	Germination (%)	Conductivity ($\mu s\ cm^{-1} g^{-1}$seed)
Urea application at flowering stage	6.9 a	358 c	6 a	91 b	9.68 a
No-nitrogen	8.0 b	372 d	4 a	89 ab	9.57 a
Diquat-direct head	8.1 bc	338 a	27 c	91 b	12.21 b
Windrow 3 week before harvest	8.7 c	350 b	25 c	91 b	12.37 b
Windrow 5 wk before harvest	8.2 bc	342 a	16 b	83 a	10.41 a

[1] Values within the same columns followed by the same letter are not significantly different at $P<0.05$ value by Duncan's multiple range test

germination after accelerated ageing (91%) or germination after the cold test (54%). Electrical conductivity was low in all seed lines, but did differ significantly between treatments: electroconductivity was greatest for the desiccated and '3-week windrow' treatments, which were the two treatments with >25% mechanical damage. The correlation between the ferric chloride test and electroconductivity was 0.97. There were no significant differences between treatments in field emergence in either the March (89%) or June planting (87%).

There were no significant correlations between seed vigour and FE in March Correlations between different seed vigour assessment techniques and field emergence in June indicated that 94% of the variability in field emergence could be accounted for by laboratory germination or cold test; accelerated ageing accounted for 86% of the variability in field emergence. The electroconductivity test and the ferric chloride test were negatively and poorly related with FE.

Nutrient concentration in the five seed lines examined was little affected by treatment (N = 3.68%, phosphorus = 0.42%, sulphur = 0.22%, magnesium = 0.13%, calcium = 0.10%, potassium = 1.05%, manganese = 12.8 µg/g, copper = 6.6 µg/g, iron = 70 µg/g, boron = 9.8 µg/g, and molybdenum = 0.23 µg/g. In contrast, zinc concentration ranged from 30-50 µg/g and accounted for 54% of the variability in FE in June, and 92% of the variability in AA (Table 2). When concentration of both Zn and Ca were considered, 95% of the variability in AA was explained (Table 2). No other nutrient had a significant effect on vigour.

Garden peas

SMC varied from 5.6-9.3%, TSW varied from 183-309 g and mechanically-damaged seed ranged from 6-62% (Table 3). Germination in eleven of the thirteen seed lines was over 90% (Table 4). The two lines with low germination (79%) also performed poorly in all other tests, and had approximately 60% mechanically damaged seed (Table 3). There were significant differences in the performance of the high germination seed lines in all vigour tests, the percentage of hollow heart, and in FE (Table 4). In most cases, the poor performance was associated with mechanical damage; when the mechanically damaged seed lots were removed from analysis, there were no significant correlations between any of the vigour tests and field emergence in March or June (data not presented).

Seed nutrient status in garden peas tended to be higher and range more (Table 5) than in the field peas surveyed. Concentration of Cu explained 69% of the variability in FE in June; the relationship was significant and negative (Fig. 1). No other nutrient explained more than 36% of the variability (data not presented).

Table 2. Linear regression relationships between field emergence in June, accelerated ageing and zinc and calcium concentration in field peas.

Regression equation	R^2	P
FEJune = 105 - 0.423 Zn	0.54	0.097
AA = 112 - 0.510 Zn	0.92	0.007
AA = 93.8 - 0.488 Zn + 180 Ca	0.95	0.026

[1] Accelerated ageing

Table 3. Seed moisture content, thousand seed weight, and mechanically-damaged seed of 13 garden pea seed lines.

Seed lines	SMC[1] (%)	TSW[2] (g)	Mechanically-damaged seed[3] (%)
Alderson (A)			
A1[4]	6.33e[5]	309b	20bc
A2	8.40bc	293c	18c
A3	8.06cd	329a	62a
Greenfeast (G)			
G1	6.17e	199i	6e
G2	7.87d	227g	7e
Princess (P)			
P1	9.30a	239f	24b
P2	8.29c	214h	10de
Small-sieve freezer (S)			
S1	6.26e	239f	10de
S2	6.32e	248e	25b
Early type			
Massey	7.70d	212h	15cd
Victory freezer	8.70b	224g	58a
Meteor	6.43e	253d	8e
Late type			
Greenshaft	5.60f	183j	8e

[1] Seed moisture content
[2] Thousand seed weight
[3] Tested with ferric chloride
[4] Different seed lines in each variety have been denoted by a different number
[5] Values within the same columns followed by the same letter are not significantly different at P<0.05 value by Duncan's multiple range test

Table 4. Seed germination, seed vigour and field emergence of different varieties of garden peas.

Seed lines	Germination (%)	Germination after accelerated ageing (%)	Seedling weight (mg/seedling)	Cold test (%)	Conductivity (µS cm^{-1} g^{-1})	Hollow heart (%)	Field emergence (%) March	June
Alderson (A)								
A 1[1]	99a	37e	27.59b	31cd	25.50cd	28b	80ab	87a
A 2	94a	39e	26.99bc	29cde	28.58c	49a	81ab	94a
A 3	79b	28e	28.27ab	8e	49.38a	28b	69cd	70b
Greenfeast (G)								
G 1	99a	80c	20.82g	72a	16.90f	8de	74bc	88a
G 2	97a	33e	22.66e-g	53ab	22.33e	15cd	78ac	89a
Princes (P)								
P 2	99a	73c	24.65de	17de	26.30cd	25b	87a	93a
Small-sieve freezer (S)								
S 1	98a	96a	27.94ab	47bc	15.2f	4e	84a	96a
S 2	96a	97a	30.02a	71a	21.10e	0e	86a	93a
Massey	98a	76c	23.97d-f	66ab	26.23cd	6e	86a	87a
Victory freezer	79b	61d	24.87c-e	13de	28.70c	2e	65d	60b
Meteor	94a	93ab	27.57b	74a	15.83f	0e	73b-d	84a
Greenshaft	97a	75c	21.88fg	65ab	38.20b	20bc	80ab	84a
F-test	**	**	**	**	**	**	**	**

[1] Different seed lines in each variety have been denoted by a different number

[2] Values within the same columns followed by the same letter are not significantly different at a given P value by Duncan's multiple range test

Regression analysis (Table 6), which excluded the two severely damaged seed lines, indicated that zinc and calcium concentration explained 67% of the variability in accelerated ageing, and, with TSW, 67% of the variability in seedling weight. These three factors, with nitrogen concentration, explained 82% of the variability in the presence of hollow heart.

Cate-Nelson analysis indicated a critical threshold for copper concentration of 7 µg/g, which was associated with a field emergence in June of >91% (Fig. 1). A critical concentration of zinc of 47 µg/g was required to achieve a germination of >78% after accelerated ageing (Fig. 2). The relationship between calcium concentration and cold test was also significant, and a critical threshold of 0.1% Ca was identified for a germination after the cold test of >65% (Fig. 3).

Discussion

Most seed lines in this survey had a field emergence of over 90%. The two lines of garden peas which achieved field emergence of less than 70% also had laboratory germinations of below 80%. Thus in this survey, laboratory germination was a good indicator of likely field emergence.

With only five samples of field peas it is difficult to make definitive statements about methods to assess seed vigour; accelerated ageing, seedling weight and the cold test should receive further investigation. In the garden peas the ferric chloride test has potential for further investigation.

In all peas physical damage and associated decrease in germination under cold stress was apparent. This damage was visible as cracking in the seeds; it is likely that the damage occurred at harvest and was a result of hard threshing of an 'unfit' crop. However, the damage did not result in a dramatically-reduced field emergence (although it was associated with poor germination in the cold test, where the cracking may have increased seed susceptibility to pathogens). This suggests that further work on moisture at harvest and rate of drying to 'safe moisture' is required.

Table 5. Nutrient concentration of seed lines from different varieties or types of garden pea.

Variety/Type	N (%)	P (%)	S (%)	Mg (%)	Ca (%)	Na (%)	K (%)	Mn (μg/g)	Zn (μg/g)	Cu (μg/g)	Fe (μg/g)	B (μg/g)	Mo (μg/g)
Alderson (A)													
A1[1]	3.65	0.45	0.20	0.14	0.05	0.01	1.46	11	42	9	85	8	0.18
A2	3.97	0.38	0.22	0.12	0.04	0.01	1.33	10	34	5	85	10	0.18
A3	3.78	0.46	0.22	0.13	0.04	0.01	1.45	11	52	9	86	10	0.25
Green feast (G)													
G1	3.87	0.41	0.22	0.15	0.12	0.01	1.24	16	47	8	118	14	0.23
G2	4.16	0.30	0.22	0.13	0.10	0.01	1.27	11	40	7	93	10	0.48
Princess (P)													
P1	4.27	0.49	0.22	0.15	0.08	0.02	1.44	9	53	10	96	10	0.80
P2	3.56	0.49	0.20	0.15	0.14	0.03	1.41	17	40	6	83	9	0.15
Small -sieve freezer (S)													
S1	4.25	0.45	0.20	0.13	0.08	0.01	1.18	10	52	7	91	14	0.57
S2	3.71	0.35	0.18	0.17	0.16	0.01	0.95	13	50	6	110	15	0.26
Early type													
Massey	4.11	0.41	0.22	0.14	0.11	0.04	1.30	18	40	8	83	9	0.79
Victory freezer	4.25	0.57	0.23	0.16	0.14	0.01	1.27	14	56	11	104	14	0.11
Meteor	4.01	0.48	0.20	0.12	0.10	0.01	1.02	13	55	9	75	18	0.20
Late type													
Greenshaft	3.85	0.42	0.19	0.13	0.10	0.02	0.22	9	42	9	97	15	0.92

[1] Different seed lines in each variety have been noted by a different number

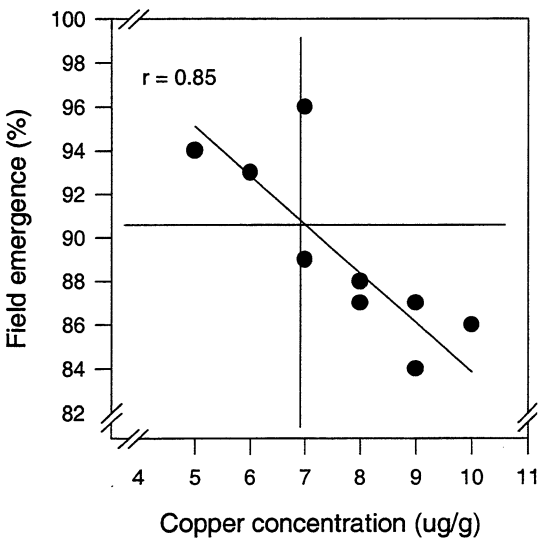

Figure 1. Relationship between copper concentration (μg/g) and field emergence (%) in June in garden pea (*Pisum sativum* L.).

Despite suggestions in the literature that maternal plant nutrition of P and N is an important factor in seed vigour in peas (e.g., Browning and George, 1981; Hadavizadeh and George, 1988; Padrit *et al.*, 1996) no evidence in support was found in this study. Nitrogen concentration ranged from 3.57-3.86 in field peas and 3.56-4.27 in garden peas; phosphorus concentration ranged from 0.38-0.45 in field peas and 0.35-0.57 in garden peas. Calcium concentration has been proposed as an important factor in membrane integrity, but there was no significant correlation (r=0.17) between electroconductivity and calcium concentration (range 0.04-0.16%) in the survey.

In contrast, the concentration of copper was negatively related to field emergence in garden peas and the concentration of zinc was implicated in seed vigour in both field and garden peas; relationships were improved with the inclusion of calcium concentration in the regression equation. Increasing zinc content in seed has been associated with increased grain yield in wheat (Yilmaz *et al.*, 1998) and increased dry matter in rape (Grewal and Graham, 1997) in zinc deficient soils. High

Table 6. Regression relationships for accelerated ageing, seedling weight, hollow heart and nutrient concentration in garden peas.

Regression equation[1]	R^2	P
AA = 88.7 - 1.21 HH	0.53	0.007
AA = -50.2 + 2.69 Zn	0.54	0.006
AA = -60.6 + 2.34 Zn + 269 Ca	0.67	0.005
AA = -82.7 + 6.2 N + 286 Ca + 2.25 Zn	0.62	0.019
AA = -22.7 - 0.133 TSW + 177 Ca + 2.40 Zn	0.66	0.014
SdWt = -2.28 + 0.0788 TSW +36.8 Ca + 0.123 Zn	0.67	0.012
Cold test = -28.2 + 1.83 Zn	0.30	0.046
HH = 95.7 - 1.80 Zn	0.63	0.002
HH = 79.8 + 0.0781 TSW - 112 Ca - 1.62 Zn	0.76	0.004
HH = 175 + 0.0044 TSW - 224 Ca - 1.28 Zn - 20.6 N	0.82	0.005

[1] AA - Accelerated ageing; HH - Hollow heart; TSW - Thousand seed weight; SdWt - Seedling weight

zinc seed in rape was associated with increased seedling vigour, increased growth, and increased chlorophyll concentration (Grewal and Graham, 1997). In contrast, soybean germination has been reported to be negatively correlated with zinc concentration, but positively correlated with calcium concentration (Smiciklas *et al.*, 1989). No data have been reported on copper and seed vigour. Calcium availability is unlikely to be a problem when soils are weakly acid, but the availability of both copper and zinc is pH dependent, decreasing with increasing pH. The results of this survey indicate that a deficit in zinc (the potential for which has already been identified on the Canterbury Plains (Haynes, 1995), where 94% of peas are grown (MAF Certification Statistics)), could have a detrimental affect on seed vigour in peas. A deficit in copper, which is often associated with a deficit in zinc because of over-liming (Haynes, 1995), could be advantageous (within limits,

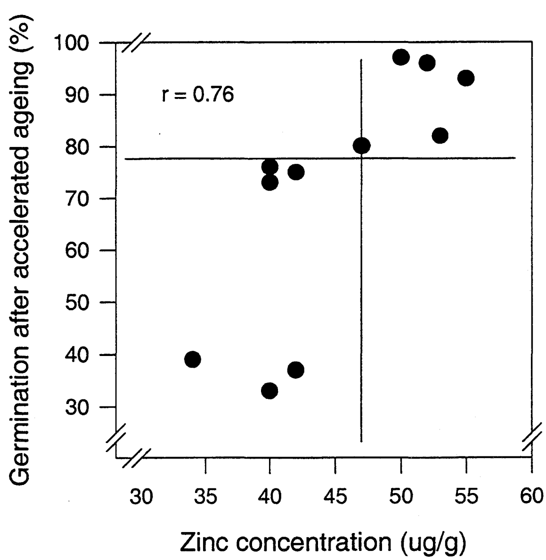

Figure 2. Relationship between zinc concentration (μg/g) and germination (%) after accelerated ageing in garden pea (*Pisum sativum* L.).

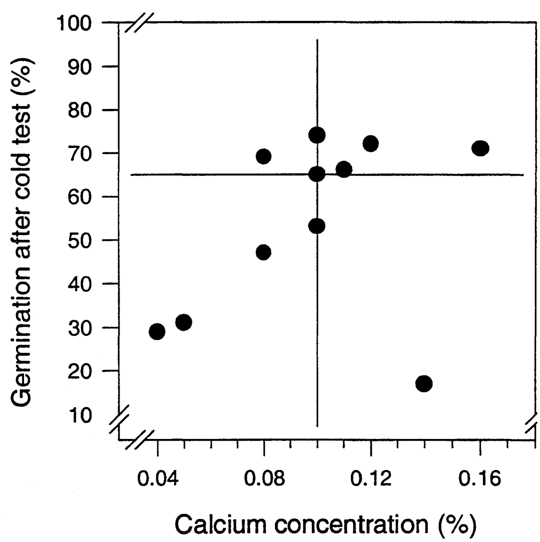

Figure 3. Relationship between calcium concentration and germination (%) after the cold test in garden pea (*Pisum sativum* L.).

not yet identified. This suggests that a zinc deficiency should be corrected through zinc fertiliser rather than amendment of pH status.

Research is required to investigate the relationship between copper and zinc concentration in soil, plant and seed, and the concomitant effects on seed vigour, under controlled, rather than survey conditions.

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