A STUDY OF SEED DEVELOPMENT, SEED COAT STRUCTURE

AND SEED LONGEVITY IN "GRASSLANDS PAWERA"

RED CLOVER (*TRIFOLIUM PRATENSE* L.)

A Thesis Presented in Partial Fulfilment of
the Requirements for the Degree of
Doctor of Philosophy at
Massey University
NEW ZEALAND

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November, 1978

ABSTRACT

'Grasslands Pawera' tetraploid red clover is an important
agricultural legume through its agronomic roles in soil enrichment
by nitrogen fixation and the production of high yields of quality
herbage. Since this cultivar was only recently released in
New Zealand, many of its agronomic aspects, and particularly those
relating to seed production are still not fully understood. The
present study was conducted to investigate the pattern of seed
development, attainment of seed quality components, time of harvesting,
cell structure in the seed coat, hardseededness and related
problems, and the longevity and germination characteristics of
seeds buried in the soil.

Peak flowering date varies with time of sowing and climatic
conditions, a sufficiently large number of effective bee pollinators
(*Bombus* species) being essential during the flowering period for
successful seed production.

The pattern of seed development in 'Pawera' red clover is
similar to that of its diploid counterparts and may be divided into
three distinct stages. The first stage lasts for 10 days after
pollination. The second stage occupies a period of 16 days,
and the third stage takes a further 10-14 days. Seed dry weight
is maximal 26 days after pollination (physiological maturity).
Maximum seed viability is attained 22 to 26 days after pollination.
Therefore if seed is harvested during the third or ripening stage,
seed quality components such as viability, seedling vigour,
seed weight, and storage life will not be adversely affected.
The correct time of harvesting can be decided by using seed
coat colour and seed moisture content as seed maturity indices.

Generally the sequences of both embryo and endosperm develop-
ment in 'Pawera' red clover show close similarity to some other
Trifolium species. The processes of cell degeneration and
differentiation occur throughout the seed developmental period.
Cell structure in the seed coat is also similar to corresponding
structures in the testa of other small-seeded legumes. The
present investigation highlights the relationship between individual
seed coat structures and their respective role in affecting

seedcoat permeability and impermeability mechanisms. The
results fail to implicate the micropyle or hilum as permeable
sites on the seed coat. In originally permeable seeds, water
conduction occurs at random sites on the seed coat. However, when
a hard seed is softened by mechanical impaction or under natural
environmental conditions, the strophiole is the only initial
permeable site on the testa. Observations in the present study
have clearly implicated the cell structure of the strophiolar
region as a unique and most sensitive area of the seed coat.

The findings of the present study suggest that the rate of
breakdown of hardseededness varies inversely with the depth of
seed burial. Seed samples containing mature seeds maintain their
viability in the soil longer than immature seeds. The rapid
depletion of seed numbers in the soil is mainly due to germination
in situ. Certain proportions of the seed population persist
in the soil for extended periods due to the influence of different
types of dormancy mechanisms. Of these, enforced dormancy plays
a most important role in maintaining the viability of buried
seeds, especially with increased burial depth. 'Pawera' red
clover seeds show a distinct periodicity of germination at different
times of the year. This is an effective genetically controlled
and environmentally modified seed survival mechanism.

ACKNOWLEDGEMENTS

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INTRODUCTION

For many years pasture production in New Zealand has relied heavily on a grass:legume association. Although such pastures are based traditionally on perennial ryegrass and white clover the use of red clover as a complement or addition to the white clover component has become common (Ulyatt *et al*. 1976).

Red clover, in addition to its nitrogen fixing role produces herbage of high nutritive value for animal production. It is a short lived herbaceous perennial plant comprising three to ten or more erect leafy stems arising from a well developed crown. Plants grow to a height of 35-90 cm, the three oblong leaflets being characterised with a pale crescent-shaped mark on the upper surface. The aerial parts, unlike white clover, are distinctly hairy (Martin and Leonard 1967; Smetham 1973; Anon. 1976b).

Generally red clover thrives in a cool, moist climate in well drained soils of high fertility. The tap root, with its numerous lateral branches, may penetrate the soil to a depth of 90-180 cm, giving the plants pronounced drought resistance (Martin and Leonard 1967; Anderson 1971). Red clover is winter dormant, generally producing up to 90% of its annual production in the spring and summer (Smetham 1973). Flower buds are developed both terminally on primary stems and on stem branches. Each head contains 50 to 275 flowers or florets (Anon. 1976b). Red clover seeds are about 2 mm long and vary in colour from yellow to deep purple. The seed is compressed, ovoid and asymmetrical with the radicle lobe much shorter than the cotyledonary lobe and appearing as a projection on one side of the seed. In diploid varieties 1,000 weed weight is approximately 2.1 g (Evans 1962) compared with a figure of 3.2 - 3.4 g in tetraploid varieties (Anderson 1971, 1973a).

Prior to 1974, only two certified cultivars of red clover were produced and used commercially in New Zealand. These were the diploid cultivars "Grasslands Hamua" red clover (also known as cowgrass, early flowering or double cut red clover) and "Grasslands Turoa" red clover (known as Montgomery, late flowering or single cut red clover) (Smetham 1973).

In 1974 a new cultivar "Grasslands Pawera" tetraploid red clover was released into the New Zealand Seed Certification Scheme (Anon. 1978). "Grasslands Pawera" was developed by selection and breeding after colchicine treatment of certified and uncertified lines of "Grasslands Turoa" red clover and some varieties introduced from Sweden (Anderson 1973a). Although it possesses some general characteristics similar to other red clovers there are some aspects of its production which give it superiority over existing New Zealand diploid cultivars (Plate 1).

"Grasslands Pawera" is a high forage yielding cultivar, particularly during the summer and autumn. It is capable of producing high herbage yields in pastures and produces high quality hay (Anon 1978). Palatability is equally acceptable to stock as other red clover cultivars but because of its larger seed and seedlings, early production after sowing is better than that of diploid varieties. It also has greater dry matter production and persistence than its diploid counterpart "Grasslands Turoa" red clover. It is winter dormant, but has better overwintering ability than diploid varieties, as well as superior drought resistance under dry summer conditions (Anderson 1971, 1973b). The larger flower head of tetraploid 'Grasslands Pawera' red clover is accompanied by greater corolla tube length (11.3 - 12.6 mm) compared with diploid varieties (10.3 - 10.8 mm) (Anderson 1973a). This creates problems in obtaining acceptable seed yields in the absence of sufficient numbers of long-tongued insect pollinators. Seed set per head varies from 29-68, with a mean of 40 seeds (Anderson 1973a).

At the time of its release, many aspects of the most suitable seed production management of 'Grasslands Pawera' red clover were not fully elucidated. This resulted in a situation where farmers and seed producers tended to manage their crops using management systems which had proved successful with the previously available diploid cultivars. This led to sometimes unfair performance comparisons being made between 'Grasslands Pawera' tetraploid red clover and 'Grasslands Hamua' and 'Grasslands Turoa' diploid red clovers, particularly since these latter two varieties often showed doubtful persistency and yield.

Because of the lack of information concerning time of harvesting, attainment of maximum seed quality components and the lack of basic studies of seed development, hard-seededness, (including seedcoat structure) and the longevity and germination characteristics of seed in the soil a study was carried out with 'Grasslands Pawera' red clover at Massey University commencing May 1974. The main objectives of this study were:

a. to observe the pattern of flowering, pollination requirements and seed development in Pawera red clover crops in the field.

b. to monitor changes in seed quality components and hard seed content during seed development and during short term storage.

c. to investigate the embryology of seeds during their development.

d. to study changes in the structure of the seedcoat during seed development with a view to determining the role of the seed coat generally, and specific regions of the seedcoat in particular, in affecting the development and the reduction of hardseededness.

e. to investigate seed longevity and the rate, extent and causes of the breakdown in hardseed content following seed burial in the field and in dry storage.

f. to monitor seed germination *in situ* following seed burial and patterns of seedling emergence in the field.

g. to suggest criteria for determining the optimum time of harvesting of Pawera red clover seedcrops which would be of value to seed producers.

Plate 1: "Grasslands Pawera" tetraploid red clover plant at the flowering stage.

CHAPTER I

INVESTIGATION ON POLLINATION, SEED DEVELOPMENT AND ATTAINMENT OF VIABILITY

INTRODUCTION

Various workers (Hyde 1950; Hyde et al. 1959; Griffiths et al. 1967; Hill 1971; Teng and Hor 1975; Kowithayakorn 1978) have carried out seed development investigations on a number of grass and legume species. Even though some information on seed development and viability is available on diploid red clover (Trifolium pratense) and white clover (Trifolium repens)(Hyde 1950; Hyde et al. 1959) few, if any, similar studies on tetraploid clover varieties (Trifolium species) appear in the literature. Since it is not known whether the results obtained with diploid clover varieties is the same in their tetraploid counterparts, it was thought desirable to carry out a seed development and production study with the new cultivar 'Grasslands Pawera' tetraploid red clover. This study was designed to obtain agronomically useful information, with particular emphasis on the sequence and period of seed development, the time of attainment of maximum viability and the optimum time of harvesting of 'Grasslands Pawera' red clover.

Seed formation follows as a result of pollination and fertilization, seed development continuing until the seed is ready to harvest after the attainment of maximum dry weight. Such development is accompanied by physical, physiological and biochemical changes. Some of the physical and physiological changes occurring in 'Pawera' red clover seed during different developmental stages were monitored in the present study. In addition, studies were carried out on the attainment of seed viability - the most important seed quality component. Moreover, the formation of hard seeds in both freshly harvested seed and dry stored seed samples, the occurrence of fresh ungerminated seeds in freshly harvested seed samples and possible causes of the germination failure of fresh ungerminated seeds were also investigated.

LITERATURE REVIEW

1. Pollination: Seed formation and successful seed production depend on the effectiveness of pollination. Wind is the principal pollinating agent of grasses and insects are the chief agents for pollination of legumes (Gurr 1962). Red clover is self-sterile (Fergus and Hollowell 1960; Gurr 1962, 1972, 1975; Griffiths et al. 1967; Martin and Leonard 1967; Palmer-Jones 1967; Free 1970; Anon. 1976b) and therefore, successful   seed production relies on the presence of successful pollinators.   (Weaver and Weihing 1960; Gurr 1962; Martin and Leonard 1967; Scott 1975).   It has long been accepted that a bumble bee species with a long, tongue or proboscis which can reach down to the base of the red clover corolla tube is the ideal pollinator (Griffiths et al. 1967; Free 1970). Red clover has a long corolla tube which surrounds the anthers and stigma with the nectary situated at the base (Gurr 1975). The development of tetra-ploid red clover varieties results in bumble bees assuming particular importance since the corolla tube of such varieties is longer than in diploid varieties, resulting in the nectary being inaccessible to honey bees (Friden et al. 1962; Clifford 1973).

Three of the four bumble bee species present in New Zealand have tongues long enough to reach the nectar of any variety of clover yet produced (Gurr 1975). All long-tongued bees (e.g. *Bombus hortorum*, *B. ruderatus*, *B. subterraneus*) visit red clover always at the front of the flower and effect pollination (Gurr 1961). These bees are known as "positive" pollinators (Free 1970). Conversely, short-tongued bees (e.g. *Bombus terrestris*) are less effective as pollinators of red clover and are referred to as "negative" pollinators (Free 1970). This latter species may resort to "robbing" florets of their supply of nectar by biting a hole in the side of the corolla tube adjacent to the nectary. They thus obtain the nectar without effecting cross-pollination (Cumber 1953; Gurr 1975; Lawes 1975). The queens of *B. terrestris* are however, much larger than other castes and their tongues are also long enough to reach the nectar directly through the mouth of the corolla tube. Moreover, all castes of *B. terrestris* that gather or eat pollen must obtain it through the mouth of the corolla tube and thus transfer pollen from flower to flower and effect cross-pollination (Gurr 1975). Some workers (Gurr 1961, 1962; Clifford 1973; Lawes 1975; Anon. 1976b) have claimed that honey bees, because of their

relatively short tongue length, are of limited value as effective
pollinators of red clover. Other workers however (Morrison 1961;
Palmer-Jones et al. 1966; Griffiths et al. 1967) have reported that
honey bees are capable of effecting an appreciable degree of pollin-
ation in diploid red clover species.

Successful seed production of red clover depends not only on
the presence of suitable pollinators but also on sufficient numbers
of pollinators at the time of pollination (Griffiths et al. 1967). A
high density of suitable pollinating bees, therefore is the first
basic need (Gurr 1962; Griffiths et al. 1967; Anderson 1973a).
There are however, some variations in the reported number of bees
considered necessary for efficient pollination. Umaerus and
Grasi (1962) have claimed that populations of 1400-1580 bumble bees
per hectare are insufficient for efficient red clover pollination.
However, other reports have suggested that 800 positive bee pollinators
(Anon 1976b) or 1600 bumble bees (Free 1970) per hectare are
perfectly adequate for the production of adequate seed yields of
diploid red clover. Clifford (1973) has reported that about 3000
bumble bees/ha are required to obtain acceptable levels of pollin-
ation in tetraploid red clover crops in New Zealand.

Generally, red clover seed yields are directly proportional to
pollinator activity (Anon 1976b). Bee activity and population in
turn depend on weather conditions. Friden et al. (1962) recorded
as many as 5950 bumble bees per hectare in a year with excellent
weather conditions but only 2560 per hectare in the following year
under poor weather conditions. Fergus and Hollowell (1960) have
reported that bees do not pollinate efficiently during cloudy, rainy
weather. According to Free (1970) weather conditions not only
influence the flight of bees directly but also indirectly by affecting
floret attractiveness to bees by reducing the levels of production of
pollen and nectar. Temperature seems to be particularly important
as a limiting factor in this regard. Foraging flights by bees may
take place at 12^0 to 14^0 in the early spring but slightly higher
temperatures are needed later in the year, especially on cloudy days.
In general, in Western Europe, with increases in temperature from
10 to 30^0C, there is a corresponding increase in pollen collection,

although levels of light intensity, rain and relative humidity are also important in determining bee activity. (Free 1970).

2. Seed development: The term "development" has been defined as the progress of a series of internal qualitative changes, (with or without external changes) governed by factors of the environment, and which lead ultimately to the production of fruit, and in annual plants to death (Whyte, 1946 cited by Salter and Goode, 1967). The term "maturity" has been described as the point at which maximum grain development is first attained (Aldrich, 1943) or alternatively the point at which maximum dry weight is first reached (Hyde 1950; Austin 1972; Schwass 1973). The latter definition has also been referred to as "physiological maturity" (Shaw and Loomis 1950; Harrington 1972) or "morphological maturity" (Anderson 1954). The term "ripeness" has been defined as the point when the seed has dried to a moisture content in equilibrium with the surrounding atmosphere (Hyde 1950) or the point when the seed has dried to a moisture content suitable for harvesting (Hill 1971).

The course of seed development in both grasses and legumes has been studied by many workers (Hyde 1950; Lowenberg 1955; Grabe 1956; Hyde et al. 1959; Griffiths et al. 1967; Hill 1971). The general pattern of changes in seed components during development shows similarity in most cases. Hyde (1950) and Hyde et al. (1959) have described the development of seed in both clover and ryegrass species and proposed three developmental stages.

(a) Growth stage: This stage lasts for the first 10 days immediately following pollination and is characterised by rapid growth rate. The moisture content of the seed is high (75-80%) and no viable seed is formed during this stage.

(b) Food reserve accumulation stage: This stage occupies a period of 10 to 14 days following the first stage. It is characterised by a uniform rate of growth which is presumably determined by the food reserve transfer rate from the parent plant into the seed. During this period, the dry matter in the seed increases approximately threefold and reaches its maximum at the end of the stage. The actual amount of water in the seed declines slightly and its weight falls to about 63%. Seed

attains viability early in this stage. In legumes, due to the
occurrence of hard seed, germination percentage begins to decline
during the later part of this stage.

(c) Ripening stage: The third or last stage occupies a period
from 3 to 7 days. During this stage the
seed dries out rapidly and shrinks in size. The dry weight remains
approximately constant but fresh weight falls to less than half
of its former maximum and the moisture content of the seed declines
from 63% to 10%. (Hyde 1950, Hyde et al. 1959).

3. Formation of hard seed: Hardseededness in Leguminosae, espec-
ially in small-seeded legumes occurs quite promiscuously in any and
every seed lot, but varies in both degree and number from one
species to another and from one sample to another in a species.
The development of hard seed may be due to various factors, including
the function of the hilum, the nature of the seed coat, the level of
seed maturity, genetic factors, environmental conditions during seed
development, and some other factors.

(a) The function of the hilum: According to Hyde (1954) the hilum
performs a function essential for
the development of the hard seed condition in some Papilionaceous
seeds. He reported that the hilum functions as a hygroscopic
valve, opening when the relative humidity is low and closing when the
latter is high. Due to this mechanism, progressive drying takes
place intermittently and hard seeds tend to acquire a moisture
content in equilibrium with the lowest relative humidity to which
they have been exposed. During seed ripening, when the moisture
content falls to about 15%, the epidermis becomes impermeable and
further drying of the seed takes place only by diffusion of water
vapour through the hilum. Thus as the seed ripens there is a cont-
inuous increase in the duration and degree of impermeability. It is
evident therefore that the conventional definition of hard seed is
an arbitrary one which takes no account of the great variation in
the intensity of the impermeable condition resulting from differences
in the degree of desiccation which occurs during the later stages
of seed development.

(b) The nature of the seed coat: Since a detailed account on the nature of the seed coat and its effect on seed impermeability is presented in Chapter (2), no review of this subject is presented in this section.

(c) Seed maturity: It has been reported that seed coat impermeability increases with seed maturity (Hyde 1950; Brown 1955; Hyde et al. 1959; Winter 1960; Williams and Elliott 1960; Quinlivan 1971a; Chow and Crowder 1974; Egley 1974). Hyde (1950) and Hyde et al. (1959) found that hard seed begins to appear in clover seed samples harvested 12 days after flowering, 96% becoming hard in white clover by the 25th day after pollination (Hyde 1950). Barrett-Lennard and Gladstones (1964) have reported that seeds of Ornithopus compressus become impermeable as soon as they are mature but the degree of hardness is not influenced by the stage of seed maturity.

(d) Genetic factors: Hardseededness is primarily genetically controlled but the rate of appearance and degree of hardness is influenced by environmental conditions (Crocker 1948; Crocker and Barton 1953; Barton 1965a; Mayer and Shain 1974; McComb and Andrews 1974). Stevenson (1937) and White and Stevenson (1948) were able to produce permeable seed in sweet clover by continuous selection and breeding. Similarly, Weihing (1962) recorded that the hard seed percentage in Persian clover was increased by selection from 0.5% in the parental stock to 63% for the 8th progeny generation. Other workers (Bennett 1959; Donald 1959; Martin and Yarnell 1961; Everari et al. 1966; Halloran and Collins 1974) have also agreed that the characteristic of hardseededness is an inherited one. Quinlivan (1971a) considers that impermeability is a varietal characteristic and that it is possible to breed or select for a level of hardseededness appropriate to a particular environment or ecosystem.

(e) Environmental conditions: It has been reported by various workers (Nelson 1926; Witte 1931a; Hill 1944; Grant Lipp and Ballard 1964; McComb and Andrews 1974; Sidhu and Cavers 1977) that the environmental conditions under which seeds of various legumes develop and ripen can affect the percentage of hard seed which form. Some workers (Aitken 19 ; Andrew 1956;

Povilaitis 1956; Williams and Elliott 1960; Quinlivan 1965, 1971a)
that seed which develops under more favourable conditions, in terms
of availability of moisture and nutrients, has a high hard seed
content. Other workers (Crocker 1948; Hyde 1950; Bacin-Mielaus
1970) however, have reported that more hard seed is formed if the
seed ripens during dry, hot weather, and especially when the relative
humidity is low (Bacin-Mielaus 1970; Quinlivan 1971a). Quinlivan
(1965) suggested that a relatively long growing period in the spring
months appears to cause not only a higher proportion of hard seeds
to develop but also increases the resistance of hardness to the
softening effect of the summer environment.

(i) <u>Other factors</u>: Factors, such as size of seed and type of
fertilizer are also reported to be related to the
extent of formation of hard seed. Black (1959) reviewed the
literature on seed size in herbage legumes and concluded that the
proportion of hard seeds in any one harvested seed lot seems to
increase as seed size decreases. Similar findings have been reported
in *Medicago sativa* (Gavrielit-Gelmond 1957).

High levels of calcium have been implicated in increasing the
production of hard seed. High levels of potassium have the opposite
effect and phosphorus has apparently no significant effect on the
formation of hard seed in crimson clover (James and Bancroft 1951).
Similarly, El-Bagoury and Nivazi (1973) found that high levels of
potassium and nitrogen fertilizers produce crops exhibiting lowest
percentages of hard seed in Egyptian clover.

Marbach and Mayer (1974) reported that the seed coat of *Pisum elatius*
is normally impermeable to water but becomes permeable after drying the
seed in the absence of oxygen. The presence of oxygen has also
been shown to increase impermeability of the seed coat of *Pisum* paludosum
at a certain stage of seed development. Some *Pisum* species with
normally impermeable seed coats contain higher phenolics and catechol
oxidase than species which exhibit more permeable seed coats. It
is thus suggested that the permeability of the seed coat to water
may be related to the content of phenolic compounds in the seed coat
and to their level of oxidation (Marbach and Mayer 1974).

4. Seed viability: The embryos of seeds of a number of species become viable at a very early developmental stage. Grabe (1956) working with smooth bromegrass found viable seeds in samples harvested as early as 5 days after flowering. Similarly, viable seeds are first formed 7, 10 and 4 days after anthesis in ryegrass, timothy and prairie grass respectively (Hill 1971). Hyde et al. (1959) also reported that in ryegrass viable seeds are first observed 12 days after anthesis and full germination capacity is reached by the fourteenth day when moisture content is 65% and seed dry weight is only 44% of its final value in perennial ryegrass. At this stage in Italian ryegrass the corresponding figures are 59% moisture and 48% of final seed weight. Negis (1964) stated that seeds of cereals can germinate as early as 7 to 14 days after fertilization.

Information about the onset of seed viability in dicotyledonous species has been reported by many workers. Brown and Porter (1942) recorded that bindweed (_Convolvulus arvensis_ L.) seeds are capable of germinating 10 to 15 days after pollination when the moisture content is still over 80%. Impermeability then becomes pronounced from 23 to 25 days after pollination as the moisture content reduced to 15%.

Hyde (1950) and Hyde et al. (1959) found that white clover (_Trifolium repens_) seeds become viable 10 to 12 days after pollination when moisture content is 77% and the dry weight of the seed still only 44% of its final weight. Similarly, viability in red clover (_T. pratense_) seed is first acquired about 12 days after pollination at a seed moisture content of 76% and at a level of dry weight only 45% of the weight of the mature seed (Hyde et al. 1959).

According to Kinch and Termunde (1957) seeds of perennial sow thistle (_Sonchus arvensis_) become viable 4 days after flowering and reach maximum viability at 9 days, while the seeds of Canada thistle (_Cirsium arvense_) can germinate 6 days after flowering and reach maximum germination 3 days later. Kinter (1960) reported that 9 day-old seeds of _Abutilon theophrasti_ imbibe water but none of them are viable. However, 14 day-old seeds showed 25% viability and 20 day-old seeds produced 100% germination when they are scarified.

In some *Desmodium* species only a small percentage of seed becomes viable even at about 20 days after fertilization but viability increases rapidly until maximum seed dry weight is obtained (Chow and Crowder 1974). Teng and Hor (1975) working with groundnut (*Arachis hypogaea* L.) reported that 10 days after tagging of the gynophores, seeds are viable (as determined by tetrazolium test) but not germinable. However, 20 days after tagging seeds show 86-90% germination and a peak germination of 100% is achieved by 40 days. Sidhu and Cavers (1977) found that the seed of *Medicago lupulina* becomes viable starting 10 days after anthesis. Maximum viability of 100% is attained after a further 4 days.

5. <u>Optimum time of harvest</u>: It is extremely important to harvest a crop at the correct time in order to obtain maximum yields of high quality seed and to minimise seed losses through cleaning, processing or shedding. Schwass (1973) stated that the optimum time to harvest a crop is largely a matter of experience. However, he also mentioned that problems may often arise which cause reduced seed yield and quality. The timing of harvest is likely to be most accurately determined by measurement of changes in seed moisture content. In most species, maximum seed yield corresponds closely with the attainment of seed maturity or with the completion of the transfer of food reserves into the seed. (Schwass 1973).

There are a number of reports on the optimum time of harvesting grass seed-crops (Davies 1960; Stoddart 1965; Roberts 1971; Hill 1973; McWilliam and Schroeder 1974; Hill and Watkin 1975) and vegetable crops (Harrington 1972). However, information about the correct time of harvesting legumes is still comparatively limited.

According to Wheeler and Hill (1957) and Martin and Leonard (1967), the best time to cut red clover for seed is when the heads have turned brown and the flower stalks turn a deep yellow. At this stage, the seed has begun to show a distinct violet colour (Wheeler and Hill 1957). If cutting is done when the atmosphere is damp, the crop may be left even though the heads become black (Martin and Leonard 1967). However, Wheeler and Hill (1957) suggested that if cutting is delayed until the heads are black or until the seeds have completely coloured, the loss of heads or the

loss of seed through shattering is certain to be heavy.

Jolly (1958) and Davies (1960) reported that white clover is ready to harvest when the majority of heads are light brown and the seeds are yellow and quite hard. They also added that in this species there is little or no danger of seed shedding because both early and late settings will be held in the head for several weeks after the seed is ripe. Hyde (1950) and Hyde *et al.* (1959) suggested the need to cut white clover seed crops not earlier than the third stage of seed development. However, they preferred late to early harvesting during that stage in order to secure high seed quality.

Scott (1973) described that white clover crops can be cut when about 80-90% of the heads are brown and matured seeds can be rubbed out in the hand. Moreover, cutting can also be done when the seed begins to change colour. In wet seasons, it is better to cut when about 70% of the heads are ripe (Zaleski 1970). However, in some varieties and climatic situations it may be necessary to harvest at an earlier stage to avoid premature sprouting and seed shedding (Ozkan 1975). Nangju (1977) found that the optimum time of harvest for soyabean was when between 85% and 95% of the pods had ripened. However, he added that the optimum time of harvest varies with different cultivars. Any delay in harvesting resulted in a decrease in seed germination.

According to the reports of various workers, the optimum time of harvest has been determined by assessing seed, stalk and flower head colour changes. However, Hill (1973) has suggested some drawbacks in judging seed ripeness by eye assessment. In wet seasons although the crop appears fit for cutting, the seeds give the appearance of immaturity. On the other hand, seed and straw colour changes tend to occur earlier under dry conditions and crops may give a premature appearance of ripeness, and be cut too early. In consequence, seed losses will be heavy in shattering varieties if crops are cut too late and the quality of seed will be affected if cutting is too early.

6. After-ripening period: After-ripening is a type of low intensity dormancy. Seed which has this characteristic will not germinate immediately after harvest but recovers its ability to

germinate following an often relatively short period of storage
(Barton 1952, 1961b, 1965a; Crocker and Barton 1953; Mayer and
Poljakoff-Mayber 1963; Thomas 1972). "After-ripening" may be
defined as those changes that occur in seed during storage as a
result of which germination is improved. An alternative definition
suggests that certain processes must occur in the embryo with time
and that these changes cannot be caused by any known means other
than by suitable seed storage (Mayer and Poljakoff-Mayber 1963,
1975). After-ripening occurs in some species during dry storage
while for other species moisture and low temperatures may be required
(Courtney 1968; Delvin 1975; Mayer and Poljakoff-Mayber 1975).

The after-ripening requirement is common in freshly harvested
seeds of a wide range of plant species (Kozlowski and Gunn 1972;
Hess 1975), including some of the common cereals (Crocker and
Barton 1953; Barton 1961b), many flower and vegetable crops
(Barton 1952, 1961b; Cathey 1975), and some weed species (Brown
and Porter 1942; Povilaitis 1956; King 1966; Chawan and Sen 1973;
Popay 1973; Villiers 1975).

There is very little published literature on the after-ripening
requirement of seed of clover species. Hill (1944) reported that
seed lots of subterranean clover require 3 to 12 months dry storage
to reach their maximum speed of germination. On the other hand,
freshly harvested seeds of some clover species, including red and
white clover, may take up water but not germinate and remain as
fresh ungerminated seed at the end of a laboratory germination test.
Apparently such a condition can be overcome by reducing the germination
temperature slightly (Anon. 1954). Nakamura (1962), in reporting the
occurrence of fresh ungerminated seeds in red clover during the
period from 2-5 weeks after harvest, suggested that this effect
may have been due to both seed coat restriction and embryo dormancy.
Maximum germination was obtained with these seeds when mechanical
treatment and prechilling treatments were applied. The use of
prechilling to break the dormancy in the seed of red, white and
some other clovers has been prescribed in the ISTA Rules (Anon. 1976a).

There are several reports (Povilaitis 1956; Barton 1961b;
Hess 1975; Villiers 1975) on the requirement for a short period
of dry storage to after-ripen the seeds of various species including

many arable weeds. Similarly, many vegetable and ornamental seeds reach their maximum potential germinability when held for about a three months after-ripening period (Cathey 1975).

The seeds of some plant species can effectively after-ripen only in a moist, low temperature environment. Freshly harvested seeds of *Polygonum aviculare*, for example, can after-ripen when they are exposed to a temperature of $4^{\circ}C$ (Courtney 1968). Similar reports about the completion of after-ripening in the seeds of many Rosaceous species during stratification have been made by Mayer and Poljakoff-Mayber (1975). Wareing and Saunder (1971) however, have suggested that although the seed coat may appear to interfere with gaseous exchange or to act as a mechanical resistance to germination, there are possibly interactions between seedcoat effects and hormonal factors. They suggest that such interactions cannot be excluded in the case of seeds with a chilling requirement.

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MATERIALS AND METHODS

The trial site used for this experiment was situated in a small valley on the Massey University campus. The site (0.3 ha) was cultivated out of pasture in May 1974. Half of the area was autumn sown with 'Pawera' red clover, the other half being utilized for a spring sowing (Fig. 1). These two crops were used to study the effect of sowing date and consequently the time of seed ripening on changes in the physiological components of seed yield.

(a) Autumn sown crop: In early June (4/6/74) about 1 kg/ha of "Grasslands Pawera" red clover seed was sown in 50 cm rows. The area was sprayed with 2,4-DB at the rate of 1.5 litres in 100 litres of water in early November 1974 to control weeds. About the middle of November the crop was topped and top-dressed with an NPK fertiliser (5-7-5) at the rate of 250 kg/ha.

Before flower initiation, the area was divided into two plots i.e. plot A and plot B (Fig. 1). Plot A (about 0.03 ha) was employed for the seed development study and plot B (about 0.14 ha.) was used for seed production. This plot B was divided again into 32 sub-plots of approximately 1 m x 8 m each. In order to harvest the crop at six different stages of seed maturity, 7 sub plots for each harvest were selected randomly.

(b) Spring sown crop: The remaining half of the trial site plot C (about 0.13 ha) was cultivated and spring sown at the rate of 1 kg/ha of "Grasslands Pawera" red clover seed in early September (4/9/74). This area was also sprayed with weedicide and top-dressed with the same manure at the same time, using identical rates employed for the autumn sown crop.

In both autumn and spring sown crops the number of internodes of 50 random plants from each crop (i.e. from autumn crop and spring crop) were counted at weekly intervals starting just before the time of floral initiation through until after peak flowering.

The crops from the plot A of the autumn sown area and also from the plot C of spring sown area were used for seed development studies.

FIGURE 1 : EXPERIMENTAL SITE FOR SEED DEVELOPMENT STUDY
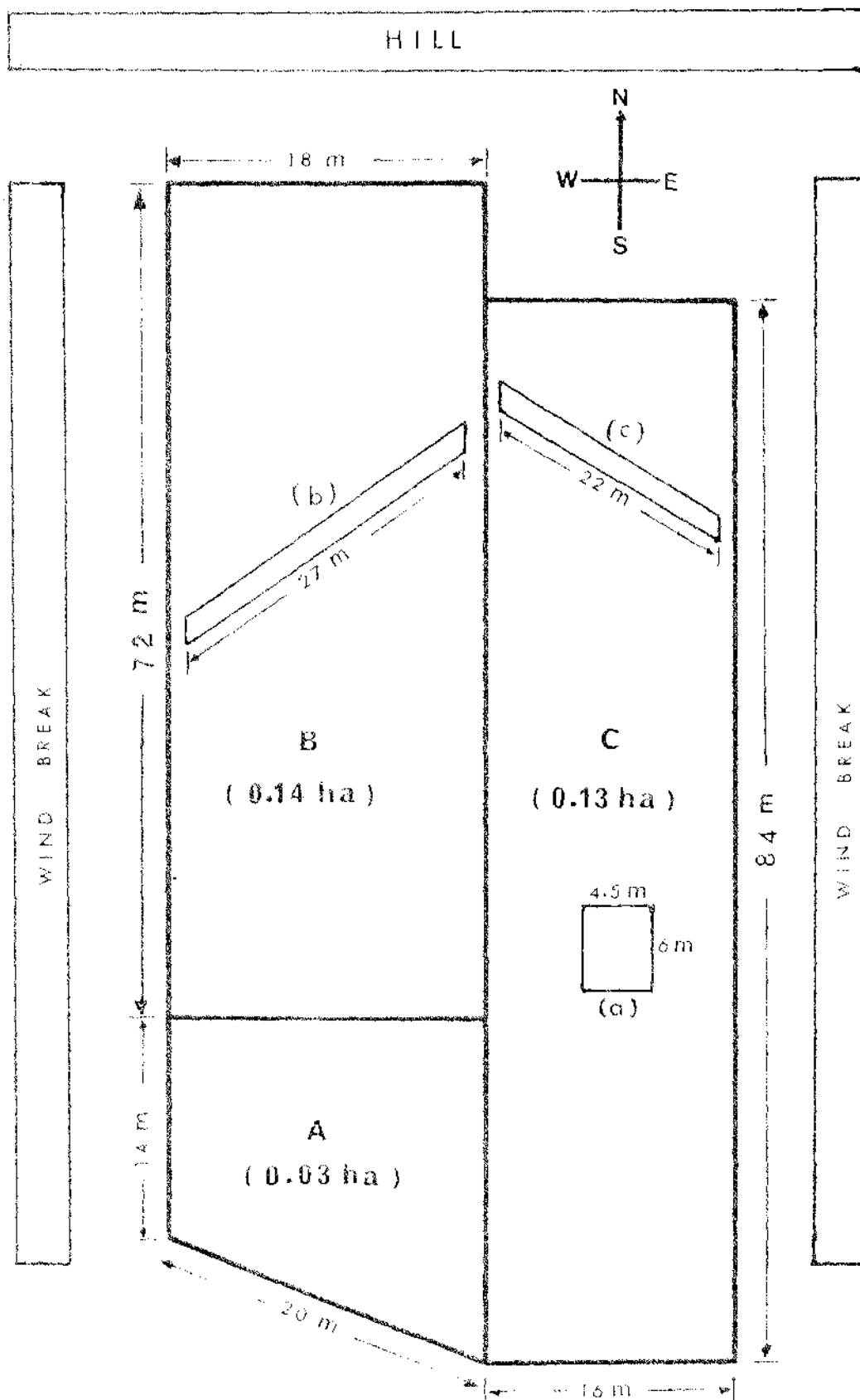
Individual flower heads (inflorescences) were tagged at the fully
flowering stage (with 85-100% flowering florets).   About 4,000
heads from both crops were marked at intervals with plastic tags,
a different coloured tag being used at each marking time.

Five of the 42 sub-plots from plot B of the autumn sown area,
were selected randomly and used for determining peak flowering date.
Fully flowering heads (with 85-100% flowering florets) from each sub-
plot were counted on alternate days from the commencement of the
flowering period until after peak flowering. At the same time, the
numbers of totally withered flower heads (with 95-100% withered
florets) in each plot were also recorded.

To investigate the length of time occupied by different stages
of floral development within an individual flower head, 15 heads
from each crop (i.e. autumn crop and spring crop) were tagged at an
early stage of floral initiation. The development of each head was
recorded daily from tagging until seedheads became totally withered.

The accumulation of nectar occuring at different stages of the
development of individual florets and during the development of
entire heads, was studied. Thirty flower heads from the autumn-sown
crop were tagged at the early bud stage and the presence of nectar
in individual florets was investigated by removing florets at
various stages of flowering. The nectar was squeezed out from the
bottom of each floret and the amount of nectar was determined by
visual observation.

Some observations were carried out in the summer of 1976 to
obtain information about pollinating agents, especially the bumble
bee (Bombus species) population, and in particular to monitor their
activity pattern and their methods of pollination.   To count the
number of bee visits to a particular site, three small areas of the
crop, i.e. sites (a) 6 m x 4.5 m, (b) 1 m x 27 m and (c) 1 m x 22 m,
were selected (Fig. 1).   Observations were made at up to three
times daily, between 8.30 a.m. and 10.00 a.m., 11.00 a.m. and 1.00 p.m.
and 4 and 5 p.m. approximately.   At each observation, general

weather conditions of the area were also recorded in order to correlate with bee activity. At Site (a) the numbers of bees visiting the area were counted and recorded three times within 5 minutes at each time of the day. At the other two sites (b) and (c), the bee number within 0.5 m on either side of a central track were recorded at each time while walking through these areas. Different species of bees and their methods of visiting the flower were also recorded (Plates 3 and 6).

Two types of insect pollination i.e. open and controlled pollination were employed in this study. First year crops (i.e. autumn and spring sown crops in 1974) harvested in 1975 summer, were open pollinated by insects. However, controlled pollination methods were used with seed crops harvested from the same areas in the summer of 1976 and 1977. In 1976, individual flower heads were covered with glassine bags at the early bud stage. At each time of covering, flower heads with almost the same stages of development were selected to obtain uniform flowering heads. At the full flowering stage of most flower heads, all bags were removed in the morning and the flowering florets were allowed to be pollinated for about 8-10 hours. In the afternoon, when most heads had been visited by bees, the flower heads were re-covered to prevent further insect pollination. These heads remained covered until the time of harvest. Small wire cages were also used for controlled pollination studies. Some flower heads with similar stages of development were covered with wire cages. At the time of full flowering, bumble bees (and/or) were put into the cages for pollination. These bees were allowed 8-10 hours in the cages and then released. After pollination, flower heads were covered with glassine bags until the seed was harvested. Similarly in 1977 flower heads were covered with both small and large wire cages (Plate 2) for controlled pollination. The cages were covered with nylon mosquito nets. At the fully flowering stage, bumble bees were put into these small cages for pollination as done in the previous year. However, in the case of large cages pollination was more effective when the cages were removed from the crop on the date of pollination and flower heads were accessable to be pollinated by visiting bees. In both cases flower heads were allowed 8-10 hours for pollination. After pollination, the heads were re-covered with cages until the time of harvest.

Plate 2:   Cages used for controlled pollination.

Attempts were made to determine whether self-pollination of
florets occurred.   Twenty flower heads were covered with glassine
bags and tagged at the bud stage.   Ten of them were left with the
bags on without any further treatment for about four weeks.   The heads
were then harvested and examined to detect the presence of seed
in each floret.   The other 10 bagged heads were shaken vigorously
at the time of full flowering to determine whether pollination could
be induced by mechanical disturbance.   These heads were left for
three weeks after the shaking treatment and were harvested.   They
were then also examined for the presence of seed.

The autumn sown crop in 1974 was successively harvested in the
summer of 1975 commencing 10 days after tagging or peak flowering
date and continuing at 4 day intervals up to 30 days.   The crop
sown in the spring of 1974 was successively harvested starting eight
days after tagging at two or four day intervals for 32 days.
Similarly, in 1976 and 1977, samples of seed from tagged heads
were harvested beginning eight days after pollination and continuing up
to 40 days at two or four day intervals.   Moreover, in 1976, florets

immediately prior to flowering (pollination) and florets 0, 2, 4 and 6 days after pollination were also harvested and used for studying the early development of the embryo.

In order to avoid any mechanical damage to the seed coat during harvesting, all harvests were done by hand.

After harvest, flower heads were spread on a wooden floor and dried at room temperature. The seed was threshed by gently rubbing the dry flower heads between the hands to avoid any mechanical damage. Flower heads harvested from plot B of the 1974 autumn sown crop, were removed by rubbing them against a smooth wire sieve, care being taken to avoid mechanical threshing damage.

In 1975, 60 dry flower heads harvested randomly from the 1974 autumn sown crop were examined to determine the numbers of seeds setting in each head. In 1976, to determine the number of seeds setting in different positions on flower heads, 30 flower heads from both the open pollinated crop and the controlled pollination crop were harvested separately. Each flower head was divided into four equal portions, top, mid-upper, mid-lower and bottom. The number of seeds in each portion of individual flower heads was recorded.

Seed colour changes during seed development in different years were also observed. The percentage and weight of different coloured seed categories from different samples of the 1977 harvested crop, was also recorded.

Each year at each harvest seeds were removed by hand from 20-50 heads on the date of harvest for the determination of seed fresh weight, dry weight and moisture tests. The number of seeds used for each determination ranged from 100 to 300 seeds at each test depending on seed availability. All moisture tests were carried out using the air oven method at 105°C for 18 hours as prescribed in the I.S.T.A. Rules (Anon. 1966).

To investigate the viability of seeds harvested at different stages of seed development, standard germination tests and tetrazolium

tests were carried out using methods prescribed in the I.S.T.A. Rules (Anon 1966).

At each harvest, seeds were immediately removed from heads by hand on the date of harvest. They were placed on blotters and subjected to four days prechilling at $5^{\circ}C$ before being transferred to a $15^{\circ}C$ constant temperature for up to 30 days. Germination counts were made at 7, 10, 20, and 30 day intervals. In the case of seeds which had been air dried, germination tests were carried out at $20^{\circ}C$ for 10 days according to I.S.T.A. Rules (Anon. 1966). Seeds obtained from different parts of flower heads were also tested for germination to find out their viability according to their position within the head.

In order to investigate the effectiveness of different durations of prechilling in breaking dormancy in freshly harvested seeds, seed samples obtained 18 and 22 days after pollination were prechilled at $5^{\circ}C$ for intervals from 0 to 8 days and then germinated at $15^{\circ}C$ for 30 days.

Gibberellic acid was also used to try to improve the germination percentage of freshly harvested seed samples. Seed samples were soaked in GA_3 solution (1000 ppm) for 24 hours at $30^{\circ}C$ (Baskin and Baskin 1970) or blotters were soaked with the same solution and seed then placed on them (Bekendam 1975; Anon. 1976a). Treated seeds were germinated at $15^{\circ}C$ after four days prechilling at $5^{\circ}C$.

Similarly oxygen gas was also used in an attempt to increase the germination percentage of freshly harvested seed samples. Seeds were germinated in plastic boxes. Each box containing 50 seeds was placed in a plastic bag which was filled with oxygen gas. Oxygen treatment was given either before or after prechilling. These treated seeds were germinated at $15^{\circ}C$.

To determine the structure of the seed coat which was responsible for low germination with freshly harvested seeds, seed coats of some seed samples were cut before germination tests. In one seed lot, the seed coat (only malpighian and outer layers) was cut while in the other lot the seed coat (including both nutrient and aleurone layers) was cut. Germination results were compared with

corresponding results using intact seed samples. Germination tests
were also carried out with mature seed samples which belonged to
different seed coat colours. These tests were made immediately
after harvest and also after three months storage. The standard
germination tests were carried out using 2 x 50, 4 x 50, 2 x 100
or 4 x 100 seeds, depending on the availability of seeds for differ-
ent tests and in different years.

The viability of freshly swollen ungerminated seeds and of freshly
harvested young seed samples, was determined using the topographical
tetrazolium method (Anon. 1976a). Seeds were soaked in a 1%
aqueous solution of tetrazolium chloride at $30^\circ C$ for 24 hours. Seeds
were then rinsed in water and each seed dissected under the micro-
scope to examine stained areas of the embryo and cotyledons.

In order to determine the agricultural value of hard seed samples
and those seeds that remained hard after the germination test period,
such seeds were scarified using the techniques and apparatus described
by Ballard and Grant Lipp (1965). Pressures ranging from 4 psi
to 14 psi at a constant duration of 2 min. and durations of treatment
starting from 1 min. up to 6 min. at a constant pressure of 10 psi,
were employed to determine the optimum intensity of scarification.

RESULTS

1. <u>Vegetative growth during the flowering period</u>: The number of internodes present on stems at the time of floral initiation and peak flowering shows some variation. Most stems in the autumn sown crop attained 7-8 internodes at floral initiation and 8-9 internodes at peak flowering. In the spring sown crop, when floral initiation began in the field, stems bore only 5-6 internodes whereas at the time of peak flowering most of them possessed 10-11 internodes. However, the variation in internode numbers was quite wide (Fig. 2, Appendix 1). When a stem produced a terminal flowerhead, no further increase in internode numbers occurred.

2. <u>Peak flowering date</u>: Floral initiation in the autumn sown crop started in early January 1975. The numbers of fully flowering heads increased steadily from the 16th of January, to a peak on the 7th of February 1975 (Fig. 3, Appendix 2). Thereafter the numbers of fully flowering heads decreased in most plots although wide variation occurred. Totally withered flower heads also began to appear about mid-January. However, the rate of formation of withered heads was relatively slow until the end of January. Commencing from the early February, the number of withered heads increased more rapidly even though there was quite wide variation between plots. The variation in withered head numbers became more pronounced starting from about the date of peak flowering (Fig. 4, Appendix 2).

3. <u>Flowering sequence</u>: It was observed that floret opening over the whole flower head in the autumn sown crop took about 7 to 8 days. In the spring sown crop, heads required a longer period (8-10 days) to reach the fully flowering stage (Table 1). Similarly, the length of time from the fully flowering stage to the totally withered stage was also longer in the spring sown crop (5-9 days) than in the autumn sown crop (7-8 days) (Table 1).

Fig 2: Frequency histograms showing the numbers of internodes on
50 random stems at the time of floral initiation and peak
flowering in both autumn and spring sown crops.

(a) Autumn Sown Crop

At floral initiation

At peak flowering

(b) Spring Sown Crop

At floral initiation

At peak flowering

Internode numbers

Internode numbers
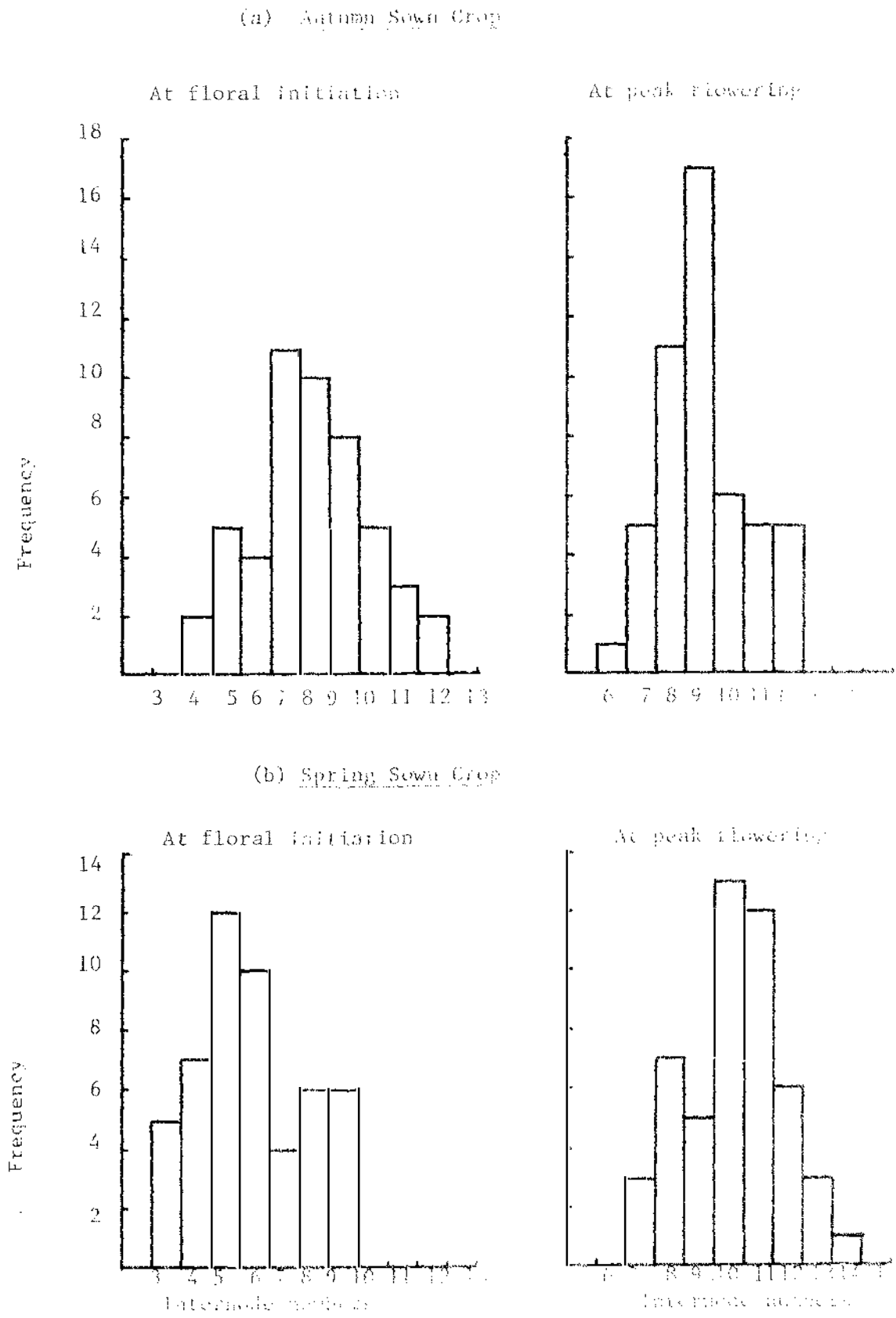
FIG. 3: Mean number of fully flowering heads, and totally withered heads present in five random plots (1975).
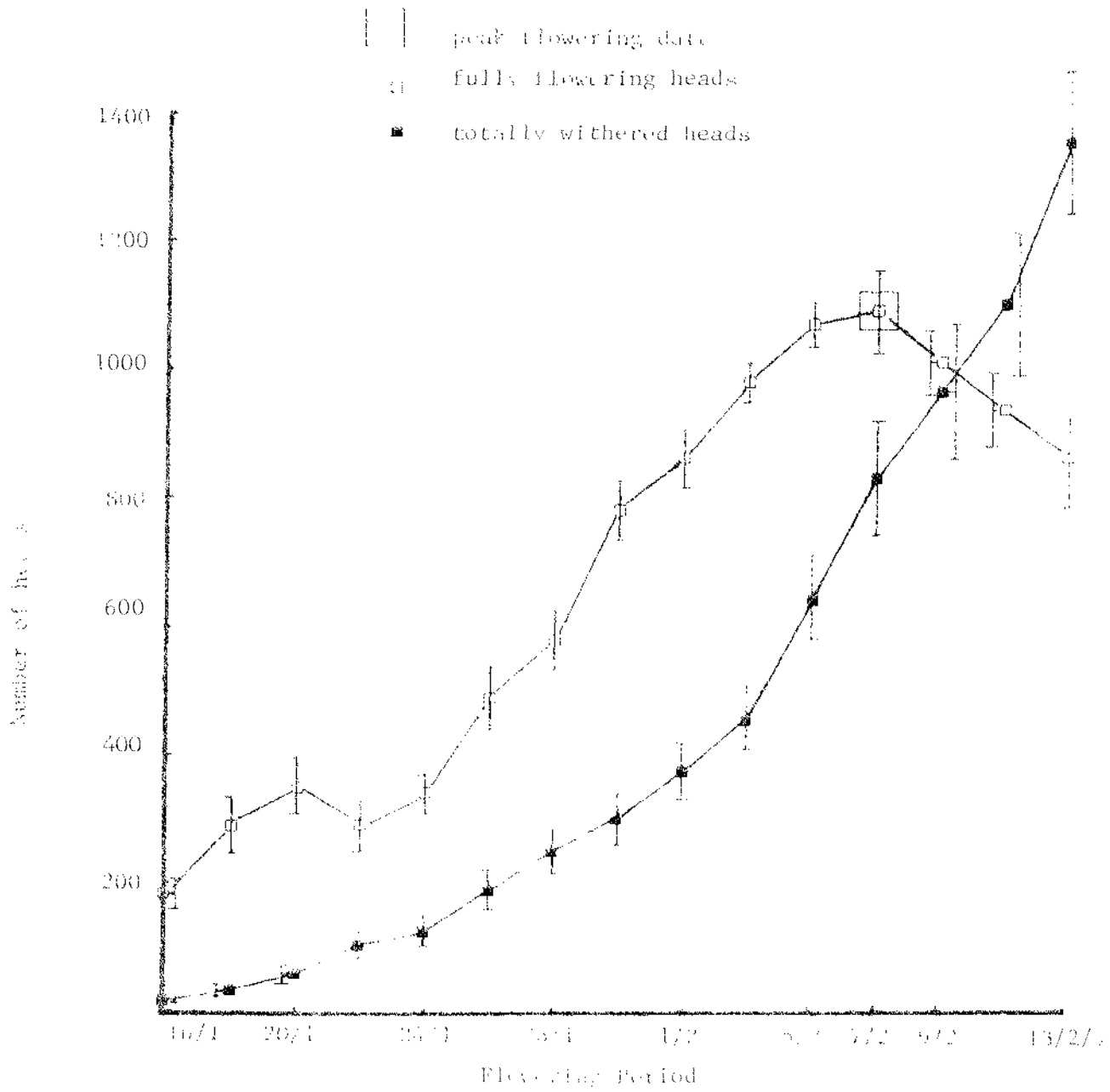
TABLE 1: Flowering sequence in a flower head

Stages of floral development	Period occupied by each stage (days)	
	Autumn sown crop	Spring sown crop
1. From early bud (red hair stage) to the appearance of pink coloured bud stage	Not recorded	2-3
2. From pink coloured bud stage to half fully flowering (40-50% flowering florets) stage	3-4	4-5
3. From half fully flowering to fully flowering stage (85-100% flowers)	3-4	3-5
4. From fully flowering stage to totally withered (95-100% withered florets) stage	7-8	8-9

4. Nectar production: The presence of nectar in individual florets showed less dependence upon the progressive floral development of the whole head than upon the development of individual florets. An individual floret contained more nectar on the first day of floret opening than on later dates. The maximum accumulation of nectar over the whole flower head appeared to occur during 3 to 6 days from the pink coloured bud stage (i.e. during the later part of stage 2 and most of stage 3 in the flowering sequence shown in Table 1).

5. Pollination: In 'Pawera' red clover, seed was formed only by cross-pollination and not by self-pollinating methods, all attempts to obtain seed by selfing being totally unsuccessful. Bumble bee populations recorded in experimental plots during the flowering period of mid-February to mid-April 1976 are shown in appendix (3). Expression of this data with temperature and relative humidity during the flowering period is shown in Figures 4 and 5 respectively. A considerable number of bees were recorded when the temperature was 17°C or higher (Fig. 4).

Fig. 4: Bumble bee populations(%) observed at different temperatures during the flowering period in 1974. (Perpendicular lines are drawn at temperatures at which 3 or more observations (x) of bee populations were recorded).
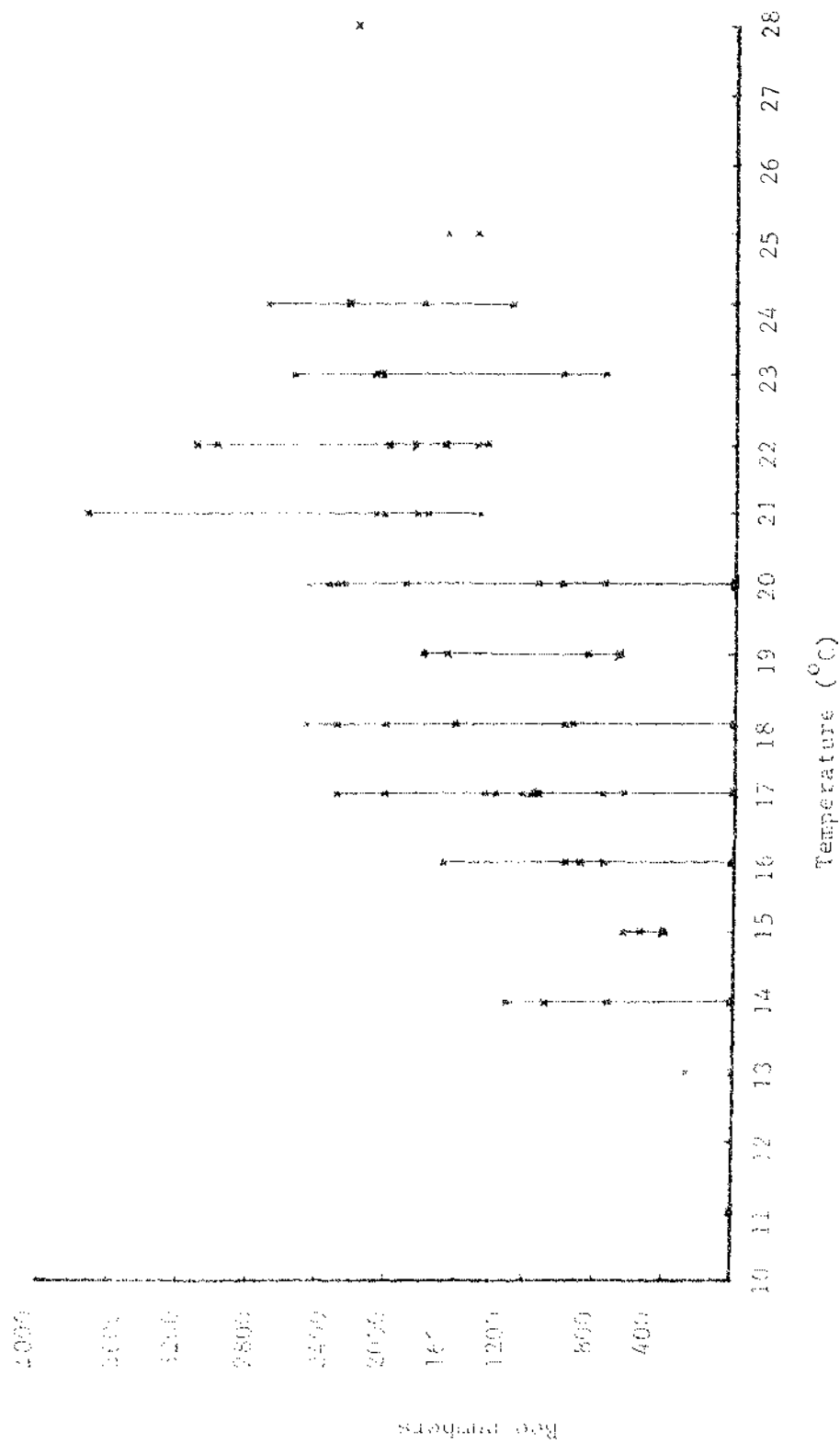
Fig. 3. Market bug populations (%) observed at different percentages of relative humidity during the flowering period in 1970. (Perpendicular lines are drawn at humidities at which 3 or more observations were recorded).
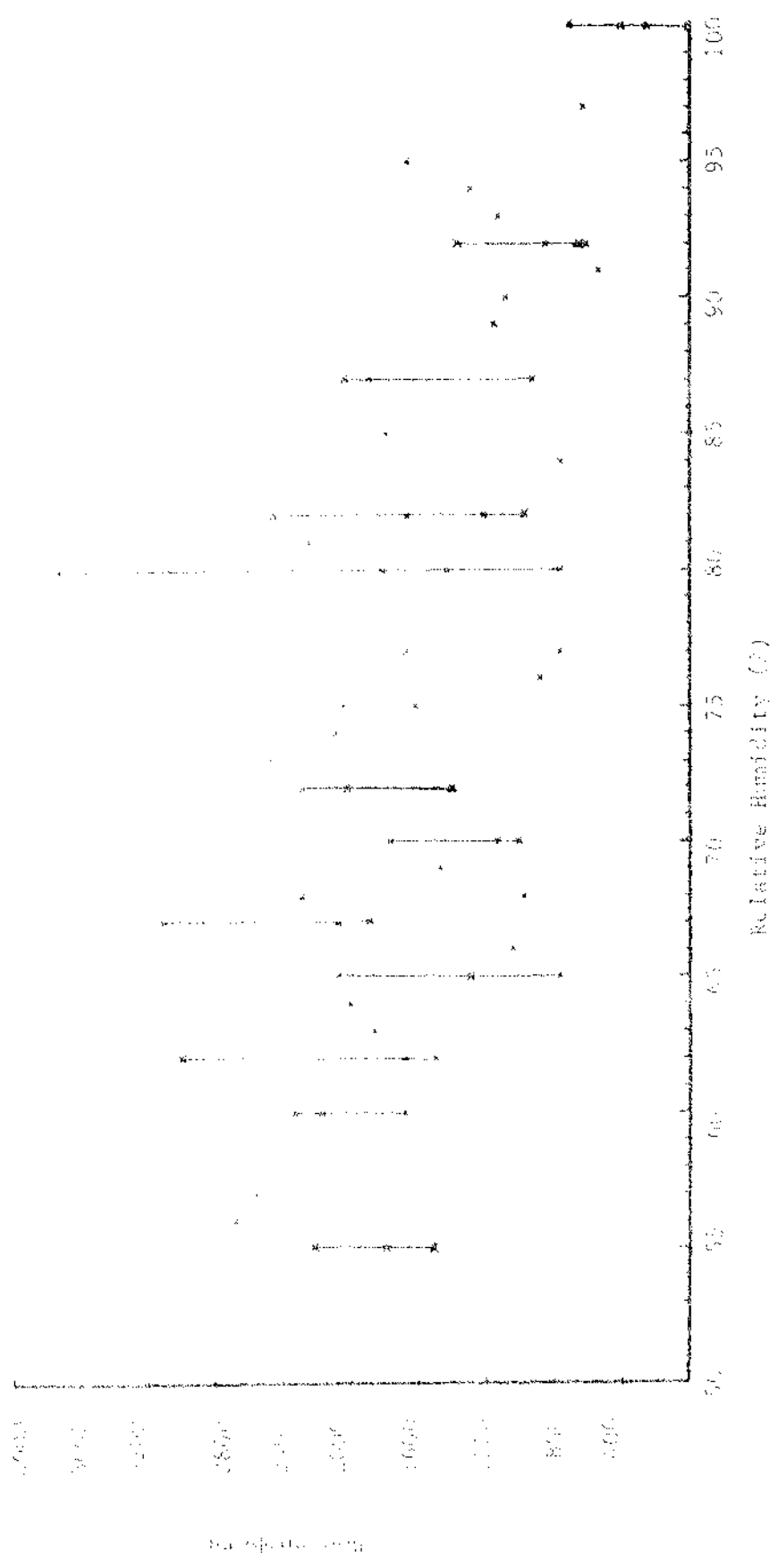
Relative humidity (%)

Similarly, a fairly high bee population was observed over a wide
range of relative humidites from 55% to 95% (Fig. 5). There seemed
to be considerable differences in the number of bees present at
different times during the flowering period of the crop. (Appendix 3).
At the beginning and end of the observed period, the bumble bee pop-
ulation on those days with fairly high temperatures varied from
about 1000 to 2000/ha. However, a fairly high bee population of
2000-3000/ha was observed during a one week period from the 18th
to 25th of March when weather conditions appeared to be favourable to
bees. In February, the species of bees present were mainly *Bombus*
hortorum and *B. ruderatus* rather than *B. terrestris*. However,
starting from early March, the *B. terrestris* population increased
gradually. During late March and early April, a large number of
young queens and other castes of *B. terrestris* appeared in the
field. Similarly, the population of *B. hortorum* seemed to increase
slightly while that of *B. ruderatus* decreased later in the season.

As shown in Plate (3), *B. hortorum* is an effective (positive)
pollinator since it collects nectar through the mouth of the
corolla tube. *B. ruderatus* also shows a similar habit of visiting
the flower and causing pollination. Both *B. hortorum* and *B. ruderatus*
have a long proboscis or tongue (Plate 4) enabling them to reach the
nectar at the bottom of the long corolla tube of the 'Pawera' red
clover floret. (Plate 5). However, *B. terrestris*, because of
its relatively shorter tongue is a far less effective pollinator
since the majority of this species rob the nectar by cutting a
hole at the base of the corolla tube (Plate 6) rather than entering
through the mouth of the floret. Nevertheless the queens and pollen
collectors of *B. terrestris* are more effective as pollinators since
they visit the flower from the front.

6.   Seed setting: In 1975, the mean number of seeds set per head
was 96 over six harvests (Table 2), with a range
from 46-132 in individual flower heads (Appendix 4).   The mean number
of seeds per 100 florets was 68 (Table 2), although a variation from
38 to 87 occurred in individual heads (Appendix 5).
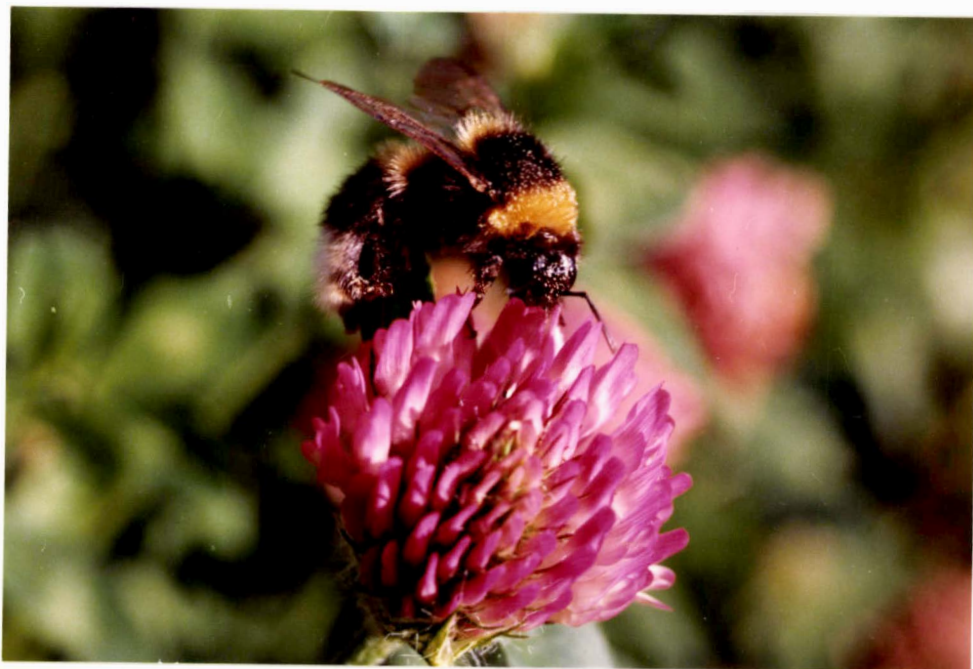
Plate 3:   A long-tongued bumble bee *(Bombus hortorum)* collecting
           nectar through the mouth of the corolla tube of a floret
           of 'Pawera' red clover.
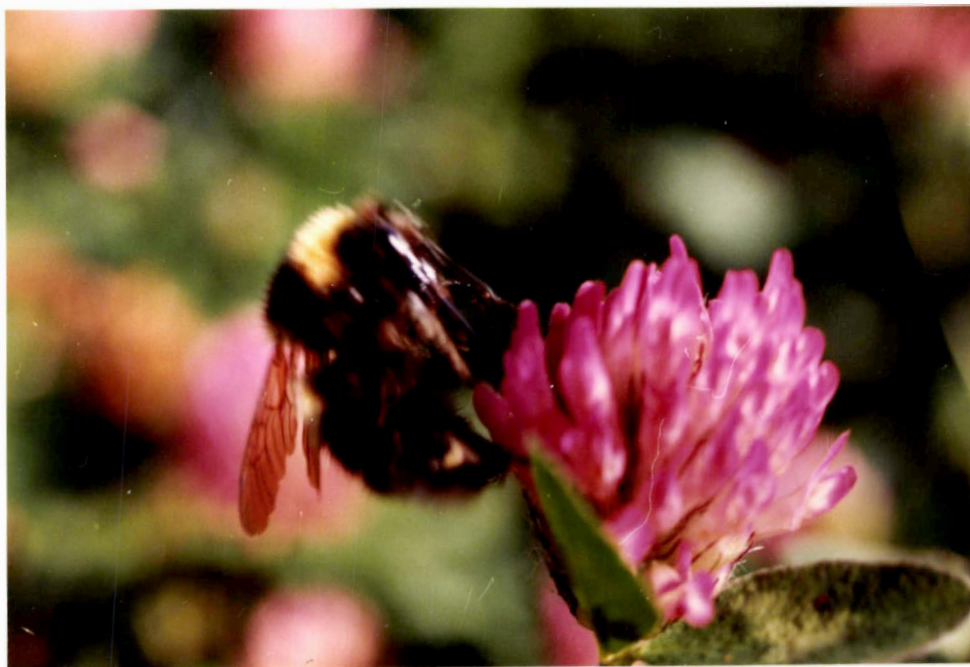
Plate 4:   A long-tongued bumble bee visiting a flower head of 'Pawera'
           red clover.

Plate 5: Partially dissected flower head of 'Pawera' red clover showing arrangement and length of florets and corolla tubes.
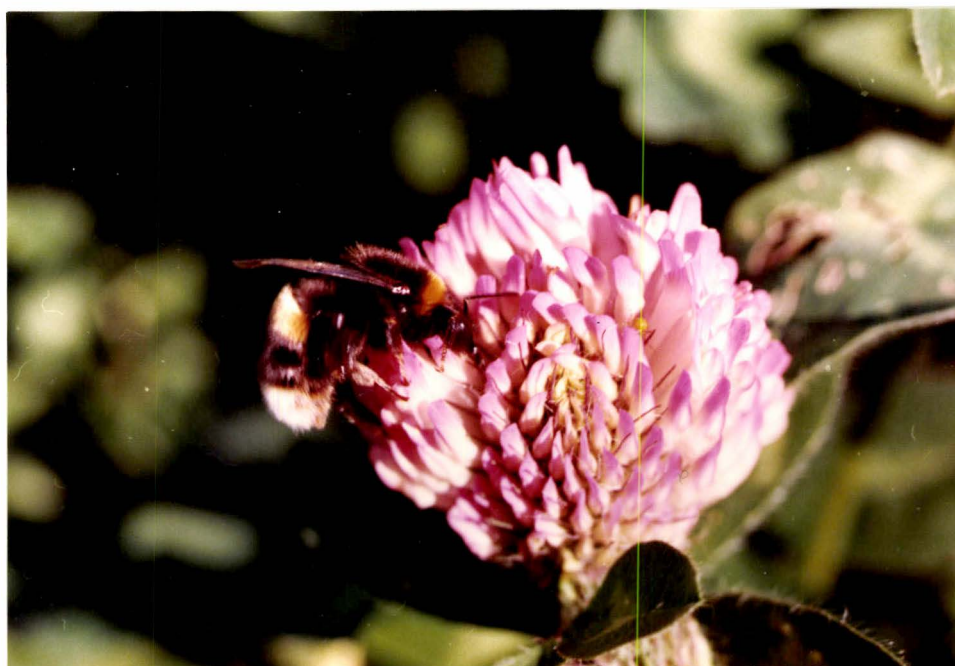
Plate 6: A short-tongued bumble bee *(Bombus terrestris)* robbing nectar from the side of a corolla tube.

TABLE 2:   Mean number of seeds per flower head and per 100 florets in
           an individual head.

Number of harvest	Mean number of seeds	
	Per flower head	Per 100 florets
1	89	67
2	105	73
3	78	62
4	89	67
5	79	64
6	100	75
Average	90	68

In 1976, the mean
seed set was only 59 seeds per head in the controlled pollinated crop
and 46 in the open pollinated crop (Appendix 5).   Similarly, the number
of seeds per 100 florets was about 45 in both controlled and open
pollinated crops.   These results are substantially lower than results
obtained in the 1975 crop.

The mean numbers of seeds set per head (Appendix 5) and the
mean percentage of seeds set in different parts of individual flower
heads are shown in Fig. (6).   The mean percentage of seeds setting
in the middle portion of individual flower heads was obviously
greater than in the top and bottom parts (Fig. 6a).   Similar trends
in the number of seeds setting 100 florets can also be observed
(Fig. 6b).   The different percentage of seeds set in different
parts of a head was due to either lack of fertility or ineffective
pollination, since the numbers of florets actually formed in each
part of the head was approximately the same (Appendix 5).   Although
some slight variation occurred, the numbers of seeds/100 florets in
individual flower heads was similar in both controlled and open
pollinated crops (Fig. 6b).

7.   Seed colour: In freshly harvested seed samples about 30% of
                    seeds began to change colour from green to yellow
or purple 23 days after pollination when seed moisture was 66%
and dry weight was 94% of its maximum weight in 1976;   and 24
days after pollination with a seed moisture content of 64% and
dry weight of 98% in 1977.   Nearly 96% of these seeds were yellow,
purple or brown at maturity (i.e. 26 days after pollination) in both

(a) Mean percentage of seeds developed in different parts on an individual flower head.

(b) Mean number of seeds/100 florets in different parts on an individual flower head.

Different position in a head

Different positions in a bud

Percentages
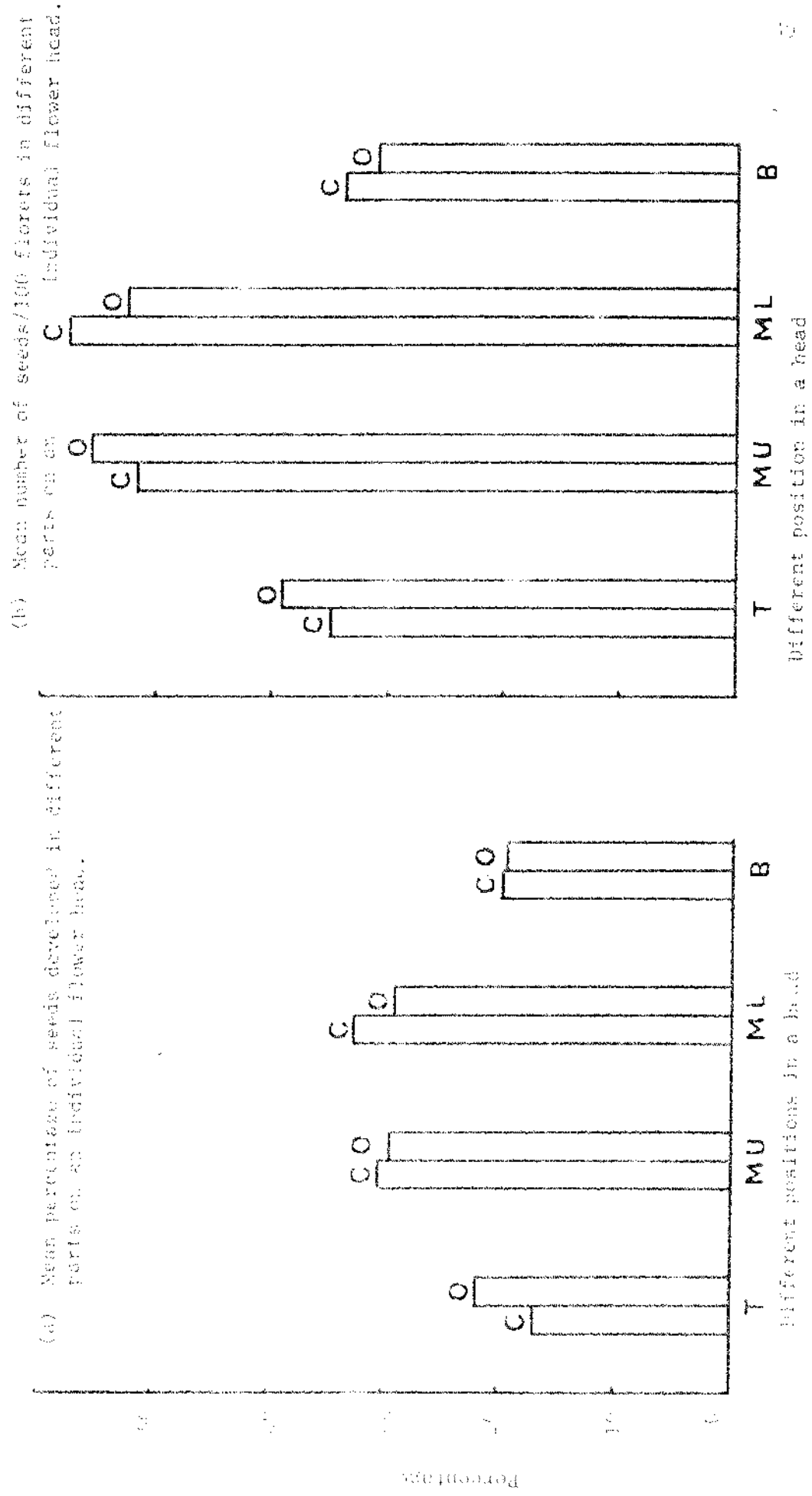
years.

When samples of different seed ages were stored under room temperature conditions for three months, all seeds turned either yellow, purple, or brown. This situation occurred even in seed samples harvested as early as 10 days after pollination (Appendix 6). Although five different seed colour categories were nominated, three distinct colours, viz.yellow, purple and brown were used to monitor changes in the proportion of different seed colours at different developmental stages(Fig. 7). The observed seed colours were standard-ised using standard colour plates as suggested by Ridgway (1912) (Appendix 7). The percentage of yellow seeds declined from more than 90% at 10 days after pollination to less than 10% 26 days after pollination. In contrast, the percentage of purple and brown coloured seeds increased slowly in samples of 10 to 18 day-old seed and then rapidly up to 26 day old seed in the brown category and 30 day old seed in the purple category. However, the percentage of brown and purple coloured seeds declined slightly while the yellow coloured seeds showed a reverse trend in seed samples harvested 40 days after pollination (Fig. 7).

8.   Changes in seed components: Changes in seed fresh weight, dry weight, absolute moisture and percentage moisture of the samples harvested at different develop-mental stages are presented in Fig. (8) and Appendices (8 and 9) for the 1976 and 1977 harvests, and in Appendices (10-12) for the 1975 harvest from autumn and spring sown crops respectively.

Changes in the size of seeds of different developmental stages immediately after harvest and after three months' storage are shown in Plates (7 and 8).

(a) Fresh weight: As shown in Fig. (8a), in the 1976 harvest the fresh weight of an individual seed was only about 3 mg (31% of its maximum weight) 10 days after pollination. Fresh weight increased markedly to a maximum individual seed weight of 9.6 mg 26 days after pollination. Following this peak, fresh weight decreased rapidly with the loss of moisture until 30 days after pollination and then more slowly up to 40 days by which stage individual seed fresh weight was only about 40% of its

Fig 7: Percentages of different seed colour categories occurring during seed development (after three months' storage)

- ■——■ yellow coloured seed
- ▲——▲ purple coloured seed
- ●——● brown coloured seed

Seed colour (%)

Days after pllination
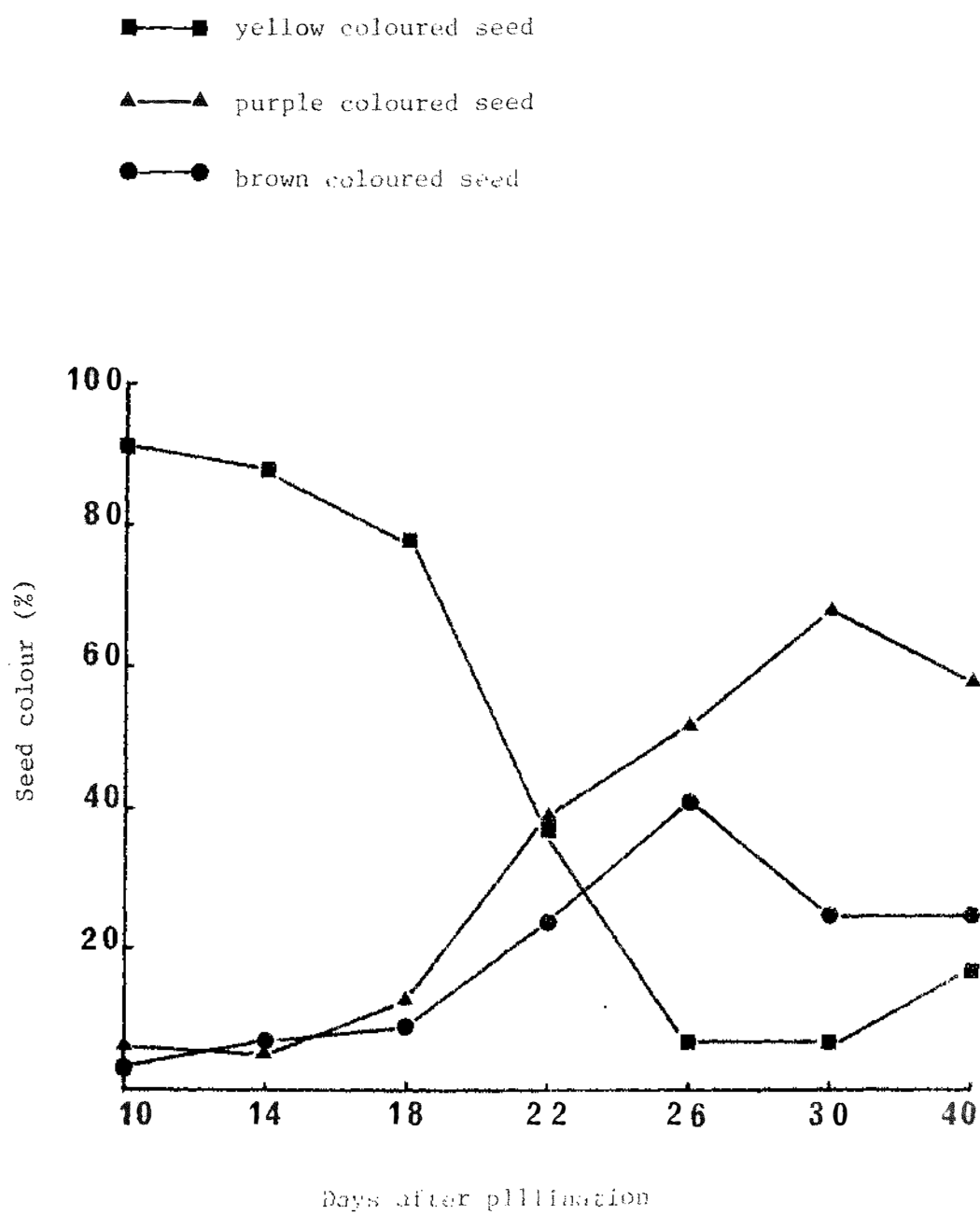
Plate 7:   Changes in seed size occurring during seed development in
freshly harvested samples.
A-F represent seed samples harvested 10, 14, 18, 22, 26
and 30 days after pollination.
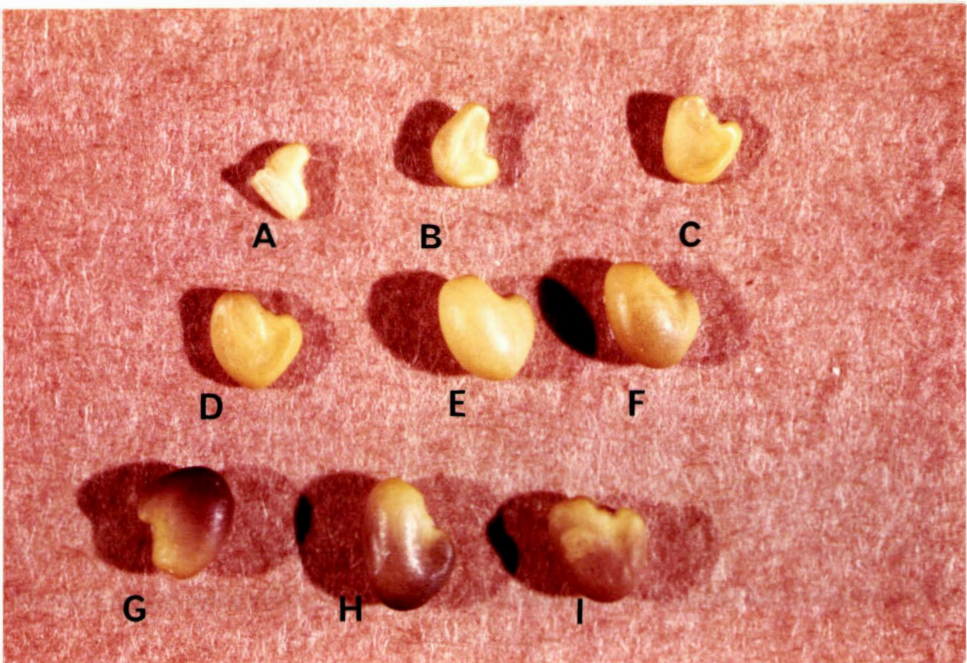
Plate 8:   Changes in seed size and appearance occurring during seed
development in samples following 3 months storage.
A-I represent seed samples harvested, 10, 12, 14, 18,
22, 24, 26, 30 and 40 days after pollination.

maximum value. A similar trend occurred in the 1977 harvest as
shown in Fig. (8b). However, in 1977 the fresh weight of individual
seeds harvested 10 days after pollination was 3.5 mg (40% of its
maximum weight) and 8.8 mg at the peak point (24 days after pollin-
ation). It was 41% of its maximum value when removed from the plant
40 days after pollination.

The fresh weight of the seeds harvested in 1975 from the 1974
spring sown crop showed a similar pattern except that it decreased
very slowly after reaching the peak point (Appendix 11).   However,
maximum individual seed fresh weight was reached at a much earlier
developmental age in seeds harvested from the 1974 autumn sown crop
(Appendices 10 and 12). This may have been due to different climatic
conditions occurring during the seed developmental period (Appendix
13).

(b) <u>Dry weight</u>:   The dry weight of seed 10 days after pollin-
ation was only about 18% of its maximum
weight in the 1976 harvested crop.   However, it increased steadily
reaching a peak point ("physiological maturity") 26 days after
pollination (Fig. 8a). During this period the dry weight of the
seed increased more than 5 times (Appendix 8).   Although, seed
dry weight subsequently remained relatively constant, it was only
about 90% of the earlier maximum weight 40 days after pollination
(Appendix 8).

In 1977 the dry weight of individual seeds increased steadily
from 24% of its maximum value 10 days after pollination to a
maximum dry weight 26 days after pollination (Fig. 8b). The dry
weight of seed 40 days after pollination was again 90% of its
maximum weight (Appendix 9).

Similar patterns of dry weight changes in seeds from tagged
flower heads of the 1974 spring and autumn sown crops were also
recorded (Appendices 10 and 11).

(c) <u>Moisture content</u>: The moisture content of seed was
expressed as both absolute moisture and
percentage moisture on a wet weight basis (Figure 8a).   Changes
in absolute moisture content show essentially the same trend as the

FIG. 8: Changes in fresh weight, dry weight, absolute moisture and percentage moisture during seed development
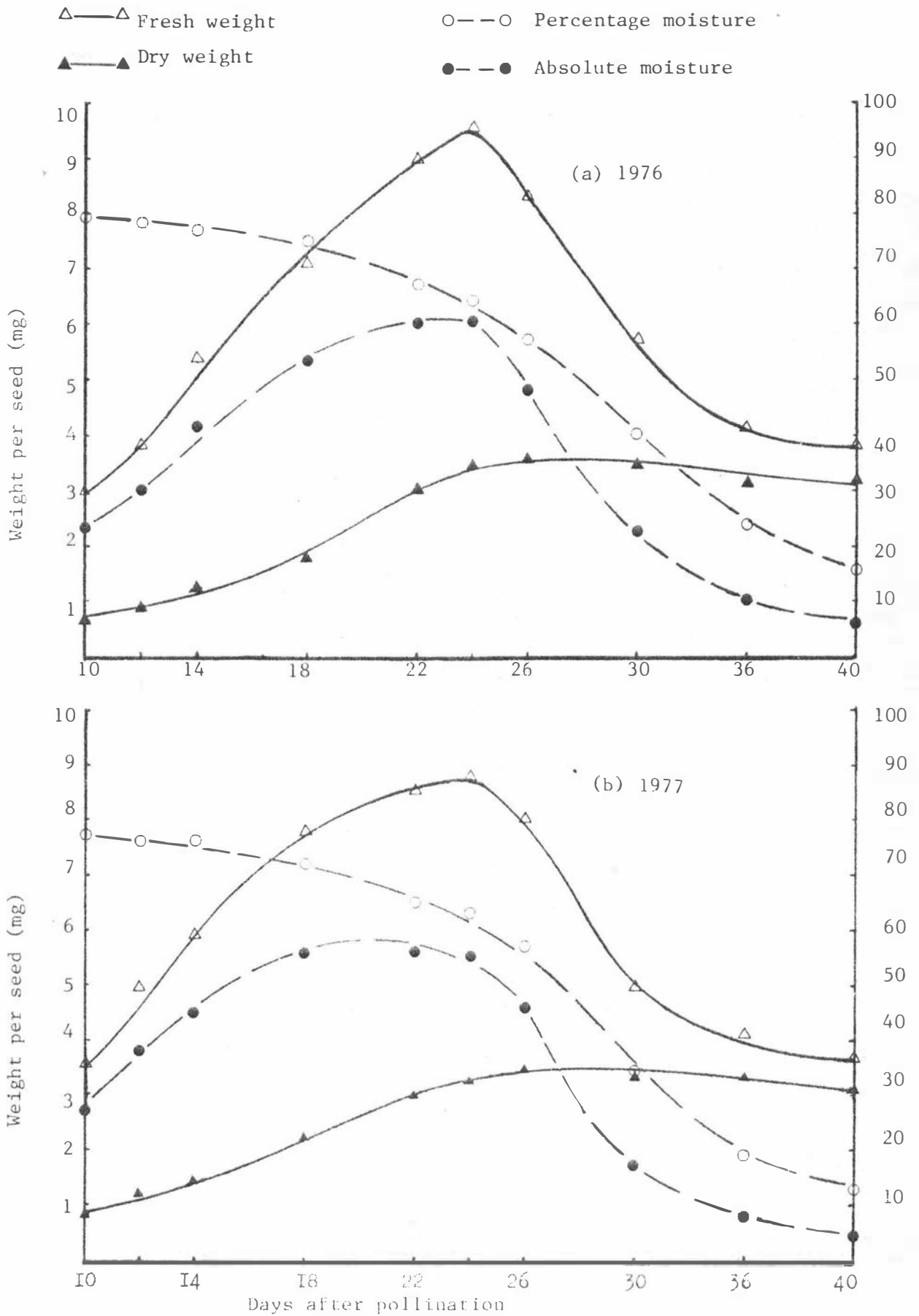
changes occurring in fresh weight throughout the developmental
stages in the 1976 harvested crop.   The results of these two
components were more closely related during the early stages of
seed development.   Subsequently the differences became greater with
the progress of seed maturity.   Highest absolute moisture content
was recorded about 2 days before seed maturity.   Subsequently, a
decrease in moisture content occurred as the seed entered the ripening
stage.   A similar trend, but a lower level of absolute moisture, was
observed in the 1977 harvest.   Seed maximum absolute moisture content
was again reached prior to the point of maximum dry weight but
a distinct plateau effect occurred between 18 and 24 days after
pollination.   Absolute moisture then declined rapidly as drying
occurred during seed ripening.   The results of absolute moisture of
seed samples harvested in 1975 are also shown in Appendices (10-12).

In the 1976 harvest seed moisture content expressed in terms
of percentage moisture was generally found to be relatively high
(over 70%) up to 18 days after pollination.   Then it declined
gradually until 26 days (the point of maximum dry weight) when the
moisture content was approximately 57% (Appendix 8).   Finally it
dropped sharply to about 16% 40 days after pollination (Fig. 8a).
The rate of reduction of moisture was about 1.4% per day during the
period from 10 to 26 days after polliantion and then increased
to 3% per day from 26 to 40 days after pollination.   A similar
trend, but with slightly lower moisture levels particularly in the
latest stages of development, occurred in the 1977 harvested crop
(Fig. 8b).   However, the moisture content of the seed at maturity
(the point of maximum dry weight) was again 57%.   Finally it
dropped sharply to about 13%, 40 days after pollination.   The rate
of moisture loss was about 1.3% per day during the 10 to 26 day
period and then increased to 3.2% per day between 26 and 40 days
after pollination, (Appendix 9).   The values of percentage moisture
for seeds from tagged flowerheads harvested in 1975 are given in
Appendices (10-11).

(d)   Changes occurring in different parts of a flower head:

The fresh weight, dry weight, and moisture content of seeds
removed from different parts of individual flower heads at different
developmental stages was also determined (Appendix 14).   Generally,

the fresh weight of seeds from both the top and bottom parts of
the head was lower than corresponding values in the mid-upper and
lower parts. These differences were more pronounced in the open
pollinated crop than in the controlled pollinated crop. Similar
patterns of seed dry weight change were also recorded. The results
for percentage moisture were reasonably consistent, particularly in
the early stages of development. In the open pollinated crop, the moisture
content of seeds from florets in the top and bottom portions
of individual heads were higher than corresponding values from the
middle portions of individual heads. (Appendix 14).

The values for moisture content of seeds of the same develop-
mental age but harvested at different times also show some variation.
(Table 3). The moisture values in seeds from all portions of heads
harvested late in the season are obviously higher than corresponding
results obtained in an early harvest. Although the moisture results
are fairly consistent at any one harvest time there was a suggestion
that seeds in the bottom portion of the head had a higher moisture
content than seeds from florets in other positions.

TABLE 3: Differences in moisture content of seeds with the same
maturity (30 days after pollination) harvested at different
dates (controlled pollinated crop).

Position of seed in the flower head	Date of harvest	
	17/4/76	6/5/76
Top	55.2	65.8
Mid-upper	58.8	65.0
Mid-lower	58.1	66.1
Bottom	59.7	67.0

9. Germination and viability tests: Germination capacity may be
expressed as the ability of
seed to germinate when placed in optimum conditions of moisture,
temperature and aeration. On the other hand, the viability of a
seed may be determined on the basis of the staining pattern revealed
in tetrazolium tests. As the present study is concerned with Legumin-
osae, the percentage of hard seeds was also included in the reported
percentage of viable seeds (Anon. 1976a).

(1) <u>Standard germination tests</u>: The results of germination
tests on freshly harvested
seeds from the 1976 crop are shown in Fig. (9a) and Appendix (15).
During the 10 day laboratory germination period, no germination was
recorded in the seed samples harvested 10-18 days after pollination
(Fig. 9a).   Germination occurred in a small percentage of 22
day-old seeds but remained low even at later stages of development.
However, the germination percentage increased markedly if the laboratory
germination test period was extended from 10 to 30 days (Fig. 9a).
Germination occurred during the later part of the germination test
period in seeds harvested 18 days after pollination. Thereafter
variable results occurred in the later stages of development. A high
percentage of hard seed appeared in 36 day old seeds when the
moisture content of the seed had fallen to 24%.   Viable seeds were
first detected in seed samples harvested 14 days after pollination,
when seed dry weight was 34% of its maximum value and moisture content
was 77%. These viable seeds appeared as fresh ungerminated seeds
and abnormal seedlings, neither of which can be classed as seeds of
agronomic value.   However, total viability rose rapidly up to
96% when seeds reached maturity 26 days after pollination. There-
after germination level dropped slightly in later maturities (Appendix
15).

As illustrated in Fig. (9b), in the 1977 harvest, the germination
capacity of seeds tested immediately after harvest showed a different
pattern from the corresponding values of the 1976 harvest.   There
was a very small percentage of germination in 18 day old seeds which
increased slightly in 22 day-old samples during the first 10 days
of the laboratory germination test period. The germination then fell
to lower levels in later seed maturities. When the germination test
period was extended to 30 days, some few seedlings were produced
even from 14 day old seed samples which showed 76% moisture and 41%
of its maximum dry weight. The highest germination result of over
40% was recorded in the seed samples 18 days after pollination.
Germination percentages declined again with the progress of seed
maturity (Fig. 9b). Hard seed was first formed in 30 day old
seeds when seed moisture was about 34%. Nearly 80% of hard seeds
were recorded in the 40 day old samples.   Viable seeds were first
observed in the seed samples harvested 12 days after pollination. Due
to the rapid increase in viability about 95% of viable seeds were

Fig 9: Percentages of normal seedlings, abnormal seedlings, fresh ungerminated seeds, viable seeds and hard seeds in samples harvested at different developmental stages.

△− −△ Fresh ungerminated seeds

▲− −▲ Hard seed

×− −× Abnormal seedlings

●———● Normal seedlings (10 days test period)

○———○ Normal seedlings (30 days test period)

□———□ Total viability (Tetrazolium test)

■———■ Total viability (Standard germination test)

(a) 1976 harvest (freshly harvested seeds)

(b) 1977 harvest (freshly harvested seeds)

(c) 1977 harvest (after 3 months' storage)

Days after pollination

Percentage

44
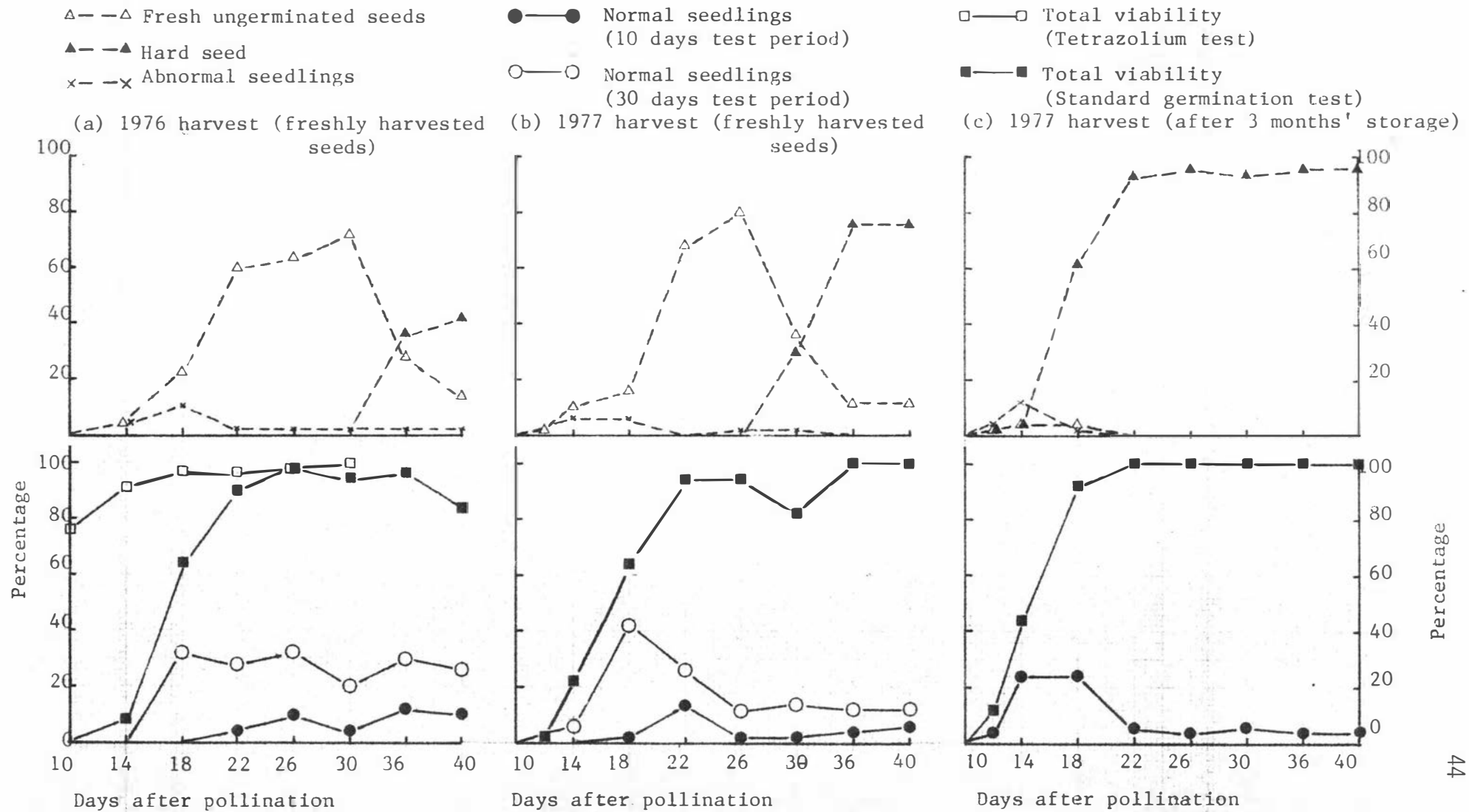
formed in 26 day old seeds reaching a maximum viability of 100%
in 36 and 40 day old seed samples. (Fig. 9b, Appendix 16). This
rapid increase was due almost entirely to the contribution of the fresh
ungerminated seed category, rather than to any increase in germination
capacity.

When seeds of different developmental ages were stored under the
room temperature conditions (Appendices 17 and 18) for about 3 months,
the percentages of germination, hard seed, total viability and fresh
ungerminated seed obtained in laboratory germination tests changed
dramatically (Fig. 9c, Appendix 19).Even 12day old seeds gave some
germination and maximum germination results following a 10 day germin-
ation test period were recorded in seeds harvested 14 and 18 days after
pollination. However, the percentage of germination dropped in more
mature seed samples due to the presence of increasing numbers of
hard seeds. Small numbers of hard seeds first appeared in 12 day old
seeds increasing to more than 90% in seed samples harvested 22 days
after pollination. This high percentage of hard seed was maintained
in progressively more mature seed samples (Fig 9c). A small percentage
of viable seeds was observed in seed samples harvested 12 days after
pollination, over 90% viability being recorded 6 days later. Maximum
viability of 100% was attained 22 days after pollination and maintained
throughout the later periods of maturity (Fig. 9c). The general pattern
of seed viability was similar to the situation with freshly harvested
seeds, major differences being only on a time basis. However, the most
dramatic difference occurred in the fresh ungerminated seed component.
Obviously in stored seed samples, a high proportion of the fresh ungerm-
inated seeds which had been previously observed in freshly harvested
samples had become hard. These seeds therefore contributed to the earlier
development and more extensive nature of the hard seed component
observed in tests carried out on samples following 3 months' storage.

The laboratory germination test results for seeds harvested in
1975 are also presented in Appendices (20-22). As shown in Appendices
(20 and 21), seeds harvested from the 1974 autumn-sown crop gave high
percentages of viability in the very early stages of seed development
especially from heads harvested after peak flowering date (Appendix 21).
On the other hand, seed harvested from the 1974 spring-sown crop attained
viability more gradually (Appendix 22). Seeds from the 1974 autumn-
sown crop produced hard seeds starting from 22 days after tagging (full
flowering) or peak flowering (Appendices 20 and 21) whereas seeds from

spring-sown crop contained no hard seeds even in samples harvested
30 days after tagging (Appendix 22).

(2) Biochemical tests: As shown in Fig. (9a) and Appendix (23),
nearly 80% of the seeds harvested 10
days after pollination were evaluated as viable seeds according to
staining in tetrazolium solution. More than 90% of the seeds were
viable 4 days later, reaching a maximum of 97% 18 days after pollin-
ation. Starting from 26 days after pollination a certain percentage
of seeds were not stained due to seedcoat impermeability to tetrazolium
solution. However, they were shown to be viable when the seedcoat
was chipped, allowing penetration of the tetrazolium solution.

(3) Additional tests on seed viability:

(a) Germination results of seeds taken from different parts
of individual flower heads: As shown in Figure (10)
and Appendix (24), the
percentages of total viability of seeds from different parts of the
head from the controlled pollinated crop were slightly higher than
corresponding results from the open pollinated crop. However,
the percentage of normal seedlings produced in samples harvested
from the open pollinated crop was considerably higher than in the
controlled pollinated crop. This was a direct reflection of the
greater number of fresh ungerminated seeds produced in tests from
seeds from the controlled pollinated crop. Between the different parts
of the head in the controlled pollinated crop, the top and mid-upper
portions gave similar germination results. There was a progressive
increase in germination capacity in seeds produced towards the bottom
of the head (Fig. 10). Although the same trend was not observed in
the open pollinated crop, seeds from the bottom portion of the head
still showed the highest percentage of normal seedlings. In all
cases the percentages of fresh ungerminated seeds in different parts
were reciprocal to their respective germination results. Abnormal
seedlings appeared at a variable but low level in all portions of
the head.

(b) Effect of prechilling on germination of freshly
harvested seeds: The germination results of freshly
harvested seeds following different
periods of prechilling are presented in Figure (11).   The seeds

FIG. 10: Percentage of total viability of freshly harvested seeds taken from different positions in flower heads harvested 30 days after pollination.

T, MU, ML and B represent top, mid-upper, mid-lower and bottom portions of an individual flower head respectively.
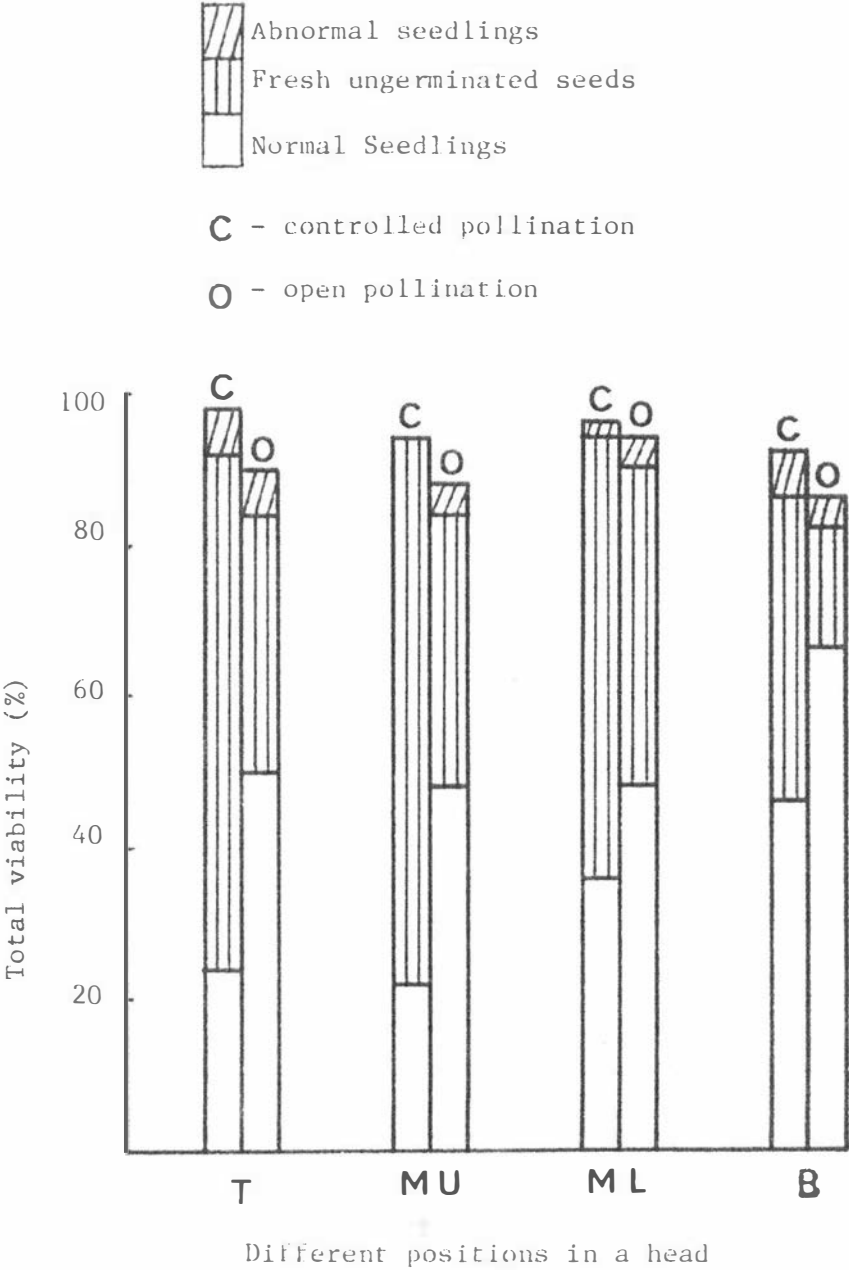
FIG. 11: Percentage of total viability of freshly harvested seed samples after prechilling at 5°C for different periods.

48
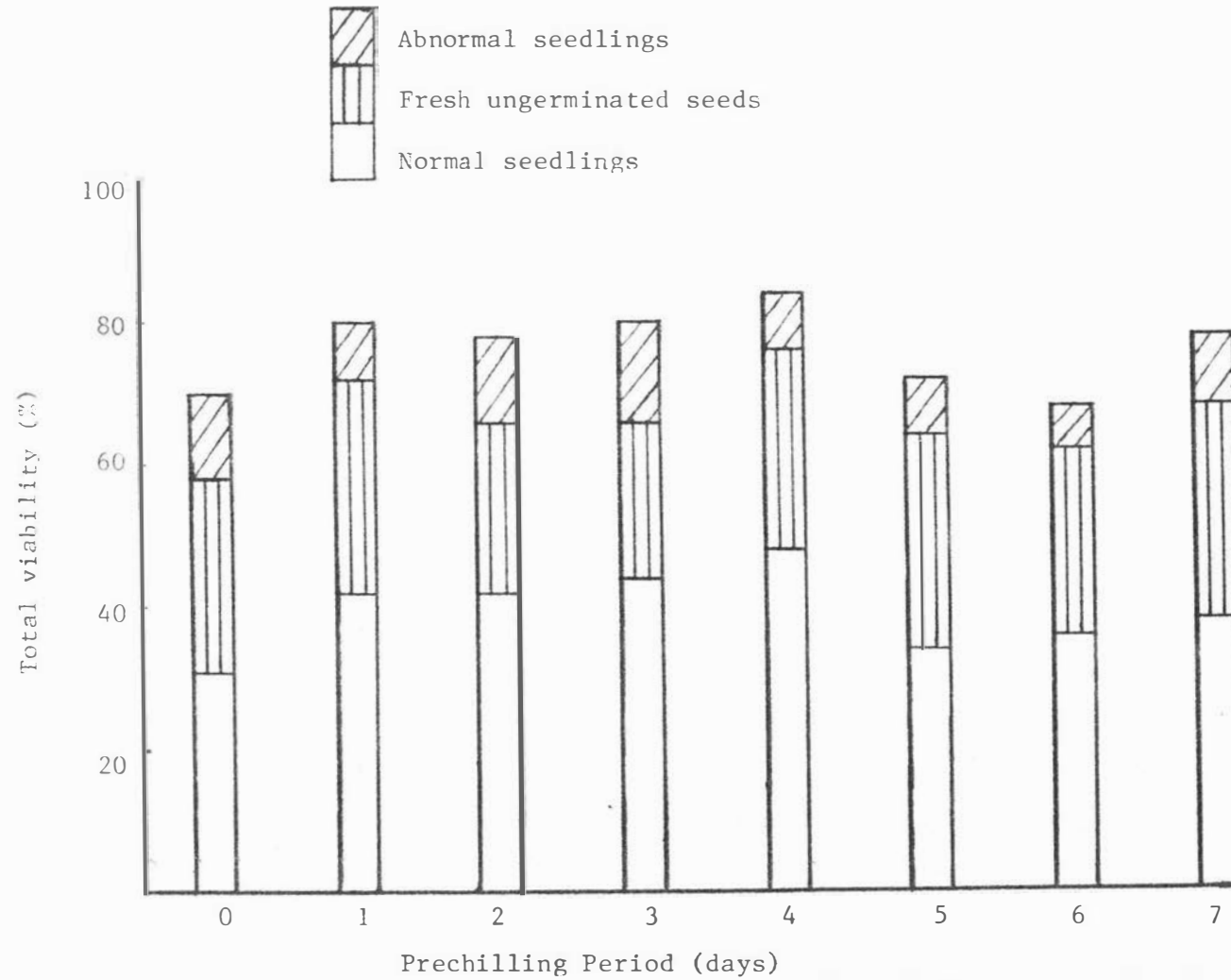
used were harvested 22 days after pollination from the 1977 crop.
The percentage of total viability and germination was similar in all
treatments, although there was an indication that samples prechilled
for 1-4 days gave slightly higher values than the others. The
proportion of fresh ungerminated seeds present was fairly consistent.
(Appendix 26). A similar pattern occurred in the 1976 crop although
there was a trend for the percentages of fresh ungerminated seeds
and dead seeds to alter with seed age (Appendix 25).

(c) Effect of gibberellic acid on germination: In the present
investigation,
neither germination nor total viability were improved by soaking
seeds in the gibberellic acid (GA_3) solution or by germinating
seeds on blotters soaked in GA_3. However, when 22 day old seeds
were soaked in GA_3 solution without receiving any prior prechilling
treatment, a much higher germination percentage was recorded.
Nevertheless, the percentage of total viability was lower than in
other treatments. When 18 day old seeds were treated with GA3
solution, they produced about 50% more dead seeds than untreated
seeds (Appendix 27). These results show that no consistent
improvement in the germination or total viability of 'Pawera' red
clover seeds is obtained by the use of exogenous gibberellic acid.

(d) Effect of oxygen gas on germination: The germination
of seed in an
oxygen enriched atmosphere had no obvious effect on germination
percentage, although there was a suggestion of a slight improvement
in the percentage of normal seedlings when samples received oxygen
at prechilling and again during germination. However overall,
oxygen treatments had no dramatic effect in improving seed
germination capacity (Appendix 28).

(e) Effect of depth of seed coat cutting on germination:
The results of germination tests following seed
coat cutting treatments are shown in Figure (12) and
Appendix (29). In 22 day old seeds, the percentage of total viability
was lowest in shallow-cut seed samples while the corresponding
values in intact and in deep-cut seed samples were similar. However,
the deep-cut treatment gave the highest percentage of normal seedlings
and contained no fresh ungerminated seeds (Fig. 12). This suggests
that the production of fresh ungerminated seeds while it is not

Fig 12: Percentage of total viability of freshly harvested seed samples
following different intensities of seed-coat injury.

Abnormal seedlings

Fresh ungerminated seeds

Normal seedlings

A- Intact seed

B- Cut through seed coat only (shallow cutting)

C- Cut through seed coat and underlying membrane
(deep cutting)
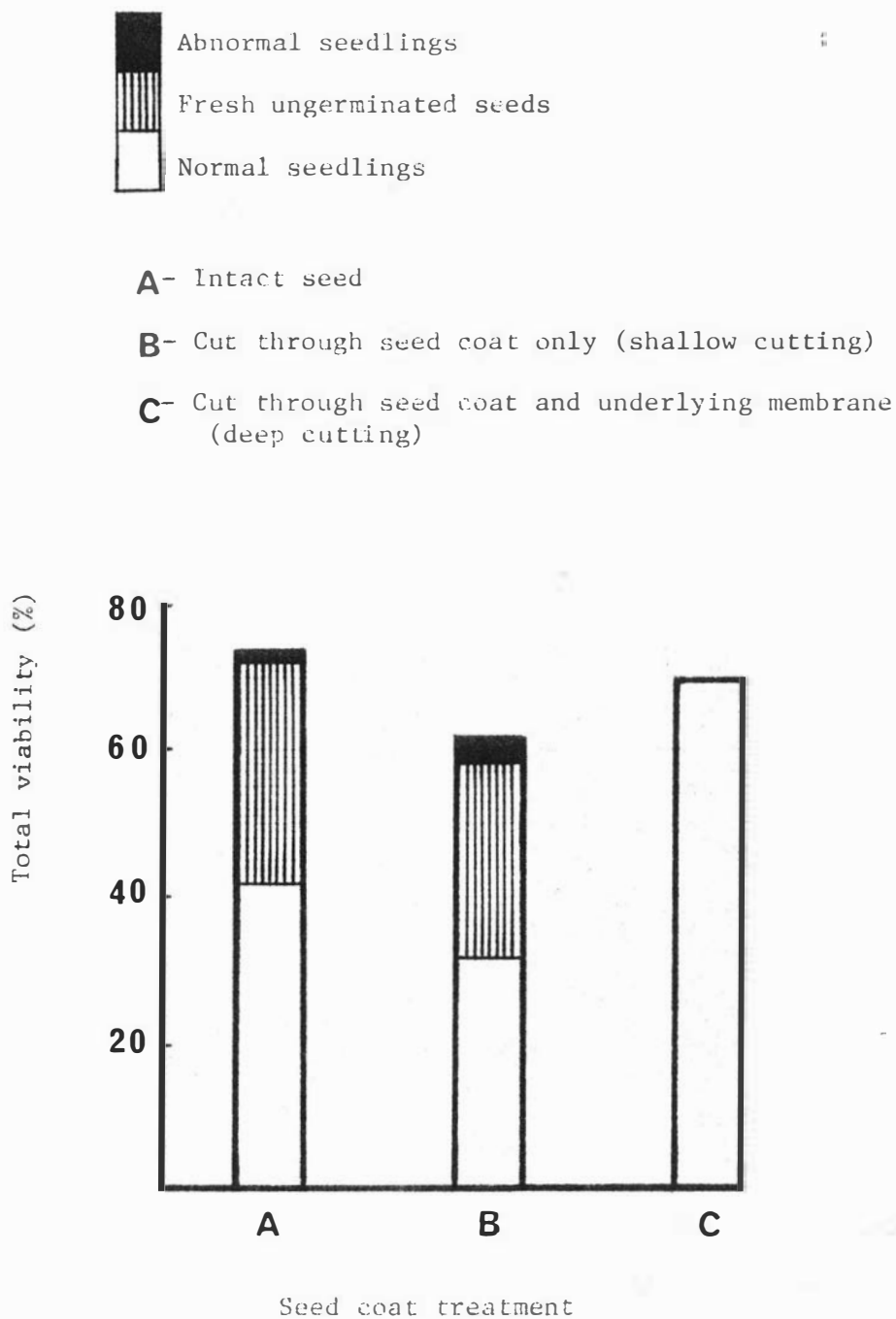
caused by the restriction imposed by the seed coat itself may well be due to a germination restriction caused by the membrane underlying the seed coat.

(f) Germination capacity of seeds in different colour categories: The germination results of seeds before and after storage are given in Appendix (30). There was a trend for yellow seed to produce a higher germination percentage than brown and purple seeds. This was a reflection of the higher hard seed contents in brown and purple seed samples. A similar situation occurred when the same seed colour categories were stored for 3 months (Appendix 30).

(g) Effect of mechanical scarification on germination: The germination results of scarified and unscarified seeds following 3 months storage are shown in Figure (13) and Appendix (31). Unscarified samples contained 96% hard seed. However, germination tests carried out on samples which have been scarified for a relatively short duration and at relatively light pressure showed a dramatic drop in hardseededness.

A small percentage of seeds still remained hard when the duration of treatment at constant pressure was short (1-2 min.) or the intensity of pressure for constant duration was low (4-6 psi). On the other hand, a small increase in the percentage of abnormal seedlings occurred when the duration of treatment or intensity of pressure was increased to 6 min. of 10 psi or 14 psi for 2 min. respectively (Fig 13, Appendix 31). The best pressure and duration combination which resulted in the highest percentage of normal seedling was recorded at 8 psi for 2 min. or 10 psi for 3 min. (Fig. 13).

Fig 13: Germination percentage of unscarified and mechanically scarified 'Pawera' red clover seeds.

Non-viable seeds

hard seeds

Abnormal seedlings

Normal seedlings

(a) Different scarification durations
(constant pressure 10 psi)
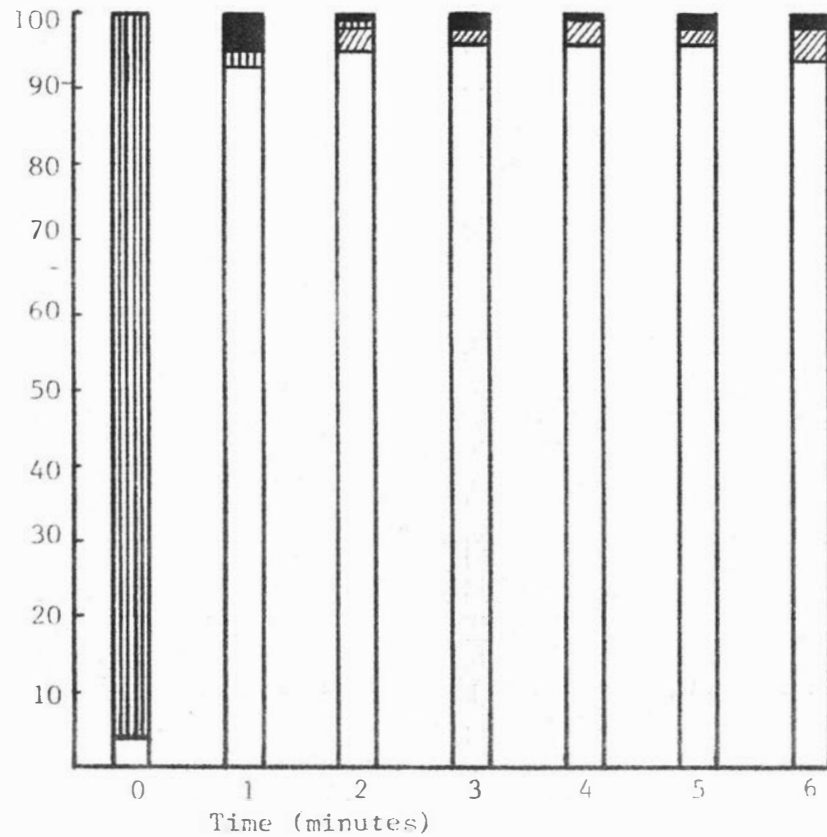
(b) Different scarification pressures
(constant duration 2 mins)

Time (minutes)

Pressure (psi)
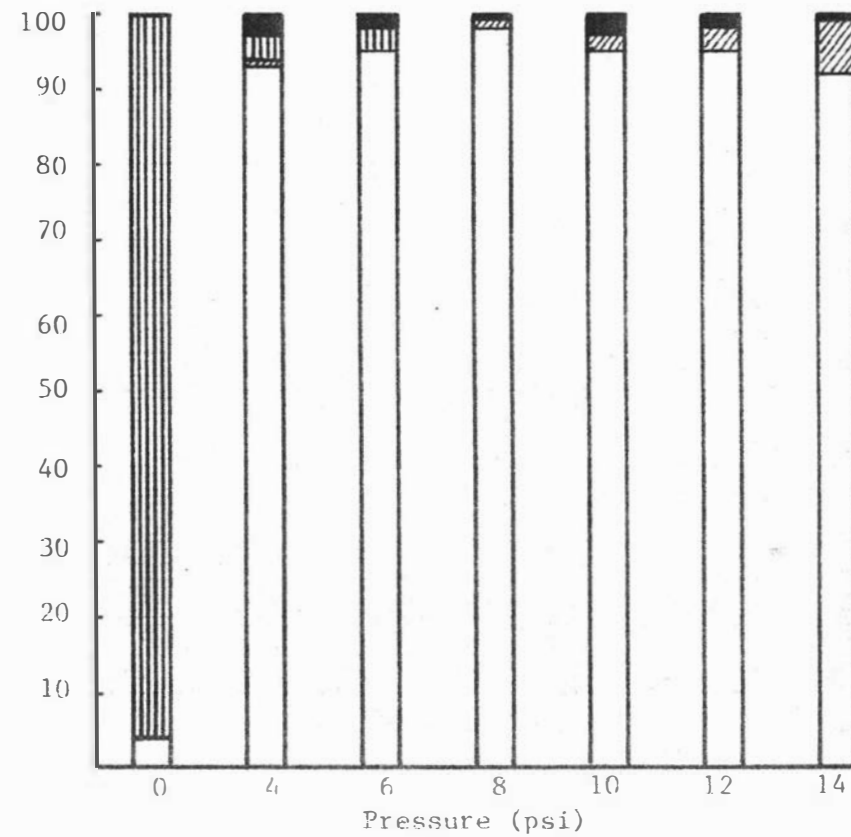
DISCUSSION

It has been suggested that internode numbers can be used to distinguish between 'Grasslands Hamua' red clover (cowgrass, broad red, early flowering diploid variety) and 'Grasslands Turoa' red clover (Montgomery, late-flowering diploid variety) (McNeur 1960). However, the numbers of internodes present on stems at the time of floral initiation and peak flowering in 'Grasslands Pawera' red clover show a wide range depending on time of sowing, and also presumably on climatic conditions as suggested by McNeur (1960). This variation suggests that the recording of internode numbers is not necessarily a reliable criterion for varietal identification in this cultivar.

Observations of the flowering pattern and sequence in autumn sown 'Pawera' red clover crops showed that the date of peak flowering in the present studies occurred slightly earlier than the date reported by Clifford (1974) who obtained a maximum number of flowering heads in 'Pawera' red clover about mid-February. This slight variation may be due to regional and climatic conditions, date of sowing or date of closing. The rate of increase in the numbers of totally withered heads appeared to be accelerated by slightly increased temperatures in early February. However, the number of days required for floret opening over the whole head in the autumn sown crop in the present study closely agrees with findings by Clifford (pers. comm.), this process taking about 7 days with a floret opening rate of 18 flowers/day. Free (1970) also suggested that it takes 6-10 days for all the flowers on an individual head of red clover to open. Since pollination begins in individual heads at the time of the half fully flowering stage (i.e. stage 2 in the flowering sequence) seeds that develope from early pollinated florets would be 3-4 days older than those produced in late flowering florets. The age of individual seeds can therefore vary according to the position of the floret from which it originated on the head.

Nectar secretaion is greatest on the first day or first few days of floret opening, being influenced by the maturation of the stigma and stamens (Free 1970). His reports generally agree with observations in the present study on nectar production. He added that the duration of nectar secretion in some species may be very limited. Moreover, factors such as soil moisture, air temperature, atmospheric

pressure, size of nectary and position of the flower on the plant may
influence the amount of nectar produced. It is also recognised that
tetraploid red clovers produce more nectar/floret than diploid varieties
(Anon. 1976b).

In clover crops, successful seed production seems to depend
strongly on the population of effective bee pollinators and on climatic
conditions that influence bee activity during the flowering period.
It has been reported by a number of workers (Griffiths *et al.* 1967;
Free 1970; Clifford 1973; Gurr 1975) that bumble bees are effective
pollinators for various red clover varieties. Such a situation certainly
appears to be the case in 'Pawera' red clover seed crops. However,
tongue length has a profound effect on foraging behaviour. All long-
tongued bumble bees visit red clover flowers always at the front of
the floret and hence brush against the sexual parts of the flower and
cause pollination (Gurr 1961). On the other hand, since the majority
of short-tongued bees use different methods for nectar collection they
do not effect pollination (Cumber 1953; Free 1970; Lawes (1975).
However, as suggested by Gurr (1975) young queens of *B. terrestris*
can be important in seed production as they are actively involved in
nectar collection late in the season. Moreover, *B. terrestris* when
gathering or eating pollen also effects cross-pollination by transferring
pollen from flower to flower (Gurr 1975). Compared with the effective
bee numbers quoted by Umaerus and Grazi (1962) and Clifford (1973), the
bumble bee population in the experimental plot during most of the
flowering season in 1976 was insufficient for the production of high
seed yield. However, the numbers of bees recorded does compare favourably
with the levels reported as adequate for the production of acceptable
levels of seed yield by Free (1970) and Anon (1976b). It should be
noted however that the bumble bee population in the present study
included both effective and ineffective pollinators.

Although some slight variation in the bee population occurred due
to seasonal differences, daily variations were enormous. Day to day
bee numbers and activity within the crop depended greatly on prevailing
weather conditions. Bee numbers in the crop fell to low or zero
levels when it was raining, or windy, when relative humidity was too
high (90-100%), or when the temperature was relatively low. In addition
the presence of moisture on the plants, whether by rain or heavy dew,
seriously discouraged bee activity. These observations generally
support findings by other workers (Fergus and Hollowell 1960;

Friden *et al.* 1962; Free 1970). Conversely, bees worked very actively
and intensely when a favourable day followed unfavourable foraging
weather conditions. This may be due to the need for pollen and ·nectar
by their colonies and because they respond readily to improved weather
conditions as suggested by Free (1970). Although foraging flights
may take place at $12-14^{o}C$ as stated by Free (1970), bee activity is
adversely affected by low temperatures. When temperatures fell to
below $17^{o}C$ the bee population decreased, but increased again with an
increasing temperature. The highest maximum temperature of $28^{o}C$ observed
in the present study apparently had no adverse effect on bee activity.
Conversely, low relative humidities appeared to favour bee activity while
very high relative humidities had an adverse effect. However, the
realtionship between bee population and relative humidity was not always
consistent. Although a clear sky seemed to favour bee activity,
cloudy and calm conditions did not appear to affect their activity
adversely if plants were dry. If it was raining or plants were thoroughly
wet, no bees appeared to work. Wind velocity can also affect bee
activity considerably if the crop is exposed to the wind directly.
While these factors of climate interrelate in affecting bee activity,
temperature effects showed a reasonably direct and positive relationship
with the number of bees present in the crop.

The length of the corolla tube of a floret of 'Pawera' red clover
was found to be about 11-12 mm which is approximately the same length
reported by Anderson (1973a) in the same cultivar and in other tetraploid
red clover varieties by other workers. (Umaerus and Grazi 1962; Anon.
1976b).

The failure of seed setting in flower heads protected from insect
pollination needs no further explanation as it has also been reported
by numerous workers (Fergus and Hollowell 1960; Griffiths *et al.* 1967;
Palmer-Jones 1967; Anon 1976b) that red clover is self-sterile.
Similar findings have also been recorded by Hyde *et al.* (1959) who
found no seeds in a number of 'Grasslands Hamua' red clover flower
heads which were covered by bags to prevent access by insects.

The higher number of seeds set (both seeds per flower head and
per 100 florets) in the 1975 harvest compared with 1976 may have been
due to higher bee populations and favourable weather conditions
experienced during the 1975 summer period. It seems that the low bee

population during most of the flowering season in 1976 was responsible
for the reduction of seed yield. However, the mean number of seeds
set per head in 1976 is still slightly higher than the rate recorded
by Anderson (1973a). The present investigation therefore stresses
the importance of adequate numbers of suitable pollinators at the time
of flowering to produce high seed yields in red clover crops as shown
by other workers. (Gurr 1962; Griffiths *et al.* 1967; Anderson
1973a). The higher percentage of seeds setting in any particular
portion of an individual head is apparently not due to higher numbers
of florets in different positions. The proportion of florets present
in different parts of individual heads is similar. Thus the higher
seed numbers present in the middle portions of individual heads
compared with the top and bottom portions may be because the easier
accessibility of more central florets for bee visitation. On the other
hand, florets in the top and bottom portions of flowerheads do not
open uniformly and bees can only pollinate a limited number of florets
at each visit.

The changes in seed components during seed development follow the
typical pattern of growth reported by previous workers (Hyde 1950;
Hyde *et al.* 1959; Griffiths *et al.* 1967; Adam *et al.* 1970). Since
the changes in the present study were monitored starting from 10
days after pollination, those changes that occur during the "growth
stage" (Hyde 1950; Hyde *et al.* 1959) or "positive acceleration phase"
(Adam *et al.* 1970) were not included. According to Hyde (1950)
and Hyde *et al.* (1959) the period from 10 to 26 days after pollination
is occupied by a food reserve accumulation stage. During this stage,
the rate of increase in fresh weight is rapid up to 14 days and is
followed by a more uniform rate of growth as suggested by Griffiths
et al. (1967). These two phases of fresh weight changes were more
distinct in the 1977 crop than in the 1976 crop. More favourable
growing conditions, possibly involving greater availability of moisture
and nutrients, seemed to contribute to higher values of individual
seed fresh weight in 1976 than in 1977. During the food accumulation
stage, seed size, measured in terms of fresh weight, also increased
uniformly and reached its maximum about 2 days before the date of seed
maturity. Differences in seed size between seeds of different ages
could also be seen clearly after 3 months storage. Those seeds harvested
earlier than the date of maximum fresh weight (approximately 24 days
after pollination) were not fully developed while those from later
harvests showed no obvious variation in seed size.

The lower amount of absolute moisture compared with fresh weight during the later part of the food reserve accumulation stage was due to increased accumulation of seed dry weight. The rate of reduction of moisture during this stage was similar in both years. Moreover, at the end of this stage the moisture content of seeds recorded in both 1976 and 1977 were approximately the same. It is essential to consider seed moisture values along with the data for other parameters in order to be able to evaluate the developmental sequence on a common basis since the age of seeds in terms of days from flowering varies widely with environmental conditions (Pollock and Roos 1972). Similarly, the role of seed moisture levels in the development of seed impermeability and in the comparison of differences in impermeability has been emphasised by Quinlivan (1971a).

In the present study, the amount of dry matter increase during the food reserve accumulation stage was much higher than the results reported by Hyde *et al.* (1959). This may be due to a slightly longer developmental period which results in heavier seed weight (Aitken 1939) or to varietal differences. Similar observations have also been reported by McKee *et al.* (1955).

Variation in the relative proportions of time occupied by different developmental phases may be due to different species, varieties and climatic conditions (Griffiths *et al.* 1967; Pollock and Roos 1972; Schwass 1973). In lucerne (*Medicago sativa* L), the periods occupied by each stage of seed development takes considerably longer than those of clover crops (Kowithayakorn 1978). Similarly, in bean seeds (*Phaseolus vulgaris* L.) fresh weight reaches its maximum in about 36 days and maximum dry weight occurs about 44 days after flowering (Lowenberg 1955). According to Al-Tikrity *et al.* (1974) the numbers of days required from pollination to seed maturity in crownvetch (*Coronilla varia* L.) seeds varies significantly between years, fields and dates of pollination. The differences reported by various workers are believed to be associated with different temperatures, precipitation and solar radiation. The later part of the growing season in the present study was cold and moist, such conditions being implicated in retarding the rate of seed maturation and ripening.

Although the growth of the seed ceases concomitantly with the loss in fresh weight (Loewenberg, 1955), further increase in seed dry weight following the decline in fresh weight and moisture content could be due to continuing deposition of food reserve materials beyond the time of seed enlargement (Harrington 1972; Pollock and Roos, 1972). However, it is quite clear that the process of food transference from the parent plant to the seed ceases by the end of the food reserve accumulation stage since there is no further increase in dry weight.

The last or ripening stage (Hyde 1950, Hyde *et al.* 1959) occupies about 14 days following the food reserve accumulation stage. According to Griffiths *et al.* (1967) the first half of this ripening is characterised by a rapid loss of fresh weight due to drying of the seed. The rate of reduction in seed moisture and the final values of this component seems to depend mainly upon weather conditions, especially relative humidity, during the ripening period. However, in two successive years the rates of loss of seed moisture during this stage show close similarity. Moreover, in both 1976 and 1977, seed fresh weight at the end of this stage is approximately the same, falling to less than half of its maximum value. It is generally stated that dry matter content is realtively constant during this stage (Hyde 1950; Hyde *et al.* 1959; Griffiths *et al.* 1967). However, in the present investigation dry matter content showed a detectable drop when the crop was harvested at a later date (40 days after pollination) in both years. The possible cause of this reduction in seed weight is not known. Even though seeds shrink after storage, they show no obvious differences in size. Generally maximum seed viability is maintained throughout the ripening period and hard seeds develop in freshly harvested seed samples a few days following the attainment of seed maturity when the moisture content ranges between 24% and 34%.

The effect of environmental conditions on changes in seed components is quite remarkable. The rates of increase or decrease in all seed components in seeds harvested from tagged flower heads from the 1974 autumn sown crop are markedly faster than those from the 1974 spring sown crop. This delayed rate of seed development in the spring sown crop may be mainly due to low temperatures, low light intensities, high atmospheric relative humidities and high rainfall. All of these conditions can directly influence the rate of pollen tube growth

(Maheshwari 1950; Hutton 1960; Povilaitis 1960; Gerassimova-navashina *et al.* 1969 ; Maun *et al.* 1969), the rate of fertilization (Johansen 1940; Evans 1961; Free 1970) and consequently seed development and production (Thomas 1961; Skerman and Humphreys 1973; Schopmeyer 1974; Clarkson and Russell 1975). Cold and moist climatic conditions during seed development are also assumed to be responsible for the higher percentages of seed moisture in the spring sown crop. The slow reduction in seed moisture during the ripening stage seems to be mainly related to high relative humidity conditions as suggested by Quinlivan (1971a).

In determining seed characters it is important to consider not only the role of environmental conditions but also differences in the position and time of formation of seeds on a plant (Grant-Lipp and Ballard 1964). Similarly, differences in seed permeability depend partly on ecological conditions during seed maturation and partly on the position of seed on the plant (Marbach and Mayer 1974). As an example, in fodder beet, the 1000 seed weight varies from 2.4 to 40.8 g according to the position seeds occupy in the inflorescence (Potekha 1974). In the present study, however, no such extreme variation occurred, although some slight differences, especially in seed moisture levels, were recorded.

The number of hard seeds formed in freshly harvested seed showed some variation from year to year. According to these observations there seemed to be no close relationship between seed moisture content and the formation of hard seed during the ripening period. The slight variation in the rate of seed development recorded in different portions of a flower head may be related to variation in the number of hard seeds formed during the ripening stage. It has been suggested by various workers (Hyde *et al.* 1959; Winter 1960; Chow and Crowder 1974; Egley 1974) that seed coat impermeability may be connected with seed maturity. The first appearance of hard seed in freshly harvested seed samples of Pawera red clover seems also to depend upon climatic conditions during the seed developmental and ripening period. Hard seeds seem to form in the early part of the seed ripening stage, particularly if the weather is warm and dry during seed development and ripening as also reported by Baciu-Miclaus (1970) and Quinlivan (1971a).

Furthermore, as suggested by numerous workers (Stevenson 1937; White and Stevenson 1948; Weihing 1962; Quinlivan 1971a) the

permeability of the seed seems to be pre-determined genetically. If seed colour is linked with genetic factors, the above proposal is supported by the findings of the present study that lighter coloured seeds produce more germination than darker ones. On the other hand, the percentage of hard seed (Witte 1931a; Grant-Lipp and Ballard 1964; McComb and Andrews 1974; Sidhu and Cavers 1977) and the degree of hardness (Crocker and Barton 1953; Barton 1965a; Mayer and Shain 1974) also seem to be influenced by environmental conditions during the ripening and storage period. Other factors, such as seed size (Black 1959) and fertilizers (James and Bancroft 1951) may also be concerned with variation in hard seed formation in different seed lots. In machine-harvested commercial seed samples, the mean percentage of hard seed in 'Pawera' tetraploid red clover is about 4% compared with mean figures of 8-9% hard seed in 'Turoa' and 'Hamua' diploid red clovers (Scott 1978 pers. comm.). Since tetraploid seeds are larger than diploid seeds, they may be more vulnerable to mechanical abrasion during harvesting and threshing which can result in a reduction in the number of hard seeds.

Variations in seedcoat colour are common in seed of many crops (West and Harris 1963). Such variation occurs not only in a single genus, but also within the same species (Ambegaokar 1976). In the present study seed colour changes in Pawera red clover occurred at a slightly later date during the food reserve accumulation stage of seed development compared with the findings of Hyde *et al.* (1959) in diploid varieties. In their report, seedcoat colour began to change in both red and white clover about 18 days after pollination when the seed weight was about 80% of its final dry weight. These colour changes were complete about 6 days later which is about the same time period required for the commencement of colour changes in 'Pawera' red clover seeds. This delay may be due to a cultivar difference or to different climatic conditions. The results for two successive years, however, show close similarity in the levels of seed moisture content and dry weight at the first signs of change in seedcoat colour. There was a negative correlation between the proportion of yellow seeds and seed maturity or seed dry weight accumulation. By contrast, brown and purple seeds showed a positive correlation with seed maturity. It is therefore clear that immature seed samples contain more yellow seeds whereas mature samples have more brown and purple coloured seeds.

Seed quality components, such as viability, germination and hard seed content show some changes with the progress of seed development. These changes, especially in freshly harvested seed samples seem to vary somewhat from year to year or even within a year depending upon the climatic conditions, date of sowing or time of harvesting. The trends of viable seeds in two years however, are very similar except during the later period of ripening. The drop in seed viability in some mature seed samples, especially at the last harvest in 1976, was mainly due to the incidence of mould during laboratory germination. Wet weather conditions during the ripening period and in consequence a slightly high seed moisture content at the time of testing the germination of freshly harvested seeds seems to favour the growth of fungi on seed samples. This effect occurred despite the precautions which were taken to avoid high mould incidence by germinating freshly harvested seeds at $15^{o}C$ instead of $20^{o}C$. On the other hand, when the viability of freshly harvested seed was determined by employing the tetrazolium test method, even 10 days old seeds showed a high percentage of viability. Although the enzyme systems present in young seeds are obviously sufficiently active in the young embryo to react well with the tetrazolium salt, the seed seems to retain the inability to produce a seedling. Similar findings have been reported in lucerne (Kowithayakorn 1978) and ground nut (Teng and Hor 1975).

Data obtained from freshly harvested seed samples were compared with the performance of similar seeds tested for germination after a period of 3 months storage. The two sets of results are remarkably different. It is well known that the seeds of many species require a short period of dry storage for after-ripening (Crocker and Barton 1953; Barton 1961b; Cathey 1975; Hess 1975; Villiers 1975). This is likely to be the situation in the present study. The great reduction in the numbers of fresh ungerminated seeds which occurs following short term storage may be a reflection of the need for Pawera red clover seed to complete a period of after-ripening during dry storage as occurs in other clover varieties (Nakamura 1962) and some vegetable seeds (Cathey 1975). The increase in germination capacity in even comparatively young seeds following storage may also be due to post-harvest changes during the after-ripening period (Hill 1971). In contrast, the sharp decline in the germination capacity in more mature seeds following storage are closely correlated with a rapid increase in

the percentage of hard seeds. These results agree with similar findings by Hyde *et al.* (1959) in diploid red clover.

Compared with results by Hyde (1950) and Hyde *et al.* (1959) with diploid red and white clover, hard seeds and viable seeds first appear in seed samples of 'Pawera' red clover harvested 12 days after pollination. Although the time of onset of seed viability in 'Pawera' tetraploid red clover is similar to the situation in diploid varieties it was slightly slower than that of some grass species (Grabe 1956; Hill 1971) and weed species (Kinch and Termude 1957; Winter 1960). However it is considerably faster than some other legumes such as *Desmodium* (Chow and Crowder 1974) and some groundnut varieties (Teng and Hor 1975).

The great increase in hard seeds, especially in the early stages of seed development following storage may be due to the drying process which occurs through the hilar fissure as suggested by Hyde (1954). A similar substantial increase in hard seed after a short period of storage has been reported by Kowithayakorn (1978) working with the seeds of lucerne. There are obviously great differences in the percentages of hard seeds found in seed samples depending on whether tests are carried out immediately after harvest or after a short period of dry storage. In the present study, major differences in the rate of onset of hardseededness and the extent of hard seed in seed samples occurred depending on the time interval between harvesting and subsequent laboratory testing. In freshly harvested seeds the proportion of different seed categories (normal and abnormal seedlings, fresh ungerminated seeds and hard seeds) was in many cases very different from corresponding levels of these categories in tests carried out after even short term (3 months) storage. It is therefore important to precisely define the pretesting history of seed samples before making comparisons between results.

'Pawera' red clover seeds are first capable of germinating when the moisture content of the seeds is about 76-77% and dry weight about 35 to 40% of its maximum weight. These findings show close agreement with reports by Hyde (1950) and Hyde *et al.* (1959) with diploid red and white clover varieties. Hyde *et al.* (1959) working with clovers and Sidhu and Cavers (1977) with *Medicago lupulina,* reported

that a period of only 4 days is required between the production of the first viable seeds and the attainment of full viability. By comparison a time interval of about 12-14 days in freshly harvested seeds and about 10 days in sotred seeds is required in 'Pawera' red clover. This compares reasonably well with the 15 day interval in lucerne (Kowithayakorn 1978) but is shorter than the situation in groundnut, which varies from 20-30 days with different varieties (Teng and Hor 1975). This delay in the rate of attainment of full viability may be due to seed size differences as reported by Francis and Gladstones (1974). However, variation in the development rate to full viability may also be due to varietal and seasonal differences (Hyde *et al.* 1959), suggesting that comaprison of seed development rates in crops grown under different climatic conditions should be made with some caution.

Seed viability is one of the most important factors affecting seed quality. Seeds should therefore not be harvested until they reach their maximum viability (Hyde *et al.* 1959; Griffiths *et al.* 1967). Generally, maximum yield of viable seed and full germination capacity are not attained until the attainment of seed maturity (Austin 1972; Schwass 1973). If the crop is harvested too early, most seeds will still be immature and lose viability very rapidly (Hyde 1950; Hyde *et al.* 1959). In addition viable seeds harvested 12 and 14 days after pollination generally produce a higher percentage of abnormal seedlings. They also do not possess the necessary seed quality component of maximum weight and are liable to be removed in commercial cleaning. Even if the crop is harvested at seed maturity, seed moisture content is still high (57% in 'Pawera' red clover) which may cause heating problems unless the crop is subsequently dried. On the other hand, if harvesting is delayed beyond the point of seed maturity a slight reduction in seed dry weight and seed losses through shedding during harvesting may occur.

Some workers (Griffiths *et al.* 1967; Hill 1973) have pointed out the drawbacks of judging seed ripeness, particularly in grass species, by using seed or straw colour changes. Instead, they strongly suggest the use of seed moisture content as an index of seed ripeness. However, in contrast to grass seed crops, estimation of clover seed readiness for harvest is often more clearly defined in terms of seed colouration and consistency (Griffiths *et al.* 1967). Other workers (Wheeler and

Hill 1957; Martin and Leonard 1967; Scott 1973; Ozkan 1975) have also suggested the use of seed or flower head colour changes in predicting the proper time of harvesting the clover crops. Observations in the present study suggest that seed colour changes have some usefulness as a maturity index. The results from two successive years show that about 90% of seeds begin to change colour from green to yellow or purple at the time of seed maturity. However, colour changes may occur slightly earlier under dry and hot conditions and a little later under wet and cold conditions. Therefore, it would be necessary to adjust the date of harvest depending upon prevailing climatic conditions as suggested by Zaleski (1970) and Ozkan (1975). If there is no inter-vention of adverse weather conditions, there seems to be little danger in predicting the correct harvesting time by using seed coat colour changes as a criterion of ripeness. Normally, the crop would be ready to cut when approximately 90% of the seeds in the best yielding heads turn brown or purple.

On the other hand, the 'Pawera' red clover seedcrop can be cut when the average moisture content of seeds is around 57%. There may be some difficulties in accurately sampling seedcrops to obtain truly representative samples for testing and in accurately testing this high moisture content of seed, particularly with portable moisture meters. However seed moisture content has been used successfully to predict the optimum harvest time in some perennial grasses (Hill 1971) and in a range of grass and legume species (Klein 1967). Seed moisture content in 'Pawera' red clover varies slightly depending upon the position of the seed in the head. Also,precipitation can cause slight variation in the values of seed moisture obtained. However differences caused by these aspects are likely to be small and do not appear to be sufficiently important to prevent the use of seed moisture content as a means of determining crop suitability for harvest. The consistency of seed moisture content at the point of seed maturity in two seasons with different climatic conditions during seed development suggests that in 'Pawera' red clover 57% m.c. figure can be used with some confidence.

In the present study, maximum viability was first attained in seed samples harvested 22 days after pollination. Therefore, if a 'Pawera' red clover crop is harvested at the end of the second developmental stage (i.e. 26 days after pollination) or during the third stage of seed development, seed quality components, such as

viability, seedling vigour, storage life and seed weight will not
be affected (Hyde 1950; Hyde *et al.* 1959).

The results in the present investigation showed that freshly
harvested seeds produce a high proportion of fresh ungerminated seeds
in laboratory germination tests. Attempts to induce these seeds to
germinate and produce normal seedlings using gibberellic acid,
prechilling or increased oxygen concentration were all unsuccessful.
Despite the effectiveness of some of these techniques in other species
(Baskin and Baskin 1970, 1975; Harty and McDonald 1972; Edwards
1973; Milthorpe and Moorby 1974; Hamilton and Carpenter 1975),
it appears that the fresh ungerminated seeds of 'Pawera' red clover
are insensitive to these stimulation treatments. The dry storage of
seed for a period of 3 months was, however, very effective in
converting seeds formerly appearing in the fresh ungerminated seed
category to the hard seed condition.

When fresh ungerminated seeds are dissected and examined under the
microscope, there is a thin but firm membrane immediately inside the
seed coat, enveloping the embryo. This membrane, in younger seeds
that produce seedlings, appears slightly thinner and less firm than
that found in fresh ungerminated seeds. Both the seed coat and the
membrane of most fresh ungerminated seeds are thought to be permeable
to water to some extent since the embryo is readily stained by
tetrazolium solution and the seed also shows a swollen appearance.
The failure of germination in these seeds may therefore not be due
to restrictions imposed by the seed coat itself because the latter is
not yet hard at that stage of development. Results in the present
study suggest that freshly swollen seeds were unable to germinate
mainly due to the presence of the membrane rather than the seed coat.
According to seed coat anatomical observations, this membrane seems
to be the aleurone layer which immediately envelops the embryo.
The inability of germination of the embryo could be due to restriction
of gases by the membrane (Davis 1930) or through the membrane preventing
expansion of the embryo by mechanical restriction. Similar suggestions
have been made by Ikuma and Thimann (1963), who demonstrated the
failure of germination in lettuce seeds due to the layers of endosperm
and the inner integumentary epidermis restricting embryo growth.

All hard seeds of 'Pawera' red clover, whether freshly harvested
or after storage, germinated promptly following mechanical scarification
provided the correct germination conditions were then furnished. The
present study suggests that the failure of hard seed germination in
'Pawera' red clover seeds is not due to embryo dormancy but to seed
coat impermeability. Other workers have also reported that hard
seeds of some small-seeded legumes, such as *Melilotus* and *Medicago*
species (Kozlowski and Gunn 1972) and *Desmodium* species (Chow and
Crowder 1974) show no embryo dormancy and germinate rapidly when they
are scarified. The ease with which hard seededness was removed
by short duration and low pressure scarification without causing any
increase in the production of abnormal seedlings or dead seeds
suggests that hardseededness is a relatively easily broken condition
in 'Pawera' red clover - certainly by comparison with some other
species (Hamly 1932 and Win Pe 1974).

CONCLUSION

In 'Pawera' red clover, internode numbers at the time of floral initiation and peak flowering vary with time of sowing. Further investigation would be required before the number of internodes can be reliably used as a criterion for varietal identification. In autumn sown crops peak flowering occurs in February, although spring sown crops generally flower at a later date. In both cases it is essential that there are sufficient numbers of effective bee pollinators (e.g. *Bombus hortorum, B. ruderatus*) present to ensure successful seed production.

The patterns of change in seed components during development in two successive years showed remarkable similarity. The time interval from pollination to seed ripening was about 5 to 6 weeks. There are three stages of seed development as determined by changes in seed dry weight. The first or growth stage occupies the first 10 days after pollination; the second or food reserve accumulation stage takes from 10 to 26 days after pollination and the last ripening stage occupies about 10 to 14 days following the second stage. Seed colour changes commence a few days before the attainment of maximum dry weight and about 90% of seeds have changed colour from green to yellow or purple at the time of seed maturity. Both seed coat colour and seed moisture content have possibilities as indices for determining the optimum time of harvesting the crop.

In freshly harvested seed samples, maximum viability is attained 26 days after pollination (at maturity) but occurs slightly earlier in seed samples after storage. Although, seeds reach their maximum germination capacity during the second stage, it would be wise to delay cutting the crop until later in the ripening stage unless seed drying facilities are available. Freshly harvested young seeds show a much higher percentage of viability in tetrazolium tests than in standard germination tests. In dry stored seeds, first viable and hard seeds appear in seed samples harvested 12 days after pollination. The number of both viable seeds and hard seeds in freshly harvested seed samples show some variation in different seasons and years. Following storage, a drastic increase in the percnetage of hard seeds occurs with increased seed maturity.

The failure of germination of fresh ungerminated seeds in freshly
harvested seed samples does not seem to be due to restrictions
imposed by the seed coat, or to a requirement for gibberellic acid,
or to a need for increased concentrations of oxygen gas. It is
suggested that the presence of the membrane enveloping the embryo
may suppress its growth by mechanical restriction, or by restricting
the availability of oxygen, or both. Hard seeds of 'Pawera' red
clover seeds show no apparent embryo dormancy and germinate promptly
following scarification.

CHAPTER II

ANATOMICAL AND MORPHOLOGICAL STUDIES OF EMBRYO DEVELOPMENT AND SEEDCOAT STRUCTURE IN 'PAWERA' RED CLOVER

INTRODUCTION

In most published accounts, either the embryology of the seed (Pandey 1955; Evans 1961; Hindmarsh 1964; Chen and Gibson 1971, 1974; White and Williams 1976; Williams and White 1976; Rembert 1977) or the anatomy of the coat of mature seed of Leguminosae (Pammel 1899; Hamly 1932; Martin and Watt 1944; Reeve 1946 a,b; Corner 1951; Hyde 1954; Chowdhury and Buth 1970; McKee *et al.* 1977) has been investigated separately. However, in order to develop a clear understanding of the structure of a seed coat, studies of ontogenetic development are necessary (Esau 1965; Bhatnagar and Johri 1972; Fahn 1974).

The purpose of the present study was to investigate both early embryo development and cell structure changes in the seed coat during the seed developmental period. Although it was necessary to examine the cell structure of the seed coat generally, it was thought appropriate to give more attention to three important regions, – the hilum, micropyle and strophiole. Interest in the ways in which the strophiolar region in particular, is related to the seed softening mechanism was aroused by the reports of Martin and Watt (1944) and Ballard (1973) who emphasized the importance of the strophiole and the need for a special study of the anatomy of this structure with relation to water absorption. The competitive roles of the strophiole, hilum and micropyle in affecting the permeability or impermeability of seeds was therefore thought to warrant special attention, particularly since the anatomical and morphological structure and function of these areas has not been previously examined in 'Grasslands Pawera' red clover.

MATERIALS AND METHODS

Preparation for microtoming and scanning Electron Microscopy

(a) Paraffin methods

Seed samples harvested at different maturities were fixed in
a mixture of formalin, propionic acid and 50% ethyl alcohol (FPA)
in proportions 5: 5: 90. Specimens preserved for a few months
in this solution showed only slight discolouration. Seed samples
were washed in 50% ethyl alcohol before dehydration. The tertiary
butyl alcohol (TBA) method was used for dehydration. The series of
solutions of water, ethyl and tertiary butyl alcohols were prepared
as described by Johansen (1940). Vials were partly filled with melted
paraffin wax and allowed to stand until the paraffin solidified.
Seeds were placed on top of the paraffin wax and covered with TBA.
The vial was then placed in an oven at 60°C. About one hour after the
seeds had sunk to the bottom of the vial, the paraffin was poured off and
replaced with melted paraffin wax. This process was repeated twice
during the next six hours and finally a good quality of melted paraffin
was used.

The vial containing the specimens was removed from the oven and the
contents poured into a tinfoil embedding dish. The specimen was
covered with paraffin wax adequately and its position was arranged
using a heated needle. When the paraffin at the bottom of the dish
began to cool, the dish was placed in a cold water bath to cool
completely. The finished blocks were removed from the embedding dishes
and excess paraffin was trimmed off. The blocks were labelled and
stored in a dust proof box until required.

Each paraffin wax block was trimmed to an optimum size and attached
to a hardwood block. The paraffin block was again trimmed with
a scalpel leaving about 1 mm of paraffin surrounding the specimen. The
face was pared to a rectangular shape, the two longest edges of the
block being exactly parallel in order to obtain straight ribbons and
perfect sections.

Microtoming was carried out according to the procedures outlined by Johansen (1940). Sections were cut 10 μ in thickness on a rotary microtome. The ribbons were cut in 2 cm sections and floated on water in a hot water bath. When the sections had fully straightened, they were mounted on microscope slides. Haupt's adhesive was used for affixing sections to the slides. The mounted slides were dried on a warm plate and stored in a slide box.

Before sections could be stained, paraffin was removed by washing the slides in xylol for about five minutes. Each slide was then transferred to a jar containing equal parts of absolute ethyl alcohol and xylol. After five minutes in the absolute alcohol - xylol mixture, the slide passed through half minute immersions in each of the following alcohol solutions, 100%, 95%, 85% and 70%.

The sections were then stained in a 1% solution of safranin for 24 hours and excess stain was washed off with running water. Sections were differentiated and dehydrated with 95% alcolhol to which 0.5% picric acid crystals had been added. After 10 seconds different-iation, each slide was placed for two minutes in 95% alcohol to which 5 drops of ammonia to each 100 cc had been added to stop the action of the acid.

Other staining methods were also employed but the safranin alone or safranin and aniline blue staining methods showed most satisfactory results.

After sections had been stained, they were washed in 95% alcohol for complete dehydration. Slides were then removed and the lower side wiped dry with a clean cloth. The slide then passed through in a differentiator (made by mixing 1 part U.S.P. clove oil and 1 part of a mixture of equal parts of absolute alcohol and xylol.) After 10 seconds the slide was transferred to a jar of xylol containing a trace of absolute alcohol and moved back and forth for a few seconds. Then the slide was placed in a jar of pure xylol for some time. Just before mounting the slide was briefly transferred to a third jar of xylol.

The slide was removed from the xylol and carefully wiped with a clean dry lint-free cloth. Sections were permanently mounted using a small drop of thin balsam. A cover slip was applied gradually, allowing the balsam to spread evenly without leaving air bubbles under the cover slip. The slide was then dried gradually on a warm plate for a few hours and stored in a slide box.

Difficulties with paraffin method: Some fair sections were obtained when flower buds and young seed samples up to 10 days after pollination were cut with a rotary microtome using the paraffin method. However, it was hard to cut sufficiently thin sections (less than 10 μ) and there were also difficulties in cutting older seed samples with hard coats. When the whole seed was embedded in the paraffin wax, the cotyledons became hard and the seed coat brittle.

In some cases, an empty space developed between the cotyledons and the seed coat and sections were crushed. When cut seed or the seed coat alone was embedded, it was very hard to obtain fair sections since the specimens were harder than the embedding medium. Therefore this method was not used for sectioning hard seeds.

(b) Freezing microtome method:

Anatomical studies of diploid *Trifolium* species have been done by sectioning imbibed seeds using a freezing microtome (Hyde, 1954). Hyde's method was investigated for the present study.

Seed samples fixed in FPA solution were embedded in 10% gelatine and frozen at 12.5 ampere. It was difficult to cut sections thinner than 15 μ in thickness even with young seeds. When older seeds with hard coats were cut, sections were crushed. Even though different gelatine concentrations, different amperes and different knife angles were employed, it was impossible to obtain acceptable and sufficiently thin sections. Therefore, sectioning using this method was discontinued.

(c) Light microscopy method:

Since it was previously proposed to use the paraffin method, seed samples were already fixed in FPA solution. When the light microscopy method was employed for sectioning, seed samples were

further fixed for two hours at room temperature in a half strength Karnovsky (1965) fixative (3% glutaraldehyde, 2% formaldehyde, 0.1 M phosphate buffer, pH 7.2).

Before dehydration, seeds were washed in 0.1 M phosphate buffer solution twice (one rapid wash and the other for 30 mins). Seeds were then dehydrated with a graded ethanol series for 30 mins. in each concentration (25%, 50%, 75%, 95%, 100%, 100%). Two washings in propylene oxide for 15 minutes each followed.

Specimens were infiltrated with a mixture of 30% epoxy resin "Durcupan ACM" and 70% propylene oxide. The bottles containing the medium with the specimens inside were left uncovered overnight on a mechanical stirrer. The medium was then replaced with a freshly prepared 100% epoxy resin mixture (Resins, A, B, C and D in proportion 10: 10: 0.4: 0.2 by volume) and the bottles were left with the caps off on the stirrer for 24 hours. (The resins were thoroughly mixed by shaking on an electric shaker "Vortex mixer").

Although a number of kinds of epoxy resin were tried 'Durcupan ACM' was found to be most satisfactory in this study. Seeds were embedded in a freshly prepared 100% epoxy resin mixture. Silicone rubber moulds were used for embedding the specimens. Seeds were positioned with a fine needle and polymerized at 60°C in an oven for three days.

Polymerized resin blocks were held in a vice and trimmed with a small saw. Further trimming was done with a file and a sharp blade. It was necessary to trim the block as small as possible and to cut the edges of the block smoothly and parallel to each other.

Each trimmed block was held in the holder of the microtome and sections were cut 2-4 µ (micron) thick with a glass knife using a L.K.B. "Ultratome". Sections were picked up individually with a very fine pair of forceps from the knife edge and mounted on clean slides. Each slide was flooded with 2-3 drops of distilled water and 3-4 sections were placed on the water. The slide was then heated on a warm plate until the sections spread out and the water was allowed to run off leaving the sections on the slide. Excess water was removed with fine blotting paper and the slide dried on a warm plate for

a few minutes.

As there are no solvents at present for epoxy resins after polymeriz-
ation, staining was carried out prior to removal of the embedding
medium. Sections were flooded with a few drops of staining solution
and the slide warmed for 1-5 minutes depending on the specimens. Older
seed sections stained quicker than younger seed sections. When sections
were well stained, the staining solution was drained off by inclining
the slide. Excess stain was washed away gently with distilled water and
sections dried with filter paper. The slides were then dried further
at room temperature for a few hours.

Although a range of different staining solutions were used, only
two gave suitable results i.e. Toluidine blue and methylene blue –
azure A.

Toluidine blue (0.05% toluidine blue in 0. 1 M phosphate buffer
at pH 7.2) stained specimens a light blue. Although most parts of
the sections were stained well with clear contrast, tracheids in the
tracheid bar of older seed sections were not stained properly. However
methylene blue-azure A stained tracheids and other tissues very well.
When the sections were stained according to the staining procedures
outlined by Warmke and Lee (1976), staining was unsatisfactory partly
due to floating of some sections and partly due to poor colour contrast.
However, when a modified staining method was used, (Methylene blue
– azure A combination alone without using an acqueous safranin 0 counter-
stain), sections were stained evenly and clearly. The entire staining
procedure was repeated if staining was too light.

A small drop of Eukitt (0. kindler, Freiburg, Germany) mounting
fluid was put on top of the sections and a clean coverslip was placed
on the fluid. The coverslip was brought down slowly with the forceps
and then pressed down with a fine needle when it fully covered and
contacted the specimens. The slide was placed on a warm plate for a
few minutes and then dried at room temperature for 24 hours. Slides
were then stored in slide boxes until required.

(d) Plane of Sectioning:

In order to observe the cell structure in the seed coat, seeds
were sectioned in three different planes.

1. Longitudinal section (LS): Most seeds harvested at different
                                periods after pollination were sectioned
longitudinally to reveal the early stages of embryo development and the
cell structure changes in the seed coat occurring in the later stages
of seed development.
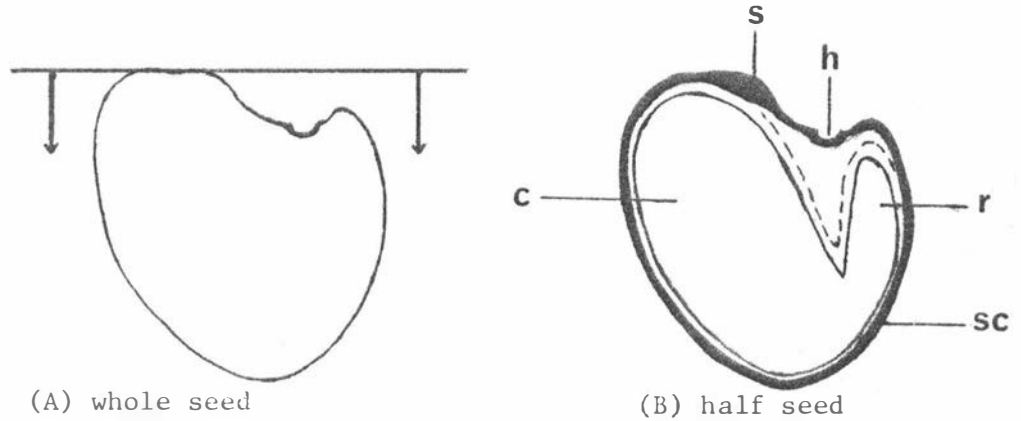
(A) whole seed   (B) half seed
Arrow (→) shows the direction of cutting –
c, cotyledon; h, hilum; r, radicle; s, strophiole; sc, seed coat

2. Transverse section (TS): In order to observe the arrangement of the
                               cells at the strophiolar region in the
seed coat some transmedian sections were made with older seeds.
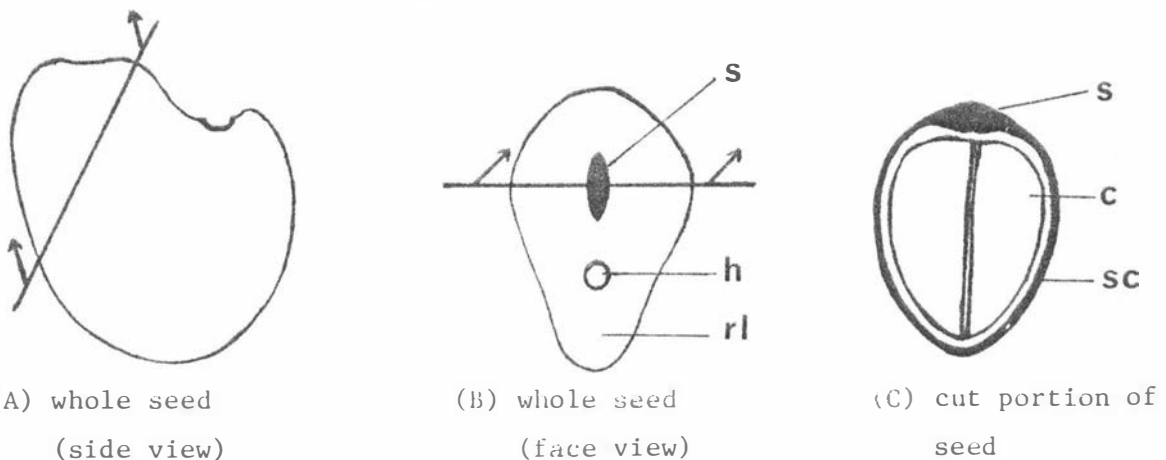
(A) whole seed   (B) whole seed   (C) cut portion of
   (side view)   (face view)   seed
Arrow (→) shows direction of cutting –
c, cotyledon; h, hilum; r, radicular lobe; s, strophiole; sc, seed coat

3.   Cross section (CS): Seeds harvested at the later stages of seed
                            development were sectioned across the upper
portion of the seed through the stropholar, hilar and radicular
regions in order to study cell structure at these regions and the
arrangement of the vascular bundles.
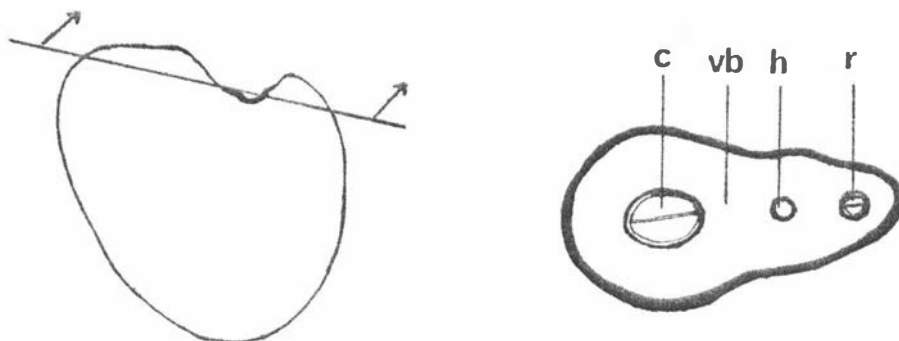
(A) whole seed   (B) cut portion of seed
Arrow (→) shows direction of cutting -
c, cotyledon;   h, hilum;   r, radicle;   vb, vascular bundle

(e)   Scanning Electron Microscopy Method:

        Whole seeds were fixed in 25% glutaraldehyde solution for 7
days.   The solution was then drained off and the seeds thoroughly
washed with distilled water.   Seeds were then soaked in 1% osmic
acid solution over-night in a cool room.   This solution was then poured
off and seeds were washed again in distilled water.   The seeds were then
soaked in distilled water after washing and kept in a cool room until
mounting.

        Before mounting, seeds were washed again with distilled water and
wiped dry with blotting paper.   They were then placed individually
in a drop of conductive cement at different sites on the scanning
electron microscope (SEM) stub.   The specimens were then frozen at -150°C
in liquid nitrogen for about three minutes.   It was necessary to freeze-
fracture the specimens in liquid nitrogen to observe the internal
structure of the seed coat.   The stubs with seeds on them were put in a
vacuum drier for about four hours.   After drying, the specimens were
kept in a desiccator until they were ready for metal coating with a thin
layer (100-150Å) of gold before being photographed at different

magnifications under the SEM.

In order to investigate the natural morphological appearance of
cell structure at different sites on the seed coat of hard seeds, some
fresh hard seeds (seeds which remained hard 10 days after soaking
in water) were examined without prior fixation and photographed under
the SEM following mounting and gold coating.

A. <u>EMBRYOLOGY</u>

<u>LITERATURE REVIEW</u>

A seed is an embryo surrounded by an envelope of stored food and
a protective seed coat. It has developed from the fertilized and
ripened ovule.   Seeds, in turn are enclosed in the fruit which is the
ripened ovary.

In red clover, each ovary contains two ovules but commonly only
one ovule develops into a seed (Pandey 1955; Povilaitis and Boyes
1959, 1960; Evans 1961). A similar situation has been reported in
subterranean clover (Aitken 1939). However, in diploid red clover,
occasionally both ovules may develop and two mature seeds are formed
(Evans 1961). Similarly, Pandey (1955) also reported that in
diploid red clover 98% of ovaries contain two normal ovules with mature
embryo sacs. Fertilization also occurs in both ovules but about 50%
of the ovules are found to be destroyed 12 days after pollination
leaving only one developing ovule in each ovary.

The abortion of one ovule within the first 10 days of seed
development results from nutritional competition between the two
developing ovules (Pandey 1955; Povilaitis and Boyes 1960; Evans
1961).   The loss of one ovule is normal and does not reduce seed
yield (Povilaitis and Boyes 1960).   These workers also observed that
in the case of ovule collapse in red clover, lower ovules in the ovary
tend to collapse slightly more frequently than the upper ones at two
days but there is almost no difference by four days after pollination.

<u>The ovule:</u>   The ovule or megasporangium is the forerunner of the seed.
               A normal ovule has a stalk called the funiculus by which it
is attached to the placenta (Foster and Gifford 1959; Bhatnagar and
Johri 1972; Fahn 1974).

In the Leguminosae, the ovule may be anatropous or campylotropous
(Corner 1951). In the campylotropous ovule the curvature is much less
than in the anatropous type, a situation common in both the Resedaceae
and Leguminosae (Bhatnagar and Johri 1972; Sprone 1974). It has been
reported that the ovule type is campylotropous in *Trifolium pratense*
(Hindmarsh 1964), *T. repens* (Chen and Gibson 1971; Rembert 1977),
T. semipilosum (White and Williams 1976) and *T. ambiguum* (Williams
and White 1976).

In both *T. pratense* (Hindmarsh 1964) and *T. repens* (Rembert 1977) the ovule contains two integuments with the outer integument overgrowing the inner. Corner (1951) reported that in leguminous ovules, the inner integument enlarges by anticlinal divisions without differentiation of its cell rows. The inner integument begins to break down in six day old seed of *T. repens* (Chen and Gibson 1971) and at eight days after pollination in *T. semipilosum* (White and Williams 1976). Chen and Gibson (1974) found that digestion of the inner integument by the endosperm was first evident at the micropylar end three days after pollination and digestion was almost complete by eight days in *T. nigrescens*. Similarly, in *T. ambiguum*, the breakdown of the inner integument is first visible adjacent to the embryo 4-6 days after pollination and is digested at the chalazal end at about 10 days (Williams and White 1976). It has been reported that the cytoplasm of the inner integument stains lighter than that of the endothelium (Chin and Gibson 1974; Williams and White 1976). Corner (1951) found that the outer integument consists of 3-5 cell rows and the inner integument of 2-3 in the leguminous ovule.

The zig-zag shaped micropyle is formed by both integuments in red clover (Hindmarsh 1964). Rembert (1967) suggested that the mature legume ovule has a micropyle with a mesostome, exostome and endostome. The mesostome is that part of the micropylar canal bounded on one side by the outer integument and on the other side by the inner. The exostome is that region of the canal surrounded on all sides by the outer integument, and the endostome is that part of the canal bounded on all sides by the inner integument.

Immediately prior to ovule maturity, the epidermal cells of the outer integument become columnar and are continuous with similar cells of the funiculus (Hindmarsh 1964). At seed maturity, the inner integument is more or less obliterated while the outer integument, in contrast becomes complicated (Corner 1951; Fahn 1974). The integuments develop to form the protective testa or seed coat (Sprone 1974), becoming tough and leathery as in broad bean, or hard as in clover and lupin (Pandey 1955; Yeates and Campbell 1960).

In red clover, the inner epidermis of the inner integument is modified into an endothelium (integumentary tapetum) (Hindmarsh 1964). The endothelium is a single layer of cells surrounding the embryo sac (Evans 1961; Chen and Gibson 1971; White and Williams 1976).

In *Datura* (Solanaceae) the cells of the endothelium absorbs the outer
adjacent layers of the integument which eventually, itself gets absorbed
by the endosperm (Bhatnagar and Johri 1972). Similarly, it has been suggested
that the endothelium participates in food transfer from the inner integument
to the endosperm in *T. nigrescens* (Chen and Gibson 1974). In the later
stages of development, the endosperm haustorium absorbs nutrients from
both the endothelium and inner integument. The endothelium begins to
degenerate when the embryo is at a heart-shaped stage (Chen and Gibson 1971;
Bhatnagar and Johri 1972; White and Williams 1976). At 8-10 days after
pollination the endothelium is almost completely digested in the ovule of
T. repens and *T. nigrescens* (Chen and Gibson 1971; 1974).

The ovule in angiosperms is vascularized by a single bundle which
often is restricted to the funiculus and terminates blindly in the chalazal
region of the nucellus (Foster and Gifford 1959). Hindmarsh (1964)
reported that in red clover the vascular strand terminates at the chalaza.
The chalaza is the basal region of an ovule or seed where the nucellus and
integument converge (Johanson 1950).

A typical ovule contains an ovular cavity or the embryo sac with eight
nuclei which is developed from the megaspore by three successive mitotic
divisions (Johansen 1950; Fahn 1974; Schopmeyer 1974). Three of the four
nuclei at the micropylar pole become organized in cells. The middle one
constitutes the egg cell while the other two lateral cells are termed
synergids. Three of the nuclei at the chalazal pole become organized into
three antipodal cells. The two remaining polar nuclei move towards the
centre of the embryo sac where they may fuse to form a diploid nucleus or
secondary nucleus (Hindmarsh 1964; Sugihara *et al*. 1969; Fahn 1974).

Embryo sac development in red clover is of the monosporic polygonum
or normal type (Pandey 1955; Hindmarsh 1964). Pandey (1955) explained
that embryosac formation in tetraploids resembles in general that in diploids.
However, some marked differences are reported, firstly a higher degree of
failure of the polar fusion at the time of flower opening occurs in the
tetraploid than in the diploid, and secondly "vegetative" ovule numbers
(without embryo sacs) as well as ovules containing abnormal embryo sacs
are more common in tetraploids than in diploids.

Fertilization: In most plants the pollen tube penetrates into the ovule
through the micropyle (Foster and Gifford 1959; Bold 1970;
Fahn 1974). When the pollen tube enters the embryo sac, usually one of the

synergids is destroyed by the penetration of the pollen tube (Foster and
Gifford 1959; Fahn 1974), while the other may remain intact until some time
afterwards (Maheshwari 1950; Kaplan 1969).   However, in *Gossypium*
(Jensen and Fisher 1968) and in *Hordeum* (Cass and Jensen 1970) one
of the synergids begins to degenerate after pollination but before the
pollen tube reaches the embryo sac.

After its entry, the tip of the pollen tube ruptures and the two
male gametes are released into the cytoplasm of the embryo sac. One
of them fuses with the egg cell to produce a zygote which later develops
into the embryo.   The second gamete fuses with the two polar nuclei or
with the secondary nucleus if the latter two have fused previously.
The polar nuclei have fused before fertilization in *Trifolium repens*
and *T. nigrescens* (Chen and Gibson 1971, 1974), in *T. semipilosum*
(White and Williams 1976) and *T. ambiguum* (Williams and White 1976).
However, Pandey (1955) reported that the behaviour of the polar nuclei
in tetraploid red clover varies from plant to plant. He observed the
fusion of polars had already occurred in about 90 per cent of the ovules
in one group of plants while only about 40 per cent fusion had occurred
in another group of plants by the time of flower opening. As a result
of the fusion of the secondary nucleus and second gamete, the endosperm
is formed.   This process of fertilization is in fact a double fertilization
which is a phenomenon known only in angiosperms (Bold 1970; Raven
and Curtis 1970).

As the embryo and endosperm develop, generally the synergids and
antipods disintegrate (Schopmeyer 1974).   The antipodal cells may
degenerate without being delimited into cells (Bhatnagar and Johri
1972) or may also persist into the post-fertilization stages (Davis 1966).
They may become large multinucleate cells or even polyploid (Davis 1966;
Bhatnagar and Johri 1972).   The commonest condition is that the antipodal
cells commence degeneration just before or soon after fertilization
(Davis 1966).

In red clover, the antipodal cells degenerate soon after their
formation and the nucellar plug disintegrates before fertilization (Hind-
marsh 1964). Pandey (1955) found there are three degenerating
antipodals at the time of flower opening in tetraploids, whereas no
trace of them is found in diploids. In some other *Trifolium* species,
the antipodal cells and much of the nucellus disintegrate before fertiliz-

tion (Chen and Gibson 1971, 1974; White and Williams 1976; Williams and White 1976). In *T. semipilosum*, the nucellus is almost completely digested four days after pollination (White and Williams 1976).

The frequency of ovules fertilized varies in different species e.g. in *T. nigrescens* 71% at two days (Chen and Gibson 1974), in *T. repens* 66% at three days (Chen and Gibson 1971) but only 45% in *T. semipilosum* four days after pollination (White and Williams 1976). Chen and Gibson (1974), also reported that in *T. nigrescens*, unfertilized ovules begin to degenerate three days after pollination.

Evans (1961) found that fertilization is often delayed in tetraploids due to the slower rate of pollen tube growth than in diploids. However, once the divisions of the embryo and endosperm begin, they proceed at an increased rate particularly in red clover.

The Endosperm: Soon after fertilization, the primary endosperm nucleus initiates a series of nuclear divisions to form endosperm nuclei (Bold 1970, Chen and Gibson 1974). The growth of the endosperm precedes the growth of the embryo (Dnyansagar 1954, 1956; Pandey 1955; Schopmeyer 1974; White and Williams 1976). In some types of endosperm no cell membranes or cell walls are laid down during the early stages of development and the young endosperm consists of a mass of relatively fluid protoplasm containing numerous free nuclei (Raven and Curtis 1970). These nuclear endosperms eventually develop cell walls and cellular endosperms are formed in the later stages of development (Chen and Gibson 1971; Cuthbertson 1974; White and Williams 1976). Generally it has been reported that most of the endosperm nuclei are concentrated around the embryo but a few migrate through the cytoplasmic threads to the chalazal end of the embryo sac (Chen and Gibson 1971, 1974; Williams and White 1976). Wall formation takes place at first in the micropylar region around the embryo and then gradually extends towards the chalazal region (Pandey 1955; Dnyansagar 1954, 1956; Chen and Gibson 1974; White and Williams 1976).

In the Papilionaceae, endosperm formation is nuclear but the extent to which it becomes cellular is variable and the development of the chalazal haustorium is usual (Dnyansagar 1954, Davis 1966). The cellular endosperm serves as a source of nutrition for the developing

embryo (Adams *et al.* 1970, Neushul 1974, Paramonova 1975).   In certain genera, e.g. *Pisum, Phaseolus* and *Arachis,* the entire endosperm tissue is digested by the developing embryo (Foster and Gifford 1959;   Fahn 1974). In *Chondrilla juncea,* the endosperm is digested during seed development, although a persistent layer of endosperm one or two cells thick encloses the embryo at maturity (Cuthbertson 1974).

In *T. repens* (Chen and Gibson 1971), the endosperm contains from 2 to 14 nuclei on the first day of pollination. On the second day it contains about 41 nuclei and the endosperm haustorium is initiated in the chalazal region.   On the fourth day, there are about 550 nuclei and the endosperm adjacent to the embryo shows signs of becoming cellular. The endosperm haustorium is well formed. In some six day-old seeds, the endosperm becomes completely cellular while in others the endosperm at the chalazal end is still free-nucleate. The endopserm haustorium disintegrates as the endosperm becomes cellular. Digestion of the endosperm by the embryo is also evident. The endosperm is completely digested in eight and ten day old material.

Williams and White (1976) found that the endosperm contains 2-4 nuclei on the first day and 32 nuclei on the second day after pollination in *Trifolium ambiguum*.   Divisions occur in "waves" passing from the embryo end to the chalazal end of the embryo sac. The endosperm becomes cellular at the embryo end five to six days after pollination. Once the endosperm becomes cellular, the cellular portion continues to proliferate ahead of the growing embryo by non-synchronous divisions. By ten days the cellular endosperm, although still dividing, has been almost completely overtaken and digested from behind by the embryo.

The haustorium becomes more conspicuous six to eight days after pollination. By eight days after pollination the haustorium appears to be burrowing into the inner integument. It is still visible as a narrow densely staining layer.

In *Trifolium semipilosum* (White and Williams 1976), the endosperm contains 2-4 nuclei on day one and 32 nuclei on three days after pollination. The endosperm is at the four-nucleate stage when the zygote begins to divide. Four days after pollination, the endosperm haustorium is formed at the chalazal end. The haustorium is fully formed when the endosperm contains about 500-560 nuclei up to twelve days after pollination. At fourteen

days after pollination, the endosperm has become cellular at the micropylar
end but remains free-nucleate in the chalazal half of the embryo sac.
Endosperm development occurs by successive doublings of the numbers of
nuclei.

In *Trifolium pratense* even though the development of the embryo and
endosperm is faster in the tetraploid than the diploid (Pandey 1955:
Evans 1961), the growth trend is very similar (Evans 1961).

The embryo: After its formation, the zygote enters a dormant stage for a
certain period. The time taken for the zygote to undergo
its first division varies with different species (Maheshwari 1950; Fahn
1974; Sprone 1974) and is to some extent dependent on environmental
conditions (Maheshwari 1950). Bhatnagar and Johri (1972) reported that the
resting period of zygote is longer when the endosperm is of the nuclear type,
as compared to the cellular type.

Some variations in resting period have been reported in some *Trifolium*
species. In *T. nigrescens* for example the zygote begins to divide on the
day of pollination (Chen and Gibson 1974) while in *T. repens* division
begins 24 hours after pollination (Chen and Gibson 1971) and in *T. ambiguum*
between 24 and 48 hours after pollination (Williams and White 1976).

Generally. the zygote begins to divide after the division of the
primary endosperm nucleus (Maheshwari 1950: Bhatnagar and Johri 1972;
Fahn 1974). In *T. nigrescens* (Chen and Gibson 1974) and in *T. semipilosum*
(White and Williams 1976), the endosperm is at the four-nucleate stage when
the xygote begins to divide while in *T. repens*, the endosperm contains
from 2-14 nuclei when the proembryo consists of 2-4 cells (Chen and Gibson
1971).

In very rare situations, the first division of the zygote is generally
followed by the formation of a transverse wall (Maheshwari 1950; Wardlaw
1955; Foster and Gifford 1959; Sporne 1974). Of the two cells thus formed,
the one lying nearest to the micropyle is called the "basal cell",
while the other one lying towards the interior of the embryo sac is called
the "terminal or distal cell" (Maheshwari 1950; Neushul 1974; Sprone
1974). The basal cell may enlarge without further division, or may undergo
a number of transverse divisions, while the terminal cell divides either
longitudinally or transversely (Maheshwari 1950; Foster and Gifford 1959;
Fahn 1974: Sprone 1974).

The early stages of embryology are similar in most angiosperms, but the later stages of development are categorized as monocotyledonous or dicotyledonous types (Raven and Curtis 1970; Neushul 1974). As the spherical mass of cells increases in size, it gradually begins to take on a characteristic shape. At this point, one of the leading differences between the Monocotyledons and the Dicotyledons becomes evident. In the Dicotyledons, the cells of the embryo proper continue to divide especially in those two areas where the cotyledons will develop. At this stage the embryo is heart-shaped in longitudinal view (Fahn 1974). As a result of further cell division, the cotyledons elongate, and the embryo resembles a torpedo in shape (Raven and Curtis 1970). During the later stages of development, the hypocotyl and cotyledons bend to conform to the shape of the embryo sac (Maheshwari 1950; Foster and Gifford 1959). Between the two cotyledons is the meristem (Plumule or shoot apex), a region of active cell division in which future growth will take place (Fahn 1974; Bracegirdle and Miles 1976).

The Monocotyledonous embryo differs in that the apical part of the embryo develops into a single (mono) cotyledon while the apical meristem (the shoot apex) is lateral in position (Raven and Curtis 1970; Neushul 1974).

During recent years, more detailed accounts have appeared of cell formation in developing embryos of some *Trifolium* species. In *T. repens*, the embryo consists of 22 cells 48 hours after pollination (Chen and Gibson 1971) while in *T. nigrescens*, it contains about 59 nuclei at the corresponding period. Three days after pollination the embryo becomes spherical but there is no indication of differentiation (Chen and Gibson 1971, 1974). On the fourth day, cotyledonary outgrowths of the embryo are conspicuous in both species. The hypocotyl and epicotyl are well differentiated in six day-old seed (Chen and Gibson 1971). In eight to ten day-old seed, the embryo is well developed and the cotyledons occupy the whole chalazal pocket (Chen and Gibson 1971, 1974).

Similar rates of embryo development have been reported in *T. ambiguum* (Williams and White 1976) even though the early developmental stage of the embryo is slow. However, in *T. semipilosum* (White and Williams 1976), it has been observed that the rate of embryo development is much slower than in the above mentioned species. The zygote is undivided 24 hours after pollination and an eight celled embryo is formed on the

third day.   The embryo contains only about 15 cells at four days and
65 cells at eight days after pollination. However, it develops rapidly
after that period. Therefore, the embryo comprises about 440 cells
by 12 days and some embryos have differentiated cotyledonary outgrowths
14 days after pollination.

Evans (1961) observed that in diploid red clover, the embryo
becomes spherical on the fourth day and cotyledonary outgrowths are
evident between the fifth and eight days after pollination. On the tenth
day, the embryo differentiates into cotyledons, hypocotyl and epicotyl
and the seed is fully formed by the fifteenth day after pollination.

Dure (1975) reported that in the Leguminosae, the growth of the
embryo is found at first to be rather slow but then to accelerate.
The final cell number of the embryo is found to be reached rather early
in its ontogeny and its subsequent increase in mass occurs as the result
of cell expansion and concomitant deposition of starch and storage
protein.   McKee *et al*. (1955) found that the final number of cells
of the pea embryo (*Pisum sativum* L) for example is reached less than
half way through the period of seed formation. Similarly, in *Phaseolus
vulgaris,* Loewenberg (1955) reported that the final number of cells
in the embryo is reached when the dry weight of the embryo is only one-
sixth of its final value. This number is reached about three weeks after
pollination whereas seed formation and maturation is not complete until
about 44 days.

The suspensor: In several plants, the suspensor of the embryo shows
                great variation with regard to its size, shape and number
of cells (Bhatnagar and Johri 1972). A variety of modifications of the
suspensor are known in the Leguminosae and have been reviewed by
Maheshwari (1950). Little attention has generally been paid to the
suspensor, since it has no special function in the majority of angiosperms
except that of pushing the embryo into the endosperm (Maheshwari
1950).

After the first division of the zygote, the cell produced at the
Micropylar end undergoes a series of transverse divisions to form a
suspensor.   In *Capsella*, the suspensor is developed from the two
basal cells of the proembryo during the formation of the octant stage
of the embryo proper (Wardlaw 1955; Foster and Gifford 1959). Johansen

(1950) reported that in *Trifolium minus*, a globular or oblate suspensor is formed as a result of the division of the basal cells.

RESULTS

Developmental sequences of the ovule, endosperm, embryo and surrounding
maternal tissues are shown in Plates (9-20).

In *Trifolium pratense* c.v. 'Pawera' each ovary generally contains
two ovules in the early stages of development (Plate 9). However,
usually only one ovule develops into a seed (Plate 11). Observations
from dissections of ovaries 8 to 10 days after pollination showed the
shrunken and aborted ovule attached to the ovary wall. There is no
tendency for ovule abortion to be confined to any one position within
the ovary. Ovules from both upper and lower positions in the ovary
collapsed with almost equal frequency.

The Ovule: The campylotropous ovule is attached to the placenta by a
              short funiculus (Plate 11). Although the stalk or the
funiculus is short in the young ovule it becomes longer in the later
stages of seed development. It has two integuments; the outer integument
consisting of 4-7 cell-rows and the inner integument of 2-3 cell-rows
(Plate 10). Immediately inside the inner integument, the single layer
of endothelium cells (integumentary tapetum) envelops the cavity (embryo
sac and nucellus). These observations show the structural appearance
of the maternal tissues just before pollination. The cells of the inner
integument and the endothelium are enlarged and their cytoplasm stains
less intensely than that of the outer integument (Plate 10). However,
from four days after pollination onwards endothelium cells stain slightly
darker than those of the inner integument.

The epidermal cells of the outer integument begin to elongate radially,
at the micropylar and chalazal ends of the ovule, approximately 48
hours after pollination (Plate 11). Elongation in other regions of the
epidermis is slower and in central areas is not obvious until eight
days after pollination. Cell elongation becomes progressively more
conspicuous and a row of cells immediately below that layer also becomes
enlarged (Plate 12).

The inner integument begins to degenerate around the embryo at the
micropylar end of the ovule six days after pollination (Plate 15).
Digestion of the inner integument proceeds towards the chalazal end and
is almost complete by the time the embryo has reached the torpedo
stage about 10 days after pollination.
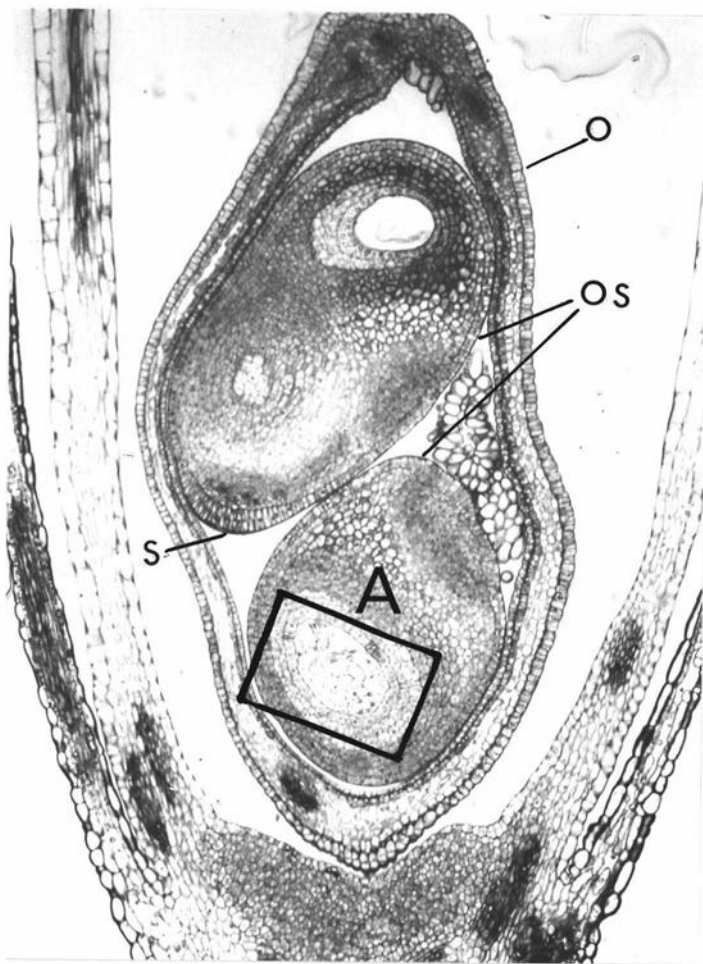
Plate 9: Two ovules in the ovary of a floret just before Pollination.
o, ovary; os, ovules; s, strophiole (LSx60)
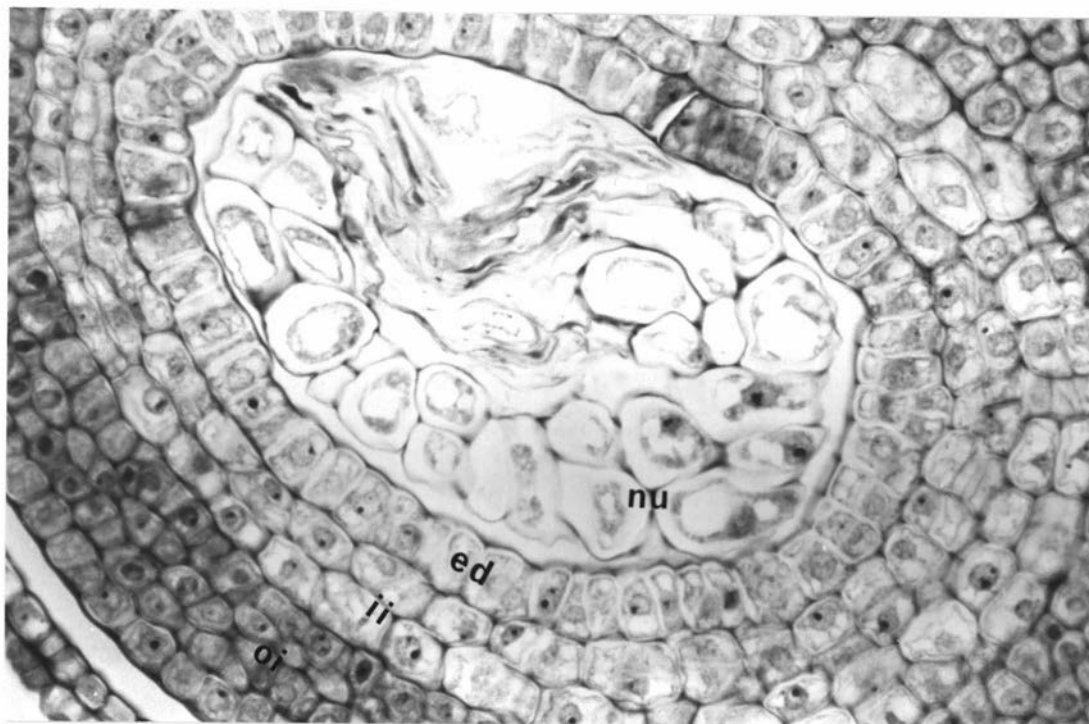
Plate 10: Section A of Plate 9.
ed, endothelium; ii, inner integument; oi, outer integument;
nu, nucellus (LS x 400).
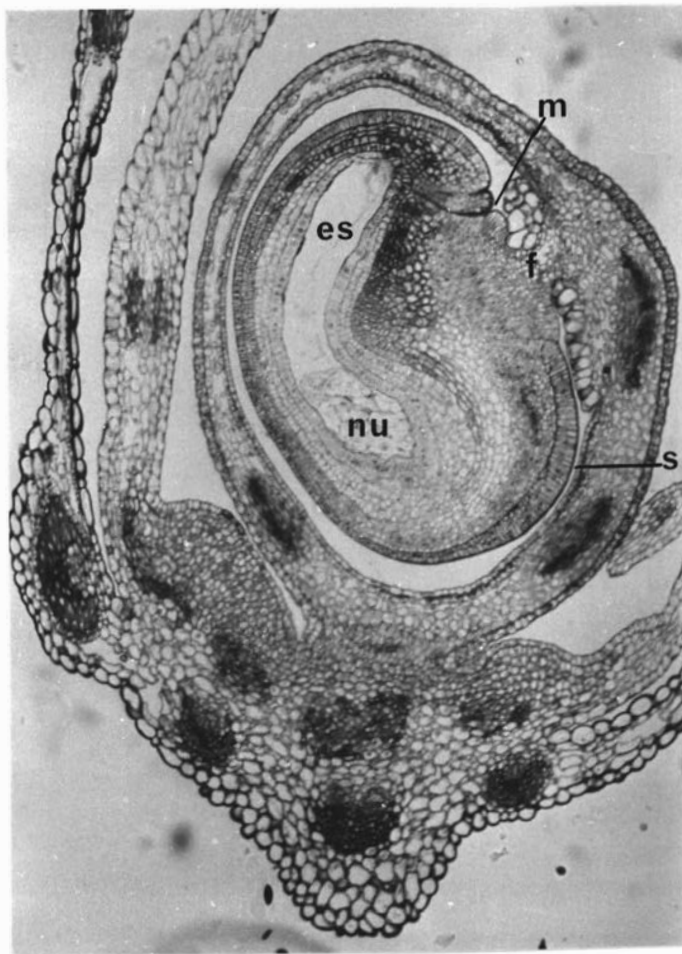
Plate 11: Two days after pollination - an ovule within the ovary.
es, embryo sac; f, funiculus; m, micropyle; nu, remains of nucellus; s, strophiole (LS x 60).
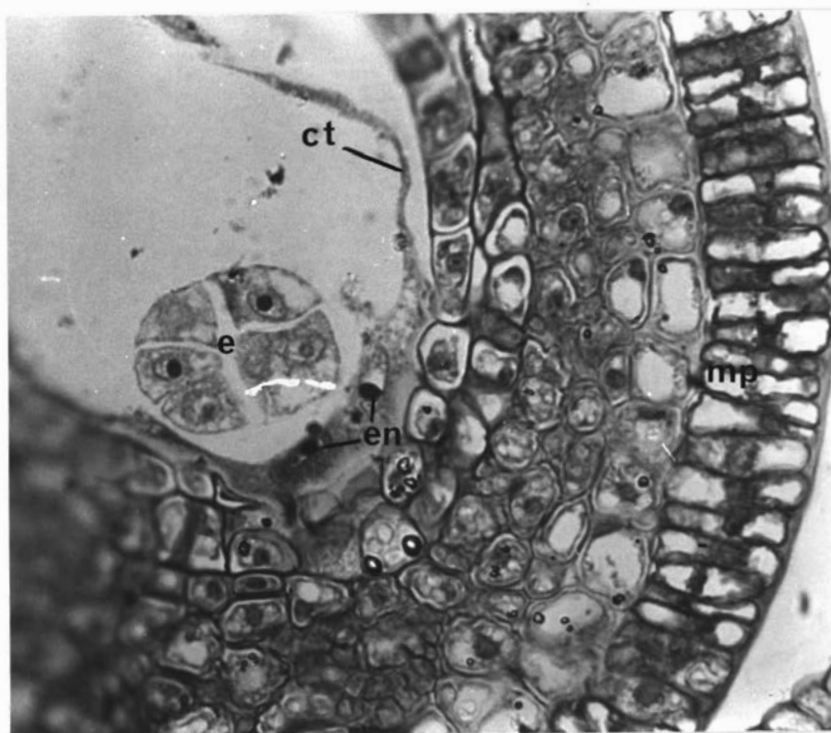
Plate 12: Four days after pollination - an early globular embryo.
ct, cytoplasm; e, embryo with six cells in this section; en, endosperm nuclei; mp, malpighian cells (LS x 400).

The endothelium also begins to break down about eight days after pollination when the embryo reaches the heart-shaped stage. The cells of the endothelium become flattened and stretched periclinally (Plate 17). Digestion starts near the embryo and is complete about 18 days after pollination.

The vascular bundle from the funiculus enters the ovule through the outer layers of the outer integument at the end of the hilum opposite the micropyle (Plate 45). This bundle terminates at the chalazal end of the ovule.

In the young ovule, the micropyle is narrow (Plate 11). However, in the later stages, it becomes an open canal at the radicle side of the hilar region (Plates 15 and 19).

Embryo sac: The embryo sac of a mature ovule can be seen in Plate (11). Two days after pollination, the remains of the nucellus are seen immediately outside the antipodal end of the embryo sac. No trace of synergids or antipodal cells were observed during this study. Until 10 days after pollination, nucellar tissue is still detectable at the chalazal end of the ovule. As embryo development progresses, the embryo sac becomes larger and its curvature becomes more conspicuous (Plates 15 and 18).

Fertilization: The early stages of fertilization, that is, the growth of the pollen tube and the fusion of male gemetes with the egg and two polar nuclei or secondary nucleus were not detected in this study.

Early endosperm and embryo development

It was observed that there were timing variations in the early development of the embryo. For example, different stages of globular embryo development were observed in six day old seed samples. Similarly, embryos ranging from heart-shaped to torpedo-shaped stages were observed eight days after pollination, and after 10 days, development ranges from the torpedo to the cotyledonary stages.

Endosperm: The primary endosperm nucleus begins to divide about two days after pollination, before the first division of the zygote. By four days after pollination, a number of free endosperm nuclei are

present in the cytoplasm around the dividing zygote (Plate 12). During the globular phase of embryo development, most of the endosperm nuclei are concentrated around the embryo, although a few migrate towards the chalazal end of the embryo sac (Plates 14 and 15).

At eight days after pollination, the endosperm becomes cellular around the heart-shaped embryo (Plate 16). The remainder of the endosperm remains free-nucleate, and an accumulation of nuclei and cytoplasm at the chalazal end of the embryo sac forms an endosperm haustorium (Plate 16). The cellular region of the endosperm forms a tube around the embryo (Plate 17) and proliferates ahead of it as it grows (Plate 18). The dividing cellular endosperm is progressively overtaken and digested from behind by the growing embryo (Plate 20).

Embryo: The zygote begins division at about two days after pollination, and the young embryo is globular in shape until about six days after pollination (Plates 13, 14, and 15). In the later globular stage, the upper end of the globe becomes flattened. The central cells of the flat area become vacuolate, while the cells on either side continue dividing to initiate cotyledonary out-growths (Plate 14).

The embryo progresses through heart-shaped and torpedo-shaped stages (Plates 17, 18 and 19) to reach an early cotyledonary stage by about 10 days after pollination (Plate 20). In Plate (20), the shoot apex is seen between the two cotyledons.

Plate (19) shows the suspensor at the base of the radicle end of the embryo near the micropyle. It is not in the plane of section of other figures.

Plate 13:   Six days after pollination - globular embryo (LS x 440).
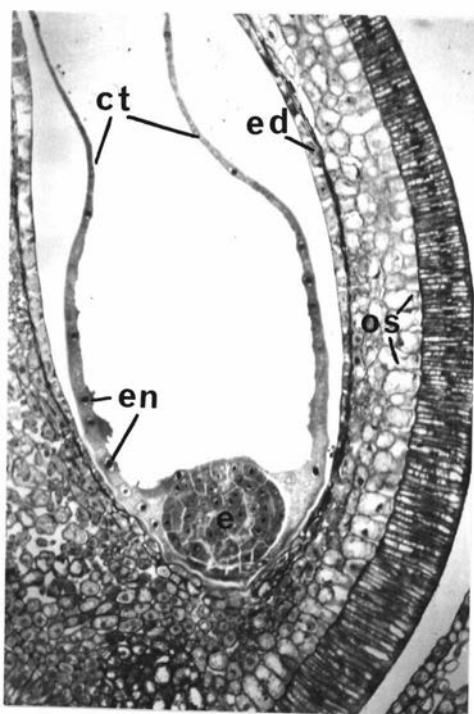
Plate 14:   Six days after pollination - globular embryo, just before the initiation of cotyledonary outgrowths.
ct, cytoplasmic threads;
e, embryo; en, endosperm nuclei;
ed, edothelium; os, osteosclereids
(LS x 120).
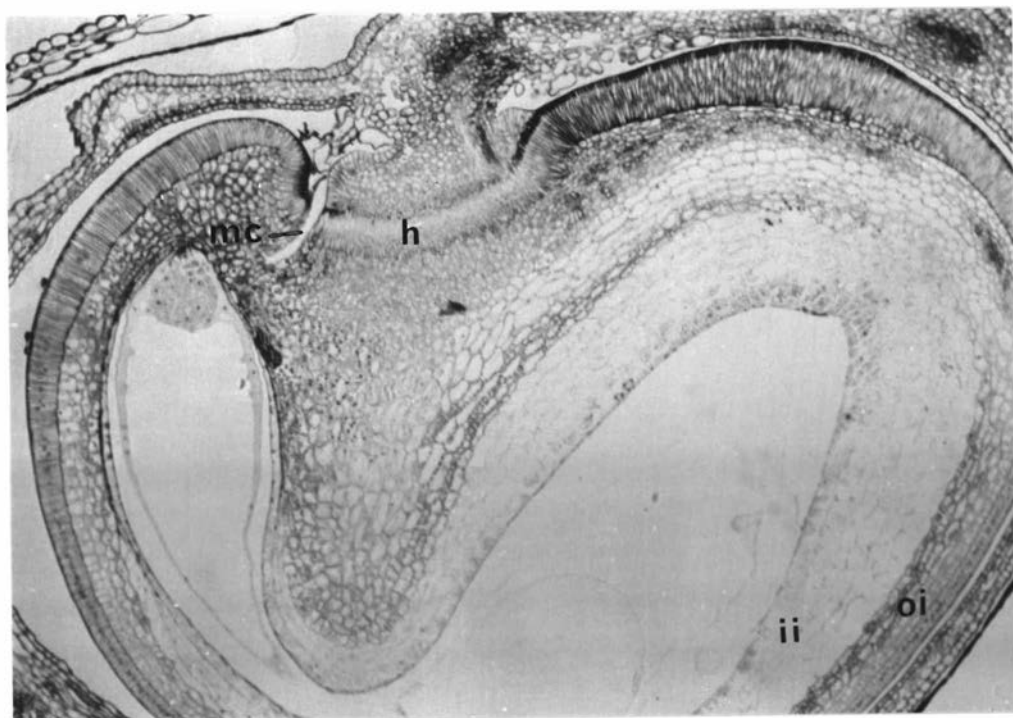
Plate 15: Six days after pollination - an ovule showing embryo and
           endosperm development and degeneration of the inner integument.
           h, hilum; ii, inner integument; mc, micropylar canal;
           oi, outer integument (LS x 160).
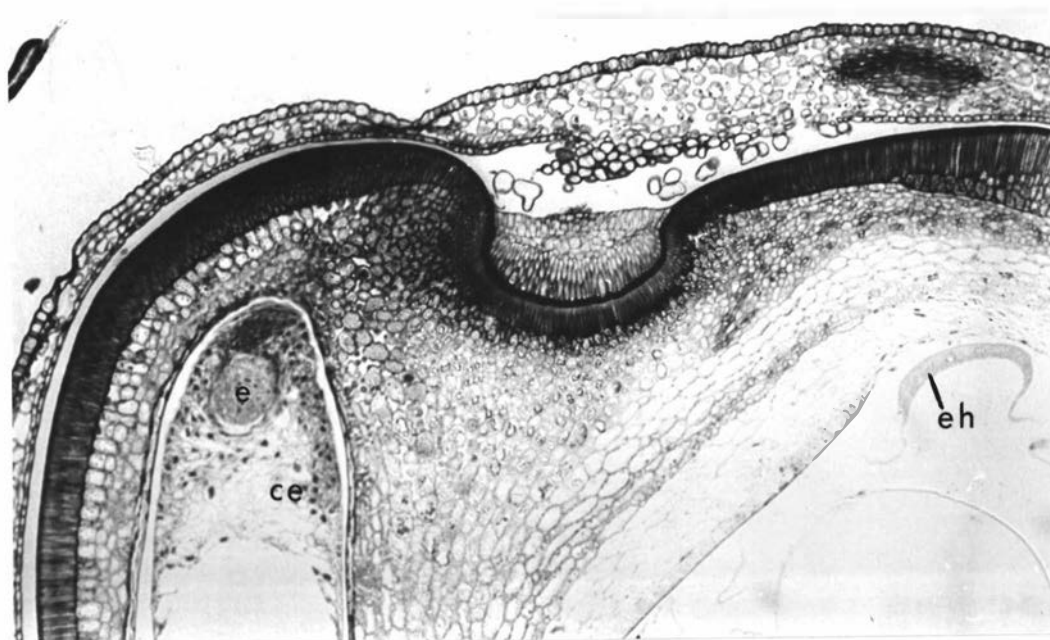
Plate 16: Eight days after pollination - oblique section through a
           heart-shaped embryo which is surrounded by cellular endosperm.
           ce, cellular endosperm; e, embryo; eh, endosperm haustorium
           (LS x 160).
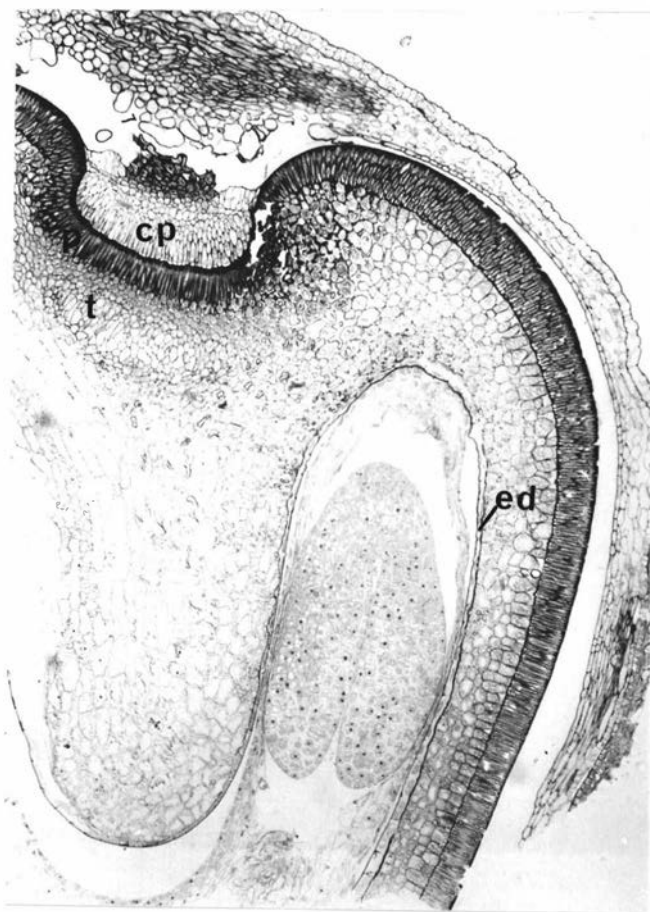
Plate 17: Eight days after pollination – torpedo shaped embryo enveloped by a tube of cellular endosperm. cp, counter palisade; ed, endothelium; p, palisade; t, tracheid (LS x 70).
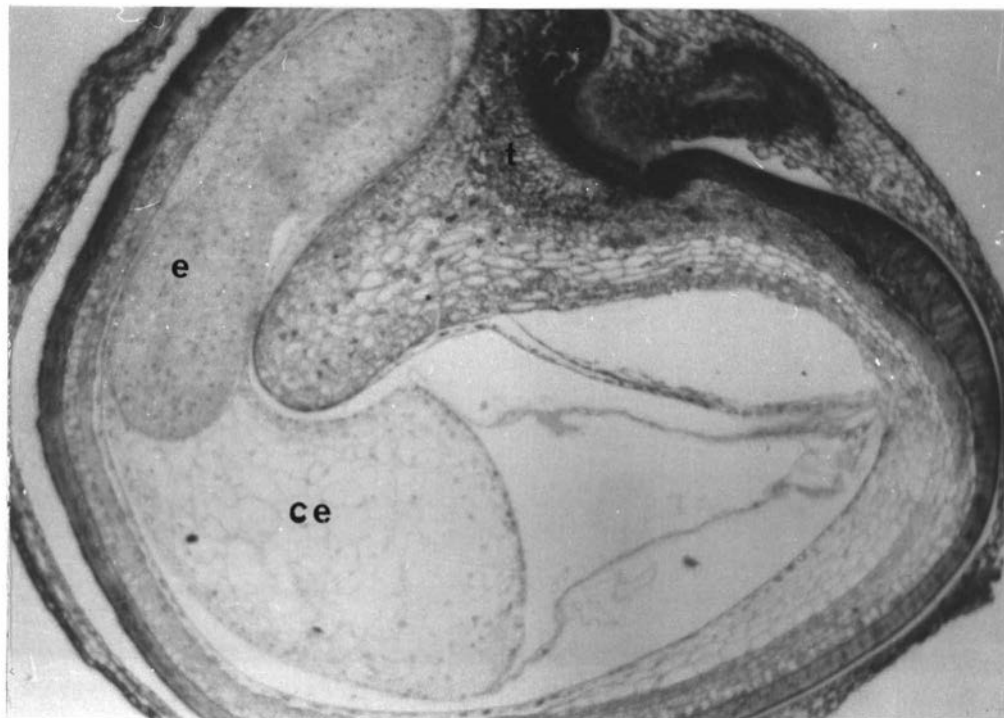
Plate 18: Ten days after pollination – cellular endosperm proliferating ahead of a torpedo-shaped embryo towards the chalazal end of the embryo sac.
ce, cellular endosperm; e, embryo; t, tracheid (LS x 60).
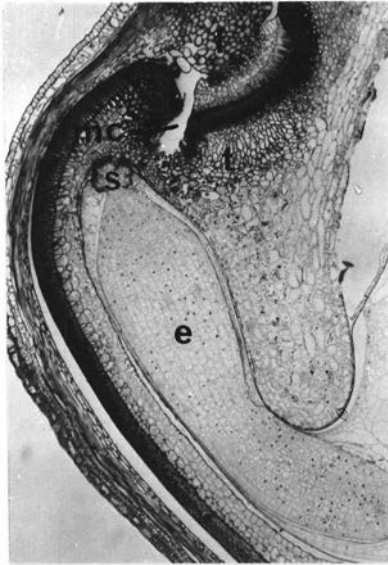
Plate 19: Ten days after pollination –
curved torpedo-shaped embryo
showing the suspensor at the
micropylar end.
e, embryo; f, funiculus; mc,
micropylar canal; s, suspensor;
t, tracheid (LS x 40).

Plate 20:   Ten days after pollination – early cotyledonary stage embryo.
ce, cellular endosperm; cs, cotyledons; pl, plumule;
r, radicle (LS x 60).

DISCUSSION

Since ovules are not always arranged in the same plane in the ovary, some serial sections show only one ovule in the young ovary even though the latter actually contains two ovules. However, in later harvests, starting from four days after pollination, most ovaries consist of only one ovule with the developing embryo. Similar observations have been reported by Chen and Gibson (1974) that unfertilized ovules in *Trifolium nigrescens* begin to degenerate three days after pollination. Ovary dissections also prove that abortion of an ovule occurs within 10 days after pollination as stated by Pandey (1955), Povilaitis and Boyes (1960) and Evans (1961). Therefore, the results in the present study agree with the findings of these workers and Aitken (1939) who reported that only one seed is formed in each ovary. However, as in diploid red clover (Pandey 1955; Evans 1961) it is only rarely in the tetraploid variety 'Pawera' red clover that two developed seeds are found in one ovary. Therefore, the formation of two seeds in an ovary may not be a unique feature of tetraploidy.

The ovule is campylotropous as found in other *Trifolium* species (Hindmarsh 1964; Chen and Gibson 1971; White and Williams 1976; Williams and White 1976; Rembert 1977). The number of cell layers in both the outer and inner integuments are in accordance with the general pattern described by Corner (1951) for leguminous seeds. With respect to development, function and stain affinity, endothelium appears to behave similarly to that of diploid red clover *Trifolium pratense* (Hindmarsh 1964), *Trifolium nigrescens* (Chen and Gibson 1974) and *T. ambiguum* (Williams and White 1976) and *T. semipilosum* (White and Williams 1976).

Degeneration of the endothelium begins at the heart-shaped stage of embryo development as found by Chen and Gibson (1971), Bhatnagar and Johri (1972), and White and Williams (1976). Chen and Gibson (1971, 1974) found the endothelium to be almost completely digested in their most advanced samples collected 10 days after pollination. However, in the present study traces of the endothelium are found to persist into the later stages of development.

The pattern of digestion of the inner integument is very similar to that observed by Chen and Gibson (1971) and Williams and White (1976).

It is somewhat less similar to that described by Chen and Gibson (1974) and White and Williams (1976). Small differences in timing may be due to species differences or to different environmental conditions during seed development.

The vascular system of the ovule shows a similar pattern to that of diploid red clover (Hindmarsh 1964). However, in tetraploid 'Pawera' red clover, the micropyle is a curved canal which does not have the zig-zag configuration as described by Hindmarsh. In the later stages of seed development, as the micropylar canal is surrounded by the outer integumentary tissue, it might be described by the term "exostome" as defined by Rembert (1967). This contrasts with the observation of three sections of micropylar canal surrounded by inner and outer integumentary tissue in *Wisteria sinensis*.

As harvesting was done on alternate days, starting from the date of pollination, the early stages of fertilization as described by other workers (Pandey 1955; Foster and Gifford 1959; Chen and Gibson 1971, 1974; Fahn 1974; Williams and White 1976) were not detected. Furthermore, since the number of ovaries used for sectioning was not large, the chances of finding sufficient correctly oriented sections for analysis of the contents of the embryo sac just before and after fertilization were very slim. Remnants of nucellus were detected as reported by White and Williams (1976). However, in the present study these nucellar remnants were apparently more persistant than those described by White and Williams.

Endosperm divisions are initiated earlier than those of the zygote as reported by Dnyansagar (1954, 1956), Pandey (1955), Schopmeyer (1974) and White and Williams (1976). The general pattern of endosperm development agrees with the findings of previous workers in some legume species including *Trifolium pratense* and *T. repens* (Pandey 1955; Raven and Curtis 1970; Chen and Gibson 1971, 1974; Cuthbertson 1974; White and Williams 1976; Williams and White 1976).

The formation of the endosperm haustorium and the initiation of cellularity is slower in 'Pawera' red clover than in *Trifolium repens* (Chen and Gibson 1971) or *T. ambiguum* (Williams and White 1976). However, cellular endosperm development occurs earlier in 'Pawera' red clover than in *T. semipilosum* (White and Williams 1976) where wall formation is delayed up to 14 days after pollination.

Since whole seeds were sectioned only up to 10 days after pollination owing to difficulties in sectioning older seeds, no observations were made of cellular endosperm development beyond this time. However, it is possible that this process follows the pattern observed in *Trifolium ambiguum* (Williams and White 1976).

The zygote apparently begins to divide between two and four days after pollination, since most four day old ovules contain early globular embryos. Compared with other *Trifolium* species studied by Chen and Gibson (1971, 1974) and Williams and White (1976), this slow development of the embryo may be due to a slow rate of pollen tube growth (Evans 1961) or to a longer zygote resting period (Maheshwari 1950; Fahn 1974; Sprone 1974). These two factors may be influenced to some extent by environmental conditions (Maheshwari 1950).

As reported by Maheshwari (1950), Bhatnagar and Johri (1972), Chen and Gibson (1974), Fahn (1974) and White and Williams (1976), the first division of the zygote occurs after that of the primary endosperm nucleus. The early stages of embryo development up to the globular-shaped stage were not observed in this study but are assumed to follow the general pattern described for other angiosperms in general texts, such as those by Maheshwari (1950), Wardlaw (1955), Foster and Gifford (1959), Fahn (1974) and Sprone (1974).

From six days after pollination the embryo begins to produce cotyledonary outgrowths following the normal pattern of dicotyledonary development as described by a number of workers (Raven and Curtis 1970; Fahn 1974 and Neushul 1974). The embryo progresses through the heart-shaped to the torpedo-shaped stages as outlined by Raven and Curtis (1970) Fahn (1974) and Williams and White (1976). At the early cotyledonary stage, the embryo also begins to bend to adapt to the shape of the embryo sac as in other species (Maheshwari 1950; Foster and Gifford 1959). At this stage, the apical meristem (plumule) is conspicuously different from that of the monocotyledonous embryo (Raven and Curtis 1970; Fahn 1974; Neushul 1974).

Although it was not possible to determine the stage at which the embryo reaches its final cell number, it attained maximum size 14 days after pollination. At this stage, the cotyledons had reached the chalazal end of the embryo sac but the dry weight of the seed was only

about 40% of its maximum dry weight. (Page 40). These findings appear to be in accordance with the results of Loewenberg (1955) and McKee *et al.* (1955) and also show that the maximum size of the embryo is reached much earlier than the maximum weight of the seed.

The pattern of embryo development in tetraploid 'Pawera' red clover is similar to that seen in diploid red clover by Evans (1961), and although development is slightly slower in 'Pawera', the pattern is also similar to that reported for certain diploid *Trifolium* species (Chen and Gibson 1971, 1974) and for tetraploid *Trifolium ambiguum* (Williams and White 1976).

The early stages of suspensor formation were not clearly observed in this study, but is assumed that development follows the general pattern seen in other legumes (Wardlaw 1955; Foster and Gifford 1959). The size, shape and number of cells in the suspensor can vary between species (Bhatnagar and Johri 1972) even within the Leguminosae (Maheshwari 1950). However, in the later stages of embryo development, the suspensor of 'Pawera' appears as a globular shaped structure which resembles the *Trifolium minus* suspensor described by Johansen (1950).

B. CELL STRUCTURE CHANGES IN THE SEED COAT DURING SEED DEVELOPMENT

LITERATURE REVIEW

The important role of the impermeable seed coat in small-seeded legumes in maintaining seed viability and causing delay in germination has been well recognized. It has been suggested by various workers that the failure of germination of viable legume seeds was mainly due to the impermeable layer in the seed coat. Although previous workers have investigated the cell structure in the seed coat of different plant species, including small-seeded legumes, no detailed accounts on the seed coat structure of red clover during different seed developmental stages have appeared in the literature. Therefore, investigation of changes in cell structure in the seed coat during seed development in 'Pawera' red clover was warranted.

As the ovule matures into a seed, the integuments undergo conspicuous changes. In legume seeds, the inner of the two integuments disappears during the ontogeny of the seed, whereas the outer integument differentuates into a variety of distinct layers (Esau 1960). Mostly these involve a reduction in thickness and disorganization but sometimes, additional layers may be formed and the thickness becomes increased (Bhatnagar and Johri 1972).

Ballard (1973) defined "seed coat" as the covering structures such as glumes, lemmas, paleas, fruit wall, testa, and even deeper layers like endosperm. In leguminous seeds, the seed coat is rather complex, conisting of a number of structures, of which one or more may be the cause of 'hard seededness'. The outermost cell layer of the seed coat known as the malpighian or palisade layer is generally assumed to be responsible for impermeability (Hamly 1932; Martin and Watt 1944; Watson 1948; Pandey 1955; Ballard 1973; McKee *et al*. 1977). However, this layer itself consists of several closely related structures namely the cuticle, sub-cuticular layer, domes, light line and cell lumen (Hamly 1932; Corner 1951; Chowdhury and Buth 1920; Martin and Watt 1944; Watson 1948). In *Lotus* species, however, there is another

layer of empty cells between the malpighian and osteosclereid cells
with regular dimensions and arrangement which is termed the intermediary
layer (Watson 1948). Scott *et al.* (1962) found that there are two layers
of osteosclereid cells with a fibrous zone and vascular tissue between
them in *Cercidium floridum* (Leguminosae).

Interior to the osteosclereid layer, there is a nutrient layer
which consists of a few layers of thin-walled cells of various sizes
(Pammel 1899; Coe and Martin 1920; Hamly 1932; Martin and Watt 1944;
Watson 1948; McKee *et al.* 1977). A band, probably made up of collapsed
nutrient cells is present just below the nutrient layer in the seed of
Lotus species and in some species of *Trifolium* and *Medicago* (Watson
1948). A few layers of endosperm cells, including the aleurone layer
are formed immediately surrounding the embryo (Hamly 1932; Watson
1948; McKee *et al.* 1977).

a.   Cuticle: The cuticle is the outermost layer of the seed coat and
                is best seen in longitiudinal sections (Chowdhury and
Buth 1970). It appears in the very early stage of ovule development
(Pammel 1899). In the seed of *Melilotus* species (Martin and Watt 1944),
the cuticle becomes distinguishable about 8 days after pollination. In
mature seed of *Trifolium pratense*, the cuticle forms a continuous even
layer with darker colour than the cuticularized layer (Pammel 1899).

Hamly (1932) reported that the cuticle of sweet clover *(Melilotus
alba)* is a very thin layer which covers the subcuticular layer externally.
Similarly, Aitken (1939) found that the outer surface of the melpighian
cells is covered by a thin cuticle in subterranean clover *(Trifolium
subterraneum)*. In beans and peas, the epidermis is highly specialized at
maturity and possesses a well-defined cuticle (Reeve 1946 a,b). The
cuticle on the seed of most *Trifolium* species (including *T. pratense)* is
thin but a few other species have a fairly thick cuticle (Watson 1948).
Corner (1951) reported that in leguminous seeds,the cuticle is generally
thin and smooth. In alfalfa *(Medicago sativa)*, a slightly thicker layer
of cutin is present on the outer tangential walls of the malpighian
cells in mature seeds than in the walls of immature ones (Anon. 1952).

b.   Sub-cuticular layer: This is a layer in which the apices of the
                              malpighian cells are projected as cones or
caps and is covered externally by the cuticle (Pammel 1899; Coe and
Martin 1920; Hamly 1932; Watson 1948). In sweet clover seed, two
distinct regions occur in this layer, one constituting the cone-like
structures and the other forming a continuous layer over these cone-like
structure.

Aitken (1939) reported that in subterranean clover, the sub-cuticular
layer (matrix) is formed in the later stages of seed development.
Martin and Watt (1944) found that this layer and domes appear in the
seed coat of sweet clover seed about 13 days after pollination. In
mature lucerne *(Medicago sativa)* seed, the radial walls of the malpighian
cells are not visible and appear as a continuous sub-cuticular layer
(Anon 1952). This layer is also known as the "cuticularised layer"
(Pammel 1899; Coe and Martin 1920; Hamly 1932; Martin and Watt 1944;
Anon 1952).

c.   Malpighian cells: In the seed coats of the Leguminosae, a layer of
                              radially elongated cells exist. These cells
are palisade-like but devoid of intercellular spaces and have been
termed malpighian cells after the investigator who first described them
(Fahn 1974). The outermost cell layer or the outer epidermis of the
outer integument in legume seeds develops into the malpighian layer
(Coe and Martin 1920; Martin and Watt 1944; Corner 1951; Anon 1952;
Chowdhury and Buth 1970; McKee *et al.* 1977). This point is emphasized
by Corner (1951) since, in the seeds of some other families, this layer
originates from the outer epidermis of the inner integument. This
latter case is true in *Gossypium herbaceum* (Malvaceae)
(Bhatnagar and Johri 1972; Fahn 1974) and *Malva sylvestris* (Malvaceae)
(Fahn 1974).

These cells have been variously designated as malpighian cells
(Pammel 1899; Coe and Martin 1920; Hamly 1932; Aitken 1939; Martin
and Watt 1974; Watson 1948; Hagon and Ballard 1970; Ballard 1973);
palisade cells (Corner 1951. 1976a.b: Hyde 1954: Esau 1960: Chowdhury
and Buth 1970; Vaughan 1970; Bhatnagar and Johri 1972; Ambegaokar 1976);
Macrosclereids (Reeve 1946 a,b; Esau 1960; Fahn 1974; McKee *et al.*
1977) and prism cells (Reeve 1946 a). Pammel (1899) pointed out that the
term 'palisade' should not be used because it is usually applied to the
elongated, thin-walled parenchyma of the leaf where it is connected

with photosynthesis. while the function of the malpighian cells is chiefly mechanical. Malpighian cells are 56 μ long in red clover seed.

The formation of malpighian cells and their components in some legume seeds has been thoroughly studied by Reeve (1946 a,b), Scott *et al.* (1962), Chowdhury and Buth (1970) and Ambegaokar (1976).

Martin and Watt (1944) working with sweet clover seeds, reported that the differentiation of the epidermal and other cell layers of the outer integument begin at the micropylar and chalazal ends of the seeds from which they spread to other parts. Within the malpighian cells the cuticularized layer, domes and light line appear in succession. These constitute a barrier to water movement in ripe seeds.

In *Pisum sativum*, Reeve (1946 b) observed that the macrosclereid cells are derived from a well defined protoderm in the young ovule. These cells follow a regional pattern of differentiation over the testa, first becoming extremely elongated in the strophiole region before appreciably elongating in the lateral walls of the ovule. Early circumferential growth of the ovule occurs while the macroscleroids undergo rapid anticlinal divisions. Later growth occurs during further elongation and also enlargement of the macrosclereid cells in a tangential direction.

The early stages of secondary wall formation begin in histologically order portions of the tests such as the strophiolar region. Secondary wall development in the macrosclereids also follows a regional pattern which mostly parallels the sequence of cell elongation. When the macrosclereid has almost approached its maximum length, secondary wall thickenings have already begun. The macrosclereid cells are characterised at maturity by fluted, twisted wall thickenings which are most pronounced in the apical parts of the cells.

Ambegaokar (1976) reported that the size of the palisade cells are uniform all over the seed of *Vigna triloba*, except at the region of the hilum and horn (strophiole). While they are small and narrow in the region of the hilum, the maximum height is in the region of the horn and gradually decrease on either side. The cells elongate tangentially and develop secondary thickening from the apex to base. In surface view these cells appear hexagonal and the lumen shows lignified thickenings.

d.   Mucilage stratum: In some leguminous seeds, the outer periclinal
                         walls of the palisade cells become more or less
gelatinous and often show successive thickening.   This feature is
very obvious on the sides of the seed of *Erythrophloeum guineense*
(Corner 1951).   The outer, firmer ends of the cell walls project as
cones or caps into the mucilage stratum, the formation of which takes
place by periclinal zones of hydration or vacuolation in the outer walls.

In *Delonix regia* and many other species this layer is absent or so
slight as to be difficult to observe.   In *Bauhinia fassoglensis* each
palisade cell is surrounded by a mucilage sheath, derived from the primary
layer of the wall (Corner 1951).   Scott *et al.* (1962) found that in
Cercidium floridum, the mucilaginous zone is formed inside the inner
hour-glass cells by bordering with transitional parenchymatous elements.

e.   Light line: A thin line which runs across the upper portion of the
                   palisade or malpighian cells and parallel to the
surface of the seed is called the "light line" (Hamly 1932;   Aitken 1939;
Martin and Watt 1944;   Reeve 1946 a,b;   Watson 1948;   Scott *et al.* 1962;
Chowdhury and Buth 1970;   Ballard 1973;   McKee *et al.* 1977) or the
"linea lucida" (Corner 1951;   Ambegaokar 1976).

Coe and Martin (1920) reported that in *Melilotus alba* and *M. offic-
inals* a well-developed light line appears immediately below the bases
of the cones.   The light-line is more conspicuous because it refracts
light much more than the regions above and below it.

The optical and chemical properties of the light line have been
thoroughly studied by Hamly (1932) and Reeve (1946a).   Hamly (1932)
considered that the light line seems to be formed by differences in
refractive index between the composition of the suberin caps and the
underlying walls of the malpighian cells.   It is therefore concluded
that the phenomenon of the light line is due to the conditions produced
by the juxtaposition of the cellulose and the suberin.

Reeve (1946 a) reported that the light line in the macroscereid
of peas, beans and other leguminous seeds may be observed in close
proximity to the termination of the lumen and where the fluted thicken-
ings converge to form the end wall.   He also found that there is no

chemical differentiation of the light line from the rest of the secondary
thickenings of the cells. Therefore, he concluded that it does not
seem logical to assign specialized functions of texture and permeability
to an optical illusion.

Scott *et al* (1962) carried out a light and electron microscopic
study on the seed coat of *Cercidium flordum* (Leguminosae). They
observed that the microfibrillar pattern throughout the length of flanges
of cells is dominantly parallel but a change to a recticulate arrangement
occurs in the light line region. Similarly Reeve (1946a) reported
that even though there is no differences in the structural orientation
of the cellulosic fibrils, the change of position caused by the convergence
of the thickenings over the apex of the lumen is observed.

According to Miller (1967), the light line may be a common phenomenon
in the seeds of legumes and certain other families e.g. Cucurbitaceae,
Labiatae, Malvaceae. He found no indications that the radial wall of
this region is chemically altered or different from the rest of the
cell. It does not contain a deposit of amyloid, callose, cutin,
lignin, suberin or wax.

f.   Osteosclereid cells: These cells are derived from the cells of
the sub-epidermal layer (Martin and Watt
1944;   Esau 1960;   Vaughan 1970;   Ambegaokar 1976) or the outer hypodermis
of the outer integument as it is called by some workers (Reeve 1946a;
Corner 1951;   Miller 1967) and in some cases from its inner epidermis
(Corner 1951). These cells lie between the malpighian cells and the
nutrient layer (Pammel 1899;   Coe and Martin 1920;   Esau 1960).

Usually these cells are in a single layer (Corner 1951:   Miller
1967:   Ambegaokar 1976) but there are two rows in the outer hypodermis
of *Delonix regia* (Caesalp.) while in the Papilionaceous *Canavalia. Ormosia*
and *Vicia*, there may be 2-6 rows near the hilum (Corner 1951). In
Voandzeia and *Canavolia* according to Ambegaokar (1976), there are 4-5
layers, the lower layers consisting of smaller cells which gradually
merge into the spongy parenchyma. In other seeds, there are no hourglass
cells or they may be so rudimentary as to be indistinguishable (Corner 1951).

Reeve (1946 a.b) reported that differentiation of the osteosclereid cells occurs in a regional pattern over the testa paralleling that of the macrosclereids. However,there is no definable hypodermal layer when the macrosclereids of the strophiole are well elongated. This is because the early stages of cell differentiation lag considerably behind the growth of the macrosclereids.

Watson (1948) explained that osteosclereids are always present in the seeds of some legume species but they are much larger and more conspicuous in some seeds than in others. In some seeds they are hourglass shaped and separated by large triangular or small prismatic intercellular spaces.

Ambegaokar (1976) identified three types of hourglass cells. Type (A) cells are somewhat elongated and dumbell-shaped and occur in *Canavalia, Voandzeia,* and *Vigna.* Type (B) cells are characterized by shorter and broader cells with basal stellate projections as in *Clitoria, Alysicarpus* and *Trigoniella.*   In *Trifolium,* bottle-like cells (Type C) are observed.

g.   Nutrient layer: This layer is termed "nutrient" because its cells are important in conducting food products during seed development and eventually collapse when the seeds mature, appearing only as a line (Pammel 1899; Watson 1948). The cells of the layer contain not only water but chlorophyll and also starch which serves to nourish the growing seed (Pammel 1899).

This tissue forms the body of the testa and develops from the middle cell-layer or cell-layers of the outer integument. During the ripening of the seed, the tissue becomes thick-walled and the cells contract into angular or condensed shapes. Comparisons are difficult to make without reference to the unripened seed (Corner 1951).

This layer is variously named the "nutrient layer" (Pammel 1899; Coe and Marton 1920; Hamly 1932; Aitken 1939; McKee *et al.* 1977), "parenchyma" (Reeve 1946a; Esau 1960; Miller 1967; Vaughan 1970) or "mesophyll tissue: (Corner 1951; Chowdhury and Buth 1970). It has also been called the "pigment layer" because of the presence of pigments (Pammel 1899).

Pammel (1899) found that in *Trifolium pratense*, this layer is unequally developed, from a few rows of cells to a dozen. The cells are thin-walled, somewhat elongated and compressed. Similarly, Coe and Martin (1920) reported that this layer ranges from four to seven cells in thickness in different parts of the seed coat of *Melilotus* species.

Miller (1967) reported that there are 3-5 layers of parenchyma cells immediately below the osteosclereid layer in *Crotalaria* seeds (Papilionaceae). He found that the cells of this relatively loosely organized tissue with intercellular spaces are thin walled and polyhedral in shape. As the seed coat matures, the cells become horizontally compressed. They are eventually crushed and disintegrate. The parenchyma is also variously modified in the hilar region.

h.　Inner integument and nucellus: The inner integument forms usually as a single row of cells during the earlier stages of seed development in Papilionaceae. However, it is very much compressed in the mature seed and is difficult to distinguish from the nucellus. In Caesalpinieae the inner integument may be observed even in mature seed (Pammel 1899).

Pammel (1899) also stated that the nucellus usually disappears in the early stages of fertilized ovule development although it may be observed for some time in the chalazal region. The cells of this layer have lost most of their structure and appear as elongated thread-like bodies.

i.　Endosperm: The formation of endosperm in the young ovule has been reviewed in the previous section. This section describes the endosperm in the mature seed. Earlier investigators regarded legume seed as being entirely exalbuminous or having no significant endosperm. However, subsequent investigations have revealed that the endosperm even though it may not be found in seeds of all species of Leguminosae, does occur in a large number of them.

Pammel (1899) reported that the seed of *Trifolium pratense* contains the thick-walled cells of the aleurone layer. Underlying this layer, primary, thin-walled cells and one or two rows of elongated, thick-walled endosperm cells are observed. Similar findings with sweet clover seed

have been reported by Martin and Watt (1944). They further stated
that the endosperm, occurring as a moist blanket about the embryo,
may possibly play an important role in the longevity and germination
of seeds.

Watson (1948) described that in *T. pratense* the aleurone layer
appears as a row of cuticularized cells, clinging to the cotyledons
instead of the nutrient layer as occurs in most seeds. No other endosperm
layers inside the aleurone cells are observed in these three genera.
Anon (1952) reported that the aleurone cells in the mature seed of
Medicago sativa, have very thick walls which are considered mucilaginous.

Miller (1967) found that all mature *Crotalaria* seeds contain a
distinct endosperm completely enveloping the embryo. The endosperm
appears opaque relatively hard and shrunken in dry seed. It becomes
gelatinoid and increases considerably in volume when in contact with
water. In its dry condition it has been described as being horny.
The endosperm tissue consists of 3-5 layers. The cells are parenchymatous,
thin walled, and compact with apparently no intercellular spaces.
They are more or less polyhedral and somewhat rectangularly elongated
around the embryo. The innermost cells often appear crushed and
disintegrated. The cells of the aleurone layer are cubical, with their
outer tangential wall thicker than the others. The aleurone is two
to three layered in the subhilar region and is often several layered
in the radicle tip region.

Paramonova (1975) reported that the endosperm in many legumes
(including peas and beans) remains non-cellular until the end of its
existence. He recorded that the embryo is enveloped by a thin film
of endosperm in the ripe seed of beans. In crownvetch seed (Leguminosae),
the presence of a single layer of aleurone cells in the testa has been
reported. The endosperm cells are also present below the aleurone
layer (McKee *et al.* 1977). Sidhu and Cavers (1977) also observed a
thin sheath of endosperm surrounding the embryo in *Medicago lupulina*.

j.   <u>Differences in the texture of cells:</u> Although no published information
                                  on differences in the texture
of cells at different positions on the surface of the seed coat apparently

occurs in the literature, the structure of the seed coats of permeable
and impermeable seeds has been examined by previous workers.

In sweet clover, there is no major difference in chemical structure
between permeable and impermeable seed coats. Those differences which
are observed are in the character and amount of thickening of the malpighian
cell wall (Coe and Martin 1922). Similarly, Stevenson (1937) failed
to detect any structural differences between the pseudo-hard and hard
seeds of sweet clover.

Aitken (1939) reported that the testas of both soft and hard seeds
of subterranean clover show the same physical and chemical organisation.
The principal difference is mainly due to the formation of various
permeable sites on the seed coats of soft seeds.

On the other hand, Ueki and Suetsugu (1958) found differences in
the morphological appearance of the strophiole in soft and hard Genge
seeds (*Astragalus sinicus* L). Seeds with transparent strophioles were
hard and those having opaque strophioles were soft. They noted that the
transparency of the strophiole could be altered by temperature treatments.
When hard seeds with transparent strophioles were exposed to alternating
temperatures of 40° and 20°C, transparent strophioles became opaque
or soft due to change in the strophiole tissue. They also observed
that the palisade layer, parenchyma and cotyledons were tightly
appressed at the strophiole in hard seeds but they were separated in
soft ones.

Some morphological differences in the coats of permeable and
impermeable seeds of *Pisum elatius* were recently reported by Marbach
and Mayer (1974). They found differences in the outer structure of
the seed coats of air dried and vacuum dried seeds. The structure of
the seed coat of air dried seed was regular and well organised while
vacuum dried seed showed seedcoat cell deformation and irregularity.
The former was impermeable to water whereas the latter allowed the
entry of moisture into the seed.

RESULTS

The morphological appearance of cell structures in the seed coat at different stages of seed development was investigated. However, emphasis was placed on three main sites i.e. hilum, micropyle and strophiole – since these areas are assumed to be responsible for the hardening or softening of seed.

Plates (26, 32-38, 45 and 46) are mainly concerned with the cell structure at the hilar region although some also include the micropyle. Plates (21-24, 42, 44 and 51) show the changes in cell structure at the strophiolar region. Plates (28, 40, 41, 46 and 50) show details of the structures of cells from other parts of the seed coat. Some of these plates are presented in Sections A and C.

The cell structure of the seed coat is extremely complex. Although it has been studied in considerable detail and there is general agreement on the form and function of the various cell types, some nomenclature variation occurs between different workers. In order to obtain uniformity of nomenclature in the present study it was decided to use the following terms to identify the cell structures of the seed coat.

a. Cuticle
b. Sub-cuticular layer
c. Malpighian cells (Palisade cells)
d. Mucilage stratum
e. Light line
f. Osteosclereid cells
g. Nutrient layer
h. Endosperm
i. Cell surface texture

a. Cuticle: The cuticle is sufficiently differentiated to be observed in the testa of sectioned seeds approximately eight days after pollination. It appears as a thin layer in young seed and becomes a little thicker as the seed matures. This smooth cuticular layer with darker colouring than the subcuticular layer, covers the latter externally (plates 28 and 33). It seems to appear at the micropylar, hilar and strophiolar regions earlier than in other parts of the seed coat.

b.   Sub-cuticular layer: This is a continuous layer in which the caps of the malpighian cells are embedded. Most of its thickness is occupied by the malpighian cell caps and the rest of the outer portion forms a continuous layer immediately below the cuticle.

This layer can be seen more clearly with a darker colour than the malpighian layer (Plates 22 and 23). The thickness of the layer increases gradually with the progress of seed maturity. It is not always a layer of even thickness, small variations in thickness being observed at most stages of seed development (Plates 23 and 24). This layer also seems to appear about the same time as the cuticle in the micropylar and strophiolar regions. In the later stages of seed development this layer can be identified in the lateral wall of the testa (Plate 28).

c.   Malpighian cells: The outermost layer of the outer integument develops into the malpighian layer. Some cells in this layer become columnar at the strophiolar region even in the ovule present in the floret just before pollination (Plate 9). Two days after pollination, malpighian cells at the micropylar region also begin to elongate radially (Plate 11) and two days later their elongation becomes more conspicuous (Plate 12). However, those malpighian cells away from the strophiolar and micropylar regions do not show any obvious changes in their shape until approximately eight days after pollination. In the later stages of seed development, the size of the ovule increases rapidly due to further cell elongation and enlargement.

It has been observed that in longitudinal section the caps of malpighian cells at the strophiolar and micropylar regions are pointed especially during the early stages of development (Plate 21) whereas those of corresponding cells in the lateral wall of the testa are more or less flattened. There is no intercellular space between the cells since they are tightly pressed together. During the early stages of seed development, the cell is wider (Plates 21 and 22) but becomes narrower and spindle shaped in the later stages of seed maturation (Plates 23 and 24).
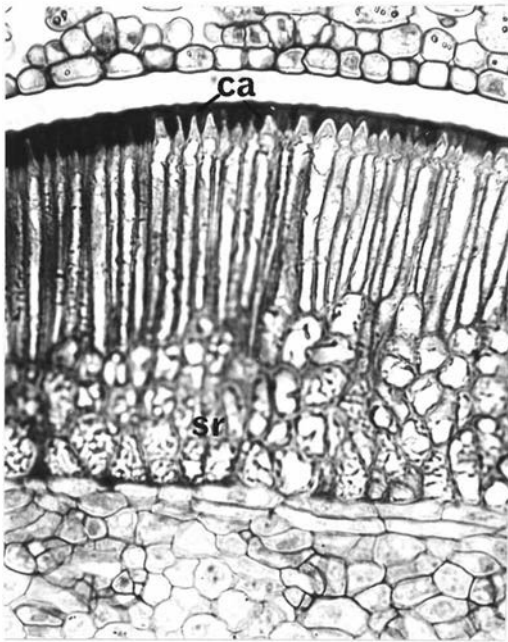
Plate 21: Eight days after pollination –
pointed caps of malpighian cells
and loose sclerenchyma cells
at the strophiole.
ca, cap of malpighian cells;
sr, sclerenchyma cells (LS x 300)

Plate 22: Fourteen days after pollin-
ation – wide malpighian
cells with thickened walls
and cell lumen.
mp, malpighian cells; cl,
cell lumen; sr, scler-
enchyma cells (LS x 350).
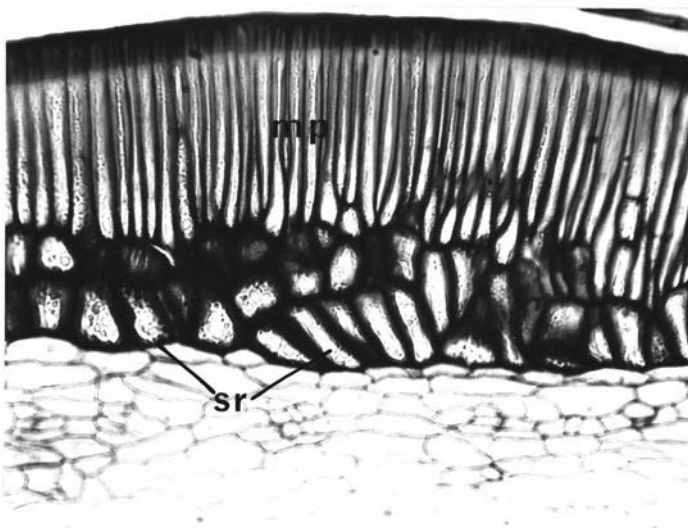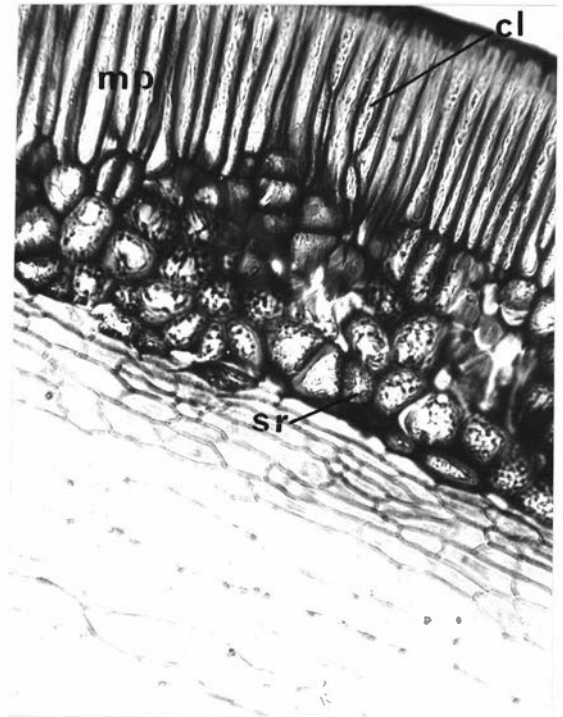
Plate 23: Twenty six days
after pollination –
spindle shaped
malpighian cells over
lying the sclerenchyma
cells.
sc, sub-cuticular layer;
mp, malpighian cells;
sr, sclerenchyma cells
(LS x 300).

The cell lumen is formed from the bottom up to the light line level (Plates 22 and 23). Secondary wall thickening seems to appear 14 days after pollination (Plate 22) but its formation is more obvious starting approximately 18 days after pollination. The margins of the malpighian caps join together just above the light line (Plates 23 and 24). In a mature seed, the average length of malpighian cells at the strophiole is about 120 μ compared with only 40 μ in the lateral wall.

d. <u>Mucilage stratum:</u> No layer of mucilage stratum was observed in the seeds of 'Pawera' red clover.

e. <u>Light line:</u> This is a translucent line that forms across the upper portion of the malpighian cells immediately below sub-epidermal layer. There is no light line in the testa of young seed but a thin faint line begins to appear about 12 days after pollination at the strophiole. This line becomes more conspicuous starting approximately 18 days after pollination when secondary wall thickening is well developed (Plates 23 and 24). Although it is a continuous line around the seed it can be seen more clearly at the hilar (Plates 26) and strophiolar regions (Plates 24 and 25) than elsewhere in the testa. This line is interrupted only by the vascular bundle and micropylar canal at the hilar region (Plate 26).

f. <u>Osteosclereid cells:</u> These derive from the cells of the sub-epidermal layer of the outer integument. Sub-epidermal cells are vacuolate and cubical in four day old seed. However, they begin to develop and to transform their shape starting from the micropylar region adjacent to the embryo about six days after pollination (Plate 14). At 10 days after pollination, the cells in the lateral wall still retain their cubical shape without having any obvious intercellular spaces (Plate 20). The intercellular space is shallow and narrow at the beginning of cell formation. However, the space between the cells becomes deeper and wider with the progress of seed development.

At the early stages of cell formation, about 10 to 18 days after pollination, these cells attain a flask or bottle shape with a narrow neck and rounded bottom having large triangular or small prismatic intercellular spaces (Plates 27). When the seeds become mature, the osteosclereid cells resemble a pillar as the intercellular space becomes much wider (Plate 50). However the shape and size of both the cell and
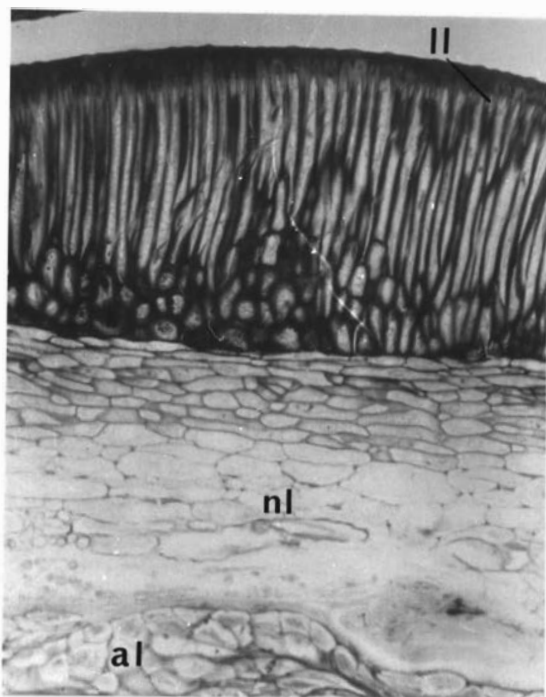
Plate 24: Eighteen days after
pollination.
al, aleurone layer;
ll, light line; nl,
nutrient layer; (LS x 210).
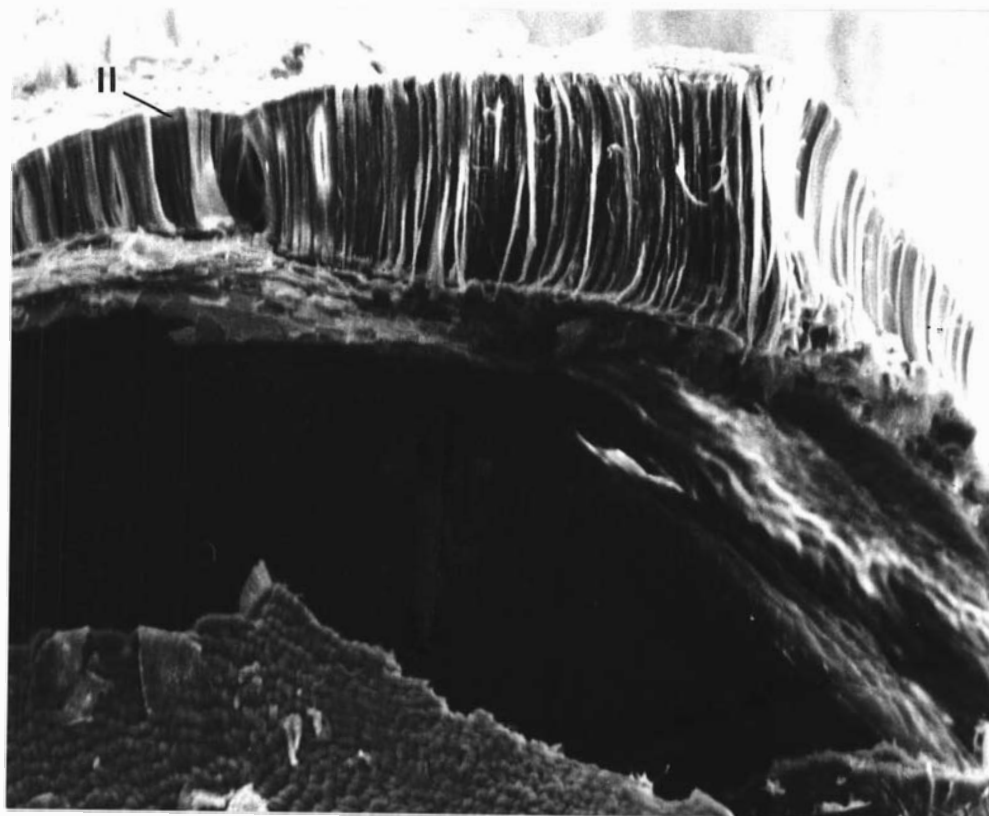
Plate 25: Eighteen days after pollination - scanning electron micrograph
(SEM x 300).
ll, light line.
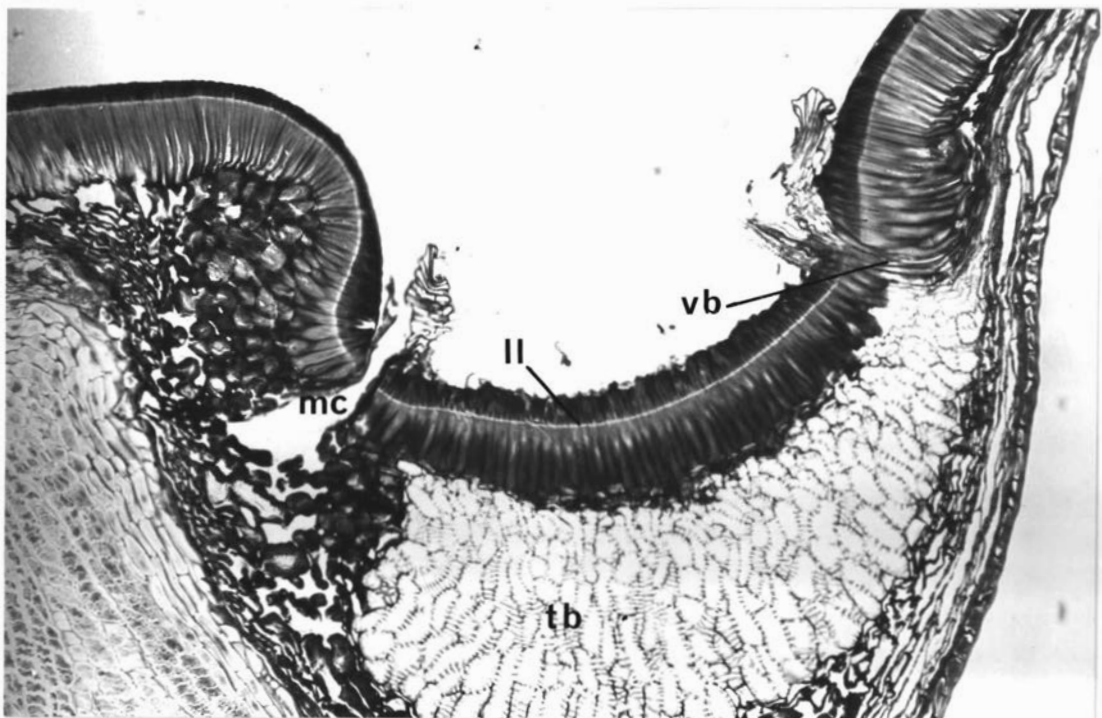
Plate 26: Thirty two days after pollination - hilar region.
11, light line; mc, micropylar canal; tb, tracheid bar;
vb, vascular bundle (LS x 160).
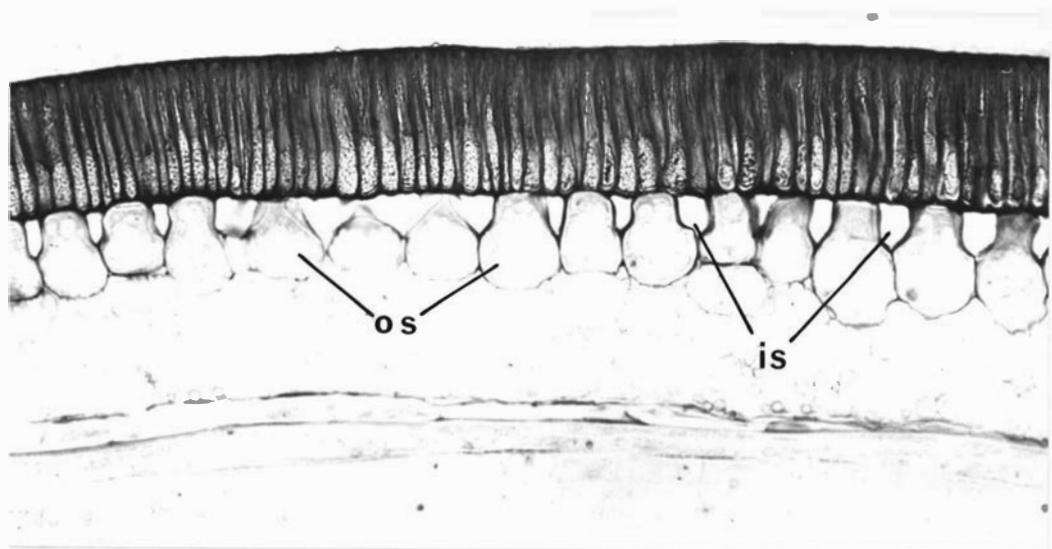
Plate 27: Ten days after pollination - lateral wall of the seed coat.
os, osteosclereid cells, is, intercellular spaces (LS x 60).

intercellular space are different at different positions in the seed coat (Plate 50). In 'Pawera' red clover, only a single layer of osteo-sclereid cells are formed between the malpighian and nutrient layers.

g.   Nutrient layer: This layer is formed between the layer of osteosclereid cells and the aleurone layer (Plates 49 and 50). It contains relatively loosely arranged parenchyma cells with inter-cellular spaces (Plate 49). These cells are polyhedral in shape with thin walls. They seem to develop from the inner layers of the outer integument. The number of layers of cells varies from about four to more than 10 depending on the position in the seed coat and the stage of seed maturity.

This layer can be seen clearly after the degeneration of the endothel-ium and the formation of the aleurone layer. The width of the layer becomes narrower gradually with the advance in seed maturity. The cells become elongated and compressed horizontally and eventually form the body of the testa (Plate 44).

h.   Endosperm: As described in the previous section, the cellular endosperm seems to be digested by the growing embryo. No endosperm cells inside the aleurone layer were observed in either transverse or longitudinal sections of seeds (Plates 44 and 49). However, in some cross sections of mature seeds, vestiges of thin-walled endosperm cells were observed clinging to the aleurone layer at the radicle end (Plate 28).

A layer of aleurone cells adhering to the nutrient layer appears obviously in the testa starting from 18 days after pollination (Plates 28, 49 and 50). The inner tangential wall of the aleurone layer seems to derive from the corresponding wall of the endothelium after degeneration of the latter. The outer tangential wall of the aleurone layer becomes thicker and cuticularised while the inner one reduces in thickness gradually with increasing seed maturity.

The aleurone cells are formed enveloping the embryo in a single layer except at the strophiolar region where 2-3 cell-layers appear (Plates 24 and 49). These thick walled cells are more or less cubical in the early stages of formation but become stretched out and elongated periclinally in mature seeds. The aleurone layer becomes appressed
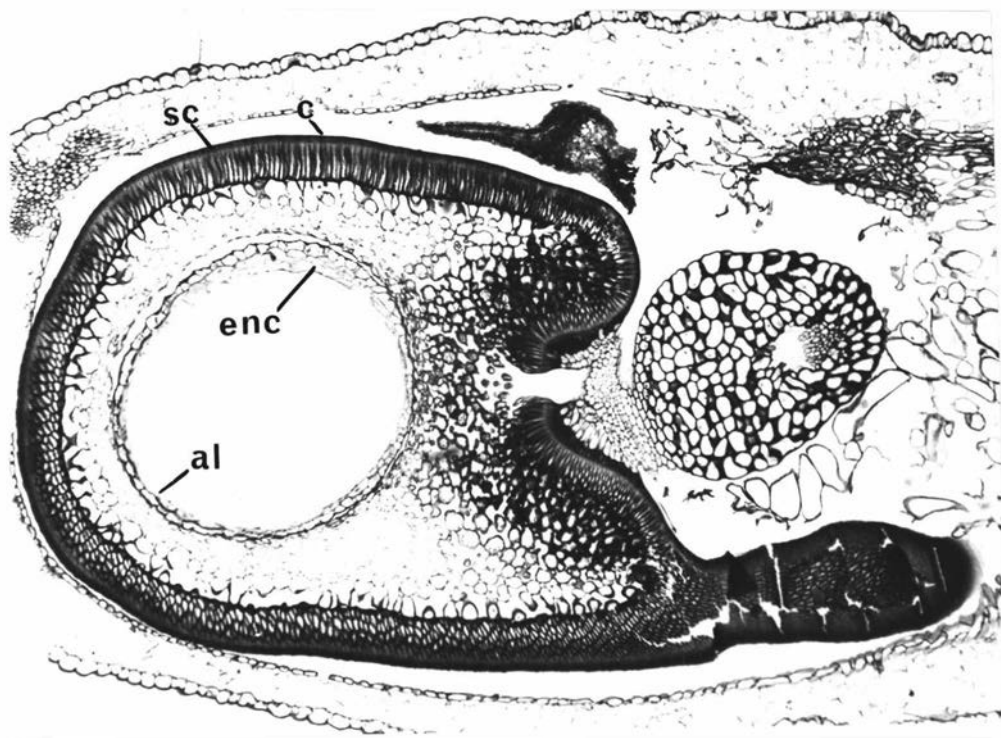
Plate 28: Eighteen days after pollination - hilar and radicular regions.
al, aleurone layer; c, cuticle; enc, thin-walled endosperm
cells inside the aleurone layer; sc, sub-cuticular layer
(CS x 70).
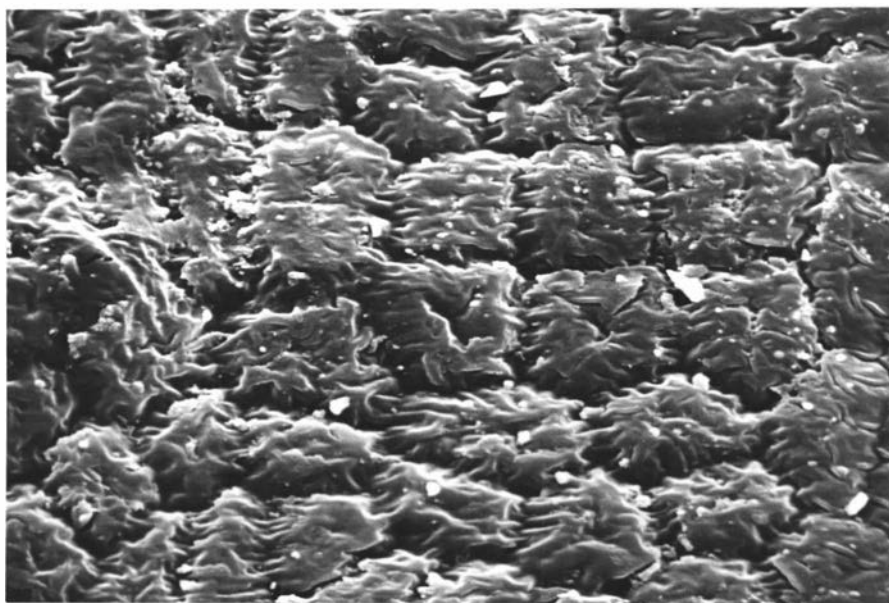
Plate 29: Scanning electron micrograph showing the morphological appearance
of a portion of the seed coat on the cotyledonary lobe (SEM x 2800).

to the testa as the nutrient layer collapses, especially at the strophiolar region when the seed is mature (Plates 44 and 50).

i. <u>Differences in cell textures on the surface of the seed coat</u>:

Different types of morphological appearance were observed at different positions on the seed coat surface of hard seeds. Cells on the outer or more exposed areas of the seed coat possess an outer covering of patches of waxy flakes (Plate 29). This is particularly evident at the area covering the cotyledonary lobes. Those cells at the less exposed areas, such as around the hilar region between the radicle and cotyledonary lobes,give the appearance of a covering of smoother or even waxy cells (Plate 30). It was also found that the patterns of surface cell arrangement and appearance at the strophiolar region was different from other parts of the seed coat. As seen in Plate (31) the cells at the strophiole seem to be arranged linearly along the plane of symmetry but the appearance of individual cells shows more corrugation with large and deep grooves occurring in the intercellular spaces.
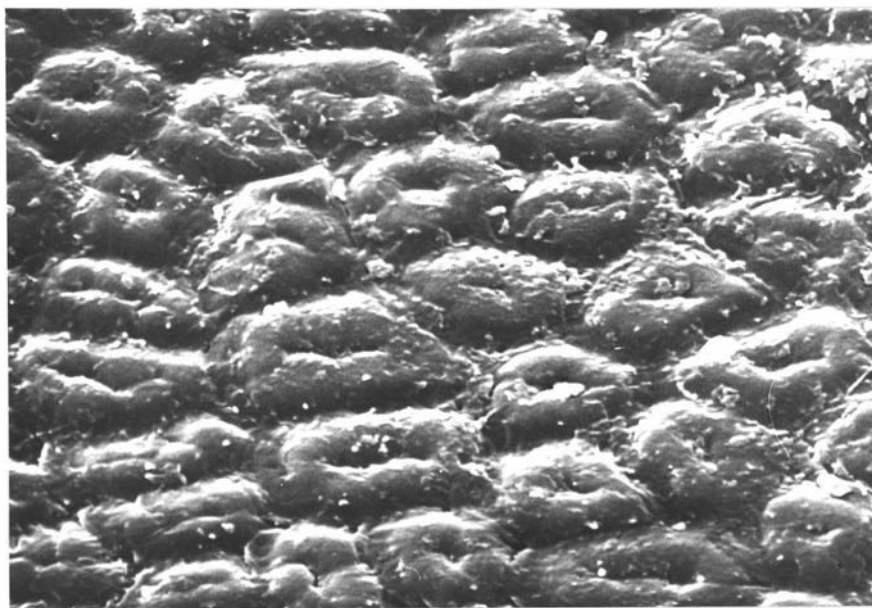
Plate 30: Scanning electorn micrograph showing the morphological appearance of an area near the hilum of the seed coat. (SEM x 2800).
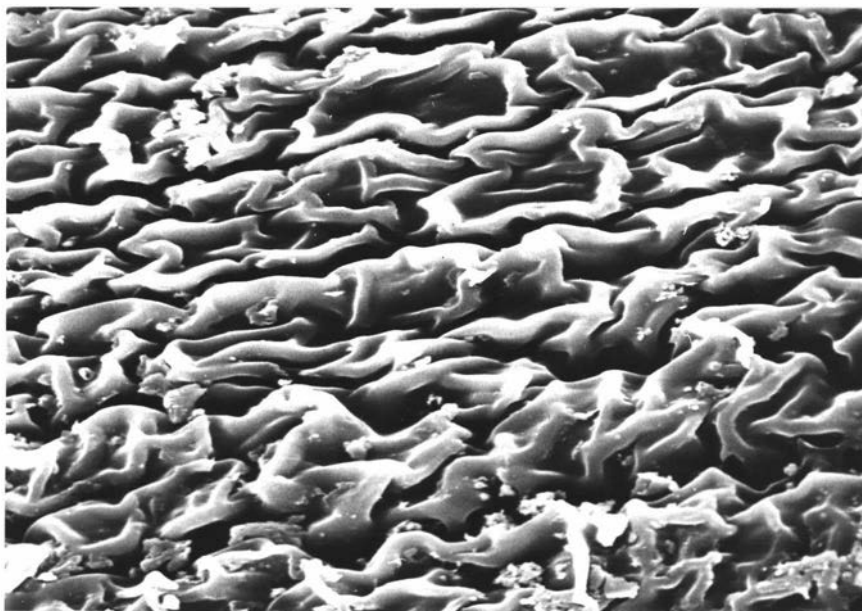
Plate 31: Scanning electron micrograph showing the arrangement of the cells at the strophiolar region (SEM x 2000).

DISCUSSION

Although small variations were noted, cell structures in the coat of 'Pawera' red clover show general similarity with corresponding structures in the seed coat of most leguminous seeds, especially with those of *Trifolium* species. No complete information appears in the literature on cell structure changes in the seed coat of red clover during seed development. The present study was designed to attempt to rectify this situation.

Since young ovules were sectioned longitudinally, in most cases similar sections were made with older seeds to observe the gradual changes of cell structures occurring in the seed coat. According to Chowdhury and Buth (1970), longitudinal sections are particularly suitable for examining the cuticle of the seed coat. However since the cuticle is not easily observed in most sections, it can not be seen clearly in most of the plates presented. As more emphasis was placed on the other structures, this particular structure was generally not well focused. However, as previous workers (Hamly 1932; Aitken 1939; Martin and Watt 1944) have pointed out, the cuticle appears as a thin darker layer in the earlier stage of seed development. Even though there is a small change in its thickness during seed development, this increase is not very obvious. Cuticular roughness as occurs in a few other legume species (Chowdhury and Buth 1970; Ambegaokar 1976) does not seem to be a common phenomenon in 'Pawera' red clover.

The general structure of the sub-cuticular layer is similar with those of some other legume species (Coe and Martin 1920; Hamly 1932; Watson 1948). As reported by Aitken (1939), this layer in the lateral wall of the testa is observed in the later stages of seed development although it can be observed in the earlier stages of seed growth in the micropylar and strophiolar regions. It seems to play a role at least partly in the impermeability of the seed coat in older seed as the thickness of this layer increases with increasing seed maturity.

Although malpighian cells are variously termed by different workers they are most commonly known as malpighian or palisade cells. According to the definitions by Pammel (1899) and Fahn (1974) these cells in the seed coat of 'Pawera' red clover should also be called malpighian cells since no intercellular spaces or connection with photo-synthesis was observed. The term malpighian cells, therefore has been

used in the present study. These cells develop from the outermost cell layer of the outer integument but not of the inner integument as recorded in some species of Malvaceae (Bhatnagar and Johri 1972; Fahn 1974).

As reported by various workers (Martin and Watt 1944; Reeve 1946b) conspicuous cell elongation is observed from the very early stages of embryo development. Secondary wall thickenings seem to form when the malpighian cells reached their maximum height (Reeve 1946b). No apparent change in the length of malpighian cells was observed during seed ripening although this has been recorded by Reeve (1946b). Instead the diameter of these cells seems to reduce a little with advancing seed maturity. In longimedian section the average length of the malpighian cells in the lateral wall of 'Pawera' red clover seed seems to be a little shorter than the corresponding length in other red clover cultivars recorded by Pammel (1899). However, in 'Pawera' red clover the length of these cells also varies from 40-50 µ in different sections. The size and length of the malpighian cells is more or less uniform on the lateral wall of the seed but they have a different morphological appearance in the hilar, strophiolar and micropylar regions as described by Ambegaokar (1976). Variations in the thickness of the wall of the malpighian cells are not always obvious.

Even though the presence of mucilage stratum in the seed coat of a few legume species has been previously reported (Corner 1951; Scott *et al.* 1962), such structure did not seem to occur in the seed of 'Pawera' red clover. This is consistent with the findings of other workers in some other *Trifolium* species. Therefore, the formation of hard seed or seed coat impermeability in 'Pawera' red clover may not be directly related to the presence or absence of this layer in the testa.

The light line in the testa of 'Pawera' red clover seed appears in the normal position as described with other legume species. This line seems to begin development earlier in the seed of 'Pawera' red clover than in *Melilotus alba* (Martin and Watt 1944). Reeve (1946a) found that in ripened seed this line can be identified easily. In 'Pawera' red clover, the light line is generally thin, although it may appear to vary in width due to the thickness of cut sections (Reeve 1946a) or due to the types of light used for observation (Watson 1948).

Although, Reeve (1946a) pointed out the importance of mounting media in highlighting the appearance and position of the light line, the media used in this study does not seem to have had any adverse effect on this aspect. The light line does not always appear very clearly in other parts of the seed coat when compared with the hilar and · strophiolar regions. This may be the reason why Corner (1951) concluded that the light line occurs only at the hilum. According to Chowdhury and Buth (1970) who did not observe the light line in Indian pulses, the occurrence of a light line may not be a common phenomenon in most legume seeds as stated by Miller (1967) although its presence seems to be a structural feature of most small-seeded legumes.

Observations made by Reeve (1946a) and more recently by Scott *et al.* (1962) have shown that the formation of a light line is most likely to be due to a change in the arrangement of the microfibrils on the malpighian cells in that region. Scott *et al.* (1962) also proved the weakness of that zone to confirm their hypothesis. On the other hand, the report of Hamly (1932) seems to confirm that the light line occurs due to differences in the refractive index between the areas above and below the light line region. Since Reeve (1946a) and Miller (1967) did not find this region to be chemically different from the rest of the cells, the most appropriate suggestion about the phenomenon of the light line may be that it is due to changes in micro-fibrillar arrangement which in turn may cause differential refraction of light at that region.

Osteosclereid cells in the seed of 'Pawera' red clover originate from the sub-epidermal layer of the outer integument as observed in most legume species (Martin and Watt 1944; Esau 1960; Ambegaokar 1976) but not from its inner epidermis as occurs in some cases (Corner 1951). It is a single-celled layer between the malpighian and nutrient layers as reported by Hamly (1932), Miller (1967) and Ambegaokar (1976). The osteosclerid cell layer has not been reported in some other *Trifolium* species to consist of more than one row of cells as found in other genera (Corner 1951 and Ambegaokar 1976).

The early stages of formation of osteosclerid cells are very similar to those occurring in *Pisum sativum* L. (Reeve 1946a,b). Reeve's findings on the pattern of cell differentiation agree with those in the present study. Ambegaokar (1976) has suggested that the bottle-shaped cells are typical in seeds of *Trifolium*. However in the present study, this type of cell shape is observed only before seeds

have reached maturity. In mature seeds the shape of these cells resembles the pillar cells described by Corner (1951). Since the shape of the osteosclereid cells varies with seed maturity it may not be appropriate to term them 'bottle-shaped' cells or 'pillar' cells. In the present study, the commonly known term 'osteosclereid' cells has been used in preference to these terms. The appearance of osteosclereid cells in 'Pawera' red clover is not similar to that of corresponding cells in *Melilotus alba* (Hamly 1932). The variation in shape and size of inter-cellular spaces in osteosclereid cells in different parts of the seed coat agrees with observations by Pammel (1899). However, Watson (1948) has suggested there may be differences in the appearance and size of the cells in different seeds.

The shape and appearance of cells of the nutrient layer in 'Pawera' red clover are very similar to those observed in seeds of other legumes (Corner 1951; Miller 1967) and in other cultivars of *Trifolium pratense* (Pammel 1899). As reported previously by Corner (1951), these cells derive from the inner layers of the outer integument. The number of layers of cells observed in 'Pawera' red clover are similar to those found in some other legumes (Coe and Martin 1920; Miller 1967) and especially in diploid *Trifolium pratense* (Pammel 1899).

The formation and appearance of the inner integument and nucellus in the early stages of seed development have been described in section A. Although the inner integument of the seeds of most species of Papilionaceae contains a single row of cells (Pammel 1899), in 'Pawera' red clover two rows of cells are observed in young ovules. However, no vestiges of the nucellus or inner integument were observed in mature seeds. Obviously these structures disappear in the later stages of seed development.

The presence of thin-walled endosperm cells immediately inside the aleurone layer in some cross sections agree with reports by Pammel (1899) and Miller (1967). Furthermore, the results of Hamly (1932) and recent reports by Paramonova (1975), McKee *et al.* (1977) and Sidhu and Cavers (1977) confirm the presence of an endosperm layer in the seeds of some leguminous species.

However, as reported by Watson (1948) with seeds of some *Lotus*, *Medicago* and *Trifolium* species (including *T. pratense*), no endosperm cells except those of the aleurone layer were observed in longitudinal or transverse sections in the present study. The inability to detect the presence of the endosperm layer in sections cut in these planes may have been due to its removal along with the embryo when the latter was removed from the seed coat at the time of embedding.

The present study confirms observations by Watson (1948) and Anon. (1952) that the aleurone cells have thick walls and that the outer tangential walls are thicker than the others. A similar effect has been reported in *Crotalaria* (Miller 1967). However, there is partial disagreement with observations by Miller (1967) that only a single row of cells occurs around the radicle tip and two or three layers of cells are present at the chalazal region instead of the sub-hilar region. Moreover, in 'Pawera' red clover the aleurone layer is not attached to the cotyledons as recorded by Watson (1948) but clings to the nutrient layer.

C.   STRUCTURE OF VARIOUS REGIONS OF THE SEED COAT AND THEIR ROLE IN
      AFFECTING SEED PERMEABILITY OR IMPERMEABILITY

      (a)   Structure of special regions of the seed coat

LITERATURE REVIEW

The general cell structure in the seed coat during seed development
has been described in Section B. However, in the seed coat there
are some more specialised regions which should be examined. In the
Papilionaceae, the three specialised regions of the testa connected
with the loss or gain of seed moisture are the micropyle, the hilum
and the strophiole (Hagon and Ballard 1970; Ballard 1973).

1.   Hilum: In the Leguminosae, when the seed breaks from the funicle,
                an abscission layer is developed so that the hilum is
clearly defined (Corner 1951; Bhatnagar and Johri 1972; Fahn 1974). The
hilum of the pulses shows a great deal of variation in shape and size
(Chowdhury and Buth 1970).   The shape varies from round to oblong,
oval or elliptical (Corner 1951; Miller 1967; Chowdhury and Buth 1970).
The hilum is at a lower level than the seed surface and is surrounded
by a small rim (Miller 1967; Chowdhury and Buth 1970). The appearance
of the hilum contributes to the general seed configuration. The hilar
sinus angle, formed between the cotyledonary and radicular lobes is
very characteristic for each species (Miller 1967).

In the long axis of the hilum there is a groove in the funicular
tissue coinciding with a fissure in the epidermis of the testa (Corner
1951; Hyde 1954; Fahn 1974). The fissure is an air passage in the
ripe seed according to these investigators. The hilar groove or fissure
is usually visible only in seeds where the funicular remnant is not
predominant (Corner 1951; Miller 1967; Chowdhury and Buth 1970) or
where the hilar sinus is not occluded by the radicular lobe (Miller
1967).

The palisade of the testa covers the face of the hilum and a layer
of the cells in the funicle is transformed into a layer of counter-
palisade cells (Corner 1951; Esau 1960; Miller 1967). This counter-
palisade layer is fused with the palisade layer of the hilum (Corner
1951; Fahn 1974). Overlying the counter palisade layer is parenchyma

with large intercellular spaces (Hyde 1954; Sidhu and Cavers 1977).

The hilar fissure or groove leads to a group of tracheids termed
the "tracheid island" (Reeve 1946b), the "tracheid bar" (Corner 1951;
Fahn 1974), or the "vascular isle" (Hyde 1954). In transmedian section,
the tracheid bar is funnel shaped (Reeve 1946b), or ovate (Corner 1951)
or U-shaped (Miller 1967) in some legume seeds.   In *Crotalaria* seed,
the tracheid bar is ensheathed by a two to three layered non-stellate
parenchyma which is in turn enveloped by a contiguous transitional
form (Miller 1967). Hyde (1954) also reported that the tracheid bar
is surrounded by stellate cells and the intercellular spaces of these
cells are continuous with those of the subepidermal layer of osteo-
sclereid cells which underlies the epidermis everywhere except at
the hilum and the strophiole.

In longimedian sectional view, the tracheid bar develops as an
elongated crescent shaped band or bar bounded above by the brachyscler-
eids and below by the stellate parenchyma (Miller 1967).   Reeve (1964b)
found that the transitional forms of tracheary elements of the tracheid
bar is connected with the xylem of the ovule bundle. Corner (1951)
also reported that the trancheid bar extends the length of the hilum
from the raphe almost to the micropyle. At the raphe end it contacts
the sheath of the vascular bundle or may stop short of the vascular
bundle.

The tracheid bar appears at an early stage in seed development
as a firm strans of watery tissue surrounded by a narrow sheath of
small-celled parenchyma (Corner 1951). Before any pronounced different-
iation of the tracheid bar occurs, early differentiation of the palisade
cells over the hilum is virtually complete. The cells of the bar are
the last to become well-defined as a tissue zone (Reeve 1946b).
At maturity the thickened tracheids contain air and the bar forms as a
white strand in the subhilar region (Corner 1951). He further stated
that the tracheid bar acts as a water-reserve for maintaining the
longevity of the embryo in the Papilionaceae. The dried out hilum
and the dried out tracheid bar may serve to aerate the germinating seed.

During the early stages of ovule development, the hilum region
is still highly meristematic in contrast to the differentiating parenchyma
cells of the rest of the ovule wall (Reeve 1946b). Elongation of the

palisade cells in the hilum region progresses from all sides towards the centre of the area. However, this differentiation appears to proceed more rapidly from the strophiolar end than from elsewhere (Reeve 1946b). The palisade cells of this region are similar to those elsewhere in the testa with the exception that they are small and narrow (Miller 1967; Ambegaokar 1976). There is a reduction in their height on the sides of the hilar groove (Miller 1967) or towards the centre of the hilum (Chowdhury and Buth 1970).

The tissues comprising the hilar and subhilar regions of the legume seed are uniquely complex. A greater variety of cell types appears here than anywhere else in the testa (Reeve 1946b; Miller 1967). This may be a characteristic feature of the hilar tissue of all Papil- ionaceae of the Leguminosae (Corner 1951; Miller 1967). Corner (1951) summarized the characteristics of the Papilionaceous seed particularly by the hilum which has typically a rim-aril, median groove, counter- palisade, tracheid bar, two recurrent vascular bundles and the vascular supply without post-chalazal extension. In Mimosoid-Caesalpinoid seeds there is often a hard, compact tissue of 2-6 rows of very thick-walled, pitted, contiguous cells just below the hilum. These serve as a strut against the contraction of the testa on to the radicle (Corner 1951).

Corner (1951) further mentioned that in many leguminous seeds there is no light line in the testa except in the hilum palisade. Miller (1967) reported that in *Crotalaria* seed there is no cuticle between the counter-palisade and palisade cells of the hilum testa, the cuticle terminating on the hilar rim slightly inside the hilar sinus and at the points of attachment of the funicular tissue. It is also absent over the surface of the hilar groove. However, Hyde (1954) explained that the counter-palisade layer is in contact with the cuticle of the epidermis of the tests in lupin seed. Osteosclereids do not occur in the hilar region (Hyde 1954; Miller 1967) but they begin to appear at a distance where the counter-palisade cells disappear (Chowdhury and Buth 1970).

Miller (1967) described the cells of the subepidermal region immediately subtending the palisade layer of the hilar rim as sclerenchymatous. They form a mechanical tissue consisting of numerous short brachysclereids. The tissue contains an abundance of intercellular spaces. In longimedian section the tissue appears as a curved mass

subtending the hilar epidermal layer. It appears as subepidermal
masses immediately below each side of the hilar rim in transmedian sectional
view.

2.   Micropyle: Anatomical observations both on mature seeds and on
                seeds at different stages of development show clearly
that the micropylar canal is blocked (Preston and Scott 1943). They
found that in *Phaseolus,* the micropylar orifice remains open but the canal
is blocked by the swollen cells of the suspensor. In *Vicia,* the lips
of the micropyle close the pore in the dry seed.

       Martin and Watt (1944) also reported that the micropyle is perfectly
closed in sweet clover seeds. When the seeds are sectioned the pressure
of the knife often causes breaks in the palisade layer at the micropyle
but in intact perfect sections, the palisade cells are closely united
about the micropyle.

       In *Crotalaria* seed, Miller (1967) found a minute micropyle opening
in the palisade tissue below the rim of the sinus. This pore occurs
on the radicular lobe side of the seed, slightly below the attachment
of the funicular remnant. The appearance of the radial walls of the
palisade layer is slightly curved around the micropyle.

       According to other workers (Esau 1960; 1965; Fahn 1974), the
micropyle may be obliterated or may remain visible as a distinct pore.

3.   Strophiole: This is a narrow elongated depression, surrounded
                by a raised border close to the hilum on the side
opposite the micropyle (Hamly 1932; Martin and Watt 1944). Hagon and
Ballard (1970) defined it as a small swelling of the testa on the raphe
between the hilum and chalaza. However, in some genera e.g. in
Bauhinia the lens (strophiole) is on the micropylar side of the hilum
(Corner 1951).

       This region is referred to as the "strophiole" (Hamly 1932;
Aitken 1939; Martin and Watt 1944; Reeve 1946b; Hagon and Ballard 1970;
Ballard 1973), "lens" (Corner 1951; Ballard 1973), "boss" (Miller
1967) or "horn" (Ambegaokar 1976).

Aitken (1939) found that in *T. subterraneum*, the malpighian cells at the strophiole are more vacuolate and have less conspicuous nuclei in the very early stages of seed development. In older seeds, the malpighian cells are bent in this area and 3-4 times longer than cells in the rest of the testa. Due to earlier and quicker development of the malpighian cells, the light line appears at this region before the seed is fully formed and dried out.

Reeve (1946b) reported that in *Pisum sativum*, the height of the malpighian cells at the strophiole in a very young ovule may be ten to fifteen times as great as those of adjacent epidermal cells.

Miller (1967) found a rounded integumentary bulge, a short distance above the hilar rim on the seed coat of *Crotalaria*. He explained that its position, degree of configuration and colouration was somewhat variable depending upon the species. The degree of the prominence of the boss (strophiole) results from the progressive elongation of the malpighian cells in this region. He found that the length of the cells is about twice that of the other cells in the testa and the lower portion of the radial wall is often curved. Lagenosclereids (osteoscler-eids) are absent in this region, occurring only on the outer margins of the hypodermis.

Miller (1967) further stated that pigmentation at the strophiolar area is often devoid or it may be of a lighter or darker hue than the remainder of the seed coat. He concluded that its colouration and prominence could be used in seed identification.

Hagon and Ballard (1970) claimed that the long, narrow malpighian cells of the central region of the strophiole have definite caps, whereas the adjacent cells have more flattened ends. A similar situation has also been reported in the testa of *Melilotus alba* by Hamly (1932).

4. Vascular bundle(s):   The vascular system of some legume seeds has been thoroughly studied by Corner (1951). He explained that there is much more variation in the vascular supply in the Papilionaceous than in the Minosoid-Caesalpinoid seed.   In *Crotalaria retusa*, the vascular supply consists of a short pre-chalazal

vascular bundle and two short recurrent vascular bundles without branches to the sides of the seed. However, in other legume seeds, he found both pre-chalazal (in *Erythrina*) and post-chalazal vascular bundles (in *Canavalia*) with short branches to the sides of the seed.

Esau (1960) also reported that the vascular system of many legume seeds is well developed. The vascular bundle extends from the funiculus to the chalazal region where it branches.

Miller (1967) found that in the seed of *Crotalaria*, a minute single vascular bundle enters the seed below the rim of the hilum on the cotyledonary lobe side opposite the micropyle. This vascular connection between the seed and the funiculus has no contact with the embryo. It consists entirely of primary xylem and phloem elements which are enveloped by thin-walled parenchyma cells. The vascular trace traverses the epidermis, brachysclereids and a portion of the stellate parenchyma before terminating within the parenchyma subtending the malpighian cells of the strophiolar region.

Fahn (1974) reported that relatively well-developed vascular bundles may be observed in the testa of certain legume seeds (e.g. *Arachis*), while in others *(Pisum* and *Lathyrus)*, they can not be easily identified.

RESULTS

1. Hilum: At seed maturity there is no more food transport from the
parent plant to the seed. At this stage the seed is disconnected
from the funicular tissue. A round sunken hilum appears between the
radicular and cotyledonary lobes (Plates 36 and 47 d,f). The overlying
parenchyma cells of funiculus can usually be seen at the hilar region
(Plates 32, 33 and 46).

A hilar groove appears in the funiculus right on top of the hilar
epidermal fissure in longitudinal view. Generally, the hilar fissure
of the epidermis is not visible due to the overlying funicular tissue.
However, the hilar region immediately below the palisade layer is
occupied by the tracheid bar, the upper end of which leads to the hilar
fissure.   Plate 32 shows the closed hilar fissure with overlying
parenchyma and funiculus. The hilar fissure in Plate 33 is about
to open and that of plate 34 is open widely.

In face view, an open hilar groove (Plate 35) and a hilar groove
partly covered by the funicular tissue (Plate 36) can be seen. In
cross section, the hilar groove is partly covered by the overlying
parenchyma cells when it is cut at a high level (Plate 46).   However,
in later serial sections the hilar groove can be seen obviously (Plate
37) and the hilar fissure of the epidermis appears clearly as the depth
of cutting proceeds to lower levels of the hilum region (Plate 38).

The shape of the tracheid bar in transmedian section is oval in
'Pawera' red clover (Plates 32, 33 and 34).   This bar is immediately
enveloped by one to two rows of thin walled cells which are in turn
surrounded by stellate cells (Plates 32 and 33). The intercellular
spaces of these cells are connected with those of the osteosclereid
cells (Plates 33 and 34).

The tracheid bar appears as a crescent shaped band bounded above
by the sclerenchyma cells (brachysclereids) and below by the stellate
(parenchyma) cells as seen in longimedian section (Plate 26).   The
tracheary elements of the tracheid bar are in contact with the vascular
bundle. As seen in Plate 26 one end of the tracheid bar stops some
distance from the micropyle while the other end extends to the vascular
bundle. The tracheid tissue begins to develop eight days after pollination
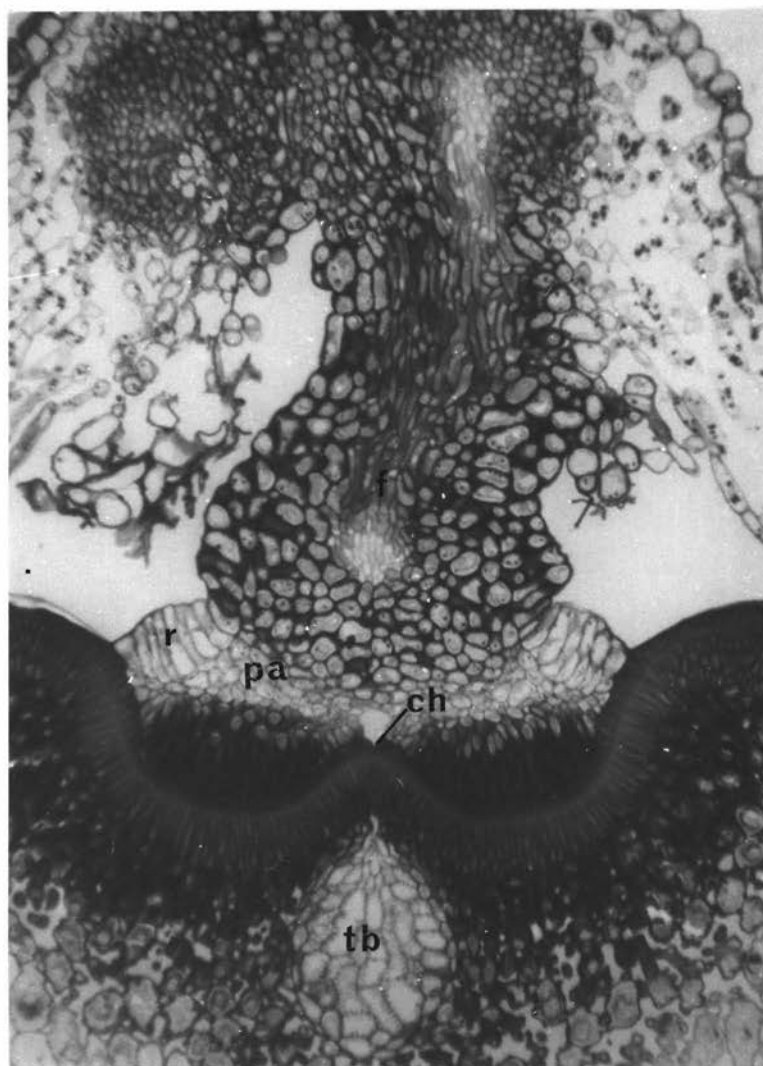
Plate 32: Eighteen days after pollination – hilar region. ch, closed hilar fissure; f, funiculus; pa, parenchyma; r, rim-aril; tb, tracheid bar (TS x 180)

Plate 33: Eighteen days after pollination – hilar region. c, cuticle; cp, counter palisade; hf, hilar fissure about to open; ll, light line; p, palisade (TS x 180).
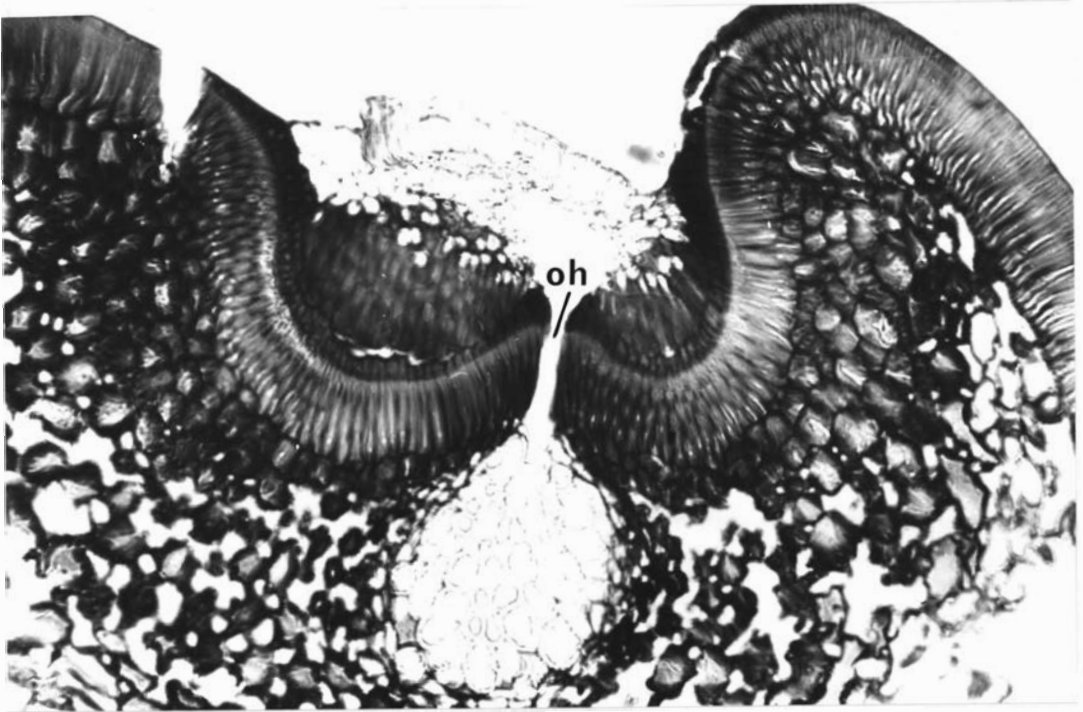
Plate 34: Thirty six days after pollination - hilar region.
oh, open hilar fissure (TS x 180).
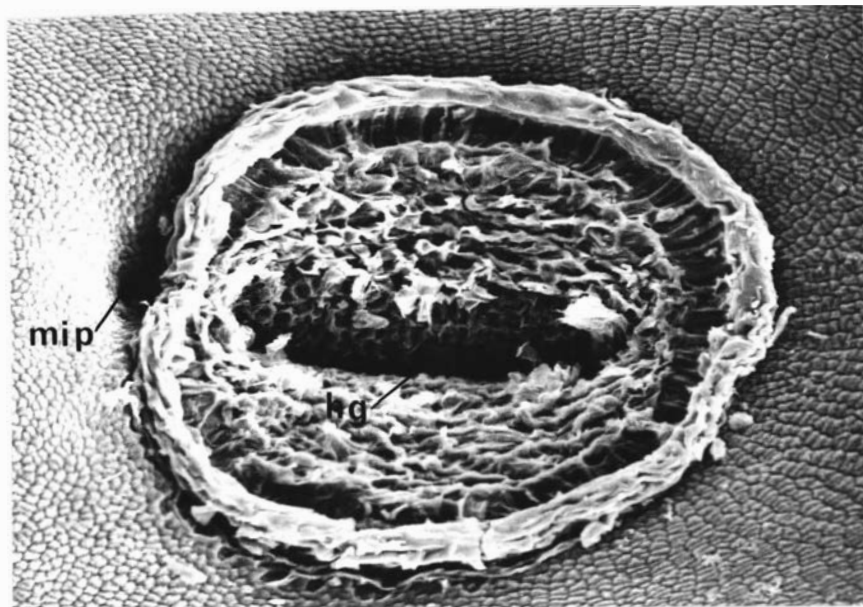
Plate 35: Surface view of the hilum.
hg, open hilar groove; mip, micropylar pore (SEM x 240).
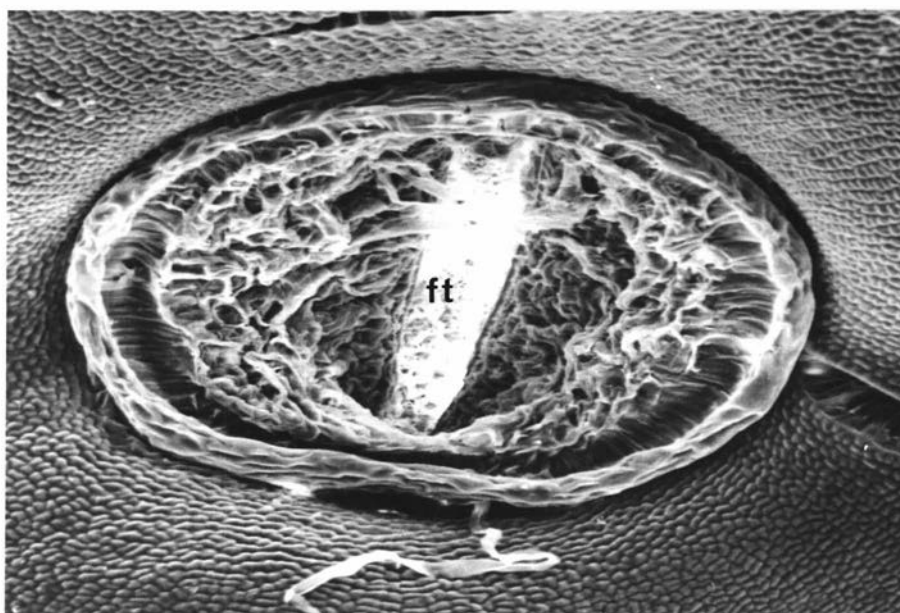
Plate 36:   Surface view of the hilum.
ft, funicular tissue covering the hilar groove (SEM x 220).
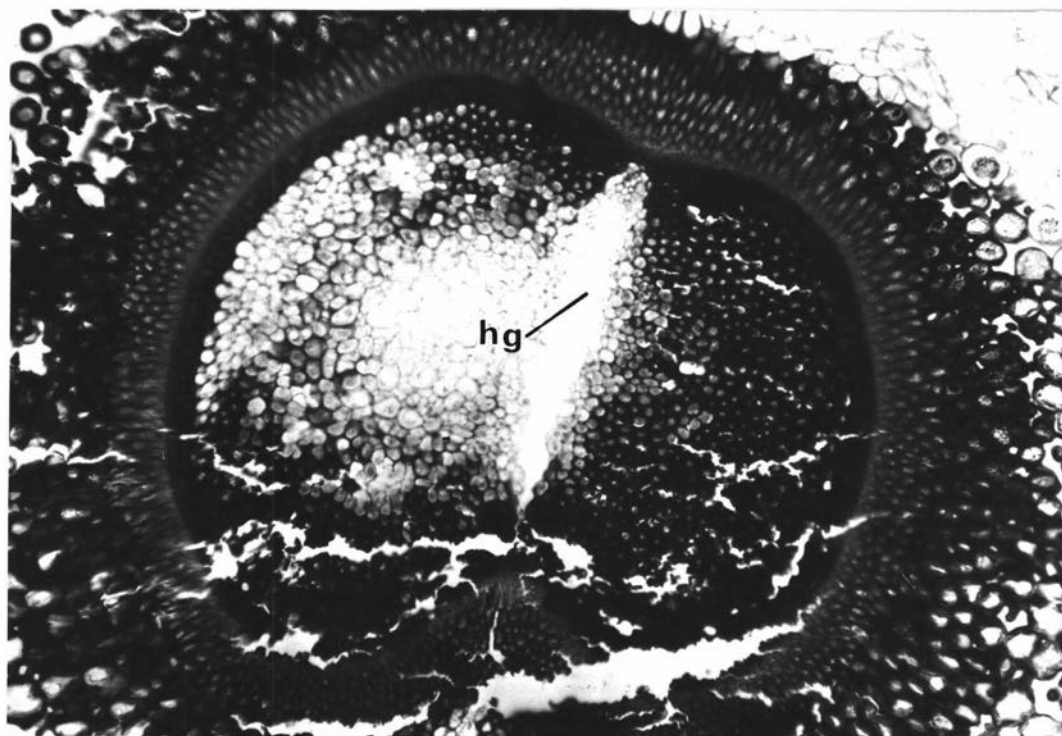
Plate 37:   Hilar groove as seen in cross section at the hilum.
hg, hilar groove (CS x 180).

(Plate 17) and differentiation of the bar becomes more pronounced two days later (Plate 19). In the early stages of development, tracheids stain well but in more mature seed these cells take up less stain (especially with toluidine blue) and the bar appears as a white band in the sub-hilar region.

Palisade and counter palisade tissue at the hilum begins to form six days after pollination and can be seen obviously in the later stages of seed development (Plates 17, 18 and 33). When the cells of the tracheid bar appear clearly, the counter palisade and palisade cells are also well defined, (Plates 17 and 33). The counter palisade seems to develop from the funicular tissue and the former is attached to the palisade of the hilum when the funiculus breaks. The cuticle can be seen between the counter palisade and palisade layers (Plate 33). The palisade cells at the hilum are small and narrow with a small reduction in their height. The counter palisade cells with thickened walls except at the outer ends, show some reduction in their height on the sides of the hilar fissure and on the edges around the hilar rim (Plate 33).

Immediately outside and above the hilar rim, a rim-aril is formed surrounding the parenchyma of the funiculus (Plate 32). No osteosclereid cells are formed at the hilar region. Instead, brachy-sclereids (sclerenchyma cells) occur as hypodermal tissue (Plates 26, 33 and 34).

2. Micropyle: The micropyle occurs immediately outside the funicular remnant of the hilum on the radicular lobe side of the seed (Plates 39, 40 and 45). In most cases the outer end of the micropylar canal of an intact seed appears as a minute pore (Plates 39 and 40). Seed sections show that neither the lips of the orifice nor the canal itself is closed or blocked but show clearly that the micropyle canal does not extend much into the interior of the seed. (Plates 15, 19, 26, 28, 41 and 45). In longimedian section it can be seen as a curved open canal, the diameter of which is about 40μ at the palisade region. The appearance of the palisade cells around the micropyle is slightly curved (Plates 28 and 45).

3. Strophiole: The strophiole is a small swelling of the testa between the chalaza and hilum on the side opposite the micropyle. The morphological appearance of the strophiole of 'Pawera' red clover seed
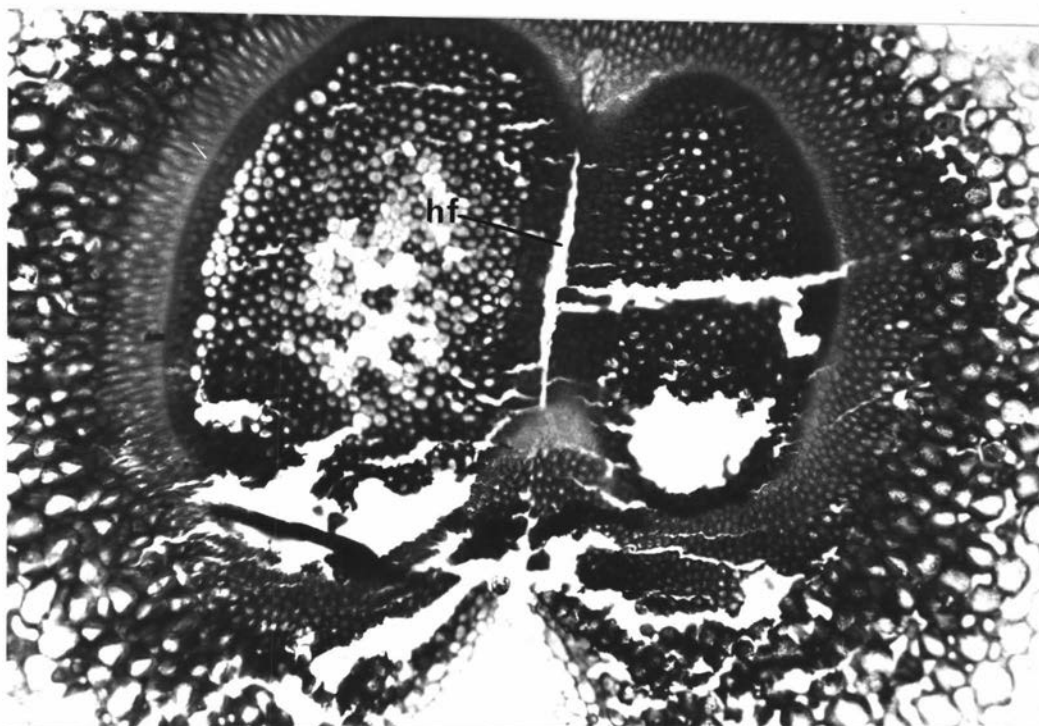
Plate 38: Hilar fissure cut at a lower plane at the hilum than in
Plate 37.

hf, hilar fissure (CS x 180).
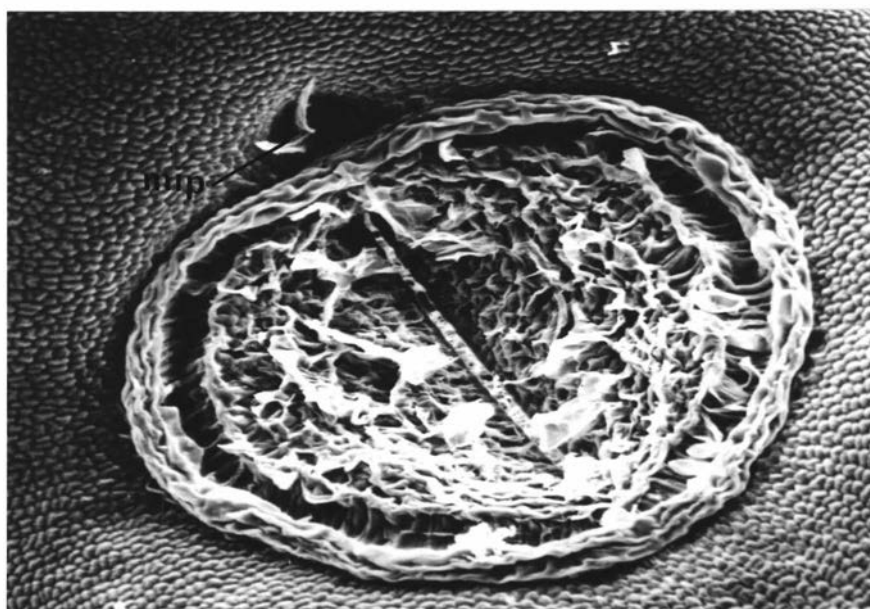
Plate 39: Micropylar pore immediately outside the hilum rim.

mip, micropylar pore (SEM x 220).
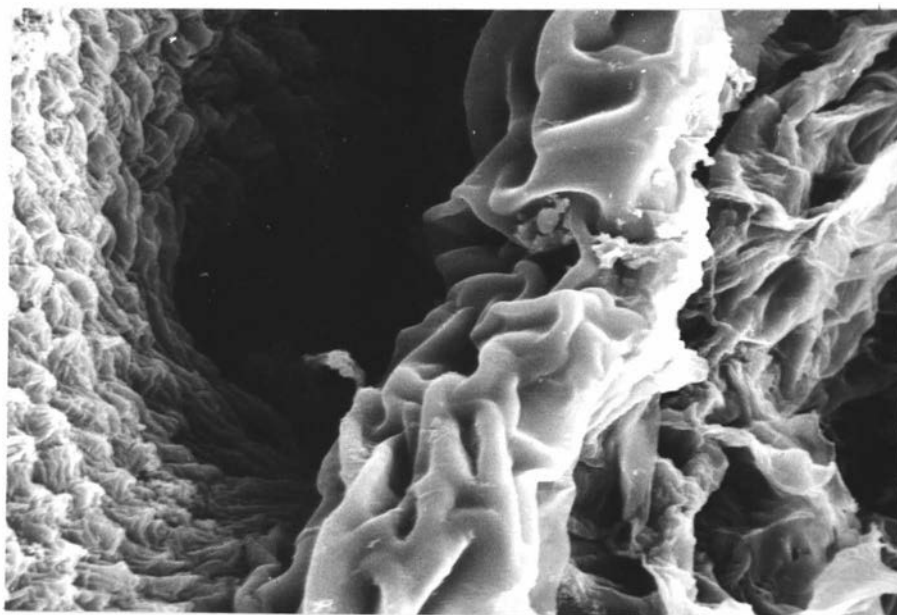
Plate 40:   Surface view of the outer end of the micropylar canal
            immediately outside the hilum rim. (SEM x 1500).

Plate 41: The outer end of the micropylar canal at the hilar region.
          (TS x 180).

can be seen in the plate (47) presented in section (c). The degree
of prominence of the strophiole varies from seed to seed. The colour
of this region is slightly darker than that of the chalazal region and
the colour of the latter is usually lighter than that of other parts of
the seed coat.

The malpighian cells at the strophiole are longer than corresponding
cells in the lateral wall of the testa. (4-5 times longer in the
young ovule and 3-4 times longer in older seed). The initiation of
cell elongation starts at this region beginning in the very early
stages of ovule formation (Plates 9, 15 and 42). The configuration of
the strophiolar region in longimedian section is shown in Plates (23, 24 and
44). The malpighian cells of this area are sited above the brachy-
sclereids (sclerenchyma cells). These brachysclereids start to appear
at this region six days after pollination (Plate 15), and become
more abundant and more obvious with thickened cell walls in the later
stages of seed development. They are mostly loosely arranged and can
be seen clearly in longitudinal section (Plates 22-24).
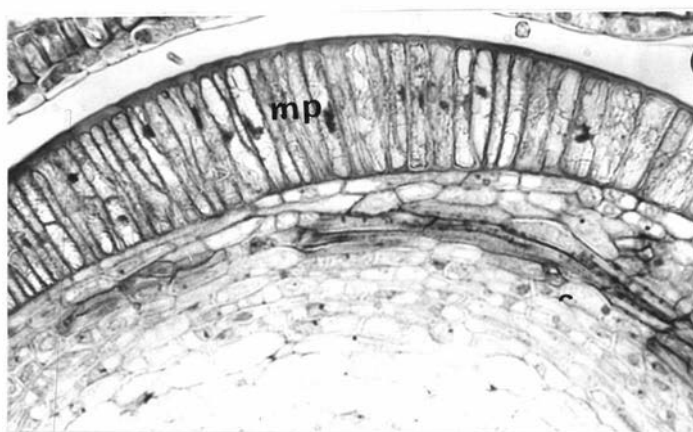
Plate 42: Malpighian cell elongation at the strophiolar region two days
after pollination.
mp, malpighian cells (LS x 300).

Transmedian sections show that the seed coat is bilaterally
symmetrical about the median plane (Plate 43). The malpighian cells
at the centre of the plane of symmetry are straight whereas those cells
on each side of the central plane are bent. The malpighian cells
along the plane are long and narrow but on both sides of the plane,
their length is reduced due to the presence of the underlying sclerenchyma
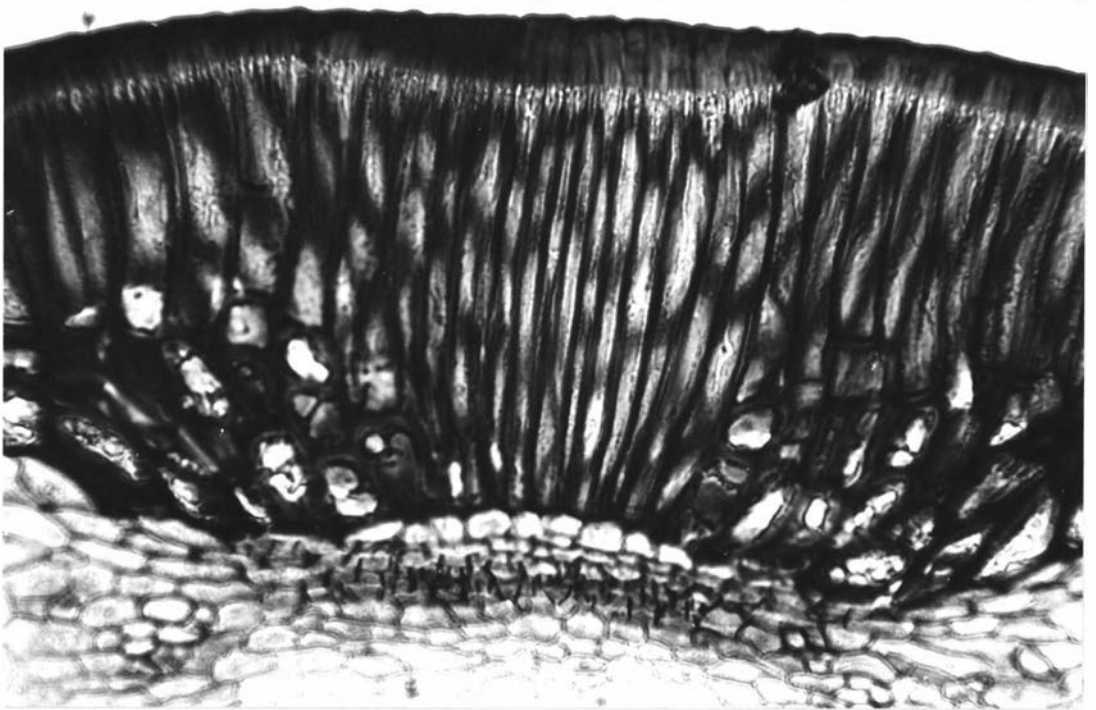
Plate 43: Eighteen days after pollination – transmedian section showing the unique structure of the cells at the strophiolar region (TS x 430).
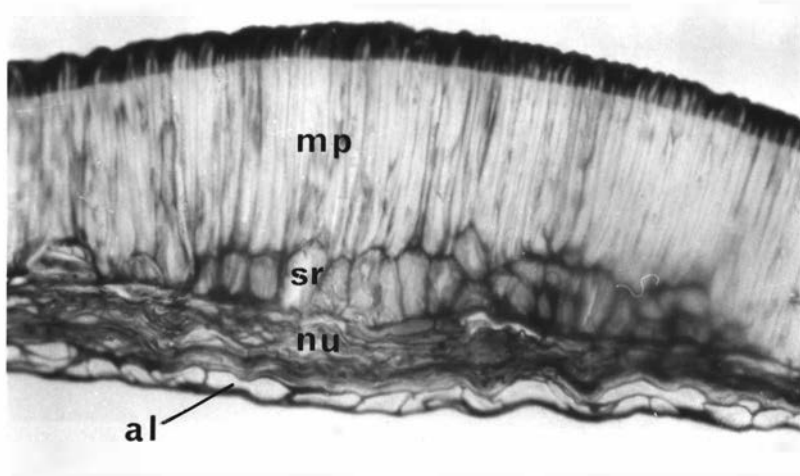
Plate 44: Forty days after pollination – cell structure at the strophiolar region of a mature seed.
al, aleurone layer; mp, malpighian cells; nl, nutrient layer; sr, sclerenclyma cells (LS x 320).

cells (Plate 43). The blackened lamella of the malpighian cells can also be easily seen. Immediately under the malpighian cells of the median plane a few compact layers of cells are formed (Plate 43). When the seed is mature the compact cell layers and nutrient layers are appressed tightly together at the base of the malpighian cells of the strophiole by the aleurone layer (Plate 44).

4.   Vascular bundle: The vascular bundle from the funiculus enters the seed at the hilum on the cotyledonary lobe side (Plates 26 and 45). The vascular elements with conducting vessels pass through the malpighian layer, brachysclereids, a portion of the stellate cells and terminate within the nutrient layer of the chalazal region. No post-chalazal vascular bundle occurs but short branches to the sides of the seed are formed between the chalaza and hilum (Plate 46). There is no direct contact between the vascular bundle and the embryo.
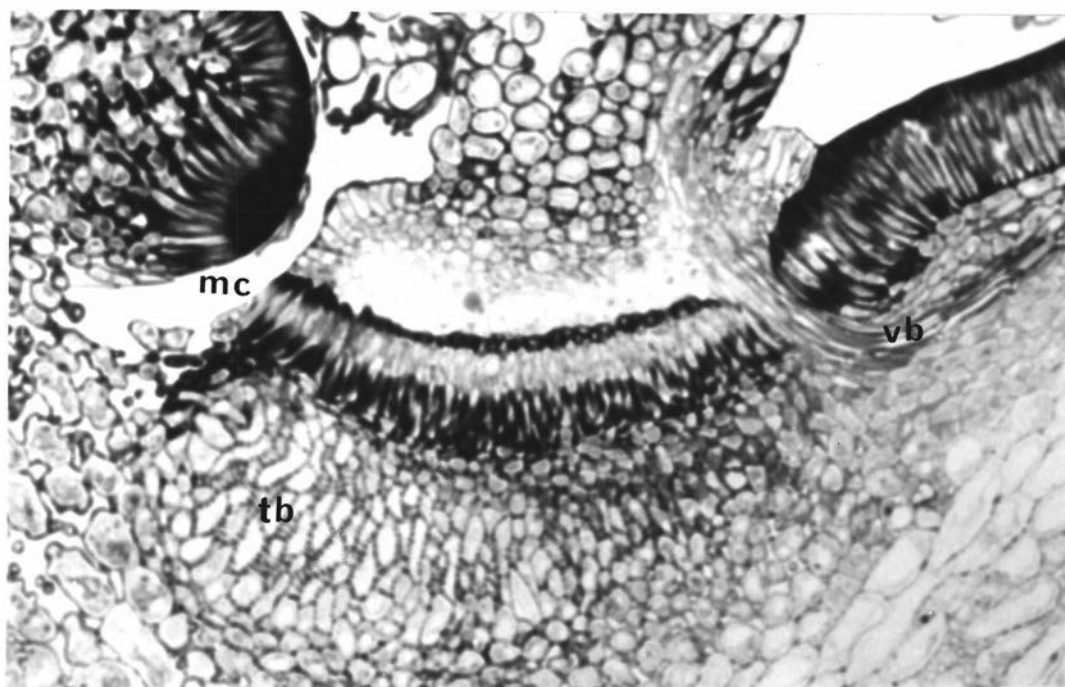
Plate 45: Ten days after pollination - hilar structure with vascular
           bundle and tracheid bar.
           f, funiculus; mc, micropylar canal; tb, tracheid bar;
           vb, vascular bundle (LS x 180).
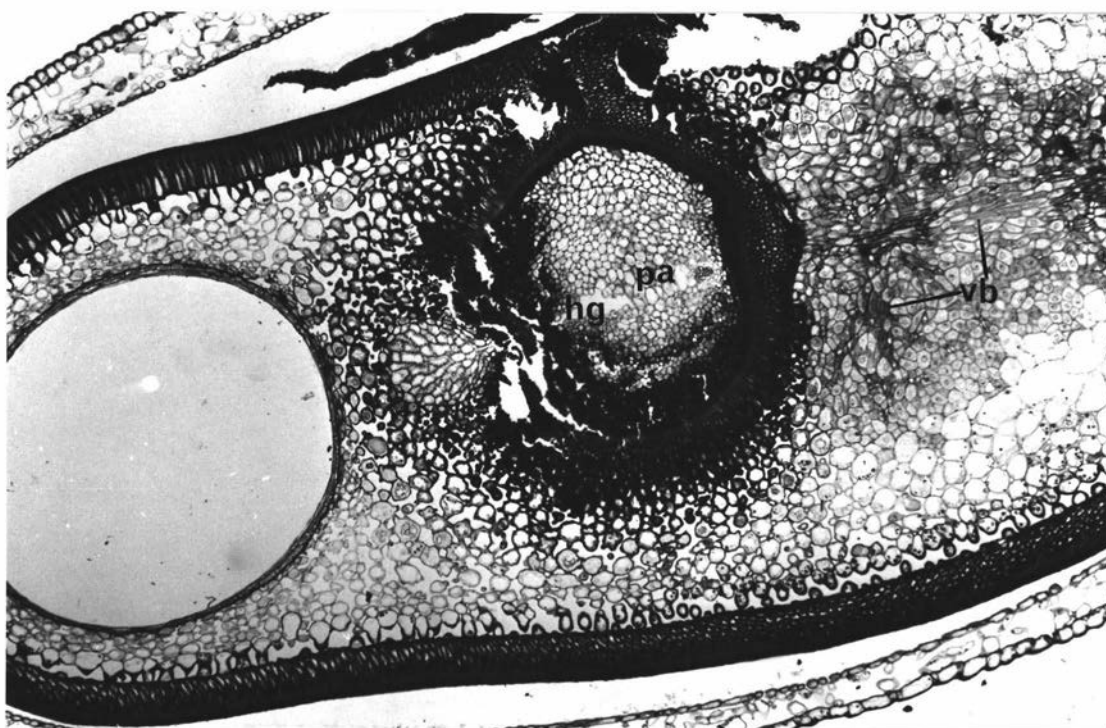
Plate 46: A cross section showing the vascular arrangement at the
           area between the hilum and chalazal end.
           hg, hilar groove partially covered by the parenchyma cells
           (pa); vb, branches of vascular bundle. (CS x 70).

DISCUSSION

In 'Pawera' red clover seed, a great variety of cell types occur at the hilar and sub-hilar regions and the tissues of these regions are extremely complex. The shape and position of the hilum is similar to that found in some other types of legume seeds (Corner 1951; Miller 1967; Chowdhury and Buth 1970), although the hilar sinus is not occluded by the radicular lobe as found in some species e.g. *Crotalaria* (Miller 1967). Therefore, the hilum can be seen clearly when the funiculus is detached from the seed. In general, cell structure and the arrangement of the tissues at the hilum is more or less the same as described in the seeds of *Crotalaria* species (Miller 1967) and *Lupinus* species (Hyde 1954).

The shape of the tracheid bar and surrounding cells also shows similarity to corresponding structures in some other legumes. Since the tracheid tissue is connected with the vascular bundle there seems to be some functional relationship between these structures. This relationship may be important particularly in the early developmental stages of the seed which depend on the supply of nutrients from the parent plant. The embryo may possibly obtain nutrients not only from the vascular supplies but also through the tracheid tissues since the latter occupies most of the sub-hilar region.

The development and differentiation of palisade cells and tracheid tissue at the hilar region occurs during the early stages of seed development as reported by Corner (1951). The present findings also show some agreement with the work of Miller (1967) and Ambegaokar (1976) on the size and shape of palisade cells and with Miller (1967) and Chowdhury and Buth (1970) on the arrangement of these cells at the hilum. The hilar region of 'Pawera' red clover seed contains the typical characteristics of seeds of members of the Papilionaceae.

In 'Pawera' red clover, a darker coloured line appears as the cuticle of the testa is observed, lying between the palisade and counter-palisade cells at the hilum. A similar effect has been reported by Hyde (1954) in diploid red clover. However, Miller (1967) reported that there is no cuticle between these layers of cells in the seed of *Crotalaria* species.

Usually sclerenchymatous tissues at the sub-epidermal region do not contain any obvious intercellular spaces. However, as stated by Miller (1967), intercellular spaces occur at the inner portion of the tissue which is integrated with the parenchyma of the sub-hilar region.

The position of the micropyle in the seed of 'Pawera' red clover is similar to that found in *Crotalaria* seed (Miller 1967) and is probably the same in some other *Trifolium* species. Although, the micropylar pore is open in most seeds the micropylar orifice appears to be blocked in some seeds. The micropyle, therefore may be blocked or may remain open as reported by Esau (1965) and Fahn (1974). In seed sections, since the micropyle appears as an open canal, observations in the present study do not agree with reports on the structure of the micropyle in *Phaseolus* and *Vicia* (Preston and Scott 1943). Moreover, in no cases were the palisade cells found to be closely united about the micropyle as found by Martin and Watt (1944). If the micropylar end is not blocked, the micropylar canal appears as an open canal in seed sections. However, the interior end of the canal terminates before reaching the embryo.

The position and shape of the strophiole in 'Pawera' red clover is similar to that found in *Melilotus alba* and *Trifolium subterraneum* (Hamly 1932 ; Hagon and Ballard 1970). As reported by Miller (1967) with seeds of *Crotalaria,* some variations in the degree of configuration was noted within seed samples. Similarly, colour variation was a constant factor. Therefore, neither of these aspects appear to be reliably useful for seed identification as previously suggested by Miller (1967). In longimedian section the configuration of the strophiole can be called doubly convex as termed by Miller. In mature seeds, the proportion of the height of the malpighian cells at the strophiole compared with corresponding cells in the lateral wall shows a similar arrangement to that which occurs in *Trifolium subterraneum* (Aitken 1939). However, even in young ovules of 'Pawera' red clover, differences in the height of the malpighian cells at the strophiole compared with the cells of the lateral wall is not as large as in *Pisum sativum* (Reeve 1946b).

The arrangement of the cells at the plane of symmetry is similar to that found in subterranean clover (Hagon and Ballard 1970). The presence of the underlying sclerenchyma cells at the strophiole seems to be a characteristic of *Trifolium* species. Moreover, as found in *Melilotus alba* (Hamly 1932) and *Trifolium subterraneum* (Hagon and Ballard 1970), the long and narrow malpighian cells of the central region of the plane seem to show definite caps when compared to their adjacent cells.

The presence of the compact cell layer detected immediately beneath the malpighian cells of the central plane in the strophiole of 'Pawera' red clover seed has not been reported by previous workers. As this structure seems to appear only at certain stages of seed development, it may not be possible to identify this structure in mature seed. Moreover, this structure can only be seen in transmedian section.

The vascular system in the seed of 'Pawera' red clover seems to be similar to that found in some other legume seeds (Martin and Watt 1944; Esau 1960; Fahn 1974), although there may have some variation in different species, genera, or sub-families (Corner 1951). In the present study no recurrent and post-chalazal vascular bundles were noted as reported by Corner (1951). However, the reports of Corner (1951) and Esau (1960) agree with the present findings that the vascular bundle stops at the chalazal region with short branches extending to the side of the seed. The vascular system in the seed of 'Pawera' red clover is therefore also very similar to that described by Miller (1967) in *Crotalaria*.

(b) <u>Seed coat structure and its relations to impermeability</u>

<u>LITERATURE REVIEW</u>

The longevity of viable seeds and their ability or otherwise to germinate when placed under conditions suitable for growth is strongly conditioned by the ability of seed to imbibe water. The seed coat of most mature legume seeds is hard, resistant to abrasion, covered with a wax-like layer and imperemable to water. This phenomenon is most widespread in the Leguminosae and some other families such as Cannaceae, Convolvulaceae, Geraniaceae, Liliaceae, and Malvaceae (Harrington 1916; Coe and Martin 1922; Watson 1948; Anon 1952; Crocker and Barton, 1953; Ballard 1973).

The seed coats in several genera of the Leguminosae have been investigated extensively to determine the structure concerned with the cause of impermeability (Pammel 1899; White 1908; Hamly 1932; Aitken 1939; Martin and Watt 1944; Watson 1948; Anon 1952; Hyde 1954; Ballard 1973; McKee *et al.* 1977). It has been agreed by most workers (Martin and Watt 1944; Reeve 1946a; Corner 1951; Hyde 1954; Esau 1960; Quinlivan 1971a; Ballard 1973; McKee *et al.* 1977) that the outer-most cell layer of the seed coat, known as the malpighian or palisade layer is responsible for the prevention of water entry into the seed. However, as the seed coat is rather complex, consisting of a number of structures, opinions are also divided over the particular structures involved.

As early as 1876, Nobbe (cited by Barton 1965b) suggested that impermeability is due to a waxy layer over the seeds. Some other early workers (Pammel 1899; White 1907) reported that the cuticle of the seed coat is impermeable to water. They recorded that the degree of impermeability is increased with the thickening of the cuticle. Rees (1911) supported their findings that the seed coats of some species of *Indigofera*, *Cytisus*, and *Acacia* are more impermeable because of the presence of a thicker cuticle. She also suggested that impermeability in *Acacia lophantha* and *Canna indica*, is due to cuticularized thickening in the malpighian cells.

Martin and Watt (1944) reported that the cuticularized layer, domes and light line constitute a barrier to water in dry ripe seeds of sweet clover. Torrey (1968) generally suggested that in some seeds impermeability

to water or oxygen or to both may be due to waxy or impervious layers in the seed coat.

Gutterman and Heydecker (1973) found that seeds of *Ononis sicula* (Leguminosae) which have matured on the parent plant during long days take a longer time to imbibe water than those matured in short days. They put forward a proposal that cuticular thickness of the seed coat is responsible for the delay in imbibition occurring in long day seeds. Smartt (1976), working with some tropical pulses, described the presence of a water-proof impervious layer in the testa and a waxy deposit over the hilum and micropyle preventing the movement of water into the seed.

Watson (1948), however concluded that in the twenty-one species of legumes he examined, variation in the thickness of the cuticle was extremely small and could not be linked with the degree of impermeability of the seed.

Rees (1911) explained that in the seed of *Melilotus albus,* the sub-cuticular layer may act as a cement substance to hold the cuticularized ends of the cells together and cause impermeability. Similarly, a report by Hamly (1932) claimed that the impermeable region of the seed coat of *Melilotus alba* is formed by a layer of tightly appressed suberin caps of the malpighian cells.

Aitken (1939) also noted that the walls of the upper ends of the malpighian cells are impermeable to water due to suberisation. She also mentioned that hardseededness is dependent on continuity of the suberin layer over the caps of the malpighian cells of the testa. This continuity depends on the degree of dehydration and thickness of deposition which in turn is based on conditions of seed ripening in the burr in *Trifolium subterraneum.* She suggested that the degree of hardness (i.e. whether seeds were capable of softening within a few weeks in the soil or whether impermeability lasted up to one or more years) is controlled by the tension of the strophiole cell walls and the toughness of the suberin. These aspects depend on the rate of seed development and degree of drying.

However, a report presented by Watson (1948) explained that the presence of very similar types of thickened, suberized or cuticularized malpighian caps occurs in both *Melilotus albus* and *M. officinalis* but there is a great difference between their germination percentages.

Coe and Martin (1920) also worked with *Melilotus officinalis* and *M. alba* and suggested that the light line in the testa prevents the absorption of water. They found that the absorption of water was not prevented by either the cuticularized layer or the cone-shaped structures of the malpighian layer. In many permeable seeds, canals between the malpighian cells are found to extend across the light line. It has also been suggested by Stevenson (1937) that the light line plays an important role in seed coat permeability in Sweet clover. Aitken (1939) and Esau (1960, 1965) also agreed that the light line was impermeable to water due to the presence of impermeable substances.

Reeve (1946a) found in the testa of *Pisum sativum* that texture and permeability are related to the pentosan-cellulosic complex of the secondary wall development of the malpighian cells. Corner (1951) suggested that hardseededness of the dried testa is caused mainly by the contraction of the walls of the malpighian cells as the seed ripens. Anon (1952) also reported that the impermeability of the seed coat of lucerne, red clover, and sweet clover is believed to be due to the presence of cutin and suberin in the cell walls of the testa.

Esau (1960) stated that the malpighian layer is assumed to be connected with a high degree of impermeability in hard legume seeds. Baciu-Miclaus (1970) found that semipermeability in hard soybean seeds is due to a large quantity of fat between the walls of the malpighian cells. He suggested that a high lignification level occurred at the base of the malpighian cells and at the top of the spool (osteosclereid) cells.

Ballard (1973) showed that the waxy cuticle is not the impermeable layer but the suberised regions of the malpighian cells are concerned with water exclusion. He found that the impermeable region in the malpighian cells still persists well below the light line to deeper levels than was previously thought.

Similar findings have been reported recently by McKee *et al.* (1977) who worked with the seeds of crownvetch *(Coronilla varia)*.

They reported that the impermeable structure is not the cuticle, or the upper part of the malpighian cells or the light line. They observed that in about 50% of the seeds studied, the impermeable region was between 59 and 72 µm below the surface of the seed coat. All seeds pierced to a depth of 98 µm or more produced normal seedlings while 87% of those pierced to a depth of 47 µm remained hard after a germination test. Therefore they suggested that the depth of penetration should include all of the outer integument and the aleurone layer in addition to portions of the endosperm layer in some seeds in order to obtain the maximum germination.

Hyde (1954) demonstrated the function of the hilum structure which proved to be related to the formation of hard seeds. In some Papilionaceous seeds, the hilum acts as a hygroscopic valve when the epidermis becomes impermeable. He showed that the fissure that formed along the median groove of the hilum, opens and closes in response to changes in the relative humidity of the surrounding atmosphere. The counter palisade of the fissure performs as a motor tissue. When the outside relative humidity is low the counter palisade dries and shrinks drawing the margins of the fissure upwards and apart. When the relative humidity is high the counter palisade swells and the fissure closes. Thus the hilum prevents the entry of water into the seed but permits the loss of moisture from the seed. This desiccation process leads to the development of an intensely impermeable testa.

DISCUSSION

It was impossible to identify the particular structure of the seed coat of 'Pawera' red clover which is responsible for impermeability just by examining cell structures. However, according to observations in the present study, some comments and comparisons can be made in relation to the findings of previous workers.

Generally it has been observed that the seed coat of an immature seed is more permeable than that of a mature one. However, no obvious difference in the thickness of the cuticle of seeds of different maturities or from different samples was observed. Therefore, the impermeability of the seed coat of mature seeds may not mainly be due to the cuticle. This assumption agrees with the findings of Watson (1948) but disagrees with those of some earlier workers (Pammel 1899; White 1907; and Rees 1911) and Gutterman and Heydecker (1973).

The sub-cuticular layer may be partly involved in impermeability as far as the thickness of that layer is concerned. In 'Pawera' red clover the sub-cuticular layer increases in thickness with advancing seed maturity. Moreover, it seems to hold the caps of the malpighian cells tightly together as reported by Rees (1911),Hamly (1932) and Aitken (1939). However, some more recent workers have exhonourated this layer as a cause of seed impermeability (Watson 1948, Ballard 1973 and McKee *et al*. 1977).

The light line in the testa has been paid some attention (Coe and Martin 1920; Stevenson 1937 and Aitken 1939) for its role in impermeability. In "Pawera" red clover, as hard seed begins to appear in 12 day old seed samples, a thin faint light line at the strophiolar region is also formed in some seeds. The light line appears more clearly in the later stages of seed development. Even though the light line is obvious in some sections (plates 24, 26 and 43), it cannot be seen obviously in other cases. It is therefore difficult to evaluate the importance of its role in relation to seed impermeability.

The impermeability of the testa of 'Pawera' red clover seed may be due to the presence of cutin and suberin in the walls of the malpighian cells. This highly suberised or lignified region may

also be present at the deeper level as suggested by Baciu-miclaus (1970) and Ballard (1973). This possibility may also agree with the results of McKee *et al*. (1977) who found that deeper mechanical piercing of the seedcoat was required to obtain a higher percentage of permeable seed.

As reported by Hyde (1954), the hilum seems to act as a one way hygroscopic valve in response to changes in environmental conditions. When 'Pawera' red clover seed with a high moisture content was placed in a sealed petridish containing silica gel, the hilar fissure opened in a few minutes. Thus, the seed lost its moisture and consequently becomes impermeable due to desiccation. This type of mechanism could lead to the formation of hard seeds if the seeds are stored in a place with low relative humidity.

(c) <u>Seed coat structure and its relation to permeability</u>

<u>LITERATURE REVIEW</u>

The ability of legume seeds to germinate is preceded by their ability to imbibe water. This uptake of water can only occur if the hard seeded condition described in the previous section has been affected in such a way that the seed coat is rendered permeable to water. In nature, the 'softening' of hard seed is considered to be influenced by the action of environmental and microbial factors, while man ensures the reduction of hardseededness to acceptable levels in commercial seedlots by mechanical scarification.

Since the 19th century there has been considerable research and debate on the structure of the seed coat responsible for the "softening" process. Among different structures, the structures and functions of the micropyle and hilum have been particularly implicated in this process. However, no detailed information, especially about the hilum, was available until the function of this region in legume seeds was conclusively elucidated by Hyde (1954). In addition, it is only in recent years that the importance of the strophiole in the softening of hard seeds has received critical attention.

1. <u>Micropyle</u>: According to Pfaefflin (1897) cited by Pammel (1899) the malpighian cells next to the micropyle take up a large amount of water and the micropyle itself allows the entry of water readily. They suggested that the micropyle acts as a hygroscopic structure, closing and opening according to environmental conditions. Exchange of gases is also accomplished more readily through the micropyle than the tracheid bar. Simpson *et al.* (1940) also found that the entry of water into cotton seed is possible through the micropyle.

Preston and Scott (1943) mentioned that the micropyle is responsible for the absorption of water. They showed that when the micropyle end of the seeds of *Phaseolus multiflorus* and *Vicia faba* was immersed in water, the weight of the seeds increased by 20% within 24 hours. In *Phaseolus*, (even though the micropyle is blocked by the cells of the suspensor) they suggested that water conduction may occur due to the high pectin content of the suspensor cells. This situation is different

in *Vicia* in that the swollen suspensor is absent in the micropyle
of mature seed. Instead the spongy tissue formed in the canal seems
to facilitate water entry although the lips of the micropyle close the
orifice. They also found that the uptake of water or rate of absorption
varies widely from lot to lot and even from seed to seed within a lot.
(Preston and Scott 1943).

Similar findings have been reported by Kyle (1959) cited by Hagon
and Ballard (1970) in work on the water absorption of the seeds of
peas and beans through the micropyle. Koller and Cohen (1959) also
found that the percentage of swelling decreases obviously when the
micropylar tip is embedded in vaseline, especially in acid treated
seeds of Convolvulus species. Corner (1976a), although he did not mention
the name of the plant, has suggested that the micropyle can act as a
permeable site.

Conversely there are some suggestions that water conduction is
unlikely to occur via the micropyle in impermeable seeds (Martin and
Watt 1944; Ballard 1973; Smartt 1976).

Villiers (1975) stated that the micropyle in legume seeds operates as
a hygroscopic valve, preventing water entry and allowing water vapour to
diffuse out of the seed. He suggests that this process thus permits
the seed to maintain its moisture content at a low level and extend
its period of viability.

2.   Hilum: As early as 1897, Pfaefflin, cited by Pammel (1899)
                 mentioned the hygroscopic action of the hilar groove.
Similar findings with some legume seeds have also been reported by
Corner (1951).

In 1954, Hyde conducted an excellent demonstration of the performance
of the hilum in some Papilionaceous seeds. He showed that the hilum
acts as a one way hygroscopically activated valve in dry seed, allowing
the seed to dry out but obstructing the absorption of moisture. However,
he also found that the hilar fissure remains open when relative humidity
is increased very gradually, permitting water vapour to diffuse into
the seed until equilibrium is reached. Similarly, Esau (1960)
states that water can enter the seed with relative ease through the hilum.

Burns (1959) in work with blue lupine *(Lupinus angustifolius)*, found that acid scarified hard seeds permit water entry through the hilar fissure due to partial hydrolysis of the counter palisade cells. In sound seeds, normally the counter palisade allows the hilum to close when the level of moisture outside the seed is higher than in the inside.

On the other hand, water conduction can apparently also be facilitated through the vascular bundles. At the hilum, where the vascular vessels enter the seed, the light line and associated structures related to hardseededness are interrupted by the vascular elements. When the outer ends of the vascular vessels are open, water can tranverse the malpighian layer through these vessels into the seed (Martin and Watt 1944; Corner 1976a).

3. <u>Strophiole</u>: Hamly (1932) was able to detect permeable sites of the seed coat of both soft and hard seeds in sweet clover *(Melilotus alba)* using osmic acid. According to Hamly, the middle lamellae of some malpighian cells at the strophiole are observed in a linear arrangement in the plane of the centre of the hilum. In mature soft seed of *Melilotus alba*, osmic acid staining occurs about these linearly arranged cells.

He also reported that the strophiole was found to be a permeable site of the seed coat when the seeds of sweet clover and alfalfa were softened with age under natural conditions. When seeds were threshed by machine a high percentage stained with osmic acid at this region. Similarly, after scarification, heat treatment or hot water treatment, seeds became permeable at this area. Naturally soft seeds of other species of *Melilotus, Medicago* and *Trifolium* (including *T. pratense*) are also stained at the strophiole when they are immersed in osmic acid solution.

Hamly (1932) concluded that the strophiole cells in hard seeds are in a state of metastable equilibrium, which when upset induces softening forming a split along the middle lamella in the plane of symmetry. He further put forward a hypothesis that rapid development of the cells at this region seems to be associated with ready separation through a reduction in the adhesiveness of the middle lamella.

Aitken (1939) also reported similar findings with the seeds of subterranean clover. She stated that clefts appear at the strophile of impermeable seeds due to temperature fluctuations or bouncing. As a rule, when hard seeds become soft, water conduction occurs only at the strophiole. She suggested that the formation of a split at the strophiole is a function of tension between the cells, the thickness of suberin at the shoulders of cells caps, and the degree of dehydration. She also agreed with Hamly (1932) that the rapid development of the cell structures at this region seems to be linked with increased tension between the cells with consequent greater sensitivity to splitting than elsewhere. However, she gave a clear statement that the strophiole is never permeable in original soft seeds. Staining occurs at the strophiole only in those soft seeds that derive from hard ones.

Martin and Watt (1944) supported Hamly's (1932) findings that the strophiole of both naturally soft and softened seeds is the place where water enters into the seeds initially. In hard seeds of sweet clover, the strophiole is impermeable until the seeds are softened by weathering or artificially.

Anon (1952) with alfalfa also described that water enters into the seed through the cleft at the strophiole but not at the hilum or micropyle. Seed sections reveal that the malpighian cells at this area are very straight and they appear as though they might split apart very readily even with a slight blow.

Ueki and Suetsugu (1958) reported that naturally soft ripened Genge (*Astragalus sinicus* L.) seeds absorb water through the strophiole. Their anatomical studies show that the palisade layer at the strophiole is thicker than at other parts of the seed coat and there are no epitherial cells (osteosclereid cells) in this region. They found no tannin in the palisade cells in the middle part of the strophiole, these cells therefore differing from those found in other parts of the seed.

Burn (1959) found that in blue lupine *(Lupinus angustifolius)*, water conduction occurs at the strophiole after the seeds have taken up water and been redried. This process initiates the formation of a cleft in the strophiole.

On the other hand, Quinlivan (1968a) with sand-plain (*Lupinus varius* L.) reported that hard seeds with a moisture content of less than 8.5% will soften at the strophiole when they are exposed to daily alternating temperatures of $15^{\circ}C$ and $65^{\circ}C$. Such temperature treatment causes a fracture at the strophiolar region. This pattern of softening is common with the seeds of some other plants, such as *Lupinus cosentini*, *Trifolium subterraneum* and possibly with other genera and species (Quinlivan 1971 a,b).

Hagon and Ballard (1970) carried out an experiment to study the effect of percussion on hardseededness and on the strophiolar structure of seeds of subterranean clover. They found that percussed seeds become permeable only at the strophiolar region but that strophioles can be resealed by storing them at relative humidities of 10% or less for a few days. It is suggested that the restoration of impermeability by exposing percussed seeds to low relative humidity may be due to shrinkage of the seed coat and internal tissues by drying. They also showed a photograph of the anatomical structure of a percussed strophiole of subterranean clover seed. According to their observations, a slit occurs extending through the malpighian cells of the median region. However, whether the formation of a fracture is due to a percussion effect or through tearing during sectioning was not determined.

Hagon (1971) confirmed the effect of daily temperature fluctuations that cause a fracture only at the strophiole of subterranean clover seeds. Ballard (1973) also demonstrated the site of initial water entry at the strophiole and different stages in the imbibition of a single seed of *Medicago truncatula* Gaertn. cv. Cyprus following percussion.

Mayer and Poljakoff-Mayber (1963) stated that the majority of seeds that possess a mechanism of softening through the strophiolar cleft belong to the Papilionaceae.

4. Permeability at random sites on the seed coat: It has been reported that staining occurs at localized areas on the testa of naturally soft seeds of sweet clover when they are soaked in osmic acid (Hamly 1932). With sweet clover, Stevenson (1937) also stated that the absorption of moisture takes place at various points on the seed coat and is not restricted to any particular region.

Aitken (1939) found that the testa of young subterranean clover seeds is rapidly permeated by osmic acid over most of the surface. However, when seeds reach their maximum size, scattered stain patches appear at the radicle tip or in nearby areas on the testa of matured dried original soft seeds. This permeability is due to incomplete formation of the suberinogenetic layer in the cell caps of those areas where the light line has not appeared. If such seeds are dried further, they may become hard due to continuity of the impermeable layer.

Burns (1959) also described that permeability in soft lupin seeds *(Lupinus angustifolius)* occurs through the seed coat as a whole, and is not limited to any particular area.

Similarly, Quinlivan (1968a) with sand-plain lupin *(Lupinus varius L)* found that naturally soft seeds or those hard seeds with a moisture content above 10% permit moisture penetration at random sites over the whole testa but not at the hilum or strophiole.

RESULTS AND DISCUSSION

Among the three specialized structures of the seed coat, the micropyle, hilum and strophiole, the latter apparently plays a most important part in the softening of hard seeds. Therefore, more attention has been paid to this region rather than the micropyle and hilum.

Micropyle: As described in the previous section, the pore and canal of the microyple of 'Pawera' red clover is not closed or blocked. Nevertheless, no water conduction through the micropyle appears to occur, unlike the situation in *Phaseolus* and *Vicia* (Preston and Scott 1943). In hard 'Pawera' red clover seeds no increase in seed weight or size occurred when they were immersed in water. It is also confirmed that the micropyle is not a permeable site for moisture uptake in both originally soft and percussed seeds because no staining due to imbitition occurred at the micropyle when such seeds were soaked in ferrous solution. This finding agrees with reports by Martin and Watt (1944) and Ballard (1973). The micropyle also does not seem to act as a hygroscopic valve as stated by Pfaefflin (1897) and Villiers (1975) since no structure at this region appears to perform as a motor tissue similar to that which occurs at the hilum.

Hilum: Since the hilum of red clover seed is thought to act only as a one way hygroscopic valve (Hyde 1954), no water conduction seems to occur through this region. However, it may be possible that the hilar fissure remains open when the outside relative humidity is increased very gradually (Hyde 1954). When both percussed seeds and original soft seeds of 'Pawera' red clover were immersed in ferrous solution, no permeability or staining was noticed at the hilar region.

Moreover, since no initial staining or swelling is observed at the vascular area in the testa when seeds are soaked in water or ferrous solution, water conduction seems to be impossible through the vascular bundles. It is possible that the outer ends of the vascular vessels are closed when the latter breaks from the funiculus during seed disarticulation. Otherwise it seems logical that these vessels, because of their structure, would be involved in water conduction as suggested by Martin and Watt (1944).

Strophiole: The strophiole of 'Pawera' red clover seed was found to
be an initial permeable site of the seed coat when hard seeds were
softened by percussion, scarification or by natural environmental
conditions.

When a percussed 'Pawera' red clover seed is immersed in ferrous
solution (0.003 MFe^{++} solution) a minute black spot starts to appear
at the centre of the strophiolar region (Plate 47a).   If a transverse
section is cut through this spot, stained malpighian cells at the centre
of the plane of symmetry are observed.   These stained malpighian
cells prove that initial imbibition occurs through a split in that
particular area.   When percussed seed is kept in the ferrous solution for
a longer period the solution spreads to other parts of the seed coat with
the sequence of staining shown in Plate (47 b-i).   The pattern of
staining follows a very similar pattern to that found in *Medicago
truncatula* by Ballard (1973).

When hard seeds of 'Pawera' red clover are scarified using the
apparatus employed by Ballard and Grant-Lipp (1965), the chalazal end,
radicle end and the bend on the lateral wall are affected severely if
higher scarification pressure (15-20 PSI for 1 Min.) or longer scarif-
ication duration (10-15 PSI for 2 Min) are used (Plate 48).   However,
if the pressure or duration of scarification is reduced (5 PSI for
1-2 Min) only a small percentage of visible damage is observed.   Even
though mechanical damage on the seed coat is not seen through the microscope,
about 98-100% of such scarified seeds become permeable and stain at
the strophiole if they are imbibed in ferrous solution.   The pattern of
staining is almost exactly the same as found in percussed seeds.
Therefore as suggested by Hamly (1932), only slight impaction is required
to make seed permeable at the strophiolar region.   Similarly Win Pe *et al.*
(1974) found that light scarification was as effective as heavy impaction
in reducing the hard seed content of *Centrosema pubescens*.
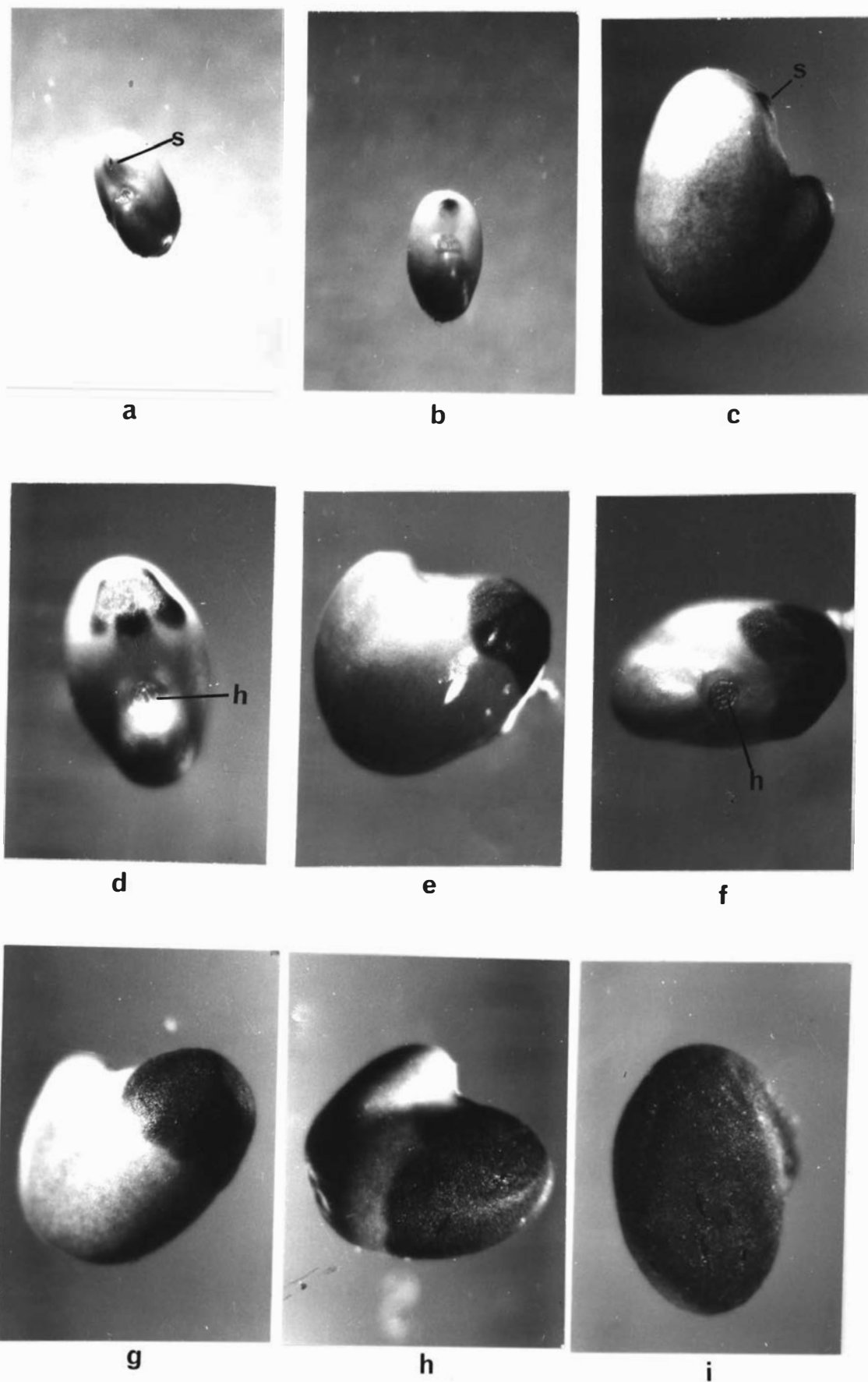
Plate 47: Plates showing the initial site of water entry at the strophiole and the pattern of subsequent staining in ferrous (0.003 MFe^{++}) solution.

h, hilum; s, strophiole.
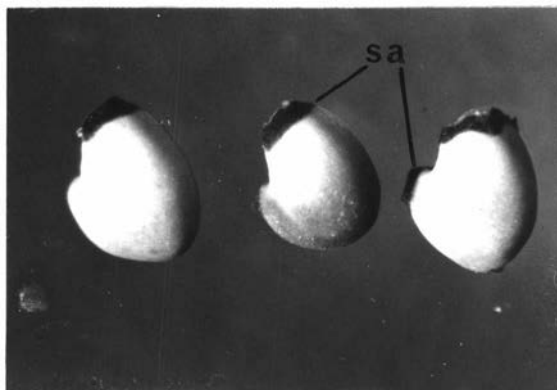
Plate 48: Black stains on the seed coat show the affected areas
          due to scarification.
          sa, staining areas.

Temperature fluctuations may affect the strophiole more severely
than elsewhere in the seed coat due to the lack of intercellular
spaces. Heat may build up quickly in that area and the bent cells
seem to respond to heat more readily and consequently pull apart as they
expand. Thus, if a seed is subjected to fluctuating temperatures, the
adhesiveness of the middle lamellae of the malpighian cells at the
centre of the plane may be reduced gradually due to the intermittent
pulling mechanism of the surrounding cells.

Seed coat anatomical observations show that the arrangement of the
malpighian cells at and around the plane of symmetry and the presence
of the foundation layers of the compact cells appear to encourage the
formation of a cleft at this area by a slight blow (Plate 49).
Since the malpighian cells in the centre of the plane are straight while
the surrounding cells are bent, the latter seem to be ready to pull
apart. Furthermore, intercellular spaces along with the osteosclereids
occur around the seed coat except at the strophiolar region (Plate 50).
Therefore, when a hard seed is percussed, scarified, or treated by
any other means, the other parts of the seed coat seem to resist the force
to some extent by the cushioning effect of the intercellular spaces
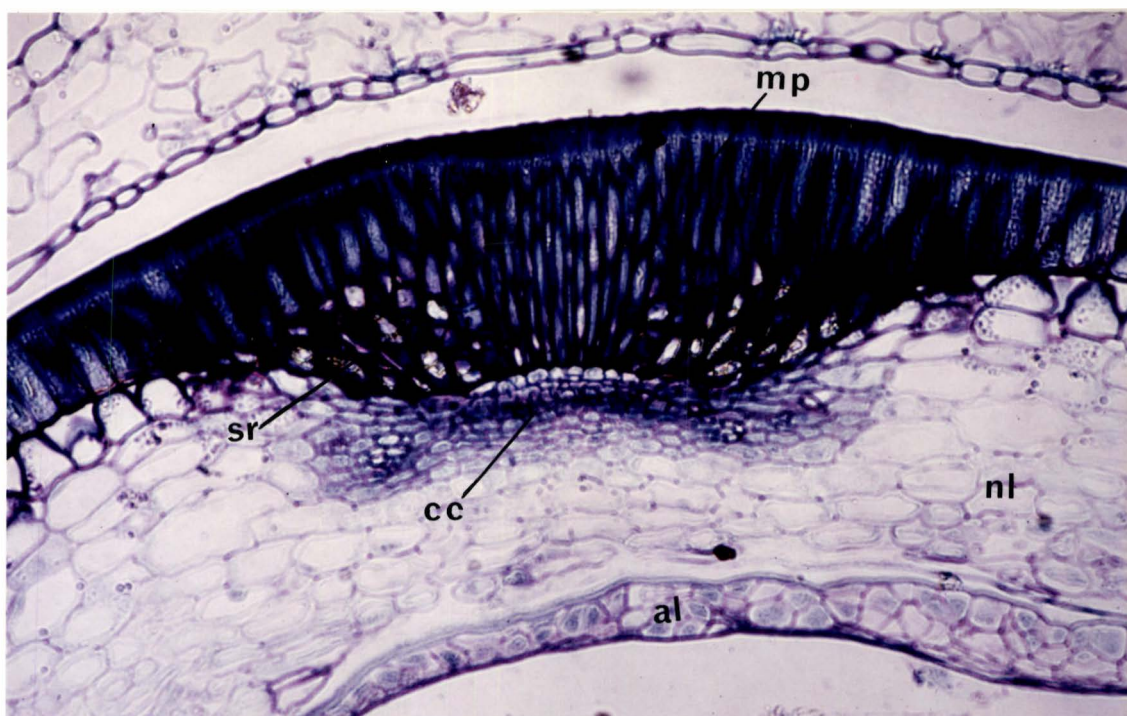
Plate 49:   Eighteen days after pollination - the arrangement of the
            malpighian and other accompanying cells at the strophiolar
            region.
            al, aleurone layer; cc, compact cell layer; mp, malpighian
            cells, nl, nutrient layer; sr, sclerenchyma cells (TS x 210).
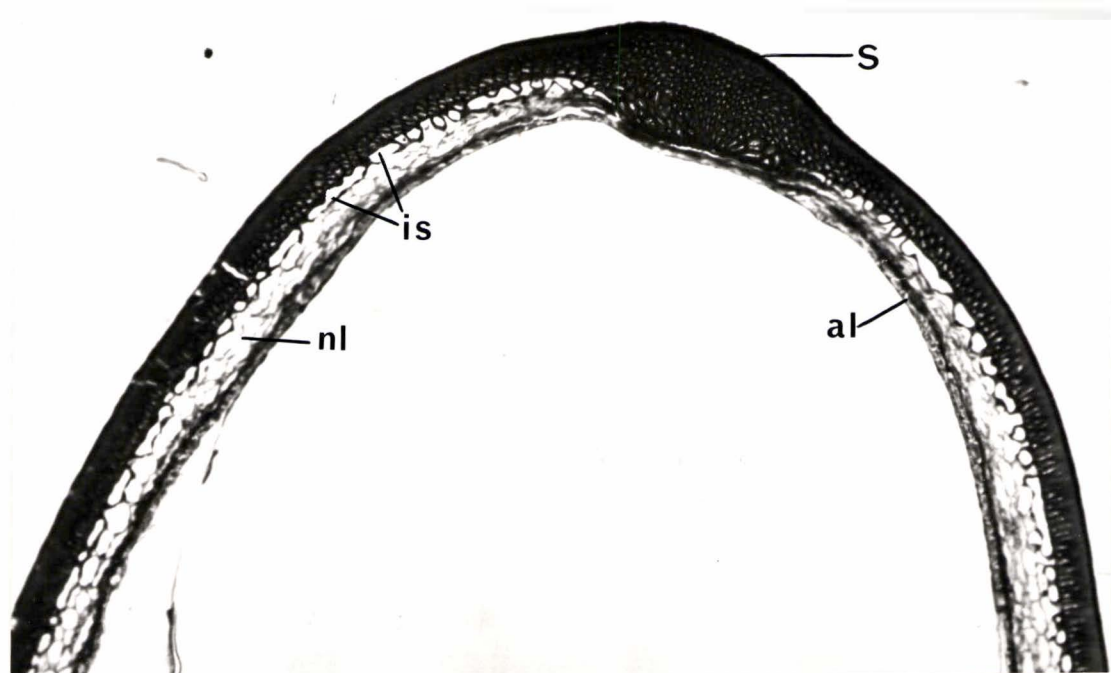
Plate 50:   A transverse section of a mature seed coat showing the inter-
            cellular spaces around the seed except at the strophiolar
            region.
            al, aleurone layer; is, intercellular spaces; nl, nutrient
            layer; s, strophiole (TS x 70).
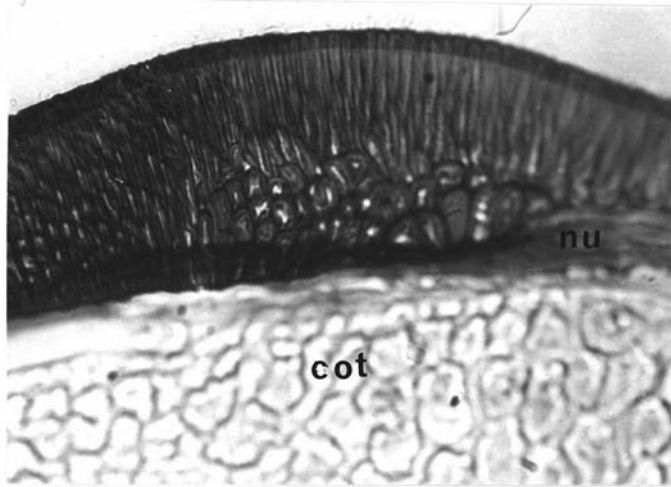
Plate 51: Anatomical features at the strophiolar region of a
mature seed.

cot, cotyledonary tissue; nu, nutrient layer (LS x 200).
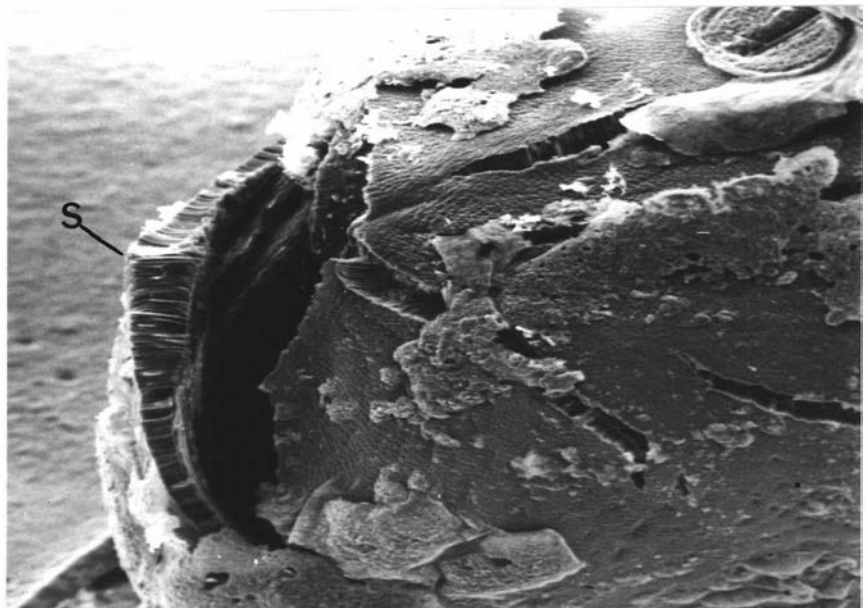
Plate 52: A fracture at the strophiole showing the general appearance
of the surface of the strophiolar region.

s, strophiole (SEM x 60).

which may assist the seed coat by developing resilience. Conversely, at the strophiole, the presence of a few layers of compact cells and the tightly appressed structure at the base of the malpighian cells in mature seeds (Plates 44 and 51) appears to act as a good hard support to any outside force. This suggests that the malpighian cells at this area are likely to be affected more severely than anywhere else.

All these situations therefore combine to increase the sensitivity of the strophiolar area to impaction and create conditions which induce this area to become an initial permeable site on the seed coat. In other words, formation of the cell structure at the strophiole is in a state of metastable equilibrium as stated by Hamly (1932). On the other hand, the rapid development of the cells at the strophiolar region may be connected with the mechanism of separation due to increased tension between the cells or due to a reduction in the adhesiveness of the middle lamella (Hamly 1932; Aitken 1939). Such an effect is easily shown in freeze fractured seeds of 'Pawera' red clover (Plate 52).

Other places of the seed coat: It has been found that water absorption takes place rapidly at random and unlocalized areas on the seed coat of young 'Pawera' red clover seeds. Similarly in well developed original soft seeds, the strophiole is not a common initial permeable site of the seed coat but water enters into the seed at random places (Plate 53). This permeability of the seed coat may be due to incomplete formation of the suberinogenitic layer in the cell caps of those areas (Aitken 1939) or due to failure of the formation of suberin or lignin in the malpighian cells.
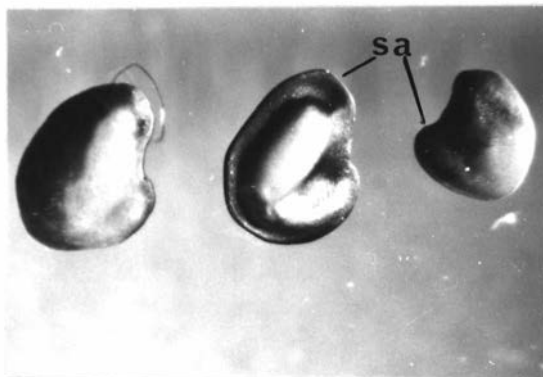
Plate 53: Staining at various sites of the seed coat.
sa, staining areas.

Occasionally water conduction occurs at the chalazal end where the circular arrangement of the surface cells are formed. These cells are arranged in such a way that this area appears as a starting or end point of the malpighian cells. (Plates 54 and 55). Since this site is very close to the strophiole, the initial site of permeability can be confused with the latter if the early stages of water energy are not detected. However, the rate of spreading of the solution is slower than from the strophiole especially in the early stages of imbibition.

In general, as reported for some leguminous species by previous workers (Hamly 1932; Stevenson 1937; Burns 1959 and Quinlivan 1968a), permeability in originally soft seed of 'Pawera' red clover is not limited to any particular area.

The results obtained in this study support observations by other workers that there is obviously a difference in the site of permeability of seeds depending on whether they are originally soft (in which case permeability occurs at random sites on the seed coat) or whether they are rendered permeable following a period of impermeability or hard-seededness (in which case permeability occurs through the strophiole only).
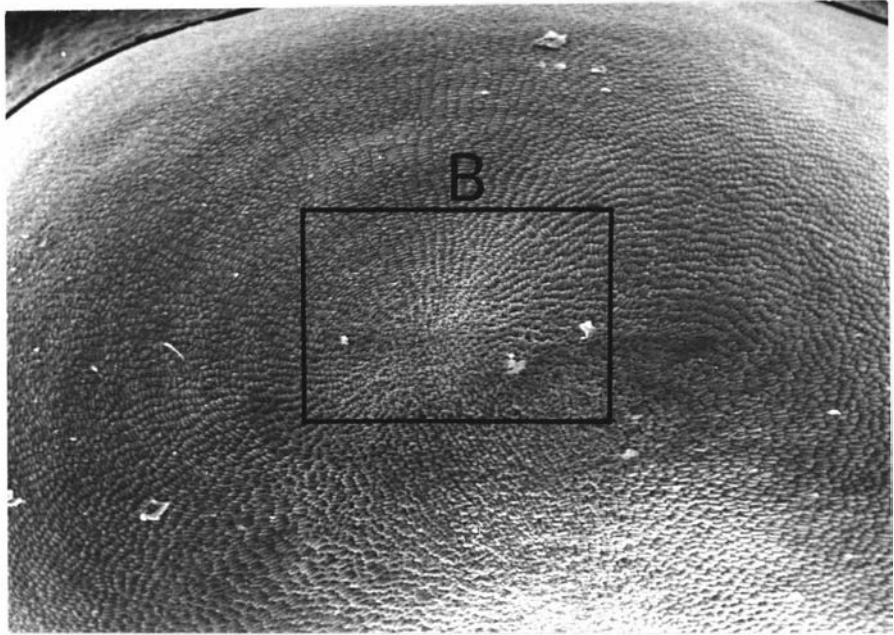
Plate 54:   Surface view of the chalazal region of a seed showing an area where the arrangement of the cells appear to meet together (SEM x 120).
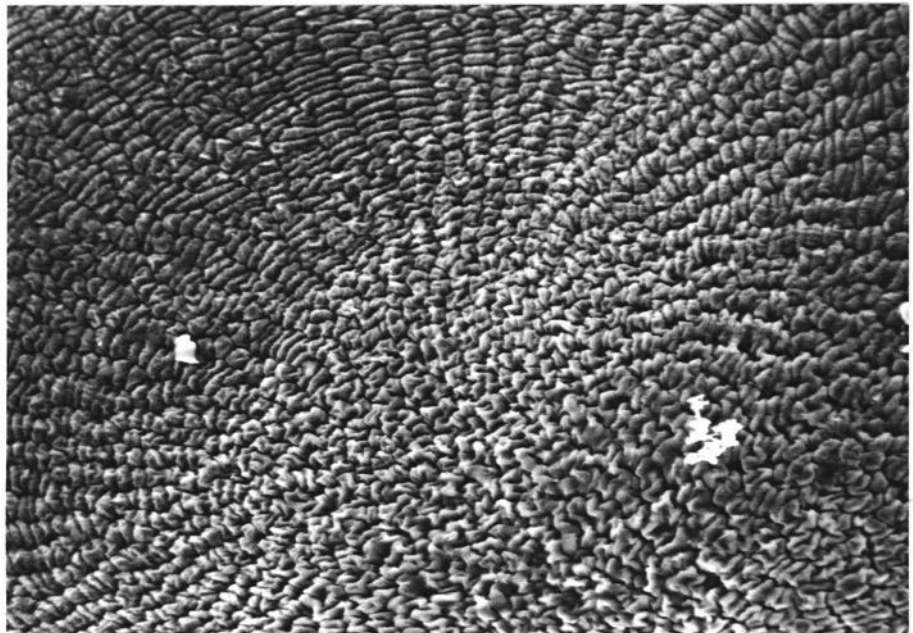
Plate 55:   Section 'B' of Plate 54 showing the meeting point of the surface cells (SEM x 300).

CONCLUSION

      Although there were some variations in the rate of development
of seeds of 'Pawera' red clover compared with similar observations on
other *Trifolium* species, the general patterns of both endosperm and
embryo development showed close similarity. Normally only one seed
was developed in a campylotropous ovule. Significant cell degeneration
and differentiation occurred during the period of seed development.

      Cell structures in the seed coat, especially at the hilum and
strophiole closely resembled corresponding structures in the seed
coat of other *Trifolium* species. However, due to lack of information
about the changes in cell structure occurring in the seed coat of
other red clover varieties, general comparisons have been made
principally with reports on seed development in other legumes.

      The formation of hard seeds in dry seed samples begin to appear
about the time of secondary wall thickening in the malpighian cells
of the seed coat. The light line was observed to appear about the
same time. The thick-walled aleurone cell layer enveloping the embryo
became more conspicuous starting 18 days after pollination and cell
differentiation proceeded during the later periods of seed development.

      As it was proposed to obtain detailed information about the
structural changes occurring during seed development, a fairly large
number of plates were presented in the interests of clarity. Further-
more, the present study described more precisely the anatomical changes
occurring at the strophiolar region. Few previous anatomical reports
have apparently mentioned this structure, although a number of workers
have highlighted the sensitivity of the strophiole. The presented
results therefore add to our understanding of the role and functions
of this area. The present investigation throws new light on the
relationship between individual seedcoat structures and their
respective roles in affecting the impermeability mechanisms of the
hard seedcoat in 'Pawera' red clover.

      The imbibition of water into originally permeable seeds has
been shown to occur at random sites on the seed coat rather than
at specific sites such as the strophiole or hilum. However the present
study has clearly implicated the strophiole as the site at which hard
seed softening (permeability) occurs following mechanical impaction.

CHAPTER III

STUDIES ON THE LONGEVITY OF HARD SEEDS AND THE NATURAL BREAKDOWN EFFECT OF ENVIRONMENTAL CONDITIONS ON HARDSEEDEDNESS IN THE FIELD

INTRODUCTION

Seeds of most species of cultivated plants germinate readily when they are fully developed and are provided with favourable conditions for germination. However, seeds of some plant species, including small-seeded legumes, may lie in the soil for varying lengths of time without germinating. Under such conditions, complete germination of a buried seed population may be spread over a period of years due to dormancy mechanisms. Although dormancy can be due to various causes, hardseeded-ness appears to be the principal factor causing delayed germination in legumes. When such hard seeds are buried near the soil surface, a proport-ion of them will germinate and emerge while in cases where hard seeds are buried deeper than the critical depth for that species, the seedling will die before reaching the soil surface. In addition, a large number of seeds will remain in the soil in a state of dormancy.

Hard seeds, even though they may remain in the soil for a considerable period of time, utlimately are induced to germinate under natural environmental conditions. Buried crop and weed seeds, thus may become a major problem in seed certification schemes. Therefore, an understanding of the longevity and characteristics of seeds of individual species in the soil is increasingly important to the seed technologist, agronomist, botan-ist, ecologist, farmer and gardener.

Naturally when seeds are dropped from the plant, they lie on the soil surface or are just covered by soil. On the other hand, the majority of seeds on arable land would be expected to be buried at various depths in the soil during cultivation. There are various factors which play an important role in affecting the population of hard seeds in the soil. However, the rate of break-down of hardseededness may vary with soil type (Evans 1959; Lewis 1961), seed maturity (Quinlivan 1971b pers. comm.) and climatic conditions, particularly daily fluctuating temperatures (Quinlivan 1961, 1966, 1971a; Taylor and Rossiter 1967; Kirchner and Andrew 1971; McComb and Andrews 1974). Seed samples of 'Pawera' red

clover were buried in the soil or stored in the laboratory to determine the effect of some of these variables on the natural softening of seed and reduction of hard seed populations in the field and in storage.

A field seedling emergence trial was also carried out to obtain further information on the pattern of breakdown of hardseededness and the germination characteristics of hard seeds in the soil.

LITERATURE REVIEW

Seed dormancy: Some workers (Barton 1961b; Koller *et al*. 1962)
claim that the term "dormancy" has not been clearly
defined in the literature. Despite this, a large amount of literature
has appeared on seed dormancy.

Seed dormancy has been described in a restricted sense as that
condition in which the seed, although viable, will not germinate under
favourable germination conditions, viz. suitable temperature, adequate
water and oxygen (Mayer and Poljakoff-Mayber 1963, 1975; Roberts
1972b; Chen and Varner 1973). In its broadest sense, the term
dormancy may be used to determine any stage in the life cycle, whether
this be a regular phase in the development process or a fortuitous
occurrence, in which active growth is suspended for a period of time
(Villiers 1972). Nikolaeva (1969) also broadly defined seed dormancy
as the absence of germination; as a greater or lesser reduction of
the germinative power of seeds or as the maintenance of the capacity
to germinate only within a small range of conditions.

Amen (1963) stated that seed dormancy is an endogenously controlled
and/or environmentally imposed temporary suspension of growth and reduced
metabolic activity which is independent of immediate environmental
conditions. Later, Amen (1968) also defined seed dormancy as an aspect
of the phenomenon of growth cessation which has as its crucial point
the problem of preserving a potential for growth without loss of
biological integrity. On the other hand, the onset of dormancy appears
to be regulated by a critical balance between growth promotors and
inhibitors. During seed maturation this balance is shifted in favour
of the inhibitor, thereby suspending embryo growth. As long as the
balance remains in favour of the inhibitor, seed dormancy is maintained
(Amen 1968; Adam *et al*. 1970).

The term seed dormancy appears commonly throughout the literature,
has received much attention, and has been defined in different ways
by various workers. However, in most cases, as pointed out by Thurston
(1960), different workers use the same term with different meanings
and different terms with almost identical meanings.

The causes of dormancy have been considered by many workers (Crocker 1916; Barton 1961b; Mayer and Poljakoff-Mayber 1963; Villiers 1972, 1975) and are generally divided into two types, namely primary dormancy and secondary dormancy. However, within the broad categories of primary and secondary dormancy, it has become more common to refer to three types of dormancy which describe more precisely the cause of the type of dormancy exhibited by buried seeds. These three types are innate, induced and enforced dormancy (Harper 1957; Thurston 1960; Roberts 1972b).

Innate dormancy which is also known as primary dormancy is present in the seed at maturity, prevents viviparous seed germination and also persists for some time after harvest or shedding (Roberts 1972b). Thurston (1960) stated that seeds exhibiting innate dormancy cannot germinate until they have undergone an after-ripening treatment or received some specific stimulus e.g. seeds of *Heracleum sphondylium* and *Avena* species. Many weed seeds in the soil do not grow even when they are supplied with conditions favourable for germination. They are therefore said to have a period of natural (innate) dormancy (Brenchley and Warington 1930).

Induced or secondary dormancy occurs as a result of seeds being supplied with water but in an environment where some other particular factor is unfavourable for germination (Roberts 1972b). The characteristic of persistence of dormancy after removing the inhibitory factor, distinguishes 'induced' dormancy from 'enforced' dormancy. Thurston (1960) has also stated that induced dormancy develops in non-dormant seeds by exposure to certain conditions, and continues after a change in these conditions or the seed is removed from them. For instance, when seeds of *Sinapis alba* are exposed to high carbon dioxide concentrations or *Avena ludoviciana* to high temperatures, dormancy develops and some special treatment is required to break it. Similarly, Barton and Crocker (1948) reported that seeds of *Xanthium* can be induced to enter a dormant state by exposure to an atmosphere enriched with carbon dioxide or lacking in oxygen. It is further suggested that this type of dormancy is most effectively induced by high temperatures.

Harper (1957) stated that an enforced condition distinct from both innate and induced dormancy is met when seeds are prevented from germination by purely external environmental limitations. King (1966) reported that non-dormant weed seeds may be forced into dormancy if placed under warm, moist conditions with an excess of carbon dioxide or deficient oxygen. Similarly, Williams (1970), 1972) also found that seeds of the perennial weed *(Agrostis gigantea)* (Gramineae) are not innately dormant but exhibit enforced dormancy following burial.

Enforced dormancy describes the condition when viable seeds do not germinate because of some limitation in the environment (Roberts 1972b). This dormancy type mostly occurs in seeds buried in the soil and has been attributed to various factors such as high carbon dioxide levels, lack of fluctuating temperature and darkness which are more pronounced beneath the soil surface. The ready germination of seeds brought up to the surface of the soil following burial at lower depths indicates that seeds buried in the soil are prevented from germination by enforced dormancy. Although all forms of dormancy are important in maintaining the viability of seed in the soil, enforced dormancy is vital to the survival of buried seeds. A very large proportion of seeds buried in the soil exhibit this dormancy type (Roberts 1972b).

Hardseededness: The dormancy mechanism which prevents the movement of water through the seed coat is referred to as "impermeability" or "hardseededness" and the seeds concerned are known as "impermeable seeds" or "hard seeds" (Quinlivan 1971a). Many workers simply defined this phenomenon as that condition where seeds do not absorb water when placed in wet or moist surroundings because of an impermeable seed coat. (Harrington, 1916; Crocker 1916; Barton and Crocker 1948; Anon 1952; Rincker 1954; Mayer and Poljakoff-Mayber 1963; Barton 1961b, 1965b; Villiers 1972, 1975).

Anon (1976a) stated that "seeds of Leguminosae and Malvaceae, which remain hard at the end of the prescribed laboratory germination test period because they have not absorbed water due to an impermeable seedcoat, are classified as hard seeds".

This type of dormancy mechanism appears to be one of the simplest but most highly effective means of delaying germination and spreading the production of seedlings from any particular lot of seeds over a period of time.

Seed dormancy due to hardseededness or impermeability belongs to the 'primary' or 'innate' dormancy type (Crocker 1916; Bibbey 1948; Barton 1962; Mayer and Poljakoff-Mayber 1963; Villiers 1972, 1975). The best known dormancy mechanism in species of agricultural crops is caused by seed coats which are impermeable to water or gases (Copeland 1976). Impermeability, although it occurs in many other plants, is common in alfalfa, clovers and other legume species. There is usually some variation in the extent of impermeability of the testa even in seeds within the same lot. A few seeds are usually permeable and capable of germination when they fall from the mother plant while others may remain in the dormant state for varying lengths of time (Villiers 1975).

In some cases, this type of dormancy mechanism has value in agricultural practice. Some workers (Gladstones 1958; Williams and Elliott 1960; Quinlivan and Millington 1962; Quinlivan 1970; 1971a, b) have claimed the importance of seed coat impermeability in the survival of forage legume crops. Some plant breeding and selection programmes have also been carried out to increase the percentage of hard seeds in Crimson clover *(T. incarnatum)* (Bennett 1959) and Persian clover *(T. resupinatum)* (Weihing 1962).

On the other hand, it has been claimed that hardseededness, especially in small-seeded legumes, may be an undesirable character from an agricultural standpoint since hard seeds are of no value for planting and special devices or techniques may be required to overcome this problem (Barton and Crocker 1948; Anon. 1952; Wheeler and Hill 1957). Seed coat impereability in some *Medicago* species for example, has been found to be the greatest single factor affecting plant populations in naturally regenerating pastures (Crawford 1971 pers. comm., Quinlivan 1971b, pers. comm.).

Breakdown effect of environmental conditions on hardseededness

Under natural conditions, the hard seed coat may be broken or eroded by natural mechanical abrasion, microbial and insect attack, passage through the digestive tract of animals or exposure to alternating high and low temperatures (Barton 1961a; Mayer and Poljakoff-Mayber 1963; 1975; Adams *et al.* 1970; Villiers 1975). However, it is impossible to estimate the proportion of impermeable seeds that will produce seedlings in any given length of time (Harrington 1916). The rate of seed softening also depends on the species, the stage of seed maturity, the maximum temperature and the daily temperature fluctuations to which seeds are exposed (Quinlivan 1965, 1966, 1971a, 1971b pers. comm).

Some early workers (Crocker 1916; Harrington 1916) initiated studies connected with the softening of hard seeds under natural and storage conditions. They reported that hard seeds became permeable very slowly in dry storage, more rapidly in germinators, and promptly under widely fluctuating natural conditions. Aitken (1939), with subterranean clover, recorded the effect of fluctuating temperatures on the softening of hard seeds. Rincker (1954) also reported the effect of regulated heat treatment in increasing the permeability of hard seeds of alfalfa and red clover. However, seed samples of the same crop showed variation in response to the same treatment.

In recent years, more research workers, especially in Australia (Quinlivan 1961, 1962, 1965, 1966, 1967, 1968a, b, 1971c pers. comm.; Quinlivan and Millington 1962; Hagon 1971; Kirchner and Andrew 1971; McComb and Andrews 1974; Ballard *et al.* 1976) have shown interest in those factors which cause the breakdown of hardseededness under natural environmental conditions. Their results generally show that daily fluctuating temperature is the major factor regulating the softening of hard seeds under both field conditions and in storage.

Quinlivan (1961) worked with the seeds of some legume species and found that a high constant temperature (60°C) was more effective than a low constant temperature (15°C) and all fluctuating temperature treatments are most effective in reducing hardseededness

Similarly, the importance of daily temperature fluctuations, especially during the summer, in inducing the softening of impermeable seeds has been reported in *Trifolium subterraneum* (Quinlivan 1962, 1965, 1966; Quinlivan and Millington 1962; Taylor and Rossiter 1967; Katznelson and Carpenter 1972), *Ornithopus compressus* (Barrett-Lennard and Gladstones 1964), Ladino white clover (Robinson 1960), *Lupinus cosentini* (Quinlivan 1962), *Medicago truncatula* (Quinlivan 1962; Kirchner and Andrew 1971), *Lupinus varius* (Quinlivan 1966, 1967) and some annual legumes (Williams and Elliott 1960; Taylor 1972; McComb and Andrews 1974).

Conversely, Gladstones (1958) has stated that seed moisture content is the main factor - and perhaps the only factor - controlling the impermeability of the seed coat in Australian blue lupin *(Lupinus digitatus)*. He showed that when the moisture content of the seed was above 14%, the seeds readily swell when soaked in water. Below 14% seed moisture, impermeability gradually increases and at 11% no more seeds swell within 14 days. Impermeability is still reversible between 14% and 11% but it is virtually irreversible below 9% seed moisture content. Similar findings have also been reported by Quinlivan (1970) who also worked with species of genus *Lupinus*.

Although it is considered that daily fluctuating temperature in the top layer of the soil is the important factor affecting the breakdown of hardseededness, the actual cause of this effect was not known until Quinlivan (1968a) reported his findings with the seeds of sand-plain lupin *(Lupinus varius)*. He suggested a relationship between the rate of softening with temperature fluctuation and seed moisture content. When the original moisture content of the seed is above 10%, a slow moisture penetration occurs through the seed coat and not through the hilum or strophiole with sudden changes of humidity or in moist soil. However he also showed that fluctuating temperatures make hard seeds with low moisture contents permeable by cracking the testa at the strophiole. Such softened seeds germinate within a few days following moistening of the soil. This type of softening does not occur in seeds with a moisture content above 8.5%.

Buried seed populations and seed longevity in the soil:

The presence of a large proportion of viable buried seeds has been reported by various workers. Buried seed populations are dynamic (Roberts 1970; Schafer and Chilcote (1970), the density of buried seeds in the soil varying enormously (Hyde 1958; Roberts 1958, 1963; Darlington and Steinbauer 1961; Roberts (1970) depending on the types of crop grown (Brenchley and Warington 1930, Hyde and Suckling 1953) and soil and climatic conditions (Brenchley and Warington 1930; Hyde and Suckling 1953; Budd *et al.* 1954; Evans 1960). Recently, Klingman *et al.* (1975) and Sagar and Mortimer (1976) also presented detailed reports on the population dynamics of buried seeds and their longevity in the soil.

The results of some workers who have attempted to determine buried seed populations are summarised in Appendix (32), the number of seeds recovered from soil samples being as high as 2400 million/ hectare.

The seeds of some species retain their viability for many years when buried in the soil. It has been reported that most of the seeds which have been found to have a long life-span possess impermeable or hard seed coats. It is assumed that this feature contributes to the longevity of seed by decreasing or preventing gaseous exchange and by reducing metabolic rate within the seed (Harrington 1916; Youngman 1952; Owen 1956; Barton 1961 a,b). On the other hand, the presence of unfavourable temperature conditions and soil atmospheres may influence the reduction of metabolic activity in seeds buried in the soil and thus retard deterioration (Darlington and Steinbauer 1961). It is however, difficult to explain the extended life-span of some species in the soil (Barton 1961 a).

Various reports on the seeds of some species possessing long life-span have been summarised in Appendix (33). These results show there is considerable variation in the longevity of buried seeds of different species. It has also been suggested that even within a species the longevity of seeds in the soil may vary with depth of burial, soil type, cultivation practices and some other factors.

<u>Depth of burial and seed longevity</u>:   The pattern of softening of
hard seeds in sweet clover
varies inversely with the dpeth to which they are buried in the soil
(Martin 1945).   He found that a large number of seeds buried at
1 and 3 inches produced *in situ* germination but no germination
occurred from softened seeds buried at lower depths until they were
brought up to the surface. At the deeper burial, the failure of
germination may be due to unfavourable conditions of the soil
atmosphere with respect to oxygen and carbon dioxide content.
Similar findings have been recorded in white clover (Robinson 1960),
red clover (Rampton and Ching 1970 and Lewis 1958) and a range of
weed species (Brown and Porter 1942; Taylorson 1970 and Stoller and
Wax 1974).

    Toole and Hollowell (1939) stated that surface soil temperatures
are subject to greater variation than those below the surface.
Therefore the average daily maximum temperatures at the surface
are doubtless higher and occur somewhat earlier in the spring than
corresponding temperatures at some distance below the surface.   This
differential soil temperature effect has been shown to have a very
marked influence upon hardening and subsequent softening of hard
seeds of a range of legume species (Kirchner and Andrew: 1971,
and Quinlivan 1971c pers. comm.).

    Stimuli, such as light, photoperiod, thermoperiod, high oxygen
tension or leaching, which may break seed dormancy are most effectively
encountered at the soil surface (Harper 1957). Therefore, most
forms of innate dormancy are broken naturally at the surface of
the soil (Chepil 1946a, b). When seeds requiring such stimuli
are buried in the soil the intensity of stimuli becomes reduced and
thus deeply buried weed seeds remain dormant for a long time (Harper
1957). As deep burial in the soil excludes light, light-stimulated
seeds such as celery, tobacco, and timothy may remain in the soil for
several years in a dormant condition (Crocker 1948).   More recently,
Harris (1959) gave a more precise explanation why buried seeds may
maintain their viability for a considerable time. Many seeds are
better preserved at depth below the surface where the soil moisture
level is more even, oxygen supply is lowered, and temperature reduced
and less prone to fluctuation. It is also believed that deep buried
seeds will exhibit suspended dormancy due to the raising of the

carbon dioxide and oxygen ratio (Moss 1959) or due to high levels
of carbon dioxide in the soil (Wesson and Wareing 1969). However,
due to fluctuations in carbon dioxide concentrations of the soil
atmosphere, seeds of some species may be able to germinate without
the soil being disturbed (Wesson and Wareing 1969).

Fate of seeds in the soil: In soil there are variable factors that
                              may simultaneously interact to
varying degrees on seeds, including changes in exposure to light,
moisture, temperature and the gaseous environment. Depending upon
the position of seeds in the soil profile and the season, the exposure
of seeds to these variables differs considerably both quantitatively
and qualitatively. The influence of all these interacting factors
upon the pattern of seed survival and germination in the soil is
not fully understood. Presumably, the physiology of buried seeds
differs considerably from that of seeds stored under laboratory
conditions (Taylorson 1970).

According to Barton (1962), gaseous exchange of imbibed pigweed
(*Amaranthus retroflexus* L.) seeds show at least a ten-fold reduction
in respiration when such seeds are held in moist storage at 20°C.
A similar effect has also been reported by Toole and Toole (1953).
suggesting that some reduction in the rate of life processes may
be responsible for the survival of seeds in the soil. Barton (1961a)
also reported that imbibed weed seeds can survive for a considerable
period of time in the soil. She also suggested (Barton 1961b)
that a greater reduction in the rate of respiration must take place
in seeds buried in the soil than in moist storage.

Lewis (1961) stated that the most rapid depletion of seed
populations takes place in the first year of burial. Although
the cause of rapid loss has not been investigated, it may be due to
soil aeration which is curtailed later by compaction (Lewis 1961;
Barton 1962). Depth of burial, the water level and soil type,
influence aeration and may affect seed longevity. However the rapid
depletion of seed populations is mainly due to *in situ* germination,
although other factors, such as fungi and bacteria may also be
involved (Lewis 1961). Lewis (1958) found some seedlings of
legumes at a depth of 10" after prolonged burial in the soil.
He stated, however, that seeds of most legumes (including red clover

and white clover) do not survive for long under wet conditions in the soil.

Hyde and Suckling (1953) observed that legume seeds rarely lose their germination capacity while they remain in an impermeable state in the soil. However, they either germinate or decay after becoming permeable. Evans (1959) also reported that germination *in situ* is the principle factor affecting the depletion of weed seed populations in loam, sand and peat soils. Similarly, Taylorson (1972) stated that the decrease in numbers of viable weed seeds in the soil is apparently due to germination *in situ* when such seeds are after-ripened and sown at shallow depths.

Some workers have attempted to explain the fate of seeds in the soil using models. Schafer and Chilcote (1969, 1970) developed a population model to analyse the persistence and depletion of buried seed of a species at any particular point in time. They described the persistent segment of the buried seed population using parameters of endogenous and exogenous dormancy with the nonpersistent segment determined by *in situ* germination and nonviability among ungerminated seed. This model was later elaborated by Roberts (1972b).

Periodicity of germination: Many seeds buried in the soil exhibit a periodicity of germination. As early as 1916, Harrington reported this characteristic in some legume species. He found that under apparently favourable conditions, only a small proportion of impermeable seeds in red clover, white clover, alsike clover and sweet clover germinated promptly in the soil when sown in warm weather. However, 50-60% of them produced seedlings in the following spring after passing through the winter in the soil without injury. Witte (1938) also reported that the germination of hard seed in some legumes occurs at two periods, the first during the first few months after sowing and the second in the following spring. According to Blaser and Killinger (1950), most of the white clover seed which falls to the ground remains hard during the warm summer months. However, a dense population of seedlings appear when the soil becomes moist after the first frost.

With the seeds of other families, some workers have also reported periodicity of germination. Brenchley and Warington (1930) stated that the period of maximum germination of weed seeds varies with the

species but mostly occurs in the autumn and winter. Maximum germination never occurs during the summer months. Apart from the periodic bulk germination in each year, only relatively few seedlings appear over the rest of the year. Barton (1945, 1961b) also reported that pigweed *(Amaranthus retroflexus)* seeds show a definite periodicity of germination which is independent of external conditions. Similarly, Chepil (1946a) described that with most species there is a typical seasonal periodicity of germination throughout the life-span of seeds. The period of maximum or peak germination varies with the species. Thurston (1960) found that seeds of some weed species germinate at a definite time of year either in spring or autumn or both. Nikolaeva (1969) recorded a similar pattern in a wide range of plants. This mechanism is important in insuring against untimely germination, although it has not yet been exhaustively explained. Roberts (1970) has observed that more information is needed about seasonal changes in the proportion of seeds under enforced-dormancy as compared with endogenous-dormancy and the factors which cause them.

Seedling emergence and depth of burial: The percentage emergence or maximum seed burial depth from which seedlings can emerge varies with species, seed size, temperature, soil moisture, soil compression, soil particle size, soil pathogens and the composition of the atmosphere surrounding the seed (Heydecker 1956; King 1966; Schafer and Chilcote 1969; Roberts 1970). Black (1959) and King (1966) defined "emergence" as the appearance of the first aerial organ or part above the soil surface.

There are a number of reports of the maximum depth of seed burial which still allows seedling emergence for various kinds of weed seeds (Brown and Proter 1942; King 1966; Courtney 1968; Roberts 1963; Roberts and Feasts 1972; Feasts and Roberts 1973), and vegetable, legume and cereal crop seeds (Andrew 1953; Lewis 1958; Hanna 1973; Smith and Cooley 1973; Onwneme and Adegoroye 1975; Hadjichristodoulou *et al*. 1977). Those seeds buried below the

critical depth for that species, may germinate but fail to emerge through the covering soil layer or remain dormant (Brown and Porter 1942; Roberts 1964, 1970; Roberts 1972b).

King (1966) stated that as a 'rule-of-thumb' for planting, seeds must be sown no deeper than four times their diameter. According to Moore (1943) seeds of red clover and other clovers almost completely fail to emerge when they are sown 5 cm deep. Murphy and Arny (1939) also found that red clover seeds produce satisfactory stands when sown at 2.5 cm depth on all soil types. However, no emergence of seedlings of alfalfa, sweet clover, alsike clover, white clover and red clover is recorded from a 7.5 cm depth of planting in most soil types.

Bolton (1962) reported that alfalfa seedlings may emerge from depths of 3.8 cm to 5 cm in sandy soils but these depths are not recommended on heavier soil types. Peiffer *et al.* (1972) stated that seedling emergence decreases significantly as the depth of planting increases. Optimum planting depth for crownvetch (*Coronilla varia* L.) is about 0.6 cm to 1.3 cm but vigorous seedlings can emerge from depths of 3.8 cm.

Permeability of seeds in dry storage: Permeability and longevity of seeds depends on the conditions of storage. It has been generally suggested (Owen 1956; Barton 1961a; Harrington 1972; Roberts 1972a) that high humidity and high temperature tend to decrease seed viability while low temperature and low humidity or low seed moisture content are beneficial to seed for maintaining their viability for a considerable period.

Witte (1931a) reported that seed hardness in small-seeded legumes increases when samples are kept under dry and warm laboratory conditions. Barton (1961a) also stated that seeds which are permeable when harvested may become impermeable during storage under certain conditions of temperature and humidity. Barrett-Lennard and Gladstones (1964) found that initially soft seeds of Western Australian serradella (*Ornithopus compressus* L.) stored at 76% relative humidity, remained soft while those kept at 44% relative humidity become impermeable. Seeds stored at 52% and 66% relative humidities resulted in 'semi-hardness' and showed delayed germination. Breakdown

of impermeability occurs in storage only under alternating temperatures but not at a constant temperature of $20^{o}C$.

Hagon and Ballard (1970), who worked with the seeds of subterranean clover, reported that some seeds revert to the impermeable condition when they are stored at a low relative humidity. It is suggested that the restoration of impermeability by exposure to low relative humidity may be caused by shrinkage which is likely to occur on drying. In the case of percussed seeds, when the slit at the strophiole does not extend to the exterior the re-apposition of surfaces over only a part of the length of the slit would probably be sufficient to restore impermeability. The strophiole can exhibit movements in response to changes in relative humidity. Such movement however operates in the opposite way to that of the hilum. The strophiole cleft is closed only by dry conditions whereas the hilum can be repeatedly opened and closed. If commercial seed samples contain some seeds which are rendered permeable at the strophiole only, the latter can be resealed by storing the seeds under low humidity conditions during the summer months (Hagon and Ballard 1970).

According to Gladstones (1958), Australian blue lupin *(Lupinus digitatus)* seeds which are air-dried in the laboratory remain fully soft for about one week after harvest and thereafter harden only slowly. However, in red clover, impermeability develops immediately after harvest and softening occurs slowly during the summer and autumn and becomes more rapid in the winter. Subsequently the rate of softening slows down again (Nakamura 1962).

MATERIALS AND METHODS

1. Seed burial sites and experimental layouts:

Two field sites of different soil types (clay loam and sandy
loam) were selected. The size of each plot was approximately 22.32
sq. meters (360 cm x 620 cm), including walking spaces between
replicates. Each replicate measured 150 x 170 cm including a space
for soil moisture and temperature measuring instruments and for soil
sampling. Along the sides of each site, field emergence trials
were carried out at both field sites.

A randomized complete block design with different randomizations
was used at each site. Three replicates were included at each seed
burial depth (Figs14&15 and Plates 56 & 57).

2. Site management:

The two seed burial sites were deep ploughed and finely tilled.
Each replicate was levelled evenly to allow uniform depths of seed
burial.

Weeds at the burial site and field emergence trial areas were
sprayed twice with Gramoxone (paraquat) at the rate of 6 litres in
300 litres of water/ha, and twice with Reglone (diquat) at the rate
of 6 litres in 700 litres of water/ha during the trial period.
Hand weeding was also done whenever necessary.

3. Seed source and preparation for burial:

Six seed samples with different maturities (harvested at 10,
14, 18, 22, 26, and 30 days after peak flowering) were taken from
the 1974 autumn-sown crop. From each sample, about 13,000 seeds
were used for seed burial trials and field emergence tests. The
remaining seed from each sample was stored in the laboratory for sub-
sequent laboratory germination tests which were carried out at similar
intervals to those from buried seed samples. These six different
maturity samples are also refered to as "developmental stages or
ages" elsewhere in the text.

FIG.14: EXPERIMENTAL LAYOUT FOR SEED BURIAL TRIAL (CLAYEY SOIL)

FIG.15: EXPERIMENTAL LAYOUT FOR SEED BURIAL TRIAL (SANDY SOIL)

A - H Seed lot removal at different time intervals
1 - 6 Seed developmental stages

M_1, M_2, M_3, M_4 Times of removal for moisture testing

M_{4-2} Removal at seed developmental stage '2' for moisture testing at time '4'
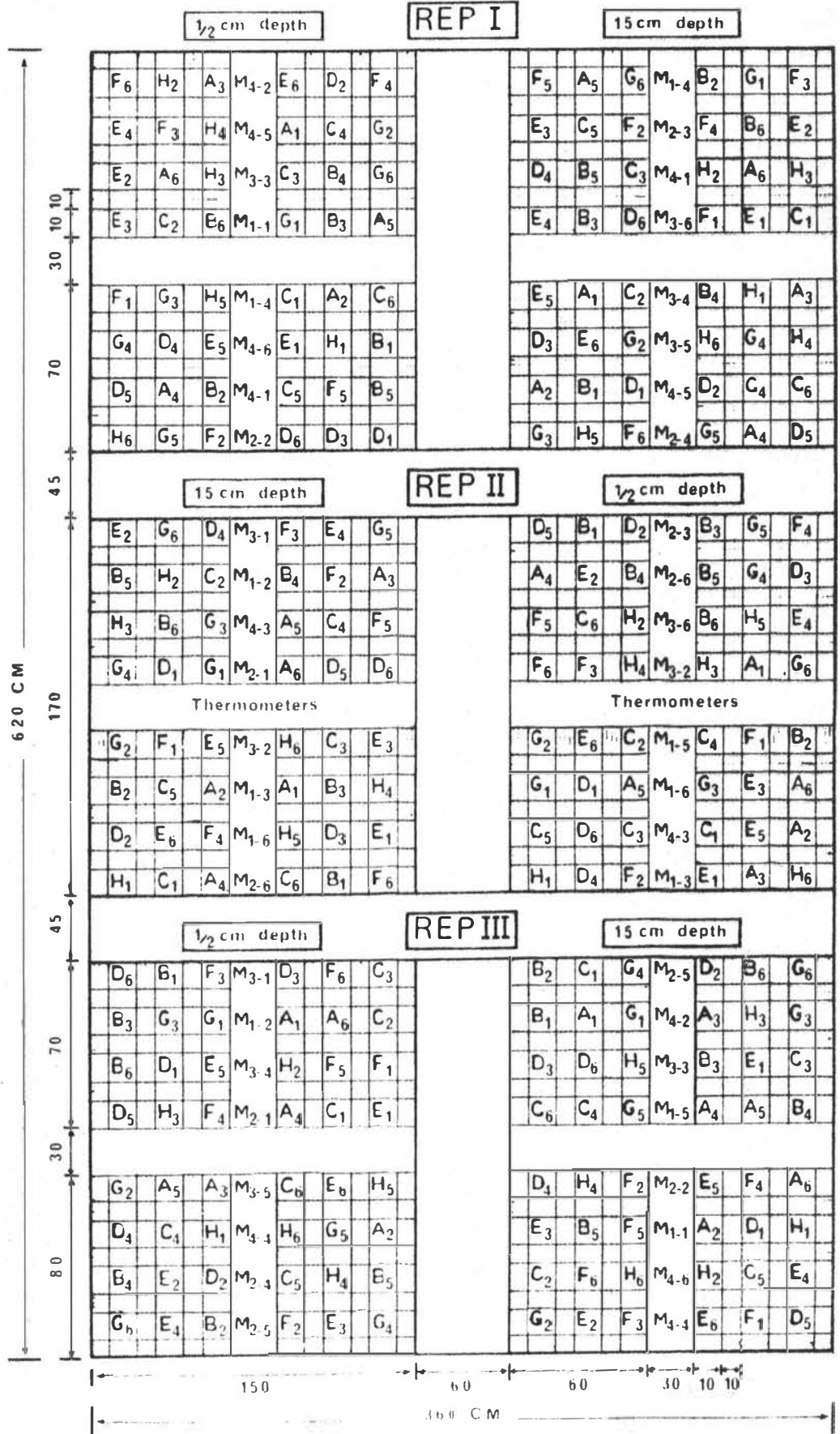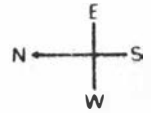
Plate 56.   Experimental layout for seed burial trial
(clay soil site)

Plate 57.   Experimental layout for seed burial
(sandy soil site)

Small nylon bags (about 6 x 8 cm) were made from open weave nylon cloth. Seeds were put into these bags before burying them in the soil. Each bag contained 100 seeds and bags were labelled with a different coloured plastic tag for different samples. Altogether 672 bags were used for the seed burial trial.

Nylon mesh bags containing seed were buried at ½ cm depth (just covered by soil) or 15 cm deep in both soil types. These bags were placed about 15 cm apart. Plastic label-pegs were placed on the soil surface to mark the position of each bag.

For the field emergence trial, 100 seeds from each maturity were buried at monthly intervals starting from July through to October. Seeds were buried at both depths and soil types described for the seed burial trial except that the seeds were not enclosed in nylon mesh bags.

4. Sample recovery and seedling counts:

Seed bags buried in both soil types were removed at intervals. Two weeks after burying, all the bags buried at both depths and soil types were removed to check the percentage germination of seed in each bag. The germinated seeds inside the bag were counted and all bags were then re-buried on the same date of removal. However, when bags were removed for germination tests at later periods, they were not re-buried again.

The first four removals for germination tests, (each removal consisting of 72 bags) were carried out initially at monthly intervals commencing one month after burial. Subsequently samples were removed at increasing time intervals, the final sample being taken after an 18 months burial period.

Seedlings that emerged from the soil in field emergence trials were counted and removed at weekly intervals. The emergence results for each seed maturity sample were recorded separately. This trial was also conducted for 18 months.

5.   <u>Measurements:</u>

At each time of removal, bags were thoroughly washed in
tap water and dried quickly between dry towels. The bags were then
cut open and the seeds remaining in each bag recorded as soft or
hard separately. Both the soft and hard seeds recovered were tested
for germination commencing on the date of removal.

Seed samples stored in the laboratory were also tested for
germination at the corresponding time of each field removal.
In all cases germination tests were carried out at $20^{o}C$ for 10 days
on top of paper (TP) in accordance with the I.S.T.A. Rules (Anon. 1966).

To determine changes in the moisture content of seed during
the burial period, additional seed samples were buried along with
corresponding samples for germination tests (Figs.14 & 15).   Four
moisture determinations were made with buried seed samples (1, 3
12 and 18 months after burial). For moisture tests, seed bags
were removed, washed and dried quickly, These bags were then opened
and seeds removed. Soft and hard seeds were separated and the latter
were dried between blotting-paper. Only those seeds which appeared
hard, were used for moisture determination.

Moisture tests on seed samples stored in the laboratory were
also carried out at the same time as those from buried seeds.
Moisture determination was conducted in an air oven at $105^{o}C$ for 18
hours.

Air and soil temperatures were recorded at both burial sites
throughout the experimental period. A recording thermograph
was stationed at each experimental site about 10 cm above the ground
to record daily ambient temperatures. Similarly, to measure soil
temperatures, minimum-maximum thermometers were buried at the ½ cm
or 15 cm depths in replicate II of each site. (Figs.14 & 15).
Minimum and maximum soil temperatures were recorded at weekly
intervals.

Soil moisture levels at different depths and burial sites
were determined gravimetrically at weekly intervals. At each

sampling about 500 grams of soil was taken from each replicate and depth at both sites. The samples of each replicate were thoroughly mixed and a 20 g sub-sample was dried in an air oven at $105^{O}C$ for 24 hours for moisture determination.

Seed samples removed from the soil in March and July 1977 were washed and dried. Each sample was placed in ferrous solution (Fe^{++} 0.003 M) for 10 days to observe the site of initial permeability of the seed coat and the number of permeable seeds.

RESULTS

A computer programme using a split plot analysis with two sub-
factors was employed to analyse data from the seed burial experiment.
Tables of analysis of variance, means of each treatment and treatment
interactions for seed recovered, hard seed and laboratory germination
results are presented in Appendices (34 - 36) for the clay soil site
and in Appendices (37-39) for sandy soil site.

A.   Seed germination and viability under field conditions

(1) Germination *in situ* in the soil: As shown in table (4)
                                        at all seed maturities,
except maturity one (i.e. seed buried in soil following removal from
plants 10 days after peak flowering), the percentages of germination
in situ in the soil after two weeks burial are similar to the
corresponding pre-burial laboratory germination results. The
germination *in situ* at all maturities at different depths and in
both soil types are similar for all of the eight seed lots examined.

TABLE 4 : Laboratory germination and *in situ* germination in the
            soil after 2 weeks burial (means of eight seedlots)

Days after peak flowering	Seed maturity (develop-mental age)	Laboratory germ-ination test immediately prior to seed burial (%)	Germination *in situ* (%)			
			Clayey soil site		Sandy soil site	
			½ cm depth of burial	15 cm depth of burial	½ cm depth of burial	15 cm depth of burial
10	1	20	9	7	7	7
14	2	12	10	7	8	8
18	3	12	11	8	9	8
22	4	9	9	7	7	7
26	5	10	10	9	9	10
30	6	10	9	8	10	9

When seed samples were removed after burial for one month (i.e. a further fortnight after the interim removal shown in Table (4)), increased *in situ* germination percentages were recorded in all seed maturities but particularly in earlier maturities at both burial depths and in both soil types (Appendix 40).   Further increases in the percentage germination *in situ* at the ½ cm depth in both soil types also occurred during the following 2 month burial period.   In contrast to the results of previous months, more obvious increases in *in situ* germination were observed in the later seed maturities (i.e. seed buried following removal from plants 18, 22, 26 and 30 days after peak flowering).   Nevertheless, the germination *in situ* at the 15 cm burial depth in both soil types decreased markedly in samples from all seed maturities.

At the third month, *in situ* germination percentages at the ½ cm depth began to decline whereas at the 15 cm depth they increased a little in the clayey soil while remaining at about the same level in sandy soil.   Similarly, at the removal after four months of burial, percentages of *in situ* germination at the 15 cm depth showed a slight increase, while those at the ½ cm depth dropped further to lower percentages.   At later times of removal during the experimental period, *in situ* germination was negligible at both depths and in both soil types except at the seventh removal (after 15 months of burial) where a small percentage of *in situ* germination occurred at the ½ cm depth in both soil types.   (Appendix 40).

(2)   Seed recovered from the soil: The percentage of seed recovered at different intervals of removal showed a sharper decline at the ½ cm depth than at the 15 cm depth in both soil types (Table 5).   A greater reduction occurred between the first and second times of removal at the ½ cm depth.   Nevertheless, at most removal times the percentage of seed recovered from the same depth in both soil types showed no obvious differences, although the percentage of seed recovered was generally much higher from the 15 cm depth than the ½ cm burial depth (Table 5).

TABLE 5 :   Percentage means of seed recovered from the soil at
             different intervals of seed removal.   Date of burial –
             10.6.75

Period of burial (month)	Date of seed removal	Clayey soil site		Sandy soil site	
		½ cm depth	15 cm depth	½ cm depth	15 cm depth
1	9.7.75	74.6	78.3	75.8	73.7
2	12.8.75	35.0	69.9	33.7	68.9
3	11.9.75	23.5	63.8	23.9	62.2
4	9.10.75	19.1	53.3	18.5	49.3
9	9.3.76	15.2	46.7	16.8	40.9
12	9.6.76	13.7	37.8	14.5	38.5
15	9.9.76	6.2	33.6	9.4	36.7
18	8.12.76	3.3	23.0	7.0	24.0

The means of both depths for seeds recovered from the clayey soil
site showed a significant ($P < 0.01$) decrease with later removals
throughout the experimental period (Appendix 41).   The results in
sandy soil showed a similar pattern but the reduction was so small from
the fourth to seventh times of removal that no significant ($P > 0.01$)
differences were recorded.   However, there was a significant ($P < 0.01$)
reduction in the percentage of seed recovered at each of the first four
removal times.   The percentage of seed recovered at the last removal
(after 18 months burial) was also significantly lower than at all
other times.   (Appendix 41   ).

On the other hand, the percentages of seed recovered are very
similar at each depth for corresponding maturities in both soil types
(Table 6).   However, higher percentages of seed were recovered from
the deeper burial for all maturities. Moreover, differences in the
percentage of seeds recovered between maturity one and six at the 15 cm
depth is about three times greater than the corresponding result at
the ½ cm depth in both soil types.   The differences between the first
five maturities of the 15 cm depth are also larger than corresponding
differences at the ½ cm depth (Table 6).

TABLE 6 :   Percentage means of seed recovered from the soil for samples of different seed maturities

Days after peak flowering	Seed Maturity (developmental age)	Clayey soil site		Sandy soil site	
		½ cm depth	15 cm depth	½ cm depth	15 cm depth
10	1	17.0	30.6	17.7	30.7
14	2	22.0	45.3	21.8	42.3
18	3	23.1	51.2	23.8	48.5
22	4	24.0	56.7	25.6	52.8
26	5	27.8	60.5	32.5	61.4
30	6	28.9	60.5	29.2	60.0

The increase in the percentage of seeds recovered (means of two depths) with stages of seed maturity was also significantly ($P < 0.01$) different for maturity levels one to five. However, no significant differences were observed between maturity levels five and six in either soil type. (Appendix 42).

(3) Hard seed recovered from the soil: At the ½ cm depth, the percentage of hard seed recovered from the soil dropped drastically from 69% to 28% in the clayey soil and from 68% to 28% in the sandy soil sites between the first and second removals. However, from the third time of removal until the last removal this decline was more gradual (Fig. 16). Similarly, at the 15 cm depth in both soil types, the level of hard seed content also decreased during the first four months and then continued to decline more gradually with increased length of the burial period. As seen in Fig. (16), the percentage of hard seeds recovered at any one depth in both soil types was remarkably similar throughout the experimental period. However, at the second time of removal the percentages of hard seed at the ½ cm depth in both soil types were more than 50% lower than those of their counterparts at the 15 cm depth. This trend continued until the end of the burial period (18 months).

FIG. 16 :   Percentage of hard seed (means of six maturities) recovered from the soil at different times of removal
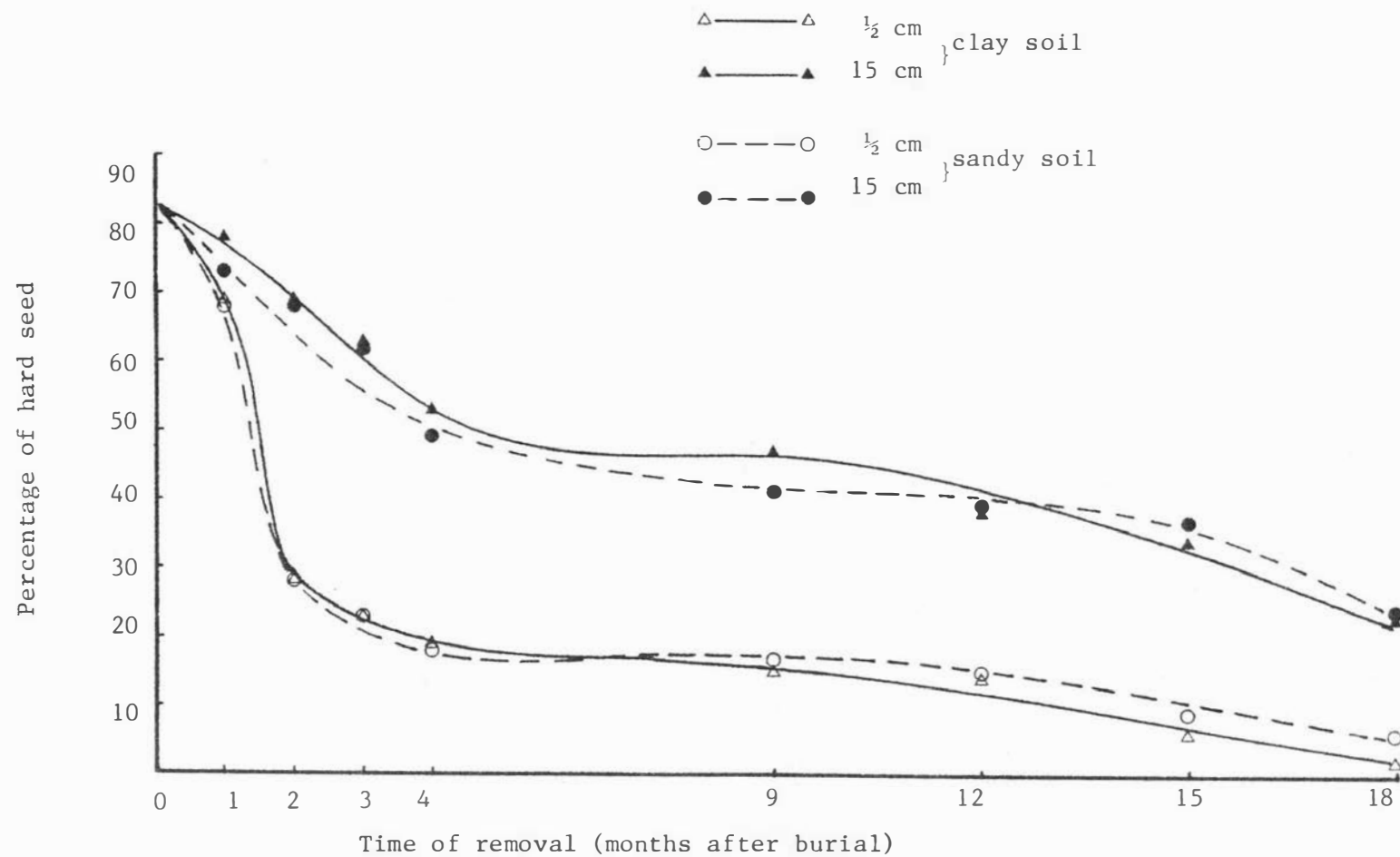
B.   Laboratory assessment of the characteristics of seed recovered
     from the soil and seed stored in the laboratory

     (1)   Germination in the laboratory: Seeds recovered from the soil
                                     showed some germination
capacity when they were tested under optimum conditions for seed
germination in the laboratory.   The percentage germination recorded
for seeds recovered from each depth of burial at each time of removal
is shown in Table (7).   A sudden drop in germination occurred at
the second removal time and again at the third removal at the ½ cm
depth in both soil types.   Germination was very low in seed samples
recovered at the third to fifth times of removal although it increased
a little again at the sixth and seventh removal.

     At the 15 cm burial depth, seed germination capacity declined
very gradually up to the fourth time of removal (after 4 months
burial) in both soil types.   It then dropped drastically at the
fifth time of removal, increased substantially at the sixth and
seventh times of removal, and finally fell to the lowest level at
the last removal (Table 7).

TABLE 7 :   Percentage germination (means of six maturities) recorded
               at different intervals of removal.
               Date of burial - 10.6.75

Period of burial (months)	Date of seed removal	Clayey soil site		Sandy soil site	
		½ cm depth	15 cm depth	½ cm depth	15 cm depth
1	9.7.75	30.2	39.0	35.1	36.7
2	12.8.75	13.2	35.1	15.7	40.1
3	11.9.75	1.8	37.8	2.6	28.3
4	9.10.75	0.7	29.8	0.7	17.3
9	9.3.76	0.3	1.1	0.3	1.9
12	9.6.76	4.8	13.0	7.8	22.3
15	9.9.76	3.6	21.6	4.0	24.1
18	8.12.76	0.0	1.8	0.0	0.2

The reduction in the percentage germination means of seed recovered from both depths in both soil types from the first to fifth times of removal showed significant differences (Appendix 41). In each soil type, these values at the fifth and eight times of removal were also significantly (P < 0.01) lower than corresponding values obtained at other removal times.

It was also observed that at both depths and in both soil types the seed germination percentage increased with increasing levels of seed maturity (Table 8 and Figs 17&18).Although a gradual and small increase in percentage germination occurred at the ½ cm depth, a more substantial increase was noted at the 15 cm in both soil types. However, at the same depth for both soil types, the germination percentages for corresponding maturities were similar although slightly higher percentages were recorded at the ½ cm depth of burial in sandy soil.

TABLE 8: Percentage germination (means of eight removals) of seeds of different maturities following seed removal

Days after peak flowering	Seed maturity (developmental age)	Clayey soil site		Sandy soil site	
		½ cm depth	15 cm depth	½ cm depth	15 cm depth
10	1	4.0	8.5	5.3	7.8
14	2	6.7	18.2	7.3	17.1
18	3	6.9	23.2	7.6	20.6
22	4	6.2	23.1	7.4	20.7
26	5	8.3	31.3	11.0	32.8
30	6	9.0	30.0	11.0	29.2

In both soil types, there was a significant increase in seed germination capacity between maturity ratings one and two (Appendix 42). No significant (P > 0.01) increases occurred between maturity levels two to four. However, the germination percentages in maturities five and six were both significantly (P < 0.01) higher than the values for other maturities in both soil types (Appendix 42).

Fig 17: Seed losses in the soil during the burial period of 18 months and the germinability of seeds recovered from the ½ cm depth.

Seed lost in the soil   germination *in situ* and dead seed

Seed recovered from the soil   Germination in the laboratory

Seed remaining hard after lab. germination tests

(a) Clay soil site   (b) Sandy soil site

Stage of seed maturity   Stage of seed maturity

Fig 18: Seed losses in the soil during the burial period of 18 months and the germinability of seeds recovered from the 15 cm depth

Seed lost in the soil   Germination *in situ* and dead seeds

Seed recovered from the soil   Germination in the laboratory
Seed remaining hard after lab. germination tests.

(a) Clay soil site

(b) Sandy soil site

Stage of seed maturity

Stage of seed maturity
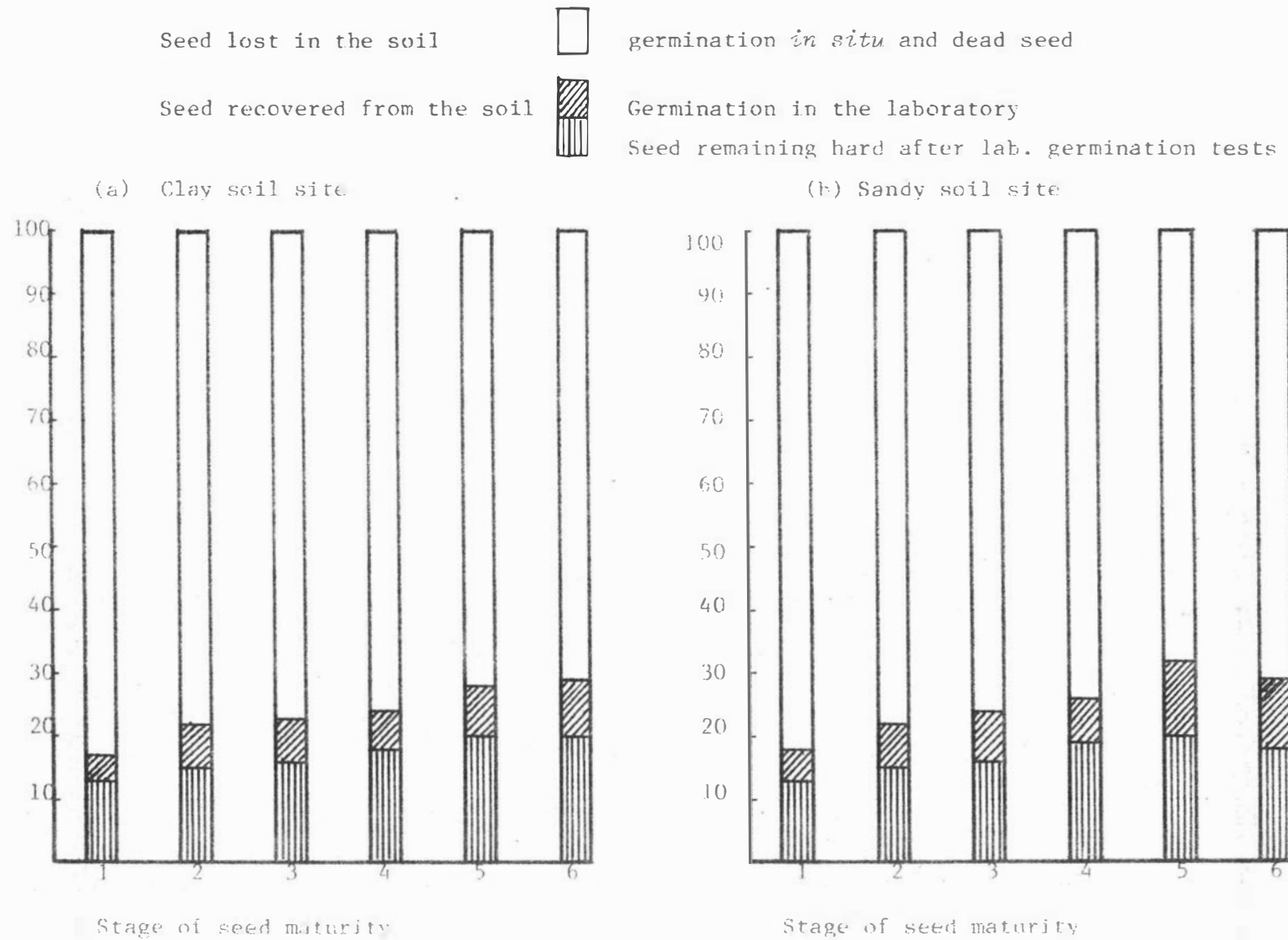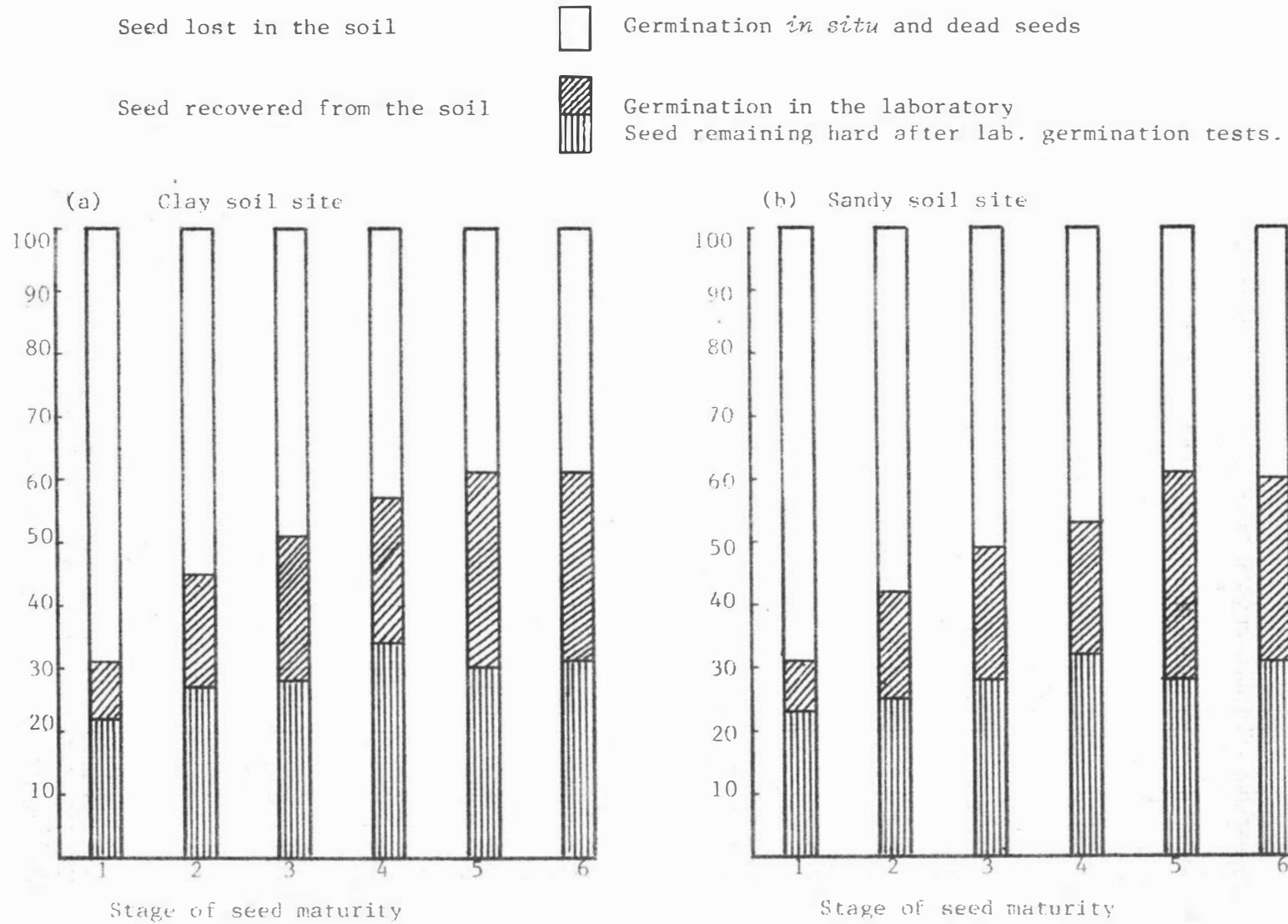
(2) <u>Levels of hard seed percentage recorded after laboratory</u>
      <u>tests</u>:

The percentage of seed remaining hard at the end of laboratory
germination tests at each time of seed removal is presented graphically
in Figure 19.   The percentage of hard seed dropped to about one
half of the original level in samples tested following removal after
one month of burial at both depths and in both soil types.   There
were also marked reductions of the hard seed content between samples
tested following one and two months of burial, especially at the ½ cm
depth.   In both soil types, the levels of hard seed recorded in
samples at the ½ cm depth were generally maintained during 2 to 9
months of burial and then declined gradually. Conversely, the
percentage of hard seed in samples buried at the 15 cm depth in
clayey soil declined sharply after one to four months of burial and
then rose to the highest level at the fifth time of removal (i.e.
following 9 months burial).   The percentage then decreased at the
following two removals (i.e. up to 15 months) and increased again at
the final removal (Fig. 19).

A similar reduction in the hard seed percentage, especially at
the ½ cm depth, also occurred in sandy soil. At the 15 cm depth,
starting from the third time of removal, the percentage of hard seed
began to increase until the fifth removal time. It then followed
a similar trend to the results in the clayey soil (Fig 19).

A significant ($P < 0.01$) reduction in hard seed percentage
(means of two depths) occurred during the first four months of burial
in seed samples recovered from the clayey soil site. (Appendix 41).
Starting from the fifth time and continuing to the final time of
removal, considerable fluctuation in hard seed content occurred. Seed
recovered from the sandy soil site showed similar but fewer significant
reductions in the percentage of hard seed (Appendix 41).

The percentage of hard seed content also increased significantly
with increasing seed maturity up to maturity four (i.e. seed harvested
22 days after peak flowering). However no further significant increases
occurred in later seed maturity samples (Appendix 42).
Moreover, no significant difference between the percentages of hard
seeds from the two soil types was observed (Appendix 43).

FIG. 19 : Percentage of hard seed (means of six maturities) remaining after normal laboratory germination tests immediately following each time of seed removal.
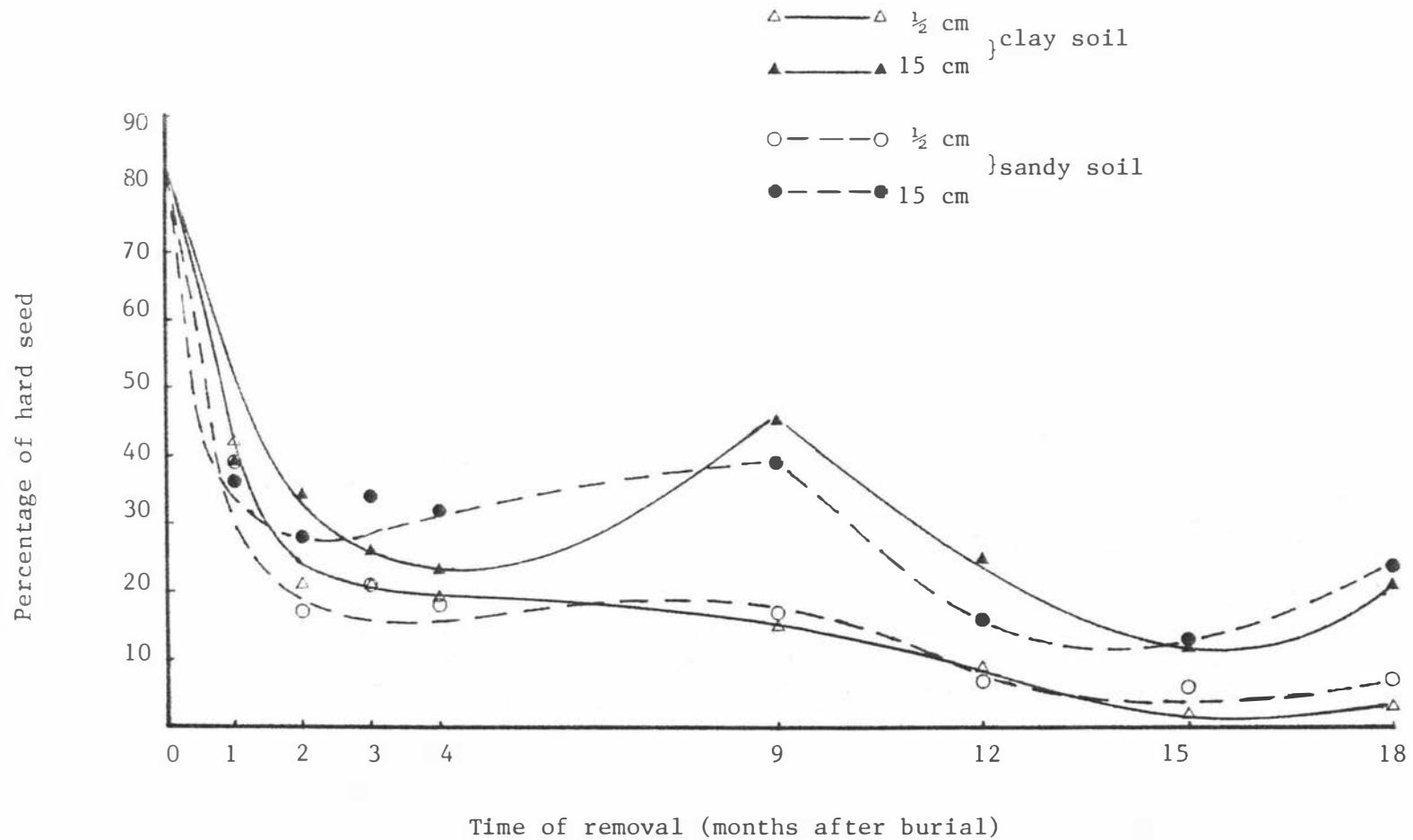
(3) Seed coat permeability test: When seeds were removed from
the soil in early autumn
(March 1977) after 21 months burial and soaked in ferrous solution,
almost all of them (99%) were hard. However, in samples removed in
the winter (July 1977) following 24 months burial, a substantially
higher percentage of seed (30%) showed permeability by absorbing the
ferrous solution through the strophiole.

(4) Seed stored in the laboratory: The percentage of hard seeds,
dead seeds, normal and
abnormal seedlings obtained from seed samples stored in the laboratory
are presented in Figure (20) ,Appendices (44 - 46) . The data
was analysed using a computer programme for three-way analysis of
variance. The statistical results, including the means of each
treatment and interactions for hard seeds, normal seedlings and
dead seeds are presented in Appendices (44-46).

The percentage of hard seeds decreased gradually with increasing
storage period from 82% at the beginning of the experiment to 65%
after 18 months storage (Fig. 20 a). Conversely, the level of normal
seedlings increased from 12% to 31% during the storage period. However,
the percentage of dead seeds showed no significant differences in most
of the tests, except at the test carried out after 9 months
storage (Fig.20 a).

The percentage of hard seeds increased with increasing seed
maturity (Fig. 20 b). By contrast, the percentage of normal seedlings
decreased as seed maturity increased. More dead seeds were recorded
in seed samples harvested at the earlier stages of seed development
(10 and 14 days after peak flowering) (Fig. 20 b , Appendix 46   ).

(5) Seed moisture content: Seed samples stored in the laboratory
generally maintained, or showed only
a small decrease in their initial moisture contents throughout the
storage period of 18 months (Fig.20a and Appendix 47). Seed samples
harvested during the early stages of maturity, generally contained a
slightly higher moisture content than those harvested at later
maturities. However, the range in seed moisture content across different
maturities during the storage period was only between 9% and 13%
(wet weight basis). The level of relative humidity in the storage

FIG. 20: Percentage of hard seed, germination, dead seed and abnormal seedlings of seed samples stored under laboratory conditions.
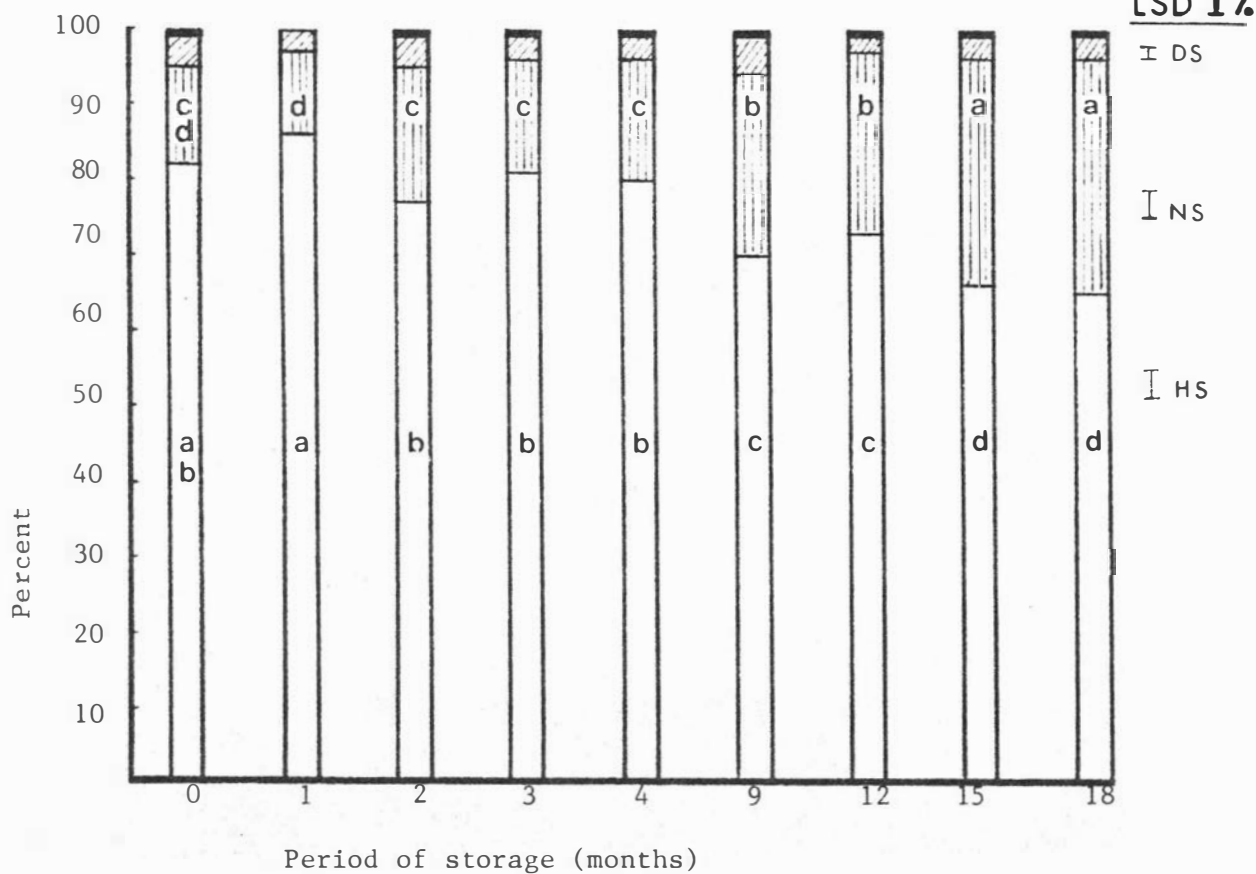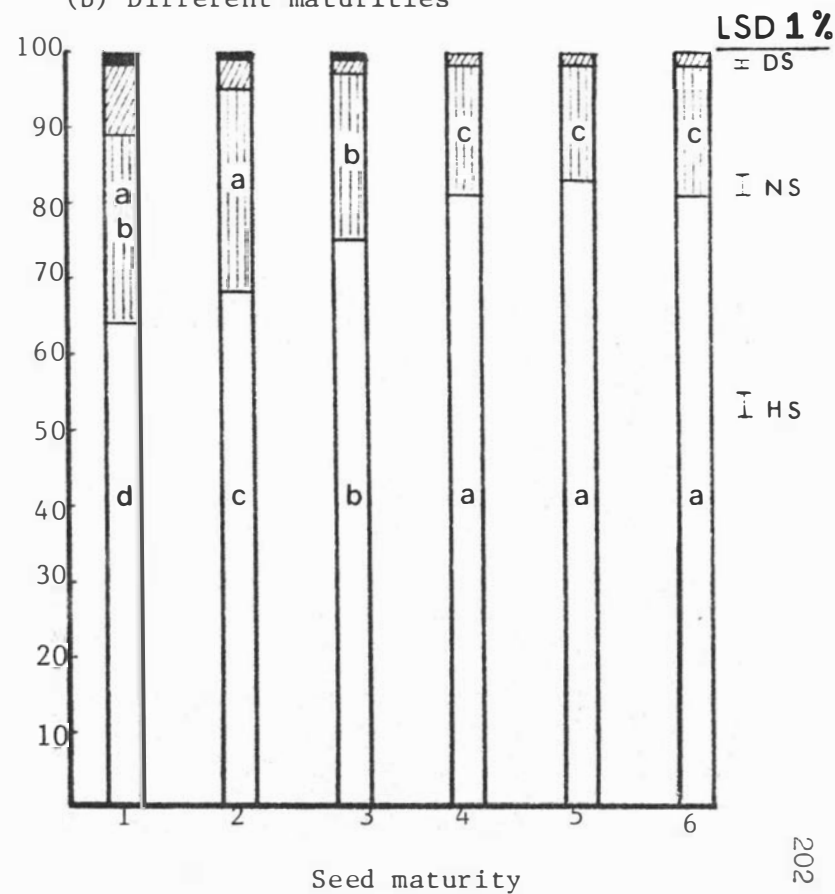
FIG. 21 :   Percentage moisture content of seed samples stored
              in the laboratory and in the soil

(a)   Laboratory storage

Seed moisture content

x——x   Initial test
△—·—△ One month after storage
▲——▲   Three "   "   "
○—·—○ Twelve "   "   "
●——·● Eighteen   "   "

(b) ½ cm depth clay soil

(c) ½ cm depth sandy soil

(d) 15 cm depth clay soil

(e) 15 cm depth sandy soil
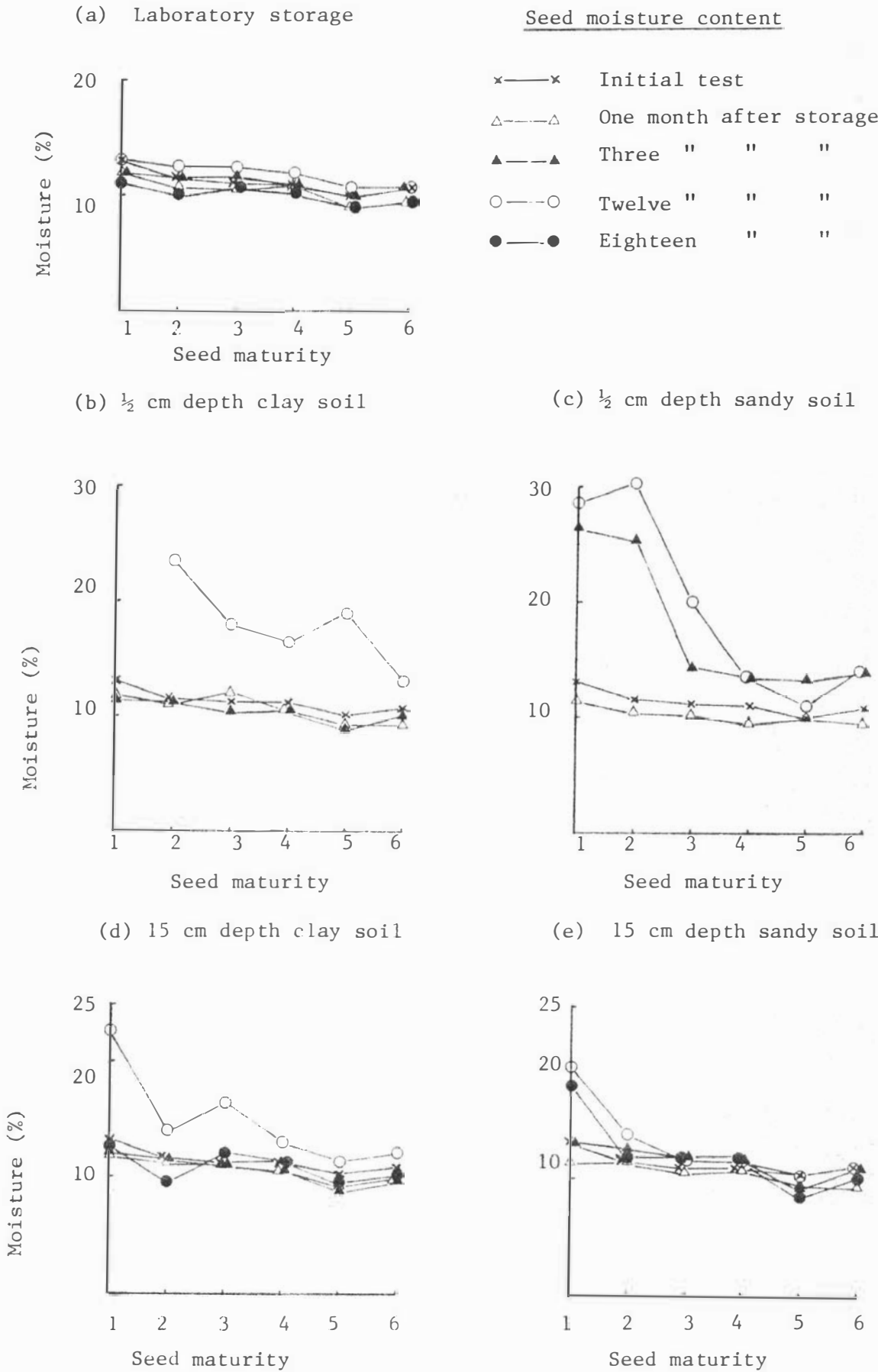
room (47-68%) did not seem to have any significant effect on seed
moisture content (Appendix 48).   Similarly, the relatively constant
storage temperature (18-26°C) (Fig. 22   and Appendix 48   ) did not
seem to affect the seed moisture levels.

As shown in Fig.(21 b), those seed samples recovered following
burial at the ½ cm depth in clayey soil also retained their initial
 moisture contents for at least three months in the soil. However,
high seed moisture contents were recorded when seed moisture
tests were carried out after 12 months seed burial (Appendix 47).
The moisture contents of seed samples recovered from the sandy soil
after one month of burial showed similarity with initial pre-burial
seed moisture levels (Fig.21 c). However, with tests after 3 and 12
months of burial, the moisture contents, especially in early maturity
samples, were substantially higher than initial seed moisture levels
(Appendix 47).

The results of moisture tests carried out on seed samples buried
at the 15 cm depth in both soil types are shown in Figs. (21 d&e).
In the clayey soil, moisture contents of seed samples tested at 1, 3
and 18 months following burial were similar to the results of the
initial test. High moisture contents in seed samples of most
maturities were noted when moisture tests were carried out after
12 months burial (Fig 21 d). In sandy soil, seed from all seed
maturities, except maturity one after 12 and 18 months burial,
maintained their initial moisture levels (Fig. 21e and Appendix 47   ).

C.   Microclimatic conditions at the seed burial sites and in the
     laboratory storage room

     (1) Ambient temperature: Monthly average ambient temperatures
                                recorded at the two burial sites in
the field and also in the laboratory where the remaining seed samples
were stored, are presented in Fig. (22) and Appendix (48).

In the field, both minimum and maximum ambient temperatures
recorded at the clayey soil burial site were generally slightly lower
than corresponding values obtained at the sandy soil burial site
from the commencement of the trial until June 1976. Subsequently,
temperatures at the clayey soil site rose to slightly higher levels

FIG.22 : Monthly average ambient maximum and minimum temperature (°C) in the laboratory and at the outdoor burial sites.
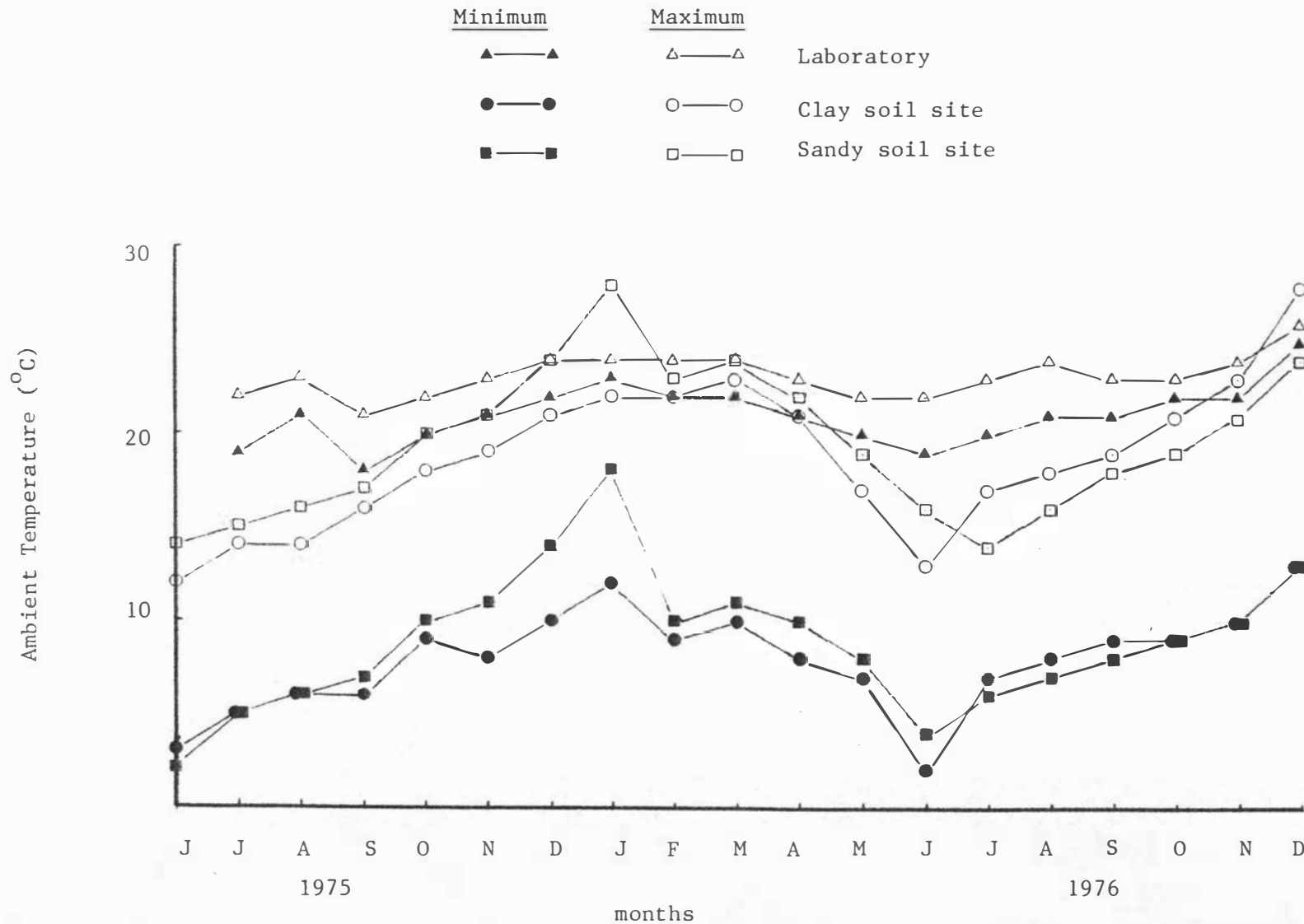
than those at the sandy soil site until the end of the experimental
period (Fig. 22). Wider fluctuations in both minimum and maximum
temperatures occurred at both field sites than in the laboratory.

As shown in Fig. (22), both minimum and maximum temperatures
in the laboratory showed only minor fluctuations throughout the
storage period. Monthly average maximum temperatures ranged from
$21^{o}C$ to $26^{o}C$ and minimum temperatures ranged from $18^{o}C$ to $23^{o}C$.
The differential between the minimum and maximum temperatures remained
relatively constant during the storage period. The most extreme
daily ambient temperatures occurring during the experimental
period in the laboratory and the field are summarised in Table (9).

TABLE 9 : Daily lowest and highest levels of minimum-maximum ambient
temperatures (^{o}C) recorded in the field and in the
laboratory

Burial/storage site	Temperature level	Min. ^{o}C	Max. ^{o}C	Difference ^{o}C
Laboratory	lowest	15	17	2
	highest	25	27	2
Clayey soil site	lowest	-3	8	11
	highest	17	30	13
Sandy soil site	lowest	-2	9	11
	highest	23	33	10

(2) Soil temperature: Monthly average soil temperatures
measured at the $\frac{1}{2}$ cm and 15 cm depths in
both soil types are shown in Fig. (23) and Appendix (49). As
seen in Fig. (23), there are four separate pairs of lines with
similar trends. The top pair of lines shows the maximum temperatures
recorded at the $\frac{1}{2}$ cm in both soil types. These temperatures increased
steadily from July until October and then rose more gradually to March
where highest temperatures of $44^{o}C$ and $40^{o}C$ were recorded in the clayey
soil and sandy soil sites respectively.

FIG. 23 : Monthly average soil temperatures (^{o}C) recorded at two depths in two soil types.
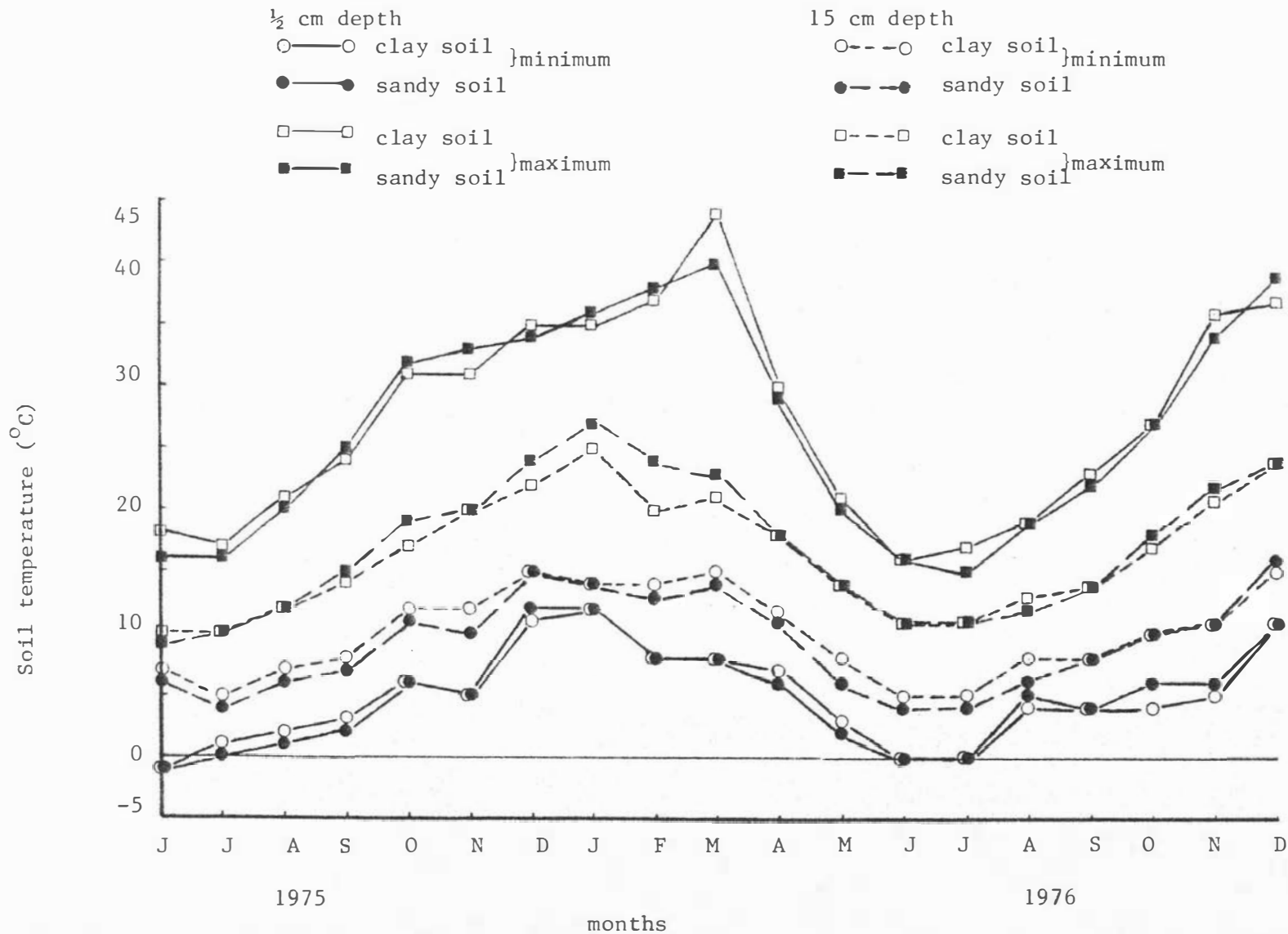
The second to top pair of lines (Fig. 23) represents the maximum temperatures measured at the 15 cm depth in both soil types. Highest temperatures were recorded in January (27°C in sandy soil and 25°C in clayey soil). The second lowest pair of lines shows the minimum temperatures recorded at the 15 cm depth in both soil types. Lowest temperatures of 5°C and 4°C in clayey and sandy soil sites were measured. The bottom pair of lines represents the minimum temperatures recorded at the ½ cm depth. Lowest temperatures of -1°C was recorded in both soil types (Fig 23 and Appendix 49).

(3) Soil moisture: Monthly average soil moisture percentages measured at the ½ cm and 15 cm depths in both soil types are shown graphically in Fig. (24) with raw data in Appendix (50). The soil moisture percentages at the ½ cm depth during the first four months of burial were rather high and fairly constant. However, they began to fall from September and then showed wide fluctuations until the beginning of the following winter (June 1976). The lowest monthly moisture contents of 4% in sandy soil and 7% in clayey soil were recorded in December 1975. Highest moisture contents of 29% in both June and July for clayey soil and 28% in August 1976 for sandy soil were measured.

As shown in Fig. (24), the moisture percentage at the lower soil levels showed less fluctuation, differences between the two soil types being more or less consistent throughout the burial period. It can also be seen that soil moisture levels at both depths in the clayey soil (and especially at the 15 cm depth) are generally higher than in sandy soil.

D. Field emergence trial

Although seeds were buried at ½ cm and 15 cm depths in both clayey soil and sandy soil sites, no seedling emergence in the field was recorded from seed samples buried at the 15 cm depth at either site. Since seed samples buried at the ½ cm depth in sandy soil produced only small numbers of emerging seedlings (Appendices 51-54),especially in the early dates of burial, field emergence data is not presented graphically.

FIG. 24: Monthly average soil moisture percentage measured at two depths in two soil types.
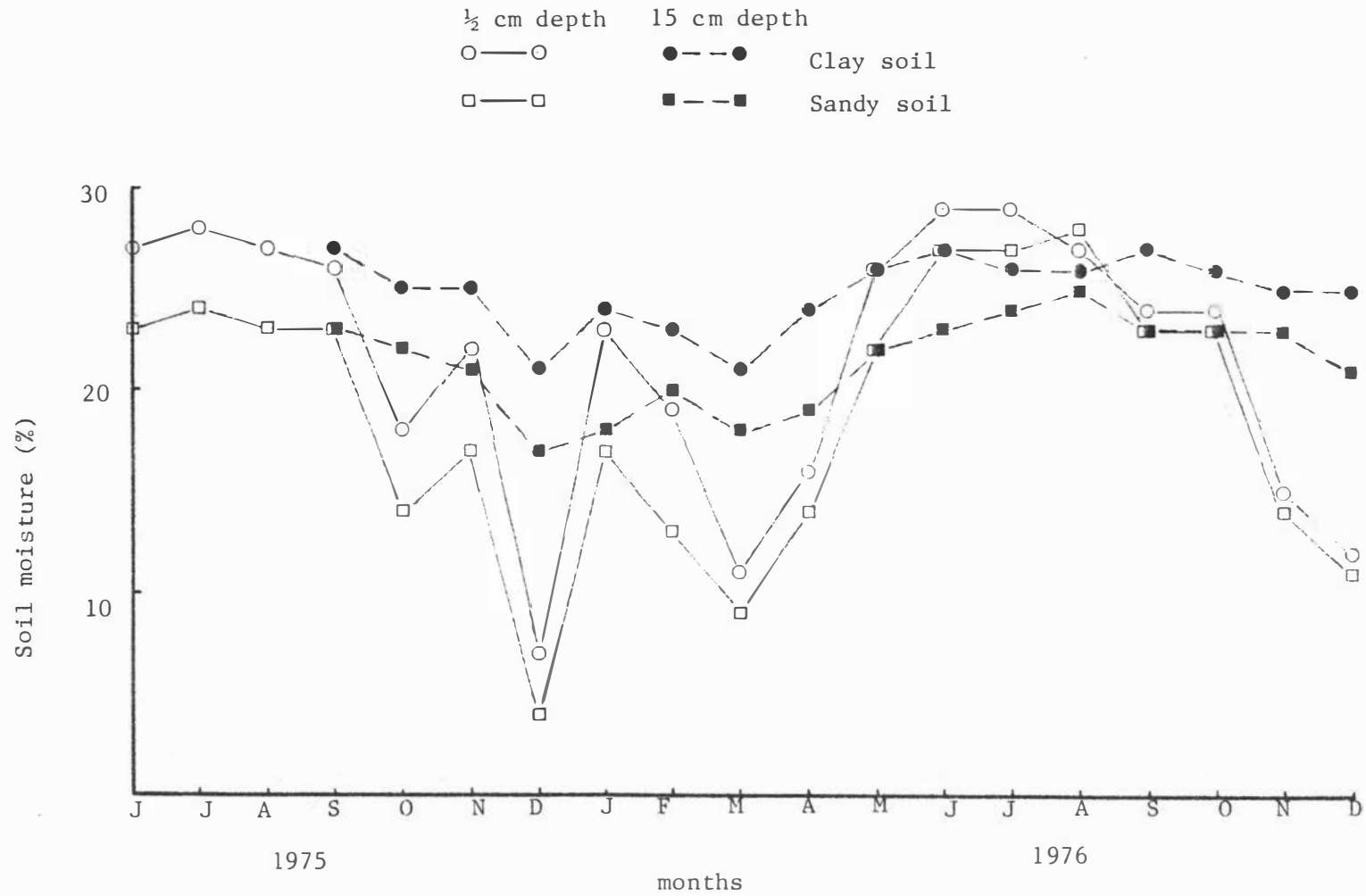
Seed samples buried at the ½ cm depth at the clayey soil site
produced a considerable number of seedlings from all the maturities
and at various dates of burial during the experimental period (Fig 25
and Appendices 51-54),When seed samples were buried in mid winter
(July), they produced considerable percentages of emerging seedlings
during the first two months of burial (Fig 25 a). Subsequently, the
percentage of seedling emergence declined to very low levels during
the spring. Almost no emergence was recorded during the following
summer and autumn months. A very small number of emerged seedlings
appeared during the following winter (Appendix 51).

Seed samples buried in August showed a similar pattern of seasonal
seedling emergence although the percentage of seedlings emerging
during the following months of burial showed some reduction (Fig.25 b
and Appendix 52). In this case, most of the seed maturity samples
produced similar percentages of emergence for the first two months
of burial. Although no seedling emergence was recorded during the
summer, a small percentage of seedlings appeared during the following
autumn and winter.

Seed samples buried in September showed a generally similar
pattern of emergence, especially during the first two months. Most
maturities produced a high percentage of seedling emergence during the
month of burial. In the following autumn and winter, seed samples
from most seed developmental ages showed a small percentage of seedling
emergence (Fig.25 c and Appendix 53).

When seed samples were buried in October, the percentage of
seedling emergence from all maturities was again fairly high during
the month of burial (Fig.25d and Appendix 54). In the following
month, the percentage of seedling emerging dropped about three-fold
compared with a less extreme reduction in samples buried in July, August
or September. However, a few seedlings appeared even in the early summer,
followed by a slightly higher and more sporadic emergence during the
following autumn, winter and spring (Fig.25 d).

FIG.25:Percentage field emergence at different times of the year with various burial dates.
(Seed samples with different developmental ages buried at 0·5 cm depth, clay soil site)
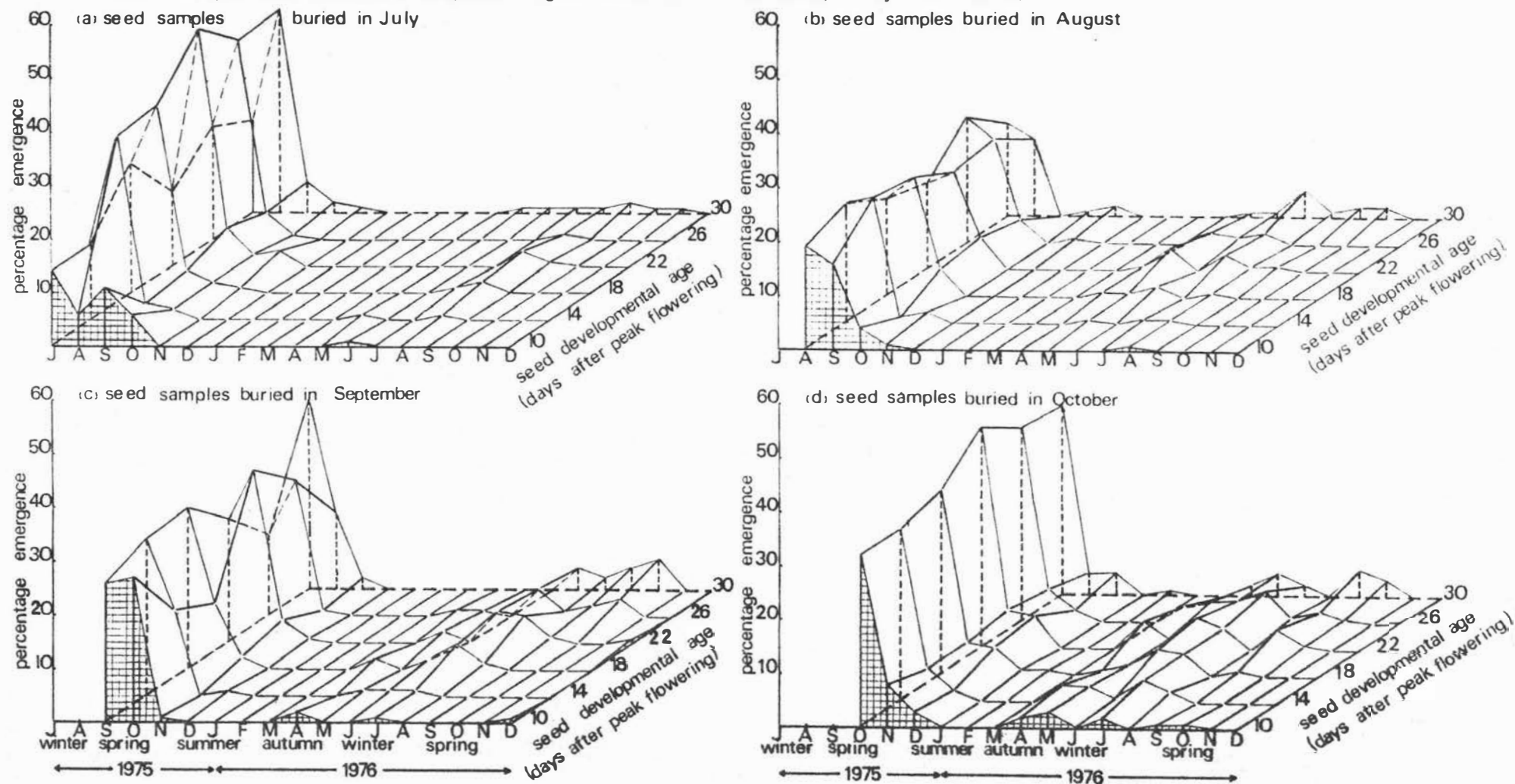
DISCUSSION

Seed growers cannot afford to overlook the important role buried seeds play in crop production. Volunteer crop plants and weeds arising from buried seeds constitute one of the main sources of contamination in seed crops. The demands by commerce for high quality seed both by domestic seed growers and in international trading make the role of buried seeds as crop contaminants of considerable importance.

It is not surprising that seeds in a dry state and consequently of low metabolic activity are capable of germination after years of storage. It is more difficult however to explain the longevity of seeds buried under natural conditions in an often moist medium such as soil and often in an imbibed state. 'Pawera' red clover seed exhibits a relatively high degree of persistency under such field conditions.

In the present study, with 'Pawera' red clover, many of the originally permeable seeds were germinable within the fortnight following burial provided they were buried in moist soil under suitable environmental conditions. It is suggested that these germinated seeds were not dormant at the time of burial and did not enter into a state of dormancy following burial. Thereafter further seed germination *in situ* in the soil would have been contributed mainly from the population of hard seeds present. A substantial breakdown of hardseededness seemed to occur during the first few months after burial under natural environmental conditions.

The high initial *in situ* germination results, particularly at the ½ cm burial depth support the reports of previous workers (Evans 1959, 1960; Lewis 1961; Schafer and Chilcote 1970, Taylorson 1972) that the depletion of seed populations in the soil is apparently due to germination *in situ* although other factors may also be involved. However, the loss of seed viability due to physiological ageing, particularly for hard seed would probably be negligible during the relatively short time of burial period in the present experiment.

It is possible that soil conditions may also influence the longevity and population of buried seeds. In the present study *in situ* germination accounted for the rapid depletion of buried seed populations, although other factors such as fungi and bacteria may also have played a part. Although, soil fungi and bacteria have long been thought to be involved in reducing the germination of buried seed, there seems to be little direct research evidence to validate this suggestion (Crocker 1938; Barton 1965b).

Persistence and viability tend to increase with increasing depth of burial. Presumably the lower and more stable soil moisture and temperature levels, characteristic at greater soil depths assist in maintaining seed dormancy and therefore seed persistence. In the present study, the decline in seed germination *in situ* at the 15 cm burial depth extending from the second to the fourth time of removal may have been due to the presence of unsuitable conditions for germination although the hardseededness of some seeds may have been broken. High levels of carbon dioxide (Martin 1945; Wesson and Wareing 1969) low and relatively small fluctuating temperatures (Harris 1959; Taylorson 1970) and consequently low intensity of stimuli (Harper 1957) at this burial depth have all been implicated as factors resulting in the poor efficiency of the soil environment in breaking dormancy and in low levels of seed germination. Another reason for declining seed germination levels *in situ* after the first month of burial may have been the limited soil aeration resulting from soil compaction (Lewis 1961; Barton 1962). It is also likely that the very low or no *in situ* germination observed at the 15 cm depth during the later periods of burial may possibly be due to warm soil temperatures and soil dryness during the summer and autumn followed by a high carbon dioxide concentration, and lack of aeration during the winter and spring seasons. It is also probable that soil water levels were important in affecting the seed survival pattern. The sandy site was prone to winter flooding, an effect which Lewis (1961) has shown maintains and extends the period of buried seed dormancy. Such a situation possibly also implicates the problems of lack of aeration occurring in waterlogged soils.

The higher percentages of germination of seeds buried near the surface (½ cm) was possibly due to better aeration and more variable temperatures. In addition, it is possible in light requiring species

that exposure to light will shorten the dormant period as suggested by Hyde (1958). Moreover, as reported by Kirchner and Andrew (1971), a thin layer of soil, covering the seed seems to intensify those conditions which are responsible for the breakdown of hardseededness. Seed buried at the 15 cm depth seemed to remain in a state of dormancy under less favourable conditions for germination. These findings agree with reports by other workers (Robinson 1960; Ramptom and Ching 1966, 1970) who found that the seeds of white and red clover maintain their viability longer when buried at depth in the soil than at or near the soil surface. Similar results have also been reported by Taylorson (1970), Stoller and Wax (1974) and Klingman *et al.* (1975) in work with weed seeds. Such an effect may also have been due to an unfavourable soil atmosphere which reduces the rate of seed metabolism and consequently deterioration (Youngman 1952; Barton 1961a; Darlington and Steinbauer 1961).

Seed samples from maturity level one (seed harvested 10 days after peak flowering) contained immature seeds which seemed to produce maximum germination only under optimum conditions provided in the laboratory. When they were buried in the soil, most immature seeds were apparently more severely affected by environmental conditions and either lost their viability or germinated within a few days. Generally, as suggested by some workers, (McAlister 1943; Hyde 1950; Bass 1965; Shands *et al.* 1967) the rate of loss of viability was found to be greater in seed samples harvested at earlier rather than later stages of maturity. This effect is presumably because mature seeds possess a higher potential vigour and larger food reserves and can therefore maintain their viability longer than immature seeds (Hyde 1950; Austin 1972; Pollock and Roos 1972).

Although environmental conditions at the two burial sites were similar in many respects, the soil conditions at the ½ cm burial depth in clayey soil at the time of burial seemed to be more favourable for germination. However, in general, marginally higher levels of seed germination *in situ* occurred at the 15 cm depth in sandy soil than in clayey soil. This may have resulted from slightly better aeration or better oxygen supply as suggested by Kirchner and Andrew (1971) and Klingman *et al.* (1975). It could also be due to slight differ-ences in environmental conditions at the two burial sites and to different reactions by samples of different ages since differences were not always consistent. This effect is consistent with findings by

Lewis (1961), that soil type has little effect on seed persistence. However he did find that soil type can influence species differentially.

The rate of breakdown of hardseededness was more rapid under natural environmental conditions in the field than in laboratory storage. The greatest reduction in seed numbers in the field occurred mainly at the $\frac{1}{2}$ cm depth of burial, presumably because the dormancy breaking stimuli were more active than at deeper levels.

It has been reported by overseas workers (Aitken 1939; Quinlivan 1961, 1968a; Hagon 1971; McComb and Andrews 1974), that daily fluctuating temperatures are the most important single factor in breaking hard seed dormancy, although other environmental conditions may also be involved. According to some of these workers (Quinlivan 1961, 1968a, 1971c pers. comm; Hagon 1971), high summer temperatures and wide temperature fluctuations ($15^{o}C$ to $60^{o}C$ or higher) are more effective than low temperatures in inducing the germination of impermeable annual legume seeds. However, in 'Pawera' red clover, the present investigation suggests it is unlikely that ambient and soil maximum temperatures in the summer are major factors in breaking hardseededness since they are not nearly as extreme as the conditions which are implicated as effective in some overseas countries, e.g. Australia (Quinlivan 1961). Hyde (1950) has also suggested that the temperature extremes in the New Zealand climate may not have a marked effect on the rate of reduction of hard seed. On the other hand, the effect of summer temperatures was unable to be determined precisely in the present study, since seed samples were originally buried in the winter and most germination occurred during the few months following burial. This suggests that maximum temperatures may not be the sole important factor; cold and fluctuating temperatures also being responsible at least partially, if not entirely, in affecting the breakdown of hardseededness.

The initial moisture content of seed at the time of burial seems to play an important role in seed softening and the subsequent germination of hard seed (Gladstones 1958; Quinlivan 1968a, 1970). According to these workers, the impermeability of seeds of some lupin species is reversible if the moisture content of the seed is above 10% (dry weight basis). In the present study, the initial moisture

contents of most seed maturities of 'Pawera' red clover seed were
above this moisture level, possibly allowing a slow moisture penetrat-
ion into the seed through the seed coat but not through the hilum
or strophiole as suggested by Quinlivan (1968a).

After burial the moisture content of seed from later maturities
fell to about 9% (wet weight basis). This may be low enough to
prevent the absorption of moisture through the seed coat. However,
the moisture contents of seed increased greatly in earlier and slightly
in later maturities during the wet season. This suggests that seed
moisture content appears to be influenced by soil moisture content
and therefore by season. The further increase in seed moisture content
following reduction to a fairly low level may not only be due to the
entry of moisture through random sites in the seed coat but also
through the hilar fissure and/or strophiolar cleft.

As suggested by Hyde (1954), the hilar fissure remains open
under environmental conditions involving gradual increases in
relative humidity. Although the soil may be wet during the winter,
the relative humidity of the soil atmosphere surrounding the seed
may vary. Since, more frequent and extreme fluctuations occur at or
near the surface, the function of the hilum of seeds buried at the $\frac{1}{2}$ cm
depth may well be more active than in seeds buried at the 15 cm
depth.   Any such increase in moisture content either through the
hilum or strophiole, did not seem to increase the size or obvious
appearance of the seed, all seeds used for moisture tests giving the
external appearance of hardness when they were removed from the soil
during the wet season. Nevertheless, such seeds, and especially
those of earlier maturities, contained 20-30% moisture.

According to some workers (Wesson and Wareing 1967, 1969;
Taylorson 1970) light is the principal factor controlling the germin-
ation of buried weed seeds.   They further state that even if seeds
have no initial requirement for light, such a requirement may develop
after a period of burial in the soil.   In the present study, light
did not appear to be important in controlling the dormancy of buried
"Pawera" red clover seeds. Seed of this species normally does not
require light for germination (Anon. 1976a) and softened seed
had the ability to germinate *in situ* at the 15 cm depth.   Moreover,
if light is the principal factor governing dormancy, then seeds removed

from the soil would be expected to germinate readily in the laboratory. Such a situation did not occur in the present study, supporting the contention by Crocker and Barton (1953) that many legumes can germinate equally well in either light or darkness.

The patterns of seedling emergence, especially from seeds buried in the soil early in the season, are very similar to the results of germination *in situ* in the soil at the ½ cm depth. The monitoring of seedling emergence patterns was carried out to obtain more information on the rate of breakdown of hardseededness and the pattern of germination in the soil as a complement to the seed burial experiment. However the results also reveal that hard seeds become permeable and germinate sporadically at different times of the year but not during the dry and relatively warm conditions prevailing in the late summer and early autumn. They also clearly show that the numbers of seedlings emerging at various times during the experimental period depend on the time or date of seed burial during the season but not on the length of the burial period, as previously suggested by Roberts (1964).

As reported by Witte (1938), most seedling emergence occurs during the first few months after burial followed by often low but sporadic germination in the following growing seasons. If seeds are buried in the soil early in the growing season, more seedlings emerge immediately following sowing followed by less emergence in the next growing season. Conversely, as reported by Witte (1934), low seedling emergence is obtained in the first growing season and more emergence appears in the following season when the date of burial is extended nearer to the end of the first growing season. Similar findings with *Agropyron repens* have been reported by Williams (1970).

'Pawera' red clover exhibits a periodicity of seed germination as shown by seeds of some legume and weed species. (Harrington 1916; Brenchley and Warington 1930; Chepil 1946a; Thurston 1960). Since, little if any germination in 'Pawera' red clover occurred during the summer months, this character insures against untimely germination. The findings of the present investigation agree with similar reports on white clover and some weed species by Brenchley and Warington (1930) and Blaser and Killinger (1950).

The low percentage of seedling emergence from seeds buried at the $\frac{1}{2}$ cm depth in sandy soil may be due to wet soil conditions and poor aeration. As the sandy soil burial site was a low land area, it was often flooded throughout the winter season. Increases in the percentage of seedling emergence in mid-spring seemed to be due to improved soil conditions following a reduction in soil moisture levels and improved aeration as suggested by Perry (1973). Conversely, seedling emergence in clay soil site was considerably higher possibly due to more favourable soil conditions since no flooding of the clay site occurred throughout the experimental period. A similar effect has been explained by Chepil (1946b) as being due to the fact that clay is less liable to develop a hard surface crust which would seriously impede seedling emergence.

According to work by Murphy and Arny (1939), Moore (1943) and King (1966), with legumes and weed species there seems little likelihood that 'Pawera' red clover seedlings are capable of emergence from a 15 cm burial depth. This was certainly the situation in the present study. Although, some workers (Bolton 1962; Milthorpe and Moorby 1974) have reported that the maximum depth from which seedlings can emerge varies with size of seed, soil type, etc., no emergence seems likely to occur if 'Pawera' red clover seeds are buried at a depth of more than 5 cm in the soil. This has been reported to be the maximum depth from which emergence can occur for some clover species (Murphy and Arny 1939; Moore 1943).

The failure of germination of viable 'Pawera' red clover seeds stored in the laboratory is assumed to be due to innate dormancy (Brenchley and Warington 1930; Harper 1957; Roberts 1970; Roberts 1972b) or primary dormancy (Crocker 1916; Anon. 1952; Schafer and Chilcote 1969; Villiers 1972; Maguire 1975). As innate dormancy or hardseededness is not permanent, the coat of individual seeds becomes permeable at different periods after harvest and the number of hard seeds gradually declines. However, under more favourable storage conditions as reported by Barret-Lennard and Gladstones (1964), the rate of reduction of hardseededness is very gradual even though seeds may have been after-ripened in dry storage.

When 'Pawera' red clover seeds were buried in the soil, some germinated shortly after burial while others persisted in the soil longer,

representing different types of dormancy mechanisms. Previous workers
(Schafer and Chilcote 1969, 1970; Roberts 1970; Roberts 1972b)
have developed models to trace the persistence and depletion of
buried seed populations. A model similar to that illustrated by
Roberts (1970) can be developed, based on information from the
present study, to monitor the fate of 'Pawera' red clover seeds and
dormancy mechanisms in the soil.

FIG. 26 :   Diagramatic model showing the dormancy mechanisms and
             fate of buried seeds (after Roberts 1970).
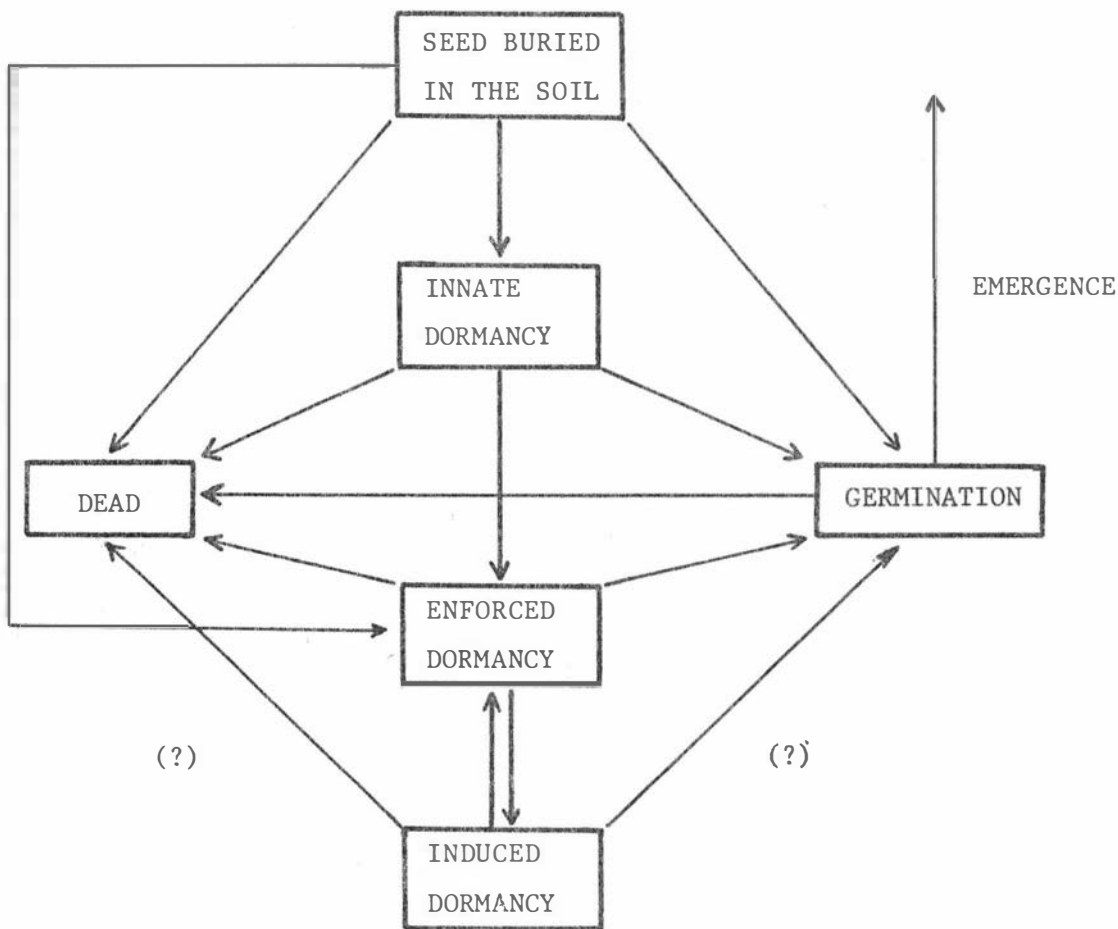
The hard seeds of 'Pawera' red clover are generally in a state of
innate dormancy at the time of incorporation into the soil and some
of them, especially those near the soil surface, may become permeable
gradually. Some originally soft seeds and some hard seeds subsequently
softened by environmental conditions will germinate and emerge from
the soil if they are buried near the surface. Those seeds buried below
the 'critical depth' for that particular species may germinate but
not emerge and consequently die later. Some extremely immature seeds
with low vigour may die shortly after burial due to the adverse effect
of natural environmental conditions and also possibly due to attack
by soil micro-organisms.

However, a certain percentage of hard seeds seem to be still innately dormant since some seeds do not germinate when removed from the soil and germinated in the laboratory. Those seeds that become permeable in the soil may enter a period of enforced dormancy if they do not germinate or die immediately. Similarly, as previously suggested, some originally soft seeds which fail to germinate immediately following burial may also develop enforced dormancy due to unfavourable environmental conditions, such as high carbon dioxide and low oxygen concentrations, lack of moisture, or light for light requiring seeds and smaller temperature fluctuations (Harper 1957; King 1966; Williams 1970, 1972; Roberts 1972b; Mayer and Poljakoff-Mayber 1975).

When buried seeds were removed from the field and germinated under favourable germination conditions in the laboratory, enforced dormant seeds germinated promptly indicating a release from the restraining environmental conditions imposed in the soil. Such types of hard seeds can also be described as 'conditionally hard' seeds. They appear hard but hardseededness seems to be broken by natural environmental conditions. The remaining viable seeds recovered from the soil failed to germinate probably due to innate dormancy. This type of viable seed can generally be termed as 'genuine' hard seed.

As suggested by Brown and Porter (1942), Crocker (1948) and Harper (1957), enforced dormancy appears to be more pronounced in seeds buried at depth in the soil than in seeds 'at or near the soil surface. Among enforced dormant seeds, a few which are not buried so deeply, may produce seedlings coincident with their periodicity of germination and a few may possibly die due to the action of soil organisms and/or physiological ageing.

Some seeds appear to develop induced dormancy possibly due to high temperatures (Barton and Crocker 1948; Thurston 1960; Roberts 1970) and dry environmental conditions or other particular factors unfavourable for germination (Roberts 1972b). Similar findings have also been reported by Toole and Hollowell (1939) who showed that high soil temperatures can inhibit the germination of seeds of some winter annual *Trifolium* species. Induced dormancy may be maintained through-out the summer or dry season and seems to be removed during the late autumn, winter and early spring due to cold temperatures and a moist soil environment (Courtney 1968; Roberts 1970).

Induced dormancy is different from enforced dormancy, being characterised by the persistence of dormancy after seed is removed from unfavourable conditions and the failure of such seed to germinate under subsequently favourable conditions (Thurston 1960; Roberts 1972b). When seeds are in a state of induced dormancy, a special treatment or stimulus is required to break the dormancy (Roberts 1970). 'Pawera' red clover seeds do not seem capable of producing seedlings when they are in the 'induced' dormancy condition unless they receive a scarification treatment or other extreme stimulus. It is also doubtful that these dormant hard seeds will die due to the action of environmental conditions or micro-organisms. However, when induced dormancy is removed, seeds seem to develop an enforced dormant state again and some of them may germinate or lose their viability. Those seeds that do not develop enforced or induced dormancy, still seem to remain hard or in a state of innate dormancy.

Since most seeds softened near the soil surface produce *in situ* germination in the soil during the time interval between any two removals, a great reduction in the germination capacity of seeds recovered from the soil and tested for viability in the laboratory occurred during the first few removal times. By comparison, softened seeds at the 15 cm burial depth did not produce any significant percentage germination *in situ* until they were removed and tested in the laboratory. In this latter case, it was therefore not surprising that laboratory germination results were fairly high and constant for the first four months of the trial. Deeply buried seeds that germinate promptly when brought to the surface may be reacting favourably to increased oxygen supply (Klingman *et al.* 1975). However, buried seeds were capable of producing only a very low percentage of germination in the laboratory when they were removed from the soil in early summer and early autumn.

Seed samples tested for germination in the laboratory following removal from the soil in the winter, and samples stored in the laboratory under more even temperature and relative humidity conditions and tested for germination in the winter both showed a markedly increased germination capacity compared with comparable tests carried out in the summer and autumn. This peridocity effect suggests that 'Pawera' red clover seeds,while they may lose their dormancy in the winter (presumably in response to moist, cool conditions) may also re-enter into a dormant state at the beginning of the dry season

as suggested by Courtney (1968) with *Polygonum aviculare*.   In
addition, it suggests that the periodicity of germination exhibited
by 'Pawera' red clover is also at least partially genetically controlled,
since a similar periodicity effect occurs irrespective of the storage
environment (field or laboratory storage).

The increase in the percentage of hard seeds removed from the
soil during the dry season could possibly be due to two factors.
Firstly, physiological changes or induced dormancy mechanisms as
discussed previously may inhibit seed germination. Secondly, physical
changes or resealing of the strophiolar fracture may prevent water
conduction through the seed coat and consequently germination of the
seed.

The restoration of seedcoat impermeability is thought to be
possible by exposure to warm temperatures and dry environmental cond-
itions (Hagon and Ballard 1970).   Under dry conditions, the hilar
fissure of the seed coat opens and allows drying until the moisture
content of the seed is in equilibrium with the lowest relative
humidity of the surrounding atmopshere (Hyde 1954).   According to
Hagon and Ballard (1970), very low relative humidity levels (10% or
lower R.H.) can cause the strophiolar cleft to reseal in subterranean
clover seed. The extremely low moisture levels (4% and 7% in sandy
and clay respectively) suggest this mechanism may have been involved
in the present study. However, according to Aitken (1939), if the
strophiolar split extends through the light line, there is no
reversion to impermeability on further drying.

The significant reduction of hard seed content during laboratory
storage in samples of early-maturities shows that if the seed is
removed from the mother plant less than 22 days after peak flowering,
the rate of breakdown of hard-seededness and deterioration is greatly
increased. However, most seeds seem to attain their maximum viability
and become impermeable if they are allowed to remain on the mother
plant until 22 days or later after peak flowering date.

The present study has a number of agricultural implications. The
role of the contribution of buried seeds is important in determining
the efficiency of pure seed production and also in deciding the value
of crop rotations, fallowing and cultivation techniques. One of the

requirements under seed certification schemes is the specification
of a minimum number of years between consecutive plantings of seed
of different varieties of the same cultivar in the same field.   In
New Zealand the current certification regulations specify this
requirement as three years for legumes, including red clover.   This
requirement is checked by examining paddock history data supplied by
the seed grower.   Although the present study was terminated before
two years it suggests that the survival of seeds of 'Pawera' red clover,
particularly when buried at depth in the soil, may well exceed the
minimum intervals specified in the New Zealand certification scheme.
It also suggests that further studies on the effect of crop rotations,
fallowing and 'stale seedbed' or minimum cultivation techniques, would
be fruitful as avenues of research designed to reduce the hazards
to pure legume seed production caused by residual buried seed
populations.

CONCLUSION

The rate of breakdown of hardseededness varied inversely with the depth of seed burial. More pronounced breakdown effects of environmental conditions on hardseededness were recorded at the ½ cm than at the 15 cm burial depth. The results of both germination *in situ* in the soil and laboratory germination results of seeds recovered from the soil generally represented the number of seeds softened at different time intervals during the burial period. On the other hand, the percentage of seeds remaining hard at the end of laboratory germination tests also expressed the extent of dormancy breakdown. All observed results from the two burial sites were similar, the effects due to small differences in micro-climatic conditions between the two seed burial sites and soil conditions were being generally insignificant. Seed samples containing more immature seeds showed rapid and greater loss of viability in the soil than samples of more mature seed.

The results of the field emergence trial also showed the rate of breakdown of hardseededness and the general germination characteristics of hard seeds in the soil at different times of the year. These findings suggest that a large number of seeds normally tend to germinate within a few months after sowing. However, since 'Pawera' red clover exhibits a periodicity of germination, very little, if any germination will occur during the following summer months.

The germination capacity of hard seeds in the soil was mainly influenced by three types of dormancy - i.e. innate, enforced and induced dormancy. The failure of germination of viable seeds lying near the soil surface under favourable germination conditions was thought to be due to innate dormancy while the inability of germination of viable seeds buried at depth was assumed to be due to enforced dormancy. The latter seemed to be superimposed by induced dormancy due to high temperatures or other factors unfavourable for germination. However, under cold temperatures and moist soil environmental conditions, this dormancy could be removed and the seeds resumed an enforced dormant condition.

Within a cropping rotation, buried seeds can be brought up to the soil surface during subsequent cultivation and produce unwanted volunteer plants. Such types of crop contamination from buried seeds may affect varietal purity; cause rejection of seed fields from certification; result in the production of lower seed classes or produce inseparable mixtures of seeds. Therefore, the requirement of legume seed certification schemes necessitates the elimination of unwanted cultivars from seed fields. When a carry-over of crop seeds occurs, there may be difficulties in maintaining pure stands of similar crops for certification purposes.

Seed certification standards are developed and precautions are taken to minimise the volunteer plant population in seed crops. However, generally these standards and precautions have been established without adequate knowledge of the longevity and dormancy of seeds buried in the soil. The present findings show that 'Pawera' red clover seeds buried in the soil, especially at deeper soil levels, remain dormant for extended periods and intensify this situation. Obviously, more information on the longevity and germination characteristics of the seeds of plant species buried in the soil is still needed to solve these problems.

GENERAL CONCLUSION

"Grasslands Pawera" tetraploid red clover, is a relatively new but nevertheless important forage crop in New Zealand agriculture. The information obtained in the present study is of both agronomic and economic value since detailed work on the seed production and hard seed characteristics of New Zealand tetraploid red clover has previously been very limited.

'Pawera' red clover crops sown in autumn and spring tend to show some variation in internode numbers and period of floral development, this latter aspect taking relatively longer in spring sown crops than in autumn sown crops. Similarly, the peak flowering date of the crop also varies depending on the time of sowing and climatic conditions. The majority of the florets on individual heads are pollinated from the half-fully flowering stage to the stage when the head is fully flowering, maximum nectar production occurring over this period. As with other red clover varieties, successful seed production in 'Pawera' red clover seems to depend chiefly on the population of effective bee pollinators (e.g. *Bombus hortorum* and *B. ruderatus*) and on those climatic conditions occurring during the pollination period which influence bee activity. Temperature effects show a reasonably direct and positive relationship with the number of bees present in the crop while bee numbers are negatively correlated with relative humidity. The higher numbers of seeds setting in the middle portions than the top and bottom portions of individual heads are possibly due to easier accessibility of central florets for bee visitation.

The pattern of change in seed components follows a typical trend of seed development. There are three stages of development; the first or growth stage occupies a period of 10 days after pollination, the second or food reserve accumulation stage takes about 16 days following the first stage and the third of ripening stage lasts for a further 10-14 days. Maximum seed dry weight (seed maturity) is reached 26 days after pollination when seed moisture content is 57%. At maturity about 90% of seeds in freshly harvested samples have turned yellow, brown or purple.

First viable seeds appear in seed samples harvested 12 days after pollination but most of these produce abnormal seedlings. Maximum viability is not attained until 22 days after pollination in dry stored seed and 26 days after pollination in freshly harvested seed samples. However, when viability of freshly harvested seeds is determined by tetrazolium test, nearly 80% of seeds are shown to be viable, but not germinable, even in seed samples harvested 10 days after pollination. In dry stored seeds, hard seed is first detected in samples harvested 12 days after pollination.

Seed moisture content and seed coat colour can both be used as maturity indices in predicting the correct time of harvesting. The crop can be cut when seed moisture conent is 57% or when most seeds from the majority of the highest yielding heads begin to change colour from green to yellow, brown or purple. Harvesting at this stage can be carried out without affecting seed viability, seedling vigour, seed weight or storage life. However, the moisture content of seed tends to show slight variation depending on the position of seed in the flower head.

Fresh ungerminated seeds in freshly harvested seed samples do not respond to a number of laboratory stimulation (prechilling, gibberellic acid or high oxygen concentration) treatments. The germination failure of fresh ungerminated seeds is thought to be due to the presence of the membrane surrounding the embryo rather than to restrictions imposed by the seed coat itself. Most fresh ungerminated seeds develop the hard seed condition following a short period of dry storage. Hard seeds in both freshly harvested and dry stored seed samples show no apparent embryo dormancy since they germinate readily following scarification.

The sequences of embryo and endosperm development in 'Pawera' red clover seeds follow the pattern occurring in some other *Trifolium* species. The growth of the endosperm precedes the growth of the embryo. Endosperm formation is nuclear but becomes cellular in the later stages of development. The zygote begins to divide between 2 and 4 days after pollination and progresses through the globular, heart-shaped, torpedo-shaped stages reaching an early cotyledonary stage about 10 days after pollination. The embryo occupies the entire ovular cavity 14 days after pollination.

Although the young ovary contains two ovules, normally only one ovule develops into a seed, the other aborting possibly as a result of nutritional competition. The inner integument degenerates during the ontogeny of the seed while the outer integument differentiates into a variety of layers.

The seed coat is rather complex, consisting of a number of structures. The outermost cell layer of the seed coat, known as the malpighian or palisade layer, is assumed to be responsible for impermeability. This layer itself consists of several closely related structures namely the cuticle, sub-cuticular layer, cell caps, light line and cell lumen. Immediately below this layer, there is a layer of osteoclereid cells with large intercellular spaces. Interior to the osteosclereid layer, there is a nutrient layer consisting of a few rows of thin-walled parenchyma cells. Below this, an aleurone layer of thick walled cells is formed enveloping the embryo. This layer is thought to be responsible for suppressing the growth of the embryo in fresh ungerminated seeds.

Although the cell structure of the seed coat was studied generally, emphasis was placed on three important regions (micropyle, hilum and strophiole) which are possibly connected with seed hardening or softening mechanisms. The micropyle occurs immediately outside the hilum on the radicular lobe side of the seed. Although the pore or canal of the micropyle in 'Pawera' red clover seed is not closed or blocked, no water entry into the seed appears to occur through this structure.

A round, sunken hilum is formed between the radicular and cotyled-onary lobes when the seed is disconnected from the funiculus. This region is thought to act as a one way hygroscopic valve, opening when the outside relative humidity is low and closing when the latter is high. This mechanism allows the seed to dry to a moisture level equivalent to the lowest relative humidity of the surrounding atmosphere. The seed thus becomes impermeable due to desiccation.

The strophiole is a small swollen area of the testa between the chalaza and hilum on the side opposite the micropyle. The malpighian cells at the strophiole are 3-4 times longer than corresponding cells in the lateral wall of the testa. The malpighian cells in the centre of the plane of symmetry are straight whereas those cells on each side of the

central plane are bent. No intercellular spaces occur at the strophiolar region. Instead, a tightly appressed structure is formed at the base of the malpighian cells. All of these structures combine to increase the sensitivity of the strophiolar region to impaction and create conditions that induce this area to become the initial permeable site of the seed coat.

In originally soft seeds, water absorption takes place at random sites on the seed coat rather than at any particular area. However, when a hard seed is softened under natural environmental conditions or by any mechanical treatment, initial water conduction occurs only at the strophiole.

In the field, a significant reduction in a buried seed population, occurs throughout the burial period. A sharp drop in seed numbers occurred during the first few months following burial, especially at the ½ cm depth. The amount of seed recovered from a shallow (½ cm) burial depth in both soil types was approximately one half of the amount recovered from a deeper (15 cm) burial depth. Similarly the number of immature seeds recovered following burial at both depths and in different soil types was also about 50% of the number of mature seeds recovered.

Seeds recovered from the soil during the first few months of burial produce a considerable amount of germination in the laboratory. The percentage of germination increases with increased seed maturity especially in seed samples recovered from the 15 cm depth.

The percentage of hard seeds drops drastically following the first two months of burial at the ½ cm depth and then declines gradually. A similar but smaller reduction in hard seed content occurs at the 15 cm depth. The percentage of seeds remaining hard after laboratory germination tests on recovered seed samples also increases significantly with increasing seed maturity.

Similarly, the percentage of hard seed increases and germination decreases with increased seed maturity in seed samples stored in the laboratory. Conversely, the percentage of germination increases while that of hard seed declines significantly with increasing storage period.

When seeds are stored in the laboratory, initial seed moisture content is maintained throughout the storage period of 18 months. However, when seeds are buried in the soil, seed moisture content increases considerably, especially in immature seeds during the wet season. This situation was more obvious in seed samples buried at the ½ cm depth in both soil types.

The greater rate of breakdown of hardseededness occurs at the ½ cm depth of burial presumably because of the more active role of dormancy breaking stimuli near the soil surface. The results indicate that cold and fluctuating temperatures and moist soil conditions in the winter are primarily responsible for the softening of hard seeds.

The findings of both seed burial and field seedling emergence trials clearly suggest that the rapid depletion of buried seed populations in the soil is mainly due to germination *in situ*. However, a certain proportion of the seed population in the soil, especially when buried at deeper levels, persists. This longevity is closely linked with different types of seed dormancy mechanisms - namely innate, enforced and induced dormancy. When such dormant seeds are brought up to the surface, especially during the growing season, some of them produce seedlings. This type of regeneration often results in the production of unwanted volunteer plants which intensify the crop contamination problems associated with the production of pure seed within a seed certification scheme. A knowledge of seed dormancy mechanisms and their effect on the longevity of buried seeds would be of great agronomic value in enabling the development of seedcrop management systems designed to maintain high levels of varietal purity in commercial seed crops of 'Pawera' red clover.

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Appendix 1: Frequency distribution of number of internodes on
50 random stems at the time of floral initiation and
peak flowering in both autumn and spring sown crops.

Internode Number	Autumn sown crop				Spring sown crop			
	at floral initiation		at peak flowering		at floral initiation		at peak flowering	
	Frequency	%	Frequency	%	Frequency	%	Frequency	%
3	–	–	–	–	5	10	–	–
4	2	4	–	–	7	14	–	–
5	5	10	–	–	12	24	–	–
6	4	8	1	2	10	20	–	–
7	11	22	5	10	4	8	3	6
8	10	20	11	22	6	12	7	14
9	8	16	17	34	6	12	5	10
10	5	10	6	12	–	–	13	26
11	3	6	5	10	–	–	12	24
12	2	4	5	10	–	–	6	12
13	–	–	–	–	–	–	3	6
14	–	–	–	–	–	–	1	2
Total	50	100	50	100	50	100	50	100

Appendix 2: Mean number of fully flowering heads and totally withered heads in 5 ($8m^2$ each) random plots.

Date of counting	Fully flowering heads		Totally withered heads	
	Mean	Standard error	Mean	Standard error
16.1.75	183	21.73	18	5.98
18.1.75	291	43.90	35	7.59
20.1.75	353	42.24	61	13.36
22.1.75	288	40.23	105	20.60
24.1.75	343	32.17	126	23.30
26.1.75	488	49.84	190	29.65
28.1.75	582	47.37	248	34.62
30.1.75	778	45.66	297	40.74
1.2.75	859	46.13	374	44.75
3.2.75	981	30.80	458	51.13
5.2.75	1068	34.75	645	66.20
7.2.75	1087	63.94	827	89.76
9.2.75	1011	49.04	965	104.16
11.2.75	936	57.15	1100	110.95
13.2.75	857	73.69	1351	109.71

Appendix 3: Bumble bee population visiting the crop at different times of the day during the flowering period (1976)

Date	Time	Mean bee population per hectare	Weather condition				
			Temp °C	R.H. %	Sky	Rain (mm)	Wind
18.2.76	4.30 pm	1530	25	55	clear	-	-
21.2.76	4.00 pm	1040	23	66	partly cloudy	-	slightly cloudy
22.2.76	11.00 am	1800	19	85	"	-	"
23.2.76	12.10 pm	1290	24	65	clear	-	"
"	3.00 pm	2180	28	60	"	-	"
24.2.76	5.00 pm	770	23	65	"	-	"
25.2.76	12.10 pm	770	20	77	cloudy	7.3	-
27.2.76	5.00 pm	1680	19	60	partly	-	-
28.2.76	11.00 am	990	18	68	"	-	-
29.2.76	11.30 am	770	17	84	"	-	-
1.3.76	10.00 am	1380	17	92	clear	-	-
2.3.76	10.30 am	2300	20	72	"	-	-
3.3.76	9.30 am	1680	16	95	"	-	-
4.3.76	11.00 am	1500	22	62	"	-	light wind
"	5.30 pm	1800	24	55	"	-	"
"	7.00 pm	1010	20	70	"	-	"
6.3.76	10.00 am	2300	17	68	"	-	"
7.3.76	10.00 am	1630	18	75	"	-	"
9.3.76	10.00 am	-	20	100	overcast	0.2	windy
10.3.76	9.30 am	1440	17	72	clear	-	slightly windy
11.3.76	9.30 am	2050	17	87	cloudy	-	calm
12.3.76	9.30 am	1140	17	93	"	-	"
14.3.76	11.30 am	2030	21	72	clear	-	light wind
16.3.76	11.00 am	1780	21	70	partly cloudy	-	calm
18.3.76	9.30 am	2470	20	82	clear	-	light wind
19.3.76	10.00 am	3120	22	67	"	-	calm
20.3.76	1.30 pm	2700	24	56	"	-	"
21.3.76	11.00 am	3000	22	62	"	-	"
22.3.76	10.00 am	2230	24	55	"	0.5	"
24.3.76	12.10 pm	2350	20	60	partly cloudy	0.8	"
25.3.76	12.30 pm	2080	23	65	clear	-	light wind

Date	Time	Mean bee population per hectare	Weather condition				
			Temp °C	F.H. %	Sky	Rain (nun)	Wind
27.3.76	2.00 pm	890	16	76	clear	3.7	slightly windy
28.3.76	10.00 am	–	11	100	raining)		"
"	5.00 pm	–	13	100	")	36.0	"
29.3.76	10.00 am	–	17	100	drizzling	25.1	light wind
30.3.76	9.30 am	940	18	87	partly)	8.2	"
"	1.30 pm	860	19	92	cloudy)		calm
1.4.76	9.40 am	990	16	82	partly cloudy	–	calm
"	11.30 am	2480	18	73	"	–	light wind
2.4.76	9.45 an	2010	18	74	clear	–	calm
5.4.76	10.30 am	2300	18	72	"	–	light wind
6.4.76	8.30 am	270	13	100	cloudy	–	calm
"	12.30 pm	1680	22	77	partly cloudy	–	light wind
"	4.15 pm	1680	25	62	clear	–	calm
7.4.76	8.45 am	1310	14	94	cloudy	–	"
"	1.00 pm	1860	22	82	partly cloudy	–	light wind
"	4.30 pm	3750	21	80	"	–	"
8.4.76	8.45 am	720	14	100	clear)		calm
"	12.50 pm	2560	23	57	")	1.9	"
"	4.30 pm	2260	20	81	cloudy)		light wind
9.4.76	8.45 am	–	18	100	cloudy)		calm
"	12.50 pm	1440	22	80	")	1.4	light wind
"	5.30 pm	670	19	92	")		"
10.4.76	8.30 am	400	15	100	"	–	"
"	12.45 pm	1830	21	80	clear	–	"

Appendix 3 contd:

Date	Time	Mean bee population per hectare	Weather condition				
			Temp °C	R.H. %	Sky	Rain (mm)	Wind
11.4.76	8.45 am	–	14	100	partly cloudy	–	calm
"	12.40 pm	2300	20	87	"	–	slightly windy
"	4.45 pm	1160	17	89	clear	–	light wind
12.4.76	9.00 am	1220	17	82	"	–	"
"	12.50 pm	1900	20	67	"	–	"
"	4.20 pm	2000	22	64	"	–	"
13.4.76	8.50 am	550	15	91	"	–	"
	12.45 pm	2080	21	67	"	–	"
	4.20 pm	1480	21	69	"	–	slightly windy
14.4.76	9.00 am	640	14	92	cloudy	–	light wind
"	1.00 pm	1140	20	70	clear	–	slightly windy
"	4.50 pm	770	16	80	"	–	"
15.4.76	8.55 am	1090	14	90	"	–	light wind
"	12.40 pm	1860	22	63	"	–	"
"	5.00 pm	–	16	100	"	–	"

Appendix 4: Number of seeds per flower head and number of seeds per 100 florets of an individual flower head harvested at six different random times (1975)

Head number	Number of harvests											
	1		2		3		4		5		6	
	No. of seeds		No. of seeds		No. of seeds		No. of seeds		No. of seeds		No. of seeds	
	per head	per 100 florets	per head	per 100 florets	per head	per 100 florets	per head	per 100 florets	per head	per 100 florets	per head	per 100 florets
1	94	62	108	76	46	44	125	71	85	54	105	69
2	84	67	98	69	93	82	113	78	88	67	78	63
3	93	77	105	70	51	43	65	62	77	71	103	85
4	68	60	74	66	76	73	74	70	108	75	106	83
5	112	81	119	75	81	73	51	38	70	59	132	80
6	114	87	96	74	72	55	101	71	105	72	113	77
7	95	67	123	70	99	63	83	78	55	51	87	70
8	96	63	114	75	89	55	102	73	79	65	85	69
9	62	52	104	75	110	83	102	65	59	56	88	74
10	71	56	108	80	67	54	74	63	68	74	100	79
Average	89	67	105	73	78	62	89	67	79	64	100	75

Appendix 5: Mean numbers and percentages of seeds and florets in different parts of individual flower heads (1976)
(Average of 30 flower heads of each pollination method)

Method of Pollination	Portion of flowerhead examined	Seeds		Florets		Number of seed per 100 florets
		No.	%	No.	%	
Controlled Pollination	Top	9.2	17.2	26.4	24.5	34.9
	Mid-upper	14.1	30.4	27.3	24.9	51.7
	Mid-lower	15.6	32.6	27.1	24.5	57.6
	Bottom	9.7	19.8	28.6	26.1	33.9
	Whole head	48.6	100.0	109.4	100.0	44.5
Open Pollination	Top	10.1	21.9	25.8	24.6	39.2
	Mid-upper	13.9	29.6	25.0	24.1	55.6
	Mid-lower	13.4	29.0	25.5	24.4	52.6
	Bottom	8.8	19.5	28.3	26.9	31.1
	Whole head	46.2	100.0	104.6	100.0	44.6

Appendix 6: Weight of seed samples with different maturities and different seed colour categories after 3 months storage (1977)

Days after pollination	Yellow		Light brown		Dark brown		Light purple		Dark purple	
	%	mg/100	%	mg/100	%	mg/100	%	mg/100	%	mg/100
10	91	42	3	–	–	–	6	–	–	–
12	91	76	4	–	–	–	5	–	–	–
14	88	110	7	–	–	–	5	–	–	–
18	78	175	6	–	3	–	9	216	4	–
22	37	310	12	341	12	342	20	347	19	341
26	7	302	11	348	30	355	15	352	37	355
30	7	342	6	346	19	351	12	350	56	353
40	17	319	8	347	17	350	30	344	28	348

Appendix 7: Colour standards and colour nomenclature (R. Ridgway 1912).

Seed colour	Reference		Colours of plates coinciding with seed colour
	Plate No.	Tone	
1. yellow	4	i	sulphine yellow
	16	i	olive lake
2. light brown	30	i	Isabella colour
	40	–	dark olive-buff
3. dark brown	45	k	dark grayish brown
	50	k	deep slaty brown
4. light purple	44	k	anthracene purple
	"	i	dull Indian purple
5. dark purple	11	m	blackish purple
	26	m	dusky auricula purple
	37	m	dark livid purple
	44	k	dark slate-purple

Appendix 8: Fresh weight (F.W.) dry weight (D.W.) and moisture content
of freshly harvested seeds at different developmental stages
(1976).

| Date of harvest | Days after pollination | mg/seed | | | Moisture |
		F.W.	D.W.	Absolute moisture	%
26.2.76	8	2.75	0.57	2.18	79.3
28.2.76	10	2.95	0.62	2.33	79.0
1.3.76	12	3.82	0.82	3.00	78.5
3.3.76	14	5.38	1.22	4.16	77.3
7.3.76	18	7.06	1.75	5.31	75.2
11.3.76	22	8.93	2.96	5.97	66.9
17.3.76	24	9.56	3.46	6.10	63.8
19.3.76	26	8.30	3.54	4.76	57.4
28.3.76	30	5.69	3.43	2.26	39.7
9.4.76	36	4.13	3.14	0.99	24.0
7.4.76	40	3.79	3.20	0.59	15.6

Appendix 9: Fresh weigh (F.W.) dry weight and moisture content of freshly
harvested seeds at different developmental stages (1977).

| Date of harvest | Days after pollination | mg/seed | | | Moisture |
		F.W.	D.W.	Absolute moisture	%
14.2.77	8	2.46	0.54	1.92	78.1
16.2.77	10	3.53	0.81	2.72	77.1
18.2.77	12	4.98	1.18	3.80	76.3
20.2.77	14	5.87	1.40	4.47	76.2
24.2.77	18	7.84	2.22	5.62	71.7
9.3.77	22	8.51	2.95	5.56	65.3
11.3.77	24	8.76	3.23	5.53	63.1
13.3.77	26	7.99	3.44	4.55	57.0
17.3.77	30	4.95	3.28	1.67	33.7
23.3.77	36	4.10	3.31	0.79	19.3
27.3.77	40	3.56	3.10	0.46	12.9

Appendix 10:   Fresh weight (F.W.), dry weight (D.W.) and moisture
content of freshly harvested seeds of different flower
head tagging dates (1974 autumn sown crop).

| Date of harvest | Days after tagging | mg/seed | | | Moisture |
		F.W.	D.W.	Absolute moisture	%
31.1.75	10	4.63	1.27	3.36	72.6
4.2.75	14	7.00	2.23	4.77	68.1
11.2.75	18	7.57	3.00	4.57	60.4
15.2.75	22	7.17	3.20	3.97	55.4
27.2.75	26	3.26	2.86	0.40	12.3
3.3.75	30	3.04	2.71	0.33	10.9

Appendix 11:   Fresh weight (F.W.), dry weight (D.W.) and moisture content of
freshly harvested seeds at different flowerhead tagging
dates (1974 spring sown crop)

| Date of harvest | Days after tagging | mg/seed | | | moisture |
		F.W.	D.W.	Absolute moisture	%
28.3.75	8	3.24	0.74	2.50	77.2
30.3.75	10	3.75	0.90	2.85	76.0
1.4.75	12	5.51	1.34	4.17	75.7
3.4.75	14	6.02	1.61	4.41	73.3
7.4.75	18	6.98	2.07	4.91	70.3
11.4.75	22	7.81	2.57	5.24	67.1
13.4.75	24	8.48	3.13	5.35	63.1
15.4.75	26	8.00	3.04	4.96	62.0
19.4.75	30	7.20	3.01	4.19	58.2

Appendix 12:   Fresh weight (F.W.), dry weight (D.W.), and moisture
                content of freshly harvested seeds at different dates
                after peak flowering (1974 autumn sown crop)

Dates of harvest	Days after peak flowering	mg/seed			moisture %
		F.W.	D.W.	Absolute moisture	
17.2.75	10	7.23	2.47	4.76	65.8
21.2.75	14	6.53	2.35	4.18	64.0
25.2.75	18	6.54	2.59	3.95	60.4
1.3.75	22	4.55	2.88	1.67	36.7
5.3.75	26	3.37	2.74	0.63	18.7
9.3.75	30	3.44	2.79	0.65	18.9

Appendix 13:   Climatic conditions during seed developmental periods.

Season of harvest	Year	Month	Number of rain-days	Total rainfall (mm)	Relative humidity % (mean daily)	Ambient temperature (mean daily)		Number of sunshine hours (mean daily)	Wind speed km/h (mean)
						max.	min.		
First	1974	Dec	7	77.9	73	22.8	12.5	7.5	10.3
season	1975	Jan	7	37.7	71	25.0	15.0	7.5	10.6
	1975	Feb	8	29.6	76	24.0	14.6	5.7	13.2
	1975	Mar	13	53.2	76	22.4	13.3	4.8	12.2
	1975	Apr	14	71.4	80	18.8	10.4	4.6	9.0
	1975	May	20	129.5	85	15.2	8.8	3.0	12.6
Second	1975	Dec	12	101.7	74	18.9	11.0	6.2	15.5
season	1976	Jan	16	90.2	79	20.6	13.7	4.2	14.2
	1976	Feb	9	60.4	80	18.9	10.1	5.8	12.4
	1976	Mar	9	85.4	75	21.2	11.3	6.2	12.4
	1976	Apr	9	51.6	77	18.0	9.5	4.5	10.8
Third	1976	Dec	11	82.3	76	19.9	13.1	4.9	13.2
season	1977	Jan	16	65.9	76	26.2	16.1	5.6	15.8
	1977	Feb	6	43.8	77	22.2	12.4	7.5	10.6
	1977	Mar	8	45.9	75	21.1	12.1	5.6	12.4
	1977	Apr	18	68.5	78	19.9	9.3	4.5	10.0

Appendix 14: Mean fresh weight, dry weight and moisture content of seeds taken from different parts of flower heads (10 flower heads at each harvest) harvested 8, 10, 18 and 30 days after pollination (1976).

Method of pollination	Position of seed in the flower head	Fresh weight (mg/seed)				Dry weight (mg/seed)				Moisture content (%)			
		8	10	18	30	8	10	18	30	8	10	18	30
Controlled pollination	Top	1.74	4.47	8.32	7.73	0.29	0.93	2.90	3.46	83.5	79.1	65.1	55.2
	Mid-upper	1.86	4.50	8.29	8.47	0.33	0.96	2.83	3.49	82.1	79.1	65.9	58.8
	Mid-lower	1.87	4.67	8.11	8.64	0.37	0.97	2.60	3.62	80.4	79.2	67.9	58.1
	Bottom	1.80	4.18	8.02	8.43	0.32	0.90	2.58	3.39	82.0	78.7	67.9	59.7
Open pollination	Top	1.73	3.72	7.18	8.71	0.35	0.83	1.98	2.94	79.9	77.8	72.5	66.2
	Mid-upper	1.98	4.56	8.09	8.94	0.40	1.03	2.40	3.15	79.8	77.3	70.3	64.8
	Mid-lower	1.89	4.75	8.26	8.78	0.36	1.09	2.54	3.09	80.8	77.0	69.3	64.8
	Bottom	1.50	4.54	7.22	8.24	0.29	1.05	2.11	2.70	80.8	76.9	70.7	67.3

Appendix 15: Percentages of germination and viability of freshly harvested seeds at different developmental stages (1976)

Date of harvest	Days after pollination	Normal seedlings Germination period (days)			Abnormal seedlings	Fresh ungerminated seeds	Hard seeds	Total viable seeds	Seed (non-viable) seeds
		7	10	30					
26.2.76	8	-	-	-	-	-	-	-	100
28.2.76	10	-	-	-	-	-	-	-	100
1.3.76	12	-	-	-	-	-	-	-	100
3.3.76	14	-	-	-	4	4	-	8	92
7.3.76	18	-	-	32	10	22	-	64	36
11.3.76	22	-	4	28	2	60	-	90	10
19.3.76	26	4	10	32	2	64	-	98	2
28.3.76	30	4	4	20	2	72	-	94	6
9.4.76	36	6	12	30	2	28	36	96	4
7.4.76	40	6	10	26	2	14	42	84	16

Appendix 16: Percentages of germination and viability of freshly harvested seeds at different developmental stages (1977).

Date of harvest	Days of pollination	Normal seedlings Germination period (days)			Abnormal seedlings	Fresh ungerminated seeds	Hard seeds	Total viable seed	Dead (non-viable) seeds
		7	10	30					
14.2.77	8	-	-	-	-	-	-	-	100
16.2.77	10	-	-	-	-	-	-	-	100
18.2.77	12	-	-	-	-	2	-	2	98
20.2.77	14	-	-	6	6	10	-	22	78
24.2.77	18	-	2	42	6	16	-	64	36
9.3.77	22	2	14	26	-	68	-	94	6
13.3.77	26	-	2	12	2	80	-	94	6
17.3.77	30	-	2	14	2	36	30	82	18
23.3.77	36	2	4	12	-	12	76	100	-
27.3.77	40	4	6	12	-	12	76	100	-

Appendix 17:   Daily maximum and minimum temperature $^{\circ}$C recorded in the laboratory

Date	Temperature	Days of the week							Weekly Average
		1	2	3	4	5	6	7	
21.3.77	Max.	24	24	22	21	22	22	24	23
	Min.	20	20	20	19	18	17	20	19
28.3.77	Max.	23	23	24	25	25	23	23	24
	Min.	19	18	20	20	20	21	19	20
4.4.77	Max.	23	24	24	23	21	21	21	23
	Min.	21	20	18	19	18	18	18	19
11.4.77	Max.	21	22	21	21	21	21	21	21
	Min.	19	18	18	17	17	19	16	18
18.4.77	Max.	22	22	21	19	20	19	20	20
	Min.	17	17	16	16	15	15	15	16
25.4.77	Max.	18	21	19	18	18	20	19	19
	Min.	15	16	15	12	14	17	12	14
2.5.77	Max.	19	19	18	20	20	19	18	19
	Min.	13	14	14	13	14	13	15	14
9.5.77	Max.	17	17	17	17	20	19	18	18
	Min.	13	11	12	12	12	16	15	13
16.5.77	Max.	17	15	15	18	19	20	17	17
	Min.	13	12	11	10	12	15	15	13
23.5.77	Max.	19	19	19	18	16	17	17	18
	Min.	15	16	16	16	13	12	15	15
30.5.77	Max.	18	18	19	18	20	17	17	18
	Min.	15	14	14	16	17	16	15	15
6.6.77	Max.	17	17	18	20	20	18	18	18
	Min.	16	15	15	15	15	14	15	15
13.6.77	Max.	21	19	19	20	20	18	17	19
	Min.	17	16	16	16	18	15	15	16
20.6.77	Max.	21	17	19	17	20	16	17	18
	Min.	16	15	13	15	14	13	13	14
27.6.77	Max.	18	18	19	19	20	19	17	19
	Min.	15	15	15	15	13	16	13	15

Appendix 18: Daily maximum and minimum relative humidity percentage
recorded in the laboratory

| Date | Humidity | Days of the week | | | | | | | Weekly Average |
		1	2	3	4	5	6	7	
21.3.77	Max.	60	60	67	68	62	59	65	63
	Min.	48	56	60	58	54	56	60	56
28.3.77	Max.	58	62	73	70	64	70	68	66
	Min.	52	52	60	63	60	62	64	59
4.4.77	Max.	64	64	63	58	60	68	70	64
	Min.	60	54	49	52	54	60	68	57
11.4.77	Max.	70	72	69	70	70	72	68	71
	Min.	67	63	61	64	65	68	60	64
18.4.77	Max.	68	68	60	64	59	62	60	63
	Min.	62	48	54	54	47	53	54	53
25.4.77	Max.	62	67	67	64	68	68	62	65
	Min.	60	58	63	60	59	63	58	60
2.5.77	Max.	67	65	69	68	65	65	68	67
	Min.	62	62	63	63	62	60	61	62
9.5.77	Max.	69	68	61	64	70	70	67	67
	Min.	58	58	58	50	55	58	58	57
16.5.77	Max.	72	68	65	60	61	60	60	64
	Min.	64	60	58	50	50	55	58	56
23.5.77	Max.	72	72	64	62	60	60	64	65
	Min.	62	62	58	58	58	58	61	60
30.5.77	Max.	68	62	59	68	68	64	55	63
	Min.	62	50	50	56	62	61	49	56
6.6.77	Max.	64	58	58	58	54	48	58	57
	Min.	58	54	56	50	44	43	50	51
13.6.77	Max.	58	56	60	64	60	58	64	60
	Min.	49	52	56	55	58	54	55	54
20.6.77	Max.	66	55	60	59	59	58	60	60
	Min.	48	52	52	54	48	50	55	51
27.6.77	Max.	65	68	64	65	62	61	63	64
	Min.	56	62	60	59	54	54	55	57

Appendix 19. The effect of seed storage for 3 months on the percentages of germination and viability of seed samples harvested at different developmental stages (1977)

Date of harvest	Days after pollination	Normal seedlings Germination period (days)		Abnormal seedlings	Fresh ungerminated seeds	Hard seeds	Total viable seeds	Dead (non-viable) seeds
		7	10					
14.2.77	8	–	–	–	–	–	–	100
16.2.77	10	–	–	–	–	–	–	100
18.2.77	12	4	4	4	2	2	12	88
20.2.77	14	20	24	12	4	4	44	56
24.2.77	18	20	24	2	4	62	92	8
9.3.77	22	6	6	–	–	94	100	–
13.3.77	26	4	4	–	–	96	100	–
17.3.77	30	6	6	–	–	94	100	–
23.3.77	36	4	4	–	–	96	100	–
27.3.77	40	4	4	–	–	96	100	–

Appendix 20:   Percentages of germination and viability of freshly harvested seeds at different flowerhead tagging dates (1974 autumn sown crop).

Date of harvest	Days after tagging	Normal seedlings Germination period (days)			Abnormal seedlings	Fresh ungerminated seeds	Hard seeds	Total viable seeds	Dead (Non-viable) seeds
		7	10	30					
31.1.75	10	12	20	26	10	–	–	36	64
4.2.75	14	9	25	75	3	12	–	90	10
11.2.75	18	4	16	53	–	43	–	96	4
15.2.75	22	2	14	40	–	56	1	97	3
27.2.75	26	–	1	5	–	4	91	100	–
3.3.75	30	–	–	3	1	–	95	99	1

Appendix 21: Percentages of germination and viability of freshly harvested seeds at different dates after peak flowering (1974 autumn sown crop)

Date of harvest	Days after peak flowering	Normal seedlings Germination period (days)			Abnormal seedlings	Fresh ungerminated seeds	Hard seeds	Total viable seeds	Dead (Non-viable) seeds
		7	10	30					
17.2.75	10	4	28	51	7	24	–	82	18
21.2.75	14	3	22	39	2	53	–	94	6
25.2.75	18	4	14	42	2	52	–	96	4
1.3.75	22	1	10	24	2	38	35	99	1
5.3.75	26	1	4	11	–	11	77	99	1
9.3.75	30	3	5	14	–	16	66	96	4

Appendix 22:   Percentages of germination and viability of freshly harvested seeds at different flowerhead tagging dates. (1974 spring sown crop)

Date of harvest	Days after tagging	Normal seedlings Germination period (days)			Abnormal seedlings	Fresh ungerminated seeds	Hard seeds	Total viable seeds	Dead (non-viable) seeds
		7	10	30					
28.3.75	8	-	-	-	-	-	-	-	100
30.3.75	10	-	-	-	-	-	-	-	100
1.4.75	12	-	-	2	-	-	-	2	98
3.4.75	14	-	12	28	3	11	-	42	58
7.4.75	18	1	11	45	3	25	-	73	27
11.4.75	22	10	27	61	2	28	-	91	9
13.4.75	24	9	29	52	6	28	-	86	14
15.4.75	26	25	32	65	5	17	-	87	13
19.4.75	30	6	16	33	-	58	-	91	9

Appendix 23:   Tetrazolium test results on freshly harvested seeds at
               different developmental stages

Date of harvest	Days after pollination	Well stained seeds (%)	Doubtful seeds (%)	Unstained seeds (%)	Impermeable seeds (%)
26.2.76	8	44	38	18	0
28.2.76	10	76	12	12	0
1.3.76	12	80	10	10	0
3.3.76	14	91	5	4	0
7.3.76	18	97	2	1	0
11.3.76	22	97	1	2	0
17.3.76	24	97	2	1	0
19.3.76	26	88	2	0	10 *
28.3.76	30	18	0	0	82 *

* viable seeds: unstained due to seedcoat impermeability

Percentages of germination and viability of freshly harvested seeds taken from different positions in flower heads harvested 30 days after pollination (1976)

Method of pollination	Position of seed in the head	Normal seedlings	Abnormal seedlings	Fresh ungerminated seeds	Hard seeds	Total viable seeds	Dead (non-viable) seeds
Controlled pollination	Top	24	6	68	-	98	2
	Mid-upper	22	0	72	-	94	6
	Mid-lower	36	2	58	-	96	4
	Bottom	46	6	40	-	92	8
Open pollination	Top	50	6	34	-	90	10
	Mid-upper	48	4	36	-	88	12
	Mid-lower	48	4	42	-	94	6
	Bottom	66	4	16	-	86	14

Appendix 25: Percentages of germination and viability of freshly harvested seed samples with different maturities, prechilled at 5°C for different periods (1976).

Days after pollination	Prechilling period (days)	Normal seedling Germination period (days)			Abnormal seedlings	Fresh ungerminated seeds	Hard seeds	Total Viable seeds	Dead (non-viable) seeds
		7	10	30					
18	0	–	–	22	10	18	–	50	50
	2	–	–	34	2	22	–	58	42
	4	–	2	40	6	22	–	68	32
	6	–	–	30	6	16	–	52	48
	8	–	2	36	6	8	–	50	50
22	0	–	2	30	2	62	–	94	6
	2	–	2	16	–	80	–	96	4
	4	2	8	24	–	60	–	84	16
	6	4	10	26	–	64	–	90	10
	8	4	8	24	–	62	–	86	14

Appendix 26:   Percentages of germination and viability of freshly harvested seed samples prechilled at 5^OC for different periods (1977)

Days after pollination	Prechilling period (days)	Normal seedlings Germination period (days)			Abnormal seedlings	Fresh ungerminated seeds	Hard seeds	Total viable seeds	Dead (non-viable) seeds
		7	10	30					
	0	–	2	36	12	22	–	70	30
	1	–	4	42	8	30	–	80	20
	2	–	2	42	12	24	–	78	22
22	3	–	2	44	14	22	–	80	20
	4	2	6	48	8	28	–	84	16
	5	2	6	34	8	30	–	72	28
	6	2	6	36	6	26	–	68	32
	7	–	4	38	10	30	–	78	22

Appendix 27: Percentages of germination and total viability of freshly harvested seed samples treated with gibberellic acid (1977)

Prechilling	GA$_3$ treatment (1000 ppm)	Days after pollination	Normal seedlings Germination period (days)		Abnormal seedlings	Fresh ungerminated seeds	Hard seeds	Total viable seeds	Dead (non-viable) seeds
			10	30					
5°C for 4 days	No treatment	18	2	42	6	16	-	64	36
		22	14	26	-	68	-	94	6
		26	22	44	-	34	-	78	22
	Seeds placed on blotters soaked with GA$_3$ solution	18	2	16	4	12	-	32	68
		22	6	28	-	60	-	88	12
without prechilling	Seeds placed on blotters soaked with GA$_3$ solution	18	2	14	10	8	-	32	68
		22	2	22	2	66	-	90	10
	Seeds soaked in GA$_3$ solution before germination	18	-	16	4	8	-	28	72
		22	4	60	6	14	-	80	20
		24	16	36	-	50	-	86	14
		26	4	26	4	64	-	94	6

Appendix 28: Percentages of germination and total viability of freshly harvested seed samples (24 day-old) germinated in an O_2 enriched atmosphere (1977)

Prechilling	Oxygen treatment	Normal seedlings Germination Period (days)		Abnormal seedlings	Fresh ungerminated seeds	Hard seeds	Total viable seeds	Dead (non-viable) seeds
		10	30					
Without prechilling	No treatment. Add O_2 at germation.	16	44	–	52	–	96	4
		16	36	–	58	–	94	6
	Add O_2 at germination and again 4 days later	22	40	–	58	–	98	2
Prechilled at $5^{O}C$ for 4 days	Add O_2 at prechilling and at germination	28	48	–	48	–	96	4

Appendix 29: Percentages of germination and viability of freshly harvested seed samples receiving different intensities of seed coat injury (1977)

Days after pollination	Treatment	Normal seedlings Germination period (days)			Abnormal seedlings	Fresh ungerminated seeds	Hard seeds	Total Viable seeds	Dead (non-viable) seeds
		7	10	30					
	Intact seed	–	8	42	2	30	–	74	26
22	Cut through seed coat only. (shallow cutting)	2	10	32	4	26	–	62	38
	Cut through seed coat membrane (deep cutting)	16	44	70	–	–	–	70	30

Appendix 30: Percentages of germination and viability of mature seed samples with different seed colours (1977).

Conditions of seed	Seed colour	Normal seedlings Germination period (days)			Abnormal seedlings	Fresh ungerminated seeds	Hard seeds	Total viable seeds	Dead (non-viable) seeds
		7	10	30					
Freshly	Yellow	4	4	22	–	16	62	100	–
harvested	Brown	2	4	18	–	4	78	100	–
seeds	Purple	–	2	4	–	10	86	100	–
After	Yellow	10	12	–	–	2	86	100	–
three months	Brown	4	5	–	–	–	95	100	–
storage	Purple	3	3	–	–	–	97	100	–

Appendix 31: Germination percentage of unscarified and mechanically scarified 'Pawera' red clover seed following 3 months' storage.

Scarification treatment		Normal seedlings	Abnormal seedlings	Hard seeds	Dead seeds
Unscarified control		4	–	96	–
Mechanical scarification					
Duration (min)	Pressure (psi)				
1	10	93	–	2	5
2	"	95	3	1	1
3	"	96	2	–	2
4	"	96	3	–	1
5	"	96	2	–	2
6	"	94	4	–	2
2	4	93	1	3	3
"	6	95	–	3	2
"	8	98	1	–	1
"	10	95	2	–	3
"	12	95	3	–	2
"	14	92	7	–	1

Appendix 32 :   Buried seed population in the soil

Name of crop	Numbers of seed (mil/ha)	Source	Comments
Red clover (*Trifolium pratense*)	1	Milton 1943	Grassland Fields
" " " "	6	Harris 1959	Arable land
" " " , "	127	Hyde & Suckling 1953	Pasture land
White clover (*T. repens*)	28	Milton 1943	Grassland fields
" " "	20	Champness & Morris 1948	Grassland fields
" " "	490	Hyde & Suckling 1953	Pasture land
Suckling clover (*T. dubium*)	343	" " "	" "
Various clover species (*Leguminoseae*)	32–385	Raffar 1971	Sheep pasture
Gorse (*Ulex europaeus*)	29	Moss 1959	Unploughable hill country
Fathen (*Chenopodium album*)	59	Hyde 1958	Dairy pasture
" " "	548	Roberts & Strokes 1966	Under vegetable crops
Dock (*Rumex* species)	40	Hyde 1958	Dairy pasture
Poppies (*Papaver* species)	280	Brenchley & Warington 1930	Wheat plots
Agrostis (*Agrostis* species)	141	Champness & Morris 1948	Grassland fields
Weeds	425–2400	Raffar 1971	Sheep pasture
"	76– 884	Brencley & Warington 1930	Barley oats
"	11– 328	Robinson 1949	Cropfield
"	37– 128	Roberts 1963	Vegetable crops
"	566	Roberts 1958	Arable field
Various plant species	42– 267	Milton 1943	Grassland fields
" " "	20– 215	Champness & Morris 1948	Grassland fields
" " "	860	Roberts & Strokes 1966	under vegetable crops
" " "	395	Hyde 1958	Dairy pasture

Appendix 33 : Long life-span of seeds of different species in the soil

Common name	Scientific name	Family	Longevity (years)	Source
Arctic tundra lupine	*Lupinus arcticus*	Leguminosae	10,000	Porsild *et al* . 1967.
Lotus	*Nelumbo*	Nymphaeaceae	2,000	Went 1963.
Fathen	*Chenopodium album*	Chinopodiaceae	1,700	} Ødum 1965.
Spurry	*Spergula arvensis*	Caryophyllaceae	1,700	
Achira	*Canna* species	Cannaceae	500	Sivori *et al* . 1968.
Weeds	-	-	300	Turrill 1957.
Moth mullein	*Verbascum blattaria*	Scorphulariceae	90	Kivilaan and Bandurski 1973.
Curled dock	*Rumex crispus*	Polygonaceae	80	} Darlington & Steinbauer 1961
Evening primrose	*Oenothera biennis*	Onagraceae	80	
Moth mullein	*Verbascum blattaria*	Scorphylariceae	80	
Gorse	*Ulex europaeus*	Leguminosae	30	Moss 1959.
Red clover	*Trifolium pratense*	"	4-39	} Goss 1924; Kjaer 1940, 1948;
White clover	*Trifolium repens*	"	4-30	Toole & Brown 1946;
				} Madsen 1962.

Appendix 34 :   Seed burial trial: Analysis of variance for seed recovered from clay soil site.

SOURCE	DF	MS	F CAL	F REQ 1 PC	F REQ 5 PC	LSD 1 PC	LSD 5 PC
Blocks	2	47.2535	1.4878	99.00	19.00		
Treatment A	1	52488.0000	1652.6246	98.49	18.51		
Main plot error	2	31.7604					
Treatment B	5	2946.8889	110.6160	3.11	2.26	2.7151	2.0658
Treatment C	7	14716.6191	552.4109	2.73	2.05	3.1427	2.3912
Interaction AB	5	671.7833	25.2164	3.11	2.26	3.8382	2.9204
Interaction AC	7	1180.1905	44.3003	2.73	2.05	4.4320	3.3722
Interaction BC	35	46.2476	1.7360	1.74	1.49	7.6765	5.8408
Interaction ABC	35	41.9452	1.5745	1.74	1.49	10.8560	8.2601
Residue	187	26.6407					

Coeff var =   13.84

Block means	36.6781	37.1781	38.0635								
A means	23.8066	50.8066									
B means	23.8135	33.6468	37.1260	40.3552	44.1677	44.7302					
C means	76.4454	52.4177	43.6399	36.1954	30.9454	25.7510	19.8899	13.1677			
AB means	17.0010	30.6260	22.0010	45.2927	23.0843	51.1677	24.0427	56.6677	27.7927	60.5427	
AB means	28.9177	60.5427									
AC means	74.5566	78.3343	34.9454	69.8899	23.4454	63.8343	19.0566	53.3343	15.1677	46.7232	
AC means	13.7232	37.7788	6.2232	33.5566	3.3343	23.0010					
BC means	61.5010	72.3343	79.0010	83.3343	80.8343	81.6677	36.5010	52.1677	55.1677	52.0010	
BC means	58.8343	59.8343	32.1677	43.6677	43.8343	46.1677	46.6677	49.3343	23.5010	34.1677	
BC means	35.1677	38.3343	42.6677	43.3343	14.3343	25.1677	29.8343	39.3343	36.6677	40.3343	
BC means	10.6677	18.1677	23.1677	30.0010	35.6677	36.8343	8.1677	14.3343	18.5010	21.1677	
BC means	29.5010	27.6677	3.6677	9.1677	12.3343	12.5010	22.5010	18.8343			
ABC means	60.0010	63.0010	71.0010	73.6677	76.0010	82.0010	79.0010	87.6677	82.0010	79.6677	
ABC means	79.3343	84.0010	26.0010	47.0010	37.6677	66.6677	37.0010	73.3343	29.3343	74.6677	

ABC means	35.6677	82.0010	44.0010	75.6677	22.3343	42.0010	24.6677	62.6677	21.6677	66.0010
ABC means	20.6677	71.6677	26.3343	67.0010	25.0010	73.6677	12.3343	34.6677	17.0010	51.3343
ABC means	17.6677	52.6677	19.6677	57.0010	25.3343	60.0010	22.3343	64.3343	6.3343	11.3343
ABC means	11.3343	39.0010	12.6677	47.0010	20.0010	58.6677	17.0010	56.3343	23.6677	57.0010
ABC means	5.6677	15.6677	9.0010	27.3343	12.6677	33.6677	12.6677	47.3343	21.3343	50.0010
ABC means	21.0010	52.6677	2.6677	13.6677	3.6677	25.0010	3.3343	33.6677	8.0010	34.3343
ABC means	9.6677	49.3343	10.0010	45.3343	0.6677	6.6677	1.6677	16.6677	3.6677	21.0010
ABC means	3.0010	22.0010	5.0010	40.0010	6.0010	31.6677				

Main factor 'A' is depth, factor 'B' is maturity, factor 'C' is interval between seed removals.

Appendix 35 : Seed burial trial: Analysis of variance for hard seed from clay soil site

SOURCE	DF	MS	F CAL	F REQ. 1 PC	5 PC	LSD 1 PC	5 PC
Blocks	2	61.9306	0.3759	99.00	19.00		
Treatment A	1	9753.3889	59.1962	98.49	18.51		
Main plot error	2	164.7639					
Treatment B	5	510.3472	26.1862	3.11	2.26	2.3210	1.7660
Treatment C	7	4100.5060	210.3994	2.73	2.05	2.6816	2.0404
Interaction AB	5	68.0389	3.4911	3.11	2.26	3.2829	2.4978
Interaction AC	7	979.5635	50.2620	2.73	2.05	3.7908	2.8843
Interaction BC	35	115.7536	5.9394	1.74	1.49	6.5657	4.9957
Interaction ABC	35	67.3183	3.4541	1.74	1.49	9.2854	7.0649
Residue	187	19.4892					

Coeff var =   19.66

Block	means	22.7510	21.5427	23.0635							
A	means	16.6329	28.2718								
B	means	16.9385	20.8760	21.9802	25.5427	24.1885	25.1885				
C	means	40.8343	27.6954	23.6399	20.8899	30.2510	16.8621	7.2788	12.1677		
AB	means	12.3343	21.5427	14.5843	27.1677	16.0427	27.9177	17.6677	33.4177	19.3343	29.0427
AB	means	19.8343	30.5427								
AC	means	42.6121	39.0566	21.0566	34.3343	21.3899	25.8899	18.4454	23.3343	14.8343	45.6677
AC	means	8.9454	24.7788	2.6121	11.9454	3.1677	21.1677				
BC	means	39.1677	41.0010	41.5010	48.3343	33.8343	41.1677	23.5010	27.8343	30.5010	30.6677
BC	means	25.8343	27.8343	24.8343	25.3343	22.0010	23.5010	20.8343	25.3343	17.3343	19.6677
BC	means	18.8343	24.1677	22.8343	22.5010	13.8343	24.5010	29.3343	38.3343	36.1677	39.3343
BC	means	8.5010	13.0010	15.3343	19.6677	23.0010	21.6677	6.1677	8.0010	7.0010	7.6677
BC	means	8.5010	6.3343	2.1677	7.6677	11.3343	12.0010	22.5010	17.3343		
ABC	means	38.0010	40.3343	39.6677	42.3343	45.6677	37.3343	48.3343	48.3343	40.0010	27.6677
ABC	means	44.0010	38.3343	16.3343	30.6677	18.6677	37.0010	22.3343	38.6677	20.0010	41.3343

ABC means	24.3343	27.3343	24.6677	31.0010	20.0010	29.6677	22.0010	28.6677	20.0010	24.0010
ABC means	20.3343	26.6677	23.3343	18.3343	22.6677	28.0010	11.6677	23.0010	16.3343	23.0010
ABC means	16.6677	21.0010	19.3343	29.0010	24.6677	21.0010	22.0010	23.0010	6.0010	21.6677
ABC means	11.0010	38.0010	12.6677	46.0010	19.0010	57.6677	16.6677	55.6677	23.6677	55.0010
ABC means	4.3343	12.6677	5.6677	20.3343	6.6677	24.0010	8.6677	30.6677	15.3343	30.6677
ABC means	13.0010	30.3343	1.6677	10.6677	2.0010	14.0010	1.3343	12.6677	2.6677	12.6677
ABC means	5.3343	11.6677	2.6677	10.0010	0.6677	3.6677	1.3343	14.0010	3.0010	19.6677
ABC means	3.0010	21.0010	5.0010	40.0010	6.0010	28.6677				

Main factor 'A' is depth, factor 'B' is maturity, factor 'C' is interval between seed removals.

SOURCE	DF	MS	F CAL	F REQ 1 PC	F REQ 5 PC	LSD 1 PC	LSD 5 PC
Blocks	2	88.3229	1.5724	99.00	19.00		
Treatment A	1	17453.3472	310.7229	98.49	18.51		
Main plot error	2	56.1701					
Treatment B	5	1206.1917	45.3992	3.11	2.26	2.7100	2.0619
Treatment C	7	4812.1012	181.1199	2.73	2.05	3.1298	2.3814
Interaction AB	5	541.1639	20.3686	3.11	2.26	3.8331	2.9164
Interaction AC	7	1487.7837	55.9978	2.73	2.05	4.4261	3.3676
Interaction BC	35	85.7679	3.2282	1.74	1.49	7.6659	5.8328
Interaction ABC	35	63.4575	2.3884	1.74	1.49	10.8414	8.2489
Residue	187	26.5686					

Coeff var = 35.29

Block	means	13.5427	15.4073	14.8656							
A	means	6.8204	22.3899								
B	means	6.2510	12.4177	15.0218	14.6468	19.8135	19.4802				
C	means	34.5843	24.1677	19.7788	15.2510	0.6954	8.8621	12.5843	0.9177		
AB	means	3.9593	8.5427	6.6677	18.1677	6.8760	23.1677	6.1677	23.1260	8.2927	31.3343
AB	means	8.9593	30.0010								
AC	means	30.1677	39.0010	13.2232	35.1121	1.7788	37.7788	0.6677	29.8343	0.3343	1.0566
AC	means	4.7788	12.9454	3.6121	21.5566	0.0010	1.8343				
BC	means	19.8343	30.3343	37.3343	34.0010	45.8343	40.1677	11.6677	23.5010	24.0010	21.0010
BC	means	33.0010	31.8343	6.6677	17.8343	21.8343	22.5010	25.8343	24.0010	6.0010	14.1677
BC	means	16.3343	14.3343	19.8343	20.8343	0.5010	0.6677	0.5010	1.0010	0.5010	1.0010
BC	means	2.0010	5.1677	7.8343	10.3343	12.6677	15.1677	2.0010	6.3343	11.5010	13.5010
BC	means	20.8343	21.3343	1.3343	1.3343	0.8343	0.5010	0.0010	1.5010		
ABC	means	17.6677	22.0010	27.3343	33.3343	31.0010	43.6677	29.6677	38.3343	40.6677	51.0010
ABC	means	34.6677	45.6677	9.0010	14.3343	18.0010	29.0010	13.3343	34.6677	8.6677	33.3343
ABC	means	11.3343	54.6677	19.0010	44.6677	1.6677	11.6677	2.0010	33.6677	1.6677	42.0010

Appendix 36 cont.:

ABC means	0.0010	45.0010	3.0010	48.6677	2.3343	45.6677	0.6677	11.3343	0.6677	27.6677
ABC means	1.0010	31.6677	0.6677	28.0010	0.6677	39.0010	0.3343	41.3343	0.3343	0.6677
ABC means	0.3343	1.0010	0.0010	1.0010	1.0010	1.0010	0.3343	0.6677	0.0010	2.0010
ABC means	1.3343	2.6677	3.3343	7.0010	6.0010	9.6677	4.0010	16.6677	6.0010	19.3343
ABC means	8.0010	22.3343	1.0010	3.0010	1.6677	11.0010	2.0010	21.0010	5.3343	21.6677
ABC means	4.3343	37.3343	7.3343	35.3343	0.0010	2.6677	0.0010	2.6677	0.0010	1.6677
ABC means	0.0010	1.0010	0.0010	0.0010	0.0010	3.0010				

Main factor 'A' is depth, factor 'B' is maturity, factor 'C' is interval between seed removals.

Seed burial trial: Analysis of variance for seed recovered from sandy soil site

SOURCE	DF	MS	F CAL	F REQ		LSD				
				1 PC	5 PC	1 PC	5 PC			
Blocks	2	62.0001	2.4759	99.00	19.00					
Treatment A	1	42632.0001	1702.4451	98.49	18.51					
Main plot error	2	25.0416								
Treatment B	5	3376.0417	119.1488	3.11	2.26	2.7988	2.1295			
Treatment C	7	12901.4584	455.3241	2.73	2.05	6.9962	2.4592			
Interaction AB	5	519.2167	18.3244	3.11	2.26	3.9583	3.0117			
Interaction AC	7	1433.1428	50.5791	2.73	2.05	4.5706	3.4777			
Interaction BC	35	43.7655	1.5446	1.74	1.49	7.9168	6.0237			
Interaction ABC	35	33.3119	1.1757	1.74	1.49	11.1958	8.5186			
Residue	187	28.3347								

Coeff var =	14.35									
Block means	36.5218	36.7718	38.0218							
A means	24.9385	49.2718								
B means	24.1885	32.0843	35.6052	39.2093	46.9385	44.6052				
C means	74.7510	51.2788	43.0288	33.8899	28.8621	26.4732	23.0566	15.5010		
AB means	17.7093	30.6677	21.8343	42.3343	22.7510	48.4593	25.6260	52.7927	32.5010	61.3760
AB means	29.2093	60.0010								
AC means	75.7788	73.7232	33.6677	68.8899	23.8899	62.1677	18.4454	49.3343	16.7788	40.9454
AC means	14.5010	38.4454	9.4454	36.6677	7.0010	24.0010				
BC means	63.8343	69.1677	73.8343	75.3343	83.5010	82.8343	42.3343	49.3343	53.3343	50.3343
BC means	56.6677	55.6677	31.6677	34.8343	40.1677	48.1677	52.1677	51.1677	20.8343	26.6677
BC means	33.0010	36.0010	43.1677	43.6677	11.0010	26.3343	28.1677	32.6677	40.8343	34.1677
BC means	10.6677	22.8343	23.1677	28.1677	36.0010	38.0010	8.5010	17.6677	18.6677	26.6677
BC means	34.0010	32.8343	4.6677	9.8343	14.5010	16.3343	29.1677	18.5010		
ABC means	65.6677	62.0010	71.3343	67.0010	77.6677	70.0010	78.3343	72.3343	81.3343	85.6677
ABC means	80.3343	85.3343	30.6677	54.0010	32.6677	66.0010	33.0010	73.6677	28.3343	72.3343

Appendix 37 contd:

ABC means	38.6677	74.6677	38.6677	72.6677	19.0010	44.3343	18.6677	51.0010	15.6677	64.6677
ABC means	28.6677	67.6677	33.3343	71.0010	28.0010	74.3343	11.0010	30.6677	11.0010	42.3343
ABC means	18.0010	48.0010	19.0010	53.0010	27.6677	58.6677	24.0010	63.3343	6.0010	16.0010
ABC means	16.3343	36.3343	16.0010	40.3343	16.3343	49.0010	25.6677	56.0010	20.3343	48.0010
ABC means	5.0010	16.3343	11.3343	34.3343	9.6677	36.6677	17.3343	39.0010	21.3343	50.6677
ABC means	22.3343	53.6677	2.6677	14.3343	9.3343	26.0010	6.0010	31.3343	9.3343	44.0010
ABC means	16.3343	51.6677	13.0010	52.6677	1.6677	7.6677	4.0010	15.6677	6.0010	23.0010
ABC means	7.6677	25.0010	15.6677	42.6677	7.0010	30.0010				

Main Factor 'A' is depth, factor 'B' is maturity, factor 'C' is interval between seed removals.

Appendix 38 : Seed burial trial: Analysis of variance for hard seed from sandy soil site.

SOURCE	DF	MS	F CAL	F REQ 1 PC	F REQ 5 PC	LSD 1 PC	LSD 5 PC
Blocks	2	86.9410	51.4156	99.00	19.00		
Treatment A	1	9327.5035	5516.1463	98.49	18.51		
Main plot error	2	1.6909					
Treatment B	5	518.7118	23.2206	3.11	2.26	2.4853	1.8910
Treatment C	7	3291.2257	147.3347	2.73	2.05	2.8697	2.1834
Interaction AB	5	66.3618	2.9707	3.11	2.26	3.5147	2.6742
Interaction AC	7	493.9638	22.1127	2.73	2.05	4.0585	3.0880
Interaction BC	35	136.2483	6.0993	1.74	1.49	7.0294	5.3484
Interaction ABC	35	56.9269	2.5484	1.74	1.49	9.9408	7.5637
Residue	187	22.3384					

Coeff var = 21.49

Block means	22.7510	20.9281	22.3135							
A means	16.3066	27.6885								
B means	17.1468	19.2093	21.4802	25.0218	24.8343	24.2927				
C means	37.6399	22.5843	27.4454	24.8343	27.6954	11.3899	9.0288	15.3621		
AB means	11.9593	22.3343	13.8760	24.5427	15.0010	27.9593	18.0843	31.9593	21.0843	28.5843
AB means	17.8343	30.7510								
AC means	39.0566	36.2232	16.8899	28.2788	21.2232	33.6677	17.7232	31.9454	16.5010	38.8899
AC means	6.6121	16.1677	5.5010	12.5566	6.9454	23.7788				
BC means	42.1677	31.5010	38.0010	40.3343	34.1677	39.6677	25.0010	22.5010	22.6677	26.6677
BC means	20.1677	18.5010	25.0010	23.0010	26.1677	31.3343	27.8343	31.3343	17.5010	20.1677
BC means	24.0010	27.8343	28.3343	31.1677	10.5010	24.5010	27.0010	31.6677	39.6677	32.8343
BC means	6.8343	11.8343	11.5010	14.0010	10.8343	13.3343	5.6677	10.8343	8.1677	12.0010
BC means	8.5010	9.0010	4.5010	9.3343	14.3343	16.3343	29.1677	18.5010		
ABC means	41.3343	43.0010	32.6677	30.3343	42.0010	34.0010	43.0010	37.6677	37.0010	31.3343
ABC means	38.3343	41.0010	16.0010	34.0010	16.3343	28.6677	17.0010	28.3343	19.3343	34.0010

ABC means	17.3343	23.0010	15.3343	21.6677	17.0010	33.0010	17.0010	29.0010	14.3343	38.0010
ABC means	25.0010	37.6677	29.3343	26.3343	24.6677	38.0010	9.6677	25.3343	10.3343	30.0010
ABC means	17.3343	30.6677	18.6677	37.0010	27.0010	29.6677	23.3343	39.0010	6.0010	15.0010
ABC means	16.3343	32.6677	15.6677	38.3343	16.0010	47.3343	25.6677	53.6677	19.3343	46.3343
ABC means	2.3343	11.3343	6.6677	17.0010	4.0010	19.0010	9.6677	18.3343	8.3343	13.3343
ABC means	8.6677	18.0010	2.0010	9.3343	7.6677	14.0010	3.6677	12.6677	5.3343	18.6677
ABC means	8.3343	8.6677	6.0010	12.0010	1.3343	7.6677	4.0010	14.6677	6.0010	22.6677
ABC means	7.6677	25.0010	15.6677	42.6677	7.0010	30.0010				

Main factor 'A' is depth, factor 'B' is maturity, factor 'C' is interval between seed removals.

Appendix 39 : Seed burial trial: Analysis of variance for lab. germination from seeds buried in sandy soil site.

SOURCE	DF	MS	F CAL	F REQ		LSD	
				1 PC	5 PC	1 PC	5 PC
Blocks	2	109.7118	7.0861	99.00	19.00		
Treatment A	1	12337.5868	796.8660	98.49	18.51		
Main plot error	2	15.4826					
Treatment B	5	1482.9618	74.8748	3.11	2.26	2.3400	1.7805
Treatment C	7	5426.4360	273.9809	2.73	2.05	2.7022	2.0560
Interaction AB	5	536.3618	27.0809	3.11	2.26	3.3094	2.5180
Interaction AC	7	1000.7614	50.5285	2.73	2.05	3.8215	2.9076
Interaction BC	35	101.5491	5.1272	1.74	1.49	6.6188	5.0361
Interaction ABC	35	40.9650	2.0683	1.74	1.49	9.3604	7.1221
Residue	187	19.8059					

Coeff var = 30.04

Block means	13.5843	15.3760	15.4906							
A means	8.2718	21.3621								
B means	6.5635	12.1885	14.0843	14.0427	21.8968	20.1260				
C means	35.9177	27.8899	15.4454	9.0010	1.1121	15.0566	14.0010	0.1121		
AB means	5.3343	7.7927	7.2510	17.1260	7.5843	20.5843	7.3760	20.7093	11.0427	32.7510
AB means	11.0427	29.2093								
AC means	35.1121	36.7232	15.7232	40.0566	2.5566	28.3343	0.7232	17.2788	0.2788	1.9454
AC means	7.8343	22.2788	3.9454	24.0566	0.0010	0.2232				
BC means	19.8343	35.0010	35.6677	34.5010	48.1677	42.3343	16.0010	24.6677	30.6677	23.1677
BC means	36.3343	36.5010	6.3343	11.6677	14.0010	16.6677	24.1677	19.8343	3.3343	6.3343
BC means	8.8343	8.1677	14.8343	12.5010	0.3343	1.6677	1.1677	1.0010	1.1677	1.3343
BC means	3.8343	10.8343	11.6677	14.1677	25.1677	24.6677	2.8343	6.8343	10.5010	14.6677
BC means	25.3343	23.8343	0.0010	0.5010	0.1677	0.0010	0.0010	0.0010		
ABC means	22.6677	17.0010	36.0010	34.0010	34.3343	37.0010	35.0010	34.0010	42.0010	54.3343
ABC means	40.6677	44.0010	13.6677	18.3343	13.6677	35.6677	16.0010	45.3343	8.0010	38.3343
ABC means	21.0010	51.6677	22.0010	51.0010	1.6677	11.0010	1.6677	21.6677	1.3343	26.6677

Appendix 39 contd.

ABC means	3.6677	29.6677	3.6677	44.6677	3.3343	36.3343	1.3343	5.33.43	0.6677	12.0010
ABC means	0.6677	17.0010	0.3343	16.0010	0.6677	29.0010	0.6677	24.3343	0.0010	0.6677
ABC means	0.0010	3.3343	0.3343	2.0010	0.3343	1.6677	0.0010	2.3343	1.0010	1.6677
ABC means	2.6677	5.0010	4.3343	17.3343	5.6677	17.6677	7.6677	20.6677	13.0010	37.3343
ABC means	13.6677	35.6677	0.6677	5.0010	1.6677	12.0010	2.3343	18.6677	4.0010	25.3343
ABC means	8.0010	42.6677	7.0010	40.6677	0.0010	0.0010	0.0010	1.0010	0.0010	0.3343
ABC means	0.0010	0.0010	0.0010	0.0010	0.0010	0.0010				

Main factor 'A' is depth, factor 'B' is maturity, factor 'C' is interval between seed removals.

Appendix 40 : Seed Burial Trial: Percentage germination *in situ* of
seed buried in clay and sandy soil

Date of seed Removal	Maturity	Clayey Soil		Sandy Soil	
		½ cm Depth	15 cm Depth	½ cm Depth	15 cm Depth
	1	17	12	19	15
	2	19	16	19	14
9.7.75	3	18	13	18	15
	4	16	9	18	12
	5	11	10	13	11
	6	15	10	14	11
	1	15	2	21	3
	2	18	4	28	6
	3	24	3	44	4
12.8.75	4	45	2	52	9
	5	61	2	46	5
	6	45	2	46	5
	1	11	6	5	5
	2	21	6	6	6
	3	21	5	12	2
11.9.75	4	31	7	15	7
	5	28	5	19	4
	6	11	3	14	3
	1	8	6	9	8
	2	8	7	4	9
	3	4	8	4	12
9.10.75	4	8	5	2	9
	5	9	9	3	11
	6	4	7	3	10
	1	–	1	1	–
	2	–	1	–	1
	3	–	1	–	–
9.3.76	4	–	1	–	–
	5	–	–	–	–
	6	–	1	–	–

Date of seed Removal	Maturity	Clayey Soil		Sandy Soil	
		½ cm Depth	15 cm Depth	½ cm Depth	15 cm Depth
9.6.76	1	1	–	1	–
	2	–	–	–	–
	3	–	1	–	1
	4	–	1	1	–
	5	–	1	2	–
	6	–	1	–	–
9.9.76	1	1	–	1	1
	2	2	–	3	1
	3	4	–	3	2
	4	4	2	3	1
	5	6	–	2	–
	6	4	–	6	–
8.12.76	1	–	2	–	–
	2	–	1	–	–
	3	–	1	1	–
	4	–	1	–	–
	5	–	–	–	1
	6	–	–	–	1

Appendix 41 : Percentage of seed recovered, laboratory germination of recovered seed and seeds remaining hard after laboratory germination recorded at different intervals of seed removal (means of two depths).

		Date of seed removal							
		9.7.75	12.8.75	11.9.75	9.10.75	9.3.76	9.6.76	9.9.76	8.12.76
Seed recovered	Clay soil	76.445 † a	52.418 b	43.640 c	36.195 d	30.945 e	25.751 f	19.890 g	13.168 h
	Sandy soil	74.751 a	51.279 b	43.029 c	33.890 d	28.862 d	26.473 de	23.057 de	15.501 f
Lab. germination	Clay soil	34.584 a	24.168 b	19.779 c	15.251 d	0.695 f	8.862 e	12.584 d	0.918 f
	Sandy soil	35.918 a	27.890 b	15.445 c	9.001 d	1.112 e	15.057 c	14.001 c	0.112 e
Hard seed remaining after lab. germination	Clay soil	40.834 a	27.695 b	23.640 c	20.890 d	30.251 b	16.862 e	7.279 g	12.168 f
	Sandy soil	37.640 a	22.584 bc	27.445 b	24.834 b	27.695 b	11.390 e	9.029 e	15.362 d

† Figures in each row of any column showing the same letter are not significantly different at 1% level.

Percentage of seed recovered, laboratory germination of recovered seeds and seeds remaining hard after laboratory germination tests recorded for different maturities of seed samples (means of two depths).

		Maturity (developmental age)					
		1	2	3	4	5	6
Seed recovered	Clay soil	23.814 † e	33.647 d	37.126 c	40.355 b	44.168 a	44.730 a
	Sandy soil	24.189 e	32.084 d	35.605 c	39.209 b	46.939 a	44.605 a
Lab. germination	Clay soil	6.251 c	12.418 b	15.022 b	14.647 b	19.814 a	19.480 a
	Sandy soil	6.564 c	12.189 b	14.084 b	14.043 b	21.897 a	20.126 a
Hard seed remaining after lab. germination	Clay soil	16.939 c	20.876 b	21.980 b	25.543 a	24.189 a	25.189 a
	Sandy soil	17.147 c	19.209 c	21.480 cb	25.022 a	24.834 a	24.293 a

† Figures in each row of any column followed by the same letter do not differ significantly at 1% level.

Appendix 43 : Seed Burial and laboratory storage trial:
              Percentage hard seed means for different sotrage conditions.

Laboratory storage   75.617 ± 11.219   a

Buried in clayey soil   22.451 ± 14.288   b

Buried in sandy soil   22.997 ± 13.158   b

Appendix 44 : Seed burial trial: Analysis of variance for hard seed from seed samples stored in the laboratory

SOURCE	DF	MS	F CAL	F REQ	STD ERROR	LSD 1PC	LSD 5PC
Replicate	2	66.3767	3.199	5.18	3.23			
Treatment A	5	1690.0692	81.458	3.51	2.45	1.2397	3.2443	2.4546
Treatment B	8	966.9368	46.605	2.99	2.18	1.5183	3.9734	3.0063
A*B interaction	40	43.6608	2.104	1.79	1.51	3.7191	9.7329	7.3638
Error	106	20.7476						

Coeff var = 6.02

Means for A*B interaction   Means for B

66.001	81.334	86.001	87.668	87.001	86.668	82.445
77.334	82.334	87.668	89.334	86.334	91.334	85.723
67.334	72.001	74.334	78.668	88.668	82.001	77.168
69.334	75.001	83.668	86.001	85.001	86.668	80.945
70.334	72.668	77.334	86.668	87.334	83.668	79.668
55.001	62.001	71.668	76.668	78.334	78.668	70.390
65.668	57.668	73.668	82.334	82.668	75.334	72.890
56.334	51.668	60.001	78.334	75.334	76.668	66.390
52.334	56.001	62.668	69.668	77.668	71.334	64.945

Means for A   64.408   67.853   75.223   81.705   83.149   81.371

Factor A is maturity Factor B is interval between germination tests.

Appendix 45: Seed Burial Trial: Analysis of variance for laboratory germination from seed samples stored in the laboratory.

SOURCE	DF	MS	F CAL	F REQ		STD ERROR	LSD 1PC	LSD 5 PC
Replicate	2	13.6543	0.798	5.18	3.23			
Treatment A	·5	668.0062	39.048	3.51	2.45	1.1257	2.9460	2.2289
Treatment B	8	995.4043	58.186	2.99	2.18	1.3787	3.6080	2.7298
A*B Interaction	40	41.6284	2.433	1.79	1.51	3.3771	8.8379	6.6866
Error	106	17.1072						

Coeff var = 20.48

Means for A*B interaction

						Means for B
20.334	12.334	12.001	9.001	10.001	10.334	12.334
14.334	14.334	10.334	8.001	10.001	7.668	10.779
20.334	21.001	19.668	16.334	10.668	17.001	17.501
20.668	20.668	13.001	11.668	14.001	12.001	15.334
18.001	20.001	20.668	11.668	12.334	15.001	16.279
31.001	30.334	25.334	21.334	19.001	18.668	24.279
25.668	39.668	25.334	15.668	15.668	23.334	24.223
33.334	44.668	38.668	20.001	23.334	20.334	30.057
35.668	39.001	35.334	27.668	21.668	26.334	30.945

Means for A	24.371	26.890	22.260	15.705	15.186	16.742

Factor 'A' is maturity, Factor 'B' is interval between germination tests.

Appendix 46 :   Seed Burial trial: Analysis of variance for dead seeds from seed samples stored in the laboratory

SOURCE	DF	MS	F CAL	F REQ	STD ERROR	LSD 1PC	LSD 5PC
Replicate	2	8.6852	2.055	5.18	3.23			
Treatment A	5	227.3296	53.792	3.51	2.45	0.5595	1.4642	1.1078
Treatment B	8	16.7083	3.954	2.99	2.18	0.6852	1.7933	1.3568
A*B Interaction	40	5.0213	1.188	1.79	1.51	1.6785	4.3926	3.3234
Error	106	4.2261						
Coeff var =	64.90							

Means for A*B interaction   Means for B

10.668	4.001	1.334	1.668	2.668	3.001	3.890
6.001	2.668	1.668	1.668	3.668	0.334	2.668
10.334	4.668	2.001	2.668	0.668	1.001	3.557
8.334	3.001	2.668	1.668	1.001	0.668	2.890
8.668	5.668	2.001	0.668	0.334	1.001	3.057
14.001	7.334	2.668	1.668	2.668	2.668	5.168
5.334	0.668	0.334	1.668	1.334	1.001	1.723
7.668	3.001	0.334	1.668	0.668	2.668	2.668
8.334	3.001	1.334	2.334	0.668	1.668	2.890

Means for A	8.816	3.779	1.594	1.742	1.520	1.557

Factor A is maturity Factor B is interval between germination tests.

Appendix 47: Seed Burial Trial: Percentage moisture content of seed samples stored in the laboratory or buried in the soil.

Treatment	Storage/depth of burial	Storage/burial period (month)	Maturity					
			1	2	3	4	5	6
Control	Laboratory Storage	0	13.1	11.5	11.2	11.0	10.2	10.7
		1	11.7	10.7	10.6	10.8	9.3	9.6
		3	11.9	11.5	11.6	11.3	9.9	10.8
		12	13.0	12.6	12.4	12.1	10.7	10.8
		18	10.9	10.3	10.6	10.2	9.2	9.6
Buried in clayey soil	½ cm	1	11.7	11.0	11.9	10.5	9.3	9.4
		3	11.3	11.2	10.3	10.5	8.9	10.0
		12	NT	23.5	17.9	16.5	19.0	13.1
		18	NT	NT	NT	NT	NT	NT
	15 cm	1	11.8	11.2	11.1	10.5	9.1	9.7
		3	12.1	11.5	11.0	10.6	8.8	9.6
		12	22.7	13.9	16.3	13.0	11.3	12.1
		18	12.9	9.5	12.0	11.3	9.3	10.4
Buried in sandy soil	½ cm	1	11.4	10.5	10.3	9.6	10.2	9.4
		3	26.4	25.4	14.4	13.6	13.3	14.0
		12	28.6	30.5	19.8	13.7	11.0	13.9
		18	NT	NT	NT	NT	NT	NT
	15 cm	1	11.3	11.5	10.6	10.9	9.4	9.3
		3	13.2	12.7	11.9	11.7	9.2	10.9
		12	19.6	13.7	11.7	11.7	10.1	10.9
		18	18.1	11.7	11.8	11.8	8.5	10.1

NT - not tested due to a very small number of seed recovered at the time of removal.

Appendix 48 : Seed burial trial: Monthly average maximum and minimum ambient temperatures and relative humidities recorded at two burial sites and in the laboratory

Year	Month	Temp & R.H. record	Temp.(^{O}C) at the burial sites		Laboratory storage	
			clay soil	sandy soil	temp (^{O}C)	R.H. %
1975	June	max.	12	14	–	–
		min.	3	2	–	–
	July	max.	14	15	22	–
		min.	5	5	19	–
	August	max.	14	16	23	58
		min.	6	6	21	51
	September	max.	16	17	21	55
		min.	6	7	18	48
	October	max.	18	20	22	59
		min.	9	10	20	50
	November	max.	19	21	23	59
		min.	8	11	21	50
	December	max.	21	24	24	63
		min.	10	14	22	52
1976	January	max.	22	28	24	68
		min.	12	18	23	60
	February	max.	22	23	24	66
		min.	9	10	22	57
	March	max.	23	24	24	64
		min.	10	11	22	55
	April	max.	21	22	23	65
		min.	8	10	21	56
	May	max.	17	19	22	62
		min.	7	8	20	53
	June	max.	13	16	22	59
		min.	2	4	19	50
	July	max.	17	14	23	61
		min.	7	6	20	50

Appendix 48: continued

Year	Month	Temp & R.H. record	Temp. ($^{\circ}$C) at the burial sites		Laboratory storage	
			clay soil	sandy soil	temp ($^{\circ}$C)	R.H. %
1976	August	max.	18	16	24	56
		min.	8	7	21	49
	September	max.	19	18	23	55
		min.	9	8	21	48
	October	max.	21	19	23	57
		min.	9	9	22	50
	November	max.	23	21	24	55
		min.	10	10	22	47
	December	max.	28	24	26	62
		min.	13	13	25	54

Appendix 49: Seed Burial Trial: Monthly average soil temperatures recorded
at different depths in two soil types

| Year | Month | Soil type (clay or sandy soil) | ½ cm Depth | | 15 cm Depth | |
			min °C	max °C	min °C	max °C
1975	June	C.S	−1	18	7	10
		S.S	−1	16	6	9
	July	C.S	1	17	5	10
		S.S	0	16	4	10
	August	C.S	2	21	7	12
		S.S	1	20	6	12
	September	C.S	3	24	8	14
		S.S	2	25	7	15
	October	C.S	6	31	12	17
		S.S	6	32	11	19
	November	C.S	5	31	12	20
		S.S	5	33	10	20
	December	C.S	11	35	15	22
		S.S	12	34	15	24
1976	January	C.S	12	35	14	25
		S.S	12	36	14	27
	February	C.S	8	37	14	20
		S.S	8	38 .	13	24
	March	C.S	8	44	15	21
		S.S	8	40	14	23
	April	C.S	7	30	12	18
		S.S	6	29	11	18
	May	C.S	3	21	8	14
		S.S	2	20	6	14
	June	C.S	0	16	5	11
		S.S	0	16	4	11
	July	C.S	0	17	5	11
		S.S	0	15	4	11
	August	C.S	4	19	8	13
		S.S	5	19	6	12
	September	C.S	4	23	8	14
		S.S	4	22	8	14

Year	Month	Soil type	½ cm Depth		15 cm Depth	
			min	max	min	max
1976	October	C.S	4	27	10	17
		S.S	6	27	10	18
	November	C.S	5	36	11	21
		S.S	6	34	11	22
	December	C.S	11	37	15	24
		S.S	11	39	16	24

Appendix 50: Seed Burial Trial: Monthly average soil moisture percentage measured at two depths in two soil types

Year	Month	Soil type (clay or sandy soil)	Moisture (%)	
			½ cm Depth	15 cm Depth
1975	June	C.S	27	–
		S.S.	23	–
	July	C.S	28	–
		S.S	24	–
	August	C.S	27	–
		S.S	23	–
	September	C.S	26	27
		S.S	23	23
	October	C.S	18	25
		S.S	14	22
	November	C.S	22	25
		S.S	17	21
	December	C.S	7	21
		S.S	4	17
1976	January	C.S	23	24
		S.S	17	18
	February	C.S	19	23
		S.S	13	20
	March	C.S	11	21
		S.S	9	18
	April	C.S	16	24
		S.S	14	19
	May	C.S	26	26
		S.S	22	22
	June	C.S	29	27
		S.S	27	23
	July	C.S	29	26
		S.S	27	24
	August	C.S	27	26
		S.S	28	25
	September	C.S	24	27
		S.S	23	23

Appendix 50 : Continued

Year	Month	Soil type (clay or sandy soil)	Moisture (%)	
			½ cm Depth	13 cm Depth
1976	October	C.S	24	26
		S.S	23	23
	November	C.S	15	25
		S.S	14	23
	December	C.S	12	25
		S.S	11	21

Appendix 51: Field Emergence Trial: Percentage of seedlings from seed samples buried at ½ cm depth in two different soil types.

Date of Burial -1.7.75

| Burial Period | | Clay Soil Maturity | | | | | | Sandy Soil Maturity | | | | | |
Year	Month	1	2	3	4	5	6	1	2	3	4	5	6
1975	July	14	14	24	14	21	17	1	2	4	5	2	3
	Aug	6	34	35	44	37	38	–	2	–	–	–	1
	Sept	11	3	4	7	5	6	3	3	1	1	1	1
	Oct	6	2	2	2	1	2	3	1	–	1	1	1
	Nov	–	–	1	3	–	1	–	–	–	–	–	–
	Dec	–	–	–	–	–	–	–	–	–	–	–	–
1976	Jan	–	–	–	–	–	–	–	–	–	–	–	–
	Feb	–	–	–	–	–	–	–	–	–	–	–	–
	Mar	–	–	–	–	–	–	–	–	–	–	–	–
	Apr	–	–	–	–	–	–	1	–	–	–	–	1
	May	–	1	–	–	–	1	–	–	–	–	–	–
	June	1	–	–	–	–	1	–	–	–	–	–	–
	July	–	–	1	–	–	1	–	–	–	–	–	–
	Aug	–	1	2	3	1	1	–	–	1	1	1	1
	Sept	–	–	–	1	–	2	1	2	3	–	1	1
	Oct	–	–	–	–	–	1	–	–	–	1	6	–
	Nov	–	–	–	–	–	1	–	–	–	–	–	–
	Dec	–	–	–	–	–	–	–	–	–	–	–	–
		38	55	69	74	65	72	9	10	9	9	12	9

Appendix 52 : Field Emergence Trial: Percentage of seedlings from seed samples buried at ½ cm depth in two different soil types.

Date of Burial - 3.8.75

Burial Period		Clay Soil Maturity						Sandy Soil Maturity					
Year	Month	1	2	3	4	5	6	1	2	3	4	5	6
1975	July	–	–	–	–	–	–	–	–	–	–	–	–
	Aug	19	22	18	16	23	17	–	1	3	2	2	3
	Sept	16	23	22	18	19	14	2	3	3	2	3	2
	Oct	4	1	5	6	4	–	1	2	–	–	–	–
	Nov	1	2	–	–	–	1	–	–	–	–	–	1
	Dec	–	–	–	–	1	2	–	–	1	–	1	–
1976	Jan	–	–	–	–	–	–	–	–	–	–	–	–
	Feb	–	–	–	–	–	–	–	–	1	–	–	1
	Mar	–	–	–	–	–	–	–	–	–	–	1	–
	Apr	–	–	1	1	–	–	3	1	–	1	–	–
	May	–	1	1	4	2	1	–	–	–	1	1	–
	June	–	–	2	–	1	1	–	–	1	–	–	–
	July	–	1	–	3	2	5	–	1	–	–	–	–
	Aug	1	1	1	1	–	1	–	–	1	1	2	–
	Sept	–	–	–	1	–	2	2	2	2	3	4	2
	Oct	–	–	–	–	–	2	–	–	1	5	1	3
	Nov	–	–	–	–	–	–	–	–	–	–	–	–
	Dec	–	–	–	–	–	–	–	1	–	–	–	–
		41	51	50	50	52	46	8	11	13	15	15	12

Appendix 53 :   Field Emergence Trial: Percentage of seedlings from seed samples buried at ½ cm depth in two different soil types.

Date of burial -1.9.75

Burial Period		Clay soil						Sandy soil					
Year	Month	Maturity						Maturity					
		1	2	3	4	5	6	1	2	3	4	5	6
1975	July	–	–	–	–	–	–	–	–	–	–	–	–
	Aug	–	–	–	–	–	–	–	–	–	–	–	–
	Sept	26	29	30	23	15	35	3	1	3	7	7	3
	Oct	27	16	12	32	25	14	2	1	3	1	6	–
	Nov	1	–	2	4	1	2	1	–	–	–	1	–
	Dec	–	1	–	1	–	–	1	–	1	–	–	–
1976	Jan	–	–	–	–	–	–	–	–	–	–	–	–
	Feb	–	–	–	–	–	–	–	–	–	–	–	–
	Mar	–	–	–	–	–	–	–	–	–	–	–	–
	Apr	2	–	2	–	1	–	–	–	–	–	–	–
	May	–	1	–	2	1	–	–	–	1	–	1	–
	June	–	1	2	–	–	–	–	–	–	–	–	–
	July	1	1	4	6	5	4	–	–	–	–	–	–
	Aug	–	–	1	5	1	2	2	2	1	1	2	2
	Sept	–	–	–	1	2	4	5	1	4	6	3	5
	Oct	–	–	–	–	3	6	–	–	6	–	4	1
	Nov	–	–	–	–	–	–	1	2	–	–	–	–
	Dec	1	–	–	1	–	–	–	–	–	1	–	–
		57	49	53	75	54	67	15	7	19	16	24	11

Field Emergence Trial: Percentage of seedlings from seed samples buried at ½ cm depth in two different soil types
Date of Burial – 3.10.75

Burial Period		Clay soil Maturity						Sandy soil Maturity					
Year	Month	1	2	3	4	5	6	1	2	3	4	5	6
1975	July	–	–	–	–	–	–	–	–	–	–	–	–
	Aug	–	–	–	–	–	–	–	–	–	–	–	–
	Sept	–	–	–	–	–	–	–	–	–	–	–	–
	Oct	32	32	34	41	36	35	5	26	23	16	22	22
	Nov	8	6	6	7	6	4	1	1	1	4	1	–
	Dec	3	2	5	6	2	4	–	–	–	3	1	2
1976	Jan	–	–	1	–	–	–	–	–	–	–	–	–
	Feb	–	–	1	–	–	1	–	–	–	–	–	–
	Mar	–	–	–	–	–	–	1	3	–	–	–	–
	Apr	2	3	1	5	3	–	–	–	2	1	–	–
	May	3	3	2	2	4	2	2	–	1	2	3	1
	June	–	1	5	6	4	4	–	–	1	–	–	1
	July	2	4	4	4	6	2	–	1	1	–	–	1
	Aug	–	2	–	–	1	–	1	2	4	3	2	1
	Sept	1	–	1	4	2	5	–	6	3	6	4	5
	Oct	1	–	–	1	1	3	1	3	4	5	4	4
	Nov	–	–	–	–	–	–	1	–	–	–	–	–
	Dec	–	–	–	–	–	–	–	–	–	–	–	–
		52	53	60	76	65	60	12	42	40	40	37	37