بسم الله الرحمن الرحيم

In the Name of Allah,
the Compassionate, the Merciful,

INTERACTIONS BETWEEN SIZE GRADING AND THE PHYSIOLOGICAL FACTORS LIMITING THE GERMINATION OF SUGAR BEET FRUITS

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INTERACTIONS BETWEEN SIZE GRADING AND THE PHYSIOLOGICAL FACTORS LIMITING THE GERMINATION OF SUGAR BEET FRUITS

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ABSTRACT

The quality and quantity of the sugar produced from sugar beet is strongly dependent on optimizing plant spacing in the field. Poor germinability and, in particular, low plant establishment, has long been a problem in sugar beet production, particularly in precision drill sowing systems designed to omit thinning, which is a time consuming process with high labour costs. In addition to physical and environmental stresses accruing in the field during germination and seedling emergence, fruit size and the physiological characteristics of the fruit itself have also been considered as very important factors involved in poor plant establishment.

This experiment was carried out using samples of three lots of a diploid monogerm cultivar (9597) which was released in Iran in 1985 and continues to be produced by the Sugar Beet Seed Institute of Iran (S.B.S.I.). According to the germination capacity of ungraded fruit, these lots were categorized as medium (LOT A), low (LOT B) and high (LOT C) quality lots and were selected to determine whether there was any similarity in the relationships between fruit size and quality within different lots.

Despite there being a linear and highly significant correlation (r= 0.96***) between fruit diameter and germination as well as fruit diameter and plant establishment in the low quality lot, the nature of these relationships in the medium and high quality lots were different in that the large fruits showed equal or lower germination and planting value than the medium fruit sizes. No apparent relationship was found between fruit thickness and germination performance of the seed lots. Although there was no significant correlation between the laboratory standard germination result for both thickness and diameter graded fruits and plant establishment of the high quality lot, highly significant correlations were found between the laboratory germination and plant establishment of the size grades of the medium and poor quality lots (r= 0.91 and 0.99 for Lots A and B, respectively). This appeared to be a function of the variation in germination performance of the size grades and suggests that, although in poor and medium quality lots the germination percentage of the fruit can sometimes be used as an index of field performance, in high quality lots more emphasis should be placed on the vigour of the seed.

The results obtained via size grading of the seed lots used in this study illustrated that 60% of harvested fruits of each lot were either too small or too big to be used for the precision drill sowing system. Further, it was also found that 24% of the fruits within the suitable size grades were either seedless (seeds aborted) or contained under-developed seed. X-radiography of the size graded fruits of the medium quality lot (A) illustrated that, despite the fact that immaturity was mostly associated with the smallest fruits (where 64% of the fruits were immature), about 18% of the larger fruits (4-5mm diameter) also contained immature seed.

An important point to note is that 11-12 % of fruits with fully mature seed in the small and larger size grades did not germinate when incubated in optimal conditions. In contrast, only 3% of the fruits of the medium size grade did not germinate. This indicates that other germination limiting factors besides immaturity are involved and that they may vary between size grades. Because of its role in impeding radicle emergence and/or oxygen entrance into the seed cavity, cap tightness is known to be an

important germination limiting factor in sugar beet. Thus the tighter the seed cap, the lower the germination of the fruit. Direct measurement of the force required for cap movement indicated that cap removal in larger fruits required a greater force than in small fruits. These results were in a similar range to those found by Morris *et al.* (1985) via indirect estimates, suggesting that enzymatic action on cap loosening is unlikely to play an important role in cap removal and therefore the direct method used in this study may be useful for selection of progenies with a reasonably loose seed cap.

Chemical inhibitory substances in the fruit pericarp have been shown to be the other important factors inhibiting germination of the seed. As they are water soluble, germination improvement may be obtained following prewashing of the fruits. Despite significant germination improvement on pleated paper after prewashing of the fruits of the high quality lot (C), no improvement was obtained via prewashing the size grades of the medium and poor quality lots. However, a significant germination improvement was achieved when prewashed fruits of the medium seed quality lot were incubated on a wetter substrate in Petri-dishes. It was found that the pericarp base is the main entry route of oxygen to the seed cavity and removal of this resulted in a 29% increase in germination percentage of the thick fruit of lot A when incubated in Petri-dishes. This is attributed to shortening the path for oxygen transfer to the seed cavity. However, a similar improvement in germination was also obtained via prewashing the intact fruit and a synergistic improvement in germination (45% increase) was found as a result of prewashing plus pericarp base removal. This could be explained on the basis of increased oxygen uptake into the embryo via the removal of both chemical and physical barriers to oxygen entry to the seed cavity, but requires confirmation by further research.

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CHAPTER I

1. INTRODUCTION

1.1 THE IMPORTANCE OF PLANT ESTABLISHMENT IN SUGAR BEET PRODUCTION

Sugar beet (*Beta vulgaris*), which was recognized as early as the 16th century as a plant with valuable properties (Thomas Theis 1971), is presently grown on over nine million hectares, mainly in the temperate regions of the Northern Hemisphere. The most important production areas are the former USSR with 3,526,000 ha., Europe 3,873,000 ha., Germany 418,000 ha. and USA 418,000 ha., (Durrant *et al.*, 1986).

Sugar beet is a biennial plant which accumulates a reserve food supply in the root during its first growing season to enable the plant to survive over the winter and produce flowering stems and seed, (correctly fruit) in the following year. In sugar beet, the term ›monogerm›is used when a cluster includes one fruit and ›multigerm› when more than one fruit makes a cluster. Normally sugar beet fruit are monocarpic; therefore, each fruit contains a single seed which is called a true seed or germplasm, comprising the embryo, food reserves (perisperm and cotyledons) and testa. The true seed lies horizontally in the cavity of a cup-shaped pericarp (commonly referred to as the seed coat rather than the fruit coat) and is covered by a removable cap (usually termed the seed cap) which is usually strongly attached to the pericarp.

Except when grown for seed production, the life cycle is usually interrupted by harvesting the root during the first season of growth when the sugar content is high. Besides being grown for sugar production, beet tops are used for animal feed, as is the pelleted mixture of pulp and molasses which remains after the sugar extraction process. In addition, this crop may be grown for ethanol production.

Usually the quality and the quantity of sugar produced from sugar beet is strongly dependent on the plant density and, in particular, optimizing the plant spacing. Normally 50-60 cm rows are prepared with inter- row distances of 15 - 20 cm (see section 2.1). Planting high vigour seed is an essential pre-requisite for obtaining suitable plant establishment. According to the literature (discussed in section 2.2), poor germinability and, in particular, low plant establishment, has long been a problem in sugar production, particularly in the precision drill system which has been designed to omit thinning, a time consuming process with high labour costs.

1.2 GERMINATION LIMITING FACTORS IN SUGAR BEET SEED

Because of the plants, cross pollinating nature, indeterminate flowering habit, and also extensive intra- and inter-plant competition on the parent plant, every lot of sugar beet seed comprises a wide range of seed size and maturity grades and these may not be of equal value for sowing. Owing to these problems, the producers of commercial fruit have to reject large amounts of the seed bulk during processing to provide a good quality seed lot of a standard size to use in the precision drill system (2.25-3.25mm thickness, 3.25- 4.5mm diameter and more than 85 % germination under optimal conditions). Besides losing lots which are under and over standard grades, this grading process is time consuming and also needs a high level of expertise. Thus this kind of seed is expensive to produce. Nevertheless, even after grading poor establishment still remains a problem for commercial sugar beet production.

There is some debate about the reason for poor seed germination. Snyder (1963), for example, showed that the presence of physico-chemical inhibitors in the seed coat of beet seed may delay the emergence and/or diminish final germination, while Grimwade *et al.* (1987) suggested that the presence of underdeveloped and shrivelled seed and seedless or empty fruits are the main causes of poor quality in beet seed. However, the interactions between these factors and involvement of others should also be taken into account in explaining low germination and,in particular, the poor stand establishment characteristic of the beet seed.

Although a lot of effort has been made to highlight the relative effects of the different factors involved, there is still argument in this area, no doubt because of the wide range of different variables and multiple interactions between them, and with the environmental conditions of the seed bed (see literature review). In addition,there are some aspects of seed performance which have not yet been investigated: for example, the effect of fruit thickness on germination and establishment, the rate of water and oxygen uptake in different sized fruits, and the effects of seed quality on seedling establishment after field emergence.

1.3 OBJECTIVE

By building on previous research, this study was carried out with the following objectives:

1.3.1 To assess the effect of two different size grading methods (based on thickness and diameter grading) on fruit and true seed weight.

1.3.2 To determine the relationship between size grades and percentage, speed, and uniformity of seedling emergence.

1.3.3 To investigate the relationships between fruit size, true seed weight and the seedling dry weight.

1.3.4 To investigate and determine the relative importance of the factors limiting germination, including inhibitory substances in the seed coat and the physical inhibitory functions of the seed coat and the cap.

1.3.5 To evaluate whether the X-ray technique can be use as a reliable method for predicting the germinability of seed.

1.3.6 To determine the relationship between standard germination results and plant establishment.

1.3.7 To define the relationships between the fruit diameter and thickness and plant establishment and seedling dry weight in the field.

1.3.8 To identify the factors which reduce plant establishment after emergence in the field under New Zealand conditions.

To fulfil these objectives, two series of experiments were conducted in this study:

The first stage was designed to define the relationship between fruit size and true seed, seedling dry weight, seed cap and seed coat weight and to evaluate percentage and speed of seedling emergence in both optimal laboratory conditions and also in the field. In addition, factors which may reduce plant establishment after seedling emergence were also investigated. All these results are discussed in chapter four. The second set of experiments was designed to investigate the physiological basis of the germination performance of monogerm sugar beet seed including, the relationship between the stage of seed maturity as determined by X-radiography and germination percentage in size graded fruits, the assessment of the effects of physical and chemical inhibitors in the fruits on seed germination, and also to investigate the physiological properties fruit pericarp bases in relation to germination. The results of this set of experiments are presented and discussed in chapter five.

CHAPTER II

REVIEW OF LITERATURE

2.1 THE IMPORTANCE OF PLANT ESTABLISHMENT IN SUGAR BEET PRODUCTION

According to the literature, the quantity and quality of sugar produced from sugar beet (*Beta Vulgaris L.*) is strongly dependent on optimizing plant spacing. Despite controversies about optimal plant densities for sugar beet production in different environments, there is large body of data demonstrating clear effects of plant density on root and sugar yield. It is well established that wider plant spacing in sugar beet gives larger roots with a greater root crown containing a lower percentage of sucrose and a higher percentage of impurities. On the other hand, owing to competition between plants, the tubers which are produced in a dense spacing are too small to be used in sugar beet factory and most of the plant growth is attributed to the top ground growth.

In a study of sugar beet grown at 56 cm row spacing and inter-row spacings of 15, 23, 31, and 38 cm (119000, 77600, 57000, and 47000 plant/ha, respectively), Kern (1976) reported that the lowest root yields/ha were obtained with the 15 cm spacing and the greatest root yields produced with the 31 cm spacing. In addition, Smith and Martin (1977), grew sugar beet in 56 cm rows at plant spacings of 15, 30, 60, and 90 cm (119000, 59500, 29800, and 19800 plant/ha, respectively) and reported that content of impurities increased with wider inter-row plant spacings. In an attempt to achieve the greatest sucrose production in non-thinned sugar beet with planting the seed at an optimum spacing, by sowing the seeds at 10, 15, 19, and 23 cm inter-row seed spacing for 4 years (1984-1987), Eckhoff *et al.* (1991) found that, the percentage of sucrose content in the root decreased and the impurities increased as seed spacing increased. Although yield varied across the years, increased sucrose content and decreased impurities of beet planted at 10- and 15-cm spacings generally resulted in highest gross sucrose and estimated recoverable sucrose yield. It should be noted that because of possible interaction between different factors such as climatic conditions, soil structure and genotype of the lots in sugar production it is difficult to recommend a standard plant spacing.

Traditionally two methods are used in order to achieve suitable plant density:

1. The conventional method, in which the seed is planted at high density and then manual labour is used for thinning the stand to achieve the desired plant population. However, this practice is a time consuming process and involves high seed and field labour costs.

2. Using a precision seeder and sowing monogerm seed with a high level of germination, high seed vigour and acceptable rate of establishment to obtain the required density.

It is well known that good plant establishment is the key for producing adequate density and suitable uniformity in plant emergence. This is a crucially important factor, especially in the case of sugar production in semi-arid and dry areas because, in spite of sowing monogerm seed with approximately 88-90 % laboratory germination, the rate of establishment is usually less than 50% of the seed sown. This problem not only appears in the above mentioned conditions, but also occurs in temperate climates.

Despite the fact that the quality (as indicated by germination percentage, ISTA 1976), of seed sold to farmers in England, has increased from an average of 87% in 1976 to 94% in 1987 (Fletcher and Prince 1987), sub-optimal plant densities are still common, particularly in crops grown in late March (Durrant and Loads, 1990). For example, in one season in south-east England, because of prolonged early drought, beet emerged 3 months after sowing and plant establishment was less than 30 % of the seed sown (Durrant *et al.*, 1983). Similarly, a two-year (1980 and 1981) sugar beet production study in the Gisborne plains of New Zealand where dry weather in spring, resulted in protracted and patchy establishment which did not exceed 47% of the seed sown (Gray and McCormick, 1983).

Many attempts have been made to identify the factors involved in poor performance of sugar beet seed in field conditions. In the following chapters, the relevant literature is reviewed and subjects such as fruit structure, seed development, germination limiting factors in monogerm sugar beet seed, the physiological base of poor field performance of the seed and the methods introduced for predicting field establishment of sugar beet seed will be discussed.

2.2 THE STRUCTURAL AND PHYSIOLOGICAL FEATURES OF THE REPRODUCTIVE UNITS OF SUGAR BEET

2.2.1 Flowering and seed development in sugar beet

The sugar beet flower, as a reproductive unit, is perfect (Lexander, 1986) and includes 5 sepals, 5 stamens, one style with 3-loped stigma and a tricarpelled ovary in which only one egg is formed (Artschwager, 1927). The nucellus is initiated at the base of the carpel, and two integuments grow out from the base of the nucellus (Artschwager, 1927).

The perisperm which is the main food reserve in the fruit, originates from the nucellus and therefore is genetically female (Fahn, 1982, Mayer and Poljakoff-Mayber, 1989). The position of the ovule in the ovary is at first straight but then later curves and develops horizontally (Artschwager, 1927).

Mean single fruit weight increases with maturity, the increase levelling off at around 55-60 day after anthesis (DAA) when fruit dry weight is around 20mg. There appears to be a critical fruit dry weight above which the percentage germination reduces (Grimwade *et al.*, 1987). True seed weight also tends to increase with maturity, and the increase leveling off at about 50-55 DAA with a weight of approximately 5mg.

As found by Grimwade *et al.*, 1987, starch and protein weight per seed which are normally in the ranges of 32-38% and 20-40% respectively, are significantly related to overall maturity. However, the accumulation of starch in the seed is much faster than protein.

Seeds start to become germinable at 27 DAA and this increases to 50% of the final germination at 40 DAA and finally, maximum germination occurs 55 DAA (Grimwade *et al.*,1987). Although a linear relationship between seed maturity and germination in some seed lots was found by these authors, they showed that this relationship might be non-linear in other seed lots when the increase in germinablity plateaued off as seeds matured. This indicates that, this relationship might be influenced by physical and chemical germination inhibitors accomulating in the mature fruits.

2.2.2 Fruit structure

In sugar beet, each seed (in reality fruit, Plate 2.1) includes pericarp and true seed. The pericarp is derived from the mature ovary wall (Smith *et al.*, 1990) and is inaccurately but commonly termed as the seed coat. The seed coat has a removable woody piece on the top (seed cap) and a pericarp base, by which the fruit is attached to the mother plant. The true seed inside comprises embryo, food reserves and testa. A cross section of a sugar beet seed including embryo, food reserve (perisperm, endosperm), and testa is shown in Figure 2.1.

The food reserves in the seed include perisperm and endosperm which store starch and protein. The perisperm is the main store of food reserves in sugar beet seed, while the endosperm comprises a small part of the food reserve in the seed which is derived from the fusion of one male gamete with two female polar nuclei.

A multigerm seed usually produces more than one seedling and results when two or several flowers form a cluster by coalescing at their bases. Whereas, monogerm seed are produced by a single flower and hence after emergence usually give only one seedling.

Before introducing monogerm seed cultivars which were suitable for precision drill sowing systems, multigerm seed were used by all sugar beet growers. However, monogerm seed is presently preferred in most planting system. Thus owing to the importance of plant establishment in the precision drill system, many researchers have concentrated their work on monogerm sugar beet seed.

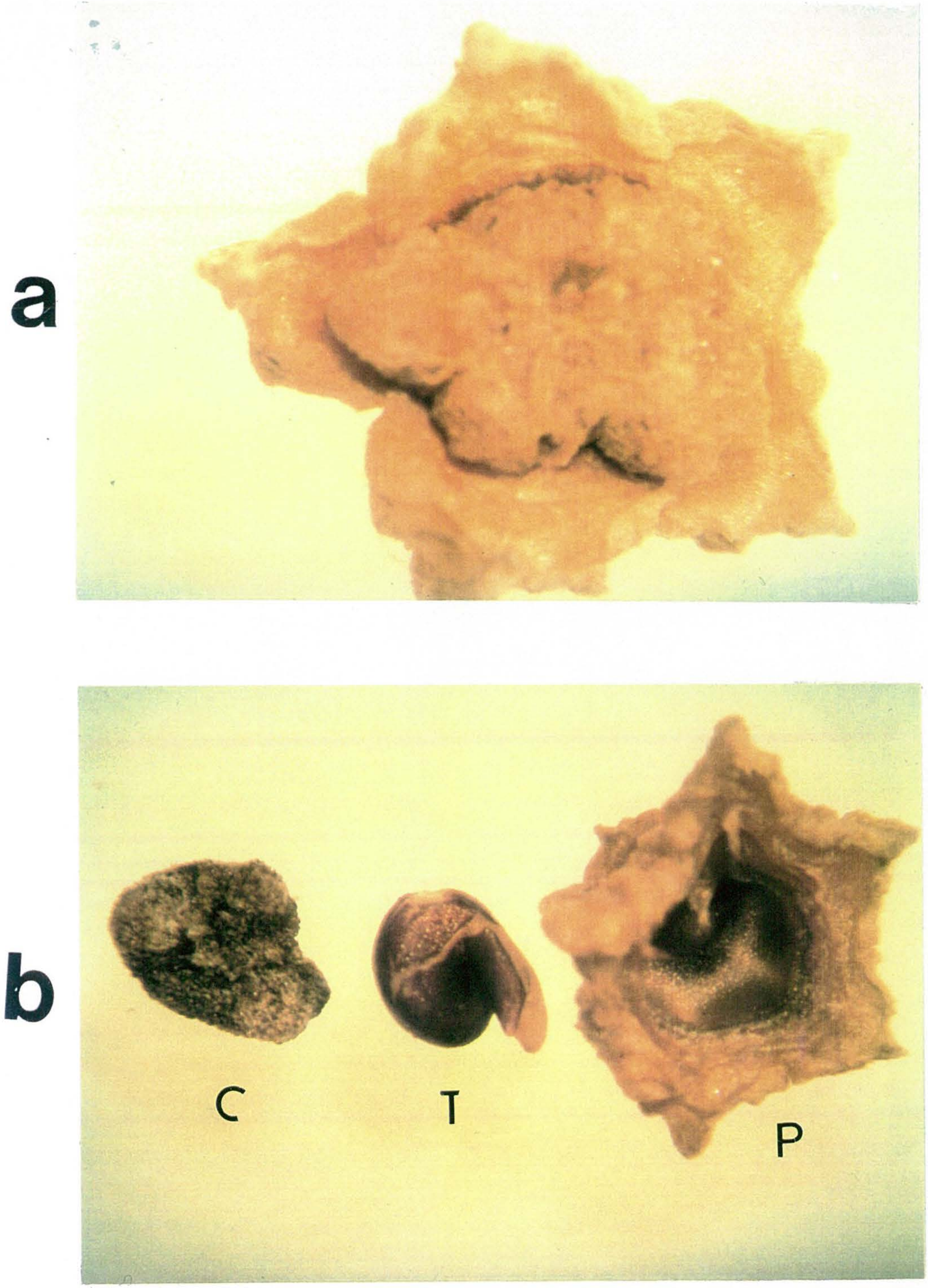


Plate 2.1 Morphological characteristics of a monogerm sugar beet fruit (a). As shown in Plate 2.1b, each fruit comprises a true seed (T), pericarp (P) and seed cap (C)

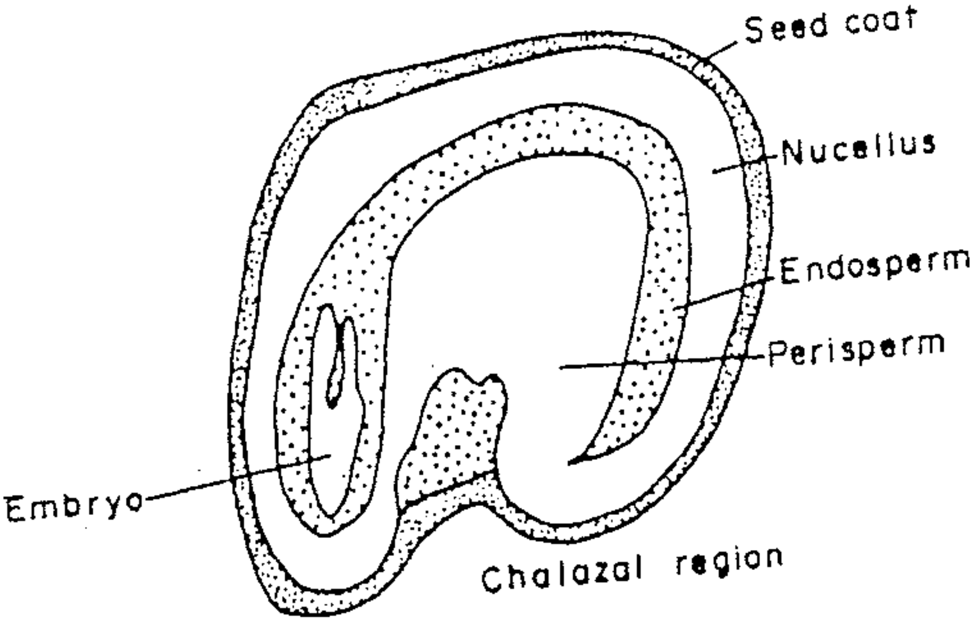


Figure 2.1 A cross section of a sugar beet true seed comprising embryo, endosperm, perisperm, newcellus and seed coat. (Addapted from Mayer, A.M. and Poljakoff-Mayber. A. 1989.

2.3 THE EFFECT OF SIZE GRADING ON GERMINATION PERFORMANCE OF SUGAR BEET SEED

According to ISTA (International Seed Testing Association) rules (1987), the following factors are considered to be associated with seed vigour:

A. Genetic constitution
B. Environment and nutrition effect on the mother plant
C. Stage of seed maturity
D. Mechanical integrity
E. Deterioration and ageing
F. Seed size, weight or specific gravity

The role of seed weight has been assessed in relation to its effect on germination and the production of vigorous seedlings. Grading of leeks (Gray and Steckel, 1986) and carrot (Gray and Steckel, 1983) seed gave improved emergence characteristics and reduced the variation in seedling weight. In leeks the emergence of the largest seeds (2.00-2.24 mm) was 13% and 21% higher than that of the ungraded and the smallest (1.6-1.8 mm) seeds respectively. These authors mentioned that, in both species, the improvement was mainly due to the increased embryo length in large seeds, the larger embryo leading to an increased percentage emergence and seedling weight and also reduced emergence time. Furthermore, large seeds of hybrid maize have been shown to germinate more rapidly than the small seeds (Hong *et al.* 1982). Although there was little difference in the final percentage of germination, increased seed size was associated with improved field emergence. The superiority of the larger seed might be related to the higher hydration potential of the seed and, possibly, faster reorganization and repair process in the cells of the seed. On the other hand, although a positive relationship was found between seed size and plant height in *Glycine Max*, no improvement was obtained by planting larger seed (Smith and Comber, 1975). Seed coat of large seeds of legumes are usually susceptible to the mechanical damage, hence the inability of larger seed to give more rapid seedling emergence might be related to mechanical damage on the seed and possibly imbibition damage (high solute leakage following rapid imbibition) which causes loss in the seed vigour (Powell and Matthews 1980 and Powell *et al.*, 1984b). The other possible cause of this failure might be related to the larger cotyledon of the seed which may be less able to overcome the impedance of soil crusts. It should be noted that, soil crusting effects on plant dry weight and lint yield of cotton has been also reported by Wanjura (1982). According to these results different factors might affect the relationship between seed size and potential performance of the seed. In addition in some crops (eg. sugar beet), the seed lies within a woody pericarp and therefore, the performance of the seed might be effected by physical and chemical inhibitors of the fruit.

Because of indeterminate flowering habit of sugar beet which is prolonged for up to six weeks (Grimwade *et al.*, 1987) , a seed bulk at harvest comprises a wide range of sizes and maturities and all of these may not be of equal value for sowing. On the other hand (Wood *et al.*, 1977) suggested that outbreeding, largely due to the self-infertile habit, is the main cause of the variation in a seed lot. These authors mentioned that, 87% of the size variation within a seed lot is related to variation between plants, 6% between racemes on a plant, and 7% within a raceme. A question arise here, is there any constant relationship between fruit size and germination performance of the seed in sugar beet?. In following sections, based on previous work, the effects of fruit and true seed size on germination, rate of emergence and seedling growth in sugar beet are discussed.

2.3.1 Fruit and true seed size effects on germination

In the literature, there are lots of contradictions about relationships between fruit size and seed germination. In agreement with the results reported by Hogaboam and Snyder (1963) and also Snyder and Filban (1970), a positive linear relationship between fruit diameter and seed germination was found by Wood *et al.* (1977). However, these last authors suggested that, it is important to recognize that it is only within a stock that an improvement in germination can be obtained via using the larger fruits. It is possible for medium or even the small fruits from one stock to be as good as or even superior to the large fruits from another stock. On the other hand, the presence of non-linear relationship between fruit diameter and germination has also been reported by other authors (for instance Akeson, 1981 and Grimwade *et al.*, 1987) in which the larger fruits showed equal and in some cases, poorer germination percentages than medium fruit size.

The contradictory results in the literature indicate that, there is no simple general relationship between fruit diameter and germination performance of sugar beet seed. Other factors such as the size of true seed within the fruit (Grimwade *et al.*, 1987), levels of chemical germination inhibitors (Bewley and Black, 1985) and physical inhibition to the seed cap (Coumans *et al.*, 1976 and Morris *et al.* 1985) might alter this relationship which will vary from seed lot to seed lot.

The size of the true seed is suggested to be a critical factor for successful germination performance (Morris *et al.*, 1985). During the imbibition phase, the embryo swells and via expansion, provides the force necessary for seed cap movement and radicle emergence (Plate 2.2). Thus, the larger the true seed within the fruit, the more the germination percentage of the fruit sown (Grimwade *et al.*, 1987). A question arises here: is there any relationship between fruit size and true seed size?.

Savitsky (1954) found that in monogerm sugar beet seed, seed weight increased proportionally with the fruit weight ($r= 0.95$) but Scott *et al.* (1974) showed the relationship to be non-linear for a wide range of stock. The non-linear relationship indicated an increase in both the relative and absolute proportion of the pericarp material in larger fruits. This is in agreement with the results reported by Snyder (1963) in which, smaller fruit tend to contain relatively larger true seed. Wood *et al.* (1980) showed that the relationship between fruit and true seed size might change in different climatic conditions during seed development. They showed that cooler temperatures favour pericarp growth whereas, in warm weather, the ratio changes in favour of the seed. A significant positive correlation between true seed weight and germination performance of the fruits was found by these authors. These results were later confirmed by Durrant and Loads (1990) who indicated that the true seed weight is the quickest measurement which is significantly correlated with germination of the fruit.

The presence of immature, under-developed and aborted seed within smaller fruits has been suggested to be the main cause of low germinability in beet seed (TeKrony, 1969; TeKrony and Hardin, 1969; Snyder, 1971; Longden, 1973; Scott *et al.* 1974; Wood *et* *al.* 1977, Coumans, 1978; Morris, 1981). These seeds may have adequate embryo development for growth, but may not have sufficient size to provide the force which is required for seed cap movement (Wood ,1975).

The X-Ray technique has been successfully used by some authors to evaluate size of the true seed within the photographed fruits and to define the relationship between the image provided via X-Ray photograph and germination percentage. For example, a significant relationship between the estimated true seed size and seedling emergence was found by Longden *et al.* (1971) and Longden and Johnson (1974). However, the latter authors reported that, some fruits containing fully mature seed, still did not emerge. This might be attributed to the imposed dormancy caused by the fruit and may be via either chemical or physical inhibition of germination, or both.

It should be noted that, despite the potentially important effect of the fruit thickness on germination of the fruit, no attempt has been made to define this relationship.

Plate 2.2 The process of seed cap movement and radicle emergence in a monogerm sugar beet fruit which occurs due to the expansion of the true seed during the imbibition phase

2.3.2 Fruit and true seed size effects on the rate of emergence and seedling size

It is well established that due to the greater amount of food reserve and larger embryo, larger fruit tend to produce larger seedlings. For example, the superiority of larger sugar beet fruit in producing vigorous seedlings with large cotyledons and high root and shoot dry weights was shown by Scott (1973) and Scott *et al.* (1977). However, there are apparently many contradictions in relationship between fruit size and speed of seedling emergence in the literature. For example, a negative correlation between fruit diameter and speed of seedling emergence was observed by Ustimenko (1957) and reported again by Hogaboam and Snyder (1963) who mentioned that, in most cases, selecting the larger fruits is not desirable for this reason. However, Wood *et al.* (1977) found that larger fruits showed more rapid emergence than small fruits. These contradictory results might be due to the differences in the size of the true seed within the fruits (Wood *et al.*, 1980) or seed cap and germination inhibitors in the fruits.

It should be mentioned that, the relationship between fruit thickness and rate of emergence has only been studied by Hogaboam and Snyder (1963), who found a negative correlation between fruit thickness and speed of the radicle emergence. However they found no evidence to indicate why the thick fruit showed slow radicle emergence.

2.3.3 The effect of fruit and true seed weight on plant establishment

Grading three commercially processed seed lots into 4 categories (2.4-2.8; 2.8-3.2 ; 3.2-3.6; 3.6-4.0mm), Akeson (1981), found that field emergence of the fruits was enhanced with increasing fruit size from 2.4 to 3.6mm, however the field emergence of the largest fruit (3.6-4.0mm) was equal to or less than that of 3.2- 3.6mm. The author noted that the influence of fruit size on field emergence was not affected by planting date. However, it seems there is no simple correlation between fruit and performance of the seed and other factors such as seed vigour and soil and climatic conditions influence on this relationship. For example, Lidgett (1976) in a sandy loam soil at 10-15% water content, more seedlings emerged from large seeds but when they were sown deeply in a wet soil, emergence percentage was similar to that for small seed suggesting that the larger fruit had a greater oxygen requirement which was not fulfilled. However, the deficiency of the larger fruits might be due to the chemical and physical factors in the fruit which restrict oxygen uptake (see section 2.5).

According to the literature, the weight of true seed and speed of germination, have been suggested to be associated with seed vigour and plant establishment (Durrant, 1988 and Durrant and Mash, 1990). In addition, by emphasizing the importance of vitality of the true seed on the rate of plant establishment, Durrant and Gummerson (1990) were of the opinion that larger true seed with faster emergence increases the prospect of high potential performance of the fruit under field conditions.

2.3.4 Relationship between germination and plant establishment

Seed germination, as defined by AOSA (Association of Official Seed Analysts, 1983), is the maximum potential of a seed lot to produce normal seedlings in optimal conditions. The germination test has been standardized so the germination results are reproducible among laboratories and is suggested as an index of quality for seed trading (Hampton 1990).

Although in recent studies (Kraak *et al.*, 1984; Durrant *et al.*, 1985), germination measured in standard conditions (ISTA Rules 1976) was closely correlated with seedling establishment, Longden and Johnson (1974) reported that seed lots with low germination percentages always gave poor emergence in the field, but some of the high germinating lots gave poor emergence in field conditions also. Durrant *et al.* (1985) found that although seeds germination tested at low temperature need a long period of incubation, this cold stress method provides a good prediction of field establishment. In confirming the results reported by Longden and Jhonson (1974), these authors indicated that there were differences in the tolerance of seed lots to cold stress. These results clearly show that the relationship between germination of the fruit under optimal and stress conditions as affected by seed vigour (Ellis and Roberts, 1980).

2.4 PHYSIOLOGICAL BASIS OF LOW GERMINABILITY IN SUGAR BEET SEED

Although a number of physical factors such as low temperature during seed development (Snyder and Hogaboam, 1963; Wood *et al.*, 1980), low spring temperature during seedling emergence (Perry and Harrison, 1975; Coumans, 1976; Morris, 1981), and soil compaction (Hammerton, 1961; Hegarty and Royle, 1978; Durrant and Scott, 1981), have been found to influence seed performance, physical and chemical inhibitors of the fruits have also been suggested to have influences on seed germination and plant establishment.

2.4.1 Physical inhibition by the seed coat and seed cap on seed germination

In sugar beet, despite comprising large true seed, some fruits may be slow to germinate or may fail to produce seedlings. Germination failure has been attributed to physical inhibition by the seed coat (Snyder 1963), and/or tightness of the seed cap (Morris *et al.*, 1985 and Grimwade *et al.*, 1987) in which the provided force by the true seed via expansion during imbibition phase, is not sufficient to remove the seed cap which is a requisite for radicle emergence. Although heavier cap weight has been suggested to be associated with low germinability of seed (Seldmayr, 1960; Snyder, 1962 and Morris *et al.*, 1985), it is not clear whether seed cap weight is related to the cap tightness.

To demonstrate physical inhibition of the cap on seed germination and also the importance of true seed size in removing the cap, two experiments were carried out by Morris *et al.* (1985). In their first experiment, incubated artificial dead seed (infiltrating fruits by vacuum, with ethanol for 30 minutes) they found that, even some of the dead seeds provided the force for cap movement. Based on this finding, they concluded that; it is unlikely that, cap movement of the dead seeds required enzymatic loosening of the cap and it is more likely that these are removed by pressure from the expanding seed. Other cap in the sample may be more firmly held and required greater pressure or enzymatic loosening of the cap or contained a smaller seed which, although capable of swelling, were unable to exert so much pressure. It is important to note that, gibberellin mediated weakening of the endosperm cells around the radicle tip of tomato seed, as reported by Groot and Karssen (1987): evidence for involvement of PGRs in loosening the tissues that might suppress seed germination.

In Morris *et al.*'s (1985) second experiment, the germination percentage of excised true seed and de-capped fruits of two cultivars of monogerm sugar beet were compared. The result of this study illustrated that, despite similar germination percentages showed by excised seed, there was a significant difference between the two cultivars in the germination of the intact fruits. A small difference was found between germination of de-capped fruit and excised seed of the cultivars. Furthermore, when the weight of the seed cap of the cultivars were compared they found that, the low germinating cultivar had a heavier seed cap than that of the high germinating cultivar. Therefore, they concluded that, germination of the seed was inhibited by the seed coat (seed cap attached) and secondly, the inhibitory characteristic of the seed coat mostly related to the function of the cap. It is important to note also that, the difference between germination of the de-capped fruit and the excised seed of the cultivar, suggests that, there is chemical germination inhibition from the seed coat itself.

The importance of finding a technique for measurement of the force required for cap movement is emphasized by Morris *et al.* (1985) who suggested that this technique has important implications for the production of high quality seed. To estimate the required force for cap movement, the authors suggested that, it was possible to make an estimate of the force if a relationship can be assumed between the osmotic potential of PEG solution which is sufficient to lower the germination of excised seed to that found in the whole fruits germinating in the water. However, they noted that firstly this hypothesis requires the true seed to fully occupy the cup cavity (as if the seeds were too small with respect to the fruit chamber, these values would be underestimates) and when different seed lots are compared, the osmotic potential of the dry seeds are equal between fruit lots.

Using two seed lots having low and high germination capacity, an attempt was made by Morris *et al.* (1985) to test the hypothesis. As the low and high germinating lots required approximately -5 bars (c.5kg cm^{-2}) vs -7.5 (c.7.5 Kg cm^{-2}) respectively to reduce the germination of excised true seed to the levels of the germination of the seed within the fruit, they conclude that, poor germination may be the result of the greater force required for cap movement. However, these results may not explain the actual level of the cap tightness, as other factors such as sensitivity of the seed to the PEG, difference in chemical component in the seed coat may be involved. Furthermore, using PEG might affect enzyme activity during germination the excised seed. Accordingly, there is a need to develop direct measurement techniques to assess the differences in seed cap tightness of the fruits, if any, between seed lots.

Oxygen is assumed to be a limiting factor for beet germination (Lexander, 1980). Evidence of oxygen shortage in excess water was given by Heydecker *et al.* (1971) and Perry and Harrison (1974) who obtained a high germination percentage even in a wet medium by applying a high oxygen pressure. Although Heydecker *et al.* (1971) were of the opinion that the entrance route of oxygen to the embryo is between the edge of the cap and the rim of the seed cavity, Perry and Harrison (1974) regarded the pore which runs from the seed cavity through the pericarp base to the outside as the main entrance path for oxygen. The pore partly consists of remainders of the vascular connection between the true seed and the mother plant, and it is filled with loosely packed dead cells. Thus, it is necessary to define whether it is the cap tightness or pericarp base structure which restricts oxygen uptake.

It should be noted that Heydecker *et al.* (1971), suggested that during wet condition a layer of Mucilage can spread over the fruit surface and hence, prevent the access oxygen to the embryo. However, Perry and Harrison (1974); could not find any Mucilage on sugar beet fruit.

2.4.2 The inhibitory effect of sugar beet fruit water soluble substances on germination of the seed

Some sugar beet seed lots perform with a low germination percentage and a low germination rate. The literature suggest that the failure of the seed could be also attributed to some water soluble materials existing in the fruit. For example Tolman and Stout (1940) found that, an extract of these substances either from the fruit or only from the pericarp tissue have a similar inhibitory effect on seed germination. They further suggested that, by soaking or washing, these substances can be removed and result in improved germination and seedling emergence. The germination inhibitory effect of these extracts on cress and sugar beet seeds were also shown by Battle and Whittington (1969). The effect of soaking sugar beet fruits has been described without the authors knowing the identity of substances removed by the water. To achieve better germination and uniform seedling emergence, Cuddy (1959), showed that most of cultivar tested germinated better after a long period of soaking. MacKay (1961) also found an increased germination rate after washing the fruit, but the effect was seen only on germination dishes not in soil. Heydeker and Chetram (1971) on the other hand, obtained an increase in field emergence after washing the fruits. The effect of prewashing was also investigated by Longden (1974; 1976) who demonstrated a 3-11 percent increase in the field emergence of the fruit by this method. In addition, the beneficial effects of prewashing on seed germination was also obtained by Lexander (1980) both in petri-dishes and also in soil.

Although several substances have been identified in the extract of beet fruit, but there are contradictions about inhibitory nature of the substances. However, no general agreement among scientists about how they inhibit seed germination. The identified water soluble materials in the extract of beet fruit include phenolic substances, abscisic acid (ABA); ammonia, oxalic acid and inorganic salts in particular potassium nitrate. Furthermore, Wheeler (1963), identified betain in water extract of sugar beet fruit, but the substance didn't affect the germination of cress seed. However, the effects on sugar beet germination were not investigated.

CHAPTER III

MATERIALS AND METHODS

3.1 RELATIONSHIPS BETWEEN FRUIT SIZE AND SEED QUALITY IN THREE LOTS OF MONOGERM SUGAR BEET SEED

3.1.1 Seed sample

This experiment was carried out by using samples of three lots of a diploid monogerm cultivar (9597) which was released in Iran (1985) and continues to be produced by the Sugar Beet Seed Institute of Iran (S.B.S.I.).

These lots have been categorized as follows on the basis of the germination capacity of their ungraded fruits.

1. The medium quality lot (LOT A)

2. The low quality lot (LOT B)

3. The high quality lot (LOT C)

These lots were chosen to assess whether there is any similarity in relationships between the fruit size and quality within lots of different germinability. Information about date of harvest and the initial quality of these lots is summarized in table 3.1

Table 3.1 Date of harvest, germination percentage, moisture content, thousand fruit weight and seedling dry weight of ungraded samples of the seed lots. Data presented are the mean of two replications for moisture content and 4 replications (± standard errors) for germination and seedling dry weight, and eight replications for 1000 fruit weight.

Lot	Harvest date	Germination (%)	Moisture content (%)	Thousand fruit dry weight(g)	Seedling dry weight (mg/normal seedling)
A	August 1988	68.8 (±4.33)	9.54	10.16 (±0.13)	1.94 (±0.03)
B	July 1986	44.4 (±1.99)	9.71	9.68 (±0.18)	1.84 (±0.07)
C	August 1990	84.5 (±1.78)	9.74	9.81 (±0.10)	1.81 (±0.04)

3.1.2 The grading process

The fruit samples were size graded by an automatic reciprocating grading machine via two methods as follows:

3.1.2.1 *Diameter Grading*

In this method, the fruits were separated by round hole screens according to diameter. The method comprised two stages as follows:

1. A preliminary stage of thickness grading in which fruits usually removed in commercial processing (less than 2.25mm and more than 3.25mm thick) were discarded. Only fruits passing through 3.25mm and retained by 2.25mm slotted screens were used.

2. The remaining fruits (2.25 - 3.25mm thickness), were then size graded with round hole screens to categorize the fruits by diameter as follows:

    A. 3.0 - 3.5 mm

    B. 3.5 - 4.0 mm

    C. 4.0 - 4.5 mm

    D. 4.5 - 5.0 mm

    E. 5.0 - 5.5 mm

It should be noted that, on a weight bases, 43 % of the fruits from lot A, 40 % from lot B and 49 % from lot C, which were either less than 2.25 mm or more than 3.25 mm thickness were rejected in the primarily stage of diameter grading. In addition, because the diameter grade of less than 3mm are too small and comprises very high percentage of seedless fruit and also fruits of more than 5.5 mm of diameter are too big to be polished and use in a commercial seed lot, these two grades have not been selected for this study. To investigate the germination performance of the heavier fruit with thick pericarp, the fruits of 5-5.5mm diameter which usually are discarded in the commercial processing, also was used in this study.

3.1.2.2 *Thickness Grading*

In this method, fruits were graded by slotted screens according to their thickness properties. This method also included two stages as follows:

1. A primarily stage of size grading by diameter with round hole screens to remove fruits which are usually omitted in commercial processing (less than 3.5mm and more than 5mm of diameter).

2. The remaining fruits (3.5 to 5mm), were then size graded with slotted hole screens to separate fruits by thickness as follows:

   A. 2.25 - 2.40 mm
   B. 2.40 - 2.75 mm
   C. 2.75 - 3.00 mm
   D. 3.00 - 3.25 mm
   E. 3.25 - 3.50 mm

It should be noted that, on a weight bases, 52 % of seed lot A, 73 % from lot B and 31 % of lot C were either less than 3.5mm or more 5 mm diameter and were rejected in the primarily stage of thickness grading. In addition because the size grade of less than 2.25mm comprises a very high percentage of seedless fruit, therefore,this grade was omitted to be studied in this work.

The proportion of the diameter and thickness fractions within ungraded samples of the seed lots are given in the Tables 3.2 and 3.3.

3.1.3. Thousand fruit weights of the size grades

To assess 1000 fruit weight of the size grades, eight replicates of one hundred fruits of each sample lots were weighed and corrected to the same moisture content determined according to the ISTA rules (1985). The seed moisture content are shown in Tables 3.4 a and b.

Table **3.2** The proportion of diameter graded fruits with 2.25-3.25mm of thickness within the ungraded samples of the seed lots

LOT	<3mm (%)	3-3.5mm (%)	3.5-4mm (%)	4-4.5mm (%)	4.5-5mm (%)	5 -5.5mm (%)	>5.5mm (%)
A	1.84	2.80	13.39	24.41	32.47	13.07	12.02
B	4.12	5.32	14.08	21.79	28.76	9.32	16.61
C	2.83	2.31	13.22	24.46	30.1	14.49	12.68

Data provided by SBSI, Iran

Table 3.3 The proportion of thickness graded fruits with 3.5-5mm of diameter within the ungraded samples of the seed lots

LOT	<2.25 (%)	2.25-2.4 (%)	2.4-2.75 (%)	2.75-3 (%)	3-3.25 (%)	3.25-3.5 (%)	>3.5 (%)
A	6.23	2.48	41.06	27.15	21.09	1.99	0
B	3.98	1.78	19.75	31.00	35.46	8.03	0
C	9.15	1.67	39.74	39.57	5.35	4.52	0

Data provided by SBSI, Iran

Table 3.4 (a) Moisture contents of the diameter graded fruits of the seed lots. Data are mean values of two replications.

LOT	3 - 3.5mm (%)	3.5 - 4mm (%)	4 - 4.5mm (%)	4.5 - 5mm (%)	5 - 5.5mm (%)
A	9.21	8.92	8.60	9.01	9.25
B	8.85	8.60	8.90	8.70	8.97
C	8.40	8.90	8.60	8.60	8.90

Table 3.4 (b) Moisture contents of the thickness graded fruits of the seed lots. Data are mean values of two replications.

LOT	2.25-2.4mm (%)	2.24-2.75mm (%)	2.75-3.mm (%)	3-3.25mm (%)	3.25-3.5mm (%)
A	9.30	9.30	9.25	8.79	8.92
B	8.70	8.85	9.10	9.30	8.70
C	8.40	8.70	8.70	8.50	8.76

3.1.4 Weight measurements of the true seed, seed cap and the seed coat of the size graded fruits

To measure the true seed weight, seed cap and seed coat weight of the lots, three replicates of 40 fruits of each size grade were soaked in water for 12 hours at room temperature (Scott *et al.*, 1974) to facilitate the excision of the true seed. The seed caps were removed with a needle and then the seed coat, seed cap and the true seed were separately oven dried at 65°C for 4 d.

3.1.5 To determine germination performance of the size graded fruits

To investigate germinability of the size graded fruits in each category, 5 replicates of 50 fruits of every seed sample were washed with tap water for 4 hours to remove any germination inhibitors and then, air dried in room temperature for 24 hours. To prevent fungal infection which is usually a cause of difficulty for seedling evaluation, the seed samples were dusted treated with the fungicide (Thiram 1% w/w) and than germinated on pleated paper at 20°C (ISTA, 1985). Using the guidelines of the ISTA handbook for seedling evaluation (ISTA, 1979), the seedling were counted 4 and 14 days after incubation and the numbers of normal and abnormal seedlings and also ungerminated seeds were recorded.

3.1.6 Seedling dry weight measurements

To assess the relationships between size grading and seedling dry weights, normal seedlings of emerged fruits were removed after final evaluation of germination and oven dried at 65°C for 4 d and then weighed.

3.1.7 Median radicle emergence times and uniformity of germination of the size graded fruits

In this experiment germination was conducted in 15cm petri-dishes. The dishes were disinfected with a solution of 33% Janola (1.15% Sodium Hypocholorite) for 15 minutes and were then washed with distilled water before testing. For every petri-dish two layers of filter paper (Whatman No.1) were used to support the seeds with 10 ml of distilled water as the germination substrate .

This experiment was carried out in four replications of 50 prewashed fungicide dusted fruits (Thiram 1% w/w) from 5 diameter and 5 thickness grades of lot A (see section 3.1.2). The fruits were incubated in a dark cabinet at 15°C (approximately the average soil temperature during seedling emergence in the field). The relative humidity of the cabinet was 92%-95%; nevertheless, to make sure that the petri-dishes did not lose water, dishes were placed in an unlidded plastic box which was covered with three layers of plastic bags. Fruits were set up in dry dishes and then, as far as possible, all imbibition started at the same time by adding water preincubated at 15°C.

The method used for evaluation of the median radicle emergence was the T50 test, which is calculated as the time when the middle seed of the germinants in the sample shows radicle protrusion through the seed cap (Coolbear, 1990). The uniformity in the radicle emergence was calculated as the interval between 10% and 90% radicle emergence (T90 - T10). The formulae which were used in this assessment are cited in Appendix 1. An important point to be noticed is that in each case, values are based on number of seeds germinating, not of seed sown. Seeds showing radicle emergence were counted and removed twice each day during the period of high germination activity (14 d) and then once every two days. Counting of emerged radicles continued until there was no further germination.

3.1.8 To investigate the potential performance of size graded fruits in field conditions

To evaluate the potential performance of the size graded fruits in field conditions, a Randomized Block Design with 6 replications was used (see the experimental lay out in Appendix 2). This field experiment was carried out in the experimental plots of the Seed Technology Centre, Massey University, the soil description being cited in the Appendix 3 of this thesis.

The land was cultivated out of a desiccated perennial rye grass and white clover and was thoroughly prepared by firstly ploughing the land (three months before sowing time) and secondly using harrowing twice until a good tilth (10-15 cm) was attained. To avoid the possible effect of herbicides on seed germination and seedling growth, no herbicide was used before and after seedling emergence therefore, the field was hand weeded during the experiment.

One hundred fungicide (Thiram 1% w/w) dust treated fruits of each grade of each lot were sown by hand at a depth of 5 cm, 5cm apart in a single row. The field was irrigated the same day after planting by a sprinkler and to make sure the soil moisture was sufficient for seedling emergence and plant establishment, the soil moisture content was assessed every day. However, because of high rainfall and cold weather no more irrigation was required. Soil temperature and moisture, ambient temperatures plus relative humidity were monitored twice a day throughout the experiment (Appendices 4 - 7).

After seedling emergence different factors might reduce the rate of plant establishment, and evaluation of these was an important part of this study. To clarify the factors limiting successful establishment, all of the seedlings were labelled as far as possible after seedling emergence (Plate 3.1). To assess the involvement of field fungi in seedling damage, the damaged plants were collected during plant growth and were incubated on agar media (20 g agar and 30 g streptomycin/l of distilled water) at 25°C. Having the antibiotic compound, this media is suitable to control bacterial growth - which cause difficulties for identification of the field fungi.

Plants were sprayed with the fungicide (Benomyl 2 kg/1000 l of water) twenty days after planting when the first symptoms of fungal (Damping-off group) infection was observed and this was followed by two applications of Thiram (150 g /100 l of water). Plants were also sprayed twice with an insecticide (Diazinon 8% E.C.100ml/100 l of water), commencing when the first symptoms of insect damage were observed.

At harvest time the established plants were counted and via counting the markers without plants, the percentage of the dead plants from total emerged plants was measured.

For the final evaluation of plant establishment, when the surviving plants were at the 6-8 leaf stage (50 days after sowing), shoot dry weights were assessed (by oven drying at 65°C for 4 d). Because of the length and morphology of roots at this stage, it was not possible to harvest intact plant roots at this time and therefore, below ground growth was not evaluated.

Plate 3.1 Labeling the emerged seedling in the field trial. This was carried out for monitoring the post emergence limiting factors of sugar beet plant establishment

3.2 THE PHYSIOLOGICAL BASIS OF LOW GERMINATION CAPACITY IN MONOGERM SUGAR BEET SEED

3.2.1 Relationships between stage of seed maturity and germination percentage in size graded fruits.

A radiographic technique as a rapid germination test for sugar beet seed was successfully used by Longden *et al.* (1971). This technique can be used to assess the stage of seed maturity and to define whether there is any relationship between fruit size, stage of maturity and germination performance of sugar beet seed. To apply this method to size graded fruits a study was carried out using an X-ray machine (43804N X-ray SYSTEM FAXITRON SERIES) in which the procedure was as follows:

Four replicates of 50 fruits from 5 diameter graded fruits of lot A were prewashed in running tap water for 4 hours and then blotted dry. Each replicate of the size graded fruits were then arranged on a single sheet of Kodak T-Max 100 film in 5 rows of 10 fruits and then carefully placed in to the machine for radiography. To facilitate comparison between the germination test and X-ray results of individual fruit, care was taken to make sure that the fruits remained in their rows and did not become mixed.

A preliminary study was undertaken to assess the best X-ray rate and exposure time, and it was shown that, a rate of 25 kv, 2.8 - 3.1 mA for a 2.7 min exposure provided the best photographs in terms of clarity in showing the internal structure of the fruits. Following ISTA guidelines (1985), a positional germination test was then conducted on the photographed fruits using the pleated paper method as previously described. The radiographed films were processed and printed by the Photographic Unit, Massey University.

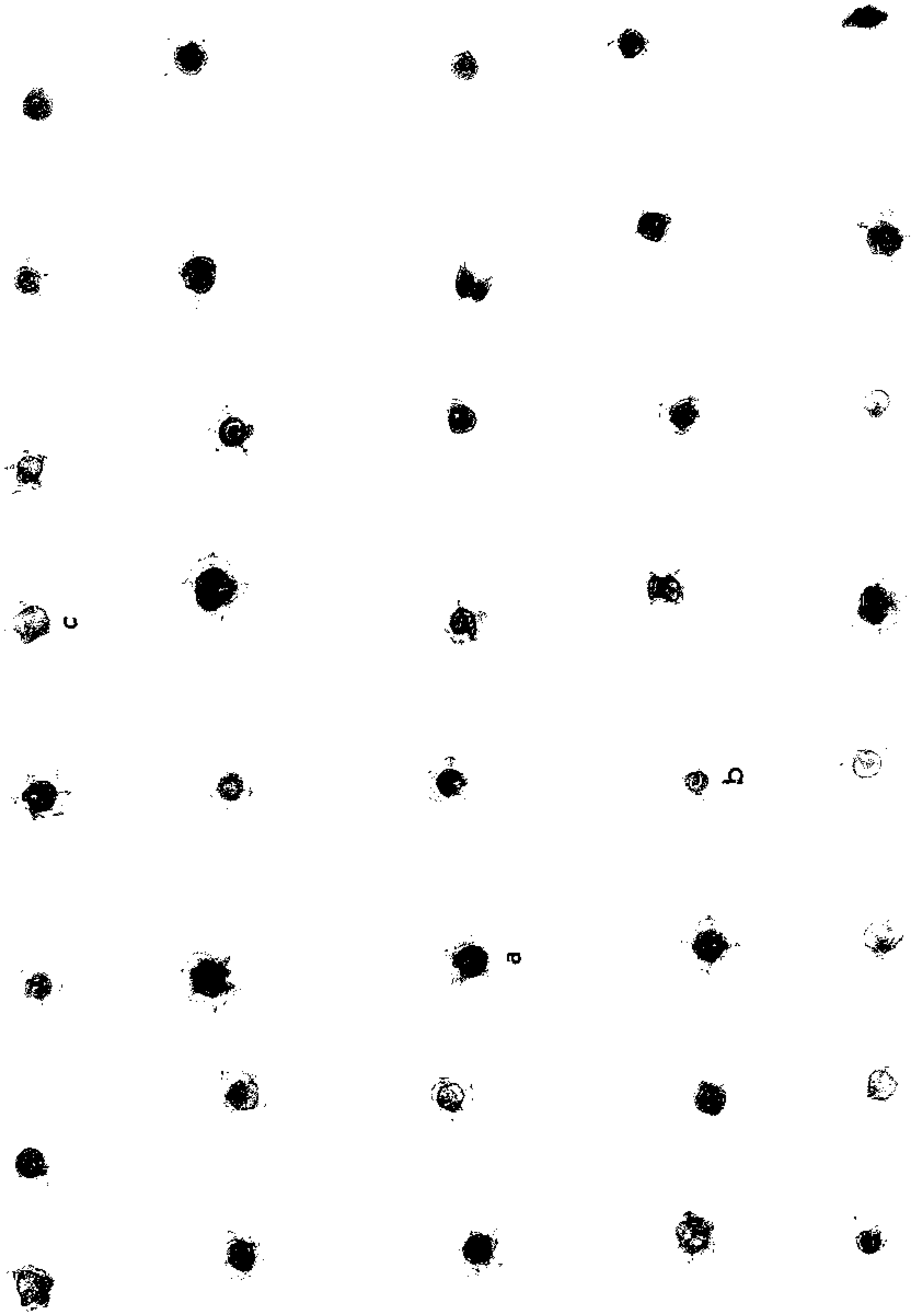
Using the photographs, the X-rayed fruits were classified as follows according to their true seed sizes.

1. **Fully mature seed, in which the cup cavity was completely filled by the true seed (Plate 3.2, a).**

2. **Underdeveloped seed, in which only a small part of the cup cavity was filled by the true seed (Plate 3.2, b).**

3. **Seedless fruit, in which the fruit is entirely empty (Plate 3.2, c).**

Comparisons were made between subsequent germination and images of individual fruits recorded on the X-ray photograph to determine whether there was a significant relationship between the prediction of seed maturity via X-ray photography and subsequent germinability.


c
b
a

3.2.2 Assessment of the effect of water soluble chemical substances in the fruit on seed germination

To assess the effect of these substances on seed germination, four replicates of 50 prewashed (4 h in running water) and unwashed fruits of the size graded fruits of the lots were compared by germinating them on pleated paper. In addition, the possible effect of the inhibitors on the mediam radicle emergence time (T50) of the unwashed fruit were assessed using the petri-dish germination method (described in section 3.1.).

3.2.3 To determine physical inhibitory effect of the cap via measuring the force required for the seed cap movement at the time of radicle emergence

To measure the force required for the cap movement, an instrument was designed and provided by Agriculture Engineering Dept., Massey University (courtesy Dr. C.J.Studman). The construction of this device and also the method used in this experiment were as follows:

3.2.3.1 *Construction*

This instrument comprises 3 separate pieces shown in Plate 3.3. The descriptions of the pieces are as follows:

1. Piece no.1 consists of a flat piece of Perspex with a perpendicular aluminium rod attached which has a blunt needle protruding from the end.

2. Piece no.2 consists of a rectangular cube of perspex which has a hole drilled through it. The aluminium rod passes through this hole keeping the rod firmly in position above the fruit.

3. Piece no.3 consist of a third piece of the device. It has 3 holes of varying diameter to allow for varying the fruit size. The tapered part the hole holds the fruit in position while, the rest of the hole allows the seed cap to be pushed out from the fruit when sufficient force is applied by the needle.

3.2.3.2 *Fruit preparation and the process of measuring the force required for cap movement*

Holes were drilled in the pericarp base area of fruits to allow the true seed to be removed so that when the force was measured the needle rests directly against the inside surface of the seed cap (Plate 3.4a). When sufficient force was applied by adding water into a container placed on top of the first piece of perspex (Plate 3.4b), the seed cap will be pushed out from the seedless fruit and will drop down to the narrow part of the hole with the rest of fruit remaining in the tapered part. By weighing the volume of water, the container and the first piece of perspex, the force required to remove the seed cap can be measured.

In this experiment, seedless fruits of the smallest (3-3.5mm) and the biggest (5-5.5mm) diameter grades of Lot A were incubated on pleated paper for 12, 18, 24, 30 and 36 hours at 20^{O}C, and the force required to remove the seed cap for at least 8 fruits in each grade at each incubation time were measured.

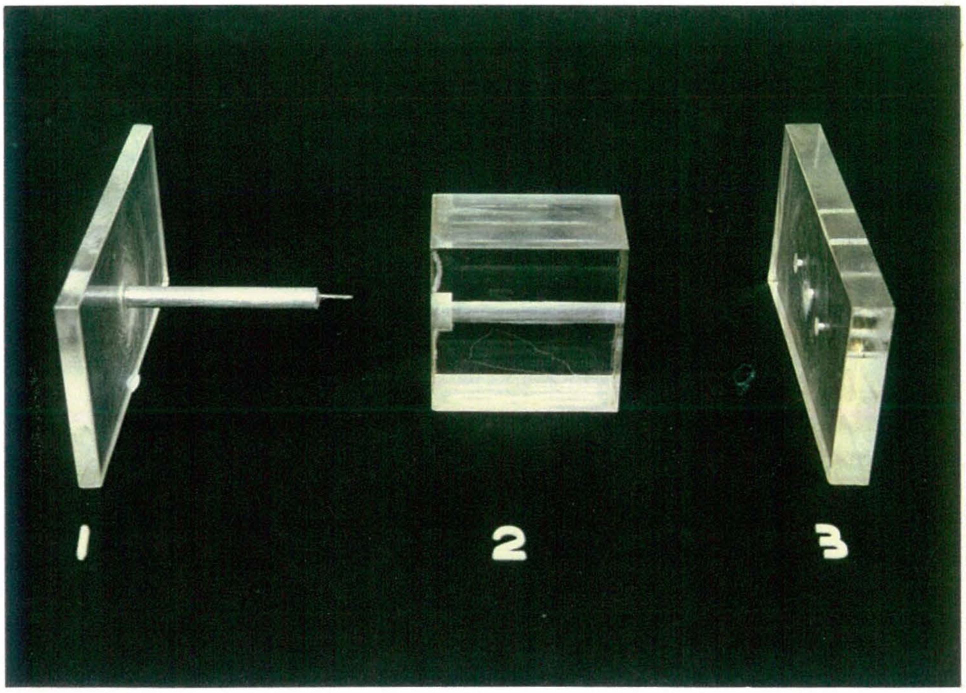


Plate 3.3 Three pieces of the device used for direct measurement of the fore reqired for the seed cap movement

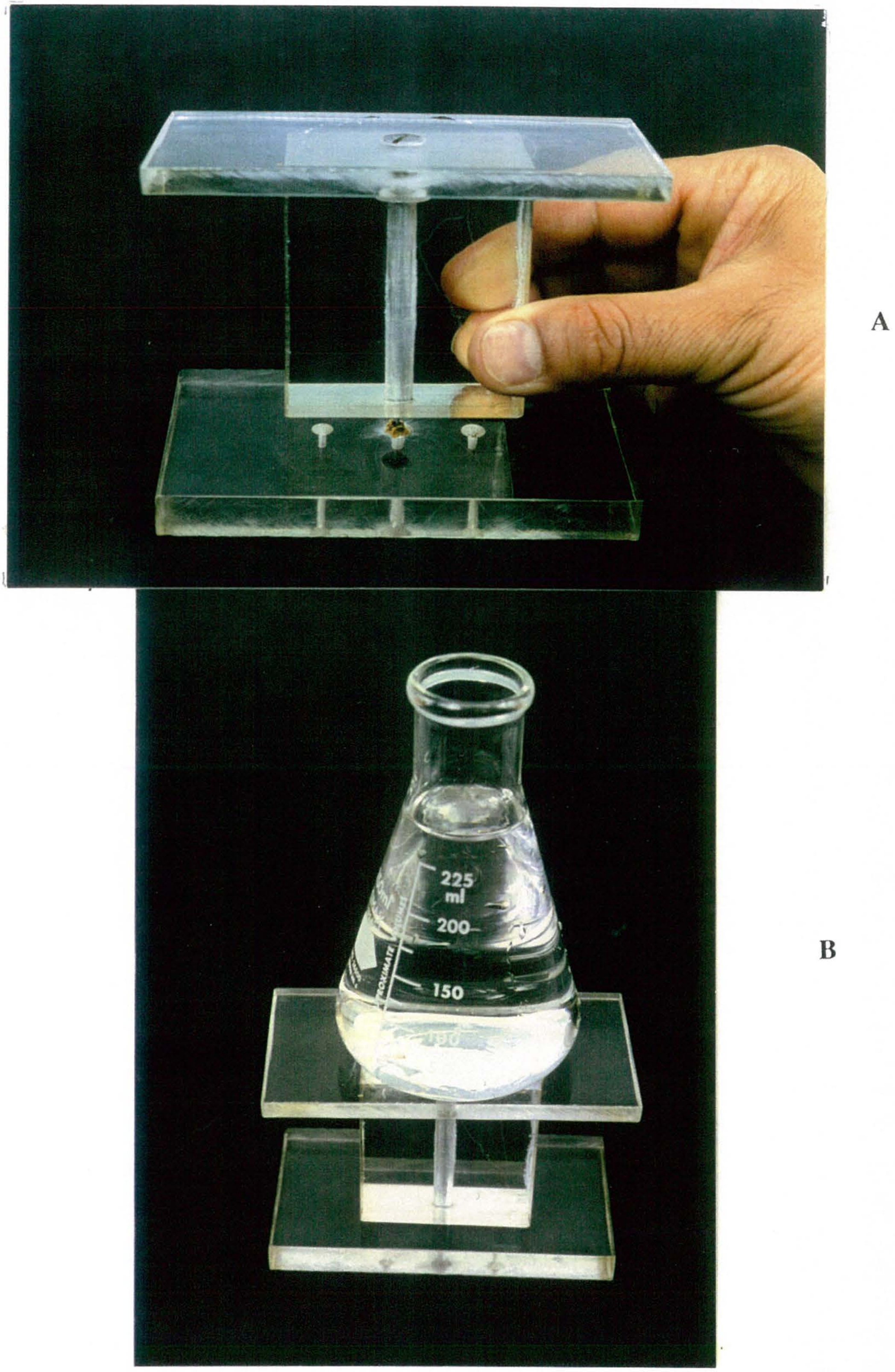


Plate 3.4 Placement of the needle of the device on the cap of seedless fruit (A) and adding water into the container placed on the top of the device designed for direct measurement of the force required the cap movent (B)

3.2.4 To investigate the physiological function of the pericarp base of the fruit

Experiments as follows were carried out to define the actual involvement of the pericarp base on the germination performance of the fruits:

3.2.4.1 *Relationship between the thickness of the pericarp base and germination performance*

To assess the effect of the pericarp bases on the germination percentage, uniformity and median radicle emergence times (T50) of prewashed and unwashed fruits, the pericarp bases of the thickest fruits were removed by scalpel to determine whether germination of the fruits could be improved. This experiment was carried out in 3 replications of 50 fruits of lot A and with 4 treatments as follows:

1. Prewashed fruits with removed pericarp bases.

2. Unwashed fruits with removed pericarp bases.

3. Intact and unwashed fruits.

4. Intact and washed fruits.

3.2.4.2 *To assess whether the pericarp bases reduce the germination percentage via limiting water uptake or via the rate of oxygen uptake during germination*

Two complementary experiments were carried out as follows:

A. In the first experiment for assessing whether the low germination percentage of the thickest fruits in the petri-dish was as a result of lack of water uptake, the water uptake capacity of the thinnest (2.25-2.4mm) and thickest (3.25-3.5mm) fruits of Lot A at 15°C and for different period of times (0.5, 1, 2, 4, 6, 8, 10 ,14, 18 , 22, 26 and 30 hours) were compared. In this experiment 3 replications of 50 fruits from each of the size grades were incubated on the substrate (either from seed cap or the pericarp bases) and weighed before and after the incubation periods.

B. Perry and Harrison (1974) regarded the pore which runs through into the fruit pericarp bases which is the main entrance path for oxygen and they suggested that this pore is filled with loosely packed, dead cells (see section 2.5.2). Based on their suggestion, it was hypothesized that the thickness of pericarp bases of the thickest fruits might be a limiting factor for oxygen uptake of the fruits, especially due to the interaction between available moisture in the germination media and oxygen uptake which happens when the fruit is oriented so that their pericarp base is in contact with the germination substrate. To test this assumption; using the petri-dish method (as described for the T50 test, section 3.1.7), and incubating 3 replications of 50 fruit in each treatment, the effect of fruit orientation on the substrate from the seed cap or upside down (pericarp base was on the media) was assessed. In this experiment the treatments were as follows.

B.1 Thickest fruits which their pericarp bases were in contact with the germination media.

B.2 Thickest fruits oriented so that their seed caps were on the germination media.

B.3 Thinnest fruits with their pericarp bases were in contact with the germination media.

B.4 Thickest fruits oriented so that their seed caps were on the germination media.

It should be mentioned that, Scanning Electron Microscopic and Energy Dispersal X-ray Spectroscopy were used to demonstrate the structure of the base of the pericarp and also to determine the components of the pericarp base area. The procedure of this processes which were carried out by DSIR Fruit and Trees E.M. Unit (courtesy Doug Hopcroft), was as follows: Using a razor blade, dry fruits of the thickest grade (3.25-3.5mm) of Lot A were cut vertically, and glued to an Aluminium SEM stub using conductive silver paint, sputter coated with 20-30nm of gold, and studied using a Cambridge 250 MK3 SEM.

3.2.5 Data analysis of the experiments

Analysis of variance was used to determined the field performance of the size grades (section 4.2.1) and simple linear and non-linear correlation analysis in determining the relationships between plant establishment of the size grades and germination percentages in optimal conditions (4.2.1). T tests and analyses of variance were employed to determine relationships between fruit size and physical and physiological characteristics of the fruits (4.1.2-4.1.5) and also to identify germination limiting factors in sugar beet fruits (5.1-5.5). It should be mentioned that, since only one grading process was carried out on each specific sample of the seed lots, all replicates are replicate sub-samples of the graded material.

CHAPTER 4

EFFICIENCY OF SIZE GRADING BY THICKNESS OR DIAMETER AND THE CONSEQUENCES FOR GERMINATION PERFORMANCE

4.1 RESULTS

4.1.1 Fruit size and fruit weight relationships

Round hole screen grading resulted in progressively greater thousand fruit weights with increasing diameter in each of the lots (Figure 4.1). However, as shown in Figure.4.2, despite the fact that there were significant differences in the weight of thickness graded fruits(slot graded), there was no clear correlation between fruit thickness and thousand fruit weight. There were differences in weights for the same size grades for different lots, as shown in Figures 4.1 and 4.2; LOT C had the heaviest and LOT B had the lightest 1000 fruit weight in the size grades (eg. 3.67 vs 5.68 g for the smallest and 14.88 vs 15.73 g for the largest diameter fruits, Figure 4.1) and similarly, for thickness grading: 5.90 vs 7.14 g for the thinnest and 12.12 vs 14.85 g for the 1000 fruit weight of the thickest grade (Figure 4.2).

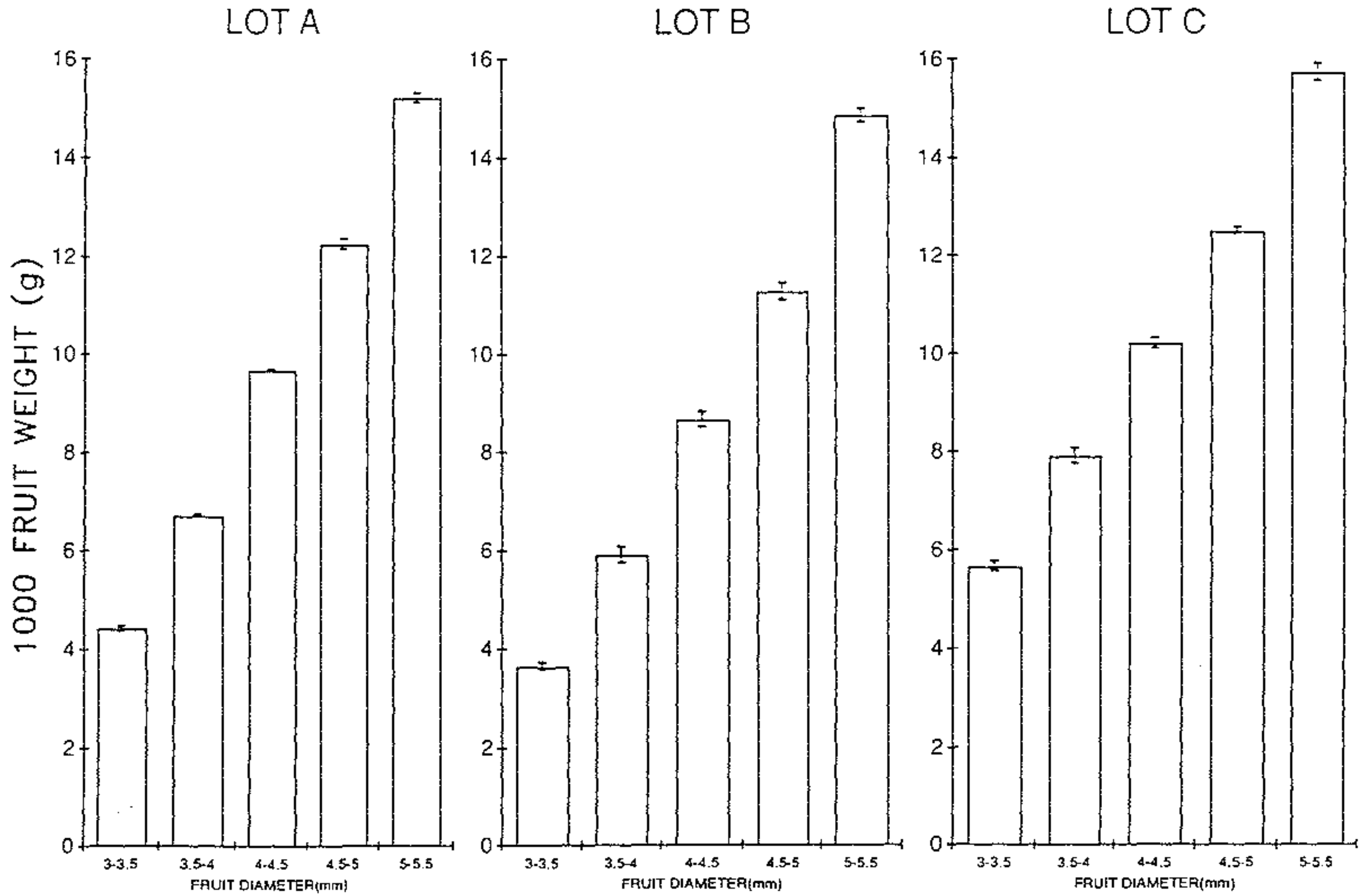


Figure 4.1 The relationship between fruit diameter and 1000 fruit weight of the lots. Each data point is the mean of eight replications and vertical bars represent standard errors of the means for each grade.

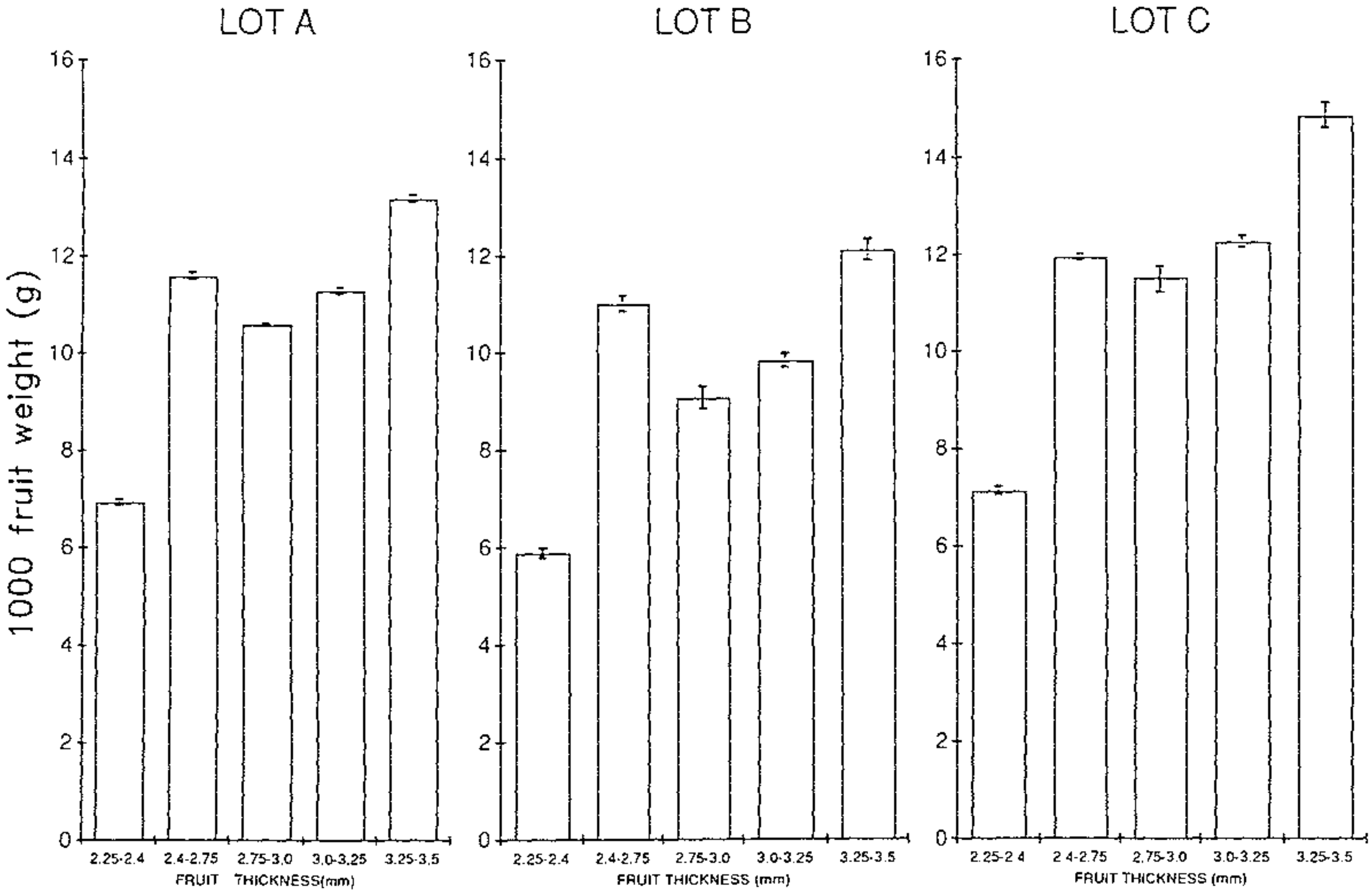


Figure 4.2 The relationship between fruit thickness and 1000 fruit weight of the lots. Each data point is the mean of eight replications and vertical bars represent standard errors of the means for each grade.

4.1.2 Relationship between fruit size and true seed, seed cap and seed coat dry weights

4.1.2.1 *Fruit diameter and fruit fraction relationships*

Measurement of true seed, seed cap and seed coat dry weights indicated that the true seed comprised a small proportion of fruit weight while the seed coat comprised most of the fruit weight. Data obtained in this experiment (Figure.4.3) indicated that there were significant increases in both absolute weights and relative proportions of seed coat material in the larger fruits of the lots (eg. from 46 % of total weight for 3-3.5mm to 64.4 % for 5-5.5mm fruits of lot A).

As shown in Figures 4.4 and 4.5, there were positive correlations between fruit diameter and both true seed weight and seed cap weight in the each of the lots. However, as shown in Figure 4.3, the relative proportion of seed cap to total weight was significantly reduced with increasing fruit size for example; from 19.3 % for 3-3.5mm to 14.9 % for fruit of 5-5.5mm diameter in Lot A.

4.1.2.2 *Fruit thickness and fruit fraction relationships*

Despite there being significant differences among thickness graded fruits in true seed and seed cap weight, as expected from Figure 4.2, no clear relationships were found between fruit thickness and the weight of different fruit fractions. As shown in Figure 4.6, the true seed comprised only a small part of the thickness graded fruits while the ratio significantly differd among the size grades; for example, in the thinnest fruits of lot A, true seed or germplasm (GP) comprised 28.3 % of the fruits vs 23.4 % in the thickest fruit with the highest proportion of seed coat. In the case of the seed cap, the thinnest fruits had relatively heavier seed caps than the thickest fruits (for instance 19.9% vs 14.9 % in LOT A).

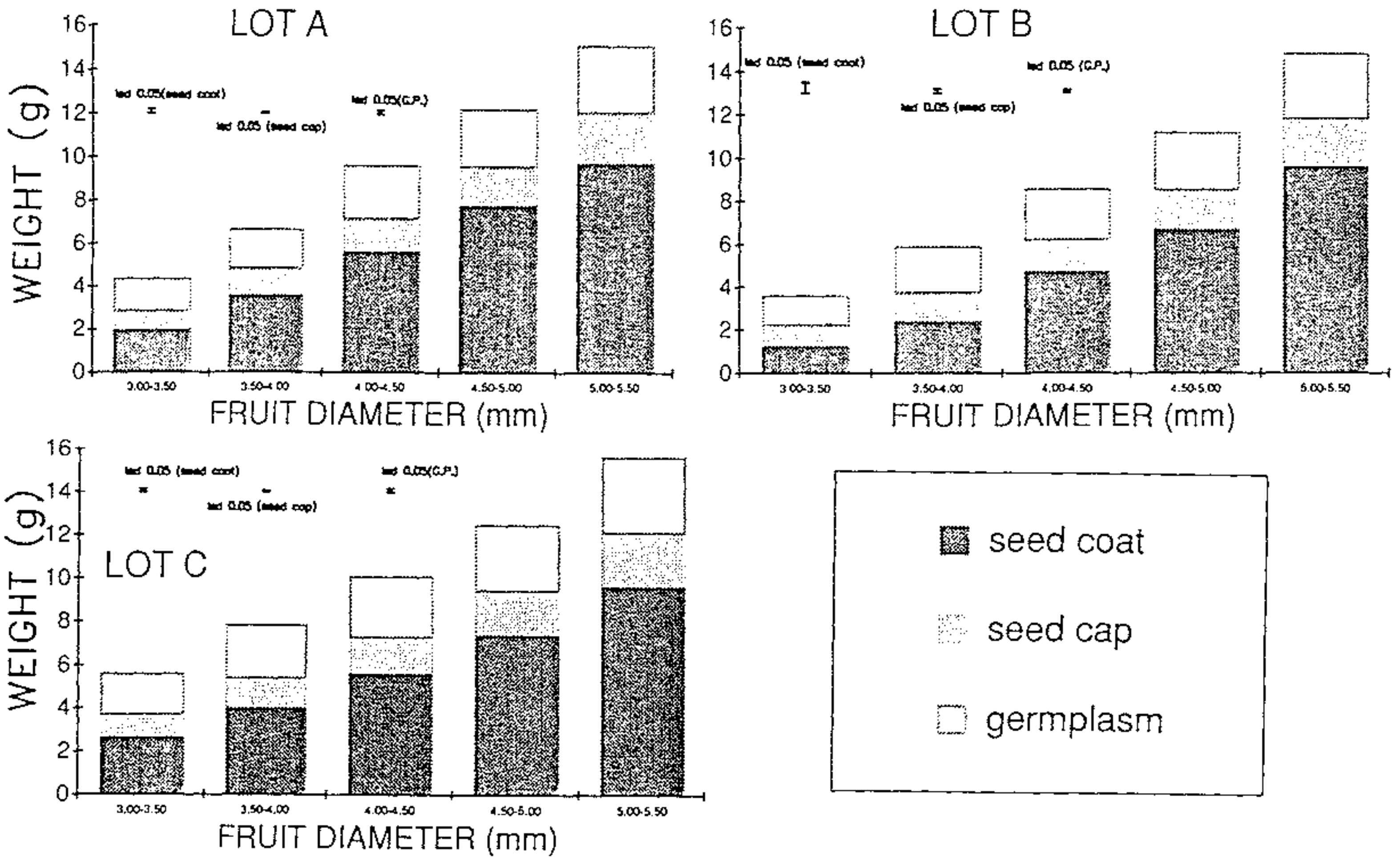


Figure 4.3 Proportions of seed coat, seed cap and true seed or germplasm (GP) to total weight of diameter graded fruits. Data presented are the mean of 3 replications.

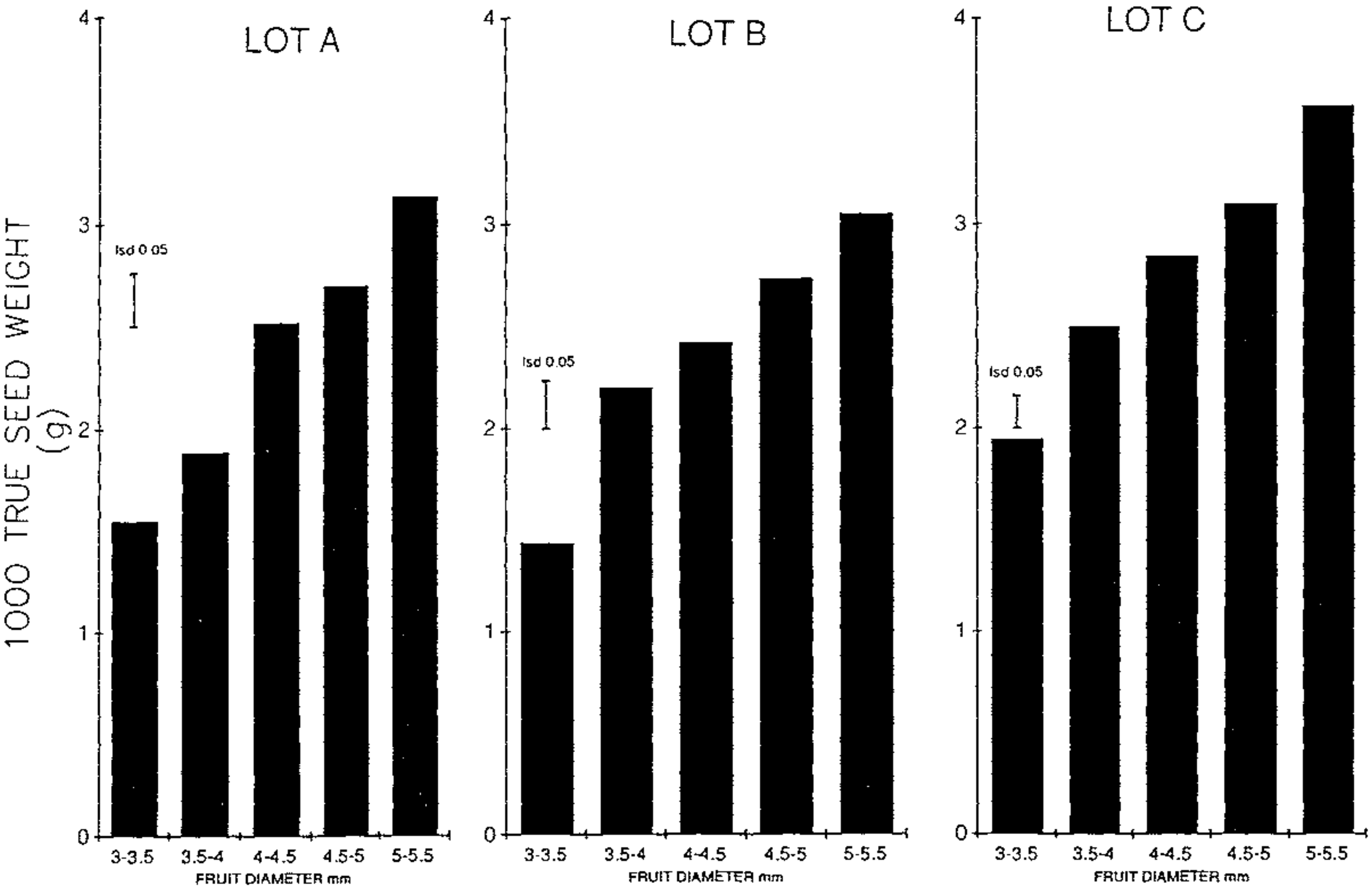


Figure 4.4 The relationship between fruit diameter and 1000 true seed weight of each of the lots. Data presented are means of 3 replications.

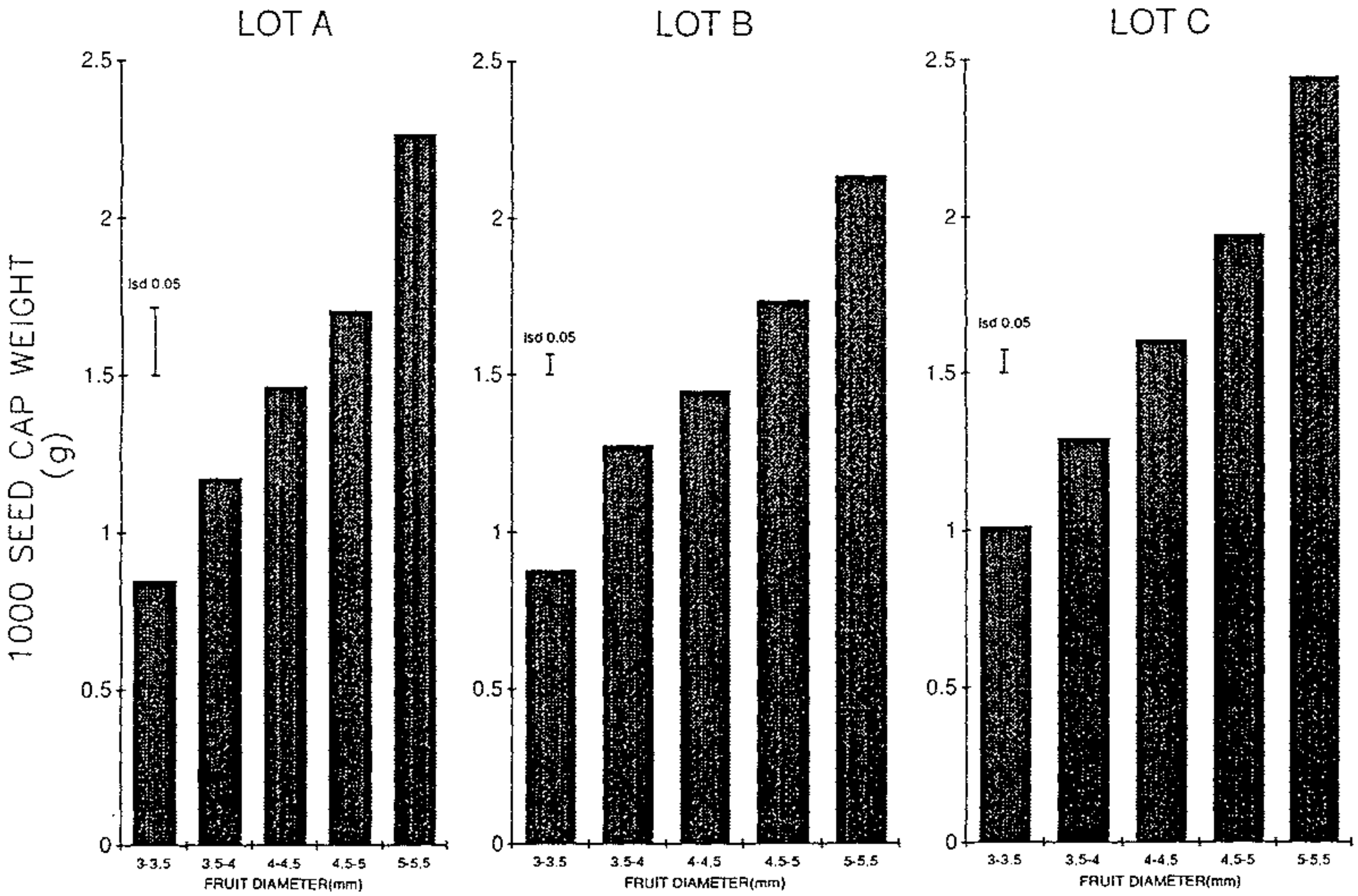


Figure 4.5 The relationship between fruit diameter and 1000 seed cap weight of each of the lots. Data are means of 3 replications.

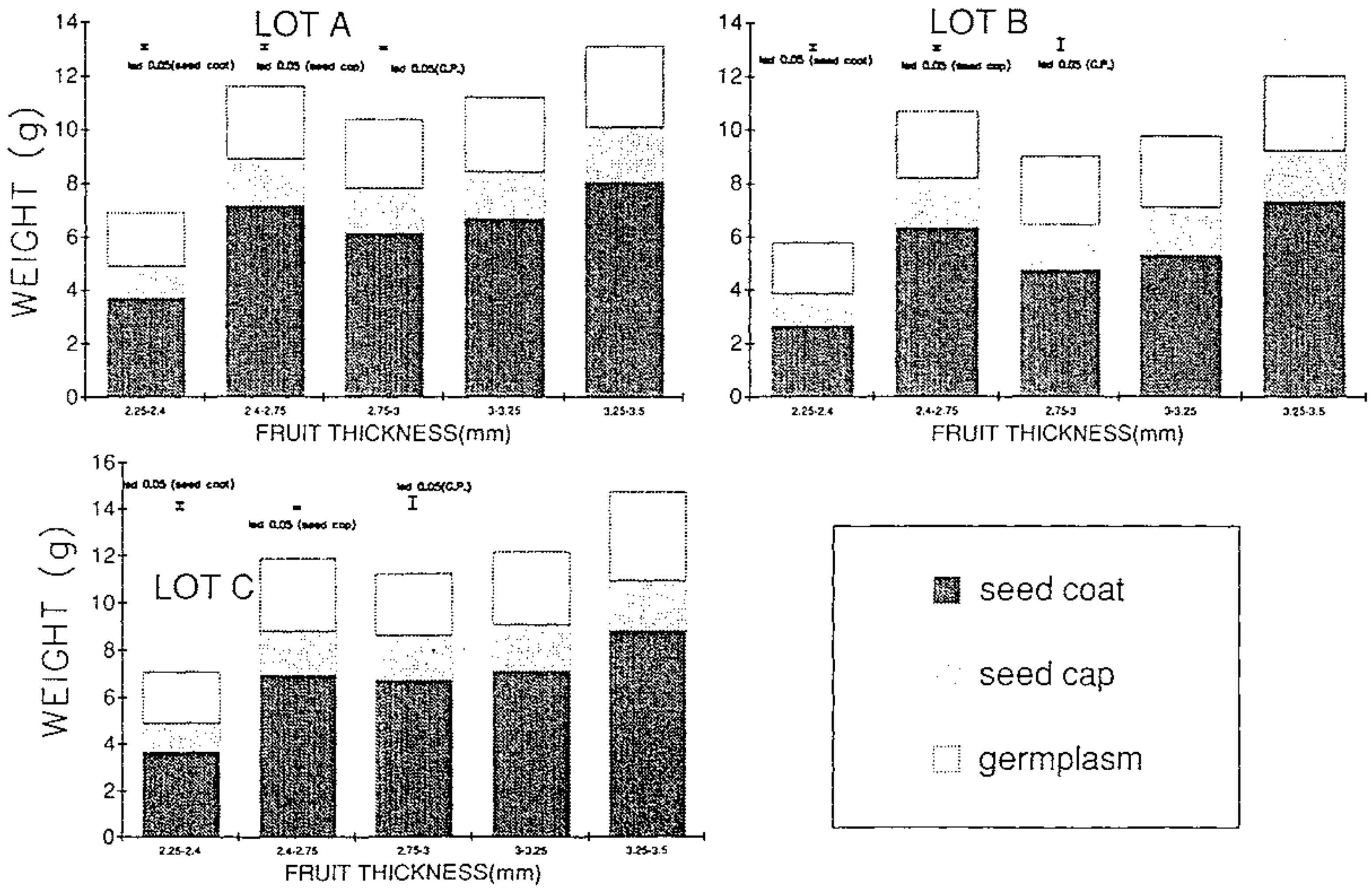


Figure 4.6 Proportions of seed coat, seed cap and true seed or germplasm (GP) to total weight of thickness graded fruits. Data presented are the mean of 3 replications.

4.1.3 Relationships between fruit size and germination performance

4.1.3.1 *Fruit diameter and germination*

There were significant differences in germination performance within the lots (Figure 4.7), lot C being the best in all grades while lot B was the worst. Furthermore, variation in the germination performance of the size grades of the lots diminished by increasing the quality; for example, in the poor quality lot (B), there were significant differences amongst the size grades whereas in the high quality lot (C), there were no significant differences between grades except for the relatively poorer smallest grade.

As shown in Figure 4.8, there was a linear and highly significant correlation (r= 0.96***) between fruit diameter and germination percentage in the poor quality lot whereas, as shown in Figure 4.9, the nature of this relationship in the medium and high quality lots was non-linear.

4.1.3.2 *Fruit thickness and germination performance*

As shown in Figure 4.10., despite significant differences among the size grades, there was no systematic relationship between fruit thickness and seed germination performance. In each lot fruits ranging from 2.4mm to 2.75mm in thickness had the best germination percentage while with the exeption of Lot B, the thinnest fruits (2.25-2.4), performed as well as all size grades of more than 2.75mm thickness.

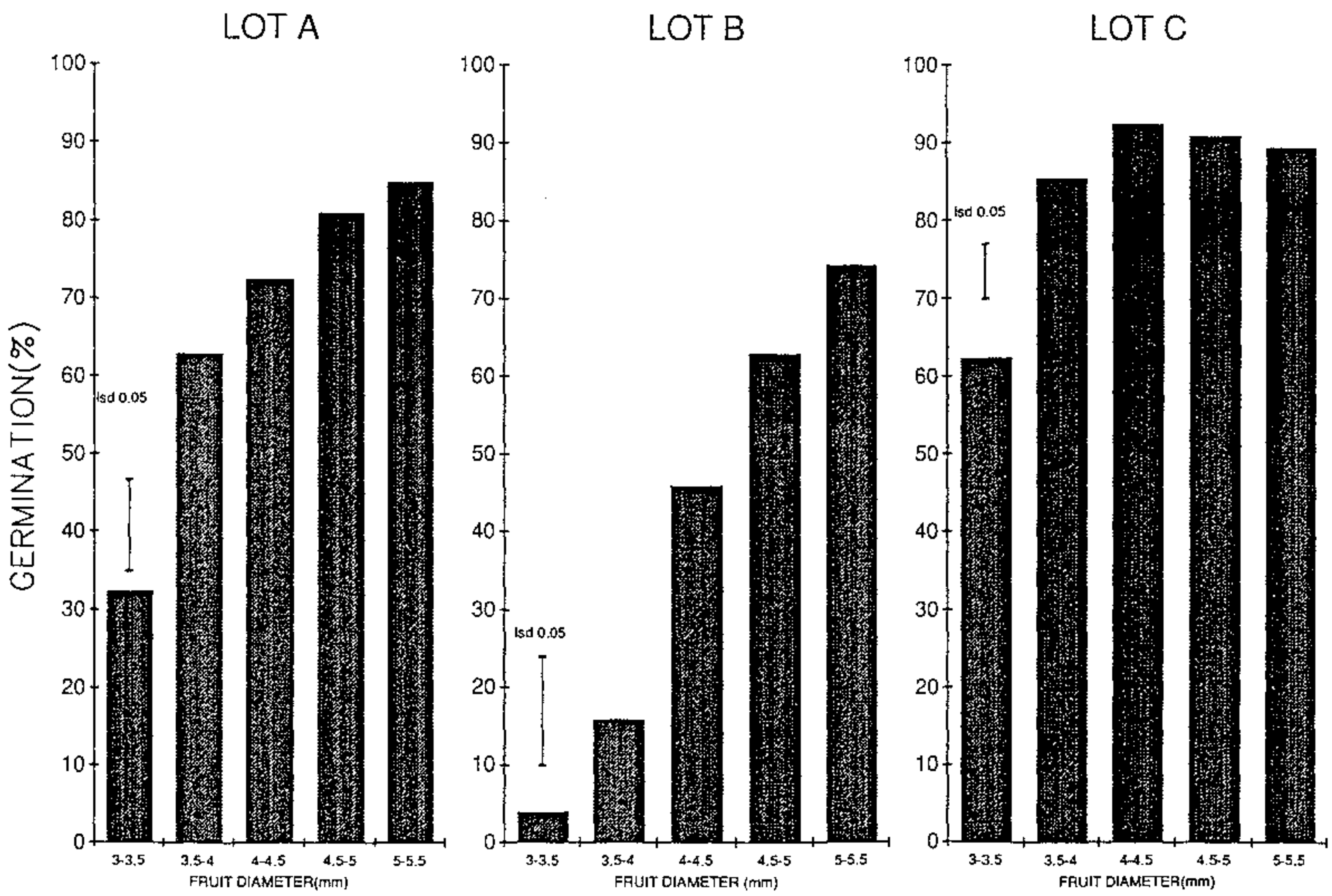


Figure 4.7 Germination percentage of diameter grades at 20°C. Data presented are means of 5 replications.

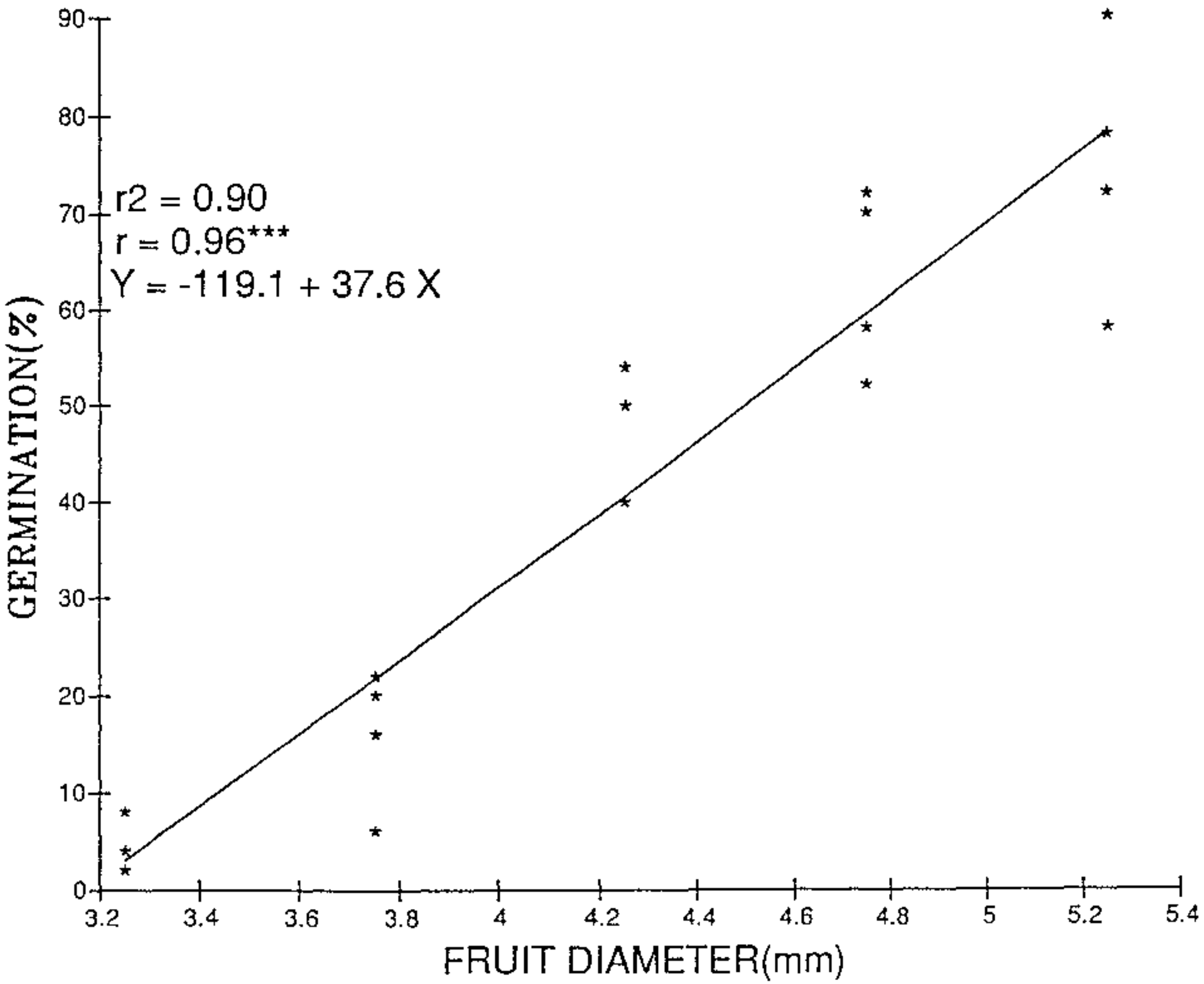


Figure 4.8 The linear correlation between fruit diameter and germination percentage in the poor quality lot (LOT B). Using 4 replications of 50 fruits of each grade, the germination test was conducted on paper at 20oC.

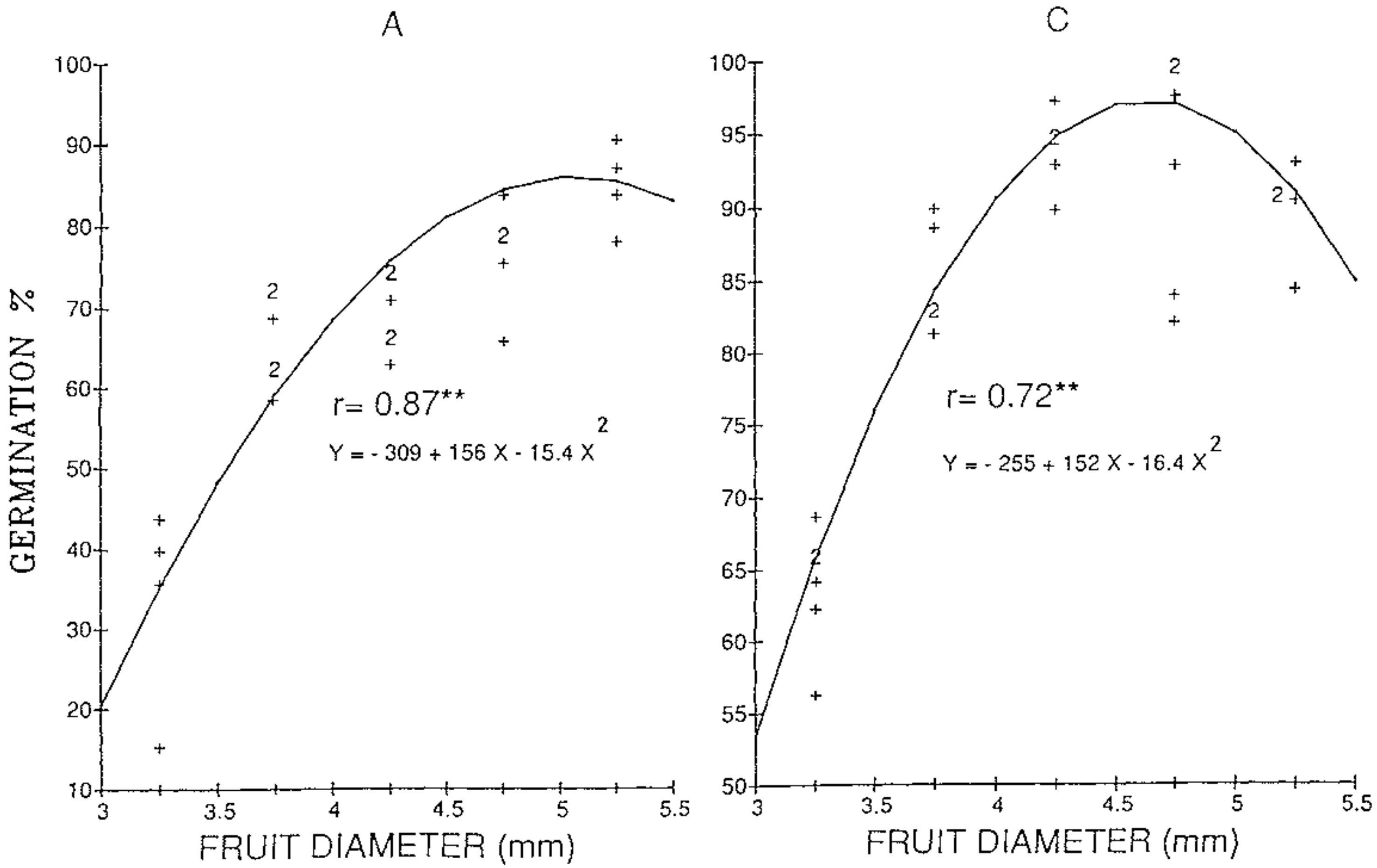


Figure 4.9 The relationships between fruit diameter and germination percentage in LOT A and LOT C. Using 4 replications of 50 fruits of each grade, the germination test was conducted on paper at 20^{o}C. (+ = individual replicate data point)

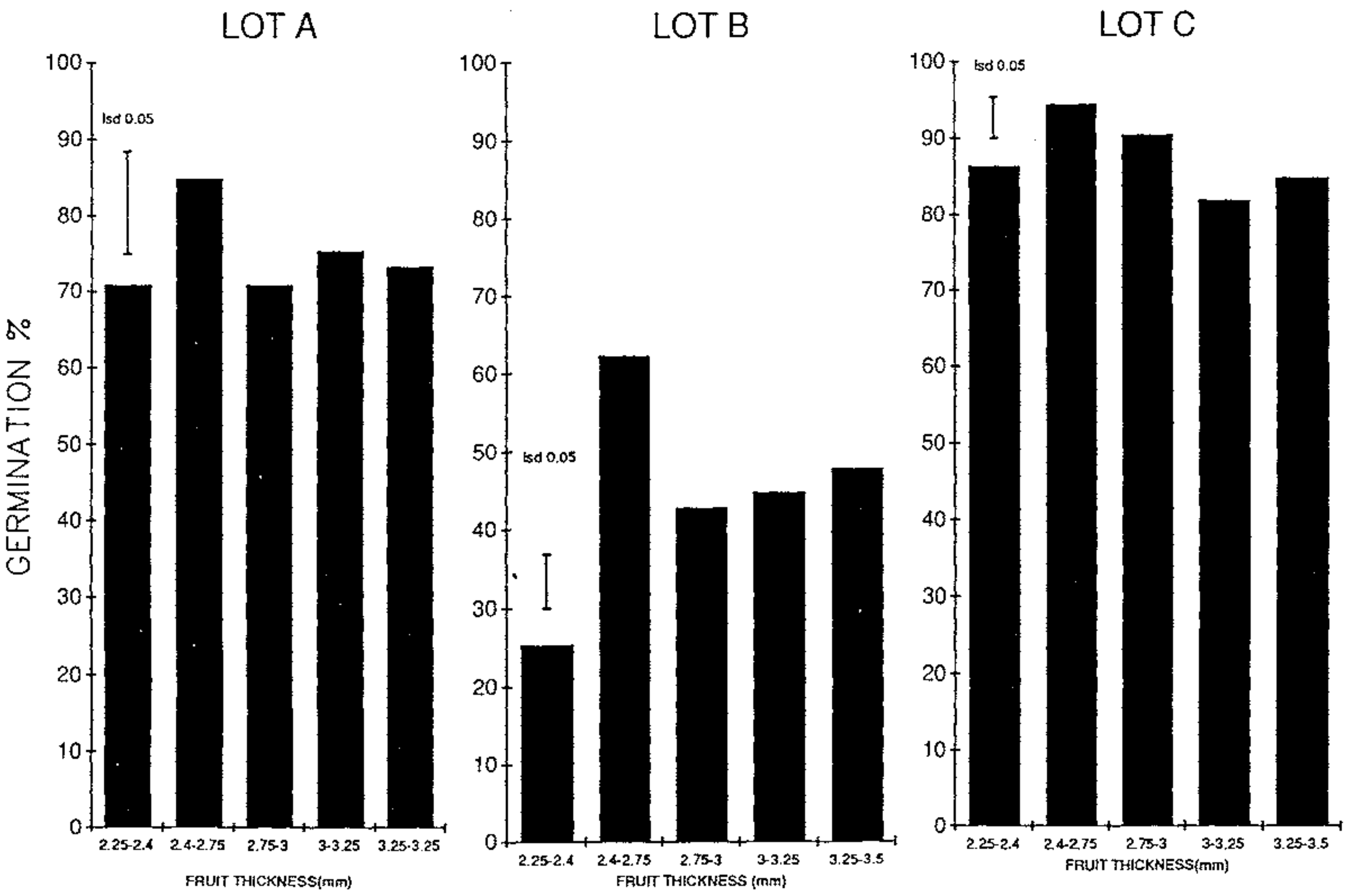


Figure 4.10 Germination percentage of thickness grades at 20°C. Data presented are means of 5 replications.

4.1.4 Seedling dry weight and fruit size

A highly significant linear correlation was found between true seed weight of each lot and seedling dry weight after 14 d growth in pleated paper. An example of this relationship for thickness and diameter graded fruits in lot A (r= 0.95**) is shown in Figure 4.11.

Although a positive relationship was found between fruit diameter and seedling dry weight (Figure 4.12), no systematic relationship was found between fruit thickness and seedling dry weight (Figure 4.13).

4.1.5 Median radicle emergence time and uniformity of germination of the size graded fruits

The test results for the medium quality lot (LOT A) indicated that there was no relationship between fruit size (thickness and diameter) and median radicle emergence times (Table 4.1a and 4.1b). Nevertheless, results obtained from analysis of variance indicated that there were significant differences in median radicle emergence times between thickness grades i.e. the thinnest showed the best rate whereas the thickest fruits were slowest and the other grades were of the equal value. Similarly, significant differences also were found amongst the diameter grades where the largest fruits (5-5.5mm) showed the slowest rates the fruits which ranged from 4-5mm had the most rapid radicle emergence.

No systematic relationship was found between fruit thickness and uniformity of radicle emergence. However, as shown in Table 4.1a, the fruit of 2.40-2.75 in thickness showed poorer uniformity of radicle emergence than other grades. No significant differences were found between uniformities of radicle emergence in different diameter grades (Table 4.1.b)

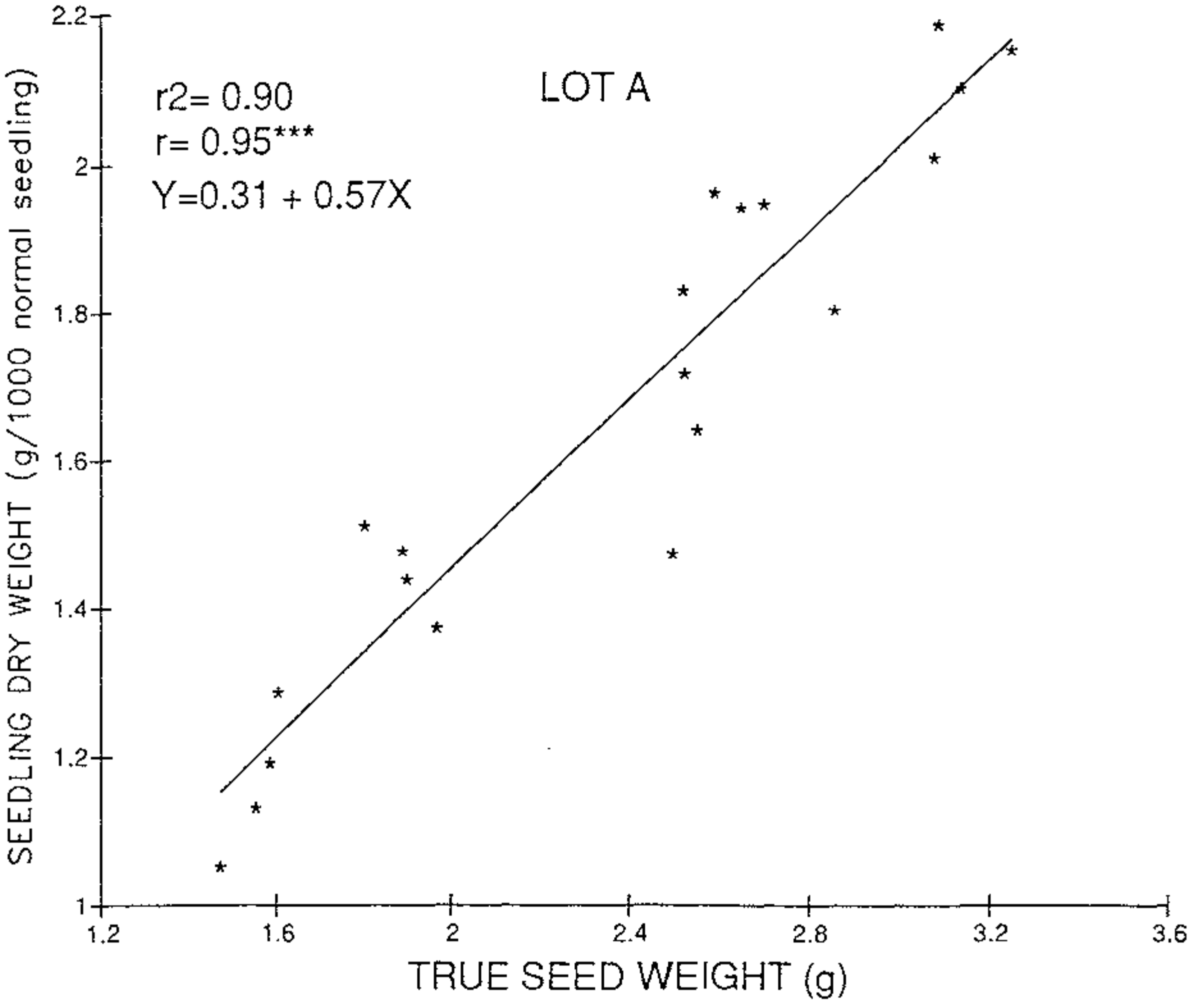


Figure 4.11 Example of the relationship between true seed weight and seedling dry weight of the thickness graded fruits in the medium quality lot (LOT A).

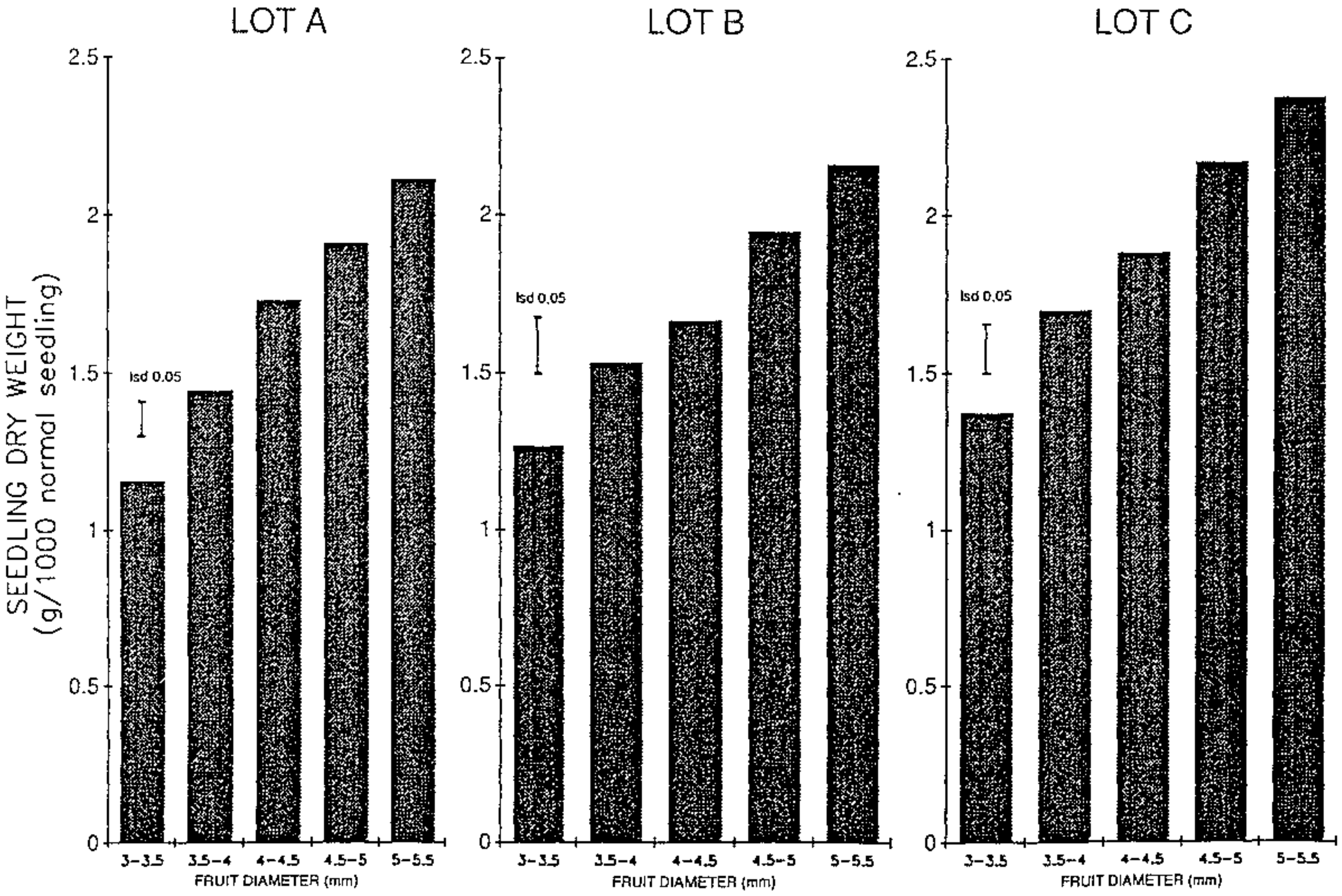


Figure 4.12 Fruit diameter and 1000 seedling dry weight relationships of the lots. Data presented are means of 4 replications of seedling dry weight after 14 d growth on paper at 20^{0}C.

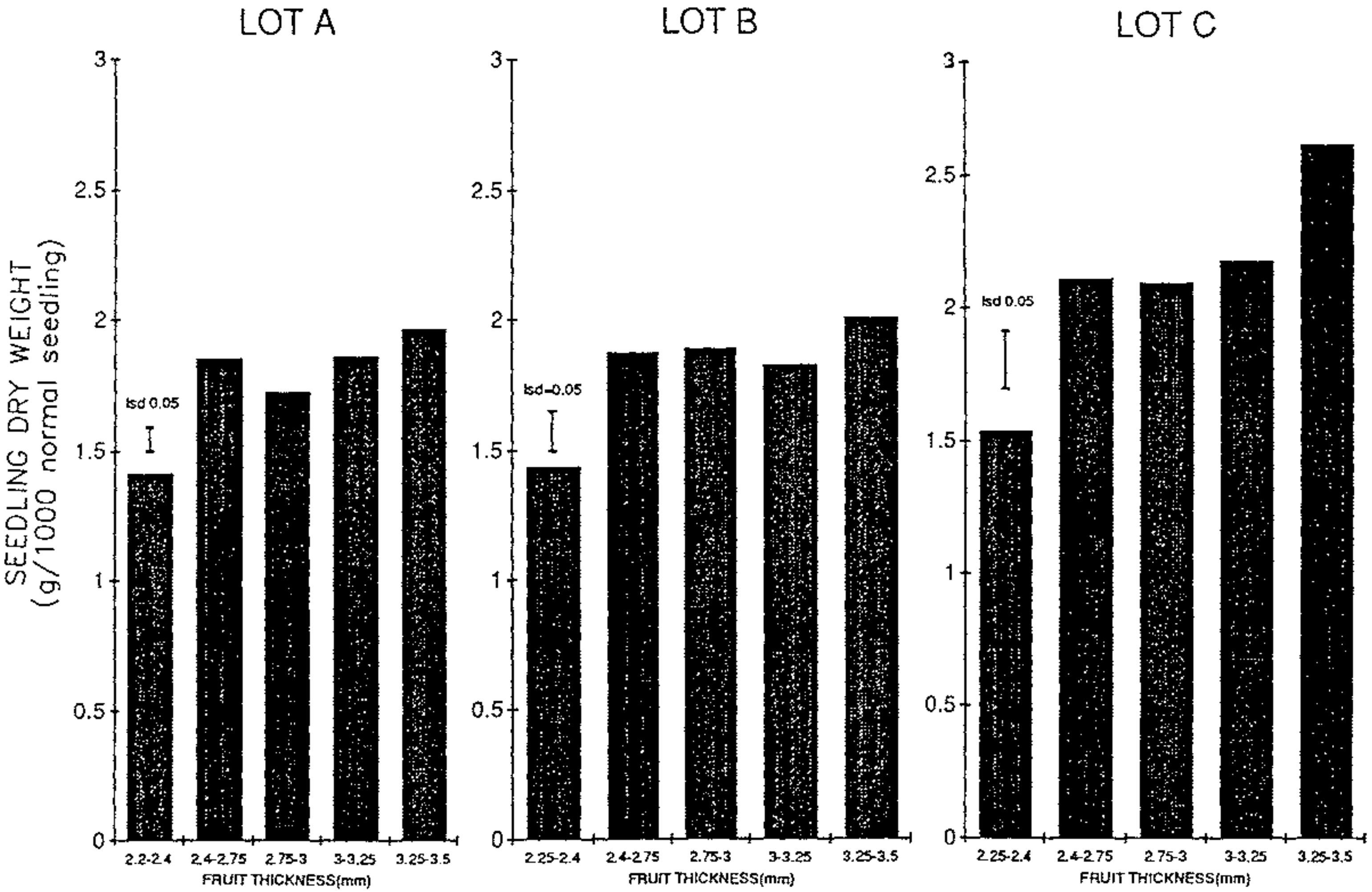


Figure 4.13 Fruit thickness and seedling dry weight relationships in the seed lots. Data presented are means of 4 replicates of seedling dry weight after 14 d growth on paper at 20°C.

Table 4.1a Median radicle emergence times and uniformity of germination of thickness graded fruits of the medium quality lot (A) incubated in petri-dishes at 15^{O}C. Data presented are means of 4 replications.

FRUIT THICKNESS (mm)	T50 (h)	UNIFORMITY (T90-T10) (h)
2.25-2.4	126	294
2.4-2.75	137	489
2.75-3.0	144	333
3.0-3.25	136	346
3.25-3.5	163	306
Lsd 0.05	29.3	125.7

Table 4.1b Median radicle emergence times and uniformity of germination of diameter graded fruits of the medium quality lot (A) incubated in petri-dishes at 15^{o}C. Data presented are means of 4 replications.

FRUIT DIAMETER (mm)	T 50 (h)	UNIFORMITY (T90-T10) (h)
3.0-3.5	162	358
3.5-4.0	172	412
4.0-4.5	135	361
4.5-5.0	143	302
5.0-5.5	204	438
Lsd 0.05	51.6	NS

4.1.6 Interaction between size grades and germination substrate

It is important to note that as shown in Tables 4.2a and 4.2b, the final germination percentage of the size graded fruits in petri-dishes on top of the paper at 15°C was less than that of those those on pleated paper at 20°C. In addition, as shown in Table 4.2.a, for the peridishe method there was a reduction in germination percentage with increasing fruit thickness; for example, the thickest fruits in the petridish method had a a germination 49 % less than those in the pleated paper method, while there were no significant differences between germination percentage of the thinnest fruits in the two methods.

To determine whether these differences were caused by different temperature or were due to the methodology used in T50 testing, germination performance of the thickest and the thinnest fruit was compared using the pleated paper and petri-dishes methods at the same temperature (15°C). The results of this experiment (data are not shown) confirmed that this problem was not temperature related and it was therefore hypothesized that this matter might be related to differences in the physiological behaviour of the size graded fruits in the two methods (see section 5.2.2 - 5.2.4 for more information).

Table 4.2a Comparison between germination performance of the thickness graded fruits in petri-dishes at 15^{o}C and on pleated paper at 20^{o}C. Data presented are means of 4 replicates and figures in parenthesis represent standard errors.

FRUIT THICKNESS (mm)	FINAL GERMINATION (Petri dishes)	FINAL GERMINATION (Pleated paper)
2.25-2.4	66 (± 4.3)	71 (± 5.4)
2.4-2.75	67 (± 2.1)	96 (± 4.3)
2.75-3.0	58 (± 8.3)	71 (± 1.6)
3.0-3.25	52 (± 7.6)	75 (± 2.5)
3.25-3.5	39 (± 3.7)	73 (± 1.5)

Table 4.2b Comparison between germination performance of the diameter graded fruits in the petri-dishes at 15°C and on pleated paper at 20°C. Data presented are means of 4 replicates and figures in parenthesis represent standard errors.

FRUIT DIAMETER (mm)	FINAL GERMINATION (Petri dishes)	FINAL GERMINATION (Pleated paper)
3.0-3.5	28 (± 1.6)	32 (± 5.5)
3.5-4.0	48 (± 4.8)	63 (± 2.5)
4.0-4.5	62 (± 1.4)	72 (± 2.5)
4.5-5.0	66 (± 4.7)	81 (± 3.2)
5.0-5.5	58 (± 3.3)	85 (± 1.5)

4.1.7 Fruit size and plant establishment relationships

Linear regression analysis between fruit diameter and plant establishment of the poor quality lot (B) showed a significant coefficient of 0.96*** (Figure 4.14) whereas, as shown in Figures 15a and c, the nature of this relationship in the high and medium quality lots was non-linear.

Despite significant differences among the thickness graded fruits of the lots (Figure 4.16), there was no systematic relationship between fruit thickness and plant establishment. Fruits of 2.4 - 2.75mm thickness in lot A and B, and 3.25 - 3.5mm in lot C showed the best establishment whereas, fruit of 2.25-2.4 thickness showed the lowest field performance.

Although there was no significant correlation between the laboratory standard germination result for both the thickness and diameter graded fruits and plant establishment of the high quality lot, highly significant correlations were found between the laboratory germination and plant establishment of the size grades of the medium and poor quality lots (Figure 4.17).

4.1.8 Relationship between fruit size and field seedling dry weight

No systematic relationship was found between fruit thickness and shoot dry weight of established plants of the lots (Figures 4.18). Nevertheless, as shown in Figure 4.18, despite there being no significant difference in the poor quality lot (Lot B), there were significant differences in the final shoot dry weight of established plants grown from the thickness graded fruits of Lot A and Lot C. In Lot A, the thinnest fruits produced the smallest plants whereas, there was no significant difference in the shoot dry weight of the other thickness grades of this lot and in lot C, fruits of 2.75-3mm and 3.25-3.5mm of thickness produced the most vigorous plants whereas, no significant difference was found in shoot dry weight of the other thickness grade of this lot.

No clear relationships were found between fruit diameter and plant shoot dry weight of the lots (Figure 4.19). As shown in this figure, despite there beinig no significant differences between shoot dry weight of the poor quality lot (Lot B), there were significant differences between shoot dry weight of plant emerged from diameter graded fruits of the medium and high quality lots (Lot A and C resp.). In LotA, fruits of 4.5-5mm of diametre were superior while no significant difference was found between shoot dry weight of the other size grades, and in the high quality lot (Lot C), fruits of more than 4.5mm were superior whereas no significant difference was found between shoot dry weight of the plants grown from the medium and small fruits (3-4.5mm of diameter) of this lot.

4.1.9 Post emergence factors limiting plant establishment

Despite applications of fungicides and insecticide during the plant growth period, 3-4% of total emerged seedlings were lost, 26 % of the damaged seedlings being cut by an insect which was identified as the Maize Seedling Beetle (*Clivina- rugithorox* ,plate 4.1), and 74 % of them were infected by fungi (*Cercospora belicola saccardo* and also *Rhizoctonia solani Kuhn*). The fungi and the insect were identified by the Plant Health Department, Massey University. In the second replicate of the field experiment of lot C, most of the plants were retarded, possibly as a result of residual effects of the plant growth regulator (Paclobutrazol or PP 333) which was applied to the previous crop; therefore the data for this replicate have not been included in the field results.

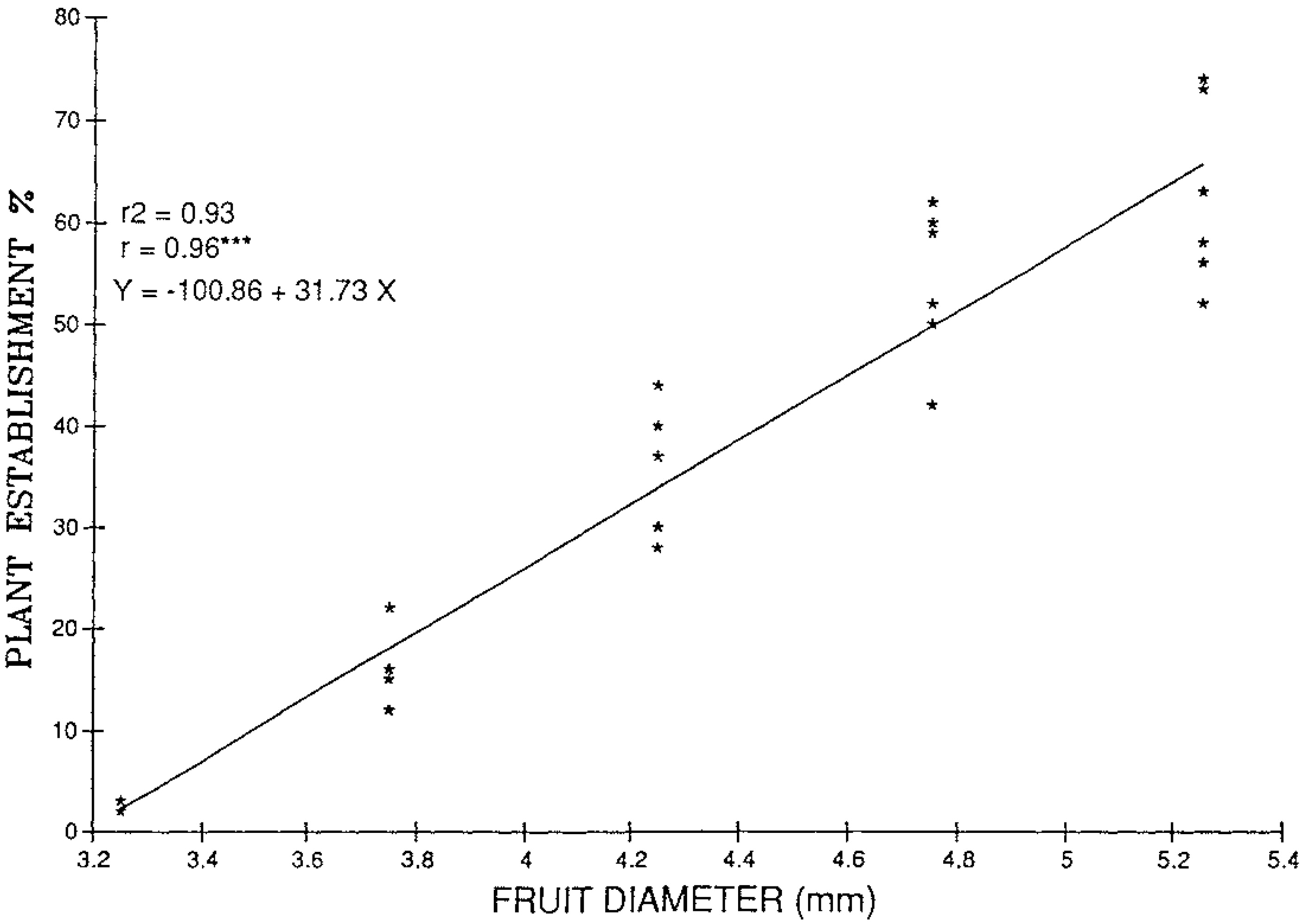


Figure 4.14 The linear correlation between fruit diameter and plant establishment in the poor quality lot (LOT B).

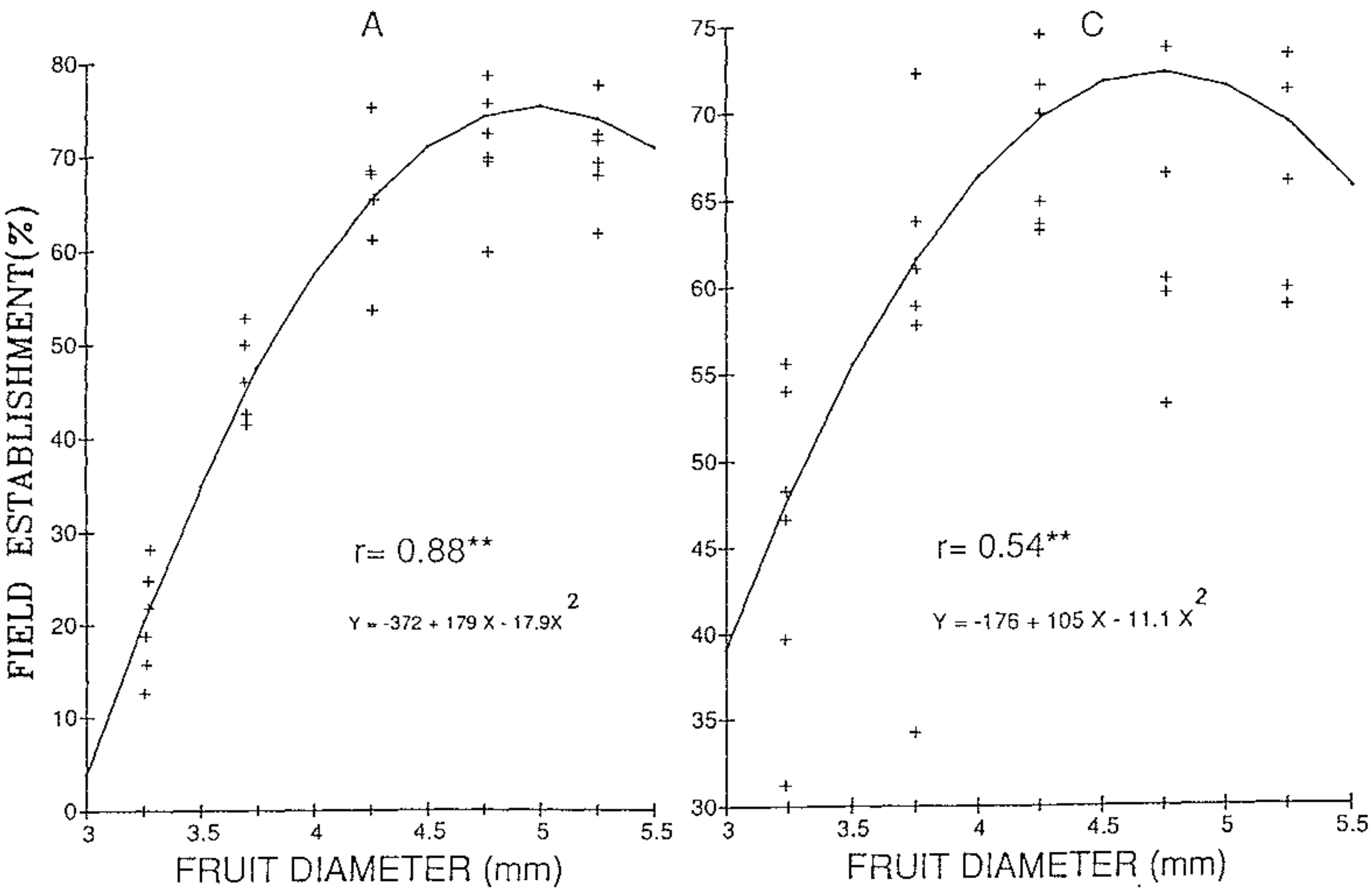


Figure 4.15 The relationship between fruit diameter and plant establishment in the medium (A) and high quality lot (C). (+ = individual replicate data point)

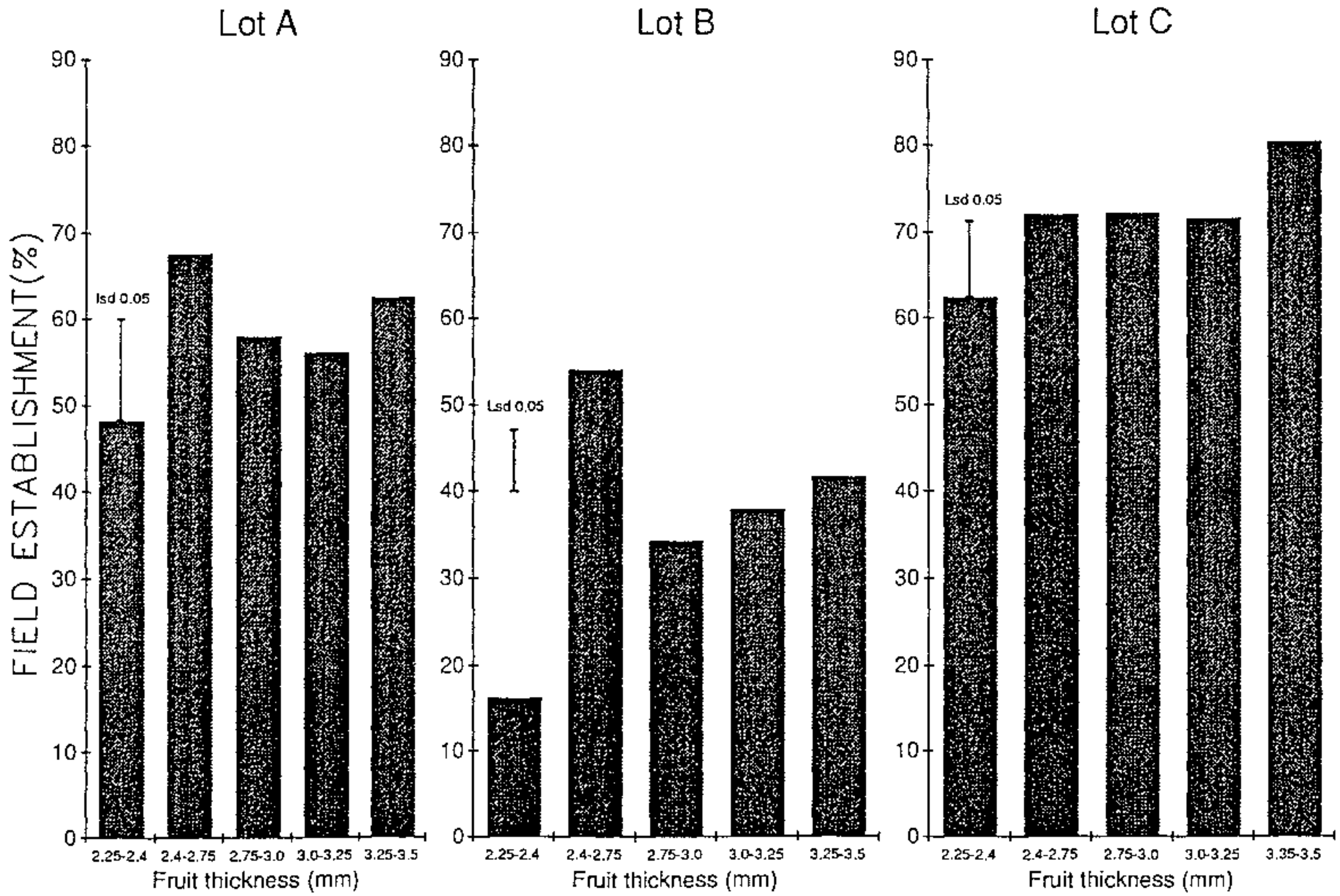


Figure 4.16 Field establishment of the thickness graded fruits in the seed lots. Data presented are the mean of 6 replications.

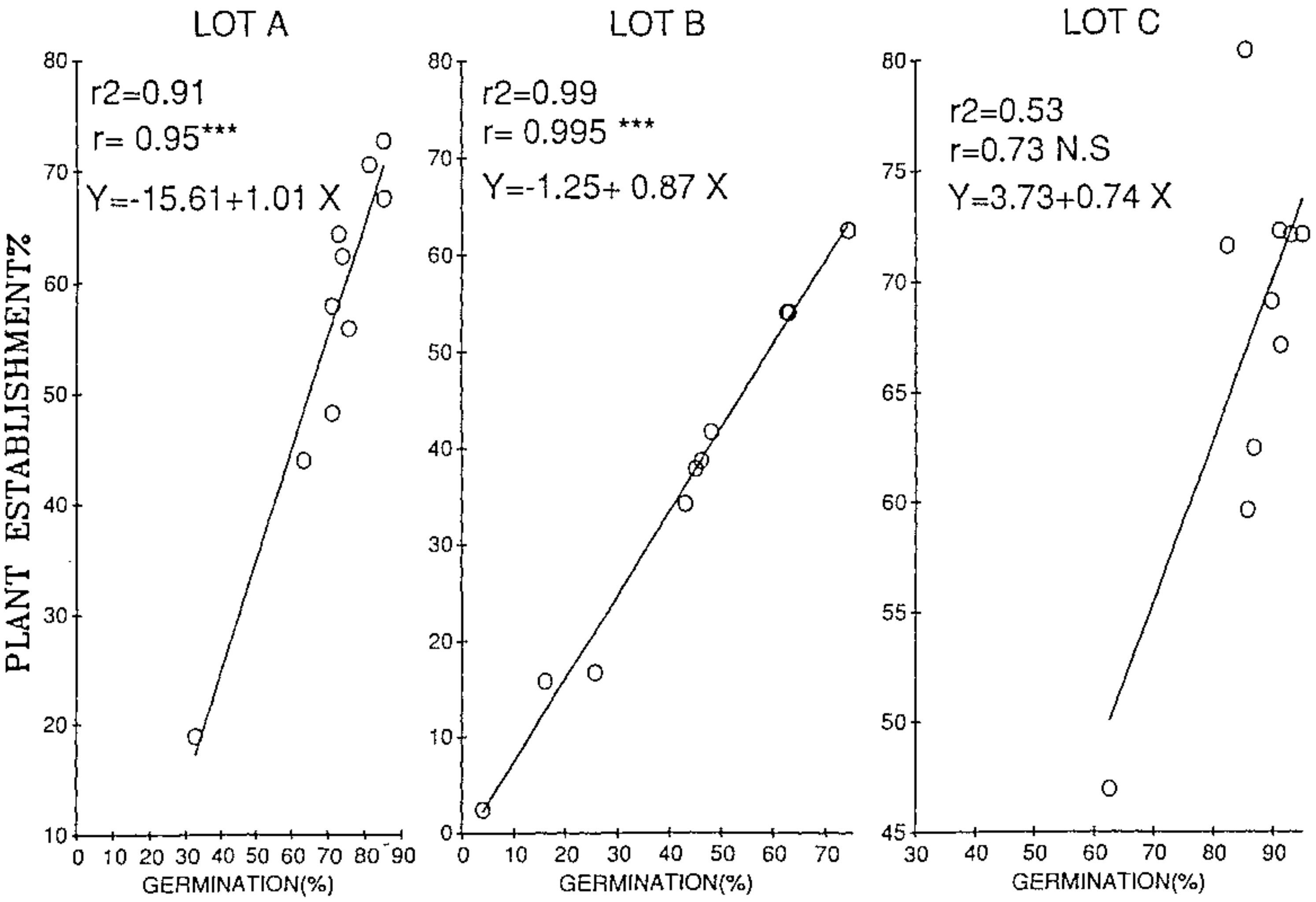


Figure 4.17 The correlation between laboratory germination and plant establishment of different size grades for each of the lots. Data presented are based on the mean values of 5 replications of laboratory germination and 6 replications of plant establishment of the size grades.

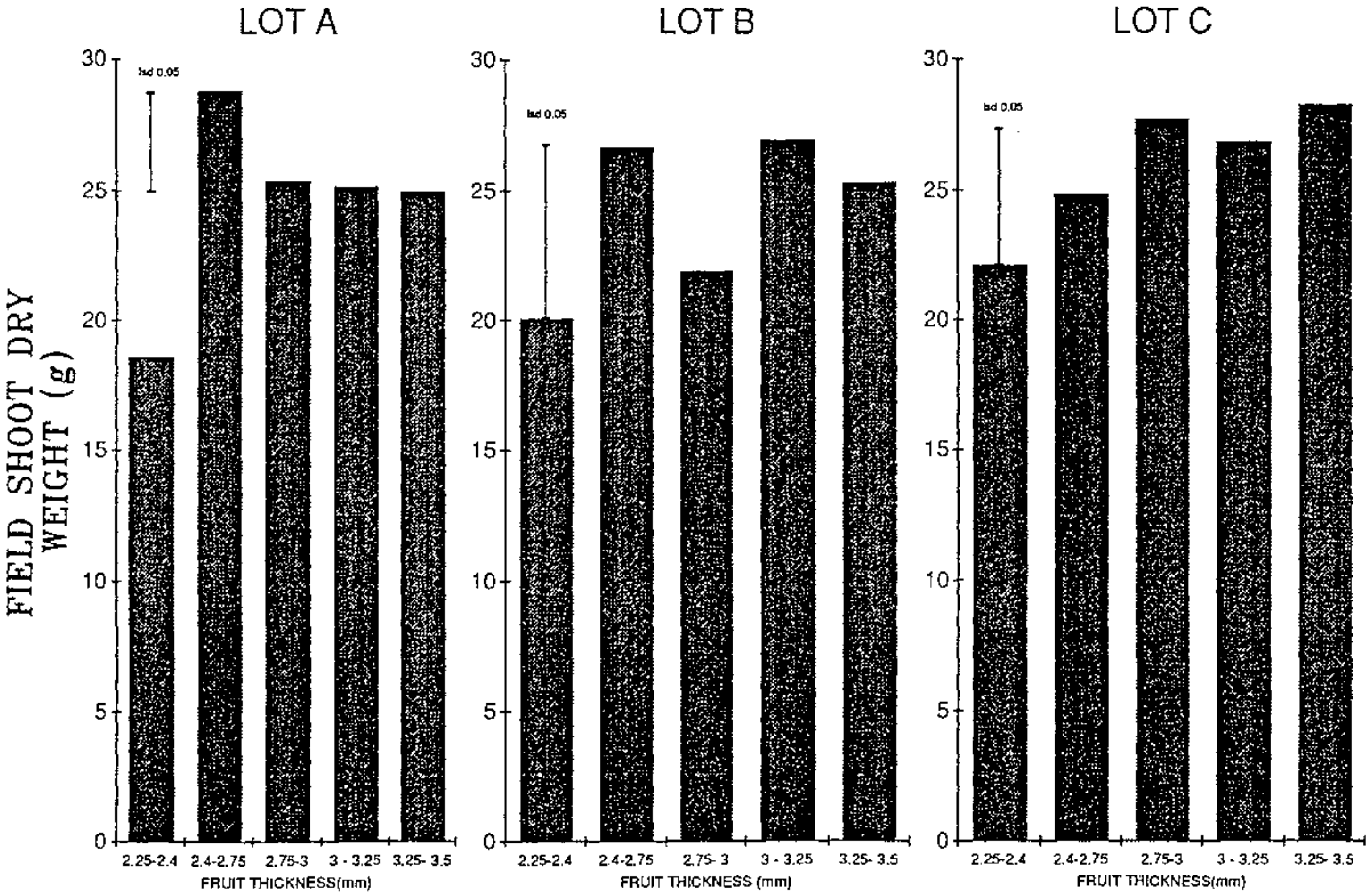


Figure 4.18 Shoot dry weights of seedlings 45 d after field sowing and fruit thickness relationships of the lots. Data presented are the means of 6 replications of shoot dry weight of LOT A and B and 5 replications of LOT C

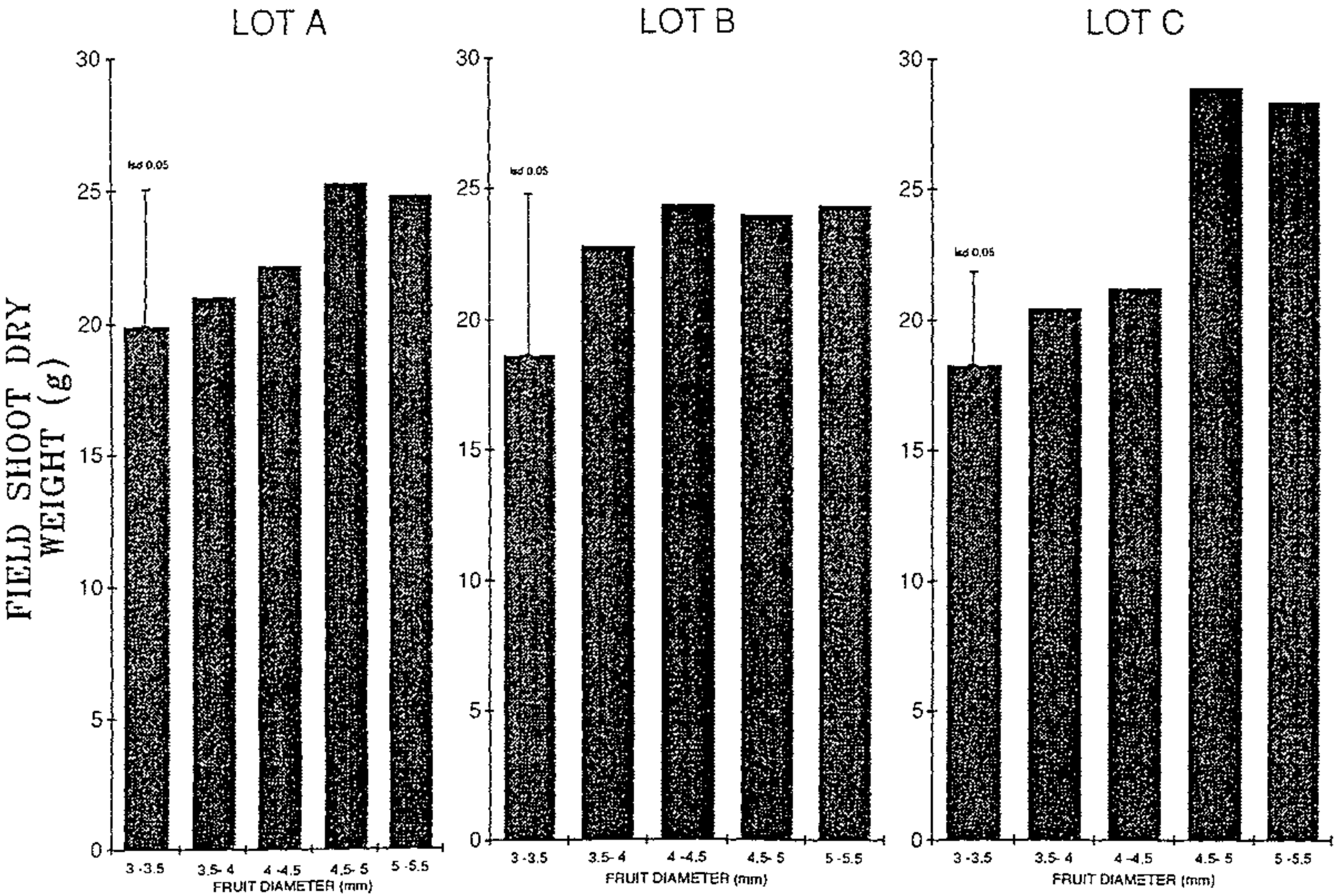


Figure 4.19 Shoot dry weights of seedlings 45 d after field sowing and fruit diameter relationships of the lots. Data presented are the means of 6 replications of shoot dry weight of Lot A and B and 5 replications of LOT C.

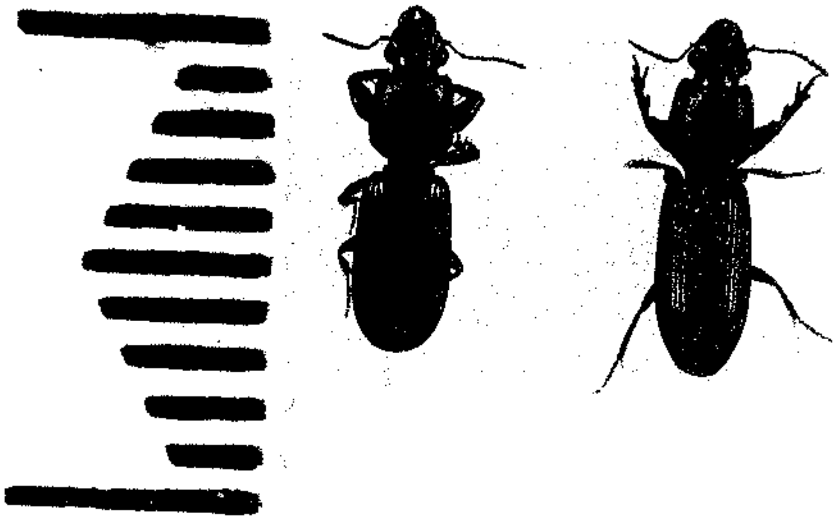


Plate 4.1 Maize Seedling Beetle (*Clivina-rugithrox*) which caused some plant establishment losses through cutting shoot of growing seedlings.

4.2 DISCUSSION

4.2.1 Fruit size and fruit weight relationship

Diameter grading is traditionally used for selecting commercially valuable sugar beet fruit. However, evidence in the literature (see chapter 2 and section 4.2.2 below) indicate that due to the variations in fruit and true seed weight relationships, this method may not always be capable of separating fruits according to true seed weight and thus improve potential germination performance. As there are no reports in the available literature about fruit thickness and true seed weight relationships, it was hypothesized that grading by thickness might provide a better correlation with true seed weight. Thus, in this present study, the relative efficiency of both diameter and thickness grading on seed performance were compared on three lots of different quality of the cultivar 9597 which was released in Iran (1985) and continues to be produced by the Sugar Beet Seed Institute of Iran (S.B.S.I.).

A positive relationship was found between fruit diameter and fruit weight in all three lots of the (Figure 4.1). These results agree with Hogaboam and Snyder (1963), Snyder (1963), Wood *et al.* (1974), Wood *et al.* (1977) and Akeson, (1981) and show that, size grading fruits using round hole screens successfully categorizes the fruits according to their weight. However, grading fruits using slotted hole screens (thickness grading) indicated that there is no precise relationship between thickness and weight of fruits despite the fact that the thinnest fruit of the lots were the lightest and the thickest fruits the heaviest (Figure 4.2).

4.2.2 Relationships between fruit size and fruit fractions

4.2.2.1 *Fruit size, pericarp and true seed size relationships*

In agreement with the results reported by Hogaboam and Snyder (1963), Snyder and Filban (1969), a positive relationship was found between fruit diameter and true seed weight (Figure 4.4), although the relative proportion of the seed to the whole fruit reduced as fruit diameter increased.

Suggesting that the relationship between fruit and true seed weight varied in different seed lots, both linear and non-linear relationship between fruit and true seed weight were found by Scott *et al.* (1974). They showed that the non-linear relationship formed a convex shaped curve where the fruit weight reached 20mg and true seed weight reached 3.2mg at the peak. In the literature, variation in the relationship between fruit and seed weight is attributed to environmental effects on true seed and pericarp growth (Wood *et al.*, 1980). These authors showed that low temperature resulted in greater yields of larger fruits with proportionally heavier pericarps and smaller true seeds. Clearly then diameter grading will not always be capable of categorizing large fruit based on their true seed size and seed quality.

No clear relationship was found between fruit thickness and true seed weight (Figure 4.6). This was supported by other findings in this study (see chapter 5) which shown that, fruit thickness in sugar beet is mainly related to the size of the pericarp base which is the vascular connection between the floral part and the pedicle. Accordingly, thickness grading cannot be reliably used to separate fruits according to their seed weight.

4.2.2.2 *Fruit size and seed cap relationships*

In sugar beet the seed cap is considered to be the most important physical barrier to radicle emergence (see section 2.5.2). In the literature, a heavier seed cap is suggested to be associated with low germinablity in monogerm sugar beet seed (Grimwade *et al.*, 1987). These authors suggested that because small fruit contain small true seed, the force provided by seed expansion during imbibition, is not sufficient to remove the cap therefore, poor planting value of small fruits may related to the inhibitory function of the cap in small fruits. On the other hand, other evidence in the literature indicates that despite the fact that large fruits also contain a large true seed, germination of larger fruit may be lower than that of the medium size grades (see section 2.3.2). It seems likely, therefore, that cap tightness in the pericarp might be more critical than cap weight. However there appears to be no available information in the literature about the relationship between fruit size (either diameter or thickness) and cap weight.

In this present study, measurement of the cap weight of diameter graded fruit indicated that the absolute weight of the cap increases with greater fruit diameter (Figure 4.5). However, the relative proportion of the cap to the whole fruit diminishes with increasing fruit diameter (Figure 4.3). No consistent relationship was found between fruit thickness and seed cap weight in thickness graded fruit of the lots.

4.2.3 The relationships between fruit size, germination and plant establishment of monogerm sugar beet seed

In some crops, eg carrot, there is general agreement on the possible relationship between seed size and seed performance. This strongly supports that differences in seed size or seed weight are likely to be associated with seed vigour. In other crops, eg Glycine max , however, no systematic relationship has been found between seed size and seed germination performance. In sugar beet there are contradictions in the literature about these relationships (see chapter 2 for details).

In general, Lot C was the best germinating lot in all size grades, while Lot A showed medium and Lot B (the oldest lot) showed poorer germination percentages (Figure 4.7). Despite a close positive relationship between fruit diameter, germination and plant establishment in the poor quality lot (Lot B) Figures 4.8 and 4.14 show the nature of this relationship in the high (Lot C) and medium quality lot (Lot A) was non-linear reaching a plateau of performance (Figures 4.9 and 4.15). It seems likely that increased fruit diameter in the high and medium quality lots with associated large true seed size may also result in increased inhibition by the fruit pericarp.

Owing to the indeterminate flowering habit of sugar beet, the presence of prolonged favourable conditions during flowering and seed development, result in the production of high quality lots with low variation in emergence of the different fruit sizes. Usually in high quality seed lots, the medium fruit sizes have sufficient time to reach maximum germinability and hence, no apparent relationship exist between fruit diameter and seed performance. Whereas, when flowering and seed development occur in less than favourable climatic conditions, this may increase both variation in fruit size and seed quality. Furthermore, adverse conditions late in development in even a favourable season, may result in seed weathering which will again cause increased variation in germination percentage and vigour. In addition, seed deterioration and aging during storage may cause more variation in the lot.

As was expected from the lack of correspondence between fruit thickness and true seed weight, no clear relationship were found between fruit thickness and germination or plant establishment of thickness graded fruit of the lots (Figures 4.10, and 4.15).

4.2.4 Fruit size and the speed of radicle emergence

Although delayed seedling emergence from sugar beet seeds with large fruit diameter has been reported by Hogaboam and Snyder (1963), Scott *et al.* (1974) subsequently shown that emergence of sugar beet seedling is more rapid in larger fruits than in smaller fruits. However, the present study failed to show any obvious relationship between fruit diameter and speed of seedling emergence. Nevertheless, largest fruits (5-5.5 mm) did show significantly slower radicle emergence than the other size grades (Table 4.1b). This effect might be related to differences in relative amount of the germination inhibitors present in the fruit pericarp (see section 2.4.1 and 2.4.2).

Hogaboam and Snyder (1963), Battle and Whittington (1969) and Wood *et al.* (1980), have all indicated that fruits which had developed under higher temperatures in the field subsequently showed better germination than those which had developed under cooler temperatures. Wood *et al.* (1980) have shown that cooler temperatures (12oC) were more suitable for pericarp growth whereas higher temperatures (20^{O}C) favoured more rapid growth of true seed. As a result the ratio of true seed size to pericarp size is higher in those developed in cooler temperatures. This suggests that, due to the physical and chemical inhibitory functions of the pericarp on seedling emergence (see sections 2.4.1 and 2.4.2), environmental conditions during seed development may be a critical factor affecting speed of radice emergence.

The development of a large true seed within a thin pericarp produced as result of development under suitable temperatures is quite capable of providing the force necessary for cap movement (see section 2.5.2) whereas, thick pericarps usually inhibit seedling emergence.

Although no clear relationship was found between all fruit thickness grades and speed of radicle emergence in this study, thinnest fruits showed the most rapid, and thickest fruit showed the slowest radicle emergence (Table 4.1a). These results again suggest that greater germination inhibition is associated with thicker pericarps (see section 5.2.2). Surprisingly, both positive and negative correlations have been shown to occur between fruit thickness and speed of seedling emergence in seeds produced on different plants of one cultivar (Hogaboam and Snyder, 1963). It should be noted however that compared with thickness grades used in this present study (2.25-3.5 mm), these authors used relatively thin fruits (2.25-2.75 mm). As a result they may not have evaluated the actual inhibitory effect of the thickest fruits on seedling emergence.

4.2.5 The relationships between fruit size and seedling dry weight

Large seeds containing a larger embryo and greater food reserves, produce seedlings under optimal conditions which are usually heavier with larger cotyledons and first leaves than seedlings produced from smaller seeds (Wood *et al.*, 1977). However, when fruits are sown in the field, despite the growth advantage of the seed with large food reserves, the capacity of the seed to producing vigorous seedlings under stress conditions is also a critical factor for affecting yield. It has been suggested that due to the greater photosynthetic activity of the larger cotyledons and first leaves during early seedling growth, larger fruits of sugar beet are more capable of producing vigorous seedlings than small fruits under field conditions (Scott *et al.* 1974).

In this study a positive correlation was found between true seed weight and seedling dry weight of the lots when seedling were grown in the laboratory on pleated paper for 14 d. As expected, owing to the positive relationship between fruit diameter and true seed weight (Figure 4.4), fruit diameter was also correlated with seedling dry weight under these conditions (Figure 4.12).

Under field conditions, however, this relationship between fruit diameter and seedling shoot dry weight was less consistent. Nevertheless, large fruit of Lot C (4.5-5.5mm) did produce more vigorous plants than plants from medium and small fruits. Medium and small fruits, however, produced similar seedling shoot dry weights. Such a result was surprising, since medium size fruits were expected to perform better in the field than small fruits. These results may reflect differences in vigour within each size grade. Also it is possible that any initial differences between treatments were gradually compensated up to the harvest time. However, no evidences are available to be used for offering suggestion about the lake of difference in seedling shoot dry weight of the poorer quality seed lot (B, Figure 4.19).

Despite there being a significant difference in the seedling shoot dry weights of thickness graded fruits of the high (Lot C) and the medium quality lots (Lot A), no consistent relationship was found between fruit thickness and seedling shoot dry weights of these lots.

4.2.6 Post emergence limiting factors for sugar beet establishment

The results in this experiment in which the emerged seedlings were labelled as far as possible after emergence confirmed that, in spite of treating the fruits with fungicide and also despite applications of post emergence fungicide and insecticides, 3-4% of the emerged seedlings were subsequently lost because of insect and fungal damage (see section 4.1.9). It is important to note that, with an exception in the second replicate of Lot C, in which most of the emerged seedlings showed retarded growth, assumed to be a result of residual effects of the plant growth regulator (Paclobutrazol or PP 333), used in a previous season's experiments, no abnormal seedlings were observed amongst the emerged seedlings by 45 d after emergence. This was despite the fact that about 5% of fruits in the optimal conditions produced abnormal seedlings with tightly twisted shoot, loop shape shoot or spiral root. This suggests that any seedling abnormality which did occur resulted in non-emergence and that if a seedling was capable of emergence, it developed normally.

4.2.7 Seed germination and plant establishment relationships

Methods for assessing seed germination are well established and standardized (ISTA, 1985), but many seeds that pass the laboratory test for germination do not perform well in field (Escasinas, 1986). This might be attributed to seed ageing (Ellis and Roberts, 1980) and low seed vigour (Perry, 1982 and Hampton, 1990). Potentially, any seed may be of high vigour within the limitation of its genotype, but loss of these potential may begin to occur at any point of development from fertilization onwards (Coolbear and Hill, 1987).

The results in this study (Figure 4.17) indicate there was a close relationship between seed germination and plant establishment in the poor (B) and medium quality (A) lots (r= 0.91 and 0.99 for Lots A and Lot B respectively). However, no such relationship was found between germination and plant establishment of the high quality lot (Lot C).

These different relationships within the lots is more likely related to the variation in germination performance of the size grades. In the poor quality lot (Figure 4.7), this is particularly apparent with a variation in germination percentage of the size grades ranging from 7% to 74%. In the high quality lot, with the exception of the smallest fruit, the germination of the size grades ranged from 85% to 92.5% (LSD 0.05= 6.9, Figure 4.7). From this it might be concluded that, although in poor and medium quality lots the germination percentage of the fruit can be used as an index of fruit field performance, in high quality lots, more emphasis should be placed on the vigour of the seed. This suggestion is supported by work by Akeson (1981) who found there was no correlation between the percentage of the fruit germinated by the standard laboratory method (A.O.S.A. Rule for germination) and the performance of the fruits under field conditions. However, the results from a laboratory packed sand emergence test (as a stress test) were highly correlated with field emergence.

CHAPTER 5

THE PHYSIOLOGICAL BASIS FOR LOW GERMINATION CAPACITY IN MONOGERM SUGAR BEET SEED

5.1 INTRODUCTION

Grimwade *et al.* (1987) suggested that the presence of immature and under-developed seed is the main reason for low germinablity in monogerm sugar beet seed lots, while, other workers have proposed that this problem may be due to the physical constraint of tight seed caps preventing radicle emergence (Morris *et al.*,1985; Durrant, 1990). On the other hand, Lexander (1980), showed that chemical inhibitors in the fruits delay or inhibit radicle emergence. In addition, this last author has mentioned that restricted oxygen uptake during the germination phase might be one of the other possible causes of low germinability in monogerm seed (see section 2.5.2 for more information).

Despite, a lot of research effort in this area, no general agreement exists about the actual effects and relative importance of these factors on seed germination. The objective of the six experiments discussed in this chapter was to assess the physiological basis of low germinability in monogerm sugar beet seed, and by comparative studies on size graded fruits, attempt to define which factors might be the major cause of low germinability.

5.2 RESULTS

5.2.1 Relation between stage of maturity as determined by x-radiography and germination percentage in size graded fruits

A highly significant negative correlation (r= -0.88**) was found between fruit diameter and the percentage of empty fruits (Fig.5.1). Nevertheless, no correlation was found between fruit diameter and under-developed seed. However, as shown in Fig.5.2, the smallest fruits contained the highest and the largest the lowest percentage of under-developed seeds (33.4 vs 3.5%). Overall, the relationship between fruit diameter and the proportion of fully mature seed showed that the percentage of fully mature seed significantly increased in fruits of the first three size grades, while no significant differences were found among fruits of more than 4.5 mm in diameter.

A very highly significant correlation (r= 0.97***) was found between the percentage of fruit which were completely filled (fully mature seed) and germination percentage (Fig.5.3). In addition, as shown in Fig.5.4, the percentage of abnormal seedlings increased with increasing fruit diameter.

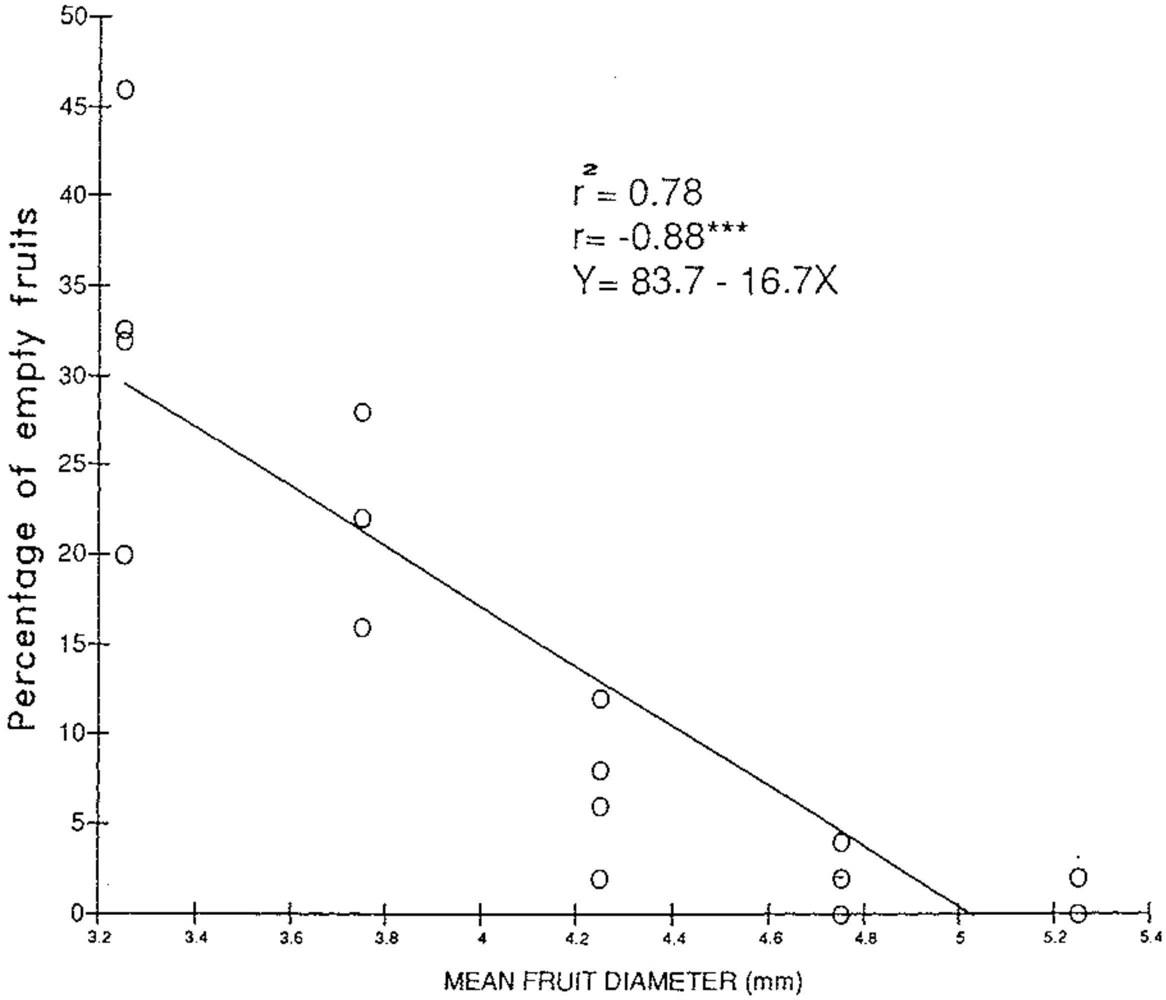


Figure 5.1 The correlation between fruit diameter and the percentage of empty or seedless fruits as determined by X-radiography on four replications of 50 fruits from each grade of Lot A.

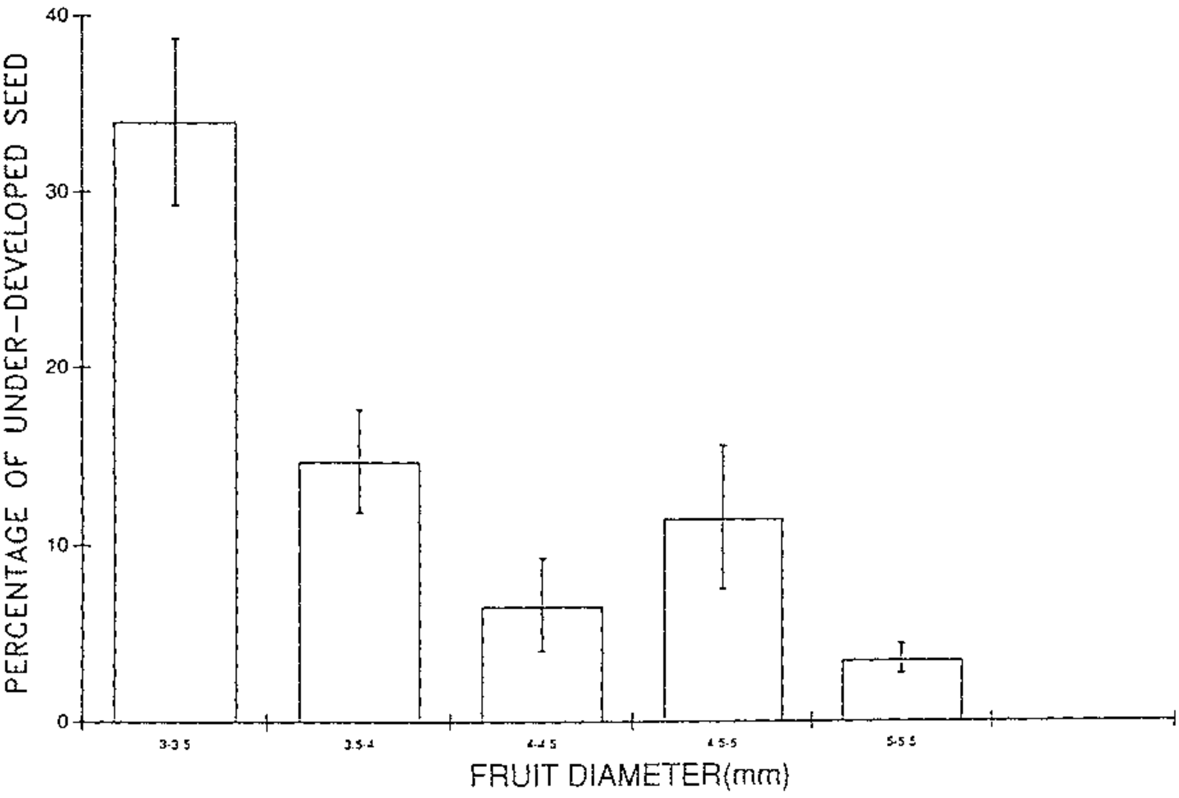


Figure 5.2 The relationship between fruit diameter and percentage of under-developed seed as determined by X-radiography. Each data point is the mean of four replications of 50 fruits of Lot A and vertical bars represent standard errors of the means for each grade.

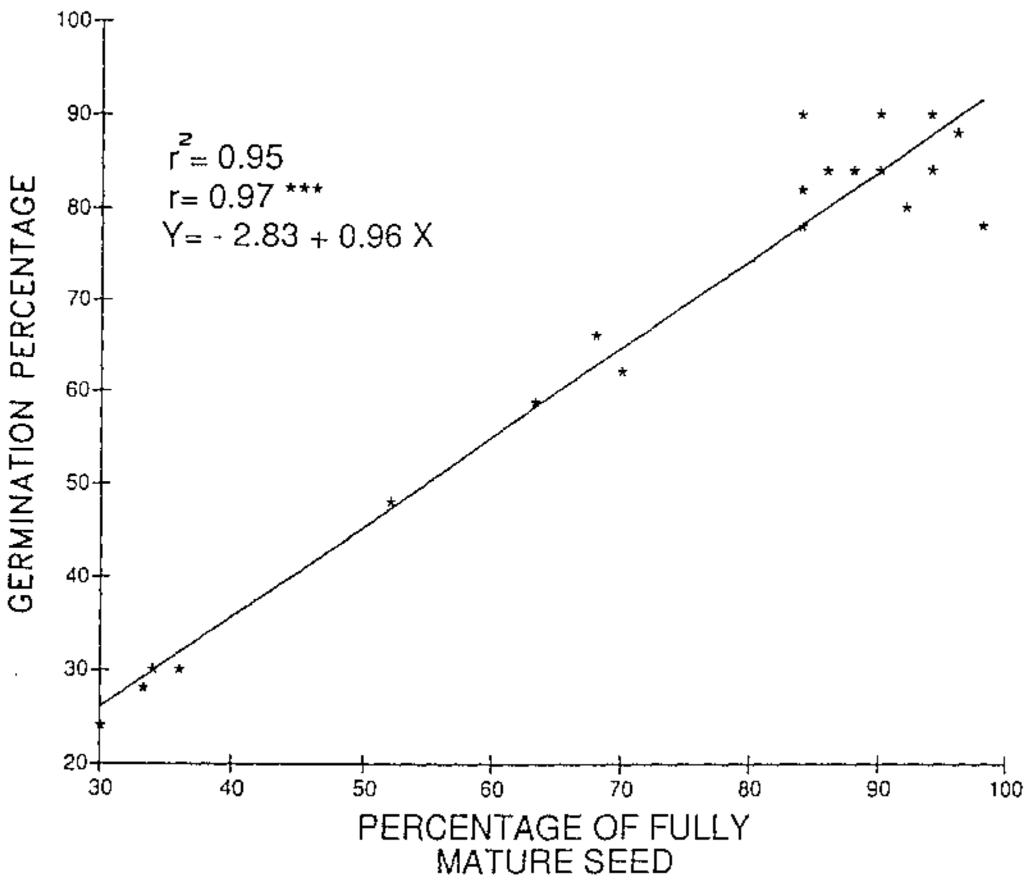


Figure 5.3 The correlation between fully mature seed, as determined by X-radiography, and percentage of germination. Each data point is the mean value of germination percentage of three replications of 50 fruits of each grade of Lot A germinated on pleated paper at 20°C.

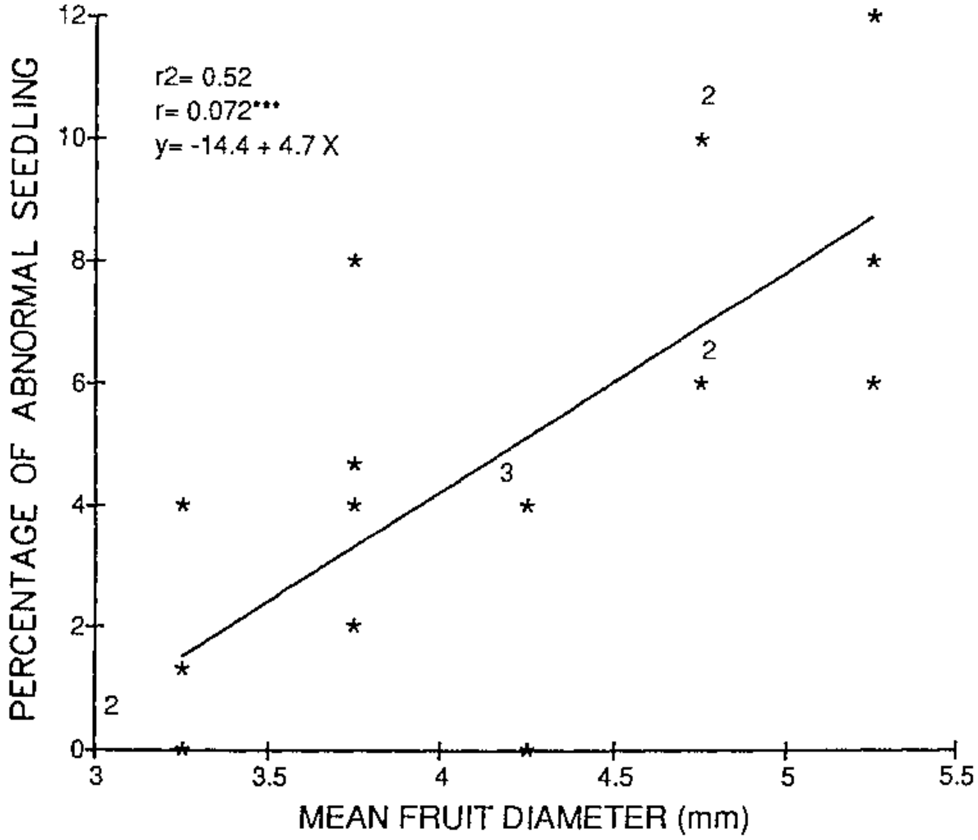


Figure 5.4 The correlation between fruit diameter and percentage of abnormal seedlings in LotA. Data points are the results of four replications of each grade.

5.2.2. Assessment of the effect of inhibitors in the fruits on seed germination

5.2.2.1 *Germination performance of prewashed and unwashed fruit on pleated paper*

As shown in Table 5.1, no significant differences were found between the germination percentages of prewashed and unwashed fruits of ungraded samples of each of the lots. Despite no significant improvements being obtained from prewashing different grades of the poor and the medium quality lots (LOT B and LOT A), a highly significant increase was found between the germination percentages of washed and unwashed fruits for the first three thickness grades of Lot C (Table 5.2). However, no significant differences were found between the germination percentages of washed and unwashed diameter grades of this lot (data not shown).

Table5.1 Comparison between germination percentage of prewashed (4 hours in running water, P.W.F.) and the control (Unwashed, U.W.F.) of an ungraded sample of each lot. Data are means of 4 replications incubated on pleated paper at 20^{o}C.

	LOT A	LOT B	LOTC
P.W.F.	72	47	86
U.W.F.	64	45	81
Lsd 0.05	**16.9**	**20.6**	**7**

Table 5.2 Comparison between germination percentage of prewashed (4 hours in running water, P.W.F.) and the control (Unwashed, U.W.F.) thickness graded fruits of lot C. Data are means of 4 replications incubated on pleated paper at 20^{o}C.

	FRUIT THICKNESS (mm)				
	2.25-2.4	2.4-2.75	2.75-3.0	3.0-3.5	3.25-3.5
P.W.F	86	94	90	82	85
U.W.F	75	77	80	82	84
	Lsd 0.05 = 6.73				

5.2.2.2 *Germination performance of prewashed and unwashed fruit in petri-dishes*

When germination percentages of diameter and thickness graded fruits of the medium lot (LOT A) in petri-dishes were studied, highly significant improvements were obtained via prewashing the fruits (from 17 % to 50 % for diameter and 20 % to 57 % for thickness graded fruits). However despite the low germinability in petri-dishes, unwashed fruits showed much faster medium radicle emergence than prewashed fruits (T50= 71 h vs 141h, $p < 0.001$).

It is important to note that, in all cases tetrazolium testing (ISTA 1985) of fruits which remained ungerminated after the test showed that the seeds were deteriorated and no viable seeds were found among them.

5.2.3 Measurement of the force required for seed cap movement.

In general, when empty drilled fruits of Lot A were incubated for different periods of time on the standard germination substrate (pleated paper, see section 3.2.3 for more information), much more applied weight was required to remove seed caps from the biggest fruits (5-5.5mm) than from the smallest fruits (3-3.5mm, overall means of 3.88± 0.1 vs 2.61± 0.11 Newtons or 0.4± 0.09 vs 0.27± 0.011 kg). In addition, as shown in Figure 5.5, incubating seeds on the germination substrate for different periods of time, did not significantly alter the mean applied force subsequently required to remove caps from the empty fruits.

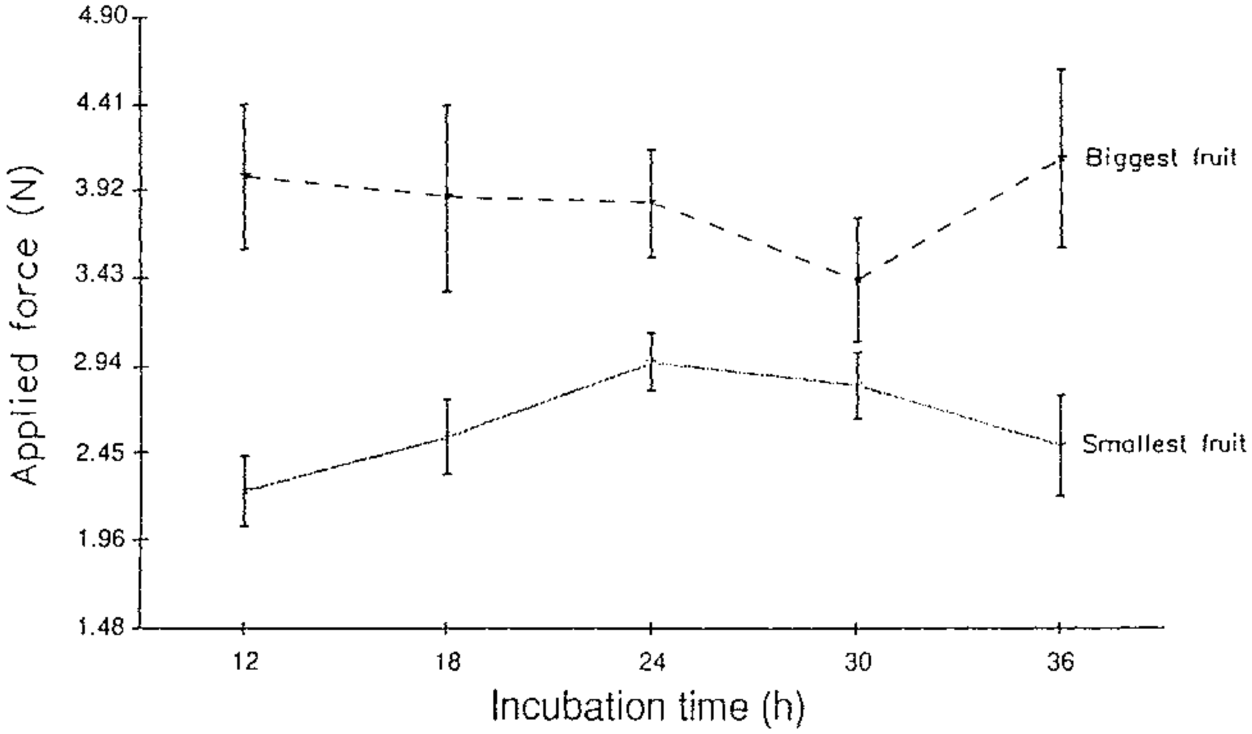


Figure 5.5 The weight (Newtons) applied to remove the seed cap from the coat of biggest (5-5.5mm) and smallest (3-3.5mm) fruit of Lot A. Each data point is the mean of 8 replications and the vertical bars represent individual standard errors.

5.2.4 To investigate physiological functions of fruit pericarp bases on germination of sugar beet seed

As discussed in section 4.1.5, the thinnest fruits showed faster radicle emergence than the thickest fruit. When the physical structures of the thickness grades were compared, it was found that the thicker the fruit the greater the pericarp bases (plate 5.1). It was thus hypothesized that the thickness of the pericarp base might have an effect on germination performance of the seeds. Therefore, as described in section 3.2.4.1, most of the pericarp bases were removed from washed and unwashed fruits of the thickest grade and in a comparison with the control (washed and unwashed intact fruits), percentage and speed of radicle emergence were measured. The results are summarized in Table 5.3.

Data cited in Table 5.3 indicate that removing most of the pericarp base significantly increased germination percentage and improved uniformity and median radicle emergence time of the fruit. An improvement was also obtained via prewashing the fruit. In general, these results clearly showed the presence of a synergistic effect between the washing process and removing the pericarp bases on germination and, in particular, median radicle emergence time of the thick fruits.

To define whether the inhibitory function of pericarp bases on germination performance of the thick fruits was via limiting water or oxygen uptake, two complimentary experiments were carried out, the results of which are summarized in the following sections:

5.2.4.1 *Comparison between water uptake capacity of the thinnest and thickest grades up to 36 hours after incubation in petri-dishes*

As shown in Fig.5.6, there was no significant difference in the water uptake of the two thickness grades beyond 18h of incubation which means that the limiting factor in the thickest fruit was not due to limitation in water uptake.

5.2.4.2 *Effect of fruit orientation on the media on germination performance of the thinnest and thickest fruits*

As discussed in section 2.5.2 , Heydecker *et al.,* (1971) were of the opinion that the main entrance route of oxygen to the embryo is between the edge of the cap and the rim of the seed cavity whereas Perry and Harrison (1974) regarded the pore which runs through into the fruit pericarp bases as the main entrance path for oxygen. Owing to the interaction between oxygen and water available in the substrate, it was suggested that orientation of the fruit either with the cap or the pericarp base on the substrate, might help to clarify which of these proposed routes is important for oxygen uptake.

As shown in Table 5.4, uniformity and median radicle emergence time of both grades significantly improved when the fruits were oriented so that their seed caps were on the media. In addition, the effect of pericarp position was greater for the thicker fruits.

Scanning Electron Microscopic and Energy Dispersal X-ray of the fruit showed that, the pericarp base was comprised of many pores filled with irregularly shaped, loosely packed, dead cells (Plate 5.2), and also a layer of lignified cells containing crystals of calcium oxalate (Plate 5.3)

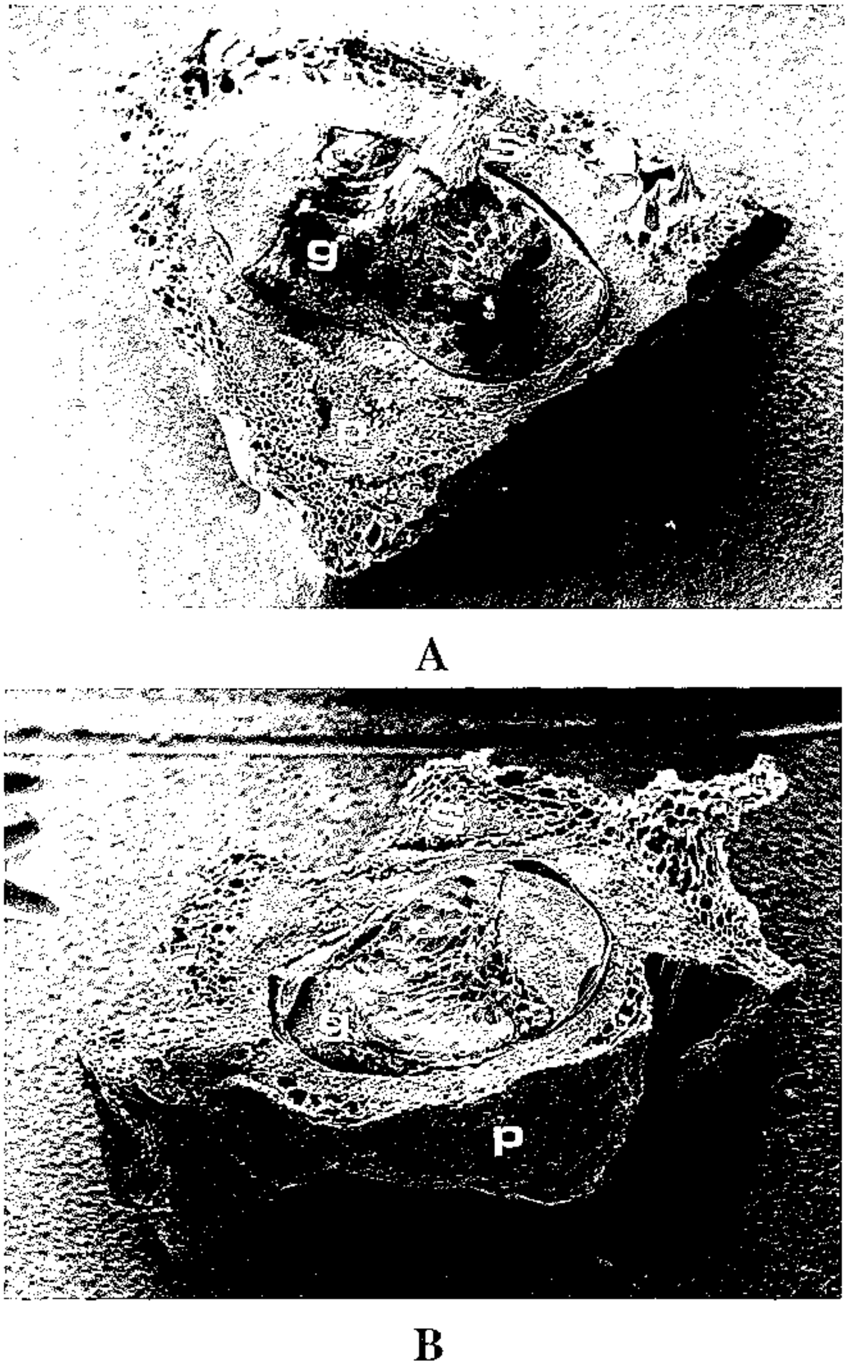


Plate 5.1 Scanning electron micrographs (X 25) of the thickest (3.25-3.5mm, A) and thinnest (2.25-2.4, B) fruits of Lot A. In this Plate the pericarp base (P), true seed or germplasm (g) and the seed coat (S) are shown.

Table 5.3 Germination percentage, uniformity and median radicle emergence times (T50) of washed and unwashed thickest graded fruit with or without the pericarp base removed. Data presented are the mean of 3 replications which were incubated in petri-dishes at 15^{O}C.

TREATMENTS	T50 (h)	UNIFORMITY (H)	GERMINATION (%)
A Prewashed pericarp removed	125	333	87
B Unwashed pericarp removed	169	367	70.6
C Prewashed intact fruits	247	416	69
D Unwashed intact fruits	338	525	42
lsd 0.05	95.8	181.3	9.9

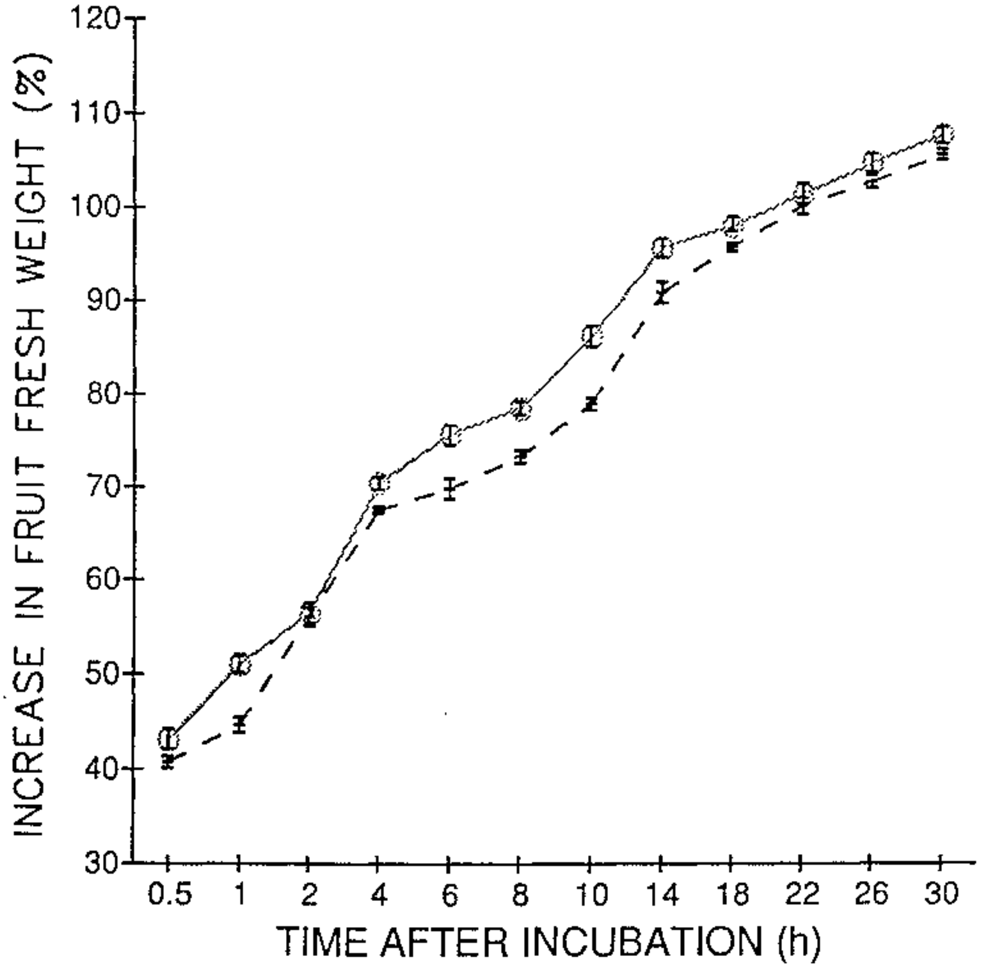


Figure 5.6 Increase in the fresh weight of the thinnest (-) and thickest fruit (O) of Lot A after different incubation times at 15°C. Each data point is the mean of 3 replications and the vertical bars represent individual standard errors.

Table 5.4 Comparison between the germination performance (median radicle emergence time, T50 or uniformity, T90-T10) of fruits of the thinnest and thickest grades of LOT A as affected by the orientation of the fruits on the germination medium. Fruits were placed so that either the pericarp bases were in contact with the medium or they were upside down. Data are means of three replications and standard errors are shown in brackets.

	T50 (h)		UNIFORMITY (h)	
	Thinnest	Thickest	Thinnest	Thickest
Pericarp base down	142 (±19.5)	192 (±27)	179 (±11)	250 (±9.7)
Pericarp base up	93 (±1.3)	107 (±5.8)	144 (±6.2)	178 (±7.4)

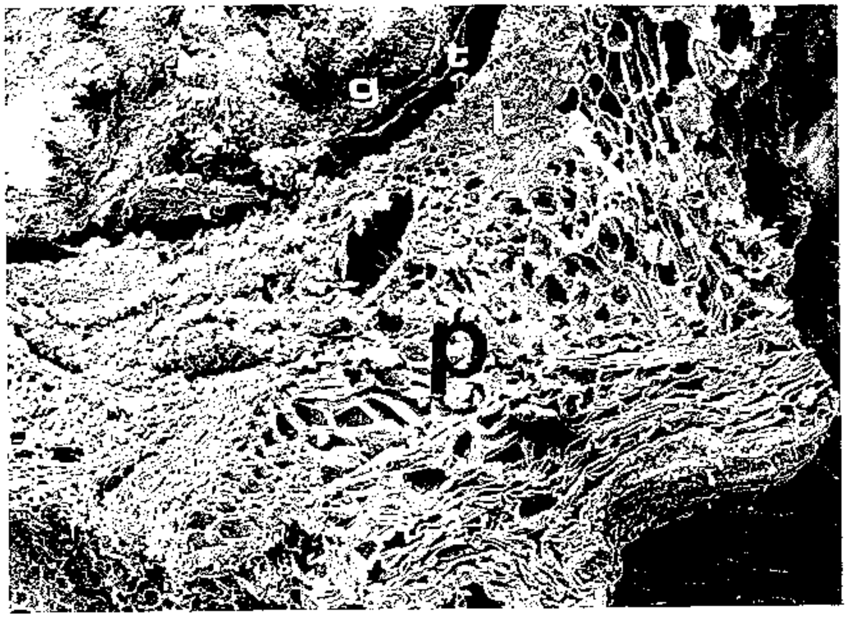
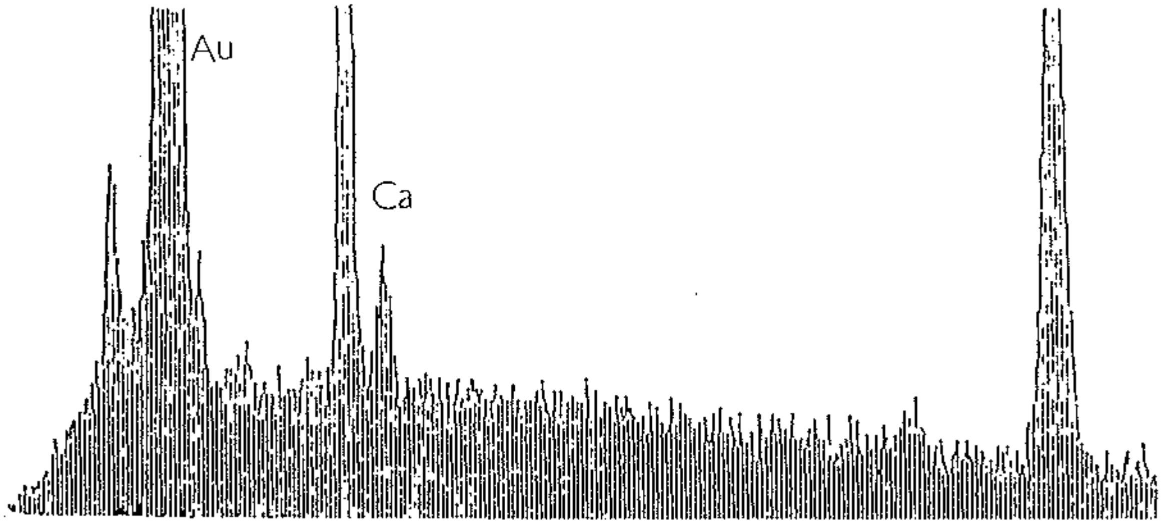


Plate 5.2 Scanning electron micrographs (X 53.75) of the pericarp base (P) of the thickest fruit of Lot A (3.25-3.5mm) comprised of many pores filled with irregularly shaped loosely packed cells. In this picture, a layer of lignified cells (L), testa (t), and germplasm (g) are shown .

A



B

Plate 5.3 Scanning electron micrographs (X 537.5) of a layer of lignified cells in the pericarp base (A) of the thickest fruit (3.25-3.5) LotA (A). Calcium (Ca) was the only metal element identified by Energy Dispersal X-ray Spectroscopy, apart from gold (Au) which sprayed onto the specimen to obtain contrast (B).

5.3 DISCUSSION

(GERMINATION LIMITING FACTORS IN SUGAR BEET)

5.3.1 Seed maturity stage, seed size and germination relationships

Sugar beet is a cross pollinating crop with an indeterminate flowering habit which is prolonged for about six weeks. Thus, a seed crop at harvest comprises a wide range of seed sizes and seed maturities. Grimwade *et al.* (1987) found that seeds younger than 31d did not fill the seed cavity. Nevertheless, when these seeds were excised and incubated, a higher number of them germinated than would have emerged from the whole fruits. From this they concluded that the germination failure of the seed within the intact immature fruits was because the true seed was too small to provide the force via expansion required for seed cap movement and radicle emergence (Grimwade *et al.* 1987).

Results obtained in this study showed that the smallest fruits contained the highest, whereas the largest fruits contained the lowest number of under-developed seeds (33.4% vs 3.5%, Figure 5.2), perhaps because the fruit produced at the end of the flowering period had insufficient time to completely mature. In addition, a non-linear relationship was found between fruit diameter and fully mature seed (the cavity entirely filled by the true seed) in the medium quality seed lot. The percentage of fully mature seed increased as the fruits increased from 3mm to 4.5 mm diameter. However no significant differences were found among fruits of more than 4.5mm diameter. This suggests that low germinability of large fruits of sugar beet reported in the literature (see chapter 2) may not be related to the size of true seed within the fruit.

In general, although seedless fruits and under-developed seeds were mainly associated with the small (3-4mm diameter) fruit (eg. Lot A Figures 5.1-5.2), there were still a proportion of fruit in the medium (4-5mm) and large fruit size (5-5.5mm) which contained under-developed and immature seeds. Two questions therefore arise: first whether diameter grading can be used as a method for discarding these fruits, and secondly is it therefore an economically practical process for increasing seed lot germinabilty?. In an attempt to answer these questions, data from seed Lot A are summarized in Table 5.3.1.

Table 5.3.1 The proportion by weight of each of the diameter class of Lot A within the harvested fruit of this lot and the percentage of immature fruits within each of the size grades.

	FRUIT DIAMETER (mm)				
	3-3.5	3.5-4	4-4.5	4.5-5	5-5.5
Proportion of each diameter class within the harvested fruit	1.6%	7.6%	13.9%	18.5%	7.4%
% of immature fruits within each class	64%	36%	20%	16%	3%

As shown in Table 5.3.2, although immaturity is mainly associated with the smaller fruit (3-4mm), these fruits are relatively a small proportion of the size grades. Whereas, about 18% of the lareger fruits (4-5mm) of the lot also contain immature seed which could not be separated from the mature fruits via diameter grading. However before any firm conclusions can be drawn, other lots would need to be examined, because the lot (A) used may not be typical of all lots.

Radiographic assessment of the diameter graded fruits of the medium quality lot used in this study (Lot A, Figure 5.1) indicated the smaller the fruit the higher the percentage of seedless fruits, and Durrant and Loads (1990) reported that the size of true seed was the most promising indicator of germination performance under optimal conditions. X-radiography may therefore be useful as a quick method for assessing germination potential of sugar beet, as a highly a highly significant and positive relationship was found between the percentage of the fruit filled with true seed (fully mature seed) and germination percentage of the fruits of Lot A (r= 0.97**, Figure 5.3). However, as only one lot was used in the experiment, trialling the technique with different seed lots will be necessary to allow any conclusions to be drawn about suitability of X-radiography as a quick method of viability testing.

5.3.2 *The inhibitory function of the fruit pericarp on seed germination*

Low germination often occurs in larger fruit sizes and Grimwade *et al.* (1987) suggested that this was attributed to the heavy thick pericarp. Thus, not only the size of true seed is important in germination performance of the seed, but also the pericarp which might have inhibitory functions, which may include the following factors:

1. Chemical inhibitors of germination (Heydecker and Chetram, 1971; Lexander, 1980; Bewley and Black 1985)

2. Physical inhibition by the seed cap, (Morris *et al.*, 1985)

3. Restriction in O^2 uptake owing to the thick pericarp base (see 5.3.2.3)

5.3.2.1 *Inhibitory effect of water soluble substances in the fruit pericarp on seed germination.*

As it contains various water soluble components, particularly, phenolic and inorganic material (Bewley and Black, 1985), the pericarp of sugar beet fruit may impose dormancy on the seed. Lexander (1980) indicated that consumption of oxygen by phenolic components in the pericarp may restrict oxygen uptake by the embryo. In addition, Heydecker and Chetram (1971) showed that washing the intact fruit increased oxygen uptake. From this they concluded that the chemical substances act as barrier to oxygen entry to the seed cavity.

Germination improvements via prewashing of sugar beet fruit have been reported by some authors (eg. Heydeker and Chetram, 1971; Longden ,1974 and Lexander, 1980). However, as also found in this present study, literature suggest that the response may varies in different seed lots (see section 2.4.2). Although a significant (6%) but very small germination improvement was obtained from prewashing the fruit of the high quality lot (Lot C), no improvement was achieved via prewashing the fruit of the low and medium quality lots (Table 5.2).

Although there were no significant differences between the germination percentage of prewashed and unwashed fruits of the medium quality lot (Lot A) on pleated paper, when germination (percentage of fruits showed radicle protrusion) of prewashed and unwashed fruit of this lot on petri-dishes was compared, the prewashed fruit showed more germination than the unwashed fruits (see section 5.2.2.2). The different response of the size graded fruits of lot A to washing with substrate might be related to better oxygen availability on the pleated paper, both for respiration of the embryo and possibly, for using in enzymatic oxidation of the chemical components in the pericarp (Bewley and Black, 1985). From this result it can be suggested that low field emergence of monogerm sugar beet seed in wet conditions might be attributed to limitations in oxygen availability and likely interactions between oxygen and the inhibitory components present in the fruit.

Although many authors agree that the improvement in germination of the sugar beet fruit via washing is only related to the leaching of the inhibitors (Snyder, 1963; Heydecker and Chetram 1971; Lexander, 1980), improvement in seed germination may also be due to advancing the germination process (Durrant *et al.* 1983). Although these authors did not mention the physiological basis of this improvement, as reported by Villiers (1974) in Lettuce seed, this might be due to the operation of physiological repair during hydration of seeds. Further work is required to define whether this is the case. Further, prewashing the fruits may assist with loosening the seed cap junction.

5.3.2.2 *The inhibitory function of the seed cap on seed germination*

As previously stated, evidence in the literature indicates that the seed cap imposes an inhibitory function on sugar beet seed germination either via impeding oxygen uptake (Coumans *et al.*, 1976) or inhibition of radicle emergence. Morris *et al.* (1985) showed that low germinability of the fruit was associated with heavier seed caps. However, no relevant evidence in the literature has been found for the actual inhibitory function of the cap with different fruit sizes, and whether the weight of the seed cap is associated with cap tightness.

Morris *et al.* (1985) noted that the application of a suitable method for determining the force which is required for cap movement may be very useful for selection of high quality seed lots. These authors are the only workers who have tried to define the force required for cap movement. The method used was an indirect estimate of the force based on the use of the osmotic potential of PEG solutions to lower the germination of the excised seed to that found in the whole fruit germinating on standard substrate. However, as noted by the authors, two major limitations were involved in this indirect method: firstly this hypothesis requires the true seed to fully occupy the cup cavity and secondly when different fruit lots are compared, that the osmotic potentials of the dry seeds are equal between fruit lots.

Using this indirect method, Morris *et al.* (1985) estimated that the force required to remove the seed cap of the low germinating lot was more than that of the high germinating lot (7.5 kg. cm^{-2} vs 5 kg. cm^{-2} resp.). Using a direct method in this present study (see section 3.2.3), the force required for cap movement of the smallest fruit (seed cap area of 0.037 cm^2) and the largest fruit (seed cap area of 0.062 cm^2) were compared and it was found that the force required for cap movement of the smallest fruits was less than that of the larger fruits (Figure 5.5). This result clearly illustrates that the inhibition in germination of heavier fruit as reported in the literature (see section 2.5.2), might be also related to the tightness of the cap of these fruits.

To compare estimates provided by Morris *et al.* (1985) and the results obtained in this present study, the mean cap area of the 20 smallest and largest fruits was assessed, and then the equivalent of the direct applied force per seed cap were calculated. The force required for cap movement of the smallest and largest fruits in this present work was 7kg. cm^2 vs 6.26kg. cm^2 respectively and despite the fact that they did not compare specific fruit sizes, results are clearly similar to Morris *et al.* (1985). These result show firstly, enzymatic activities of the true seed are unlikely to play an important role on loosening the cap junction. However, since dissecting out embryos from the cup cavity is a time consuming process, a better technique for direct force measurement (eg. possibly appling a vacuum to the out side surface of the cap of intact fruit) would be helpful for allowing the selection of progenies with reasonably loose seed caps, and consequently a more rapid seedling emergence.

5.3.2.3 *The inhibitory function of the pericarp base on germination performance of sugar beet seed*

As discussed in section 4.1.5, when germinated on petri-dishes, the thickest fruit showed lower radicle emergence and radicles emerged more slowly than for the thinner fruit. Furthermore, it was shown that the thickest fruit have thicker pericarp bases than the thinner fruits (Plate 5.1). Removing most of the pericarp base of the thickest fruits produced a significant improvement both in germination percentage and speed of radicle emergence of the fruits (Table 5.3). Thus it was hypothesized that the thickness of the pericarp base might restrict water or oxygen uptake during the germination phase (see section 2.5.2).

Comparison between water uptake rate in the thinnest and the thickest fruits showed that there was no significant difference between the two grades after 18 hours of incubation (Figure 5.6). The T50 for the thinnest and thickest fruits was 126 h and 163 h respectively which means that the rate of water uptake can not be assumed to be a limiting factor in germination of the thickest fruit.

Although there are disagreements about the exact route of oxygen uptake, two routes have been suggested to be involved; either between the edge of the cap and the rim of the cavity, or via the pore which runs through the pericarp base (see section 2.5.2). It was thus thought that in the petri-dish where the fruit were oriented so that either the thick pericarp base or the tight seed cap of the fruit was touching the wet substrate, the excess water might have interacted with oxygen uptake. The results indicated that fruits had slower radicle emergence when they were oriented so that their pericarp bases were in contact with the wet media, rather when they were upside down (seed cap in contact with the germination substrate, Table 5.4). As shown in this table, orienting the fruit on the wet substrate via the seed cap rather than the pericarp base resulted in an improvement of 44% in median radicle emergence time and 29% in the uniformity of radicle emergence. Thus the entry of oxygen to the embryo is more likely to run through the pericarp base.

When improvements in uniformity and T50 of the fruits oriented with the cap rather than pericarp base on the median in the thickest and the thinnest fruits were compared, it was observed that improvements in germination performance of the thinnest fruits were less than those of the thicker fruits (44% vs 34% for the T50 and 29% vs 20% for the uniformity). From this it might be concluded that due to the smaller pericarp bases, the thinnest fruits are more capable of emerging rapidly than the thick fruits on a wet substrate. This finding is quite important for providing high vigour seed lots. These might be obtained via either of the following methods:

1. Discarding the thicker fruits via thickness grading which would improve the speed of emergence. The loss of fruits is dependent on seed lot (eg. 2% of fruit of Lot A, 8% in Lot B and 5% of the fruit of Lot C ranged from 3.25 to 3.5 mm of thickness).

2. Selection of plants with production capacity for thin but high vigour fruit with uniform and rapid radicle emergence.

It should be noted that although polishing of the fruits during processing can be used to slightly reduce the size of the pericarp base, severe polishing may result in serious damage to the true seed within the fruits.

In contrast to Perry and Harison (1974) who suggested that there was only one pore in the pericarp base, Scanning Electron Microscopy and Energy Dispersal X-ray of the fruits illustrated that the pericarp base appears to comprise many pores filled with irregularly shaped loosely packed, dead cells (Plate 5.2). There is also a layer of lignified cells containing crystals of calcium oxalate (Plate 5.3). However, it is not clear whether possible rapid water absorption by these deposits might interact with oxygen uptake by the embryo.

In summary, the improvement in germination performance of the thickest fruits obtained via pericap removal (Table 5.3) might be due to either of these factors:

1. reducing the barrier to oxygen movement into the seed cavity

2. removing some of the chemical germination inhibitors, particularly phenolics, which restrict respiration (see section 5.3.2).

5.3.3. The relative importance of the inhibitory factors limiting germination of different fruit sizes of sugar beet

To clarify the relative importance of different inhibitory factors in different fruit sizes in one seed lot, the fruits of Lot A were categorized into three groups, small (3-4mm), medium (4-5mm) and large fruits (5-5.5mm) and using data obtained in this study (Figures 5.1-5.3), percentage of mature fruits and percentage of mature ungerminated fruits are presented in Table 5.3.2.

Table 5.3.2 Percentage of mature fruit and percentage of mature ungerminated fruits in the small (3-4mm), medium (4-5mm) and large fruit size (5-5.5mm) of Lot A

	FRUIT SIZE		
	Small fruits (3 - 4 mm)	Medium fruit (4 - 5 mm)	Largefruits (5-5.5mm)
Percentage of mature fruits in the size grade	48%	87%	96%
Percentage of mature ungerminated fruits	11%	3%	12%

It can be seen form Table 5.3.2 that 52% of the small fruits and 13% of the medium size fruits of Lot A were immature, either having no seed (seeds were aborted) or comprising under-developed seed. Thus, the main cause of low germinability of these size grades is the stage of seed maturity. On the other hand, in the large fruits only 4% of the fruits comprised under-developed and aborted seeds, whereas 12% of the mature fruit did not germinate. This might be due to more chemical inhibitors in the fruits (Grimwade *et al.*, 1987) or their thick pericarp bases which act as barrier to oxygen entry into seed cavity (see section 5.2.3). It is important to note that the possible contribution of non-viable seed in the fruit should also be taken into account. However, in this study, the viability of the seeds was not assessed.

Owing to the interaction between the cap tightness and other modes germination inhibition (Heydecker and Chetram 1971), it is difficult to define the actual inhibition level of the cap. However, the level of germination inhibition of the pericarp base and chemical inhibitor was observed in this present study.

As shown in Table 5.3.3, prewashing of intact fruit which removed the water soluble inhibitors, resulted in a significant 27% increase in germination percentage. Similar germination improvement was also obtained via removing pericarp base from the unwashed fruit. These results show that in thick fruits, the chemical substances and the thick pericarp base have relatively equal inhibitory effect on seed germination.
Both process are likely to increased the availability of oxygen in the seed cavity by removing either chemical or physical barriers to oxygen uptake (see previous discussion and literature review sections 2.4.1 and 2.4.2). Removing the pericarp of

prewashed fruit gave a further increase in germination improvement indicating that there is also an interaction between chemical substances and the pericap base as barriers of oxygen uptake to the seed. A synergistic effect between prewashing and cap removal on oxygen uptake was also reported by Heydecker and Chetram (1971). In is important to note that, not only final germination percentage of the fruit was improved by pericap base removal and prewashing, the rate of germination also improved via this process (Table 5.3). It should be note that, as dada cited in Table 5.3.2 are obtained via incubation of diameter grades of lot A, and those in Table 5.3.3 was achieved via incubation of thickest fruit of the lot, it is not possible to make a direct comparison between these results.

Table 5.3.3 The effect of pericap base removal and prewashing the fruit on germination percentage of thick fruits of the medium quality seed lot (A). Data presented are the mean of 3 replications which were incubated in petri-dishes at 15^{O}C.

	PERICARP BASE REMOVAL	
	--	+
UNWASHED FRUITS	42%	71%
PREWASHED FRUITS	69%	87%

LSD 0.05= 9.9

CHAPTER 6

GENERAL DISCUSSION AND SCOPE OF FURTHER WORK

Despite the fact that the germination percentage (ISTA, 1985) of seed sold to farmers for precision drilling systems normally has to exceed 90%, poor plant establishment is common in most sugar beet production areas of the world. Besides factors such as physical and environmental conditions during germination and seedling emergence, the physiological characteristics of the fruit itself have also been considered as a very important factor involved in poor plant establishment. The broad aims of this study were to assess the effects of size grading by diameter and thickness on germination performance of the seed, and also to investigate factors limiting germination of sugar beet seed. In this concluding chapter the achievements of this work, its limitations and scope for further work are discussed.

6.1 ACHIEVEMENTS OF THE RESEARCH

6.1.1 The relationship between fruit size, germination performance and plant establishment of monogerm sugar beet seed.

In sugar beet not only is the size of true seed critical for radicle emergence and seedling growth (section 2.5.1), but the interaction between seed size and the inhibitory functions of the other parts of fruit is also important. Another aspect is that the proportion of the fruit fractions can change due to variation in environmental conditions during seed development (Wood *et al.*, 1980).

Despite a linear relationship between fruit diameter and germination or plant establishment in the poor quality seed lot (Figure 4.8 and 4.14), the nature of these relationships in the medium and high quality lots was non-linear (Figure 4.9 and 4.15) i.e. the larger fruits showed equal or lower germination than fruits in the medium size grades. It seems likely that increased fruit size in these lots may reflect an increased level of germination inhibitor. This indicates that although it does eliminate the smallest fruits, diameter grading is not an efficient method for categorizing the fruits for germination performance.

Although there was a significant relationship between fruit diameter and germination performance, there was no such relationship between fruit thickness and germination performance, both in laboratory and field conditions (see sections 4.1.3.2 and 4.1.7). This is explained by the fact that a range of fruit diameters occurred within each thickness class.

There is much debate in the literature about the reliability of the standard germination test result as an indicator of plant establishment (see chapter. 2). The results of this study illustrated that this relationship varies among seed lots of the same cultivar. Despite there being a positive correlation between seed germination and plant establishment in the poor and medium quality lots (Figure 4.17), no such relationship was found in the high quality lot (Lot C) used in this study. This is a function of the variation in germination performance of the size grades. In the poor quality lot for instance, there was high variation in germination of the size grades (7% to 74%, Figure 4.7) whereas in the high quality lot, with the exception of the smallest fruit, the germination of the size grades only ranged from 85% to 92.5% (Figure 4.7). This suggests that, although in poor and medium quality lots the germination percentage of the fruit can sometimes be used as an index of field performance, in high quality lots more emphasis should be placed on the vigour of the seed.

In contrast with results reported by Wood *et al.* (1977), no systematic relationship was found between fruit diameter and speed of radicle emergence, although the largest fruits did produce slower radicle emergence than the other size grades. This suggests that variable oxygen uptake or variable physical or chemical inhibitor levels may also play a role in influencing the speed of radicle emergence (see section 5.3.2) and could explain the often contrary results previously published.

Although a close relationship was found between fruit size (thickness and diameter) and seedling dry weight after 14 d in optimum conditions (Figure 4.14), no consistent relationship was found between fruit size and shoot dry weight of established plants in the field. These results may reflect differences in the level of seed vigour within each size grade. Also it is possible that any initial differences between treatments were gradually compensated up to the harvest time.

6.1.2 Germination limiting factors in sugar beet seed

6.1.2.1 *Significance of seed maturity stage and true seed size on seed germination*

The size of the true seed is well known as a critical factor for successful sugar beet germination performance. During the imbibition phase, the seed swells and via this expansion, provides the force which is required for seed cap removal, a prerequisite for radicle emergence. Grimwade *et al.* (1987) showed that although under-developed seeds within fruits did not emerge, they could germinate when they were excised. The germination failure of these immature fruits was because the true seeds were small and therefore not capable of providing enough force to remove the seed cap. Thus, the larger the true seed within the fruit, the greater the germination percentage of the fruit sown.

Results obtained via diameter grading of the harvested fruits of the medium quality lot (A) illustrated that 60% of the fruits were either too small or too large to be used in precision drill systems (see chapter 3). Further as shown in Table 5.3.1, 24% of the graded fruits of suitable commercial size contained immature seed. These results clearly show that firstly: variation in fruit size is an important problem in sugar beet seed production and secondly: immaturity can be an important germination limiting factor in fruits graded to commercial specifications. In addition, even when seed was fully mature (the seed cavity entirely filled with the true seed), some proportions of the fruit in each of the size grades of seed lot (A) used in this study did not germinate (Table 5.3.2). This suggests that some germination inhibitory factors are involved. These are discussed in the following sections.

6.1.2.2 *Germination inhibitory factors in sugar beet fruit*

In the literature the inhibitory function of the seed cap is attributed to cap tightness, which suppresses radicle emergence, and also acts as barrier to oxygen entry into the seed cavity. An association between low germinability in sugar beet seed lots and heavy seed caps has been reported in the literature.

In this study it was found that the larger the fruit the larger the seed cap (chapter 4). Further, it was illustrated that the direct force required for cap removal of the larger fruits was significantly more than that required for the smallest fruits (chapter 5). These results suggest that greater seed cap tightness might be associated with heavier seed caps. It was also found that the force required for seed cap movement of empty drilled fruits was relatively similar to that obtained by Morris *et al.* (1985) via indirect estimates, based on the use of the osmotic potential of PEG solutions to lower the germination of the excised seed to that found in the whole fruit germinating on standard substrate (see 5.3.2.2). This suggests that enzymatic action on cap loosening is unlikely to play an important part in cap removal, and confirms therefore that the force provided via expansion of the true seed during imbibition is likely to be the main limitation to seed cap removal.

Water soluble components in the fruit may delay or inhibit seed germination. Evidence in the literature suggests that by consumption of oxygen, these substances act as a barrier to oxygen entry into the embryo (see section 5.3.2.1). Because they are water soluble, germination improvement might be expected via prewashing of the fruits. However, as reported in the literature and also demonstrated in this study, the response of seed lots to prewashing is variable (see chapter 5). This might be due to the concentration of the material in the fruits, or possibly, the sensitivity of the seed to the inhibitors. It is important to note that despite there being no response to prewashing in Lot A of this study when incubated on pleated paper, when radicle emergence of washed and unwashed fruits of this lot in petri-dishes was compared, a significant germination improvement was obtained via prewashing (see section 5.2.2.2). The influence of substrates on the response to prewashing is probably related to greater oxygen availability in the pleated paper, both for seed respiration and possibly the oxidation of the chemical substances in the pericarp (see section 5.3.2.1). This suggests that due to the interaction between available oxygen and water in the substrate, chemical inhibitors might be more effective in wet conditions. Thus, by improving oxygen availability through removing the inhibitors and possibly advancing the germination following prewashing and drying back (Durrant *et al.* 1983), higher seedling emergence in the field may occur (see section 2.4.2).

Contrary to the suggestion by Heydecker *et al.* (1971), the present study confirms the report by Perry and Harrison (1974) that the seed cap is not involved in oxygen uptake, and it is the pores which run through the pericarp base which provide the path for oxygen transfer to the seed cavity (see section 5.3.2.3). In addition it was found that the thicker the fruit the greater the pericarp base.

Significant germination improvement was obtained by removing the pericarp base of fruits (see section 5.2.4). This is likely to be due to reducing the barrier to oxygen movement into the seed cavity, and also by removing some of the chemical germination inhibitors. Although equal germination improvements of 29% were obtained via removal of the pericarp base or prewashing, a synergistic germination improvement of 45% was obtained via both treatments (see section 5.2.4). This result shows that thin fruits of sugar beet, because of their thin pericarp bases, are more capable of emerging in wet field conditions than thick fruits. These results may be important for selection of progenies with thinner pericarp bases and therefore potentially improved performance.

6.2 LIMITATIONS OF THE STUDY AND SCOPE FOR FURTHER WORK

6.2.1 Limitations of the study

1. Despite the fact that the environment during seed development, maturation, and seed storage has a critical influence on seed quality, and particularly on seed vigour, no information about the production history of the lots used in this study was available and thus the reasons for the poor performance of lots A and B could not be explained.

2. No apparent relationships were found between fruit thickness, seed weight, and germination performance of the seed. As previously stated, this might be due to the fact that fruits with different diameters occurred in each of the thickness classes. Thus alterations in the methodology used in this study to select fruits where diameter did not vary with thickness, and/or using seed lots from different cultivars with different physical characters might have provided more useful information about these relationships.

3. Only one seed lot (A) was used in this study for determining the relationship between stage of seed maturity (via X-radiography) and seed performance, and this lot may not have been typical of all lots. Examining other lots therefore, might have provided more information as to whether this technique might be used as a quick and reliable viability test.

4. As discussed in section 5.3.1, some mature ungerminated fruits (the seed cavity filled with the true seed) were observed in this experiment. The germination failure of these fruits might be attributed to the inhibitors in the fruits and also it is possible that some of the fruits did not germinate because the true seed was non-viable. Thus application of both X-radiography and a tetrazolium test might have been more appropriate to provide further information about the factors limiting germination.

6.2.2 Scope for further work

1. Owing to its indeterminate flowering habit, a seed lot of sugar beet at harvest comprises a wide range of fruit sizes in which usually only the medium fruit size (3.25- 4.5mm diameter) is suitable for precision drill sowing systems. This requires that the larger and smaller fruits of the harvested seed lot are rejected via size grading. This process often discards around 70-75% of the seed lot (personal experience) and can sometimes be as much as 90% of the harvested fruits (Wood *et al.* 1977). This is in fact a major problem in sugar beet production. It has been shown that, besides genotypic characteristics, time of planting and distances between mother plants have critical effects on fruit size and seed quality. Thus there is a need for further research to determine how fruit fractions and consequently seed performance are affected by these factors, and how production practices might be changed to reduce fruit size variation.

2. As it contributes both chemical and physical inhibition, the pericarp is known as an important factor affecting sugar beet germination. Because pericarp weight increases during the post maturity stage (Wood *et al.*; 1977, Grimwade *et al.* 1987),

determination of the best harvest time after seed physiological maturity to prevent further pericarp growth is necessary. In addition, harvest timing may prevent seed weathering which may reduce seed germination and in particular seed vigour.

3. The presence of aborted and under-developed seeds is an important factor limiting germination in sugar beet seed. As little is known about the actual physiological basis of seed development in sugar beet fruits, this provides an interesting area for further work.

4. The pericarp (including the seed cap) which has an inhibitory effect on seed germination, originates from the maternal ovary wall tissue. The major part of the true seed size, the perisperm, is also maternal tissue (derived from the nucellus). Plant breeding techniques based on selecting male sterile plants may therefore, be successful in creating cultivars with high seed vigour and suitable fruit size, both in terms of suitability of the fruit for standard precision drill sowing systems and also with a low level of the physical inhibitors (i.e. reasonably thin pericarps with a loose seed cap).

5. Different cultivar responses to prewashing have been reported in the literature (see Chapter 2). However, is not understood whether these differences are due to the concentration of the chemical materials in the fruit or are related to the sensitivity of the seed in the fruit to the inhibitors. This is again an interesting area for further work.

6. Although the direct method used in this present work for measurement of the force required for seed cap movement provided a reasonable estimation, dissecting out embryos from the cap cavity is a time consuming process. A better technique for direct force measurement (eg. possibly by applying a vacuum to the outside surface of the cap of the intact fruit) would be helpful for allowing the selection of progenies with reasonably loose seed caps, and consequently more rapid seedling emergence.

7. Because of interactions between the inhibitory function of the seed cap, water soluble material in the fruits and also the pericarp base, a multiway comparison between germination performance of excised seed, unwashed and prewashed fruits with or without pericarp bases might provide more useful information about the relative importance of each inhibiting factor.

8. Studies on the chemical nature and concentration of the water soluble substances in the fruit and also the effect of these inhibitors on oxygen uptake appear to be interesting areas for further research.

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Appendix 1 The formula used in this study for measurement of the median radicle emergence time (T50) and uniformity

Median Germination / Emergence Times (T50)

$$T_{50} = t_i + \frac{[(N+1)/2 - n_i]}{(n_j - n_i)} X (t_j - t_i)$$

Where ;

N = final number of germinants / emergents

n_j and n_i = total number of germinants / emergents by adjacent counts at t_i and t_j,

where : $n_i < (N+1)/2 < n_j$

Uniformity / median spread of germination /emergence times (T_{90}-T_{10}) were calculated as follows :

$$T_{10} = t_i + \frac{[(N+1)/10 - n_i]}{(n_j - n_i)} X (t_j - t_i)$$

where : $n_i < (N+1)/10 < n_j$

$$T_{90} = t_i + \frac{[9 (N+1)/10 - n_i]}{(n_j - n_i)} X (t_j - t_i)$$

where : $n_i < 9(N+1)/10 < n_j$

Appendix 2 Lay out of the field experiment using Randomized Block Design with six replications. The roman numerals indicate the block numbers and the letters identify the seed lots. In addition, the numbers show different fruit sizes which were randomly distrbuted in each replication.

	C										A										B									
I	10	9	3	6	7	1	5	8	2	4	8	1	7	6	5	4	9	10	3	2	3	10	9	8	7	6	4	5	1	2
	B										C										A									
II	3	1	4	8	9	5	7	2	6	10	6	8	9	2	5	3	7	10	4	1	9	10	5	4	8	6	1	7	3	2
	A										C										B									
III	6	3	7	1	5	9	4	8	2	10	6	7	9	10	5	4	8	3	1	2	8	9	5	6	10	4	2	7	3	1
	B										A										C									
IV	9	2	1	3	5	7	8	10	6	4	6	10	1	3	4	2	9	7	8	5	8	3	6	9	2	4	7	1	5	10
	A										B										C									
V	5	7	4	1	10	8	9	6	3	2	1	7	8	2	10	3	5	6	9	4	6	2	3	1	8	4	5	9	7	10
	C										B										A									
VI	9	8	7	3	6	4	5	10	1	2	3	9	8	6	4	2	10	1	7	5	7	5	4	8	6	1	9	10	3	2

Appendix 3. Soil analysis of the field experiment (performed by Fertilizer and Lime Research Centre, Massey University, New Zealand)

pH	Olesen P	SO_4	Exch K	Exch Ca	Exch Mg	Exch Na	CEC	NH_4 N	NO_3 N
6.2	54	5.75	0.58	8.05	0.99	13	2.6	13.5	29.8

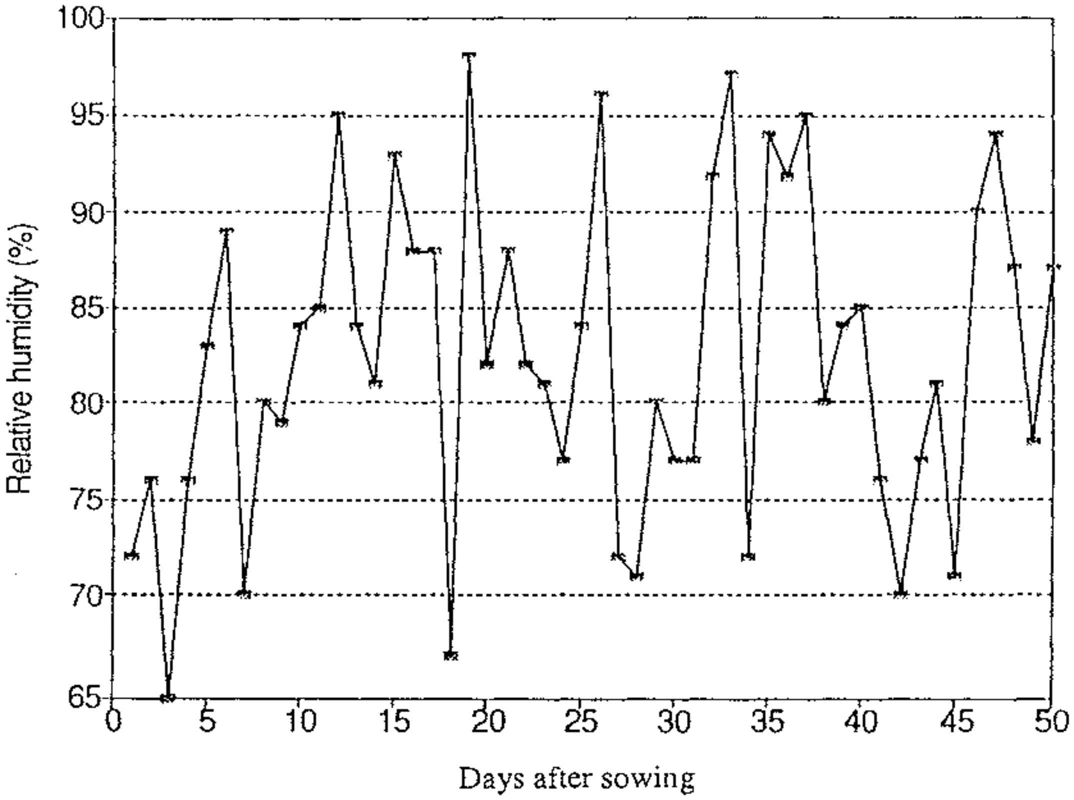
Explanation of the figures:

--NH_4-N and NO_3-N are expressed as micrograms per gram oven dry (extracted on flied moisture soil and corrected for moisture).

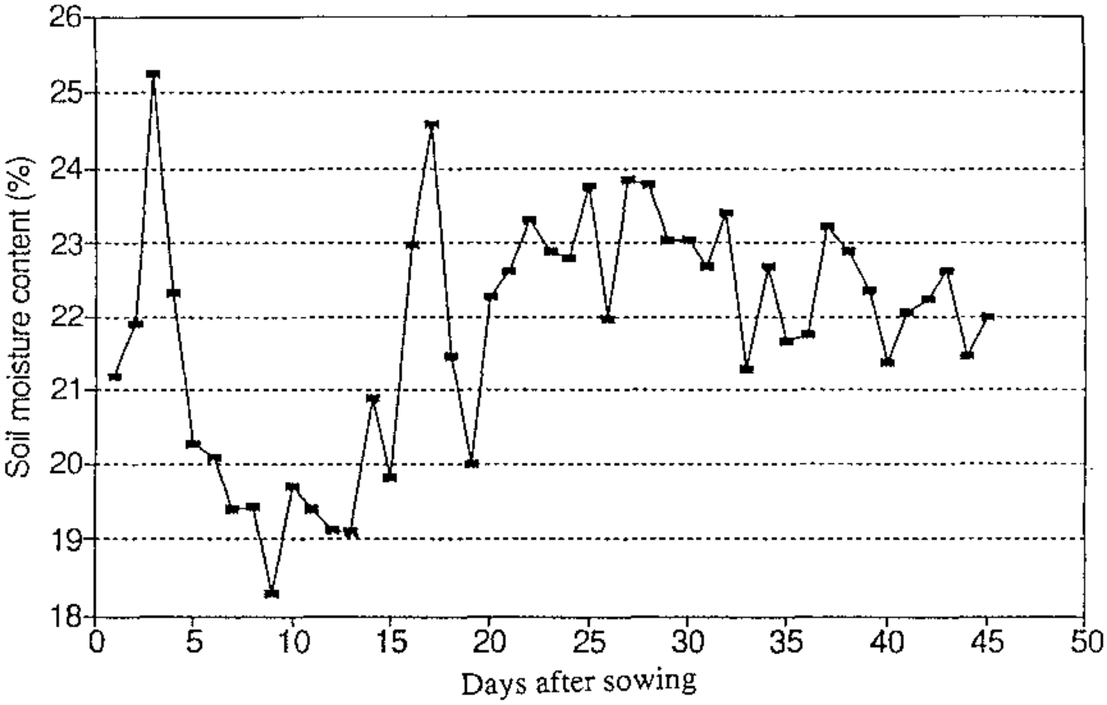
--Phosphate and sulphate values are expressed as micrograms per gram (air-dry).

--Exchangeable cations and CEC values are expressed as meq/100g (air-dry).

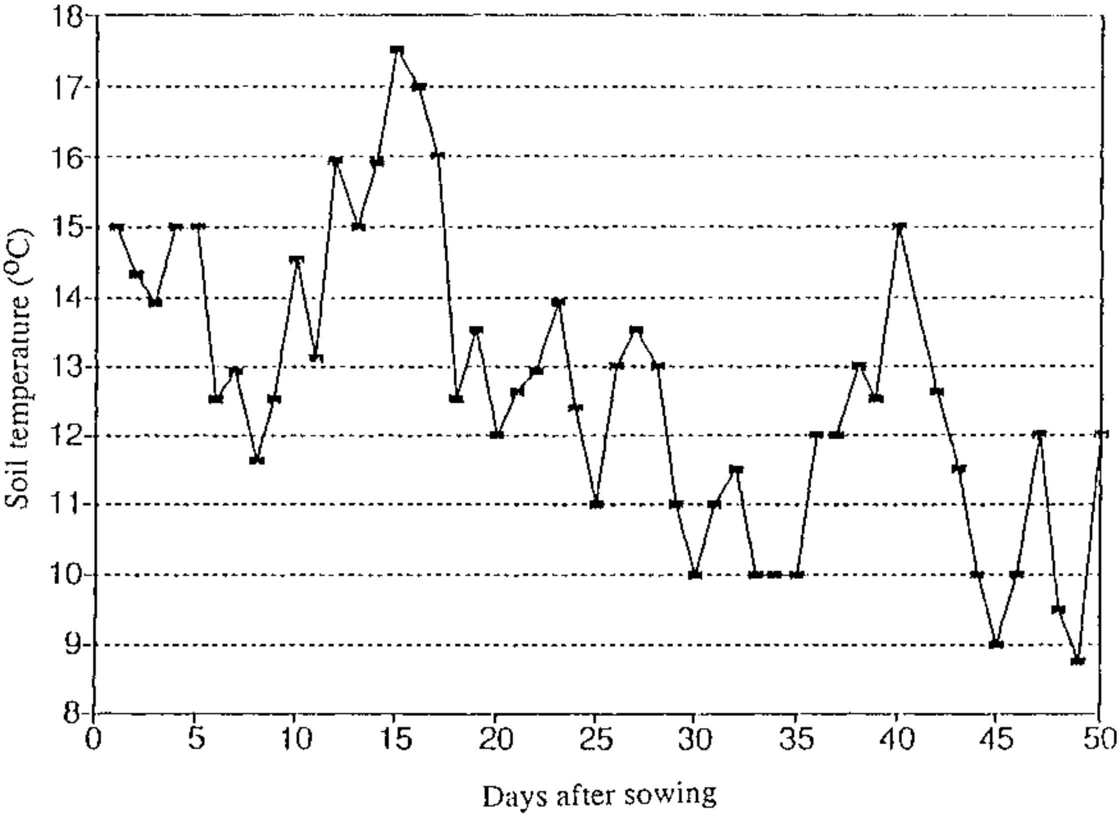
Appendix 4 Relative humidity from seed sowing to harvest time recorded at Agresearch Institute (formerly DSIR Grassland, Palmerston North, New Zealand, at 9am (1.5 Km from the field site).



Appendix 5 Seed bed moisture content during seedling emergence and growth up to the harvest time. Each data point is the mean moisture content of four soil samples which were randomly taken from a depth of 5-7 Cm at 6pm each day and then oven dried at 105°C for 16 h.



Appendix 6 Seed bed temperature from seed sowing to harvest time. Each data point is the mean soil temperature recoded at a depth of 10 Cm 3 times a day (8am, 1pm and 6pm).



Appendix 7 Maximum and minimum air temperature in the field from seed sowing to harvest time.