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NITROGEN FIXATION IN PEAS

(*PISUM SATIVUM*)

A thesis

submitted in partial fulfilment

of the requirements for the degree

of

Doctor of Philosophy

in the

University of Canterbury

by

David C. Askin

Lincoln College

1983

ABSTRACT

Field experiments were conducted at Lincoln College, Canterbury, from 1979-81 to assess the effect of peas (*Pisum sativum*) on subsequent crops and evaluate factors influencing their N-fixing activity.

In two trials, spring-sown peas harvested green or dry were grown to contrast with barley. On a low fertility soil, wheat yielded 73 and 61 per cent more grain respectively following the two pea harvests when compared with barley. Wheat after lupins yielded 85% more than after barley. Seed size in wheat was 31.8 mg after barley but 39.7 mg after legumes.

In the second trial, which was on a more fertile soil, winter-grown ryegrass produced 34 and 23 per cent more biomass after vining and seed peas respectively. Pea residues were removed and contained 6.8 and 2.7 g N m^{-2} at green and dry pea stages respectively. Nitrogen harvest index (NHI) was 0.47 and 0.85 at the respective stages. Peas harvested dry relied on soil N for 50 per cent of their N requirements. Also on a high fertility soil, winter ryegrass after spring-sown peas and lupins yielded 25 and 11% respectively more than after wheat.

Incorporation of green pea residues to the soil would further increase subsequent crop yield, but most N is removed in seed at final harvest. These trials clearly showed the benefits from growing peas compared with cereals in crop rotations. The NHI and soil N uptake, however, limits soil nitrogen increases after peas.

The following four factors influencing pea N fixation were evaluated with peas cv. Puke sown in late spring (13/11/80). Treatments were 1) 8 t ha^{-1} straw incorporated at sowing; 2) irrigation during flowering and pod filling; 3) 4.5 g N m^{-2} at nodule formation; and 4) 4.5 g N m^{-2} at flowering. Overall treatment responses were small because of adequate soil nitrogen and 114 mm rainfall during pod-filling. Nitrate (0 - 20 cm)

was 34 ppm N at sowing and straw halved this to 20 ppm N, 36 days after sowing. Straw increased, and nitrogen reduced, N-fixation but dry seed yield was unaffected by these treatments. Irrigated green pea yield (644 g m^{-2}) was increased by 8% when nitrogen was applied at flowering. Irrigation increased dry seed yield from 305 to 343 g m^{-2}.

Trial 4 examined 1) the effect of autumn (7/5/1980) and spring (12/9/80) sowing, and 2) moisture stress, natural rainfall and irrigation applied from flowering on indeterminate (cv. Partridge) and determinate (cv. Whero) peas. Available soil nitrate of more than 6.5 ppm N (0 - 20 cm) reduced reliance on N fixation. Autumn sowing increased N fixation by 27 per cent, primarily through an extended period of N fixation. Irrigation of Partridge stimulated vegetative growth at the expense of seed development. At final harvest Partridge and Whero residues contained 16.8 and 7.1 g N m^{-2} respectively. Soil moisture and sowing date did not significantly influence seed yield. Lupins cv. Unicrop sown in winter (24/6/80) and spring (12/9/80) yielded 453 and 251 g m^{-2} respectively.

In Trial 5, eight pea cultivars were grown without irrigation and seven acetylene reduction assays used to assess N-fixation during growth. Cultivars and their seed yields (g m^{-2}) were Whero (262), Partridge (96), Huka (360), Rovar (284), Puke (269), Pania (306), Tere (229), Small Sieve Freezer (270). The highest NHI was recorded in the earliest maturing cultivar, Tere (0.85) and the lowest in Partridge (0.38) because of late flowering during drought stress. Whero and Partridge reached peak N fixation activity (5 µmoles C_2H_4 $plant^{-1}$ h^{-1}) 18 and 39 days before flowering respectively. Other cultivars reached peak activity soon after the start of flowering. Field pea N fixation was twice that of garden peas. In all cultivars, reliance on N fixation for nitrogen requirements during reproductive growth diminished as soil nitrogen uptake increased.

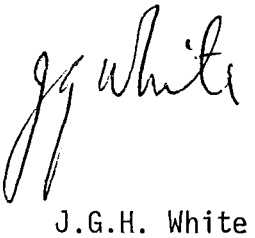
Diurnal variation in N fixation activity in cv. Whero was measured at three hourly intervals over three 24 h periods, during bud formation,

flowering and pod-filling. The optimum time for a one hour assay was between 1100 and 1400 h NZST, but this was not constant. Extrapolation from a single assay to a daily N fixation total could lead to 40% error. There was no distinct diurnal cycle at bud formation, but at flowering (during drought stress), N fixation increased during the night. In contrast, during pod-filling, N fixation decreased during the night. This was attributed to insufficient carbohydrate for nodule function. Mean N fixation increased from the first to last cycle.

Peas enhanced soil fertility when compared with cereals in all trials. Although water stress frequently limits pea yield in Canterbury, when water is adequate, available soil nitrogen will reduce the reliance of peas on N fixation.

Certificate

I hereby certify that the work embodied in this thesis was carried out by the candidate under my immediate supervision. Except for Trial One reported in Chapter Two, he planned, executed and described the research which is now submitted.



J.G.H. White
Supervisor

To my wife Virginia and daughter Sarah,
this page is dedicated to you in acknowledgement of your love, friendship, hard work and sacrifice during the course of this study.

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INTRODUCTION

Much physiological research has been done on peas because of their large viable seed, rapid germination and rapid attainment of a sufficient size to provide material for biochemical study. Although many overlapping studies have been carried out on "experimentally easy" periods such as germination and early seedling development, there has been much less attention paid to the whole life cycle (Pate, 1977a). In particular, very little research effort has been placed on field studies to assess both the effect of peas on soil fertility and factors which influence their nitrogen-fixing activity.

Canterbury is a very important cropping area of New Zealand, and in the past, soil fertility has been maintained by clover-based pastures. In recent years, however, land values have risen dramatically and crops have frequently been more economic than traditional meat and wool production. These trends have caused an intensification of crop rotations. Interest has therefore been stimulated in the ability of grain legumes to enhance soil fertility. However, the levels of mineral nitrogen in a cultivated soil are higher than in pasture for two reasons. Cultivation stimulates mineralisation of organic nitrogen (Russell, 1973), and grasses compete with the legumes for combined nitrogen. These factors increase the reliance of pasture legumes on nitrogen fixation but are likely to reduce nitrogen fixation in grain legumes. Also in contrast to pastures, grain legumes have large, protein-rich seeds and up to 90 per cent of total plant nitrogen can be removed in seeds (Rhodes, 1980). Thus, even if grain legumes were to fix all of their own nitrogen, only a small fraction of that nitrogen may remain after harvest to enhance soil fertility. Peas are more normally grown on soils of high fertility where yields are greatest, and nitrogen fixation may be reduced by the ready availability of combined nitrogen.

In view of the importance of peas in crop rotations, the studies reported here were initiated with two primary aims. The first was to establish the influence of peas on subsequent crop growth. Results for soils of low and medium fertility are reported in Chapters 2 and 3. The second aim was to identify those environmental factors which most influence nitrogen fixation in field-grown peas. At the start of the study, little was known of the factors which would most influence nitrogen fixation in Canterbury. Since drought frequently reduces pea yields it was expected that irrigation would also stimulate nitrogen fixation. The addition of fertiliser nitrogen or the reduction of combined nitrogen by the incorporation of straw was also likely to significantly alter nitrogen fixation. Peas are harvested green, as vining peas, or at maturity. In these two systems, nitrogen fixation and nitrogen removal may be different. A factorial experiment was used to test the effect of moisture, nitrogen and harvest date treatment combinations on nitrogen fixation and subsequent crop growth. Results are presented in Chapter 3.

The importance of drought on nitrogen fixation was highlighted in Chapter 3. Autumn sowing may allow peas to develop before drought limits yield. A trial was established to show the effect of sowing time and effect of changing soil moisture on nitrogen fixation in cultivars which had contrasting flowering patterns. Indeterminate or determinate flowering may influence the timing of the decline in nitrogen-fixing activity (Sinclair and de Wit, 1975, 1976). These results are presented in Chapter 4.

Cultivar differences in nitrogen fixation are important because they may allow an understanding of the factors which reduce nitrogen fixation. They also show those cultivars with the most potential for enhancement of soil fertility. The seasonal profiles in nitrogen fixation of eight cultivars were assessed and results are given in Chapter 5.

Inadequate carbohydrate supply is frequently considered the rate-limiting step in nitrogen fixation (Hardy and Havelka, 1976). Diurnal variation in nitrogen fixing activity allows a field assessment of the importance of current photosynthesis to nitrogen fixation. Three twenty-four hour cycles were measured in Maple peas cv. Whero. Results are presented in Chapter 6.

The discussion (Chapter 7) focusses on the importance of peas in crop rotations and the techniques used to measure nitrogen-fixing activity. Factors which are important in the control of fixation in peas are also discussed.

CHAPTER 1

LITERATURE REVIEW

1.1 INTRODUCTION

The genus Pisum has been used by man since the Stone Age (Cole, 1961, cited by Pate, 1977b). The pea (*Pisum sativum*) commended itself to cultivation because the seed lacked bitter or poisonous compounds, was more digestible than most other legume seeds, and the growth requirements of the plant were suited to man's early cultivation practices (Pate, 1977b). Peas rank fifth in world grain legume production with 7.9 mn ha sown annually (FAO Production Yearbook, 1982) and are now grown in many temperate regions including the higher elevations of the tropics. The genus shows very considerable adaptability, with a short growth cycle of usually 80 - 100 days (Pate, 1977b).

In New Zealand, 24,200 ha of peas were grown in 1979/80 for harvest at maturity with an average yield of 2.73 t ha^{-1}. The area sown to garden peas was 7,000 ha with an average yield of 4.96 t ha^{-1}. Canterbury is the most important pea growing area in New Zealand, with 17,600 ha sown to dry peas and 1,900 ha sown for processing as green peas in 1979/80. Dry pea and garden pea yields in Canterbury were 2.69 and 4.11 t ha^{-1} respectively (Agricultural Statistics, 1979/80, 1982). Eighty-four per cent of the total pea crop in New Zealand was exported in 1981, with a value of NZ $24.7 mn (N.Z. Official Yearbook, 1982).

Peas fix nitrogen through a symbiotic association with *Rhizobium leguminosarum*, but the amount fixed depends on a number of factors. These include cultivar, crop growth, soil mineral nitrogen, soil structure, availability of water, time of sowing, photosynthate supply to

nodules and *Rhizobium* strain (Minchin, Summerfield, Hadley, Roberts and Rawsthorne, 1981). The amount of nitrogen returned to the soil will be determined by complex interactions between the factors outlined above, the proportion of plant nitrogen removed in the seed, and the method of disposal of crop residues.

1.2 SEASONAL DEVELOPMENT OF THE PEA CROP

The symbiotic development of a New Zealand Maple pea and cv. Black-eyed Susan was detailed by Pate (1958), from research conducted in Ireland. In field trials where soil nitrogen was low, Pate (1958) found no evidence for a nitrogen hunger period after depletion of cotyledonary nitrogen, although, in a controlled environment, when pea seedlings relied totally on symbiotic nitrogen, Mahon and Child (1979) showed a short period of nitrogen hunger which affected subsequent growth.

The initiation and development of nodule tissue in legumes has been reviewed by Torrey and Zobel (1977), Bergersen (1980), and Dazzo (1980). Pate (1958) found that important events in the symbiotic cycle of peas, such as the time of appearance of nodules, took place in a precise sequence and were related to the pattern of leaf production and nitrogen accumulation in the host. Most of the nodules were present by the time of mid-vegetative growth. Nodules increased in number until the six to eight leaf stage, but they increased in size until mid-flowering. Degenerative changes in the nodule population occurred with fruit setting (Pate, 1958).

Using sloping glass panels, Salter and Drew (1965) found that root growth of peas increased rapidly until initiation of flower primordia. Shortly afterwards, new root growth declined with a slight resurgence during the flat pod stage only. Sprent, Bradford and Norton (1977) observed a similar cessation of root growth during pod fill in tick beans (*Vicia faba*). If root growth does decline or cease during pod fill, then

water supply would be crucial at that stage.

Above ground plant development in peas typically follows a sigmoidal growth curve (Dean and Clark, 1980; Rhodes, 1980), with nitrogen fixation and uptake of soil mineral nitrogen resulting in a gradual increase in plant nitrogen during vegetative growth (Dean and Clark, 1980; Rhodes, 1980). Nitrogen uptake in peas is from two sources: from nodules which fix atmospheric nitrogen, and from roots which assimilate mineral nitrogen from the soil. The mineral nitrogen is translocated to the shoots which reduce inorganic nitrogen to ammonium forms of nitrogen, via nitrate reductase (Wallace and Pate, 1965). Although leaf age and position affect the upward or downward translocation of nitrogen, Pate (1977a) concluded that upper leaves generally specialise in upward translocation to the adjacent shoot, while lower leaves generally nourish roots. The roots, however, may constitute a major obstacle to the free circulation of nitrogen within the pea plant (Oghoghorie and Pate, 1972).

The proportion of total plant nitrogen accumulated at flowering depends on cultivar and climate. Research with field pea (Pate and Minchin, 1980), white lupin (*Lupinus albus*) (Atkins, Herridge and Pate, 1978) and with blue lupin (*Lupinus angustifolius*) (Farrington, Greenwood, Titmanis, Trinick and Smith, 1977) have shown low values of final plant nitrogen and dry matter accumulated by flowering. In contrast, indeterminate and determinate soybeans accumulated 58 and 78 per cent respectively of their total dry weight at first flower, but an indeterminate soybean cultivar commenced flowering two weeks before the determinate cultivar (Egli and Leggett, 1973). However, peas grown in New Zealand show a reversed flowering pattern with determinate cultivars flowering before indeterminate. Therefore, indeterminate cultivars may have their seed development limited by drought conditions at late flowering. Total plant nitrogen in peas increased to a maximum at maturity under conditions

of adequate moisture (Pate and Flinn, 1973), although Pate's (1958) studies, under field conditions without irrigation, showed that the field peas reached their maximum nitrogen content during early fruiting. The maximum is frequently reached before final harvest and a decline in total plant nitrogen with the onset of plant maturity has been observed both in peas (Pate, 1958; Rhodes, 1980) and in lupins (Gladstones and Loneragan, 1975; Farrington *et al.*, 1977; Rhodes, 1980; Burtt, 1981). Gladstones and Loneragan (1975) considered the 20 per cent decline which they measured in lupins may have resulted from translocation from plant tops to the soil, although Rhodes (1980) suggested leaf-fall prior to harvest was the major factor in peas. Nitrogen is a very mobile element, with 66 per cent of total plant nitrogen stored in pea seeds (Pate and Flinn, 1973) and up to 90 per cent in lupin seeds (Rhodes, 1980).

Pea cultivars bred for even pod maturity, may rely more heavily on nitrogen translocation than on uptake, for seed nitrogen supplies (Pate and Flinn, 1977). In contrast, nitrogen uptake and translocation in indeterminate Maple peas may continue to supply a large portion of seed nitrogen requirements during seed filling. Thus the need for massive nitrogen translocation from leaves would be reduced (Pate and Flinn, 1973; Atkins *et al.*, 1978).

1.3 FACTORS AFFECTING NITROGEN FIXATION IN PEAS

1.3.1 Carbohydrate Supply

A link between nitrogen fixation and energy supply was suggested by Allison (1935) who studied a range of legumes, grown with adequate moisture and generally low levels of mineral soil nitrogen. In grain legumes, which produce large, protein-rich seeds, the nodulated roots and the seeds compete for photosynthates. As grain legume breeders seek to increase seed and protein yield (Jermyn, 1977), an improved output

from the nitrogen-fixing symbiosis is required because developing seeds sequester progressively larger proportions of the total photosynthate production (Sinclair and de Wit, 1976). These conflicting demands for photosynthate by the nodules and growing seeds have been studied in soybeans by Sinclair and de Wit (1975, 1976) who divided twenty-four legume and non-legume crops into four distinct groups based on biochemical composition of seed (Figure 1.1). They considered that many grain legumes are unable to meet seed nitrogen demand from soil nitrogen uptake and nitrogen fixation sources. To meet these demands, nitrogen is translocated from vegetative organs to seeds, which reduces photosynthesis and therefore nitrogen fixation (Sinclair and de Wit, 1975, 1976). Plants of this nature were termed "self-destructive". In contrast, carbohydrate-rich seeds of cereals, which require less nitrogen, do not cause self-destruction, as soil nitrogen uptake is generally sufficient for seed nitrogen requirements. Thus the duration of seed development relies on a continuous uptake of nitrogen to meet the needs of the plant. Where this rate is low, extensive translocation occurs with a shorter period of seed development and lower total yield (Sinclair and de Wit, 1976). If nitrogen fertiliser applied to legumes during seed filling is to cause an increase in seed yield, the rate of nitrogen uptake must be increased (Sinclair and de Wit, 1975). For the rate of uptake to be increased, applications of nitrogen fertiliser must be great enough to compensate for the reduced nitrogen fixation which occurs when nitrogen is applied to nodulated legumes (McAuliffe, Chamblee, Uribe-Arango and Woodhouse, 1958; Allos and Bartholomew, 1959; Hoglund, 1973; Chen and Phillips, 1977).

The four biochemical groups outlined by Sinclair and de Wit (1975) showed the unique nature of soybeans in their particularly high requirement for energy during seed production (Figure 1.1). Soybeans have between 10 (Sinclair and de Wit, 1975) and 16 (Hill, Horn and

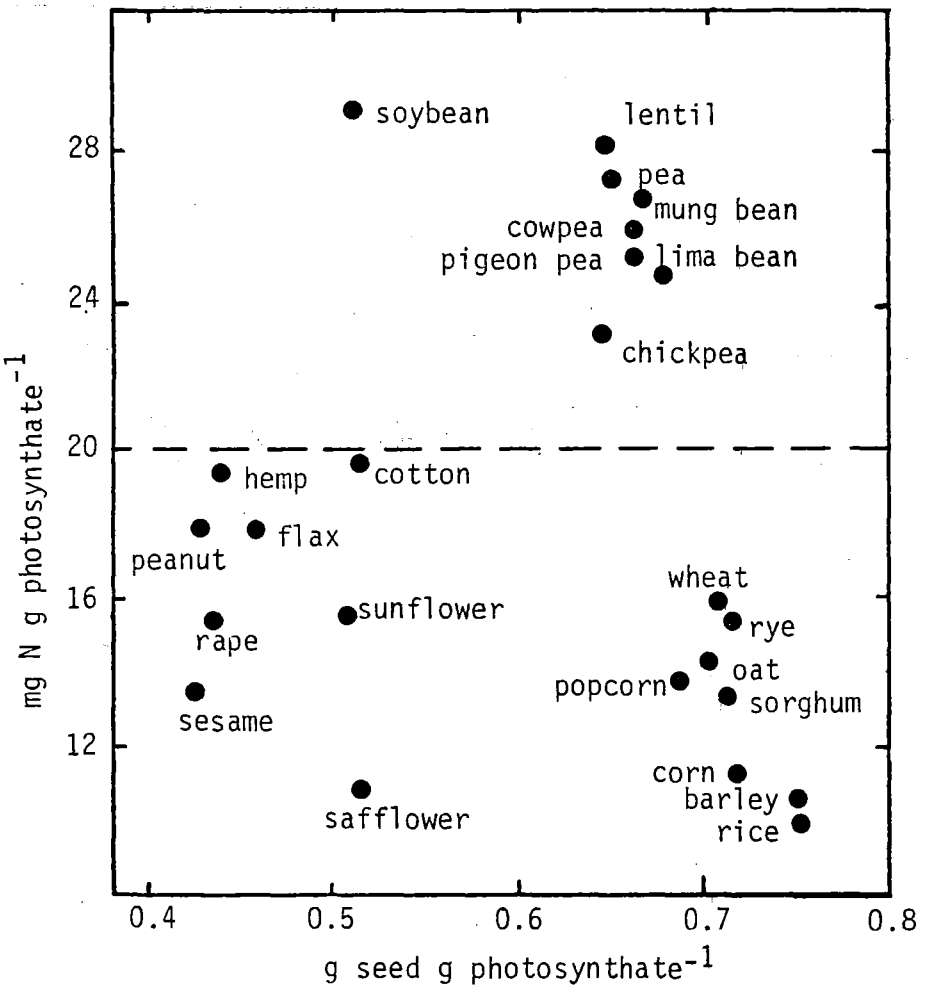


Figure 1.1: Plot of milligrams of nitrogen required and grams of seed biomass yielded per gram of available photosynthate for the 24 crop species analysed by Sinclair and de Wit (1975). The dashed line represents the nitrogen requirement when the nitrogen supply rate is 5 g ha^{-1} day^{-1} and the available photosynthate rate is 250 kg ha^{-1} day^{-1}. (Figure from Sinclair and de Wit, 1975)

Porter, 1977) times the lipid content of peas, while nitrogen contents were also higher in soybeans (5.6% N) compared with peas (3.6% N) (Hill *et al.*, 1977). Thus peas, which have lower seed requirements for nitrogen and energy in the production of lipids, may be able to continue active nitrogen fixation when soybean nitrogen fixation would be limited by insufficient carbohydrate from photosynthesis.

Much of the research which assesses the importance of carbohydrate supply for nodule function has been done with soybeans (Hardy and Havelka, 1976) which are particularly energy demanding, as shown in Figure 1.1. Experiments have also frequently operated under lower insolation than that experienced in Canterbury (Lawrie and Wheeler, 1973, 1974; Minchin and Pate, 1974; Huang, Boyer and Vanderhoef, 1975a, b; Bergersen, 1970), and this would further exacerbate inadequate carbohydrate supply by reducing photosynthesis. The initial stages of nodule development are particularly important because of the heavy demand placed on plant photosynthate for both plant and nodule growth. Minchin and Pate (1973) showed that 32 per cent of the net carbon gain by the shoot of young pea plants was utilised by nodules. Total respiration of underground organs at this stage accounted for 47 per cent of the net carbon gain of the shoot. From this, Pate (1976) considered that selection for economy in root respiration might be a sensible way to improve legume yield. Although the low insolation (5 MJ m^{-2} day^{-1}) used by Minchin and Pate may have reduced total photosynthate available for plant development, it is clear that nodule development is a direct cost in terms of plant growth. This cost has been shown in soybeans where root growth was greater in plants which relied on adequate levels of fertiliser nitrogen than those that relied on fixation. The reduced root growth limited phosphorus uptake and the nodulated plants had greater phosphorus requirements (Cassman, 1979, quoted by Munns, 1979). Photosynthate for nitrogen fixation may be further reduced

by high night temperatures, which increase losses through respiration (Pate, 1977a). Thus the conclusions reached for soybeans need to be treated with caution when extrapolation is made to peas grown in Canterbury.

To gain an understanding of the conflicting demands made by nodules and seeds, grain legumes need to be studied in the context of hormonal, genetic and nutritional responses during the growth of the plant. At present, little is known about the hormonal relationship between nodules and plants, although the work of Peat, Minchin, Jeffcoat and Summerfield (1980) indicated a positive effect of hormones, originating from flower buds, on nitrogen fixation. Further efforts need to be made in legume breeding so that improvements are made in the ability of grain legumes to fix nitrogen under drought conditions and in the high fertility soils where they are frequently grown (Oram and Brock, 1972; Jermyn, 1977; Gridley and Evans, 1979; Gibson, 1980).

The energy costs of nitrogen fixation have received considerable attention in recent reviews (Pate and Flinn, 1977; Pate and Minchin, 1980; Gifford and Evans, 1981; Pate, Atkins and Rainbird, 1981). Minchin *et al.* (1981) considered that although quantitative estimates of the relationships between carbon and nitrogen are most informative, indirect evidence based on manipulations of the plant or its environment provide insight into the competitive restraints under which the nodules function, and of their potential when relieved of these restraints.

There are many reports of manipulations which have reduced photosynthesis and nitrogen fixation of grain legumes, such as reductions in light or by defoliation (Lawrie and Wheeler, 1973; Lawn and Brun, 1974a; Bethlenfalvay and Phillips, 1977; Bethlenfalvay, Abu Shakra and Fishbeck, 1978; Schweitzer and Harper, 1980; Sheikholeslam, Fishbeck and Phillips, 1980). Nitrogen fixation is frequently increased when treatments are imposed which increase photosynthesis (Figure 1.2).

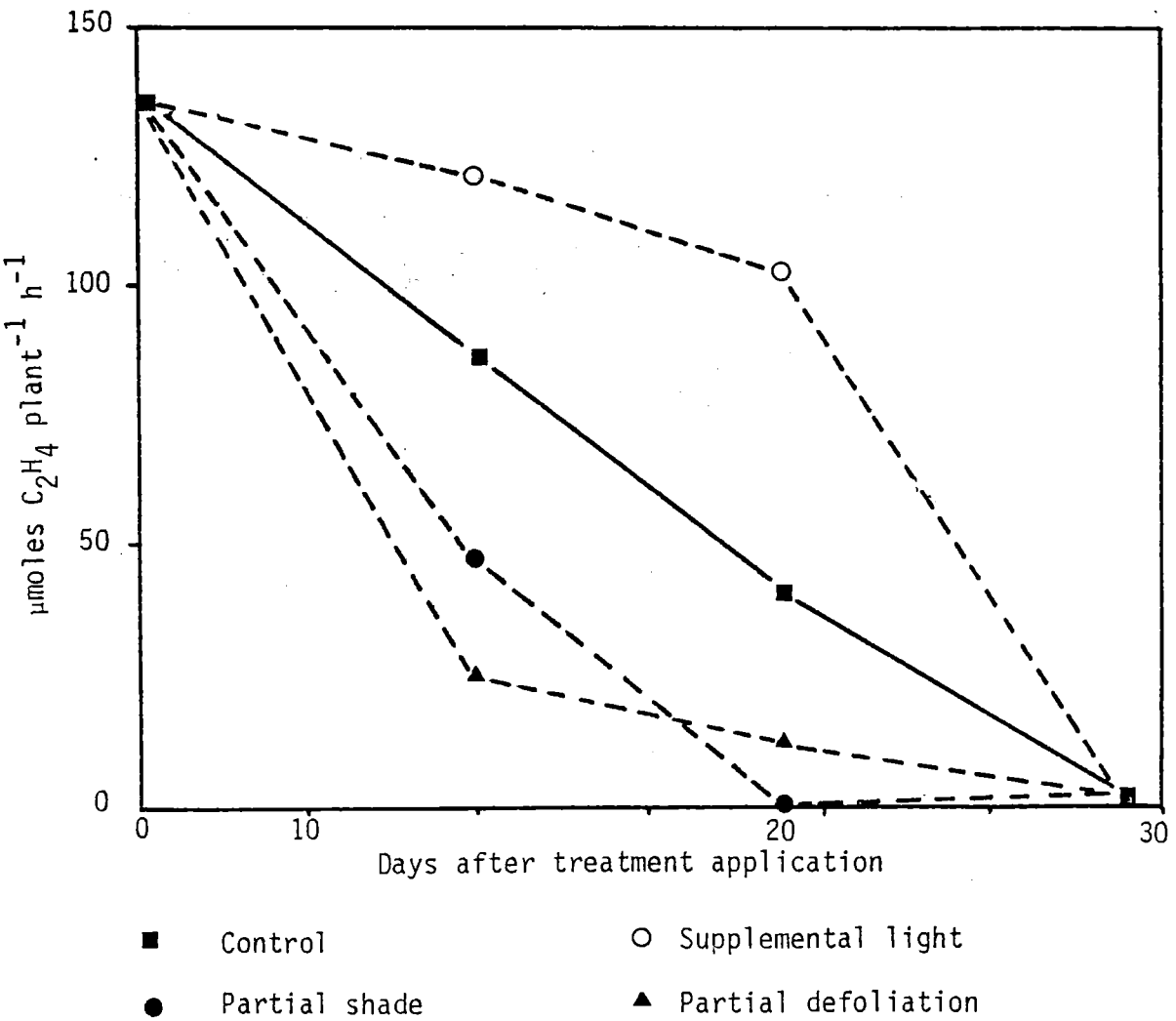


Figure 1.2: Effect of photosynthetic source sink manipulation treatments on total acetylene reduction activity of nodules per plant for soybean cv. Clay (25 plants m^{-2}; 11,200 kg ha^{-1} of ground corn cobs incorporated 4 weeks before sowing). (From Lawn and Brun, 1974)

These may include increased light or carbon dioxide, or the grafting of a second shoot onto a root (Quebedeaux and Hardy, 1973; Streeter, 1973; Lawn and Brun, 1974; Hardy and Havelka, 1976; Sheikholeslam *et al.*, 1980). The principal short-term effect of a greater carbon supply on nitrogen fixation in grain legumes is to increase nitrogen fixation efficiency. In the longer term, increases in nodule mass per plant, the rate of growth of nodules and a later onset of nodule senescence, are more important (Minchin *et al.*, 1981). The effects of supplementary carbon dioxide on nitrogen fixation and growth of grain legumes vary with concentration, and growth conditions (Minchin *et al.*, 1981). Phillips, Newell, Hassall and Felling (1976) showed that long-term carbon dioxide enrichment of peas increased growth when grown at an insolation of 5.5 MJ m^{-2} day^{-1}. Short-term responses to carbon dioxide enrichment were also observed. However, this may have resulted from increased photosynthate supplies, or from additional carbon skeletons supplied, which may have been used to remove inhibitory levels of nitrogen compounds from the nodules. The first explanation is more likely at the low insolation levels used in their experiment.

There are also reports of increased nitrogen fixation after reproductive portions of peas have been removed (Roponen and Virtanen, 1968; Lawrie and Wheeler, 1974), although the removal of fruits has also been shown to decrease nitrogen fixation, at least initially (Hardy, Holsten, Jackson and Burns, 1968; Bethlenfalvay *et al.*, 1978a; Peat *et al.*, 1980). The removal of reproductive sinks stimulates rapid vegetative growth (Roponen and Virtanen, 1968) and thus the demand for nitrogen may also be increased. Plant responses to these treatments will therefore influence the observed changes in nitrogen fixation.

The physical damage caused by treatments such as depodding may cause structural and hormonal changes in the plants (Lawrie and Wheeler, 1974). Minchin *et al.* (1981) noted that manipulations are

frequently done in controlled environments and often fail to produce plants which resemble their counterparts in the field. This limits the usefulness of conclusions from these experiments. Clearly, such manipulation studies are complicated by endogenous control mechanisms which allow the magnitude of nitrogen fixation to reflect, if not always to meet, the demands of the host plant (Minchin *et al.*, 1981).

Flinn (1974) studied the regulatory nature of the relationship between leaves and the adjacent fruit in peas. Leaflet photosynthesis rose and fell in response to changing demand for assimilate by the developing pods and peas. Pate and Flinn (1977) concluded that in general, leaves do not continuously operate at the peak photosynthetic rates of which they are capable, but are constrained by factors which have in turn influenced the size and activity of the sink regions which they supply. These factors are most likely hormonal rather than caused by a carbohydrate build-up in leaves which could reduce photosynthesis (Pate and Flinn, 1977).

1.3.1.1 Hydrogen Evolution: Theoretical estimates of the cost of nitrogen fixation in terms of energy (ATP) utilisation are complicated by the ATP-dependent evolution of hydrogen, catalysed by nitrogenase (Schubert and Ryle, 1980). During catalysis by nitrogenase, protons and nitrogen compete for electrons (Dixon, 1968). A survey by Schubert and Evans (1976) of nodulated legumes and non-leguminous nitrogen-fixing plants showed that in air, 40 - 60 per cent of the total electron flux available for the reduction of atmospheric nitrogen, by either excised nodules or intact nodulated plants, was utilised in the production of hydrogen gas. They demonstrated considerable differences in hydrogen evolution among legume species and used a term "relative efficiency", which expressed the electrons used to produce hydrogen as a proportion of the total electron flow through nitrogenase. Estimates were made in air

where electrons passed to protons and to nitrogen. In argon, instead of nitrogen, all electrons passed to hydrogen so that:

$$\text{Relative Efficiency} = 1 - \frac{H_2 \text{ (in air)}}{H_2 \text{ (in argon)}}$$

The fact that no hydrogen was produced when plants were incubated with saturating levels of acetylene, allowed the relative efficiency to be calculated by:

$$\text{Relative Efficiency} = 1 - \frac{H_2 \text{ (in air)}}{C_2H_4 \text{ (in argon)}}$$

Relative efficiencies were governed by ATP concentration, pH, and substrate concentration (Hwang and Burris, 1972; Dixon, 1975).

The theoretical relationship between acetylene reduction and nitrogen fixation resulted from two electrons being used to reduce acetylene to ethylene, and six to reduce nitrogen to ammonia, i.e. three acetylene molecules are equivalent to one di-nitrogen molecule (Sprent, 1979). Wide variations from this theoretical relationship have been found (Bergersen, 1970), and hydrogen evolution can have a dominant effect. Water stress (Minchin and Pate, 1975) and combined nitrogen (Oghoghorie and Pate, 1971) may also cause a departure from the theoretical value of 3:1.

Although theoretically hydrogen evolution could significantly reduce nitrogen fixation, the presence of hydrogenase in some cultivar: *Rhizobium* combinations allows the re-utilisation of the hydrogen produced (Schubert and Evans, 1976). Dixon (1975) suggested several possible functions of hydrogenase:

a) to prevent the build-up of inhibitory concentrations of hydrogen in nodules;

b) to protect nitrogenase from oxygen inactivation;

c) to metabolise the hydrogen evolved during nitrogen reduction and so to conserve a portion of the otherwise wasted energy.

If photosynthate supply to nodules is a major limiting factor for legume nitrogen fixation, then the recycling of hydrogen via hydrogenase may lessen the demand of nodules for photosynthate, and thus increase dry matter production. Schubert, Jennings and Evans (1978) compared the yield and efficiency of nitrogen fixation by soybean and cowpea plants with nodules which recycled hydrogen, and those which lacked an active hydrogenase and therefore evolved the hydrogen produced during nitrogen fixation. They observed a small increase in the efficiency of nitrogen fixation by a cultivar nodulated with a *Rhizobium* strain capable of re-utilising hydrogen. As they conceded, however, conclusive evidence of the role of the hydrogen recycling process in nitrogen-fixing efficiency required comparisons with strains that are genetically identical, with the exception of the presence or absence of hydrogenase.

From the preceding discussion, it appears that when photosynthate supply limits nitrogen fixation the relative efficiency of the symbiotic system becomes an increasingly important limit for dry matter production. However, when Gibson (1978) studied eight legumes, each inoculated with varying *Rhizobium* strains, he found a negative correlation between relative efficiency and dry weight, which was completely contrary to that expected by Schubert *et al.* (1978). Hydrogen evolution was positively and significantly correlated with plant size and total plant nitrogen. Similar trends were observed in subterraneum clover (*Trifolium subterraneum*), white clover (*Trifolium repens*), peas, tick beans and blue lupins. Soybeans, from three to seven weeks after inoculation, showed that relative efficiency increased with time in all symbiotic combinations (Gibson, 1978). Similar trends have been observed in peas and common beans (*Phaseolus vulgaris*) (Bethlenfalvay and Phillips, 1977a).

Relative efficiency also increased with reduced light intensity (Bethlenfalvay and Phillips, 1977b; Gibson, 1978), nitrate fertiliser applications (Gibson, 1978) and was greatest during the night in subterraneum clover (Gibson, 1978). The experiments of Gibson (1978) and Bethlenfalvay and Phillips (1977a, b) indicate that hydrogen evolution relative to acetylene reduction is greatest under conditions of maximum photosynthate supply. Thus hydrogen evolution may be a result of "spill-over" respiration - i.e., energy is produced that cannot be used in nitrogen fixation, and so hydrogen evolution is the easiest means of getting rid of this excess energy (Gibson, 1978; Li, Chin, Zhao, Zhang and Zhou, 1980).

1.3.1.2: Diurnal Cycles: The advent of the acetylene reduction technique has allowed numerous studies of diurnal fluctuations in nitrogen fixation, but Minchin *et al.* (1981) note the considerable confusion in the data now available. Fixation activities have been expressed in different units, and techniques and growth conditions have varied widely. A 'typical' diurnal profile of nitrogen fixation shows a daytime peak followed by a declining rate at night (Minchin *et al.*, 1981). This is generally taken to indicate that nitrogen fixation relies on current photosynthesis (e.g. Bergersen, 1970; Hardy and Havelka, 1976; Chunderova and Alisova, 1979), although data on the current rates of photosynthesis, below-ground respiration and on soluble carbohydrate concentrations in roots and nodules are consistently lacking (Minchin *et al.*, 1981).

Storage compounds such as glycogen and poly-B-hydroxybutyric acid in bacteroids may represent up to 50 per cent of the dry weight of soybean nodules (Rawsthorne, Minchin, Summerfield and Cookson, 1980). These compounds may be used to support nitrogen fixation during periods of darkness. The large tap-root of lupins appears to be capable of

buffering the nodule system against carbohydrate deficiency during the night (Trinick, Dilworth and Grounds, 1976). These buffering compounds may explain the frequent reports of night-time peaks in activity, while other patterns suggest the over-riding effects of one environmental factor or another (Minchin *et al.*, 1981). Clearly, differences in plant morphology and phenology (Halliday, 1976, quoted in Minchin *et al.*, 1981; Ayanaba and Lawson, 1977), variations in light intensity (Sheikholeslam *et al.*, 1980), air and soil temperatures (Minchin and Pate, 1974; Chunderova and Alisova, 1979) are important factors in determining the diurnal patterns of nitrogen fixation (Minchin *et al.*, 1981). Temperature may be particularly important as Chunderova and Alisova (1979) showed in peas, where fixation closely followed temperature changes in diurnal cycles, up to a maximum of 32°C. This is a considerably higher temperature than that normally considered optimum for pea growth and nitrogen fixation. In Minchin and Pate's (1974) study, the effect of two environments on nitrogen fixation were assessed in peas, each with a 12 h day (5.5 MJ m^{-2} day^{-1}). The first was at 18°C continuously, whereas the other was at 18°C during the day and at 12°C during the night. In the constant temperature, fixation was higher during the day, but in the changing environment, slightly less nitrogen was fixed during the day than during the night. Although during the night similar amounts of carbohydrate were available for fixation, the cooler environment allowed more efficient use of this carbohydrate. An important practical application of this study was made by Pate (1976). The apparent efficiency of consumption of translocate in nitrogen fixation during the photoperiod (13.6 mg carbohydrate mg N $fixed^{-1}$) was considerably less than at night (5.3 mg carbohydrate mg N $fixed^{-1}$). Thus, if carbohydrate supply allows fixation during the night, the efficiency of use will be greater. In the Canterbury environment, nights are often cool and with the long summer days, optimum conditions for fixation may

exist.

Appropriate agronomic studies could elucidate factors which influence carbohydrate supply, and their relationship with nitrogen fixation in peas. Nitrogen demand by pods and peas of determinate cultivars will be greater than those of indeterminate cultivars which fill pods over a longer period. When photosynthate supply is below optimum for both pod fill and nitrogen fixation, an indeterminate flowering pattern may confer significant benefits on the seasonal pattern of nitrogen fixation, due to a longer period of pod filling. With an indeterminate flowering habit, seed nitrogen demands may be able to be met largely from current nitrogen fixation and soil nitrogen uptake, and leaf nitrogen would not need to be translocated to the developing fruits. Thus, photosynthesis and nitrogen fixation would continue at high rates, at a time when determinate cultivars had entered the 'self destruct' cycle.

1.3.2 Combined Nitrogen

Nodule formation in grain legumes is depressed in the presence of combined nitrogen, particularly nitrate nitrogen (Dixon, 1969; Munns, 1977). In his review, Pate (1977a) considered that nodule initiation in peas is less sensitive to high levels of nitrate supply than is the nodulation of many other herbaceous legumes. Even so, nitrate reduces root hair curling in peas, and thus nodule numbers are reduced compared with those grown without combined nitrogen. A direct effect of nitrate on pea root hairs was shown when only one part of a divided root system was exposed to nitrate and in only that part was nodulation reduced (Gaumann, Jaag and Roth, 1945, quoted by Virtanen and Mietten, 1963). The effect of varying levels of nitrate on nodule size and number have been assessed for the field pea (Oghoghorie and Pate, 1971). All plants in their study

were inoculated at sowing and grown for one week in nitrogen-free culture solution (pH 6.5). Thereafter, plants received a range of nitrate nitrogen up to 315 ppm N in the culture solution. Red nodule numbers were reduced by approximately 40 per cent, and nodule size was reduced by approximately 70 per cent, in the range from 0 to 315 ppm N, 28 days after sowing. Nodule numbers in this experiment may have been affected more severely by the nitrate if a direct inhibitory effect of nitrate on root hair curling is important. It is possible that the peas in this study had initiated nodules before the nitrate treatments were applied, as Lie (1969) showed that pea seedlings may develop nodule initials within six days of inoculation with *R. leguminosarum*. Although the degree of inhibition on nodule formation and nitrogen fixation by combined nitrogen is open to question in field-grown peas, it is clear that nitrogen fixation is reduced by high levels of nitrate. In contrast at low levels of added nitrogen, nitrogen-fixing activity may be stimulated. Studies by Allos and Bartholomew (1959), Oghoghorie and Pate (1971) and Hoglund (1973) have shown that nitrogen fixation and plant nitrogen uptake were increased by low levels of combined nitrogen in a range of legumes. The initial peak in nitrogen yield (Figure 1.3) which resulted from the addition of combined nitrogen, was dependent on that nitrogen being added to the nitrogen demand of the host. The stimulation in growth may increase nitrogen-fixing activity in peas as shown by Oghoghorie and Pate (Figure 1.3). However, further additions of combined nitrogen actively suppressed nitrogen fixation at a level of combined nitrogen supply which was inadequate for the nitrogen demands of the host. Further increases in nitrogen supply allowed growth to increase over and above the initial peak.

When nodulated pea plants are exposed to combined nitrogen, extremely active nitrate reductases develop in all of their tissues (Wallace and Pate, 1965). These reductases allow growth on high levels of

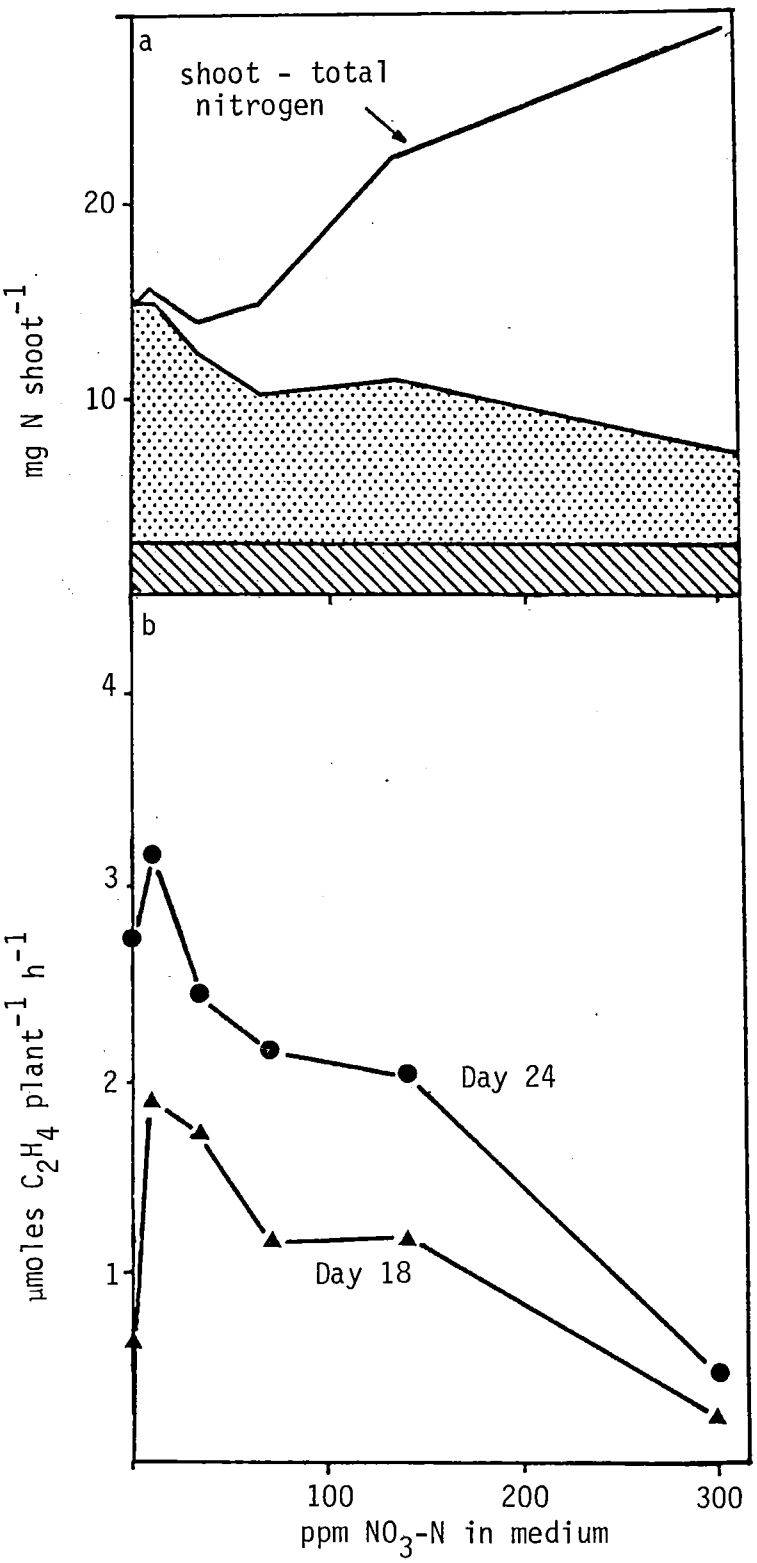


Figure 1.3a: Dependence of shoot on nitrogen from cotyledons ▨, from nitrogen fixation ⁘ and from nitrate □.

b: Acetylene reduction assays at two dates comparing the performance of nodulated roots of field pea when grown without nitrate or when exposed to a range of constantly maintained levels of nitrate (from Oghoghorie and Pate, 1971).

combined nitrogen to be somewhat superior to that on any known association with *Rhizobium* (Oghogorie, 1971, quoted by Oghoghorie and Pate, 1971). In his review, Gibson (1977) concluded that effects of combined nitrogen under field conditions are not fully understood. It is important to understand the process of mineralisation of organic nitrogen and its changes with soil depth, organic matter and climatic changes. Figure 1.4 shows the marked increase in mineral nitrogen levels Hart (1978) recorded during late spring and early summer in the Canterbury environment. These results were obtained from a Templeton silt loam soil at Lincoln, which had a pH of 5.5, per cent C and N of 4.5 and 0.24 respectively between 0 and 20 cm. Soil pH increased with depth, but per cent C, N and C:N ratios decreased with increasing depth. Both fallow and wheat treatments were established in cultivated plots. During spring and summer, ammonium levels were always less than 1.5 g m^{-2}, between 0 and 80 cm. Thus nitrate nitrogen was the most important component of total mineral nitrogen (Figure 1.4), and most of this was concentrated in the top 40 cm. The proportion at each sampling depth, however, was variable and associated with both rainfall, and plant nitrogen uptake where wheat plants grew. Other studies in New Zealand (Tham, 1971; Hoglund and Brock, 1978; Quah, 1980) and Australia (Simpson, 1962) have shown similar increases in mineral nitrogen levels during the late spring and summer periods. It is therefore likely spring crops such as peas experience increased levels of available mineral nitrogen during their growth in this locality. Peas are frequently grown after pasture where organic nitrogen levels are high and mineralisation of this would be adequate for plant nitrogen demands. In contrast, nitrogen mineralisation is reduced on soils where fertility has been depleted by intensive cropping. Under these conditions peas would have to rely more on nitrogen fixation than on mineral nitrogen. Rhodes (1980) grew peas on a fertility depleted soil where per cent C (0 - 15 cm) was 2.3

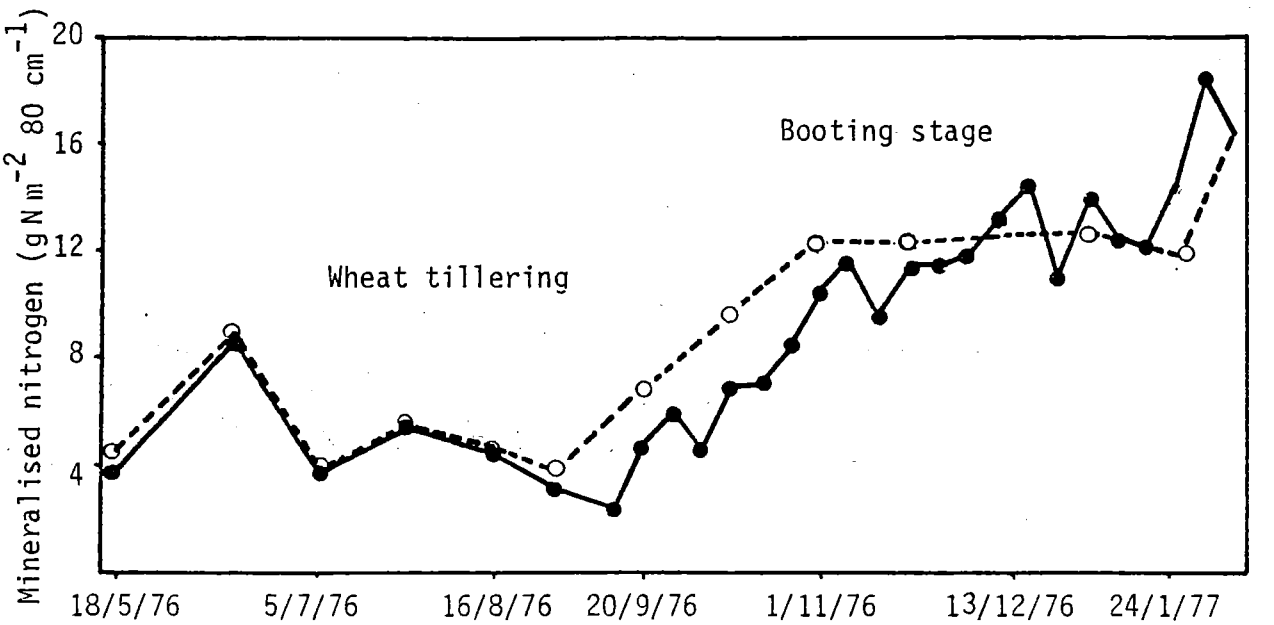


Figure 1.4: Net soil nitrogen mineralised under fallow ●——● and under wheat ○- - -○; data from Hart, 1978.

and mineral nitrogen on fallow plots (0 - 20 cm) was 0.7 and 0.5 g N m^{-2} in late spring and late summer respectively. Peas fixed approximately 100 per cent of their nitrogen in these conditions. Nodulation was depressed in the range of grain legumes studied by Dean and Clark (1980), when 20 kg N ha^{-1} were applied as ammonium nitrate. Allos and Bartholomew (1959) concluded that the capacity to fix nitrogen from the atmosphere is related to factors which are closely correlated with plant growth and that legumes use nitrogen preferentially from the soil, nitrogen fixation occurring only when the soil supply is inadequate.

Field-grown peas rarely respond to nitrogen fertiliser (Mulder, 1948; Pate, 1977a), and thus nitrogen from symbiosis or that mineralised from soil organic matter or both are able to supply requirements. Studies in Canterbury have also shown nil or only small yield increases to nitrogen fertiliser in grain legumes (Quah, 1980; Newton, 1980). The proportions of plant nitrogen which come from the soil or from fixation are frequently difficult to assess. Virtanen and Hausen (1952) attempted to show these proportions, by making use of the observation that ineffective nodules, once formed, block the formation of effective nodules in peas. Thus, differences in nitrogen uptake of peas preinoculated with effective or ineffective strains of *Rhizobium*, grown in pots with soil contaminated with resident rhizobia, could show the proportion of plant nitrogen which came from fixation. Their results suggested that the peas obtained approximately 67 per cent of the nitrogen from the symbiosis. The total amount of plant-available nitrogen in the pots used by these workers is not known, and therefore this percentage cannot be accepted as necessarily being the proportion for field-grown peas where mineral nitrogen supply may be greater. The technique could be extended to field-grown peas, however, and in those circumstances it would be a useful assay of total nitrogen fixation. The assumption that an ineffective *Rhizobium* strain would block the formation of nodules by

effective strains would require further validation as Russell (1973) noted that a legume root can carry nodules formed by several *Rhizobium* strains.

Combined nitrogen may reduce nitrogen fixation by reductions in root carbon reserves through stimulation of nitrogen reductase in shoots and roots (Small and Leonard, 1969; Pate, 1976).

1.3.3 Incorporation of Straw

The addition of straw to the soil causes changes to a number of soil properties which in turn may affect nitrogen fixation by legumes. During the decomposition of straw, carbon dioxide is produced, inorganic nitrogen is immobilised, microbial activity is stimulated, soil aeration is enhanced and bulk density is decreased (Williams and Cooke, 1961; Russell, 1973; Ham, 1978; Shivashankar and Vlassak, 1978; Waddington, 1978). Cereal residues are frequently burnt after harvest. This helps to kill weed seeds and reduce disease and pest carry-over to subsequent crops. While the wide-scale incorporation of straw would thus have some beneficial effects on soil structure and organic matter levels, it could also increase weed, disease and pest problems in subsequent crops.

In compacted soils, straw incorporation may benefit nitrogen fixation because of increased aeration. In solution culture, Virtanen and Hausen (1936) showed that pea nodules, *per se*, have a direct requirement for oxygen. It seems that peas have a greater requirement than a number of other crops (oats, potato and tulip) (Wiersum, 1979), and thus the increased aeration following straw incorporation may particularly enhance root development and nodulation of peas. The shallow rooting which is caused by poor soil aeration can restrict the uptake of nutrients. In Wiersum's experiments, phosphorus and potassium were more severely restricted than either calcium or nitrate. The stimulation of micro-organism activity (Russell, 1973) is also likely to increase *Rhizobium*

numbers (Shivashankar and Vlassak, 1978) by providing a suitable substrate for proliferation. Thus nodule numbers may be increased.

Carbon dioxide applied in a partially enclosed soybean canopy is known to increase nitrogen fixation and growth (Hardy and Havelka, 1973; Shivashankar, Vlassak and Livens, 1976a, b; Shivashankar and Vlassak, 1978), but in a field situation, carbon dioxide produced during the decomposition of straw is not likely to enhance photosynthesis. Russell (1973) considered that although the rate of carbon dioxide production is increased by the incorporation of organic matter, the air space in the soil and hence the rate of diffusion of carbon dixoide into the atmosphere is also increased.

The reduced availability of combined nitrogen after the incorporation of organic matter which has a high C:N ratio, is frequently held responsible for increased nitrogen fixation by legumes (Pinck, Allison and Gaddy, 1946; Weber, 1966a; Kohl, Shearer and Harper, 1980). Many workers (e.g. Thornton, 1929; Hardy and Havelka, 1973; Waters, Graham, Breen, Mack and Rosas, 1980) have detailed the effects of organic substances on nitrogen fixation. There is a need, however, for studies which relate both the effect of straw on the availability of combined nitrogen over time, and its effect on nitrogen fixation.

The type of straw used may be important, although this is frequently not mentioned. Rice hulls as a surface mulch have benefited nitrogen fixation in common bean (Waters *et al.*, 1980), although rice straw inhibited growth of *R. leguminosarum* and *R. japonicum* (Rice, Lin and Huang, 1981).

1.3.4 *Rhizobium*

The development of an effective symbiosis between *R. leguminosarum* and host plants is very important if nitrogen fixation in peas is to be maximised. Reviews by Vincent (1970), Bergersen (1978), Vincent (1980) and Minchin *et al.* (1981) have discussed the study of

root nodule bacteria; infection and nodule development; genetic factors controlling the symbiosis, and efficiency of carbon usage by *Rhizobium* strains. In New Zealand, peas form effective nodules very readily with resident rhizobia and crop yields are often greater than 4 t ha^{-1} without inoculation (White and Anderson, 1971; Falloon and White, 1978; Wilson, Hanson and Jermyn, 1981; White, Sheath and Meijer, 1982). Although a yield increase of 81 per cent after inoculation in peas has been obtained in Australia on a low fertility soil (0 - 10 cm < 0.05% N) which lacked resident rhizobia (Evans, pers. comm.), it is unlikely that similar increases would be obtained here. This is because in Canterbury, uninoculated peas form effective nodules a few days after emergence in the spring. Lie (1969) also observed nodule initiation only six days after inoculation. Fully adapted resident strains of rhizobia in the soil are likely to be more competitive and numerous than introduced strains (Johnston and Beringer, 1976), and thus these resident strains would be likely to form the majority of the pea nodules. In tick beans, Candlish and Clark (1975) found no difference between ethylene production of plants nodulated with 'wild type' and those with commercial *Rhizobium* strains, although Dean and Clark (1979) showed increased ethylene production and nodulation but no increase in dry matter from inoculation with selected *Rhizobium* strains.

Effective *Rhizobium* strains form few, large nodules on upper regions of the main root axis in peas (Pate, 1977a), and uninoculated peas in Canterbury generally show this type of nodulation. However, substantial differences exist between the amount of nodule tissue formed by individual *Rhizobium* strains even when these strains prove equally effective in nitrogen fixation (Pate, 1977a), while conversely cultivars may exhibit substantial differences in efficiency of nitrogen fixation when inoculated with the same *Rhizobium* strain (Pate, 1977a). Mahon's (1979) studies failed to show that *Rhizobium* induced differences in carbon usage

during nitrogen fixation, but some strains of *Rhizobium* (Bethlenfalvay, Abu-Shakra and Phillips, 1978b) exhibited improved nitrogen fixation, and induced greater photosynthetic efficiency in host plants when grown in a constant environment. The fifteen *Rhizobium* strains used by de Jong and Phillips (1981) produced pea plants with different rates of nitrogen-fixing activity and total nitrogen content at the same morphological stage of development. Nitrogen fixation interacted with leaf photosynthetic efficiency and plant growth in a manner that was dependent on the allocation of symbiotically-fixed nitrogen.

Evaluation of *Rhizobium*:cultivar interactions must be finally done in the field, and improvements in nitrogen fixation are more likely when selection is made in both *Rhizobium* and host (El-Sherbeeny, Lawes and Mytton, 1977; Sprent, 1979). Although the acetylene reduction assay can give a useful initial evaluation (Boonkerd, Bezdicek and Weber, 1978), isotopic techniques will be more reliable, as hydrogen evolution does not confound the results, while indirect measurements such as nodule mass or number may have little correlation with the actual nitrogen fixation.

When an effective strain has been isolated, various methods of inoculation are available (Vincent, 1970; Dean and Clark, 1979) and single inocula have been found to be more effective than multiple ones (Clark, 1980). Inoculation procedures aim to maximise the preferred rhizobia in the rhizosphere to give introduced strains a competitive advantage over resident strains (Bergersen, 1978). Once formed, nodules may actively prevent new nodule formation (Pate, 1977a) and in the long-term, rhizobia survival in the soil is of paramount importance for annual crops (Johnston and Beringer, 1976; Roughley, Blowes and Herridge, 1976). *R. japonicum* strains have survived ten years without soybean crops (Elkins, Hamilton, Chan, Briskovich and Vandeventer, 1976), and individual strains show varying environmental preferences (Mahler and Bezdicek, 1980).

The mineral nutrition of rhizobia is particularly affected by pH, and changes in pH have two main effects. Firstly, the availability of micro-nutrients is generally enhanced with increasing acidity; with the exception of Mo (Russell, 1973). In the second instance, processes of nodule formation may be directly retarded by acid conditions. Lie (1969) showed for peas that a pH of less than 5.0 caused nodule failure in peas, although one *Rhizobium* strain tested formed nodules and fixed nitrogen at pH 4.6. *Rhizobium* strains *per se* were not directly inhibited by low pH. Instead, Lie considered that early stages of nodule development, possibly after root hair infection, were particularly acid sensitive. In peas, the overall requirements of the symbiosis have been shown to be ten times as sensitive to acid conditions than either root or bacterial growth alone (Evans, Lewin and Vella, 1980). Coatings on pea roots change with soil acidity. Lie (1969) observed that roots growing in a neutral soil solution and inoculated with *Rhizobium* were covered with a slime layer or bacterial cells which resembled a rhizosphere. In acid conditions, however, only a thin slime layer was present. This layer may protect roots from the injurious effects of H-ions (Lie, 1969).

Nodules are rich in Mo, Co, Fe, Zn, P, S and N (Munns, 1977). Deficiencies of Mo, Co and B are particularly serious as they are important for nodule function. Molybdenum is a component of nitrogenase, and its importance for nodule function in peas has been shown by Mulder (1948). In New Zealand, many soils are deficient in this nutrient (During, 1972). Although liming increases the availability of Mo, the misdiagnosis of Mo deficiency as an acidity problem has in the past led to inflated estimates of the lime requirements of legumes (Munns, 1979). Cobalt is an important component of cobamide co-enzymes which are needed for rhizobia functions (Munns, 1977). Symptoms of born deficiency in peas are similar to nitrogen deficiency symptoms, but Mulder (1948) showed that the boron require-

ment of the growing leaves and stems in general is greater than that of the nodules. When the growth medium was totally boron-deficient, however, nodule formation was more affected than tops. Iron is an important component of leghaemoglobin. It facilitates oxygen diffusion to support vigorous respiration while protecting the oxygen-sensitive nitrogenase system. Nodule requirements for Fe are small in comparison to whole plant requirements (Munns, 1979) as are the requirements for P and S. If growth of the roots and tops is not limited by inadequate P and S, then there will be sufficient of these nutrients for the nodule requirements.

Deficiencies of Mo and Co cause nodule failure, by preventing essential enzyme formation. By contrast, Zn deficiency primarily limits pea growth (Mulder, 1948). Similarly, deficiencies of Fe, Mn and Cu produce symptoms unrelated to nitrogen deficiency and are not known to be corrected by supplying combined nitrogen (Munns, 1977).

In conclusion, it seems that in Canterbury improved nitrogen fixation is unlikely to occur because of altered *Rhizobium* strains, particularly because of the competitive ability and survival of local strains. There is scope, however, for enhancement of nitrogen fixation with the present *Rhizobium*, by adjusting agronomic practices to optimise nitrogen fixation.

1.3.5 *Water*

Water supply has a major effect on legume nodulation and nitrogen fixation, both of which are reduced in stress conditions (Sprent, 1971). In Canterbury, grain legume yield is frequently limited by drought (Anderson, 1971; Anderson and White, 1974; Stoker, 1975, 1978; White *et al.*, 1982), but there are no reports which detail effects of drought on nitrogen fixation. Legumes use various mechanisms to

escape, withstand or recover from drought (Turner, 1979). Rapid phenologic development (Fischer and Turner, 1978) may allow the legumes to escape the usual effects of drought, and this may be manipulated by time of sowing and by the use of indeterminate cultivars (Elston and Bunting, 1980; Shibles, 1980) which allow developmental plasticity (Turner, 1979). Peas chosen for rapid development will have less time in which to fix nitrogen.

Conventional peas are not specially adapted to withstand drought (Maurer, Ormrod and Fletcher, 1968; Miller, Manning and Teare, 1977). Semi-leafless peas, however, may show enhanced water use efficiency and thus ability to yield well under dry conditions (Snoad, 1980, 1981) but recent studies in Canterbury showed only small advantages in water use efficiency of these peas (Wilson, Hanson and Jermyn, 1981). Some success, however, has been achieved in breeding both for increased rooting depth in soybeans (Taylor and Klepper, 1978) and for stomatal control to minimise water loss during drought (Turner, Begg, Rawson, English and Hearn, 1978; Turner, 1979). Grain legumes also differ in their depth of water extraction, and ability to reduce soil water potential (Lawn, 1982b). In response to changing soil moisture, legumes are also able to change the proportion of total nitrogen fixation which occurs at different depths in the soil profile. Hoglund and Brock (1978) showed that the proportion of nitrogen fixation in a white clover pasture occurring in the top 75 mm soil horizon was 90 per cent in winter and 10 per cent under dry summer conditions. Identification of pea cultivars which can use some or all of the above mechanisms to tolerate or avoid drought will enable maximum production and nitrogen fixation.

Drought affects plant growth in a number of inter-related ways and these will directly or indirectly affect nitrogen fixation. As drought increases, photosynthesis decreases (Beardshell, Mitchell and Thomas, 1973; Huang, Boyer and Vanderhoef, 1975b) and these effects could

arise from changes in stomatal resistance, reduced leaf area (Sivakumar and Shaw, 1978a, b) and/or internal variables (Elston and Bunting, 1980). Drought increased leaf death in *Vicia faba* (Elston and Bunting, 1980), and reduced translocation of nutrients from older leaves to the seed in lupins (Hocking, 1982). Finally, drought decreases biomass yield and often reduces harvest index (Fischer and Turner, 1978).

1.3.5.1 Effects of Drought on Nodule Function: Drought reduces rhizobia survival in the soil (Foulds, 1971), limits their movement and reduces root hair infection which in turn limits nodule formation (Worrall and Roughley, 1976). Water stress occurs in nodules when the root system cannot supply sufficient water to maintain nodule turgidity. This reduces nitrogen fixation and prevents the export of products of fixation (Sprent, 1976a). Minchin and Pate (1973) calculated that nodules required 0.35 ml of water for the transport of each mg of fixed N to the host via the xylem, and they estimated that the flow of water to the nodule in the phloem would not be sufficient. When soils are moist, further water supply could occur via direct water uptake in the nodules, although Minchin and Pate (1973) considered that nodule surfaces are more adapted to gas than water exchange. For the nodules used by Minchin and Pate (1973), Pate (1976) calculated the nodule water requirements would have been met from the phloem (20%), surface intake (13%) and the remaining 67 per cent from root water uptake. For optimum nodule function and nitrogen fixation rates, soils need to be near field capacity (Sprent, 1972c), but not subject to waterlogging (Minchin and Pate, 1975). When soil moisture deviates from the optimum, nitrogen fixation is reduced in most legume species, e.g. cowpea (Zablotowicz, Focht and Cannell, 1981), tick bean (Sprent and Bradford, 1977; Gallacher and Sprent, 1978), common bean (Sprent, 1976), pea (Masefield, 1968; Minchin and Pate, 1975), soybean (Pankhurst and Sprent, 1975; Sprent, 1975, 1976a, b; Adjei-Twum and

Splittstoesser, 1976) and in white clover (Engin and Sprent, 1973; Hoglund and Brock, 1978).

When water loss from nodules is not more than 20 per cent of the maximum fresh weight, effects of water stress are reversible (Sprent, 1971a), but Sprent (1971b) showed irreversible damage in soybean nodules from severe drought. Less severe drought stress directly reduced bacteroid activity (Sprent, 1976) while Pankhurst and Sprent (1975) showed that resistance to oxygen diffusion increased in stressed soybean nodules. The soil surface is frequently drier than deeper layers, and nodules in the surface zone may maintain nitrogen fixation from water supplied via the xylem (Sprent, 1972c; Hume, Criswell and Stevenson, 1976; Farrington *et al.*, 1977).

Pate (1976) considered that where water stress is sufficient to cause wilting of lower leaves, photosynthesis is likely to be arrested. Because these leaves are the main providers of carbon to the nodules (Pate, 1966), it is possible that the first reduction in nitrogen fixation during drought will be caused by reduced assimilate supply to the nodules. Drought may also cause reduced transpiration and thus produce a build-up of fixation products (Minchin and Pate, 1974). These workers showed that fixation products accumulated in nodules at night when water loss from shoots was at a minimum and a similar accumulation occurred in nodules during the day when high humidity reduced transpiration. The cool, humid nights of the 18:12 degree day:night environment they used, caused a large accumulation of soluble nitrogen in the nodules. This regime did not prevent nitrogen fixation at night from occurring less effectively than in the day time when transpiration facilitated a much more rapid clearance of fixed nitrogen from the nodules (Minchin and Pate, 1974). Thus, it appears that drought may initially reduce photosynthate supply but finally nodule senescence will reduce activity.

1.3.5.2 Waterlogging: Grain legumes such as peas and common beans are intolerant of waterlogged soils (Sprent, 1979), which cause reduced plant growth (Jackson, 1979; Belford, Cannell, Thomson and Dennis, 1980) and reduced nitrogenase activity (Minchin and Pate, 1975). These effects have been attributed to inadequate oxygen supply to the roots (Ferguson and Bond, 1954; Wiersum, 1979).

Nodule function is more tolerant of low oxygen partial pressure than is nodule initiation (Pate, 1976; Sprent, 1979). Individual nodules respond to waterlogged soils by increasing their surface area, while nodule number and activity decrease (Sprent, 1976a). Thus plants growing in wet soils in winter or early spring may form some nodules, but nitrogen-fixing activity will be limited. However, if soils become fully waterlogged, plant death may occur in 24 hours. Waterlogged soils also frequently show chemical and physical changes (Buresh, Casselman and Patrick, 1980). To avoid these effects, plants have been studied in nutrient culture, so that nodule efficiency can be assessed under varying levels of oxygen. Pate (1976, from unpublished data of Minchin and Pate) showed that in a waterlogged environment, nodule efficiency is less where the nodule is located lower down the root. Insufficient oxygen supply reduced the efficiency of carbohydrate consumption (Bergersen, 1971) and thus the low nitrogen fixation resulted from a misuse of, rather than a restricted supply of carbohydrate (Pate, 1976). Early studies to assess the effect of inadequate oxygen supply to pea roots grown in solution culture (Virtanen and Hausen, 1936) showed that the addition of nitrate nitrogen increased growth. Oxygen present in the nitrate molecule may help to supply the oxygen requirements of the root (Pate, 1976).

In Canterbury, most soils used for pea crops are free draining and therefore waterlogging is not a major problem. However, autumn and early spring-sown peas may be damaged by prolonged rain which causes anaerobic conditions to develop. Even if these conditions persist for

only a day, the plants if not killed, may remain yellow with consequent yield reductions.

1.3.6 *Time of Sowing*

In Canterbury, all garden and many field pea crops are sown in the period August - November, while some field peas are sown in May. There are a number of advantages to be gained from autumn sowing. Autumn-sown crops flower earlier than spring sowings, and this confers a significant advantage for crop yield and possibly also for nitrogen fixation. Autumn-sown tick beans (Newton, 1980) flowered approximately one month earlier than spring-sown. This earlier development allowed crops to mature before the full effects of drought, which is particularly important in Canterbury. On these soils, waterlogging in winter is uncommon and thus increased root development can occur during the longer growing season. This enhanced root growth increases the volume of soil searched, for both nutrients and water. Although little information is available for peas, the yield increases possible in dryland tick beans have been shown by Newton who recorded a 105 per cent increase by autumn sowing compared with spring sowing. Lupins in Canterbury are normally autumn sown (White, 1961), but there are no reports of comparions between spring and autumn sowing for this species in Canterbury. Other advantages to be gained from autumn sowing are the minimising of soil structural damage which is a problem with cultivation of wet soils in early spring (Dawkins, Hebblethwaite and McGowan, 1980), reduced reliance on herbicides for weed control, and the spread of farmers' work schedules. Research in the North Island of New Zealand has shown considerable benefits from autumn sowing of lupins (Withers, 1973; Withers, Baker and Lynch, 1974). Withers *et al.* 1981) considered early sowing was important to maximise seed protein yield, especially from cultivars which develop slowly. Further

research in Canterbury is needed on the effect of autumn sowing of peas.

As sowing date is retarded in the spring, grain legumes generally show reduced yields because of drought stress (Withers, 1979; Martin and Table, 1981; Newton, 1980). Under these conditions, irrigation will enhance yield (Anderson, 1971; White *et al.*, 1982), but Newton showed that irrigating spring crops did not fully compensate for the greater yield obtained by autumn sowing. McCormick (1975) found for Waikato-grown soybeans, however, that mid-season sowings were best because early and late in the season, temperatures were lower than optimum. Later sowings would also reduce total insolation available during seed set, with an increased likelihood of carbohydrate stress which could cause a reduction in nitrogen fixation and growth (Sinclair and de Wit, 1976). Martin and Table (1981) conducted several experiments in Canterbury with vining peas, and concluded that early spring sowing generally resulted in increased yield.

There are a number of reports in the literature on the effect of sowing time on crop yields (Farrington, 1974; McCormick, 1975; Martin and Table, 1981), but the effect of time of sowing on nitrogen fixation has received little attention. Autumn-sown pea crops remain vegetative longer than do spring-sown crops, and thus have the opportunity to fix increased quantities of nitrogen. Cool temperatures during winter are likely to reduce nitrogen-fixing activity in all grain legumes, but during this period nodules may develop which can rapidly take advantage of warmer spring temperatures. Nitrogen fixation in grain legumes is greatest between 20 and 25 degrees, although the optimum for peas may be lower (Minchin and Pate, 1974; Roponen, Valle and Ettala, 1970) and the maximum rate of nitrogen fixation for a crop generally occurs at the late vegetative stage. Therefore, as far as these factors are concerned, peas should be sown so that the late vegetative stage occurs at the optimum temperature for fixation.

1.4 GRAIN LEGUMES IN CROP ROTATIONS

1.4.1 The Use of Pasture to Maintain Soil Fertility

New Zealand and Australian agriculture relies heavily on legume-based pastures to maintain soil fertility and structure for subsequent crop rotations. Beneficial effects of preceding pasture on subsequent crop production in New Zealand have been shown by Sears (1953), and Sears, Lambert and Thurston (1953), and Australian results have been reviewed by Russell (1980). Using a low fertility soil, Sears, Goodall, Jackman and Robinson (1965) estimated that 670 kg N ha^{-1} were fixed by white clover, and they considered that nitrogen fixation would be reduced as fertility increased, and the pasture shifted to grass dominance. In this study, an artificially infertile soil was used and clippings were removed to further deplete mineral nitrogen levels. Later studies by Edmeades and Goh (1978), based on ^{15}N, have shown that nitrogen fixation by white clover in old and fertile pastures (20 year old = 45 kg N ha^{-1} $annum^{-1}$) was indeed less than that recorded for young pastures (2 year old = 142 kg N ha^{-1} $annum^{-1}$). Also under high fertility, but generally young pastures, total nitrogen fixation by white clover, estimated by the acetylene reduction technique (Ball, Brougham, Brock, Crush, Hoglund and Carran, 1979) was closer to 184 kg N ha^{-1} $annum^{-1}$, averaged over 9 sites in New Zealand (Hoglund, Crush, Brock, Ball and Carran, 1979). In Mid-Canterbury, nitrogen fixation was estimated to be 120 and 190 kg N ha^{-1} $annum^{-1}$ respectively for dryland and irrigated, rotationally grazed three year old pastures (Hoglund *et al.*, 1979).

Cultivation reduces the levels of soil organic matter (Sears, Goodall and Jackman, 1965; Russell, 1980). As the amount and type of organic matter affects the availability of plant nutrients (such as sulphur and nitrogen), and soil physical conditions, a normal objective is to maintain soil organic matter and particularly organic nitrogen, at a high

level which can sustain moderate cropping intensity and heavy yields (Russell, 1980).

1.4.2 The Use of Grain Legumes to Maintain Soil Fertility

Although some of the effects of pasture on soil fertility have been documented (Sears, 1953; Ball *et al.*, 1979), there have been fewer reports which assess the effect of grain legumes on soil fertility. Lupins have been grown in Canterbury for seed, and as a green manure to improve soil fertility and subsequent wheat yields (White, 1961), but there were no quantitative comparisons between the effect of lupins and pasture at the time of White's (1961) report. More recently, Douglas, Sinclair and Ludecke (1972) showed increased maize yields in rotation with soybean when compared with continuous maize, and Piggot and Cooper (1980), also in the North Island, reported a significant improvement in soil nitrogen after a third crop of forage lupins when compared with oats or ryegrass. Peas and tick beans yielded well in comparison with ryegrass/oats when grown for winter greenfeed in Canterbury (Janson and Knight, 1980), and caused a following wheat crop to yield double that after tick beans or peas compared with that after subterraneum clover, blue lupin or ryegrass/oats (Janson and Knight, 1980). These studies have shown considerable yield increases in subsequent cereal crops after some grain legume crops were returned to the soil via grazing animals.

The effects on soil fertility and subsequent cereal growth, of a range of grain legumes grown for seed, however, have still to be researched in Canterbury. Rhodes (1980) and Rhodes, Askin and White (1982) have shown that peas and lupins, when compared with cereals, significantly increased yields of subsequent wheat or Tama ryegrass. As most of the fixed nitrogen was removed in the grain legume seed, the dif-

ferences in subsequent crop yields were probably caused by reductions in soil fertility after cereal crops. Yield increases of subsequent crops in rotations with grain legumes have also been measured in Australia (Wells, 1970; White, Elliot, Sharkey and Reeves, 1978); Boundy, 1978; Doyle and Herridge, 1980; Hawthorne and Lewis, 1980), and in the tropics (Sharma and Ambika Singh, 1970; Jones, 1974; Lab, Rajat De and Ram Kala Singh, 1978; Gajindra Giri and Rajat De, 1979; Ahlawat, Singh and Saraf, 1981). The increases in south-east Australia are particularly important because monetary returns from livestock industries have been lower than those from crops. In that area, many farmers have looked to grain legumes and particularly lupins to take over the fertility-building role of annual pastures (Boundy, 1978). The change has frequently been successful, as marked increases in wheat yields have been recorded from areas which previously grew lupins (Table 1.1). The poor lupin yields, however, recorded in 1976, highlight some of the risks in growing this crop and in particular, disease problems which may reduce yields when lupins are grown in succession. Boundy (1978) considered that the major boost to wheat grain yield from lupins was the increased level of soil nitrogen after lupins (Table 1.1). In a high fertility situation, no yield differences were recorded between wheat after wheat and wheat after lupins. Where diseases were a problem, however, they considered that rotating wheat and lupins had beneficial effects by interrupting disease cycles of both crops. More recently, lupins have been shown to reduce the severity of common root rot (*Cochliobolus sativus*) in subsequent wheat crops (Moore, pers. comm.). In New Zealand, lupins are not widely grown for seed, mainly because financial returns to the grower do not make them an attractive crop. Before they can be grown widely, a greater acceptance by those who formulate pig and poultry rations is required.

Table 1.1: Effects of rotating wheat and lupins on soil mineral nitrogen and crop grain yields, 1974-1976.

1974	Grain yield t ha^{-1}	Soil min. N kg ha^{-1}	1975	Grain yield t ha^{-1}	Soil min. N kg ha^{-1}	1976	Grain yield t ha^{-1}
W	2.7	35	W	2.6	36	W	2.3
						L	0.8
			L	3.1	107	W	3.8
						L	0.2
L	3.3	75	W	4.7	61	W	3.7
						L	0.7
			L	2.6	130	W	4.3
						L	0.1

W = wheat; L = lupins.
(Data from Boundy, 1978)

Grain legumes either deplete soil nitrogen less than cereals (Wood and Russell, 1979) or they may increase soil nitrogen levels above those prevailing before the crop was sown (Jones, 1974; Boundy, 1978). The removal of crop residues and nitrogen-rich seeds may reduce soil nitrogen (Wetselaar, 1967), and substantial soil nitrogen increases are likely only in legume crops with a low harvest index (Wetselaar, 1967; Arnold, 1977). Research with garden peas (Rasmussen and Pumphrey, 1977) showed that irrigation increased growth and the total uptake of nitrogen, phosphorus, potassium and sulphur. Residues contained 75, 70, 90 and 80 per cent respectively of the total plant nitrogen, phosphorus, potassium and sulphur at green pea harvest. If these residues were removed, total nut-

rient removal from the system was increased three- to four-fold (Rasmussen and Pumphrey, 1977). There is a need for New Zealand work to quantify under varying agronomic treatments, the nitrogen losses and effects of subsequent crop growth, which occur from harvests at either green pea stage or crop maturity.

When grain legume and cereal crops are followed by a non-legume crop to indicate fertility changes, the indicator crop frequently yields more after grain legumes than after the cereal. This may occur even when the indicator crop is fertilised with heavy rates of nitrogen (Bowerman and Clare, 1976; Richards, 1978). Thus, soil structural changes caused by the legume crops benefit subsequent crop growth. However, on well structured soils, grain legumes may have only small beneficial effects on soil physical properties (Russell, 1980) or soils may show no benefits (Williams, 1975). Soil physical changes after grain legumes have included reduced bulk density (Sharma and Ambika Singh, 1970), increased aggregate stability (Sharma and Ambika Singh, 1970; Arnold, 1977; Russell, 1980) and increased pore space (Sharma and Ambika Singh, 1970).

Peas are very sensitive to poor soil structure and they require a porous, well aerated soil (Low, 1973; Dawkins, Hebblethwaite and McGowan, 1980; Hebblethwaite and McGowan, 1980). When grown under conditions of poor soil structure, pea yield may be reduced because of reduced water extraction through poor root development (Hebblethwaite and McGowan, 1980), reduced plant density (Russell, 1982) and inability to make compensatory growth (Hebblethwaite and McGowan, 1980).

1.5 MEASUREMENT OF NITROGEN FIXATION

1.5.1 Increase in Dry Matter and Total Nitrogen

Nitrogen fixation may be estimated from increased dry weights, as compared with plants which have been grown in the absence of nitrogen (Sprent, 1979). Frequently good correlations between dry weight and nitrogen fixed are found, but over long periods, the relationship becomes complex, because the nitrogen percentage varies (Sprent, 1979). An increase in total nitrogen in plants grown in the absence of combined nitrogen was the first method to establish conclusively that nitrogen fixation occurred. This method is still the only absolute, and simple, method of accurately measuring nitrogen fixation. Although it can be used as a check on other techniques, it does not indicate the relative importance of nitrogen fixation in the field where plants can obtain nitrogen from the soil. Even where plants are grown on artificially impoverished soils, legumes may gain appreciable amounts of nitrogen from the soil as shown by Kohl, Shearer and Harper (1980). They incorporated 34 t ha^{-1} of corn cobs prior to sowing soybeans, but the nodulated plants nevertheless obtained approximately 47 per cent of their nitrogen from the soil.

1.5.2 Difference Methods

Nitrogen fixation may be more accurately determined when the proportion of nitrogen coming from the soil is estimated. This correction is obtained by growing a non-fixing plant and comparing its nitrogen accumulation with the nitrogen-fixing legume. Three versions of the difference technique were listed by La Rue and Patterson (1981).

1.5.2.1 Comparison of a Legume with a Non-Legume:

Soil nitrogen contribution to a nitrogen-fixing legume is estimated by growing a non-legume concurrently with a legume. This technique is particularly useful in pastures where grasses and legumes are frequently grown in association. Two major assumptions are made. First, all the nitrogen in the non-legume is assumed to come from soil nitrogen, and secondly it is assumed that the legume and non-legume take up soil nitrogen in proportion to that available. In the second instance, it is assumed that differences due to growth patterns and root morphology are insignificant. The second assumption in particular is open to question. At least one author has shown that estimates of nitrogen fixation by a pasture legume are altered by the choice of the non-fixing control plant (Wagner, 1954).

1.5.2.2 Comparison of a Legume with a Non-Nodulating Legume:

The use of near isogenic lines which differ only in fixation ability go at least part way to reducing the errors inherent in the comparison above. Growth patterns, root morphology and nitrogen uptake are more likely to be identical, but this is difficult to prove. The availability of isolines is limited to a few soybean cultivars (La Rue and Patterson, 1981).

1.5.2.3 Comparison of Inoculated and Uninoculated Legumes:

In this instance, the only variable assumed to affect nitrogen fixation is the presence or absence of rhizobia. The assumption that the presence of nodules does not affect root morphology may not be correct for peas. Nodules which form on the taproot of peas tend to inhibit the formation of lateral roots (Torrey and Zobel, 1977).

1.5.3 Isotopic Methods

The use of ^{15}N remains the generally used check for other estimates of nitrogen fixation. A truly direct estimate of fixation can be made by using $^{15}N_2$ gas, but experimental techniques are difficult to

master and equipment is expensive (La Rue and Patterson, 1981).

1.5.3.1 Isotopic Dilution: A legume and non-fixing control plant (generally a non-legume) are grown in soil to which ^{15}N-labelled nitrate or ammonium has been added. This technique involves the assumption that the nitrogen fertiliser added is equally available to both legume and non-legume. Also, if soil nitrogen is low, the non-fixing crop may not grow as well as the test crop and thus not be a useful control. Reviews by Fried and Middleboe (1977) and La Rue and Patterson (1981) and experimental work (McAuliffe, Chamblee, Uribe-Arango and Woodhouse, 1958; Goh, Edmeades and Robinson, 1978; Rennie, 1982; Wagner and Zapata, 1982) give further information on techniques. These methods were not used in the study reported here.

1.5.4 Nitrogen Balance

A nitrogen balance technique was used by Sears *et al.* (1965) to measure changes in total soil nitrogen in pastures, and Rhodes (1980) used a similar technique to estimate nitrogen fixation in peas and lupins. The assumptions that mineralisation of organic nitrogen and leaching losses are the same for all treatments in nitrogen balance studies may not be valid. If not, the accuracy of nitrogen fixation estimates will be reduced.

1.5.5 The Relationship Between Acetylene Reduction and Nitrogen Fixation

The acetylene reduction assay has resulted in an explosion of data on nitrogen fixation (Sprent, 1979), essentially because it is simple, rapid and inexpensive (Sprent, 1979). The nitrogen-fixing enzyme, nitrogenase, catalyses the reduction of atmospheric di-nitrogen to ammonia (Dilworth, 1966) and the discovery that acetylene inhibits nitrogen fixation

and is reduced to ethylene by nitrogenase (Dilworth, 1966) led to the development of the acetylene reduction assay as a sensitive measure of nitrogen-fixing activity (Hardy *et al.*, 1968). The ratio of nitrogen to acetylene reduced, of 3:1, is the theoretical value obtained from the stoichiometry of the equations (Bergersen, 1970):

$$\text{Nitrogen fixation } N_2 + 6H^+ + 6e^- \rightarrow 2NH_3$$

$$\text{Acetylene reduction } 3\ C_2H_2 + 6H^+ + 6e^- \rightarrow 3C_2H_4$$

Although good correlations have been observed (Hardy, Burris, Herbert, Holsten and Jackson, 1971), wide variations from the 3:1 relationship have been found (Bergersen, 1970; Oghoghorie and Pate, 1971; Herridge, 1982a, b).

The initial enthusiasm has led to widespread use of the assay (Hardy, Burns and Holsten, 1973), but a number of important factors need to be considered before nitrogen fixation can be accurately estimated.

1.5.5.1 Factors Affecting Acetylene Reduced to Nitrogen Fixed:

Acetylene Concentration: Nitrogenase is thought to have approximately two and a half times greater affinity for nitrogen than for acetylene (Schollhorn and Burris, 1967), but the greater solubility of acetylene enables assays to be carried out in air with between 10 - 20% acetylene. Differences in ethylene production of up to 20% were observed between soybean plants incubated in an inert gas and oxygen mixture, and those incubated in air and acetylene (Hardy *et al.*, 1968). Thus, when incubations are done in air and acetylene, some energy may continue to be used in the reduction of nitrogen even at high levels.

Nitrogen and Oxygen Concentration: Saturating concentrations of acetylene limit hydrogen evolution and the *in vivo* function of hydrogen production by

nitrogenase may be to provide reductant for oxygen, and thus decrease oxygen concentration in the vicinity of nitrogenase (Hardy *et al.*, 1973). Inhibiting levels of oxygen may therefore occur when hydrogen evolution is blocked by the presence of acetylene.

Background Ethylene Production: In some incubation systems, the background production of ethylene by higher plants, fungi and bacteria (Hardy *et al.*, 1973) and by soil (Witty, 1979) may contribute to an over-estimate of legume nitrogen fixation, but Hardy *et al.* (1968) found negligible ethylene was formed in the absence of acetylene.

Interval Between Sampling and Assay: In order to obtain values which are representative of the *in situ* activity, it is important to use intervals of no longer than two hours and preferably less, between digging and assaying plants (Hardy *et al.*, 1968; Oghoghorie and Pate, 1971).

Injury: Excised nodules of soybeans were less active than nodulated roots which in turn were less active than whole plants (Hardy *et al.*, 1968; Hardy *et al.*, 1973). This reduced nodule activity may be caused directly by root injury with consequent reductions in the transport of nitrogen fixation substrates and products to and from the nodule. Rhodes (1980) found no differences between whole lupin plants and decapitated roots, which suggested carbohydrate storage was adequate during the 70 minute assay which he used. However, if plants had been assayed *in situ*, the acetylene reduction rates may have been considerably higher, particularly if digging had caused physical damage to nodules.

Duration of Incubation: Lag periods from the start of the assay to the first appearance of ethylene occur particularly when soil cores are used, which results in under-estimates of nitrogen fixation (Sinclair, 1973). Heavily nodulated roots, which rapidly reduce acetylene, may require assays of less than one hour. Under these conditions, Hardy *et al.* (1968) found

that linear production of ethylene from soybean roots ceased shortly after one hour.

Water: Acetylene is considerably more soluble than ethylene in water. When *in situ* measurements or soil cores are used and soil moisture varies, peak heights of acetylene will vary relative to ethylene, and the use of acetylene as an internal standard will then over-estimate nitrogen fixation.

1.5.5.2 The Usefulness of Acetylene Reduction: The use of the stable isotope of nitrogen is fundamentally the most accurate and absolute method for measuring nitrogen fixation because it is a direct method and is not subject to the correction factors applied to the acetylene reduction assay. However, because of the large cost and time required for isotopic measurements of nitrogen fixation, acetylene reduction techniques are useful for the following purposes:

1) Non-destructive assays allow the elimination of major sources of biological variability (Fishbeck, Evans and Boersma, 1973; Sinclair, 1975; Sprent, 1979). That is, individual plants or groups of plants may be assayed a number of times during the season and thus differences between plants would be minimised.
2) Assessment of the influence of temperature and other climatic effects on nitrogen fixation.
3) Investigations of agronomic treatments and their effects on rates of nitrogen fixation during plant development.
4) Initial screening for nitrogen fixation in cultivar and *Rhizobium* strain evaluation, although hydrogen evolution should be calculated as well.

The extrapolation of a few one-hour assays to a total nitrogen fixation for a season must be treated with extreme caution, because of rapid nitrogen fixation responses to weather and diurnal changes. Bergersen

(1970) considered that acetylene reduction is most useful where the absolute amount of nitrogen fixed need not be measured, although within species and growth conditions Sinclair, Hannagan, Johnstone and Hardacre (1978) found the assay was very closely related to nitrogen fixation as measured by total plant nitrogen.

1.6 CONCLUSION

Although much is known of the physiological responses in peas, little is known of the factors which limit their nitrogen fixation in Canterbury. The growing season has long days with high insolation and cool nights which are known to benefit both growth and nitrogen fixation. Long dry spells are frequent and are exacerbated by north-westerly (fohn) winds which further intensify drought stress. Peas are frequently grown after pasture when soil nitrate levels are high, and under these conditions nitrogen fixation may account for a small proportion of total plant nitrogen. Harvests are taken either as green peas or at crop maturity and little is known of the effect of harvest stage on soil fertility and subsequent crop growth.

The following trials were designed to assess the importance of these factors.

CHAPTER 2

THE EFFECT OF GRAIN LEGUMES ON SOIL FERTILITY

2.1 INTRODUCTION

By far the most important grain legume grown in New Zealand is peas, although small areas of other grain legumes such as lupins and tick beans are also grown. Over three-quarters of these grain legumes are grown in Canterbury, where they are used in rotations with wheat, barley and herbage seeds. Research in New Zealand has concentrated on agronomic aspects to improve the yield of these crops, but little attention has been given to their effect on soil fertility and subsequent crop yield. Douglas *et al.* (1972) found that maize grown in rotation with soybean gave higher yields than continuous maize. The beneficial effect of autumn-sown, legume forage crops on subsequent cereal yield has been reported by Janson and Knight (1980) and Piggot and Cooper (1980). Overseas work has shown that grain legumes can improve soil physical conditions (Sharma and Ambika Singh, 1970), soil nitrogen status (Wild, 1972) and subsequent crop yield (Boundy, 1978; Ellington *et al.*, 1979; Doyle and Herridge, 1980; Hawthorne and Lewis, 1980; Russell, 1980; Ahlawat *et al.*, 1981).

With the development of intensive cropping systems and a reduction in the frequency of pasture in crop rotations, greater reliance may be placed on grain legumes to increase soil nitrogen levels. The work reported in this paper was initiated to supply information on the effects of grain legumes on soil fertility under Canterbury conditions.

P.J. Rhodes, D.C. Askin, J.G.H. White, 1982. *Proceedings of the Agronomy Society of New Zealand,* in press. (Initial legume and cereal treatments and ryegrass in trial 1 were grown by Rhodes; other crops were grown by Askin.)

2.2 MATERIALS AND METHODS

2.2.1 Trial 1

The trial was conducted on a Templeton silt loam at Lincoln College, Canterbury, where soil fertility had been depleted by cropping, and large yield responses in wheat had been recorded following application of fertiliser nitrogen (Dougherty *et al.*, 1979). A randomised block design, with four replicates, was used. Plot size was 15 x 12 m. Treatments were: field peas cv. Huka, garden peas cv. Puke, lupins cv. Uniharvest, barley cv. Manapou, and fallow, each at two levels of nitrogen (0 and 80 kg ha^{-1}).

Seed and serpentine reverted superphosphate at 300 kg ha^{-1} were sown into a cultivated seed-bed on September 24, 1978. Sowing rates for Huka and Puke peas, lupins and barley, were 170, 310, 250 and 160 kg ha^{-1} respectively. Lupin seed was slurry inoculated and barley and pea seed treated with Orthocide fungicide.

Nitrogen as calcium ammonium nitrate was applied to appropriate plots on November 14, 1978. Atrazine at 1.0 kg ha^{-1} was applied pre-emergence to lupins, and terbutryn at 0.4 kg ha^{-1} to peas at the five to six node stage. Dicamba plus MCPA at 0.1 and 0.6 kg ha^{-1} respectively was applied to barley at tillering. Diquat at 0.8 kg ha^{-1} and later glyphosate at 1.4 kg ha^{-1} were applied to fallow plots.

Puke peas were harvested at the green pea stage and other crops at maturity. Seed yield from a 5.4 m^2 quadrat was obtained before plots were header harvested. Barley and lupin residues above header height, and all pea residues, were removed.

Ryegrass - Half of each plot was cultivated, and on March 10, 1979, 'Grasslands Tama' was sown at 40 kg ha^{-1} with superphosphate at 500 kg ha^{-1}. Nitrogen as ammonium sulphate at 0, 25, 50, 100 and 200 kg ha^{-1} was

applied on April 22 to sub-plots on the fallow treatment. Three permanent sampling areas, each 1 m^2, were randomly located on sub-plots and on all other non-fallow plots. Tama ryegrass was harvested for DM yield on June 14 and regrowth cut on August 31, and again on September 26, 1979.

Wheat - The remaining area of plots not sown with ryegrass was cultivated and superphosphate at 250 kg ha^{-1} applied prior to sowing wheat cv. Kopara at 150 kg ha^{-1} on July 27, 1979. On September 24, wheat was sprayed for annual weeds with terbutryn at 1.0 kg/ha. Wireweed was not controlled effectively and bromoxynil (0.2 kg ha^{-1}), ioxynil (0.2 kg ha^{-1}) and mecoprop (1.1 kg ha^{-1}) were applied on October 24.

At maturity, components of yield were measured from twenty heads sampled at random from 0.3 m^2. A 2.25 m^2 area was cut to ground level and seed yield determined.

2.2.2 Trial 2

The trial was situated on an area which had grown two wheat crops after lucerne. A randomised block design with four replicates was used. Plot size was 9 x 22 m. Treatments were: wheat cv. Oroua with nitrogen at 0 and 85 kg N ha^{-1}, field peas cv. Huka and lupins cv. Unicrop. Lime (5 t ha^{-1}) and superphosphate (400 kg ha^{-1}) were broadcast prior to drilling. Wheat at 170 kg ha^{-1} and peas and lupins at 200 kg ha^{-1} were sown into a cultivated seed-bed on September 14, 1979. Nitrogen as sulphate of ammonia was applied to wheat on October 10, 1979. Bromoxynil (0.2 kg ha^{-1}), ioxynil (0.2 kg ha^{-1}) and mecoprop (1.1 kg ha^{-1}) were applied to wheat on October 26, 1979. Metribuzin (0.2 kg ha^{-1}) and methabenzthiazuron (0.7 kg ha^{-1}) were applied to peas on October 16, 1979, and chloroxuron (0.5 kg ha^{-1}) plus Citowett at

0.5% v/v to lupins on October 26, 1979.

At crop maturity, plots were header harvested and grain yield measured from two 1.5 x 22 m areas within each plot. All remaining vegetation was removed. The trial area was cultivated prior to sowing 'Grasslands Tama' ryegrass at 40 kg ha^{-1} on March 20, 1980. Ryegrass was harvested from ten 0.1 m^2 quadrats cut to ground level on August 2, 1980.

2.3 RESULTS

2.3.1 Trial 1

Crop Yields - Barley seed yields were increased by fertiliser nitrogen, but there was no significant effect on legume yields, and values for the no nitrogen treatments only are presented (Table 1). Seed yield from

Table 2.1: Seed yields, harvest index and nitrogen harvest index.

Crop	Seed yield (kg DM ha^{-1})	Harvest index	Nitrogen harvest index
Huka peas	2260	0.66	0.86
Puke peas	470*	0.23	0.44
Lupins	3430	0.49	0.92
Barley	1410	-	-
Barley + N	2380	-	-
LSD 5%	570	0.05	0.07

*Puke green pea yield 2590 kg ha^{-1} (TR =1109)

Uniharvest lupins was higher than for Huka peas, but harvest index was lower. Nitrogen harvest index was higher for lupins, and lupin seed had a higher nitrogen concentration (4.6%) compared with peas.(2.9%). The green pea yield of Puke peas at a TR of 109 was 2590 kg ha^{-1}. On a dry matter basis, this was 470 kg ha^{-1}, which was

considerably less than the seed yield of Huka peas at maturity. The harvest indices were also lower (Table 1).

Ryegrass - Ryegrass yield after pea cultivars was similar to that for fallow, but yield after Uniharvest lupins was higher (Table 2). Ryegrass production after barley was lower than after legumes or fallow, particularly where no nitrogen had been applied to the barley crop. Nitrogen applied to ryegrass increased yields up to the highest rate applied (Table 3).

Table 2.2: Tama ryegrass yields (Trial 1)

Treatment	Ryegrass yield (kg DM ha^{-1})
Fallow	2810
Huka peas	2650
Puke peas	2670
Lupins	3300
Barley	1860
Barley + N	2290
LSD 5%	400

Table 2.3: Effect of autumn-applied nitrogen on Tama ryegrass yield, fallow treatment

N applied (kg N ha^{-1})	Ryegrass yield (kg DM ha^{-1})
0	2810
25	3340
50	3860
100	4970
200	5960
LSD 5%	510

Wheat - Colour differences were obvious by late tillering, with plots previously sown to barley a paler green than others.

The analysis of variance of final harvest results (Table 4) incorporated single degree of freedom tests on four orthogonal comparisons, which were: cropped versus fallow; barley versus legumes; Puke versus Huka, and peas versus lupins (Appendix IV). Total biomass and seed yields were low, with a large difference in seed yield between barley and legumes. The relatively large amount of straw present in the barley plots, was reflected in the harvest index of 0.28 which was significantly different from the legumes ($P < 0.05$). Spikelets per ear were unaffected by previous treatments, with a mean of 16.5. The reductions in both ears per m^2 and 1000 grain weight had a large effect on the yield difference between barley and legumes.

Table 2.4: Wheat grain yield, components of yield and harvest index.

Previous crop	Grain yield (kg DM ha^{-1})	Harvest index	1000 grain weight (g)	Grains $spikelet^{-1}$	Ears m^{-2}
Huka peas	2480	0.43	39.1	1.42	268
Puke peas	2660	0.43	40.6	1.60	253
Lupins	2850	0.41	39.4	1.65	269
Barley (no N)	1540	0.28	31.8	1.34	211
Fallow	2630	0.43	39.2	1.57	259
LSD 5%	570	0.15	4.2	0.32	36
Significance of single d.f. orthogonal comparisons	barley vs legumes **	barley vs legumes *	barley vs legumes **	NS	barley vs legumes **
	Other comparisons N.S.				

2.3.2 Trial 2

Crop Yields - There was no response to nitrogen in wheat, which was severely affected by take-all (*Gaeumannomyces graminis* var. *triticus*). Excellent weed control was achieved in peas, but calindrinia (*Calindrinia menziesii*) and wireweed (*Polygonum aviculare*) were not controlled in the lupins, where yields were less than peas (Table 5).

Table 2.5: Seed and Tama ryegrass yields (Trial 2).

Treatment	Seed yield (kg ha-1)	Ryegrass yield (kg DM ha-1)
Peas	3350	2940
Lupins	1910	2600
Wheat	2940	2350
Wheat + N	2990	2520
LSD 5%	670	460

Ryegrass - Tama yield after peas was greater than after wheat, but yields after lupins and wheat were similar (Table 5). There was little residual effect of nitrogen fertiliser applied to wheat.

2.4 DISCUSSION

Peas are sensitive to poor soil aeration (Low, 1973; Wiersum, 1979), and high bulk density (Eavis and Payne, 1969; Hebblethwaite and McGowan, 1980). At the site used in Trial 1, continuous cropping had caused a deterioration in soil structure, which is likely to have been responsible for the low pea yields obtained (Table 1), in comparison with those reported by Stoker (1975) and Falloon and White (1978).

Lupin yields, however, compared favourably with those reported by other workers for crops grown in Canterbury (Stoker, 1975; Lucas *et al.*,

1976; Hill *et al.*, 1977). This suggests that lupins tolerate low fertility conditions better than peas, and would be more suitable for use as a break crop after successive cereal crops.

Cereal yields at both sites were low. The response to nitrogen by barley (Table 1) indicates that nitrogen deficiency limited yields in Trial 1, although poor soil structure may also have been important. Take-all depressed wheat yields in Trial 2, and could have reduced the effect of applied nitrogen.

The large response to nitrogen on fallow plots (Table 3) and the residual effect of nitrogen applied to barley (Table 2) indicates that ryegrass was highly responsive to nitrogen at site 1. This suggests that ryegrass yields were higher after legumes compared with barley due to differences in available nitrogen. Greater uptake of soil nitrogen by barley and removal of this nitrogen in grain and straw would have reduced subsequent nitrogen availability relative to lupins and peas and resulted in lower ryegrass yields. Similarly, a reduction in nitrogen supply to ryegrass after wheat relative to peas would have resulted in the lower yields obtained in Trial 2. The smaller difference between ryegrass yield after lupins and after wheat (Table 5) compared with that between lupins and barley (Table 2) may have been due to the removal of soil nitrogen in weeds harvested with the lupins.

In Trial 1, the nitrogen stress in wheat which followed barley was first observed at late tillering and became progressively more acute. This premature leaf senescence reduced all components of yield except spikelets per ear (Table 4) and also the grain-filling period, resulting in shrivelled grains. These factors resulted in the 1100 kg ha^{-1} yield difference in wheat after barley compared with wheat following legumes.

Nitrogen return from Puke and Huka peas was limited primarily to that contained in roots and nodules. Mineralised nitrogen from this source recovered by ryegrass and wheat appears to have been sufficient

only to offset removal of soil nitrogen in the harvested pea grain and straw, since yields after peas were similar to those on the fallow treatment. It is unlikely that the yields of subsequent crops would have been substantially higher had Huka pea residues been returned, since 86 per cent of the nitrogen present at maturity was in the seed (Table 1). Yields may have been higher with Puke pea residue return, since 56 per cent of the nitrogen at the green pea stage was contained in crop residues.

In contrast to peas, ryegrass and wheat yields were higher after lupins than after fallow. Mineralisation of nitrogen returned to the soil in roots, nodules, abscissed leaves and other crop residues appears to have compensated for soil nitrogen removal via lupin seed, and could account for the higher yields.

Although these trials have shown that grain legumes, when harvested at maturity, can have a beneficial effect on subsequent crop yields in comparison with cereals, their use as fertility building crops in intensive cropping systems appears limited. Total soil nitrogen can be increased via grain legumes only where fixed nitrogen added to the soil via roots and nodules, leaf fall and crop residues exceeds losses of soil nitrogen through uptake and removal in the seed. This may occur under low soil fertility conditions where nitrogen fixation supplies most of the crop nitrogen requirement. However, the net increase in total soil nitrogen, particularly in legume crops with a high nitrogen harvest index, is unlikely to be sufficient to offset nitrogen uptake and removal in subsequent non-legume grain crops. Under high fertility conditions, where soil nitrogen uptake can account for a major proportion of legume nitrogen (McAuliffe *et al.*, 1958; Allos and Bartholomew, 1959; Gibson, 1976), the removal of soil-derived nitrogen in seed may be greater than the return of fixed nitrogen, resulting in a net reduction of total soil nitrogen.

CHAPTER 3

SOIL NITROGEN, MOISTURE AND HARVEST DATE INFLUENCE ON PEA NITROGEN FIXATION, ACCUMULATION, DISTRIBUTION AND SUBSEQUENT RYEGRASS PRODUCTION

3.1 INTRODUCTION

Dryland peas removed about as much nitrogen as they fixed when grown in a low fertility soil at Lincoln (Rhodes, 1980). However, fertile soils are more commonly used for pea crops in Canterbury. Under such conditions little is known of their nitrogen fixation and effect on subsequent crop growth. Furthermore, drought frequently limits growth and yield of peas in Canterbury (Anderson and White, 1974; Stoker, 1973, 1975; White *et al.*, 1982). Most researchers suggest irrigation at flowering and pod filling overcomes this limitation. Similarly, little is known of the effect of these irrigation treatments on nitrogen fixation.

Whether the crop is harvested as green or dried peas also affects soil nitrogen removal and the amount of time which peas have to fix nitrogen. Ten per cent of the peas grown in Canterbury are harvested at the green pea stage (N.Z. Agricultural Statistics, 1979/80, 1982). Initial studies by Rhodes *et al.* (1982) showed that time of harvest influenced the growth of subsequent crops. In that study, a direct comparison of harvest date was not possible as cultivar differences confounded the results. If real, these differences have important agronomic implications.

In all legumes, nitrogen fixation can be affected by changing the available soil nitrogen and moisture. Addition of highly carboniferous material such as straw causes a decrease in available nitrogen as the straw decomposes (Russell, 1973), and this may stimulate symbiotic nitrogen fixat-

ion to replace the source of nitrogen no longer available. In contrast, addition of fertiliser nitrogen to the soil generally reduces the requirements for fixed nitrogen by legumes. The bimodal effects of a range of nitrogen fertiliser additions have been studied in pasture and grain legumes (Allos and Bartholomew, 1959; Oghoghorie and Pate, 1971; Hoglund, 1973), with the general finding that with low levels of added nitrogen, plant nitrogen uptake and fixation reach an initial peak. This peak is dependent on combined nitrogen being complementary to the nitrogen supplied from symbiosis. At a higher level of combined nitrogen, fixation is actively suppressed. At this level, combined nitrogen may not fully compensate for the loss of nitrogen from the symbiosis. A trough in nitrogen yield then occurs. Further increases in combined nitrogen allow growth and nitrogen yield to increase above the initial peak in nitrogen yield. Thus small amounts of nitrogen fertiliser may stimulate growth and nitrogen fixation when applied during periods of inadequate nitrogen supply. Peas may suffer a temporary nitrogen deficit during nodule development, and again during grain filling when carbohydrate supply for nodule function may be limited. Pate (1976) considered that it would be at these stages when peas were most likely to benefit from fertiliser nitrogen. The possible responses from nitrogen additions to peas at these stages merits further attention in the field. For these reasons, growth, yield and nitrogen fixation were examined to:

1) identify the most important factors which influence nitrogen fixation of field-grown garden peas,
2) assess the influence of irrigation, straw and nitrogen fertiliser on subsequent soil fertility as measured by growth and nitrogen uptake of annual ryegrass.

3.2 MATERIALS AND METHODS

3.2.1 Trial Site, Soil Tests, Fertiliser and Cultivation

The trial was sited on a Templeton silt loam soil in paddock H6 of the Lincoln College Henley Research area. Prior to this trial, the site grew lucerne for several years, followed by two successive wheat crops. Soil samples for Ministry of Agriculture and Fisheries quick test determinations were taken from the trial area in June, 1978. After wheat was harvested in January, 1979, the stubble was removed and the area was sprayed with glyphosate at 0.7 *l* ha^{-1} in April 1979 to control couch grass (*Agropyron repens*). Superphosphate at 400 kg ha^{-1} and lime at 5 t ha^{-1} were broadcast on 29 August, 1979, and initial cultivation commenced on 8 September.

3.2.2 Experimental Design

A 2^5 factorial design with two replicates, and four blocks was used. Blocks and fourth order interactions were confounded. The treatments and levels of each were:

Nitrogen early as ammonium sulphate at nodule formation

E_0 : Nil
E_1 : 45 kg N ha^{-1}

Nitrogen late, as ammonium sulphate at first flower

L_0 : Nil
L_1 : 45 kg N ha^{-1}

Straw

S_0 : Nil
S_1 : 8 t ha^{-1}

Irrigation

I_0 : Nil
I_1 : Irrigation from flowering

Harvest date

H_G : Harvest at green pea

H_D : Harvest at dry pea

Four plots of barley, located at the south end of each block, were also grown. A winter active, annual ryegrass cv. Tama was sown in all pea and barley plots, after final harvest, to assess fertility changes.

3.2.3 Crop Husbandry

A stack of wheat straw of about 1000 kg was covered with polythene in autumn, 1979. It was anticipated that weed and wheat seed in the straw may present a problem so the stack was sealed on 4 September and 12 cans of methyl bromide (each 450 g) were injected during the interval up to 30 September, but samples of wheat seeds placed in the straw were still viable on October 10, 1979. Straw was chaffed and spread on the surface of plots which were then rotary hoed on October 26, 1979, and volunteer wheat seedlings sprayed with glyphosate at 0.9 *l* ha^{-1} on November 8.

On November 13, 1979, garden peas cv. Puke and malting barley cv. Manapou were sown at 300 and 150 kg ha^{-1} respectively into plots measuring 22 x 1.5 m. Commercial *Rhizobium* prills containing *R. leguminosarum* were broadcast at approximately 70 kg ha^{-1} the day after drilling, just prior to 6 mm of rain. Seeding equipment did not allow even application of the prills and broadcasting was used to give more even application. Metribuzine at 0.2 kg ha^{-1} and methabenzthiazurone at 0.7 kg ha^{-1} were applied to the peas on 13 December, 1979, to control annual weeds. No herbicides were used on the barley. Nitrogen was broadcast on to E_1 plots on 5 December, and to L_1 plots on 24 December, 1979. Three trickle irrigation lines per plot were laid after drilling, and irrigation was applied to bring plots to near field capacity on 24 December, 27 December, 14 January and 22 January. Soil moistures were determined in the S x I plots from

24 December to 28 January, 1980, by taking 4 cores each 2.5 cm diameter from each plot at 0 - 20 cm. Field capacity and wilting point (0 - 20 cm) for this soil at -15 bars is 35 and 15 per cent moisture respectively, on a dry weight basis (Hussein pers. comm.).

3.2.4 Soil Nitrate

Available soil nitrate was measured in S x I plots. At each sampling date, four cores were taken from each plot at 0 - 20 cm and two cores from 20 - 60 cm; these were bulked, and analysed by the method described in Appendix III. Sampled areas were not used for further plant or soil measurements, and holes were filled with loose soil.

3.2.5 Acetylene Reduction Assays

Two methods of determining nitrogen fixation were used. In the first instance, plants were dug and assayed in jars, and in the second, plants were assayed *in situ* as outlined below.

Jars: Ten plants were dug from the inner six rows of plots and tops severed at the first node. Roots were incubated with 60 ml acetylene, using the method outlined in Appendix I. Assays were conducted 31, 38, 55, 69 and 85 days after drilling. Mean seasonal nitrogen fixation (C_2H_2) was estimated from the area under graphs derived from the five assays (main effects only).

In Situ Assays: Polythene containers of 10 *l* capacity measuring 260 mm diameter x 275 mm depth were cut 3 cm from the base, and the top portion of two containers were placed in each plot (H_G treatments only). Two cm were left above the soil surface. Containers were open at the base to allow normal root development below 270 mm. Eight pre-germinated

seeds were sown per container within one day of drilling. Assays were conducted at fortnightly intervals, as follows: Incubations commenced soon after 0500 h (NZST) by placing reflective silver painted buckets (labelled "G", Plate 3.1) over the top of the containers in the ground, and the junction sealed with plastic tape. Acetylene was supplied through a regulator (A) and via a tube (B) into a chamber of 1 *l* capacity (C and D). Taps were opened for a short period to allow gas to reach air pressure and were then closed again. Injection into the bucket (G) was through a sealing grommet (F). After periods of up to 2 hours, the gas in the bucket was mixed by pumping a 100 ml syringe (H) in and out five times via the sealing grommet. Gas samples were taken with double-ended needles and stored in 10 ml vacutainers for future analysis.

A malfunction in the gas chromatograph was repaired after the finish of the trial and samples were stored for approximately four months. As storage time may have influenced the acetylene reduction results obtained, a subsequent test to assess the effect of storage time was conducted. Vials were collected from four jars and acetylene reduction activity was measured initially and at 2, 33, 71 and at 165 days after incubation. Vials were refrigerated during storage.

3.2.6 Plant Measurements

Plants were counted from three 0.1 m^2 quadrats in each plot, 27 days after sowing. Harvests were conducted during vegetative growth, first flower, pod fill, green pea and maturity, 24, 43, 57, 74 and 105 days after sowing. The first harvest was from the factorial combination of straw and early nitrogen treatments only, as other treatments had not been applied. At this harvest, ten plants were randomly selected and dug from the south end of plots, nodules greater than 1 mm in diameter were counted, and top dry weights were recorded. Subsequent harvests

Plate 3.1: Acetylene reduction apparatus for *in situ* measurement of nitrogen-fixing activity.

A. Acetylene source and regulator.
B. Entrance into 1 *l* chamber.
C. Plunger.
D. Outlet to bucket assembly.
E. Modified 1 ml syringe.
F. Sealing grommet (not shown).
G. 10 *l* bucket placed over plants in the field.
H. 100 ml syringe used for mixing gas and taking samples.

were from quadrats to allow estimation of plant population changes and dry matter on an area basis. At first flower, plants from two 0.1 m^2 quadrats were harvested by cutting plants at ground level. Two plants, chosen at random, were separated into leaf and stem for dry weight and nitrogen analysis. Total dry weight (g m^{-2}) was calculated from all plants harvested, and nitrogen (g N m^{-2}) was calculated from the concentration of nitrogen in plant fractions multiplied by plant population and plant size. At pod fill, three 0.1 m^{-2} quadrats were harvested, and three plants were separated into leaf, stem and reproductive portions. Sampling intensity was increased to reduce residual error. At this harvest and at green pea, nitrogen samples were bulked to reduce the number of analyses which resulted in main effects having eight replicates. Nitrogen in plant parts was calculated by applying the one nitrogen concentration over the four values for dry weight.

At the green pea harvest, an area of 2.25 m^2 was harvested by hand from the central six rows of each plot; plant number and green weight determined and the crop threshed in a DSIR mini-viner to obtain a green pea yield. Three subsamples of the vined peas were taken for tenderometer measurements and a further sample oven dried for moisture determination. Twenty-five plants outside the 2.25 m^2 quadrats were randomly selected for pod count and dry matter and three of these plants were divided into stem, leaf, pods and peas; the components oven dried and stored for nitrogen analysis. These sub-samples from 25 plants were used in the calculation of the nitrogen balance because the vined pea yield did not allow for seed losses in the mini-viner. The remaining crop on all plots for green pea harvest (H_G) was then mown with a sickle-bar mower and residues removed.

The final harvest for grain yield was delayed because of moist weather and occurred on 26 February. Vines from 2.25 m^2 were

counted, pulled and stored in calico bags for later threshing in a mini-thresher. In addition, twenty-five plants were harvested and treated in the same manner as those for the green pea harvest. Correlation among the components of yield was assessed for all treatments, and separately for all irrigated and dryland treatments. Harvest index (HI) and nitrogen harvest index (NHI) were calculated from the ratio of seed dry matter and nitrogen to total dry matter and nitrogen. Samples for nitrogen analysis were not bulked. The remaining mature pea vines and barley plots were threshed on March 7, 1980, and residues removed. Barley yield was calculated from two headed areas, each 44 x 1.5 m. Due to the loss of barley samples (after seed weights were recorded), harvest index was estimated to be 0.50 and nitrogen in straw and grain were estimated to be 0.6 per cent (Rhodes, 1980) and 2.0 per cent (Drewitt and Smart, 1981) respectively.

Nitrogen translocation from above-ground vegetative organs to seeds was calculated from the difference between seed nitrogen and uptake of nitrogen between flowering and final harvest. Thus the percentage of seed nitrogen which came from translocation was estimated by

% of seed N from translocation =

$$\frac{\text{Seed N - (total above-ground N at final harvest - N at flowering)}}{\text{Seed N}} \times 100$$

3.2.7 Tama Ryegrass

The trial area was sprayed with glyphosate at 1.6 *l* ha^{-1} on March 10, 1980, and Tama ryegrass was direct drilled at 45 kg ha^{-1}, across the plots, using a 1.5 m Duncan drill on March 11. After the ryegrass emerged on 20 March, 1980, all pea seeds, pods and weeds were removed from 3 1 m^2 quadrats in each plot, including those which had grown barley. Thus pea roots and nodules were the only components able to influence

subsequent crop growth. The possible influence of above-ground residues was estimated by measuring nitrogen in these residues. The ryegrass was harvested to ground level on 5 and 6 June, 1980, from 3 0.1 m^2 quadrats from each cleared area. Green weights were recorded and subsamples taken for dry matter and per cent nitrogen. Remaining uncut areas in each pegged quadrat were cut to ground level and harvested material was removed. Regrowth was harvested on 21 and 22 August, 1980, and dry matter recorded.

3.2.8 Estimate of Nitrogen Fixation by Nitrogen Balance

At the green pea harvest, nitrogen removal in seed and non-seed residues was calculated from three plants in each plot which were partitioned into stem, leaf, pod and peas. The vined pea yield at this harvest was unsuitable because of losses of small seeds in the mini-viner. The nitrogen balance at crop maturity was based on seed and residue yields from 2.25 m^2.

3.3 RESULTS

3.3.1 Soil Moisture and Quick Test

The season was atypical in that a very heavy rainfall (108 mm) occurred 51 days after sowing, 10 days after the start of flowering (Figure 3.1). A rainfall of this type is likely to occur every 20 years (Christchurch Meteorological Service, pers. comm.). This rain markedly reduced the expected irrigation response and is likely to have caused leaching of nitrogen particularly in previously irrigated plots. The potential soil moisture deficit increased rapidly during December and had reached 170 mm when this rainfall occurred (Figure 3.1). Because straw had no effect on soil moisture levels, only the main effect of irrigation

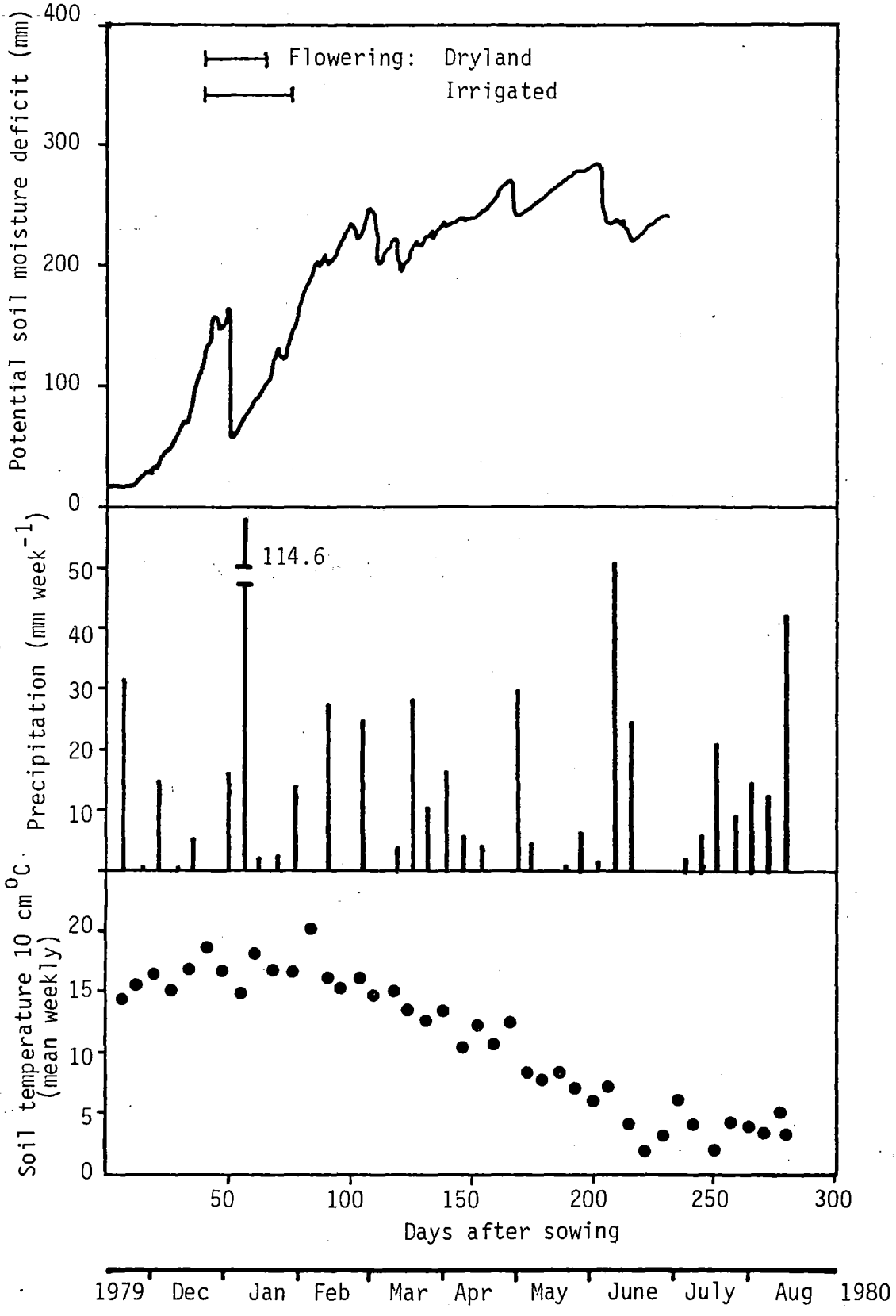


Figure 3.1: Climate data from Lincoln College meteorological station.

is shown (Table 3.1).

Table 3.1: Soil moisture as per cent of dry weight (1979/80, 0 - 20 cm).

Treatment	Days after flowering						
	41	51	55	62	66	69	76
Irrigation I_0	Mean 10.8	*	30.0	17.8	15.0	14.1	44.1
$S_{\bar{x}}$	(0.4)		(1.3)	(0.3)	(1.0)	(0.1)	(0.3)
I_1	11.5		30.0	17.9	22.6	19.0	19.4
$S_{\bar{x}}$	(0.4)		(1.3)	(0.2)	(0.5)	(0.7)	(0.2)

(* = 108 mm rainfall)

Results from M.A.F. quick test analyses are presented in Table 3.2.

Table 3.2: Soil pH and quick test nutrient concentrations (0 - 20 cm).

pH	Ca	K	P	Mg
5.4	8	6	16	18

3.3.2 Effect of Straw on Soil Nitrate and Nodulation

The incorporation of straw reduced available soil nitrate by 50 per cent in the top 20 cm between 14 and 77 days after sowing (Table 3.3). The effect of straw in reducing nitrogen availability was less at lower profile depth (20 - 60 cm) but reductions in mineral nitrogen because of irrigation became significant in the lower profile after 70 days. The presence of straw had caused an increase in nodule numbers per plant from 5 ($S_{\bar{x}}$ 1.0) to 12 ($S_{\bar{x}}$ 1.7) 24 days after drilling.

Table 3.3: Available soil nitrate (ppm N).

Treatment		Days after drilling 0 - 20 cm 14	22	29	36	63	70	77	85	92	99
Straw	S_0	34*	37*	34*	39	13	11	14	6	12	8
	S_1	18	19	15	20	9	6	4	7	10	7
Irrigation	I_0			22	32	13	9	15	8	10	11
	I_1			27	27	9	9	2	5	11	5
$LSD_{.05}$		11.0	9.8	10.0	20.0	8.5	8.8	16.0	3.5	4.2	10.8
CV		19.6	15.6	19.0	30.5	34.6	45.8	81.1	25.6	17.5	64.2
		No significant interactions									
		20 - 60 cm									
Straw	S_0		17	15	13	14	7	7	5	5	8
	S_1		16	11	11	11	7	5	6	5	8
Irrigation	I_0			14	12	16	8	8*	7*	7*	10*
	I_1			12	13	9	6	3	4	4	6
$LSD_{.05}$			6.0	9.8	3.9	9.6	3.3	3.8	1.6	2.3	3.3
CV			16.6	34.0	14.6	35.0	21.2	29.7	13.5	19.0	19.1
		No significant interactions									

3.3.3 Dry Matter Accumulation and Plant Population

Dry matter was analysed both for individual plants and on a unit area basis but only the area results are presented. As assessments of fertility changes were made on an area basis with Tama ryegrass, individual plant data did not add significantly to understanding.

Plant population counts taken 27 days after drilling showed no significant differences between treatments, with an even population of 155 plants m^2 (CV = 13.5%). At the first two harvests, dry matter production was not significantly affected by straw, but 57 days after drilling a highly significant positive response was measured (Table 3.4). The interaction of S x L for dry matter showed that in the presence of straw, yield was reduced by late nitrogen whereas without straw, yield increased. Plant population responded similarly (Table 3.5). Irrigation significantly increased plant dry matter by final harvest (Table 3.4). Nitrogen applied at nodule formation had no effect on plant dry matter.

3.3.4 Green Pea Yield and Components of Yield and Seed Yield at Final Harvest

Irrigation increased green pea yields by 19 per cent and delayed maturity, as measured by tenderometer readings. Late nitrogen increased yield of green peas with irrigation, but had no effect without irrigation (Table 3.6).

Final seed yields were increased with irrigation by 12 per cent (Table 3.7). Seed weight was increased by straw and reduced by nitrogen applied at nodule formation and at flowering. Irrigation did not influence seed weight but pods per plant were increased by 18 per cent. The correlation matrices and levels of significance for the 16 plots without and 16 plots with irrigation are shown in Table 3.8. Levels of significance were reduced when components of yield and seed yield were

Table 3.4: The effect of treatment on the dry matter of whole tops (g DM m^{-2}).

Treatment		Days after sowing 24 Vegetative	43 Flowering	57 Mid-pod fill	74 Green pea	105 Maturity
Straw	S_0	37.9	139	313**	487	555
	S_1	34.4	142	339	439	535
Nitrogen early	E_0		139	335	454	539
	E_1		141	317	473	551
Nitrogen late	L_0		141	327	465	555
	L_1		139	325	462	535
Irrigation	I_1		140	331	469	518*
	I_1		141	321	457	571
$LSD_{.05}$		13.7	13.7	19.2	76.0	42.0
CV		21.9	19.5	11.7	32.6	9.45
Significant interactions:		None	None	SxL*	None	
			S_0 L_0 302			
			1 324			
			S_1 L_0 351			
			1 326			
			$LSD_{.05}$ 27			

Table 3.5: The interaction between straw and nitrogen applied at flowering on plant density at mid-pod fill.

Plants m^{-2}		Straw S_0	S_1
Nitrogen late	L_0	154	163
	L_1	159	148
CV	11.3	$LSD_{.05}$	12.5*

Table 3.6: The effect of treatment on marketable green pea yield and mean tenderometer reading (TR) 74 days after sowing.

Treatment		Green pea yield ($g\ m^{-2}$)	TR
Straw	S_0	607	113
	S_1	625	115
Nitrogen early	E_0	618	115
	E_1	614	113
Nitrogen late	L_0	607	115
	L_1	625	113
Irrigation	I_0	561**	116**
	I_1	670	112
$LSD_{.05}$		29.1	2.9
CV		9.4	5.1
Significant interactions:		LxI*	None
		I_0L_0 569	
		L_1 554	
		I_1L_0 644	
		L_1 696	
		$LSD_{.05}$ 40.1	

Table 3.7: The effect of treatment on components of yield and final pea grain yield.

Treatment		Plants m^{-2}	Pods $plant^{-1}$	Seeds pod^{-1}	1000 seed weight (g)	Seed yield (g m^{-2})
Straw	S_0	133*	2.3	4.72	236**	327
	S_1	123	2.5	4.36	245	322
Nitrogen early	E_0	126	2.3	4.62	245**	318
	E_1	130	2.5	4.45	235	331
Nitrogen late	L_0	130	2.3	4.61	245**	331
	L_1	126	2.5	4.47	235	318
Irrigation	I_0	131	2.2*	4.48	240	305**
	I_1	125	2.6	4.59	241	343
Barley						371
$LSD_{.05}$		8.6	0.2	0.50	5.4	21.0
CV		8.9	13.8	14.8	3.0	9.06
Significant interactions:		None	None	ExL*	ExS*	None
				L_0E_0 4.95	S_0E_0 238	
				E_1 4.27	E_1 233	
				L_1E_0 4.29	S_1E_0 253	
				E_1 4.64	E_1 237	
				$LSD_{.05}$ 0.71	$LSD_{.05}$ 7.6	

correlated without regard for irrigation effects (32 plots). At both irrigation levels, seed yield was positively and significantly correlated with plant density. Pods per plant and seeds per pod were negatively correlated.

Table 3.8: Correlation matrices for components of yield, a) without irrigation and b) with irrigation.

a)	1	Plants m^{-2}	1.00				
	2	Seed weight	-0.40	1.00			
	3	Pods $plant^{-1}$	0.08	-0.18	1.00		
	4	Seeds pod^{-1}	-0.10	-0.02	-0.80**	1.00	
	5	Seed (g m^{-2})	0.59*	-0.29	0.31	0.15	1.00
			1	2	3	4	5
b)	1	Plants m^{-2}	1.00				
	2	Seed weight	0.08	1.00			
	3	Pods $plant^{-1}$	-0.05	-0.10	1.00		
	4	Seeds pod^{-1}	-0.19	-0.23	-0.68**	1.00	
	5	Seed (g m^{-2})	0.49	0.11	0.02	0.39	1.00
			1	2	3	4	5

3.3.5 Rates of Nitrogen Fixation

Nitrogen fixation (C_2H_2) activities were very low (Table 3.9) and are presented on a per plant basis, from assays conducted in 1 *l* jars. When diluted to 10 *l*, the amount of ethylene in gas samples from buckets was not measurably different between treatments, and results are not presented. The ethylene measured from assays in jars was always less than 1 umole C_2H_4 $plant^{-1}$ h^{-1}. These low values do not reflect the effect of time on vial storage as storage did not significantly influence

Table 3.9: The effect of treatment on the ethylene production (nmoles C_2H_4 $plant^{-1}$ h^{-1}).

Treatment		Days after sowing 31	38	55	69	85
Straw	S_0	21*	672	70**	269*	15
	S_1	55	569	261	407	17
Nitrogen early	E_0	58*	643	222	377	9
	E_1	17	598	109	300	24
Nitrogen late	L_0	-	-	198	400	12
	L_1	-	-	133	277	20
Irrigation	I_0	-	-	148	113**	9
	I_1	-	-	184	563	23
$LSD_{.05}$		33	457	87.8	170	19
CV		75.0	62.0	70.8	65.6	155
Significant interactions:		None	None	ExS**	None	LxIxS**
				S_0E_0 77		
				E_1 63		
				S_1E_0 366		
				E_1 156		
				$LSD_{.05}$ 124		

values of ethylene produced (Figure 3.2).

Thirty-one days after drilling, straw increased nitrogen fixation and early nitrogen depressed fixation. Although differences were insignificant 38 days from sowing, at 55 days there was a significant E x S interaction (Table 3.9). Without straw, early nitrogen had no effect, but with straw, early nitrogen decreased fixation. Irrigation increased nitrogen fixation 69 days after sowing. All rates of nitrogen fixation were very low (less than 23 namomoles C_2H_4 $plant^{-1}$ h^{-1}) 85 days from sowing and although there was a significant L x I x S interaction, very low rates made this unimportant. Total nitrogen fixation estimated from acetylene reduction was 0.6 and 0.4 g N m^{-2} in irrigated and dryland treatments respectively.

3.3.6 Plant Nitrogen and Translocation

The presence of straw increased nitrogen in whole tops during mid-pod fill (57 days after sowing) but in the E x S and I x S interactions, highest nitrogen yields were observed in the presence, or absence of both factors (Table 3.10). The E x I x S interaction at the green pea stage (Table 3.11) showed that with the addition of irrigation from flowering and incorporation of straw, the absence of early nitrogen gave maximum nitrogen yield. With irrigation but in the absence of both straw and early nitrogen, yield was depressed from that obtained with early nitrogen. Differences without irrigation were not significant. Plant residues, from control plots, contained 6.8 g N m^{-2} at the green pea harvest.

At the final harvest, an L x I interaction showed that in the presence of late nitrogen, applied at flowering, irrigation significantly increased nitrogen yield, whereas without this nitrogen, irrigation reduced yield. The nitrogen concentration of the peas showed a similar interaction (Table 3.12). The amount of nitrogen at final harvest in

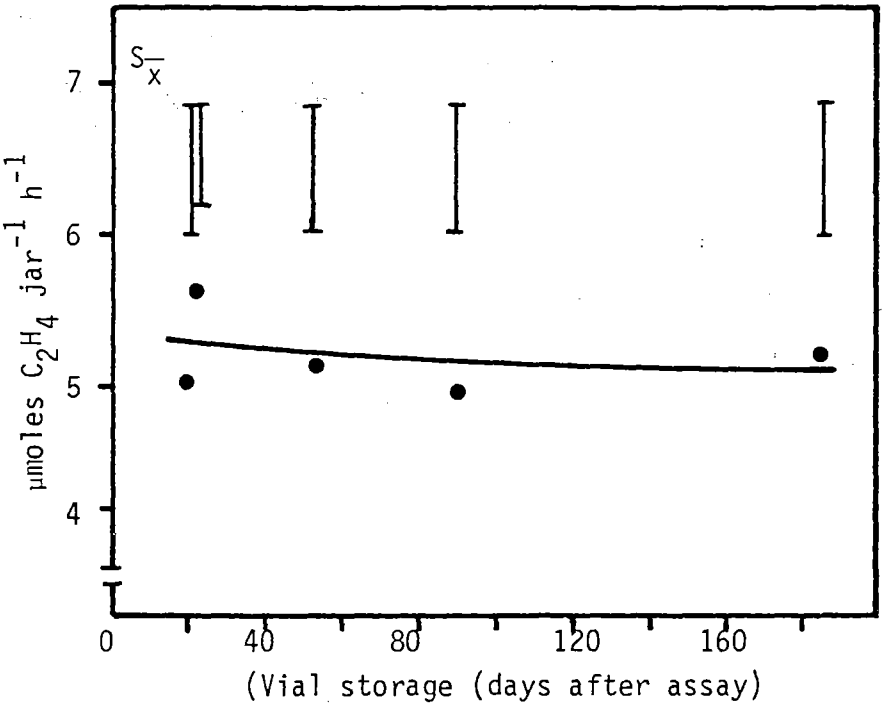


Figure 3.2: The effect of vial storage on ethylene production (curve fitted by eye).

Table 3.10: The effect of treatment on nitrogen in whole tops (g N m^{-2}).

Treatment		Days after sowing 43 Flowering	57 Mid-pod fill	74 Green pea	105 Maturity
Straw	S_0	5.34	9.6*	13.7**	17.3
	S_1	5.32	10.6	16.0	17.2
Nitrogen early	E_0	5.22	10.2	15.2	16.7
	E_1	5.44	10.0	14.6	17.7
Nitrogen late	L_0	5.59	9.9	14.6	17.3
	L_1	5.07	10.3	15.1	17.1
Irrigation	I_0	5.25	10.0	14.0*	16.5
	I_1	5.41	10.2	15.7	17.9
$LSD_{.05}$		1.13	0.9	1.50	2.89
CV		28.0	17.7	20.0	22.4
Significant interactions:		None	ES** IS**	EIS*	LI**
		S_0E_0	10.6	L_0I_0	18.7
		E_1	8.7	I_1	16.0
		S_1E_0	9.9	L_1I_0	14.3
		E_1	11.2	I_1	19.9
		S_0I_0	10.1		
		I_1	9.2		
		S_1I_0	9.9		
		I_1	11.2		
		$LSD_{.05}$	1.27	$LSD_{.05}$	4.08

Table 3.11: The interaction of early nitrogen (E), irrigation (I) and straw (S) on nitrogen in whole tops at the green pea harvest.

	I_0	I_1
S_0E_0	13.0	13.2
E_1	12.7	16.0
S_1E_0	15.4	19.2
E_1	15.0	14.6
$LSD_{.05}$	2.99	
CV	20.0	

Table 3.12: The effect of treatment on nitrogen concentration (% N) in plant components at final harvest.

Treatment		Stem	Leaf	Pod	Peas
Straw	S_0	0.91*	2.45	0.57	3.72
	S_1	0.97	2.63	0.65	3.69
Nitrogen early	E_0	0.96	2.34**	0.52**	3.76
	E_1	0.92	2.74	0.70	3.65
Nitrogen late	L_0	0.93	2.57	0.60	3.65
	L_1	0.92	2.51	0.62	3.76
Irrigation	I_0	0.96	2.53	0.58	3.76
	I_1	0.92	2.55	0.64	3.65
$LSD_{.05}$		0.05	0.26	0.11	0.14
CV		7.5	13.9	24.1	5.2
Significant interactions:		ExLxI**	None	IxS* ExS*	ExI* LxI*

	I_0	I_1
L_0E_0	0.90	1.07
E_1	1.02	0.75
L_1E_0	0.97	0.92
E_1	0.93	0.96
$LSD_{.05}$	0.10	

S_0I_0	0.60	E_0I_0	3.73
I_1	0.54	I_1	3.79
S_1I_0	0.56	E_1I_0	3.79
I_1	0.73	I_1	3.50
S_0E_0	0.55	L_0I_0	3.79
E_1	0.59	I_1	3.51
S_1E_0	0.50	L_1I_0	3.74
E_1	0.80	I_1	3.78
$LSD_{.05}$	0.16	$LSD_{.05}$	0.20

stem, leaf, pod and peas (Table 3.13) was influenced by the nitrogen concentration of these components (Table 3.12). Although a number of interactions were present in the stem, leaf and pod fractions, the NHI (nitrogen harvest index) of more than 80 per cent reduced their importance. The effect of treatments on the nitrogen yield of seeds was more important. Seed nitrogen (g N m^{-2}) was highest in the presence of both or absence of both irrigation and nitrogen applied at flowering (Table 3.13). Plant residues, from control plots, contained 2.7 g N m^{-2} at the final harvest.

Nitrogen that was translocated from stem, leaf and pods to seeds was less than 21 per cent (in the E x I interaction) of the total seed nitrogen at maturity (Table 3.14). Similar E x I interactions were observed for actual levels of nitrogen translocated and the percentage of seed nitrogen which came from translocation. When plants were irrigated and received early nitrogen, the percentage of seed nitrogen which came from translocation was only 7.6. The translocation was highest without irrigation and with early nitrogen. Without early nitrogen, irrigation had little effect on the percentage of the nitrogen from translocation.

The NHI at green pea showed an L x I x S interaction, where the greatest NHI was observed without straw and late nitrogen, but with irrigation. At both green pea and crop maturity early nitrogen reduced NHI, but late nitrogen had no effect. At maturity, in the presence of straw, early nitrogen reduced NHI; however, without straw there were no significant differences (Table 3.15).

3.3.7 Tama Ryegrass

The direct-drilled ryegrass established well and grew at a mean rate of 2.67 g m^{-2} day^{-1} from sowing to the first harvest, and at 2.24 g m^{-2} day^{-1} from the first to the second harvest. Growth rates in

Table 3.13: Partitioning of nitrogen at final harvest.

Treatment		Stem	Leaf	Pod	Peas
			(g N m^{-2})		
Straw	S_0	0.86	1.21	0.45	14.8
	S_1	0.92	1.29	0.58	14.4
Nitrogen early	E_0	0.86	1.07*	0.39*	14.4
	E_1	0.92	1.43	0.65	14.8
Nitrogen late	L_0	0.89	1.28	0.57	14.6
	L_1	0.89	1.22	0.46	14.5
Irrigation	I_0	0.83	1.22	0.44	14.1
	I_1	0.95	1.28	0.60	15.1
$LSD_{.05}$		0.14	0.28	0.26	2.50
CV		21.6	30.0	67.0	22.9

Significant interactions:	ExLxI*		LxI*		None	LxI**
		I_0	I_1			
	L_0 E_0	0.82	0.91	L_0I_0 1.42		L_0I_0 15.9
	E_1	1.03	0.79	I_1 1.13		I_1 13.4
	L_1 E_0	0.76	0.95	L_1I_0 1.01		L_1I_0 12.3
	E_1	0.70	1.15	I_1 1.43		I_1 16.8
	$LSD_{.05}$	0.29		$LSD_{.05}$ 0.40		$LSD_{.05}$ 3.54

Table 3.14: Translocation of above-ground plant nitrogen to seeds (mg N plant^{-1}) and the percentage of seed nitrogen which came from translocation.

		Translocation of nitrogen (mg N plant^{-1})	Percentage of seed N from translocation
Straw	S_0	16.7	14.8
	S_1	15.1	13.5
Nitrogen early	E_0	17.1	14.1
	E_1	14.7	14.1
Nitrogen late	L_0	17.0	14.4
	L_1	14.8	13.8
Irrigation	I_0	17.1	17.0
	I_1	14.7	11.2
$LSD_{.05}$		7.20	6.79
CV		59.7	63.5
Significant interactions:		ExI*	ExI*
	E_0I_0	14.4	E_0I_0 13.4
	I_1	19.8	I_1 14.9
	E_1I_0	19.9	E_1I_0 20.7
	I_1	9.6	I_1 7.6
	$LSD_{.05}$	10.2	$LSD_{.05}$ 9.6

Table 3.15: Nitrogen harvest index at green pea and maturity.

Treatment		Nitrogen harvest index	
		Green pea	Maturity
Straw	S_0	0.46	0.85
	S_1	0.47	0.84
Nitrogen early	E_0	0.49**	0.86**
	E_1	0.45	0.83
Nitrogen late	L_0	0.47	0.84
	L_1	0.46	0.85
Irrigation	I_0	0.45	0.85
	I_1	0.48	0.84
$LSD_{.05}$		0.026	0.017
CV		11.0	2.7

Significant interactions:

LxIxS*

	I_0	I_1
S_0L_0	0.43	0.51
L_1	0.45	0.45
S_1L_0	0.48	0.47
L_1	0.46	0.48
$LSD_{.05}$	0.05	

ExS*

S_0E_0	0.86
E_1	0.85
S_1E_0	0.87
E_1	0.81
$LSD_{.05}$	0.024

ryegrass growing after barley were 2.07 and 1.86 g m^{-1} day^{-1} respectively. Thus at the first harvest the ryegrass yield after peas was 29 per cent higher than after barley. The addition of early nitrogen increased growth and nitrogen uptake in ryegrass, whereas late nitrogen had less effect (Table 3.16).

Nitrogen yield of ryegrass showed a significant I x S interaction. In the presence of straw, irrigation of the peas had little effect, but without straw, irrigation reduced nitrogen yield. The E x I interaction indicated that in the presence of early nitrogen, irrigation reduced nitrogen yield of ryegrass to a greater extent than without early nitrogen. Nitrogen concentrations of ryegrass were above 2.76 per cent nitrogen after peas, but after barley the concentration was 2.36 per cent nitrogen. Highest nitrogen concentrations were recorded for the plots which were not irrigated but had received late nitrogen.

At the second harvest, a significant L x H interaction showed that at the green pea harvest, late nitrogen reduced dry matter yield whereas when plots were harvested at crop maturity, late nitrogen increased ryegrass growth.

3.3.8 Estimate of Nitrogen Fixation by Nitrogen Balance

Nitrogen fixation was estimated to be 4.5 and 7.5 g N m^{-2} for green and dry pea crops. This was 36 and 49 per cent of the total above-ground nitrogen respectively (Table 3.17).

Table 3.16: Dry matter production and nitrogen concentration and yield of Tama ryegrass after peas and after barley, 87 days after sowing; and dry matter production 163 days after sowing.

Treatment		Days from sowing 87 (DM gm^{-2})	87 (N gm^{-2})	87 (N %)	163 (DM g m^{-2})
Straw	S_0	227	7.4	3.20	165
	S_1	238	7.6	3.19	176
Nitrogen early	E_0	222*	6.7**	3.01**	176
	E_1	243	8.3	3.38	165
Nitrogen late	L_0	229	7.2	3.10*	168
	L_1	235	7.8	3.29	173
Irrigation	I_0	239	8.4**	3.51**	175
	I_1	226	6.5	2.89	166
Harvest	H_G	242*	7.8*	3.21	170
	H_D	223	7.2	3.18	171
Barley		181	4.3	2.36	141
$LSD_{.05}$		14.2	0.6	0.17	12.0
CV		11.9	16.6	10.80	13.9

Significant interactions:						
IxSxH*		ExI* IxS**		LxI** IxS**		LxH*
	E_0I_0	7.3	L_0I_0	3.24	HG L_0	174
	I_1	6.1	I_1	2.96	L_1	166
	E_1I_0	9.6	L_1I_0	3.77	HD L_0	161
	I_1	7.0	L_1I_1	2.81	L_1	180
	S_0I_0	8.9	S_0I_0	3.65		
	I_1	5.8	I_1	2.76		
	S_1I_0	7.9	S_1I_0	3.36		
	I_1	7.2	I_1	3.02		
$LSD_{.05}$		0.9		0.25		17.0

Table 3.17: Total nitrogen fixation estimated by nitrogen balance.

	Peas harvested: Green	Mature	Barley
	($g\ N\ m^{-2}$; control plots only)		
N inputs:			
Seed	1.4	1.4	0.3
N removal:			
Seed	5.8	13.1	7.4
Residue	6.8	2.3	2.2
Subtotal	12.6	15.4	9.6
Ryegrass	6.9	7.1	4.3
Total N removal	19.5	22.5	13.9
Net N yield (removal-inputs)	18.1	21.1	13.6
Calculated N fixed (net N yield for peas - net N yield barley)	4.5	7.5	
Per cent of plant N from fixation	36	49	

3.4 DISCUSSION

3.4.1 Effect of Peas on Soil Fertility

This trial has shown that ryegrass produced 28 per cent more dry matter and 65 per cent greater nitrogen yield after peas compared with barley. A similar result has been reported for a lower fertility soil (Rhodes *et al.*, 1982) and differences in ryegrass yield were attributed to changes in soil nitrogen status. The extent to which peas may restore or deplete soil nitrogen depends on the amount of nitrogen which they are able to obtain from the soil. If this is high, as in pea crops following grazed ryegrass/white clover pasture, then nitrogen fixation may be low, and a net decline in nitrogen status in the soil will occur. If fertility is low, almost all pea nitrogen requirements will come from fixation and a net increase in soil fertility may occur during breakdown of crop residues. A fallow treatment would be required to measure the absolute depression in fertility, but in this trial the reduction was markedly less after peas than after barley. Peas relied on soil nitrogen for more than half of their total nitrogen (Table 3.17), and thus if nitrogen is taken as a major index of soil fertility, peas reduced soil fertility. Canterbury farmers, however, generally expect peas to enhance fertility. The greater yield of Tama after peas compared with that after barley does not, however, allow for the overall loss of soil nitrogen which occurs when peas are grown.

At the second ryegrass harvest yield differences after peas and barley were reduced (Table 3.16), and Rhodes (1980) also noted that differences were reduced with time. He postulated that this was due to an inadequate availability of soil nitrogen, but reduced light and cooler temperatures depressing growth are also plausible factors.

Differences in subsequent crop yield after peas or cereals are likely to be greater when pea residues are returned to the soil as in this trial they contained 6.8 and 2.7 g N m^{-2} at green and dry pea harvests respectively. Residues were removed to simulate the effect of baling or burning, but the effect of residue return on soil fertility requires further investigation. Nitrogen release from the roots at either stage of maturity was similar, as shown by the small difference between yields of ryegrass from green pea and dry pea plots.

The translocation of plant nitrogen to seeds is important for two reasons. Firstly, the high seed nitrogen demand may limit yield by the self-destruct mechanism outlined by Sinclair and de Wit (1976), and secondly, the residues, depleted of nitrogen, may reduce nitrogen return to the soil and possibly immobilise nitrogen during decomposition. Translocation of above-ground plant nitrogen to seeds (Table 3.14) may have been underestimated because flowering, and not the start of seed growth was used as the basis for the calculation (Withers *et al.*, 1981). Thus the importance of translocation, as it influences seed yield, is difficult to assess. Applying nitrogen fertiliser at nodule formation to subsequently irrigated peas reduced translocation, however the reason for this is not clear.

The discrepancy between estimates of nitrogen fixation based on acetylene reduction or the nitrogen balance technique highlights the importance of cross checking between techniques. Frequent acetylene reduction assays are also necessary to more reliably estimate seasonal fixation. The nitrogen-fixing activity is likely to have increased very rapidly between 38 and 51 days after sowing, as other authors have observed rapidly increased fixation during late vegetative and early reproductive stages (Sosulski and Buchan, 1978; Dean and Clark, 1980). It is unfortunate that this was unable to be quantified as the *in situ* assays

were unsuccessful owing to ethylene dilution. Levels of activity were low but this was unlikely to have been caused by vial storage (Figure 3.2). Diurnal variation in nitrogen-fixing activity, however, may have significantly altered the time of mean daily activity and the diurnal cycles observed later (Chapter 6) show the need to assess this. Changes in the molar ratio of acetylene reduced to nitrogen fixed may also have contributed to error. In further studies, isotopic techniques, use of ureide analysis (Herridge, 1982a, b) and nodulating and non-nodulating isolines of peas, which are presently unavailable (Herridge, 1982c), would indicate the proportion of plant nitrogen which came from soil nitrogen. Background mineralisation and the assessment of nitrogen losses by leaching should also be determined from fallow plots, when nitrogen balance studies are used to measure nitrogen fixation.

3.4.2 Irrigation

The 19 and 12 per cent increase in green and dry pea seed yields due to irrigation were much smaller than those frequently observed in Canterbury because of high rainfall (108 mm) 10 days after the start of flowering. Mean increases in green pea yield of as high as 204 per cent have been obtained by Stoker (1973) on a soil with lower plant available water than that used here. Even on more water-retentive soils (Templeton silt loam), irrigation gave a mean increase over three seasons of 74 per cent in mature seed yield. Rainfall was 23 per cent less than normal, which may have contributed to this (Stoker, 1977). Further studies (Anderson and White, 1974; White *et al.*, 1982) have confirmed that in much of Canterbury, substantial responses to irrigation can be obtained in yield of spring-sown peas, and that these responses will be greater on shallow soils with less stored moisture. Thus although this trial was sown late to increase nitrogen fixation and dry matter responses to irrigation, the abnormally high rainfall reduced the expected response.

The intense storm 10 days after the start of flowering also reduced nitrogen fixation in all treatments. The severe physical injury the plants sustained probably caused the reduction by reducing photosynthesis and carbohydrate supply to the nodules. It is almost certain that this reduction in fixation was not due to waterlogging (Jackson, 1979) as the soil is free draining and irrigation increased fixation by 24 per cent only four days after the storm (Table 3.9). Plants rapidly recovered and it is probable that nitrogen fixation rates also increased rapidly due to leaching of soil nitrate (Table 3.3) and adequate moisture.

Although small, the seed yield response to irrigation can be attributed to an 18 per cent increase in pod set, while other parameters remained relatively constant. Other researchers have shown increases in all components of yield from irrigation (Stoker, 1973, 1977; Anderson and White, 1974; White *et al.*, 1982) but in this trial, pod number may have been increased by reduced floret abortion during the 10 days of irrigation between the start of flowering and the rain. Salter (1963) also observed a similar increase in pods per plant from two irrigation treatments which were similar to the dryland and irrigation treatments in this trial. As plant size and plant area may also influence floret abortion (Falloon and White, 1980) the interaction between soil moisture and leaf area on pods per plant merits further attention.

From the detailed studies reported below, the population established in this trial (128 plants m^{-2}) was sufficient to give optimum yields. In Canterbury, White and Anderson (1974) showed for irrigated peas grown at five densities between 52 and 358 plants m^{-2} that when irrigated, maximum yields of green peas occurred at 182 plants m^{-2} although without irrigation yields did not increase significantly above 90 plants m^{-2}. When the same plots were harvested as seed peas, although differences in yield were not large, the optimum density occurred between 105 and 182 plants m^{-2}. On a shallow Lismore soil in Canterbury, Stoker

(1975) found optimum densities increased from 71 plants m^{-2} under dryland conditions to 121 plants m^{-2} with frequent border-dyke irrigation.

Pods per plant, seeds per pod and 1000 seed weight are sensitive to variations in moisture status and population (White and Anderson, 1971; Stoker, 1973, 1975). A detailed analysis of components of yield in peas by Hardwick and Milbourn (1967) showed that the number of pods at each node is of considerable importance in determining final yield. Hill *et al.* (1977), in a season of above-average rainfall, measured 10 pods $plant^{-1}$ with garden peas sown at 66 plants m^{-2}. In this trial, the number of pods per plant was low and the greater plant density would have contributed to this. The irrigation response also suggests that inadequate water limited this component. Water stress during flowering has been shown to cause abscission of one third of the flowers and young pods in *Phaseolus vulgaris* (Stoker, 1974). Abscission in this trial was not measured but the heavy rainfall and wind may well have contributed to reduced pod set in all treatments. Pods per plant and seeds per pod were negatively correlated suggesting that competition within individual plants limited the total number of seeds that were able to develop on each plant. Mature seed weight is frequently less affected by irrigation and density treatments than other components (Stoker, 1977; White *et al.*, 1982) and in this experiment it was the least important component of final yield.

3.4.3 Straw Incorporation

Although straw halved available soil nitrate during vegetative growth, final yield of peas was unaffected. This was due to both generally adequate levels of soil nitrate even in the presence of straw (Table 3.3) and compensation for reductions in soil nitrate by increased reliance on symbiotic fixation. Plants grown with straw were initially

paler in colour and had increased nodule number than those without, which is almost certainly due to a temporary nitrogen deficit in the soil. The effect of straw in reducing soil nitrate levels is well known and has been demonstrated in both greenhouse (Waddington, 1978) and field trials (Russell, 1973). Thornton (1929) showed that nodulation in soybeans was enhanced by straw, as in this trial, while recently trials (Shivashankar *et al.*, 1976a; Shivashankar and Vlassak, 1978) also showed straw increased nitrogen fixation and final yield of soybeans. Straw contains polysaccharides which are suitable for the rapid multiplication of rhizobia (Russell, 1973) which would enhance fixation, thus countering nitrogen immobilisation.

During straw decomposition, soil micro-organisms evolve carbon dioxide (Sorenson, 1979) and increased levels of carbon dioxide have frequently enhanced photosynthesis in soybeans (Hardy and Havelka, 1976; Finn and Brun, 1981, 1982). In general, however, soil respiration contributes only 12 per cent of the total carbon dioxide assimilated by the crop (Krzysch, 1972, quoted in Shivashankar *et al.*, 1976b) and in the windy Canterbury environment, the effect on photosynthesis from straw decomposition would be minimal.

3.4.4 Nitrogen Fertiliser

Pate (1976) considered that nitrogen applied at nodule formation and during fruiting may benefit pea development. The observed green pea yields support some use of nitrogen applied at flowering (Table 3.6) when fixation was unable to supply all plant requirements. Dryland green pea yield was limited by insufficient water and showed no response to nitrogen. In contrast, nitrogen deficiency limited irrigated peas as shown by the eight per cent increase in yield of irrigated green peas after nitrogen was applied at flowering. Irrigation increases plant demand for a range of nutrients, including nitrogen (Rasmussen and Pumph-

rey, 1977) and in this trial, nitrogen supply would have been further reduced by leaching after the rainfall.

Nitrogen-fixing activities were always low, at less than 700 nmoles C_2H_4 plant^{-1} h^{-1}, because of the high levels of available nitrogen in the soil. Fertiliser nitrogen further reduced nitrogen-fixing activity (Table 3.9). The inverse relationship between fixation and soil nitrogen uptake explains the absence of a nitrogen response in seed yield (Table 3.7). Nitrogen fertilisers normally reduce nitrogen fixation in peas, but fixation may be stimulated when low levels are used (Oghoghorie and Pate, 1971). If so, this gives rise to bimodal responses in nitrogen yield where increased growth and fixation result from a small addition of nitrogen fertiliser. Further increases in nitrogen actively replaces nitrogen from fixation. Although bimodal responses may have occurred, they were not important because of the high fertility. Nitrogen-fixing activity increased very rapidly between 31 and 38 days (Table 3.9) and is likely to have continued to increase rapidly for another 13 days until the storm. The increase in growth and hence demand for nitrogen would account for the majority of this increase, as does the increase in soil temperature (Figure 3.1), which would increase fixation over the range experienced (Pate, 1977a).

The interaction between nitrogen applied at nodule formation and straw in total nitrogen yield in plants at mid-pod filling (Table 3.10) indicates the compensatory responses of peas to changes in available nitrogen. Nitrogen fertiliser is likely to have been divided four ways: some used by micro-organisms involved in the decomposition of the straw (Russell, 1973), some available to the peas and the remainder lost by leaching and volatilisation. The amount of nitrogen actually available to peas, therefore, would have contributed to the initial peak in nitrogen yield, observed in a bimodal response. In contrast, without straw, nitrogen fertiliser reduced nitrogen yield. Nitrogen fixation was reduced

by early nitrogen (Table 3.9) but the subsequent amount of nitrogen available to plants was insufficient to compensate for the reduction in nitrogen fixation. Thus, without straw a greater amount of fertiliser would have been available and this actively suppressed nitrogen fixation without supplying the total nitrogen needs of the plant.

3.5 CONCLUSIONS

Peas depleted soil fertility, as determined by a subsequent ryegrass crop, less than a cereal. Approximately 50 per cent of the pea nitrogen requirements came from fixation. This lower demand for soil nitrogen appears the principal factor for enhancing subsequent crop growth.

Irrigation increased pea yield, though only by 12 per cent due to above average precipitation. It also increased fixation, as did the addition of straw. Nitrogen fertiliser, and harvesting at the green pea stage reduced fixation. It is evident that peas are able to compensate for changes in soil nitrogen status by altering demand for fixed nitrogen.

CHAPTER 4

SOIL MOISTURE, CULTIVAR AND SOWING DATE INFLUENCE NITROGEN FIXATION AND GROWTH OF PEAS

4.1 INTRODUCTION

It is widely recognised that drought reduces pea yields (Salter and Goode, 1967; Anderson and White, 1974; Jermyn and Batey, 1982; White *et al.*, 1982). In Canterbury, yields are frequently increased by irrigation (Stoker, 1973, 1977; Anderson and White, 1974b; White *et al.*, 1982) and irrigation at flowering and pod swelling is recommended (Salter and Goode, 1967; Stoker, 1977; White *et al.*, 1982). Although considerable efforts have been made to ascertain the effect of irrigation on growth and yield of peas both in New Zealand and overseas, there are few reports of field studies which have assessed the effect of water stress on nitrogen fixation. In a controlled environment study on peas, Minchin and Pate (1975) showed either water logging or drought reduced both the growth and nitrogen fixation, due to reduced transport of nitrogen from the root to the shoot. In a field study, Mahler, Bezdicek and Witters (1979) grew peas on a soil catena which showed varying levels of plant available water. Nitrogen fixation and yield were highest in peas grown on the low lying soil where both soil nitrogen availability, water use and yield were greatest. An initial study in Canterbury (Chapter 3), assessed growth and nitrogen fixation under varied soil moisture and nitrogen treatments. Although irrigation had the greatest influence on yield and nitrogen fixation, that trial was not designed to study responses to irrigation in detail. Further research is needed to show the effect of cultural and irrigation strategies on nitrogen fixation.

A strategy frequently used by farms on drought-prone soils is to autumn-sow peas so that they mature before droughts occur. This practice may have the added benefit of allowing nodules to form in winter which are then able to fix nitrogen as soon as soil temperatures rise in spring.

This trial was designed to assess the effects of autumn or spring sowing on nitrogen fixation and crop development in two pea cultivars. To assess further the effects of sowing time and its relationship with soil moisture at flowering, the moisture regime was varied from the commencement of flowering for each cultivar and sowing date. At present, the influence of these agronomic practices on nitrogen fixation is not known.

Two cultivars were chosen because of their ability to withstand frost during the winter, and because of their contrasting flowering patterns. The self-destruct mechanism, which may limit grain legume yields (Sinclair and de Wit, 1975), is likely to be more important in determinate, than in indeterminate cultivars. These indeterminate cultivars fill seeds over an extended flowering period and have large nitrogen reserves (Pate, 1977a). Partridge is an indeterminate cultivar, which has the potential to continue nitrogen fixation during pod filling, because the slower rate of pod development would be expected to reduce competition for assimilates between nodules and seeds. In contrast, the determinate cultivar, Whero, could be expected to cease nitrogen fixation shortly after the start of flowering when competition for assimilate would be intense.

4.2 MATERIALS AND METHODS

4.2.1 Trial Site

The trial was conducted on a Templeton silt loam soil at the Henley Research Farm, Lincoln College. The area had been summer fallowed in 1978/79, followed by autumn-sown Paroa ryegrass, which was grazed in late winter and the area was summer fallowed again in 1979/80. MAF quick-test

results were obtained in April 1980 and 5 t ha^{-1} of lime was spread at the end of April. The area was grubbed twice and harrowed prior to drilling the autumn-sown crop.

4.2.2 Trial Design

A factorial design was used with the three treatments and their levels shown below:

Sowing date A : autumn-sown (7/5/80)
S : spring-sown (12/9/80)

Cultivar W : Whero
P : Partridge

Irrigation D : covered with shelters during rain from flowering to maturity.
R : natural rainfall throughout growth.
I : irrigated from flowering when soil moisture reached 60% of field capacity.

A treatment combination A W D, is therefore autumn-sown Whero kept dry from flowering to maturity. This format (A W D, S W I, etc.) is used in all tables as appropriate.

4.2.3 Drilling and Crop Husbandry

Seed was treated with methiocarb (3 g methiocarb kg^{-1} seed) to deter birds from damaging the germinating seeds, and skim milk powder mixed to a wet paste with water, was used as a sticking agent. Inoculant granules containing *R. leguminosarum* were mixed with the seed until no further granules would adhere to the seed. Although peas readily form nodules without inoculation, inoculum was used to ensure that fully effective rhizobia

had every chance to form nodules. Autumn-sown peas were sown with a 1.5 m Duncan drill in 15 cm rows, at a rate of 140 viable seeds per m^2 to allow for plant mortality during the winter. Spring-sown peas were sown with a Stanhay precision seeder to establish the same population of plants at the spring sowing, as was present in autumn-sown plots. The area was surrounded by netting to stop hare and rabbit damage.

Weeds in autumn-sown plots were controlled by a mixture of metribuzine at 0.2 kg ha^{-1} and methabenzthiazurone at 0.7 kg ha^{-1} on 19 August, 1980, applied at the 9 - 10 node stage, when Whero and Partridge were 21 and 16 cm tall respectively. Patches of Whero plots were severely damaged by the herbicide and these areas were avoided as much as possible for sampling purposes. On September 5, the areas for spring-sown peas were cultivated with a rotary tiller and on September 9, a multi-tine grubber with crumbler attached was used to kill weeds which had grown after drilling of the autumn crop. Dry conditions after drilling the spring crop reduced weed establishment and no herbicides were used.

Three trickle lines were laid in plots which were to be irrigated at flowering. Neutron probe access tubes to allow measurement of soil moisture were placed in Whero plots soon after drilling. Removeable rain shelters (2.7 x 6 m) were erected on plots just prior to flowering. The shelters consisted of a permanent semi-circular framework of 17 mm reinforcing rod over which a clear plastic sheet could be rolled when rain appeared likely, and unrolled afterwards (Anderson, 1971). Shelters were used from 30 September 1980 to 13 February 1981. Plastic covers were renewed as necessary. Soil temperatures at 10 cm were measured for spring-sown Whero in dry, rainfall and irrigated plots on 31/12/80, 1/1/81 and 8/1/81 to assess the effect of moisture treatment on soil temperature.

4.2.4 Soil Nitrate

Soil nitrate was assessed at five intervals during growth: drilling, vegetative and pod filling stages in autumn Whero plots and during vegetative and pod filling stages in spring-sown crops. Sampling depths were 0 - 20 and 20 - 60 cm from May 17 to November 1, and 0 - 20 and 20 - 40 cm from December 15. Soil samples for nitrate analysis were measured by Ravensdown Fertilizer Company by the method described in Appendix II.

4.2.5 Soil Moisture

Although neutron probe access tubes were placed in all Whero plots, later analysis of data obtained did not give reliable indications of soil moisture and only back-up gravimetric soil moistures are given. These were taken before and after irrigation was applied (Figure 4.2, Appendix 4.1) by taking 2 cores per plot at depths 0 - 20 and 20 - 60 cm and after 27/11/80 at depths 0 - 20 and 20 - 40 cm. Sampling depths were reduced in the summer because of the difficulty of extracting soil cores at lower depths.

Field capacity for this soil over 0 - 20, 20 - 40 and 40 - 60 cm depths is 35, 25 and 27 per cent respectively on a dry weight basis. Wilting point at -15 bars is 15, 15 and 11.5 per cent at the respective depths (Hussein, pers. comm.). Irrigated plots were kept between 60 and 90 per cent of field capacity, but the first irrigation of autumn Whero and an irrigation of spring Whero exceeded this (Figure 4.2).

4.2.6 Sequential Harvests

Sampling dates were timed to reflect particular growth stages, and sampling methods were changed to allow for both increased plant variability and the difficulty of sampling plants which grew up to 3.5 m long.

All treatments were sampled at flowering so that later analysis of dry matter could compare plant size, nitrogen concentration and nitrogen yield for the different cultivars and sowing times at a particular growth stage other than maturity. Estimates of nitrogen fixation (C_2H_2) from spring and autumn crops were analysed separately.

For the autumn sowing, four initial harvests were conducted to assess nodulation and the start of nitrogen fixation. At each of these harvests, 10 plants were randomly chosen from the south end of plots and roots visually assessed for pink nodules, and when pink, 78 days from drilling, acetylene reduction assays began (Appendix I). Thereafter, two 0.1 m^2 quadrats were harvested and ethylene production and dry matter per top and per root were calculated. To minimise damage to the plots, a single 0.2 m^2 area was harvested from 17/10/80 and DM per plant measured. In addition, five plants per quadrat were separated into leaf, stem and reproductive portions. Harvests from 188 days were essentially the same, but only two plants were separated into leaf and stem because of the excessive time taken to separate leaf and stem portions. The ratio of leaf: stem was used to estimate leaf weight and stem weight per plant from all plants harvested. Plant mortality was high between harvests at 188 and 238 days after sowing, and measurements were based on a count of healthy stems from 0.2 m^2. The weights of pods and peas per healthy stem were measured by removing these parts from all harvested plants.

Spring-sown peas were harvested similarly, with leaves and stems separated from five plants, 99 days from sowing. Subsequent spring harvests utilised a leaf weight to stem weight ratio from two healthy stems to calculate the leaf and stem weight per healthy top. Pods and peas were removed from all plants. Plant components were oven dried at 70^{o}C for 36 hours, weighed and stored for nitrogen analysis (Appendix III).

At the final harvest, above-ground portions of plants were sampled from 0.2 m^2 for each treatment. Healthy stems were counted, pods and peas removed from all plants and two plants per quadrat were randomly selected for division into leaves and stems. Plant parts were oven dried as above and stored for subsequent nitrogen analyses, which were conducted on three replicates at the final harvest.

Final seed yield, plants per m^2 and 1000 seed weight were obtained from 1.8 m^2 quadrats in the central 6 rows in each plot. Pods per plant were counted for all plants harvested in separate 0. 2 m^2 quadrats as at earlier harvests. Harvest index was calculated from the ratio of seed yield to total biomass from 1.8 m^2. Nitrogen harvest index was the ratio of nitrogen in seed to total yield of nitrogen (g N m^{-2}), calculated from the nitrogen content in stem, leaf, pod and peas of five plants at the final harvest. Nitrogen translocation was calculated from the difference between plant nitrogen at flowering and at final harvest. Where seed nitrogen exceeded this difference, translocation from vegetative and pod fractions must have occurred. Where seed nitrogen was less than the difference in plant nitrogen between flowering and final harvest, uptake of nitrogen (between flowering and maturity) exceeded seed nitrogen requirements and translocation was negative.

Twenty pods were chosen at random from the 0.2 m^2 quadrats and the number of mature peas and the total number of ovules per pod were determined. The percentage of ovules failing to develop was calculated for each treatment:

$$\% \text{ ovule failure} = \frac{\text{No. of ovule initials per pod} - \text{No. of mature peas per pod}}{\text{No. of ovule initials per pod}} \times 100$$

4.2.7 Influence of Row Position on Reproductive and Total Biomass Production

During pod filling of irrigated autumn-sown Partridge and at maturity of spring-sown Partridge, 1 m lengths of plots were harvested to assess the yield from each of the 10 rows. In each row the number of plants was counted, whole tops separated from intact pods and dry matter per plant calculated for reproductive and vegetative portions. Harvest index was the ratio of total pod and pea dry weight to total yield per row.

At the final harvest of irrigated, autumn-sown Partridge, two methods were employed to estimate yield. The first was a yield from 1.8 m^2 where all plants in the middle six rows were harvested. In the second, two one m^2 quadrats were laid in the middle of the plots, on top of the plants. All plant material below these quadrats was harvested without regard for plant number or where plants originated within the plot. Quadrats were square and covered the six inner rows.

4.2.8 Lupins

On 24 June 1980 and again on 12 September 1980, *L. angustifolius* cv. Unicrop was sown at 200 kg ha^{-1} into four plots of the same size as the pea plots. Final seed yield was measured at maturity from 1.8 m^2 quadrats.

4.3 RESULTS

4.3.1 Soil Moisture

Water supply was initially adequate for growth and nitrogen fixation of autumn-sown peas, but abnormally dry conditions during September and October (117 - 177 days after sowing) caused a rapid rise in the potent-

ial soil moisture deficit (Table 4.1; Figure 4.1). Soil temperatures also rose during this period and remained above 10°C for the rest of the season (Figure 4.1). Soil moisture (0 - 20 cm) was 16.6 per cent on 3 October in autumn-sown plots relying on rainfall, and although rain in early November brought the soil moisture to 23.5 per cent, rainfall thereafter was inadequate (Figure 4.2, Appendix 4.1). Rainfall which occurred at the start of flowering of autumn-sown Partridge delayed the need for irrigation by 11 days. Spring-sown Whero flowered shortly after rain in November, but spring Partridge started flowering when soil moistures in the top 20 cm were below wilting point. Rain shelters increased 10 cm soil temperatures only slightly, during early January (Table 4.2).

Table 4.1: Long-term and actual monthly precipitation, July 1980 to February 1981, Lincoln College Meteorological Station.

Month	Long-term mean (mm month^{-1})	Actual precipitation (mm month^{-1})
July	61	40
August	58	48
September	51	1
October	51	13
November	51	85
December	61	29
January	56	25
February	46	12
	(1944-1960)	

Table 4.2: The effect of soil moisture treatments on soil temperature in spring-sown Whero. (Mean of six readings between 31/12/80 and 8/1/81.)

Treatment	Soil temperature at 10 cm	$S_{\bar{x}}$
Dry	18.0	0.8
Rainfall	16.3	0.7
Irrigated	16.0	0.9

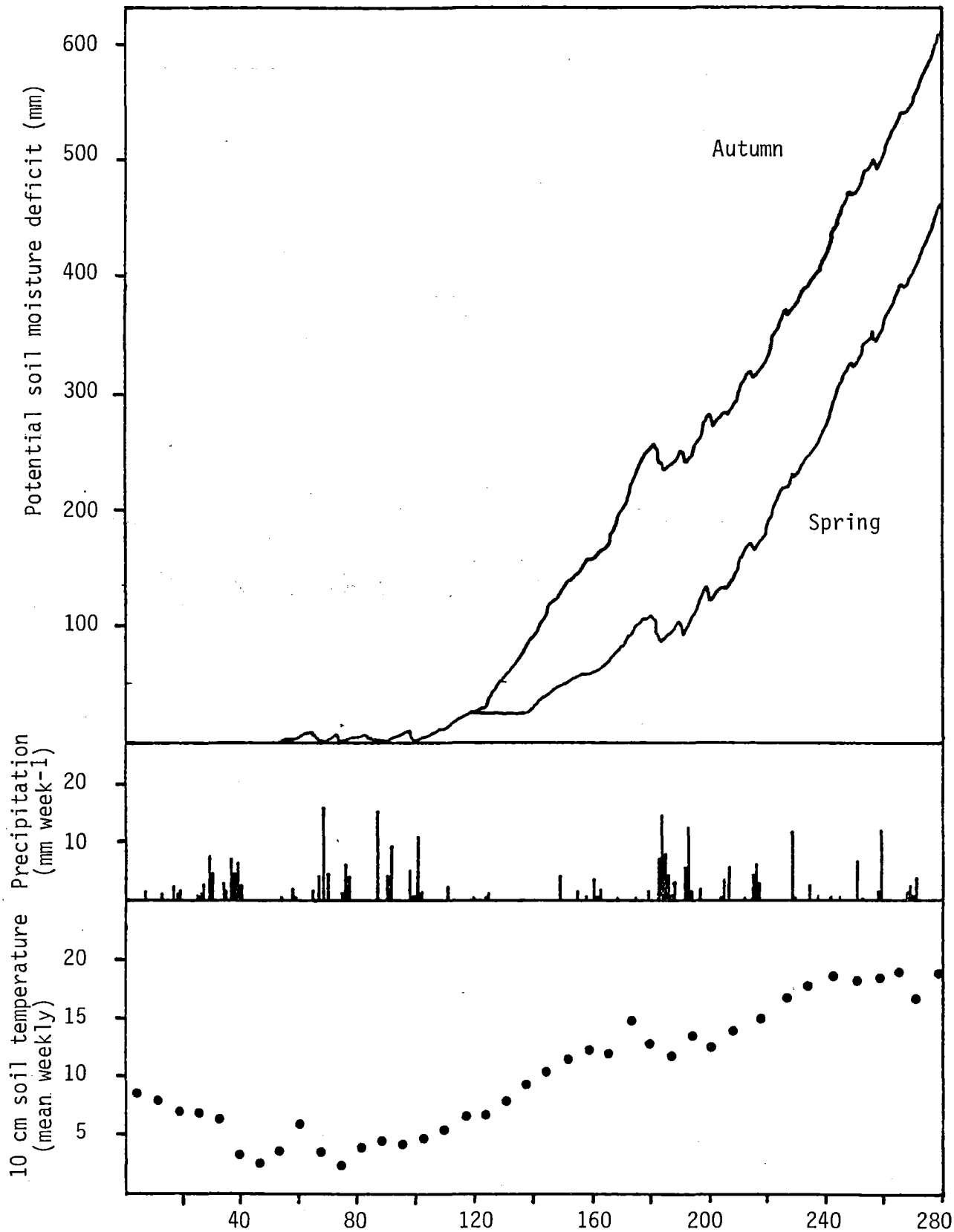


Figure 4.1: Calculated and actual climatic records for the period from May 1980 to February 1981. (Soil moisture deficit estimated to be zero in mid-winter, July 1.)

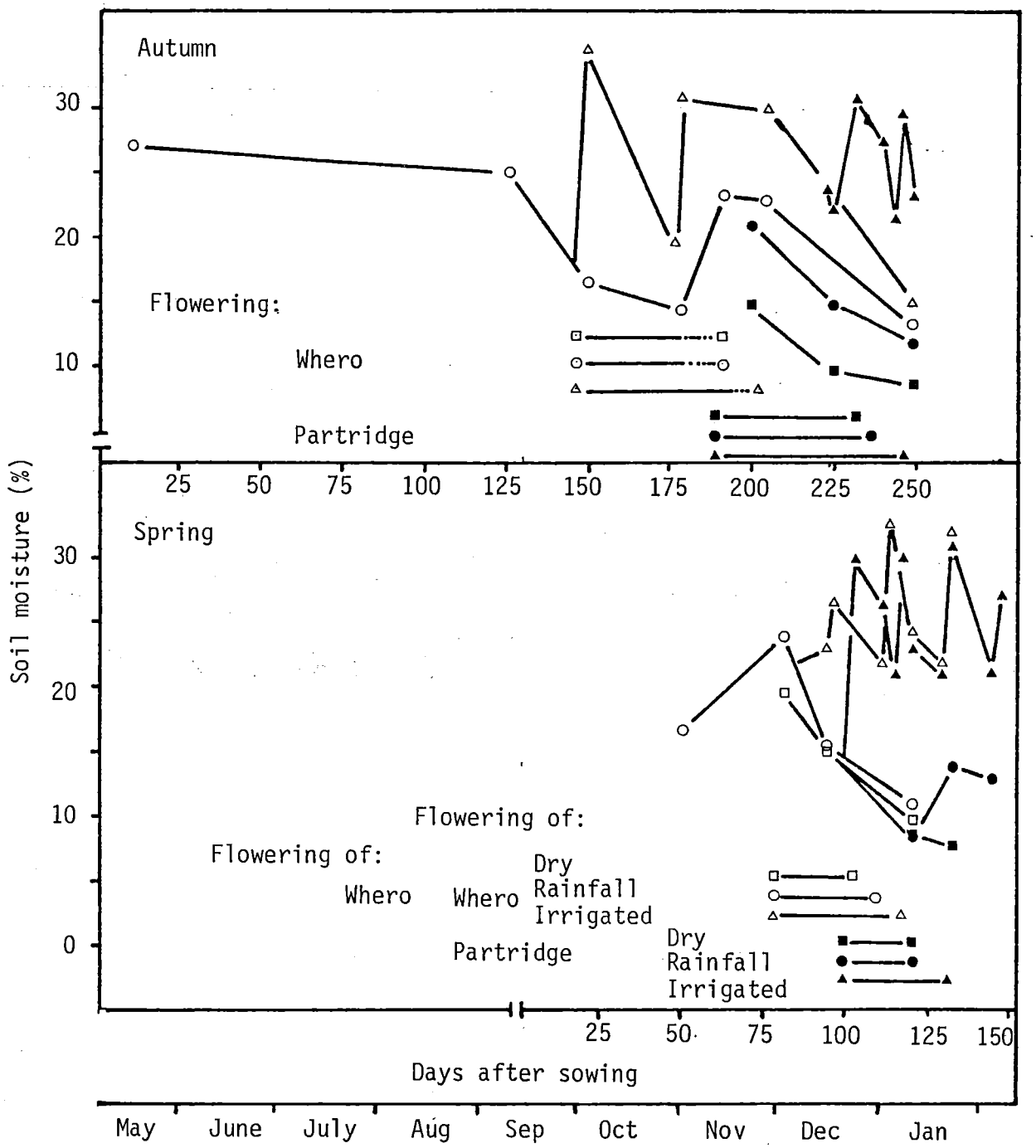


Figure 4.2: Effect of irrigation treatments on soil moisture percentage (0 - 20 cm). Dotted line refers to flowering of non-productive basal branches.

4.3.2 Soil Tests

M.A.F. quick-test results are shown in Table 4.3. Olsen test phosphorus levels were medium to high, and a response to added superphosphate was unlikely. Levels of available soil nitrogen were generally above 10 ppm NO_3-nitrogen, but irrigation of autumn-sown Whero reduced these to 4 (0 - 20 cm) and 8 ppm N (20 - 60 cm) during pod filling (Table 4.4).

Table 4.3: Soil pH and quick test nutrient concentrations.

pH	Ca	K	P	Mg
5.5	10	16	27	23

4.3.3 Nitrogen (C_2H_2) Fixation

Autumn-sown peas developed small white nodules 29 days after sowing, which did not become pink until 58 days after sowing. Twenty days later, ethylene production of 60 nmoles C_2H_4 plant^{-1} h^{-1} was observed (Figure 4.3). Mean weekly soil temperatures at this time were below 6^{o}C (Figure 4.1). In contrast, spring-sown peas produced 275 nmoles C_2H_4 plant^{-1} h^{-1}, 14 days after emergence (Figure 4.4) when mean weekly soil temperatures were above 10^{o}C.

Rates of acetylene reduction did not exceed 2400 nmoles C_2H_4 plant^{-1} h^{-1}, and peak fixation rates occurred before flowering in all treatments except irrigated autumn Whero where the peak was reached near the end of flowering. Soil moisture changes were associated with large changes in nitrogen-fixing activity. This activity tended to be greater in Partridge than Whero except where irrigation of autumn Whero increased fixation. Autumn sowing allowed an early development of nitrogen fixation

Table 4.4: Levels of available soil nitrate during growth of peas.

Treatment	Date	Stage of growth	Depth cm	Nitrate $\mu g\ NO_3\ g\ soil^{-1}$	$S\bar{x}$	Soil nitrate $g\ N\ m^{-2}$
A W R	17. 5.80	Drilling	0-20	45.5	7.4	10.3
			20-60	20.8	4.1	11.7
A W R	11. 9.80	Vegetative	0-20	16.0	1.6	3.6
			20-60	41.0	4.4	23.1
A W R	1.11.80	Pod filling	0-20	12.0	4.0	2.7
			20-60	13.0	1.5	7.3
A W I	1.11.80	Pod filling	0-20	4.0	0.3	0.9
			20-60	8.0	1.6	4.5
S W R	1.11.80	Vegetative	0-20	22.0	5.1	5.0
			20-60	22.0	1.2	12.4
S W R	15.12.80	Pod filling	0-20	6.5	0.65	1.5
			20-40	13.5	1.04	3.8
S P R	22. 1.81	Pod filling	0-20	16.5	2.33	3.7
			20-40	19.3	2.78	5.4
S P I	22. 1.81	Pod filling	0-20	14.8	2.39	3.4
			20-40	16.3	3.01	4.6

(Bulk densities used to calculate this were from data 17 May, 1980.)

(A, S = autumn and spring sown; W, P = Whero and Partridge;
D, R, I = dry, rainfall and irrigated.)

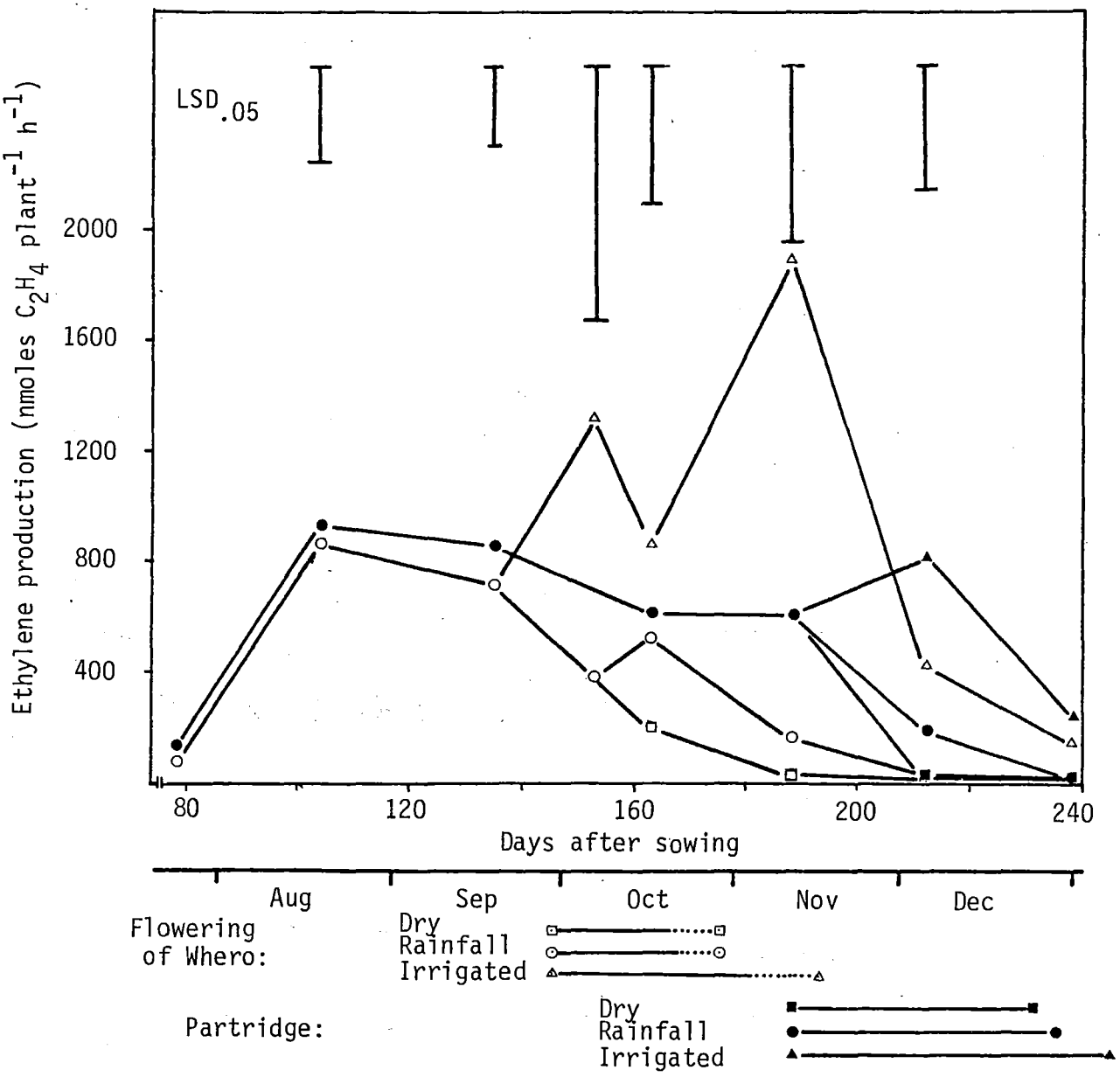


Figure 4.3: Influence of cultivar and soil moisture on nitrogen fixing activity of autumn-sown peas. (Dotted line refers to flowering of non-productive basal branches.)

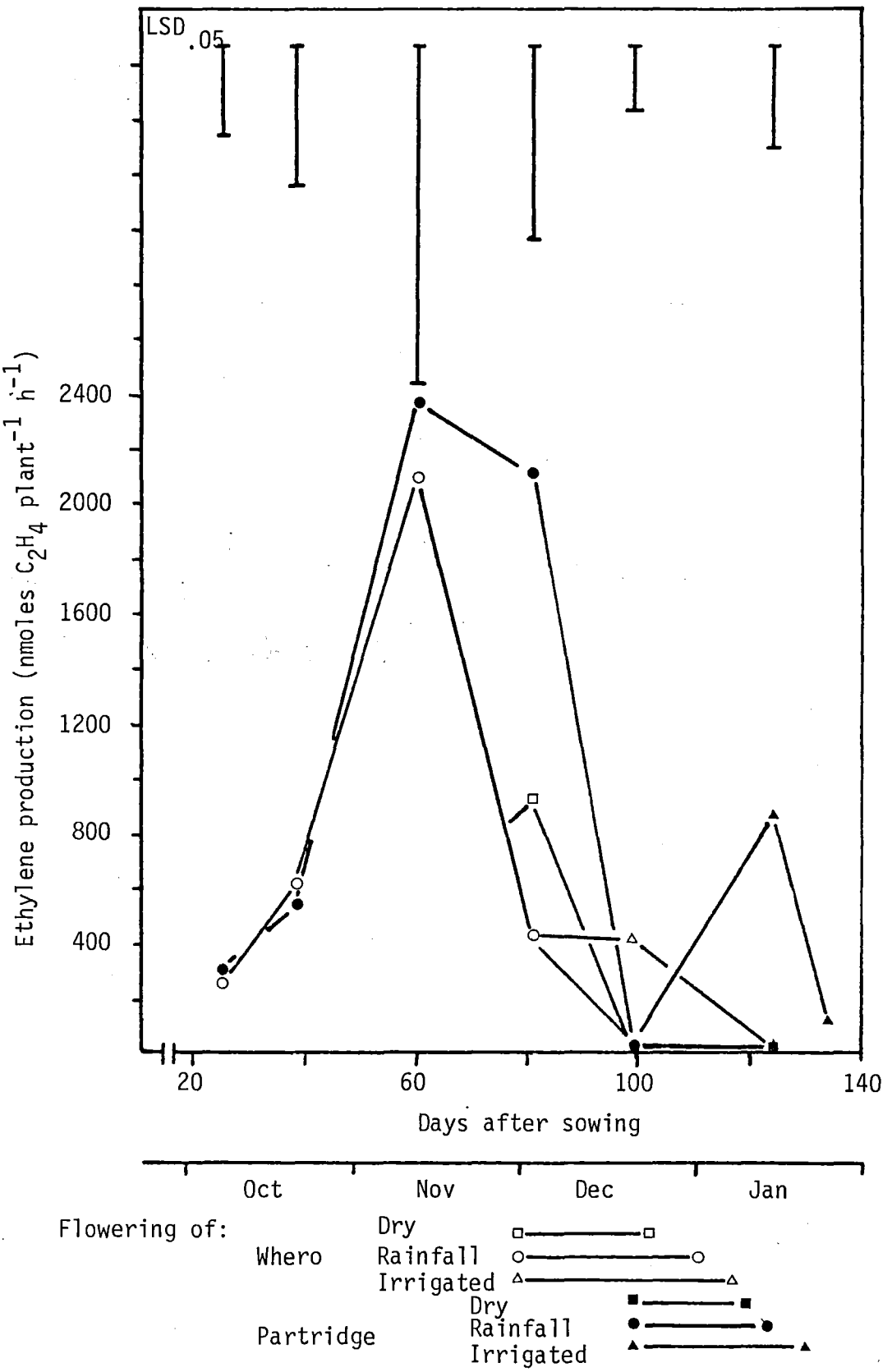


Figure 4.4: Influence of cultivar and soil moisture on nitrogen fixing activity of spring-sown peas.

(Plate 4.1), but the peak rate before flowering was less than 1000 nanomoles C_2H_4 plant^{-1} h^{-1}.

4.3.4 Effect of Inter-Row Competition

Irrigated Partridge plants, in particular, grew very long and during pod filling some vines were 3.5 m long. The outer two rows were not used for sequential harvests, but these rows became dominant and suppressed growth in the other rows. This was particularly evident in autumn Partridge and less evident in spring-sown, irrigated Partridge (Figure 4.5; Plate 4.2). Plants generally lay along the length and partially across plots so that competitive effects did not extend far into adjacent plots. Total biomass production and harvest index (HI) were greatest for outside rows. This competition was further shown by the two methods of final harvest for autumn-sown, irrigated Partridge (Table 4.5). Thus, the apparent reduction in yield per plant (Figure 4.5) and per m^2 (Table 4.6) from irrigation of autumn Partridge was more an example of intense competition with two rows becoming dominant and contributing greatly to the final seed yield.

Table 4.5: Influence of method of harvest on final seed yield of autumn-sown, irrigated Partridge peas.

Inner 6 rows only	2 x 1 m^2 quadrats cut from each plot
(g m^{-2})	
98	253**
CV 5.1%	

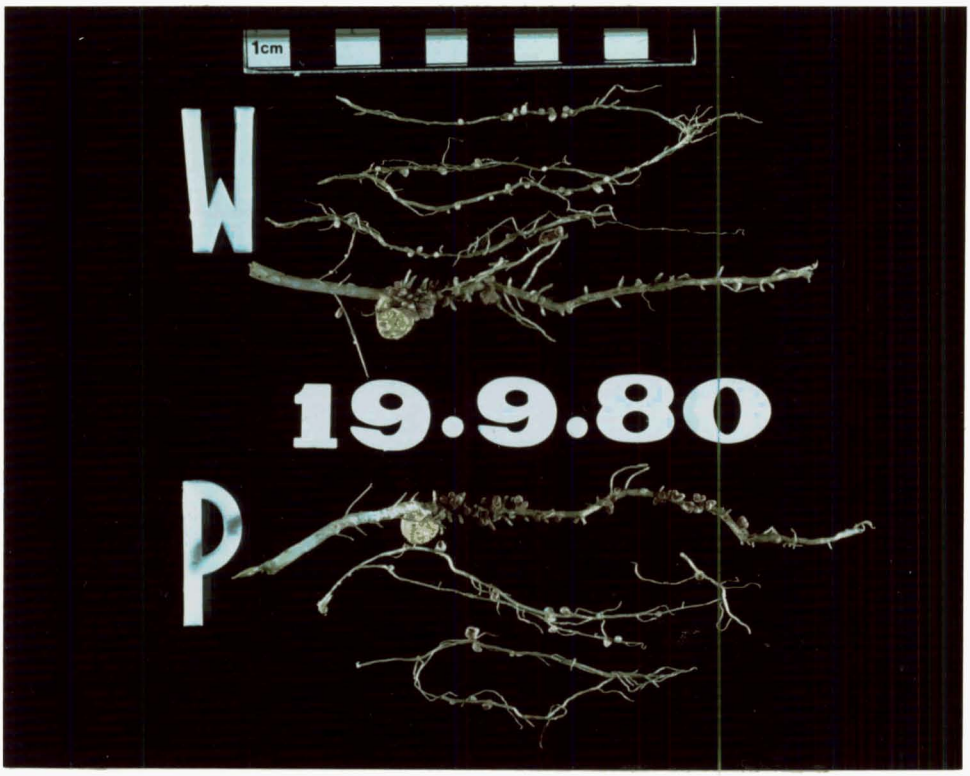


Plate 4.1: Nodule development on autumn-sown peas.
(W = Whero; P = Partridge)

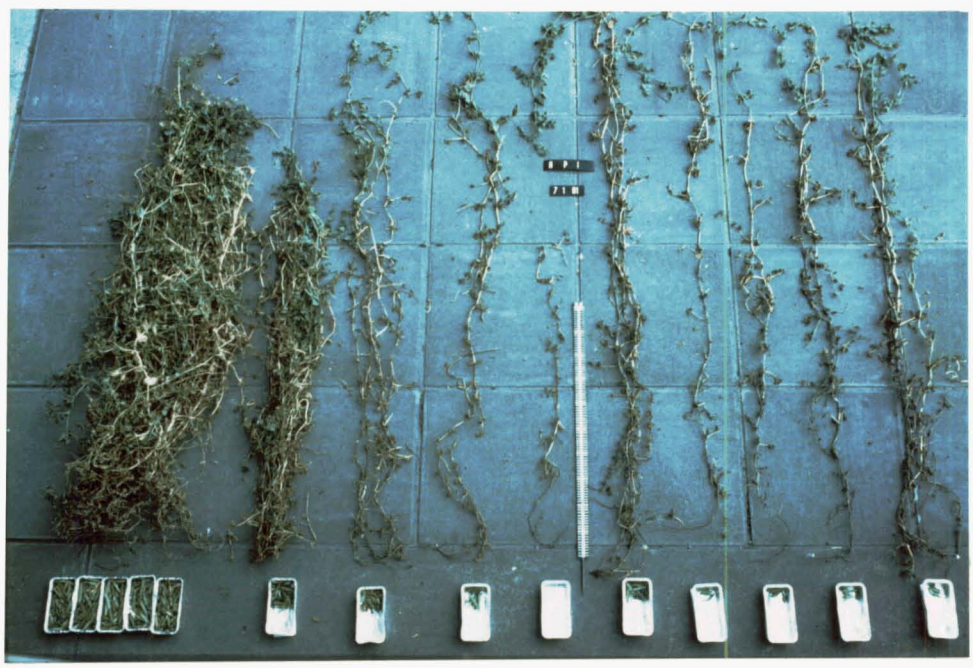


Plate 4.2: The influence of row position on vegetative and reproductive yield of autumn-sown and irrigated Partridge peas.
(Outside row on left; Rule = 1 m)

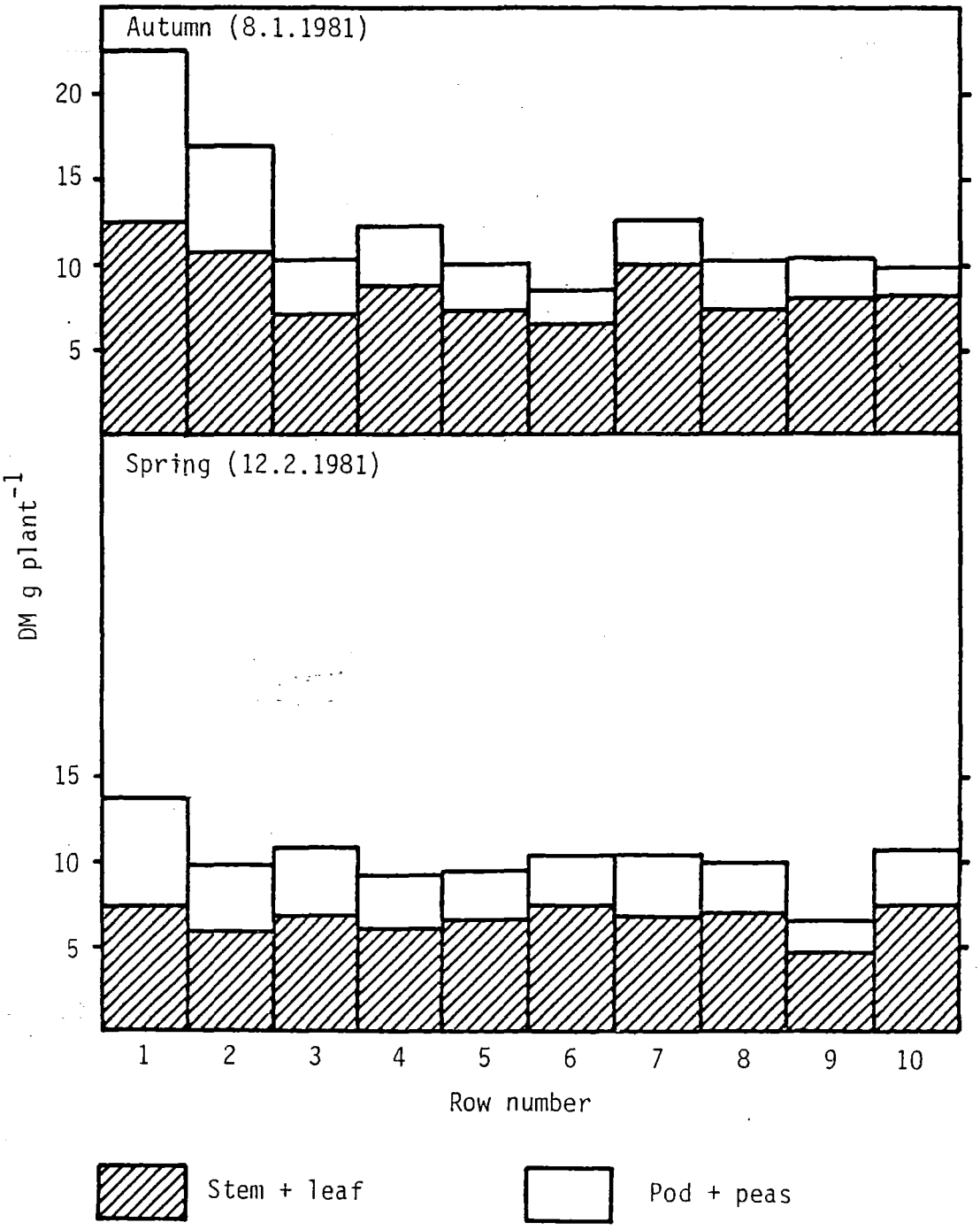


Figure 4.5: Effect of row position on dry matter production per plant in irrigated autumn and spring sown peas.

Table 4.6: Effect of all treatment combinations on time to maturity, seed yield and estimated nitrogen fixation.

Treatment		Time from sowing to maturity (days)	Seed yield ($g\ m^{-2}$)	Estimated nitrogen (C_2H_2) fixation ($g\ N\ m^{-2}\ season^{-1}$)
Autumn Whero	D	212	243	1.3
	R	240	318	1.6
	I	240	326	3.2
Partridge	D	250	166	2.7
	R	250	159	2.7
	I	254	98	3.3
Spring Whero	D	124	286	1.5
	R	130	280	1.4
	I	130	313	1.5
Partridge	D	134	205	2.3
	R	134	153	2.3
	I	154	223	2.7

(D, R, I = dry, rainfall and irrigated.)

4.3.5 Accumulation and Partitioning of Dry Matter

Initial growth rates were slower in autumn-sown than in spring-sown peas, and were also slower in Partridge than in Whero (Figure 4.6). At both sowing dates, irrigation increased biomass yield of Partridge. The later maturity of Partridge compared with Whero was shown by the sequential apportioning of dry matter into stem, leaf, pod and peas (Figure 4.7). Irrigation of autumn Partridge stimulated leaf and stem growth with less than 10 per cent of dry matter per plant as peas at the final harvest. In contrast, irrigation of spring Partridge enhanced pea more than vegetative development. The partitioning of dry matter at final harvest is presented in detail in Table 4.7.

4.3.6 Dry Matter and Nitrogen at Flowering and at Final Harvest of all Treatments

Autumn and spring Partridge peas flowered 43 and 21 days later respectively, than Whero peas. At flowering of all treatments, root and top weights were highest in the oldest plants, which were autumn-sown Partridge. Autumn-sown Whero plants, however, were generally smaller than the spring-sown Whero plants (Table 4.7). These differences were smaller on a per m^2 basis because of the greater population in autumn-sown peas (Table 4.8). At the final harvest, autumn-sown Partridge produced 921 g m^{-2} in leaf and stem which was 67 per cent more than from spring-sown Partridge. A number of interactions which were significant on a per plant basis are not shown, as they were not important on an area basis (Table 4.8).

At flowering of all treatments, roots of spring-sown peas had 67 per cent higher nitrogen concentration than did autumn peas which had 3.13 per cent nitrogen (Table 4.9). The interaction of sowing date and cultivar at flowering for nitrogen concentration of whole tops showed that when autumn-sown, Partridge had 2.79, but when spring-sown, this cultivar

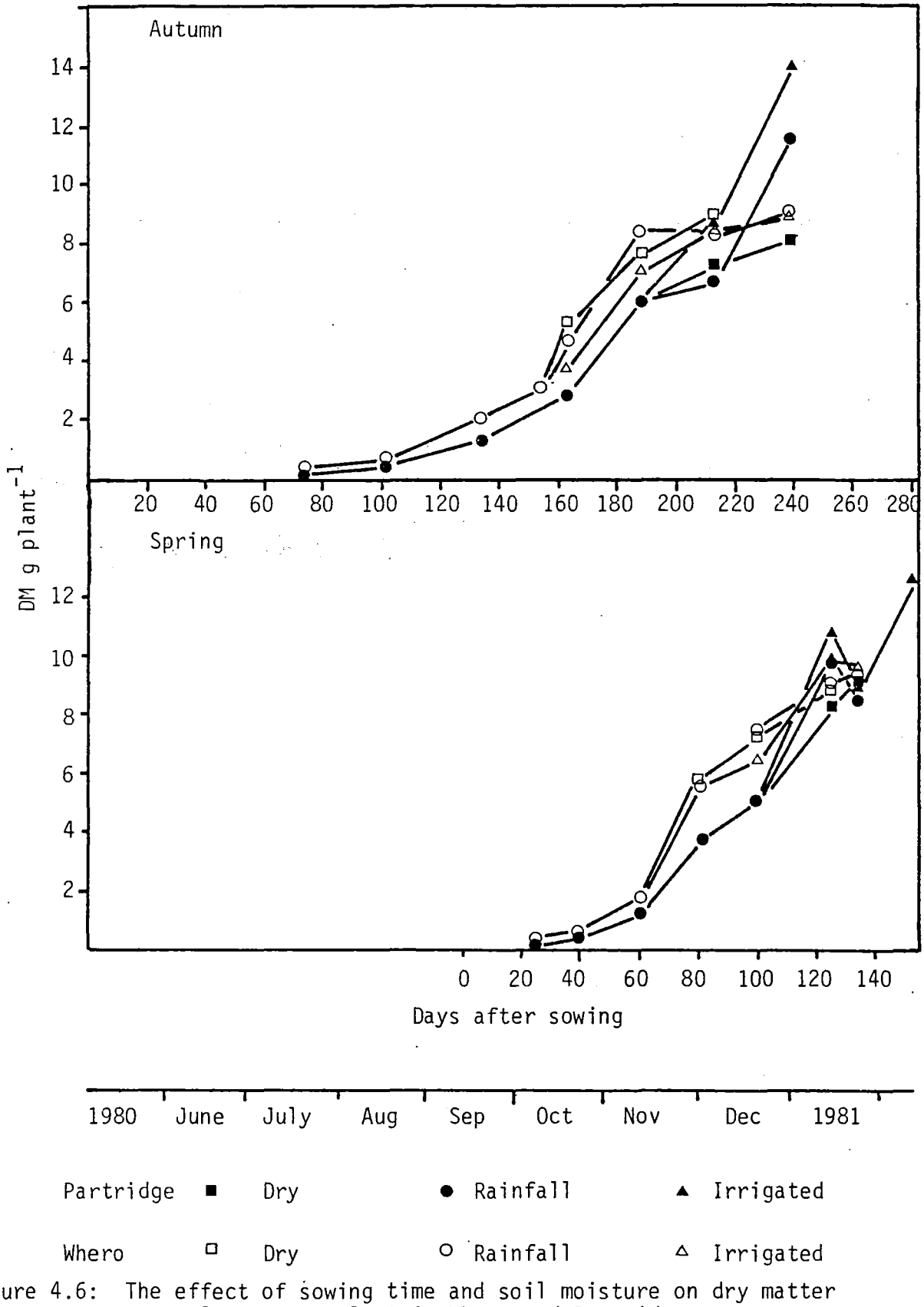
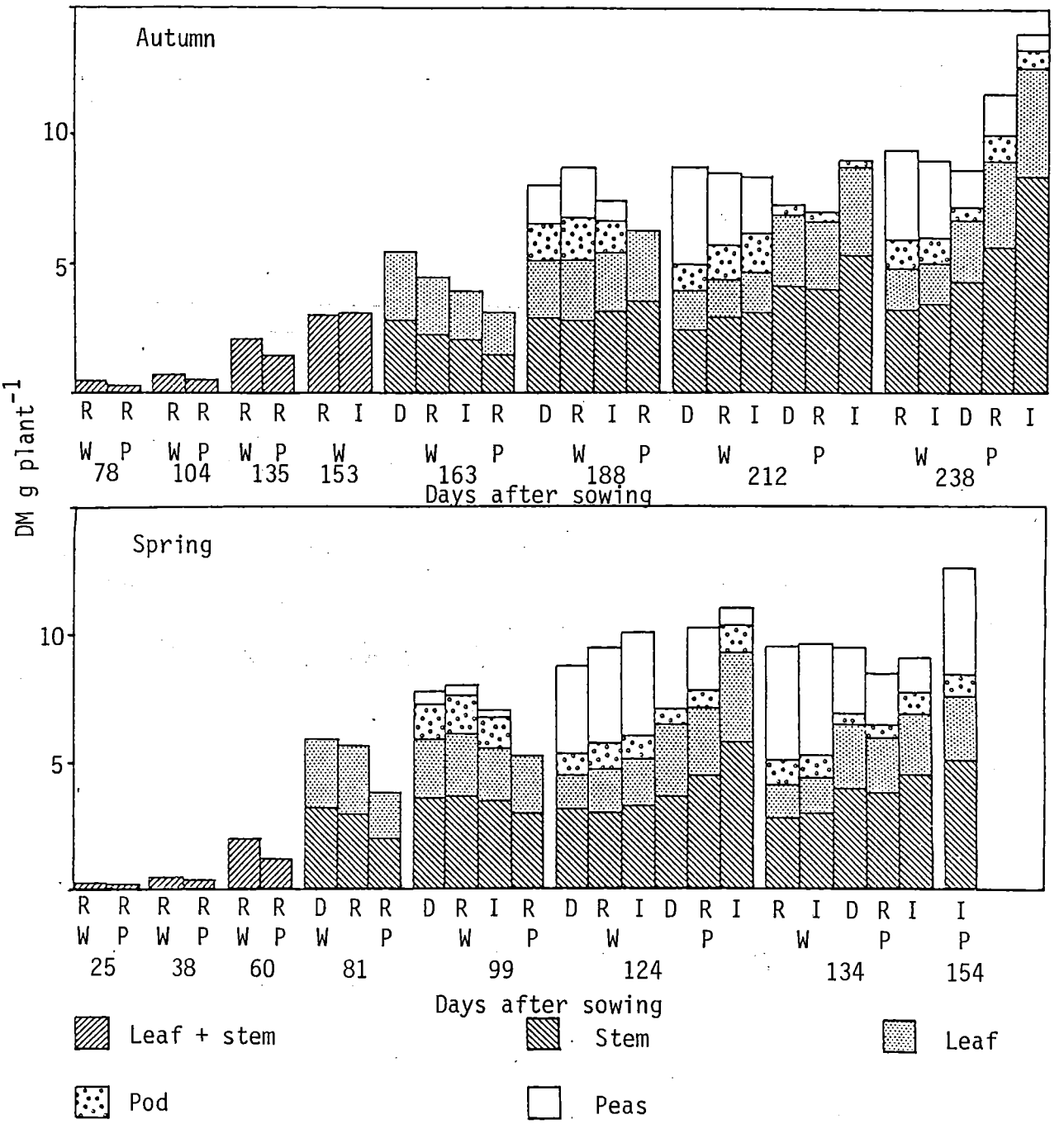


Figure 4.6: The effect of sowing time and soil moisture on dry matter accumulation per plant in Whero and Partridge.



(W, P =Whero, Partridge; D, R, I = dry, rainfall, irrigated.)

Figure 4.7: The effect of sowing time and soil moisture on partitioning of dry matter per plant in Whero and Partridge.

Table 4.7: Influence of cultivar, sowing date and soil moisture on the partitioning of dry matter per plant into root and whole tops at flowering; and into stem, leaf, pod and peas at final harvest.

Treatment		Flowering		Final harvest			
		Dry matter (g plant^{-1}) in:					
		Root	Whole tops	Stem	Leaf	Pod	Peas
Cultivar	W	0.13**	4.4	2.8**	1.5**	0.8	3.5*
	P	0.23	4.7	5.2	2.8	0.6	2.2
Sowing date	A	0.15*	4.3	4.4*	2.3*	0.8	2.3*
	S	0.21	4.8	3.6	1.9	0.7	3.4
$LSD_{.05}$		0.04	1.11	0.68	0.36	0.16	0.98
Moisture	D			3.4**	2.0	0.6	2.8
	R			3.6	2.0	0.8	2.6
	I			5.0	2.4	0.8	3.2
$LSD_{.05}$				0.85	0.44	0.20	1.20
CV		19.7	21.5	25.0	24.4	32.0	49.5
Significant interactions		None	CxS**	CxS* CxM*	CxS* CxM** MxS*	MxS*	None

		A	S	A	S	A	S
	W	3.1	5.6	2.9	2.8	1.5	1.5
	P	5.5	3.9	5.9	4.4	3.2	2.4
$LSD_{.05}$		1.56		0.98		0.51	

(A, S = autumn and spring sown; W, P = Whero and Partridge; D, R, I = dry, rainfall and irrigated)

Table 4.8: Influence of cultivar and sowing date on plant population, and dry matter in roots and plant tops at flowering; and the influence of cultivar, sowing date and moisture on the partitioning of dry matter at the final harvest.

Treatment		Flowering			Final harvest			
		(Dry matter g m^{-2}) in:						
		Plants m^{-2}	Root	Whole tops	Stem	Leaf	Pod	Peas
Cultivar	W	115	14.6**	473	248**	133**	75	313*
	P	120	26.4	568	482	254	62	210
Sowing date	A	140**	21.4	591*	426*	223*	79	220
	S	95	19.5	450	305	164	58	303
$LSD_{.05}$		23	4.73	115	90	43	27	100
Moisture	D				347	200	58	266
	R				339	186	79	253
	I				409	194	68	266
$LSD_{.05}$					111	53	34	123
CV		17.5	20.4	19.5	35.5	32.2	57.5	55.1
Significant interactions		None	None	CxS**	CxS**	CxS**	None	None
				A S	A S	A S		
				W 431 515	245 252	131 135		
				P 751 385	607 358	314 193		
			$LSD_{.05}$	162	128	61		

(A, S = autumn and spring sown; W, P = Whero and Partridge, D, R, I = dry, rainfall and irrigated.)

Table 4.9: Influence of cultivar and sowing date on nitrogen concentration in roots and whole tops at flowering; and the influence of cultivar, sowing date and soil moisture on nitrogen concentration in plant components at final harvest.

Treatment		Flowering		Final harvest			
		Nitrogen concentration (N%) in:					
		Root	Whole tops	Stem	Leaf	Pod	Peas
Cultivar	W	3.98	3.14	1.04**	2.56**	1.62**	4.48
	P	4.37	3.34	1.52	3.16	2.95	4.44
Sowing date	A	3.13**	3.04**	1.40	3.05*	2.67**	4.46
	S	5.22	3.44	1.16	2.66	1.90	4.46
$LSD_{.05}$		0.41	0.26	0.32	0.30	0.38	0.32
Moisture	D			1.19	2.32**	2.07	4.56
	R			1.26	2.95	2.59	4.53
	I			1.38	3.30	2.20	4.29
$LSD_{.05}$				0.39	0.36	0.46	0.39
CV		8.7	7.1	35.7	15.0	23.8	10.3
Significant interactions		None	CxS**	CxS**	None	MxS**	None

	A	S		A	S		A	S
W	3.29	2.99	W	1.35	0.73	D	2.20	1.94
P	2.79	3.90	P	1.46	1.58	R	2.73	2.44
						I	3.08	1.33
$LSD_{.05}$	0.37			0.45			0.65	

(A, S = autumn and spring sown; W, P = Whero and Partridge; D, R, I = dry, rainfall and irrigated.)

had 3.90 per cent nitrogen. In contrast, Whero had a higher concentration (3.29) in the autumn, and 2.99 per cent nitrogen when spring-sown. The highly significant difference between root nitrogen content per plant at the two sowing dates (Table 4.10) was associated with differences in nitrogen concentration in roots at flowering.

Final harvest nitrogen content per plant reflected the differences between the two cultivars; Partridge had more stem and leaf nitrogen per plant, whereas Whero had more nitrogen stored in pods and peas. These effects balanced each other and there were no significant differences in total nitrogen per plant (Table 4.10). Irrigation increased nitrogen yield in Partridge, but not Whero (Table 4.11). At flowering, nitrogen concentration of whole tops was greatest in spring (3.90) and least in autumn Partridge (2.79% N).

At final harvest, leaf nitrogen concentration was 23 per cent greater in Partridge compared to Whero. Increases in the availability of soil moisture were also associated with increased leaf nitrogen concentration. Nitrogen concentration in pods was 82 per cent greater in Partridge than Whero. Enhanced soil moisture in autumn-sown crops resulted in increased nitrogen concentration in pods. Nitrogen content of the seed was unaffected by treatment (Table 4.9). Nitrogen yield per plant and per m^2 in stem and leaf were highest in Partridge but seed nitrogen yield in Whero was 48 per cent greater than Partridge (Tables 4.10, 4.11). Irrigation of Partridge stimulated a significant increase in total nitrogen per m^2.

4.3.7 Components of Yield and Final Seed Yield

Whero yielded 76 per cent more seed than did Partridge, but some of this difference was caused by the harvesting technique which did not allow for the inter-row competition which occurred particularly in autumn-sown, irrigated Partridge. Plant populations were maintained by

Table 4.10: Influence of cultivar and sowing date on nitrogen content in roots and whole tops at flowering, and the influence of cultivar, sowing date and soil moisture on nitrogen content of plant components at final harvest.

Treatment		Flowering		Final harvest				
		Nitrogen content (mg N plant^{-1}) in:						
		Root	Whole tops	Stem	Leaf	Pod	Peas	Total
Cultivar	W	5.52**	119**	31**	38**	13**	155**	237
	P	10.08	184	80	91	18	98	287
Sowing date	A	4.86**	137	66*	75*	20**	100*	264
	S	10.75	166	44	53	11	153	261
$LSD_{.05}$		2.00	37.7	21.1	17.4	3.1	42	56
Moisture	D			43	49*	10**	126	229
	R			48	62	18	117	248
	I			75	81	17	136	310
$LSD_{.05}$				25.7	21.3	3.7	51	68
CV		22.6	22.0	54.9	39.1	28.7	47.2	30.4
Significant interactions		None	None	None	None	MxS*	None	None

	A	S
D	11	10
R	24	12
I	23	11

$LSD_{.05}$ 5.3

(A, S = autumn and spring sown; W, P = Whero and Partridge; D, R, I = dry, rainfall and irrigated.)

Table 4.11: Influence of cultivar and sowing date on nitrogen yield (g N m^{-2}) in roots and whole tops at flowering; and the influence of cultivar, sowing date and soil moisture on nitrogen yield in plant components at final harvest.

Treatment		Flowering		Final harvest				
				Nitrogen yield (g N m^{-2}) at:				
		Root	Whole tops	Stem	Leaf	Pod	Peas	Total
Cultivar	W	0.58**	13.1*	2.6**	3.3**	1.2	13.8*	20.7
	P	1.14	21.1	7.2	8.1	1.5	9.3	24.7
Sowing date	A	0.69*	18.4	6.1**	6.9**	1.7**	9.6	22.7
	S	1.03	15.8	3.7	4.4	1.0	13.5	22.6
$LSD_{.05}$		0.20	7.9	1.35	1.48	0.37	4.1	5.0
Moisture	D			4.3	4.8	1.1	12.1	22.2
	R			4.3	5.6	1.5	11.1	20.2
	I			5.9	6.6	1.4	11.4	25.5
$LSD_{.05}$				1.65	1.80	0.45	5.0	6.1
CV		20.4	40.7	39.8	37.6	40.1	51.3	31.4
Significant interactions		None	None	None	CxS*	MxC*	MxS*	MxC*

	A	S		W	P	A	S	W	P
W	3.6	2.9	D	1.0	1.1	11.8	12.3	21.8	22.7
P	10.3	5.9	R	1.5	1.4	11.1	11.1	21.8	18.6
			I	0.9	1.9	5.8	17.1	18.3	32.8
$LSD_{.05}$	2.10			0.64		7.11		8.6	

(A, S = autumn and spring sown; W, P = Whero and Partridge; D, R, I = dry, rainfall and irrigated.)

rain shelters in autumn-sown peas, but density of spring-sown peas was unaffected by moisture regime. Pod numbers were increased by increased soil moisture, particularly in spring-sown plots. Seeds per pod were increased 56 per cent by spring sowing, and Whero contained 30 per cent more than Partridge at both sowing dates (Table 4.12). The potential number of seeds per pod was much greater than the mean of 2.3 observed. Twenty-five per cent of autumn-sown Whero pods did not contain even one fully developed seed (Table 4.13), and nearly half the ovule initials failed to develop. Plate 4.3 shows the range of ovule failure in Whero and Partridge peas. Autumn-sown Partridge had the lowest percentage ovule failure (39%). Calculation of seeds per pod from values in Table 4.13 (obtained from 20 pods) results in higher values than those in Table 4.12, but this table was based on a calculation which incorporated a pod count from all plants in 0.2 m^2 quadrats and seed yield from all of those plants.

Individual seed weights were 59 per cent greater in Whero, compared with Partridge (Table 4.12). Seed size in Whero was increased by autumn sowing, whereas time of sowing had little effect on Partridge. Increased soil moisture reduced seed size over all treatments.

4.3.8 Harvest Indices

The HI and NHI of Whero were 75 and 97 per cent greater than Partridge. Harvest indices were lower in autumn- than spring-sown peas. NHI in autumn peas was depressed by rainfall and further depressed to 0.29 by irrigation (Table 4.14).

4.3.9 Translocation of Nitrogen

Negative values for translocation of nitrogen occurred when the total nitrogen increase in plant tops between flowering and maturity

Table 4.12: Influence of cultivar, sowing date and moisture on plant population, pods per plant, seeds per pod, seed weight and seed yield at final harvest.

Treatment		Plants m^{-2}	Pods $plant^{-1}$	Seeds pod^{-1}	1000 seed weight (g)	Seed yield (g DM m^{-2})
Cultivar	W	84	5.6	2.6**	251**	295**
	P	90	6.4	2.0	158	168
Sowing date	A	85	5.6	1.8**	210**	218
	S	89	6.4	2.8	100	243
$LSD_{.05}$		6.1	0.84	0.36	5.8	39.5
Moisture	D	105**	4.8**	2.6	216**	225
	R	81	5.9	2.3	209	227
	I	74	7.3	2.1	189	240
$LSD_{.05}$		7.5	1.03	0.45	7.2	48.3
CV		12	23.9	26.7	4.9	29.0
Significant interactions		MxCxS**	CxS** CxM* SxM*	None	CxS*	None

	A	S
WD	135	89
R	55	84
I	55	84
PD	107	89
R	89	96
I	68	88
$LSD_{.05}$	15	

	A	S
W	5.7	5.5
P	5.5	7.3
LSD .05	1.2	

	A	S
D	4.4	5.2
R	6.3	5.6
I	6.0	8.5
$LSD_{.05}$	1.5	

	W	P
D	4.8	4.8
R	5.8	6.0
I	6.1	8.4
$LSD_{.05}$	1.5	

	A	S
W	260	243
P	160	156
		8.2

(A, S, = autumn and spring sown; W, P = Whero and Partridge; D, R, I = dry, rainfall and irrigated.)

Table 4.13: Influence of cultivar, sowing date and moisture on the percentage of pods which did not contain one or more fully developed seeds, the total ovules per pod and the percentage of ovules which failed to develop.

Treatment		Percentage of pods# without one (or more) fully developed seeds	Total ovules pod-1	Ovules failure# (%)
Cultivar	W	14.8**	6.9**	52**
	P	8.5	6.5	45
Sowing date	A	16.4**	6.7	47
	S	7.0	6.7	51
$LSD_{.05}$		4.3	0.25	4.7
Moisture	D	9.0*	7.0*	52
	R	10.4	6.5	50
	I	15.7	6.6	44
$LSD_{.05}$		5.2	0.30	5.8
CV		62.0	6.2	16.4
Significant interactions		CxS**	MxCxS*	CxS**

	A	S
W	24.9	4.7
P	7.9	9.2

$LSD_{.05}$ 6.0

	A	S
WD	7.3	7.1
R	6.3	7.0
I	6.5	7.2
PD	6.7	6.8
R	6.5	6.4
I	6.7	6.1

$LSD_{.05}$ 0.6

	A	S
W	54	51
P	39	50

$LSD_{.05}$ 6.7

(# Arcsine transformation did not alter conclusions drawn from these analyses.)
(A, S = autumn and spring sown; W, P = Whero and Partridge; D, R, I = dry, rainfall and irrigated)

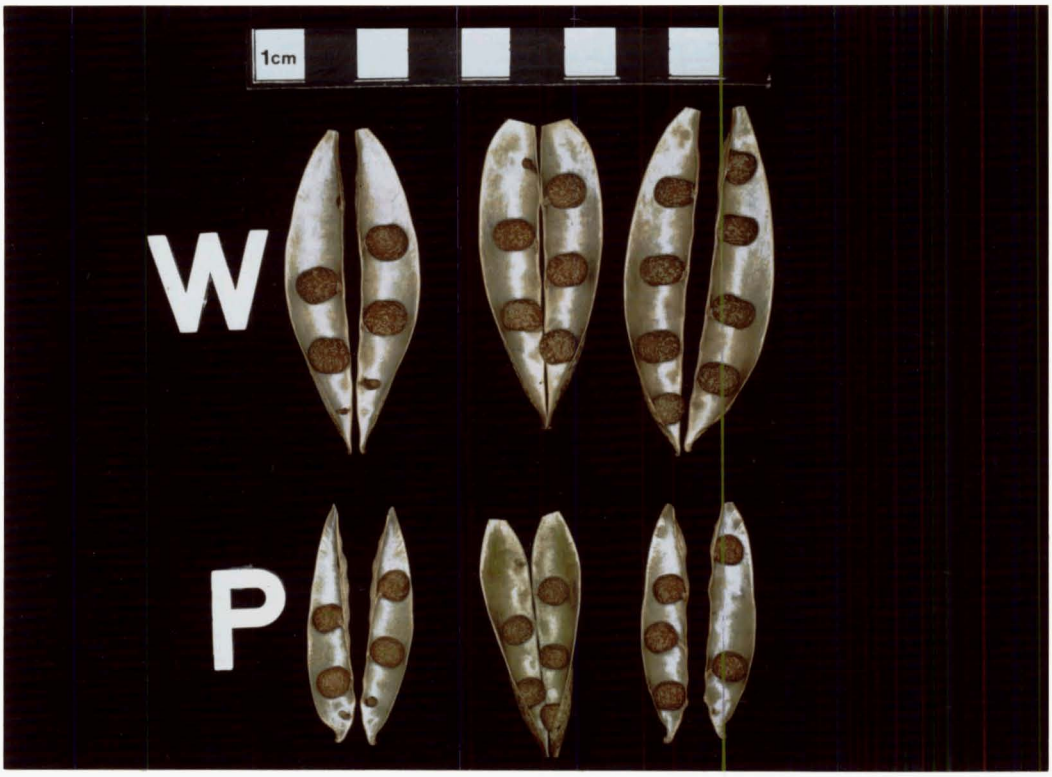


Plate 4.3: Varied levels of ovule failure in Whero (W) and Partridge (P) peas.

Table 4.14: Influence of cultivar, sowing date and moisture on dry matter, harvest index (HI) and nitrogen harvest index (NHI) at final harvest.

Treatment		HI (from 1.8 m^2)	NHI (from 5 plants)
Cultivar	W	0.42**	0.65**
	P	0.24	0.33
Sowing date	A	0.30**	0.40**
	S	0.36	0.59
$LSD_{.05}$		0.03	0.08
Moisture	D	0.35	0.55
	R	0.33	0.47
	I	0.32	0.45
$LSD_{.05}$		0.04	0.10
CV		16.1	24.1
Significant interactions		MxCxS*	SxM*

	A	S		A	S
WD	0.44	0.44	D	0.53	0.57
R	0.40	0.44	R	0.38	0.57
I	0.36	0.44	I	0.29	0.62
PD	0.17	0.33			
R	0.25	0.23			
I	0.18	0.28			
$LSD_{.05}$	0.08			0.14	

(A, S = autumn and spring sown; W, P = Whero and Partridge; D, R, I = dry, rainfall and irrigated.)

Table 4.15: Influence of cultivar, sowing date and soil moisture on translocation of nitrogen from above-ground plant components at flowering to the seed by final harvest, and the percentage of seed nitrogen from translocation.

Treatment		Translocation of N to seed mg N plant^{-1}	Percentage of seed N from translocation
Cultivar	W	27.4	17
	P	-5.2	-116
Sowing date	A	-39.1**	-146*
	S	61.3	47
$LSD_{.05}$		40	191
Moisture	D	45.7*	39
	R	10.6	5
	I	-23.0	-193
$LSD_{.05}$		48.8	234
CV		#	550
Significant interactions		None	None

	A	S
D	28	64
R	-46	67
I	-100	54
$LSD_{.05}$		69

#As mean was close to zero, coefficient of variation was not presented.
(A, S = autumn and spring sown; W, P = Whero and Partridge;
D, R, I = dry, rainfall and irrigated.)

exceeded the nitrogen content of seeds (Table 4.15). Autumn-sown peas took up more nitrogen between flowering and maturity than was required by seeds, whereas in spring-sown peas, 47 per cent of the seed nitrogen came from translocation. Irrigation of autumn crops reduced the reliance on translocation, but irrigation had no effect on spring crops. In all treatments, the percentage of seed nitrogen from translocation was greatest when soil moisture was lowest.

4.3.10 Lupin Seed Yield

Autumn lupins grew very slowly through the winter, but made rapid growth during the spring. Seed yields were 450 and 250 g m^{-2} for autumn and spring crops respectively (Table 4.16).

Table 4.16: Influence of sowing time on lupin density, seed yield, HI and seed size at final harvest.

Sowing date	Plants m^{-2}	Seed yield g m^{-2}	HI	1000 seed weight (g)
June 24, 1980	138	453**	0.42*	201*
September 12	111	251	0.35	155
CV %	34.6	6.4	6.7	3.5

4.4 DISCUSSION

4.4.1 Rates and Estimates of Nitrogen (C_2H_2) Fixation

Autumn sowing increased total nitrogen (C_2H_2) fixation by 27 per cent when compared with spring sowing (Table 4.6), but the actual proportions of plant nitrogen from fixation, calculated by acetylene reduct-

ion, were only 11 and 9 per cent for autumn and spring respectively (Tables 4.6, 4.11). However, the estimate of nitrogen fixation by acetylene reduction must be treated with caution for a number of reasons. Plants sampled were from the central rows which, during reproductive growth of Partridge particularly, were at a competitive disadvantage because of the dominant growth of the two outer rows (Figure 4.5, Plate 4.2). Thus, nitrogen fixation would have been reduced as a direct result of this competition. This inter-row competition in 1.5 m wide plots was very different from that which occurred in an adjacent area (20 x 20 m) of Partridge peas. In this large plot, similar in appearance to that occurring in a field situation, all plants near the middle were at least partially covered by other plants, and no plants became especially dominant. In contrast, the two outer rows in the 1.5 m plots became dominant because they did not compete with plants on each side. Thus the inner rows were more than usually stressed by those excessively large plants (Plate 4.2). The acetylene reduction estimates may have also been inaccurate for two further reasons; diurnal changes in activity (Chapter 6) and movement from the theoretical molar ratio of 3:1 (Burris, 1972; Goh *et al.*, 1978; Gibson, 1976, 1978; Witty, 1979; Carran *et al.*, 1982).

Although the above factors limit the preciseness of conclusions, it is clear that plants relied on soil nitrogen for a large part of their nitrogen requirements. The ready availability of soil nitrogen reduces fixation in many different legumes (Minchin *et al.*, 1981), although controlled environment studies with peas (Oghoghorie and Pate, 1971) showed that nitrogen fixation was reduced but not eliminated by levels of inorganic nitrogen in excess of those in this trial. Nitrogen applied to peas in Canada at a rate of 10.6 g N m^{-2} reduced peak fixation activity from 6.2 to a peak of only 0.8 umoles C_2H_4 $plant^{-1}$ h^{-1} (Sosulski and Buchan, 1978). The soil in their trial contained 4.4 g N m^{-2} in the surface 30 cm, whereas in the trial reported here, nitrate levels in the surface 20 cm were as

high as 10.3 g N m^{-2} at sowing of the autumn peas (Table 4.4). Even under the high nitrate nitrogen levels in both sowings of this trial, the longer growing season of autumn-sown peas could be expected to increase total fixation. However, mean temperatures in winter and early spring were below 10°C for 140 days and these cool temperatures would have limited both growth and nitrogenase activity. The far more rapid development of active nodules in the spring-sown crop is likely to have been a direct effect of temperature on the processes of nodule development and growth of the host. Temperature increased from 100 days after sowing of autumn peas but there was not a concomitant increase in nitrogenase activity (Figure 4.3), most probably because of the ready availability of mineral nitrogen.

From 100 days after sowing, availability of soil moisture in autumn Whero had a major effect on rates of nitrogen fixation (Figure 4.3) as irrigation caused a two and a half fold increase in nitrogen-fixing activity. Drought stress has been shown to reduce nitrogen fixation in both whole plants and detached nodules of a range of grain legumes (Sprent, 1972c; Minchin and Pate, 1975; Sprent, 1976a, b) but studies by Sprent (1972c) showed that water can be withdrawn from the xylem for nodule requirements. The large irrigation response in this trial, however, showed that water from root xylem sources was not able to supply all nodule water requirements. Although irrigation applied to Partridge at flowering caused an increase in fixation rate of 300 per cent, in comparison with the plots dependent only on rain, the actual nitrogen-fixing activity was low. Figure 4.5 shows that sampled plants had been suppressed by the unusually large plants in outside rows (Plate 4.2). This competition would have reduced the light available to the inner rows and thus inadequate photosynthate may have limited the overall nitrogen fixation response to irrigation.

In spring-sown Whero and Partridge, peak fixation activity did not exceed 2400 nmoles C_2H_4 $plant^{-1}$ h^{-1} and as this peak occurred before

flowering, carbohydrate stress in the nodules was unlikely to have caused the early decline (Figure 4.4). Responses to irrigation were also small, indicating that peas relied on soil nitrate supplies, which in the surface 40 cm in spring-sown crops were always more than 5 g N m^{-2} at the sample dates. Both spring and autumn crops accumulated 23 g N m^{-2} in above-ground biomass over approximately 130 and 240 days respectively (Table 4.11). Autumn-sown wheat grown in an adjacent trial with similar paddock history accumulated 28 g N m^{-2} (Majid, pers. comm.). Although rooting characteristics of wheat may have allowed it to obtain soil nitrogen more successfully and at greater depth than peas (Pate, 1976), it is clear that soil sources would have been sufficient for most of the nitrogen required by the peas.

4.4.2 Components of Yield and Final Seed Yields

Final seed yields of Whero were similar to those reported by Falloon and White (1978), and the low yield of 168 g m^{-2} for Partridge was not unexpected, because of the inter-row competition and the excessive vegetative growth. Increased soil moisture accompanied increased plant mortality, but plant densities at final harvest were still within the range where only small changes in seed yield occur (Falloon and White, 1978). Increased populations generally reduce components of yield (White and Anderson, 1971, 1974; Anderson and White, 1974b), but a measure of plant density did not allow for the very long vines on these peas. This sprawling nature limits the conclusions which can be drawn, particularly for autumn Partridge.

A number of factors altered the number of pods per plant. The indeterminate cultivar, Partridge, flowered for a longer period than Whero, and thus when irrigated it produced a greater number of pods. Only 9 per cent of these pods were non-productive, whereas in the autumn-sown Whero 25 per cent of the pods were non-productive. This was caused by an extended

flowering in Whero which produced a number of small pods even when most of the plant was dead. Most of these were formed from axillary buds at the base of the plant (Figure 4.3). These may have arisen because the herbicide caused some die-back in plants, and new growth developed from the base but it did not yield productive pods.

There are two probable reasons for the increased pod numbers which occurred after irrigation of spring crops. Floret abortion may have been reduced (Anderson, 1971), and in the case of Partridge, more pods were set during the flowering which was extended by irrigation. The failure of many ovules to develop seeds is of considerable concern and the potential yield loss of 50 per cent in most treatments of this trial is very high. As previously observed by Falloon and White (1980), the loss may be due to genetic and environmental influences. These workers found that larger plants showed reduced ovule failure and the results were confirmed in this trial where autumn-sown Partridge produced the largest plants and smallest loss.

The final seed yield was markedly influenced by the difference between Whero and Partridge in seed size. The 11 per cent increase in yield with spring sowing contrasts strongly with yield differences reported for other grain legumes (Newton, 1980) and for the lupins grown in this trial where there was an 80 per cent increase from autumn sowing (Table 4.16). Table 4.5 indicates the culmination of inter-row competition on final yields of autumn-sown, irrigated Partridge. The yields presented in Table 4.6 are thus biased in favour of the spring-sown crops which did not develop the same inter-row competition. Spray damage in autumn-sown Whero plots also contributed to the greater seed yields in spring sowings.

4.4.3 Individual Plant and Crop Development: Nitrogen Uptake and Translocation

The development stages of flowering and crop maturity were chosen for comparisons of treatments for two reasons. First, flowering is frequently considered a critical stage of development, with the amount of nitrogen in the plant an important factor in the self-destruct characteristic of grain legumes and particularly of soybeans (Sinclair and de Wit, 1975, 1976). These authors and others (Hardy and Havelka, 1976) considered that the translocation of nitrogen from vegetative tissue to the developing soybean seed could limit the length of the seed development period and therefore limit total seed production. Withers, Watkin and Forde (1981) considered that seed protein yields of lupin and tick beans are frequently limited by the self-destruct cycle and that this could be intensified by drought stress. Self-shading was a greater limit to seed protein yield in the peas than in the lupins and beans which these researchers grew. This reduction in yield, caused by self-shading, would result in reduced demand for nitrogen reserves, and thus decreased nitrogen translocation. In the trial reported here, translocation may have been underestimated. The increase in plant nitrogen between flowering and the start of seed growth, assumed to be negligible at the inception of this trial, has recently been shown to be significant (Withers *et al.*, 1981). The rate of pod and pea development influences the demand placed on vegetative tissues, and thus an indeterminate cultivar should be able to continue fixation longer than determinate cultivars.

Although nitrogen translocation may limit yield in some legumes, this trial has shown how agronomic treatments may be used to alter the dependence on vegetative nitrogen. This has implications both for final yield of seed and protein and for the amount of nitrogen remaining in crop residues after harvest. Autumn-sown peas experienced a longer period between flowering and maturity than spring-sown, and thus had longer to use both soil nitrogen or fixation nitrogen supplies for seed requirements. The

cultivar difference also reflects both the longer seed development in Partridge and reduced seed nitrogen demand in Partridge because of the smaller final yield. Soil moisture status was also an important factor controlling nitrogen translocation to seeds. Drought stress hastened plant development and thus the period of seed development was reduced and consequently greater reliance was placed on non-seed nitrogen rather than on soil and fixation sources.

The nitrogen translocation and NHI shown by these crops have important implications for the maintenance of soil fertility. Rhodes (1980) found NHI values of 0.86 and 0.91 for Huka peas and Uniharvest lupins respectively when plants were grown under low fertility. Although these were higher than values reported for peas and lupins by other workers (Roponen and Virtanen, 1968; Pate and Flinn, 1973; Farrington *et al.*, 1977; Withers, 1979), in general, very little nitrogen remains in plant residues where it can be used for subsequent crop growth. In this trial, the increased moisture in autumn sowing stimulated vegetative production at the expense of seed and thus greatly reduced the proportion of plant nitrogen stored in the seed. Thus where HI is low and seed yields are poor it is likely that crop residues will have a considerable amount of nitrogen (Partridge 16.8 g $N\,m^{-2}$) stored in them which could be used as stock feed or ploughed in to enhance soil fertility.

The low HI in Partridge suggests that a more beneficial use of this cultivar would be as an autumn-sown crop for silage or with oats as hay and could be followed by common beans (*P. vulgaris*), or irrigated spring wheat or barley, if the peas were harvested early enough in the spring. Irrigation of Partridge when at least 50 per cent of the flowers were fully open may have increased HI in this cultivar. The small change in HI for Whero caused by increased moisture was probably due to the determinate flowering pattern which limited the vegetative response to increased moisture.

The significant interactions observed between cultivar and sowing date in biomass production at flowering (Table 4.7) were caused by age

differences at flowering and herbicide damage in autumn, Whero plots. Partridge was not damaged by herbicide and thus responded to the longer growing season and increased moisture, by increased crop biomass. Although damaged rows in Whero were avoided as much as possible, some spray affected areas were sampled and this may explain the reduced yields observed with autumn Whero, particularly at flowering.

4.5 CONCLUSIONS

Nitrogen fixation was increased by 27 per cent with autumn sowing. Throughout the trial, soil nitrogen supply was sufficient to allow good cereal growth. The availability of mineral nitrogen reduced reliance of peas on nitrogen fixation.

Nitrogen translocation was increased to 47 per cent of total seed nitrogen when spring-sown. There were large differences in the percentage of seed nitrogen which came from translocation (Whero 17, Partridge -116%).

Irrigation stimulated nitrogen-fixing activity. Autumn-sown Partridge grew very well, but HI was less than 0.26 at the final harvest. The possibility of using such a crop for silage or stock feed should not be overlooked.

Lupins were grown in the trial at the two sowing dates and the June-sown crop yielded 453 kg ha^{-1}, which was 80 per cent more than the spring-sown crop.

CHAPTER 5

PEA CULTIVARS INFLUENCE NITROGEN FIXATION

5.1 INTRODUCTION

Very little is known about the differences in seasonal patterns of nitrogen fixation of pea cultivars grown in New Zealand. As peas are the major grain legume in Canterbury with an average of 19 000 ha sown annually in the 1976-1980 period (Agricultural Statistics, 1979/80, 1982), it is important to identify cultivars with superior nitrogen fixation. Many seasonal profiles of nitrogen fixation activity, based on acetylene reduction, have been reported for grain legumes overseas (Minchin *et al.*, 1981). The most common profile for these legumes is an increase in activity during vegetative growth, which may continue until early fruiting, followed by a decline which coincides with the main period of seed filling (Pate, 1958a; Roponen and Virtanen, 1968; LaRue and Kurz, 1973; Weil and Ohlrogge, 1975; Sosulski and Buchan, 1978). In contrast, nitrogenase activity of soybeans (Hardy *et al.*, 1968) continued until crop maturity, while Mague and Burris (1972) showed large cultivar differences in seasonal profiles of the same crop. The three pea genotypes evaluated by Young (1982) showed different phenological development, and fruit development, apical growth and nitrogen fixation varied independently. These differences show that a number of factors affect rates and seasonal profiles of nitrogen fixation. Cultivar differences exist in peas grown in New Zealand (Rhodes, 1980) but one of the two cultivars grown by Rhodes was harvested before crop maturity and thus a complete comparison was not made.

In this trial, eight pea cultivars were chosen to give as wide a range of development types as possible. There were two main aims:

i) To assess the range of seasonal profiles of nitrogen fixation in widely differing cultivars and identify those with the greatest nitrogen fixation potential.

ii) To establish the factors which control nitrogen fixation in dryland peas.

5.2 MATERIALS AND METHODS

5.2.1 Trial Site

The trial was conducted on a Templeton silt loam soil at the Lincoln College stud sheep farm in the 1980/81 season. Cropping history of the trial site is given in Table 5.1.

Table 5.1: Cropping history of trial area.

Years	Crop grown
1974-78	Pasture
1978/79	Fodder beet
1979/80	Oats (seed crop)
1980	Autumn-sown Italian ryegrass (grazed in winter and spring)

The trial area was cultivated to 3 cm depth with a rotary hoe to break the ryegrass crowns on September 5, 1980. The area was ploughed on September 9, and a week later cultivated once with a vibratiller and Dutch harrows. After drilling on September 16, the area was Cambridge rolled.

5.2.2 Treatments

Eight cultivars were replicated four times in a randomised block design. Cultivars and important characteristics are shown in Table 5.2.

Table 5.2: Cultivars sown and their important characteristics.

Cultivar	Important characteristics
Huka	(Field) White pea; mid season; Parentage: White Prolific x Victoria x Black eyed Susan.
Whero	(Field) Maple pea; mid season; Parentage: Elite x Partridge 73 (backcrossed to Partridge 73, three times).
Partridge 73	(Field) Maple pea; late; Parentage: Race 1 wilt resistant selection out of Tasmanian Partridge.
Rovar	(Field) Blue pea; mid season; Parentage: Rondo x Vares (backcrossed to Rondo).
Puke	Garden pea; mid season; Parentage: Jade x Small Sieve Freezer.
Pania	Garden pea; late (compared with Greenfeast); Parentage: (Greenfeast x Victory Freezer) x unnamed accession from U.S.A.
Tere	Garden pea; early; Parentage: William Massey x Victory Freezer x Sprite x Swift.
Small Sieve Freezer	Garden pea; mid season; Parentage: Navajox Famous.

(Source: W. Jermyn, pers. comm.)

5.2.3 Sowing

Seed was sown on September 16, 1980, to give 130 seeds m^{-2}. A Stanhay precision seeder was used with belt size 28 x 36 holes. To minimise bridging, chokes were not used. Rhizocote inoculant granules (E642/2) were spread during the first rain after sowing on October 4, at a rate of approximately 70 kg ha^{-1}. No herbicides were required. Lincoln College meteorological station data were used to calculate the potential soil moisture deficit. This allowed for bare ground during cultivation and incomplete crop cover early in growth (French and Legg, 1979).

5.2.4 Soil Nitrate

Soil nitrate was measured at 0 - 20 and 20 - 40 cm from each block on 11 September and 25 November, 1980.

5.2.5 Acetylene Reduction Assays

Assays were performed 27 days after drilling and at regular intervals until 129 days after sowing. To minimise damage to plots, systematic samples were taken from the south end and finished at the north end of all plots. Digging of quadrats started at approximately 0900 and finished at 1200 hours NZST. Plants within 0.2 m^2 quadrats were assayed as outlined in Appendix I. Plants were separated into roots, leaf + stem, pods and peas when appropriate. Pods were counted and all fractions oven-dried at 70^{o}C for 24 hours and weighed.

5.2.6 Final Harvest and Nitrogen Analyses

Final harvest was on January 23, 1981, except for Partridge which was harvested on February 3. An area of 1.8 m^2 was hand harvested, plants counted and seed threshed on a Vogel plot harvester. Seed loss from Rovar pods which had shattered was estimated from a count of seeds on the ground after 0.2 m^2 quadrats were harvested. Components of yield, other than 1000 seed weight, were calculated from a separate 0.2 m^2 quadrat. Seed weight was calculated from a sample of 500 seeds harvested from the 1.8 m^2 quadrats. A productive pod was defined as one which had at least one fully developed seed; productive pod counts were used to calculate seeds per pod. Harvest index (HI) and nitrogen harvest index (NHI) were calculated on both an area and an individual plant basis. Harvested components were analysed for nitrogen from harvests at 27, 70, 129 and 140 days from sowing, by the method described in Appendix III.

5.2.7 Growth Analysis

Absolute growth rates (AGR) were calculated for each replicate by fitting quadratic equations to the natural log of dry matter at each harvest (higher order equations did not significantly increase the fit of these lines). Dry matter for each harvest was then estimated from these equations. Relative growth rates (RGR) for each replicate were then calculated by differentiating the quadratic equations above, and AGR for each harvest was found by multiplying RGR by the estimated dry weights obtained. Analyses of variance were conducted on the AGRs at each harvest.

5.2.8 Nitrogen Increase in Whole Tops

Nitrogen percentage in whole tops was calculated from harvests at 43, 57, 85 and 104 days by interpolation from straight lines drawn between actual nitrogen percentage in whole tops at 27, 70 and 129 days. Nitrogen contents and uptake were calculated from the nitrogen concentration and estimated dry weights. As nitrogen uptake data were derived from interpolation, analysis of variance was not used.

5.2.9 Statistical Analysis

The results were analysed as a randomised complete block experiment with 8 cultivars and 4 replicates. Significance levels are indicated as follows: $P < 0.01$; $P < 0.05$; NS not significant at 5% level. Means were separated by using orthogonal contrasts (Appendix IV):

1.	Tere	vs	other cultivars
2.	Whero and Partridge	vs	other cultivars, not Tere
3.	Whero	vs	Partridge
4.	Rovar and Huka	vs	Garden cultivars, not Tere
5.	Rovar	vs	Huka
6.	SSF	vs	Puke and Pania
7.	Puke	vs	Pania

Contrasts 5 - 7 were normally insignificant and are labelled 'others' in tables.

5.3 RESULTS

5.3.1 Weather During the Trial

Very dry conditions prevailed after drilling (Figure 5.1), and the potential soil moisture deficit reached approximately 150 mm 70 days after drilling, when most cultivars were flowering. However, during the early flowering of all cultivars, except Partridge, rainfall was above

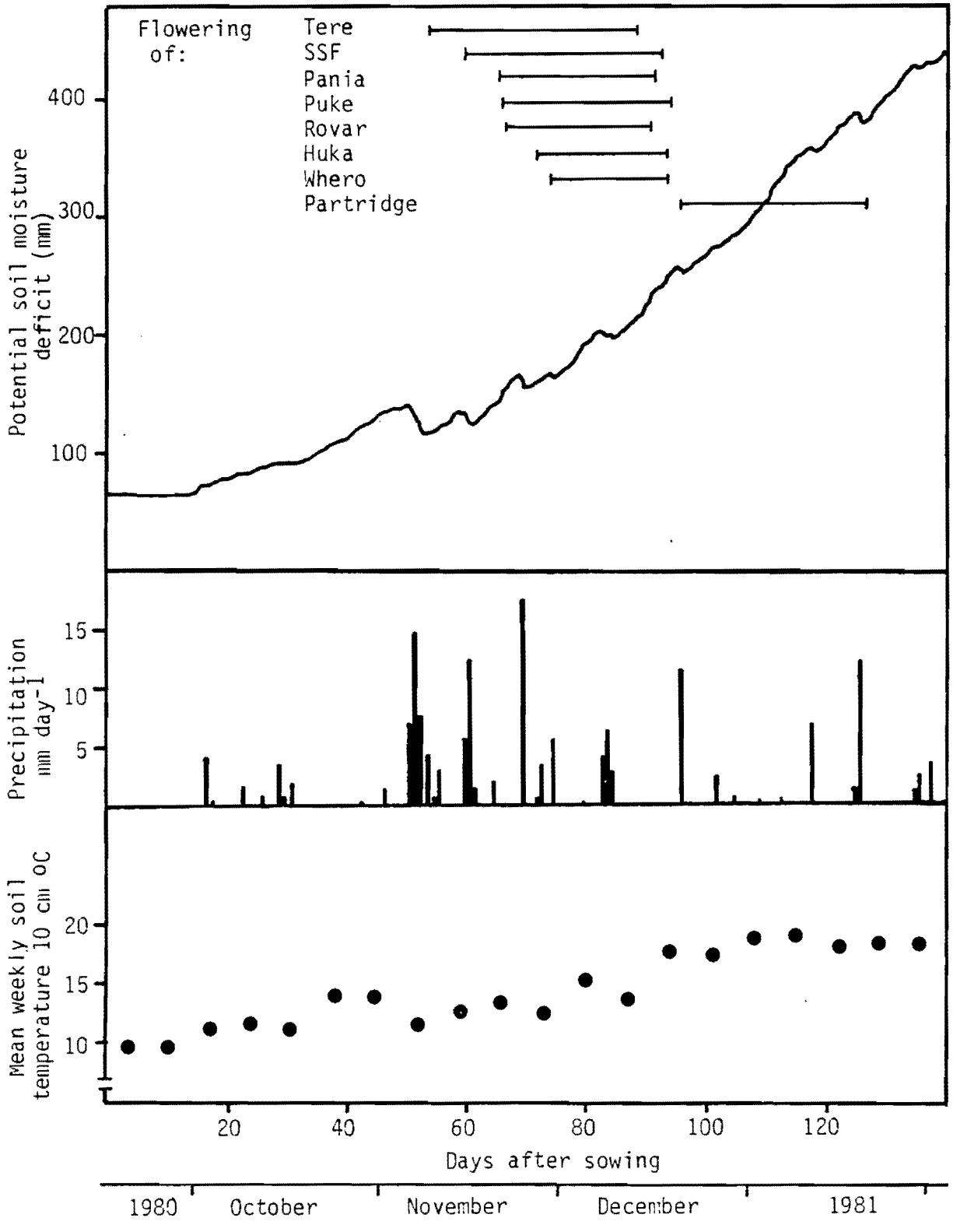


Figure 5.1: Calculated and observed meteorological data from Lincoln meteorological station (1980/81).

the long-term average (Table 5.3, Figure 5.1).

Table 5.3: Long-term and actual precipitation for Lincoln Meteorological Station, October 1980 to January, 1981.

Month	Long-term precipitation	Actual precipitation
	(mm month^{-1})	
October	51	13
November	51	85
December	61	29
January	56	25

Mean weekly soil temperature increased from the first to the second harvest and declined after rain at the third harvest. Distal portions of some buds of Rovar and Puke were brown 57 days after drilling. This coincided with a light frost 53 days after drilling.

5.3.2 Available Soil Nitrogen

Nitrate nitrogen was 14.5 and 15 ppm N in the 0 - 20 and 20 - 40 cm depths respectively, five days before drilling. Equivalent values were 12.5 and 7 ppm N 70 days after drilling.

5.3.3 Plant Density

The dry conditions after sowing reduced weed establishment, but crop establishment was also reduced. Plant counts at the first harvest from 0.2 m^2 and at the final harvest from 1.8 m^2 are given in Table 5.4. The highest plant density of 105 plants m^{-2} occurred with

Partridge. A number of small plants died between first and final harvest.

Table 5.4: Plant population 27 and 129 days after drilling.

Cultivar	Days after drilling	
	27	129
	(plants m^{-2} from 0.2 m^2)	
Huka	99	85
Whero	91	88
Partridge	105	87
Rovar	65	75
Puke	80	67
Pania	69	63
Tere	70	51
SSF	73	59
LSD .05	13.8**	8.5**
CV	11.5	8.2

5.3.4 Seed Yield and Components of Yield

Seed yields were average for Canterbury, but Partridge yield was very low at 96 g m^{-2} (Table 5.5). Rovar yields were underestimated by 87 g m^{-2} ($S\bar{x}$ 4.0) due to pod shattering. The very early maturing cultivar, Tere, and the late maturing cultivar, Partridge, yielded less than other cultivars, although the yield reduction with Tere was not significant. Non-productive pods in Tere and Partridge accounted for 7 and 8 per cent of their total pods respectively. For all other cultivars, non-productive pods accounted for less than 2.7 per cent of total pods. The low Partridge yield was caused by significantly fewer pods per plant than other cultivars, fewer seeds per pod and a much smaller seed size than other cultivars. Although garden peas had more peas per pod than field peas, they had

Table 5.5: Effect of cultivar on seed yield, plant population, number of productive pods per plant, peas per pod and seed weight at final harvest.

Cultivar	Seed yield ($g\ m^{-2}$)	Plant population ($pl\ m^{-2}$)	Productive pods $plant^{-1}$	Peas pod^{-1}	1000 seed weight (g)
Huka	360	86	4.5	3.7	241
Whero	262	79	5.4	3.4	256
Partridge	96	91	2.9	2.7	169
Rovar	284	80	5.8	2.6	260
Puke	269	63	4.7	5.3	218
Pania	306	74	4.3	5.3	204
Tere	229	76	4.0	4.4	234
SSF	270	60	4.1	5.3	218
$LSD_{.05}$	54.5	12.5	1.3	0.7	7.9
CV	14.0	11.2	19.6	12.4	2.4
Orthogonal comparisons, variance ratio, and significance:					
Cultivars (7 d.f.)	17.7**	6.3**	4.1**	21.3**	121.9**
1: Tere vs other cultivars	3.2	0.0	1.1	2.2	12.2**
2: Maples vs other cultivars not Tere	61.6**	12.3**	2.0	4.3*	50.5**
3: Whero vs Partridge	41.9**	4.3*	15.9**	3.9	519.1**
4: Rovar and Huka vs garden peas not Tere	5.9*	20.8**	4.3*	90.7**	228.0**
5-7: Others	3.9*	2.3	1.9	3.1	14.0**

slightly less pods per plant than did field peas (except Partridge). These factors, together with lower plant populations of garden peas resulted in reduced seed yields from garden peas (Table 5.5).

Calculated seed yields generally overestimated the actual yields. In Whero, the 40 per cent discrepancy was caused by one replicate. When this was omitted from the calculation, the discrepancy was reduced to 8 per cent. A similar discrepancy in Tere occurred because the area harvested for seed yield had only 76 plants m^{-2}, whereas that used for components of yield had 51 plants m^{-2} (Tables 5.4, 5.5).

5.3.5 Crop Growth and Nitrogen Fixation

Initial growth of Partridge was particularly slow, although from 100 days after sowing the rate increased relative to other cultivars, which reflects its late maturity (Figures 5.2, 5.3). Initial growth of Tere was greater than other cultivars but the major difference was between Partridge and other cultivars (Figures 5.4, 5.5; Appendix 5.1). Although Tere initially produced a large pod weight per plant (85 days after drilling), the weight of peas per plant at the final harvest was lower than all except Partridge (Figures 5.2, 5.3). Root dry matters are not presented because roots were sampled to 20 cm only and thus do not give an accurate indication of root yield. Roots have been observed to depths of 80 cm.

The higher level of nitrogen fixation in the Maple peas (Whero and Partridge) in early growth was clearly shown by the orthogonal contrasts (Table 5.6). Whero and Partridge reached peak fixation rates 18 and 39 days respectively before flowering, whereas other cultivars generally reached peak fixation shortly after flowering started (Figures 5.4, 5.5). Nitrogenase activity of Whero was greater than other cultivars later in the season, which reflected the later maturity of Whero, and 44

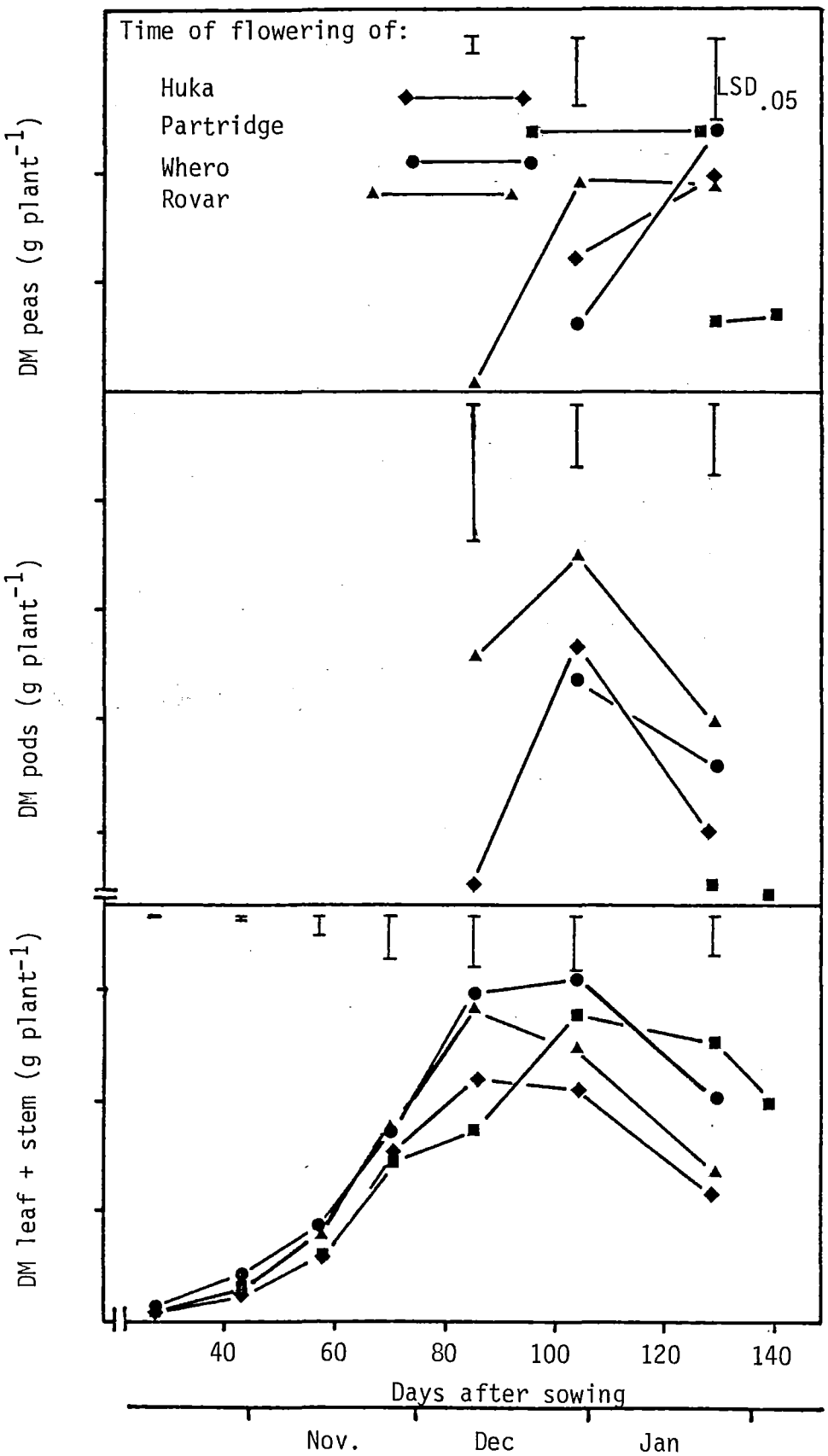


Figure 5.2: Partitioning of dry matter in field peas.

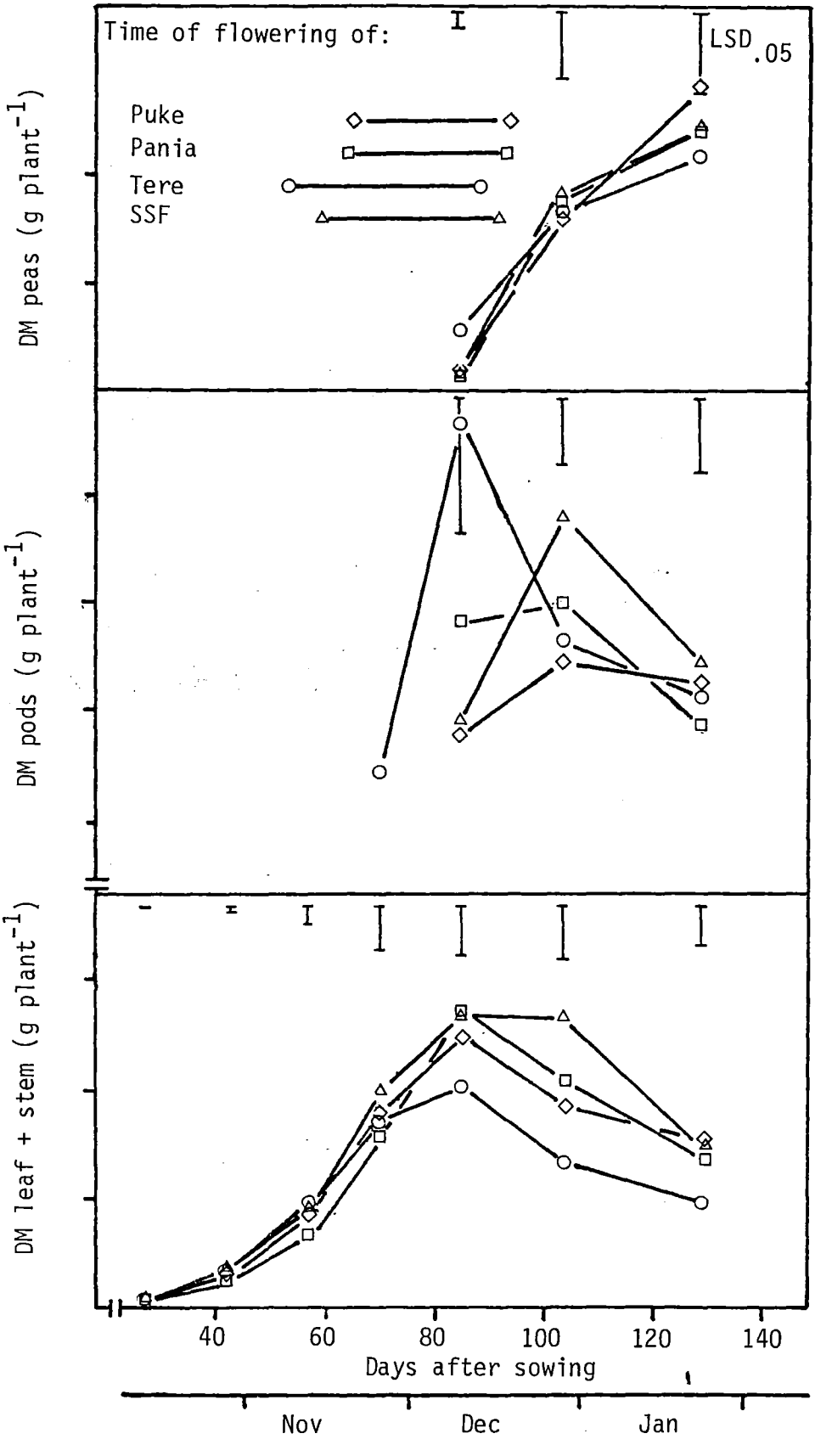


Figure 5.3: Partitioning of dry matter in garden peas.

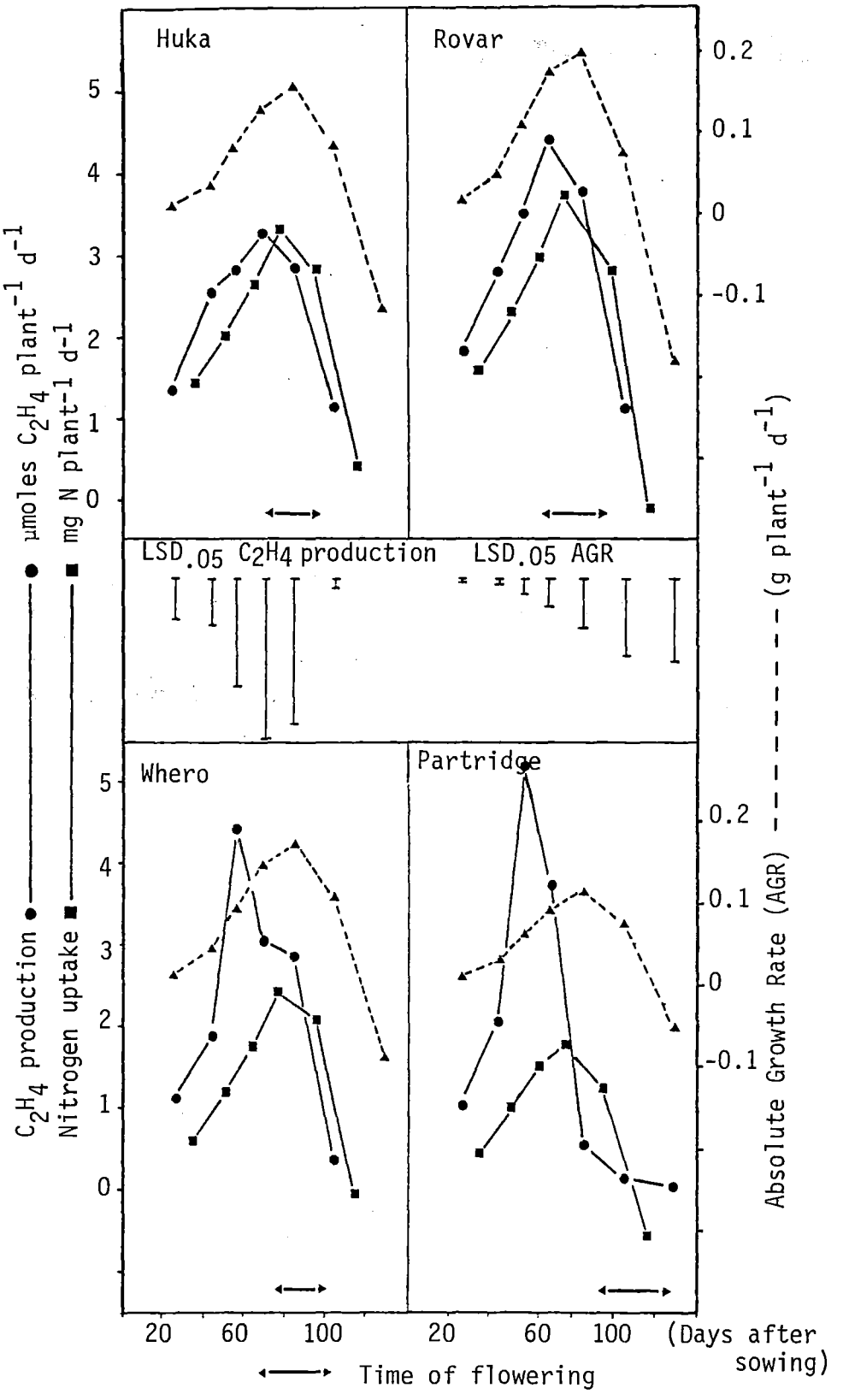


Figure 5.4: Absolute growth rate, ethylene production and rate of nitrogen uptake for field peas.

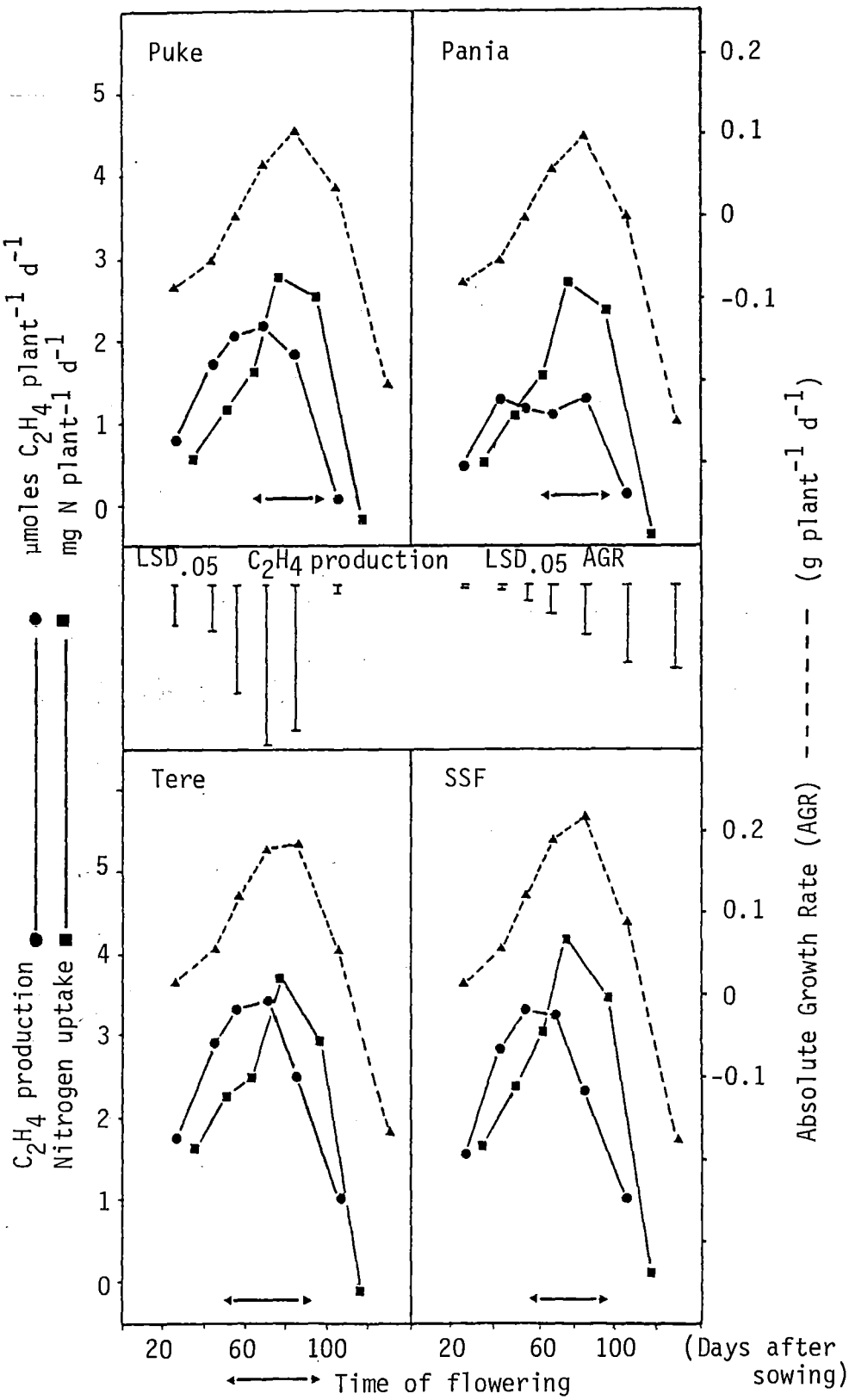


Figure 5.5: Absolute growth rate, ethylene production and rate of nitrogen uptake for garden peas.

per cent of fixation activity occurred after flowering. In contrast, only two per cent of the nitrogen fixation of Partridge occurred after flowering began. At flowering, Partridge plants were 1.2 m long and half of the leaves were at least partially chlorotic, particularly at the base of plants where shading occurred. The later maturity of Rovar and Huka compared with garden peas was shown by their continued fixation when garden peas were dead (Table 5.6). Peak rates of nitrogen uptake were closely linked in all species with peak crop growth rates (Figures 5.4, 5.5). Partridge had a considerably lower nitrogen uptake than other cultivars, which was associated with a lower plant growth rate.

The concentration of nitrogen in plant components soon after emergence, at flowering, and at final harvest, is shown in Table 5.7. Nitrogen concentration of roots in Maple peas was lower than most cultivars initially, but by 70 days after sowing, the situation was reversed. A similar trend was observed in plant tops where nitrogen concentration declined with age in all cultivars except Tere (Figure 5.6). Because cultivars flowered at different times, a direct comparison between cultivars at one date does not allow for changes in nitrogen concentration with plant development (Figure 5.6). At the final harvest, nitrogen concentration of stem plus leaf, and pods were significantly higher in Partridge than in other cultivars. Rovar and Huka had the lowest nitrogen concentrations in seed, with values of 3.92 and 3.91 per cent nitrogen respectively. The highest value was recorded for Tere which had 4.71 per cent nitrogen.

5.3.6 Harvest Index and Nitrogen Harvest Index

The differences between the two methods of calculation (on a per plant and an area basis) were insignificant and as other measurements have been on a per plant basis, Figure 5.7 and Appendix 5.2 show values

Table 5.6: Ethylene production at each harvest and estimated nitrogen fixed (from acetylene reduction assays).

Cultivar	Days from sowing: 27	43	57	70	84	104	129	Estimated nitrogen fixation g N m^{-2}
	nanomoles C_2H_4 $plant^{-1}$ h^{-1}							
Huka	331	1554	1826	2233	1817	177		2.61
Whero	1137	1913	4450	3097	2898	399		4.32
Partridge	1028	2090	5180	3747	565	167	80	4.11
Rovar	771	1746	2471	3389	2718	95		2.93
Puke	768	1699	2021	2193	1812	84		1.96
Pania	423	1209	1146	1063	1250	56		1.13
Tere	792	1928	2320	2429	1543	79		1.84
SSF	572	1826	2326	2273	1325	16		1.77
$LSD_{.05}$	432	600	1338	1979	1763	97		0.62
CV	39.5	23.3	33.5	52.8	67.8	49.0		22.1
Orthogonal comparisons, variance ratios and significance:								
Cultivars (7 d.f.)	3.7*	1.8	9.1**	1.6	1.7	13.3**		16.2**
1: Tere vs other cultivars	0.2	0.9	0.9	0.0	0.1	3.2		7.8*
2: Maples vs other cultivars, not Tere	17.9**	5.4*	56.4**	4.5*	0.0	51.7**		80.4**
3: Whero vs Partridge	0.3	0.4	1.3	0.5	7.8*	25.1**		0.3
4: Rovar and Huka vs garden cultivars, not Tere	0.1	0.2	0.6	2.5	2.2	7.9*		19.5**
5-7: Others	2.5	1.9	1.5	1.2	0.6	1.7		1.8

Table 5.7: Concentration of nitrogen in plant components 27 and 70 days after sowing, and at final harvest.

Cultivar	Days after sowing: 27		70			Final harvest		
	Percent nitrogen in: Roots	Stem + leaf	Roots	Stem + leaf	Pods	Stem + leaf	Pods	Peas
Huka	2.82	4.61	2.97	3.46		1.41	1.01	3.91
Whero	2.65	4.62	3.25	3.23		1.19	1.05	4.38
Partridge	2.93	4.73	3.53	4.00		2.30	2.03	4.29
Rovar	3.25	4.59	2.83	3.15		1.04	0.76	3.92
Puke	3.06	4.86	2.85	2.98		1.19	0.83	4.47
Pania	3.24	4.80	2.66	2.93		1.15	0.80	4.29
Tere	3.22	5.02	2.89	2.57	3.55	1.19	0.98	4.71
SSF	3.37	4.89	2.90	3.14		1.18	0.86	4.70
$LSD_{.05}$	0.4	0.36	0.34	0.58		0.35	0.25	0.47
CV	8.9	5.2	9.8	12.4		17.8	16.3	7.5
Orthogonal comparisons, variance ratios and significance:								
Cultivars (7 d.f.)	3.2*	1.6	5.7**	4.5**		11.7**	24.0**	3.5*
1: Tere vs Others	1.5	4.7*	1.3	10.9**		1.6	0.6	6.15*
2: Maples vs other cultivars not Tere	9.7**	0.5	31.9**	8.5**		31.2**	94.6**	0.3
3: Whero vs Partridge	2.0	0.4	3.1	7.6*		43.9**	67.3**	0.2
4: Rovar and Huka vs garden cultivars, not Tere	2.2	5.0*	0.9	2.6		0.3	0.5	14.8**
5-7: Others	2.4	0.1	1.0	0.6		1.6	1.5	1.0

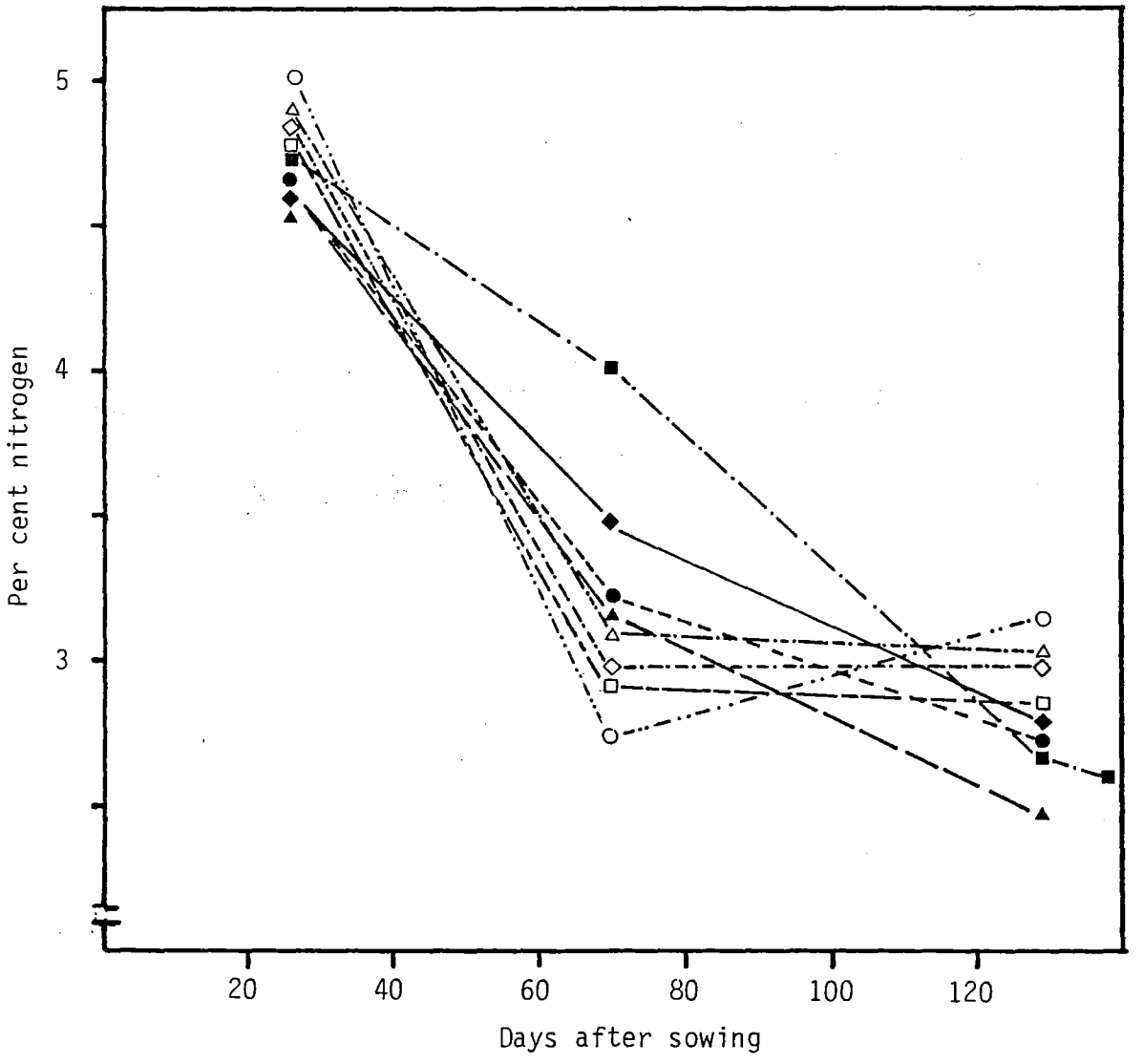


Figure 5.6: Changes in nitrogen concentration with time in whole tops of eight pea cultivars. Symbols as for Figures 5.2, 5.3.

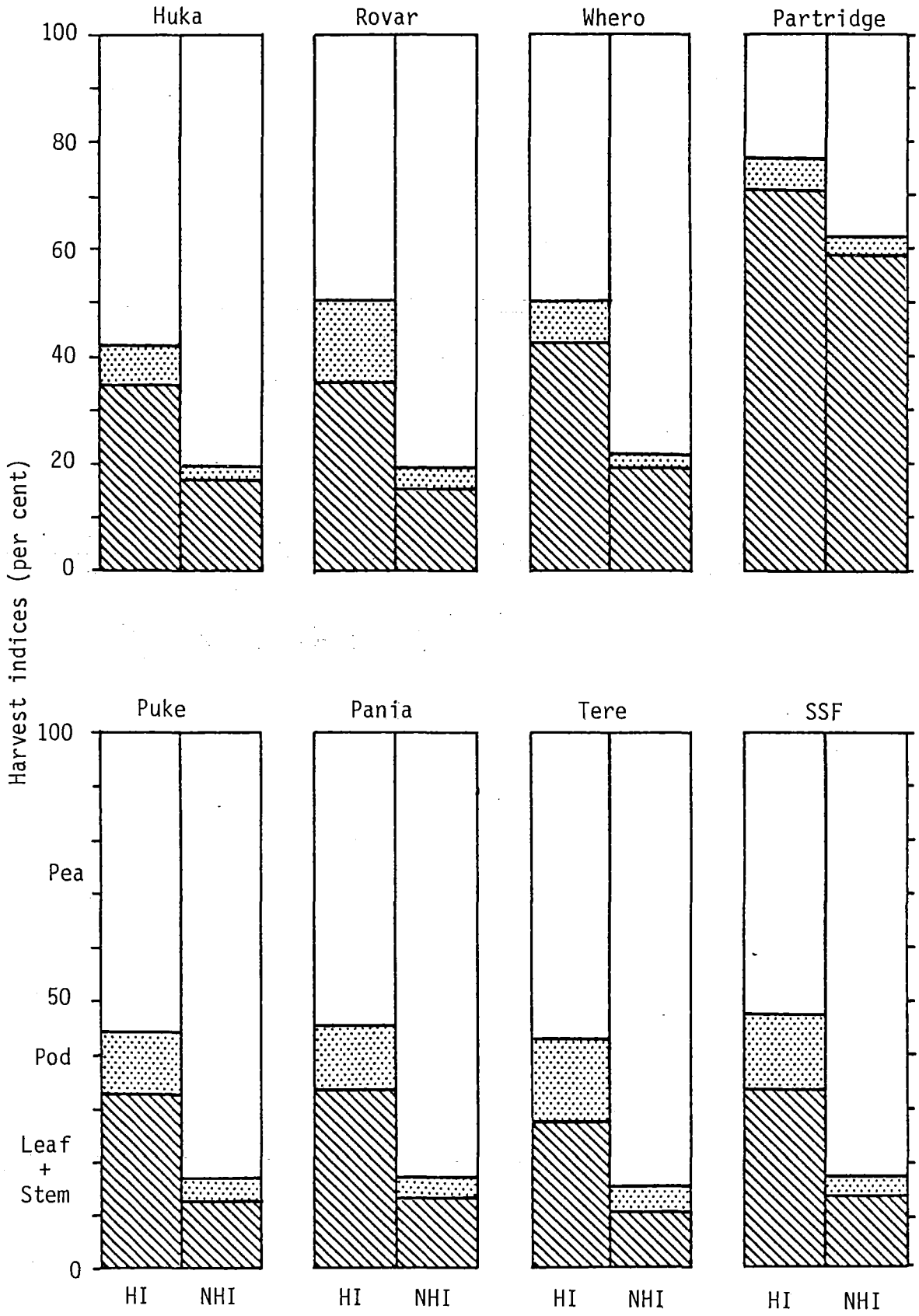


Figure 5.7: Effect of pea cultivar on HI and NHI (per plant) at crop maturity.

per plant. Maple peas and in particular Partridge were least efficient when assessed for their ability to convert crop biomass into seed yield. Trends for dry matter and nitrogen were similar. Taken over all cultivars, Tere partitioned the greatest proportion of its nitrogen and biomass into seed.

The amount of nitrogen in plant components on a per plant and per m^2 basis at final harvest is shown in Table 5.8. Garden peas accumulated more nitrogen in peas and in total than other cultivars except Whero, although on an area basis nitrogen accumulation of all cultivars was similar except for Partridge which was significantly lower. Although Rovar and Huka had less nitrogen in total on a plant basis than garden peas, the greater density of Rovar and Huka made these differences insignificant when considered on a per m^2 basis. Seed loss by Rovar also reduced the total nitrogen accumulation of this cultivar. The 8.4 g N m^{-2} in Partridge residues was 91 per cent greater than in Whero, the next highest cultivar (Table 5.8).

5.4 DISCUSSION

Nitrogen fixation of field peas was greater than that of garden cultivars, and of the field peas, Partridge and Whero fixed approximately 50 per cent more nitrogen (C_2H_2) than the mean of Huka and Rovar. The seasonal profile of nitrogen fixation in the late maturing Partridge, in particular, was marked by an early peak in nitrogenase activity, and Partridge also differed from other cultivars in two respects. Firstly, plant density was higher and secondly, growth and nitrogen uptake rates were lower than other cultivars. The lower than average initial concentration of nitrogen observed in Partridge (Table 5.7) may have two causes. Firstly, the higher density would have reduced available soil nitrogen to individual plants and secondly, the smaller plants may have explored less

Table 5.8: Amount of nitrogen in plant components at final harvest.

Cultivar	mg N plant^{-1} in:				g N m^{-2}			
	Stem + leaf	Pods	Peas	Total	Stem + leaf	Pods	Peas	Total
Huka	34	5	154	193	2.9	0.4	13.3	16.6
Whero	48	7	211	266	3.8	0.6	17.1	21.5
Partridge	88	4	60	153	8.0	0.4	5.0	13.7
Rovar	28	7	151	187	2.3	0.6	12.2	15.0
Puke	37	9	242	288	2.3	0.6	15.1	18.0
Pania	32	7	198	238	2.4	0.6	14.6	17.5
Tere	24	10	194	209	1.8	0.8	14.5	17.4
SSF	35	10	222	267	2.1	0.6	13.5	16.2
$LSD_{.05}$	12.3	2.4	67	74	1.08	0.22	5.95	6.96
CV	20.5	21.6	25.5	22.3	22.9	25.9	30.8	27.8
Orthogonal comparisons, variance ratios and significance:								
Cultivar (7 d.f.)	24.1**	6.6**	6.3**	3.5*	30.9**	2.8*	3.2*	0.9
1: Tere vs other cultivars	19.1**	6.7*	0.5	0.5	16.7**	12.0**	0.5	0.0
2: Maples vs other cultivars not Tere	100.0**	7.7*	9.3**	1.4	13.2**	0.6	2.5	0.2
3: Whero vs Partridge	46.6**	6.6*	21.8**	10.1**	64.9**	3.1	17.9**	5.4*
4: Rovar and Huka vs garden cultivars, not Tere	0.8	14.7**	10.6**	10.5**	1.0	1.3	0.8	0.4
5-7: Others	0.5	3.5*	0.6	0.7	0.6	0.9	0.1	0.2

soil. Differences in nitrogen fixation as a result of changing density in peas has not received much attention, but a number of studies with the upright growing tick bean have shown that nitrogenase activity per plant varies inversely with increasing plant density (Sprent and Bradford, 1977; Sprent *et al.*, 1977). Partridge established the highest plant density which could have increased the competition for combined nitrogen, light, and moisture, compared to other cultivars. Pea plants grown under decreasing levels of soil nitrogen showed decreased nitrogen concentrations in the experiments of Sosulski and Buchan (1978), and Mahon and Child (1979), and similar trends with lucerne, ladino clover and soybean have been observed (McAuliffe *et al.*, 1958; Lawn and Brun, 1974b). Thus, the lower nitrogen concentration in Partridge may have resulted from a soil nitrogen deficit. Although the deficit has not been shown unequivocally, the increased nitrogenase activity of Partridge plants supports this.

Later in crop development, the supply of mineral nitrogen to all cultivars is likely to have increased as it is known that in similar soils in Canterbury, mineralisation of organic nitrogen increases rapidly during the summer (Ludecke and Tham, 1971; Hart, 1978). In this trial, nitrogen may have been immobilised during the phase of active microbial activity, following initial cultivation, with subsequent release of soil nitrogen as the ryegrass decomposed (Russell, 1973). For this to be ascertained accurately, fallow plots would have been necessary, to measure available nitrogen levels without the confounding influence of nitrogen uptake by crops. Values of 13 and 7 ppm NO_3-N for 0 - 20 and 20 - 40 cm respectively show that the actual level of nitrogen under the crop was above the level where positive yield responses to N fertiliser in wheat may be expected (Ludecke, 1974). Although the actual rate of mineralisation is unknown, an increased availability of soil nitrogen may be postulated from Figures 5.4 and 5.5. Regardless of the pattern of nitrogen

fixation, all cultivars reached peak levels of nitrogen uptake simultaneously, and the reliance on nitrogen fixation was reduced in all cultivars from approximately 70 days.

At 80 days after sowing, Partridge had the lowest growth and nitrogen uptake rates, and thus the lowest demand for nitrogen. From studies with a range of pasture legumes and soybeans, Allos and Bartholomew (1959) considered that for preference legumes use nitrogen which is available in the soil, and that fixation occurs only when the nitrogen supply is inadequate. Nitrogen fixation in peas may be less sensitive to soil nitrate than the legumes used by the above workers, but Oghoghorie and Pate (1971) and Sosulski and Buchan (1978) still showed reductions in fixed nitrogen as levels of nitrate increased. In the present trial, as availability of soil nitrogen increased, the reliance on fixation of all cultivars would have been reduced. This was most significant with Partridge, most probably because it accumulated the lowest total nitrogen in above-ground herbage. The nitrogen concentration data in Table 5.7 provide further indirect evidence in support of the hypothesis that increased soil nitrogen reduced nitrogen fixation particularly in Partridge. Seventy days after drilling, when nitrogen fixation of Partridge had started to decline rapidly, nitrogen concentration was the highest of all cultivars. Only some of this difference was accounted for by the later maturity of Partridge (Figure 5.6). Thus, early in growth when fixation was required to fill a deficit in plant nitrogen, nitrogenase activity was high, whereas fixation was depressed later when plant nitrogen requirements were met by adequate levels of soil nitrogen. At the final harvest, the smaller overall requirement for plant nitrogen in Partridge was evident in the total nitrogen of only 13.7 compared with the mean of 17.5 ($g\ N\ m^{-2}$) from other cultivars.

Nitrogen fixation may also have been depressed by self shading in the tall growing Partridge and to a lesser extent in Whero. Pate and

Flinn (1973) showed that for field peas, lower leaves are an important source of carbohydrate for nodules. Lower leaves of these Maple peas became noticeably chlorotic from approximately 85 days after sowing. The reduced carbohydrate output from senescing leaves may have caused some carbohydrate stress to occur in the nodules of these cultivars.

Nitrogen fixation continued for longer in Whero than in other cultivars. This was reflected in the significantly higher nitrogen yield (21.5 g N m^{-2}) of Whero, compared with other cultivars. If protein yield of herbage is important, then Whero would be the best cultivar to grow. The low peak in nitrogen fixation in Pania may have been caused by sampling errors. The possibility that differences in rooting depth caused some of these seasonal nitrogen fixation profiles merits further study.

The early peak in nitrogen fixation of Maple peas may have another explanation. Nitrogenase activity measured by acetylene reduction incorporates hydrogen evolution; thus nitrogen fixation may be overestimated (Schubert and Evans, 1976). Gibson (1978) assessed a range of pasture and grain legumes for their relative efficiencies and concluded that hydrogen production may have resulted from "spillover" respiration as the most ready means of getting rid of excess energy. Demand for photosynthate from nodulated plants is likely to be highest when plants are small and forming nodules, and again during pod filling, when energy-rich seeds are formed. At these stages, relative efficiency is likely to be greatest. The relative efficiency of pea plants (grown in a controlled environment, but with insolation similar to levels experienced in Canterbury) was highest very early in growth (0.92) with a marked depression to 0.52 during vegetative growth and an increase to 0.99 near maturity (Bethlenfalvay *et al.*, 1978). Thus, peas in the present trial which showed large peaks in nitrogenase activity during vegetative growth may have been operating at a relative efficiency of approximately 0.5.

If this were the case, nitrogen fixation would have been overestimated. Plants with low relative efficiencies, however, are frequently the most productive in terms of dry matter and plant nitrogen (Gibson, 1978). Partridge in the present trial produced less dry matter and plant nitrogen than other cultivars, and so if a negative correlation existed between relative efficiency and plant production, Partridge was likely to be more efficient than other cultivars.

Nitrogen fixation rates recorded in this trial were low compared with some values published for peas grown without nitrogen (Bethlenfalvay *et al.*, 1978), or in low fertility (Rhodes, 1980). Other workers have shown similar values to those obtained in this trial when peas have been grown in fertile conditions (Mahler *et al.*, 1979; Sosulski and Buchan, 1978).

Estimates of nitrogen fixation over the life of a crop require a mathematical summation of many assays on replicate plots (La Rue and Patterson, 1981). Estimates of total nitrogen fixation based solely on a few acetylene reduction assays may be very inaccurate because of, 1) diurnal variation in nitrogen fixing activity (Minchin and Pate, 1974; Pate and Greig, 1968); 2) changes in the ratio of ethylene formed to nitrogen fixed (Hardy *et al.*, 1973), and 3) variation in the relative efficiency of nitrogen fixation (Bethlenfalvay *et al.*, 1978). Although cultivars may have responded differently to at least some of these factors, the values for total nitrogen fixation presented (Table 5.6) suggest that Maple peas fixed more nitrogen than other cultivars, and that garden peas fixed the least. Much plant breeding is done on soils of high fertility and, as increased fertility reduces the reliance of plants on symbiotic nitrogen, it is possible that highly bred cultivars rely more on soil nitrogen than on nitrogen fixation (Sprent, 1979). Partridge in particular, and Whero indirectly (as its parentage is based on Partridge), have not been bred as intensively as other cultivars. For this reason, Maple peas may rely more on nitrogen fixation

for their nitrogen requirements, than other cultivars. Garden peas, however, have been carefully selected for even pod and pea development, and the self destruct mechanism (Sinclair and de Wit, 1975) is likely to have a greater influence on these highly determinate cultivars when carbohydrate supply is inadequate for fixation and growth. Huka, Rovar and Whero were also determinate and if inadequate carbohydrate supply caused reduced garden pea fixation, it should have also reduced fixation in these cultivars. Thus the differences in total fixation are more likely to reflect genetic characteristics. Nitrogen fixation is also known to be sensitive to drought (Chapters 3 and 4) and the dry conditions experienced during pod development of all cultivars is very likely to have further reduced nitrogen-fixing activity.

Seed yields were highest in Huka with 360 g m^{-2}. The seed losses due to pod shattering in Rovar highlight the need for careful timing of harvest. Even at maturity, Rovar remained relatively upright which may have helped the crop to dry out more rapidly than more prostrate cultivars. The upright nature would also reduce the likelihood of stone damage in harvesting equipment. Partridge also differed markedly from other cultivars in the very low seed and total nitrogen yields obtained. As pea yields are influenced by plant density, some of the differences in seed yield may possibly be attributed to the varied densities. Population differences resulted from both dry conditions after sowing, which caused variable establishment, and from the sowing equipment used. Partridge had the smallest seed and the seed holes in the Stanhay belts may have allowed two seeds to be sown at one time. Although a number of workers have shown density yield relationships in garden peas, there are few reports for field peas. Falloon and White (1978) grew Huka and Whero in Canterbury and showed little difference in seed yield between 50 and 170 plants m^{-2} for Huka, and 50 and 130 plants m^{-2} for Whero. Anderson and White (1974) reported that the garden pea cv. Victory Freezer

reached a maximum seed yield at 105 plants m^{-2}, but differences were insignificant between 52 and 358 plants m^{-2}. White *et al.* (1982) showed that seed yields did not differ significantly between 100 and 200 plants m^{-2}, although yield responses with Victory Freezer were different in the two seasons of their trial. From this information, it seems likely that seed yields were not affected by the different plant densities, although yield components may have been influenced. Components of yield are normally inversely related to plant density (Gritton and Eastin, 1968; Meadley and Milbourn, 1970; Falloon and White, 1978, 1980) and in this trial, small differences were recorded for all cultivars, with the exception of Partridge (Table 5.5). The very late flowering of Partridge was associated with both reduced pods per plant and seeds per pod. As Partridge flowered during a drought and the percentage of non-productive pods was increased, it is reasonable to suggest drought stress reduced these yield components. The differences observed in seed size are largely of genetic origin.

The discrepancies observed between calculated seed yields based on 0.2 m^2 quadrats and actual seed yields from 1.8 m^2 quadrats highlight the need for large and representative areas to be used for the calculation of components of yield. Seed losses in the harvester, used for seed threshing, would also widen the discrepancies as no seeds were lost from hand podded 0.2 m^2 quadrats. Similar and regular plant densities were not established because of the dry conditions at sowing, which resulted in uneven plant emergence and mortality of some plants which emerged late. The application of irrigation, as a basal treatment, to all plants soon after sowing would have enhanced plant emergence. This was not available.

Hardwick and Milbourn (1967) showed that garden peas are highly plastic plants. That is, pea plants possess a number of physiological mechanisms by which components of yield are adjusted to prevailing con-

ditions. As the responses cannot be reversed, they are preserved in the plant structure until final harvest, and can then be used as a record of the plant development.

Branching ability in garden peas harvested green is an undesirable characteristic as pods on branches generally mature later than those on main stems (Hardwick and Milbourn, 1967). Peas grown as a dry seed crop may benefit from a branching ability if established densities are low. Falloon and White (1978) showed the marked superiority in branching ability of Huka over Whero at densities of up to 100 plants m^{-2}. At 25 plants m^{-2}, Huka had 2.5 compared with Whero which had 0.5 branches $plant^{-1}$. Branching ability may help to suppress weeds, but increased seeding rates are more likely to reduce weed populations (Anderson, 1971; Falloon and White, 1978).

The one branch per plant produced by Huka and Rovar helped to increase their seed yields over those of other cultivars, by increasing the number of sites for pods to develop (Plate 5.1a, b). Rovar and Huka had more pods per plant than garden peas, but fewer than Whero. The tall growth of Whero would also increase sites for pod development. Although Partridge was the tallest and produced more nodes than other peas, pod numbers were the lowest recorded. This may reflect the late flowering during a drought. Salter (1963) studied the effect of 10 combinations of wet or dry soil conditions on the yield of vining peas. Irrigation at the start of flowering increased pod and pea numbers, while irrigation during pod swelling increased pod number. Although peas in the experiment reported here were not irrigated, Figure 5.1 shows that rain which fell during flowering of garden peas was likely to enhance pod set and the number of peas per pod. Peas per pod were greatest in the early maturing cultivar, Tere, which suggests a response to rainfall at this time. Peas per pod were lower in Rovar because of seed losses through pod shattering.

Plate 5.1a: Field peas 69 days after sowing.
(from left: Huka, Whero, Partridge, Rovar)

Plate 5.1b: Garden peas 69 days after sowing.
(from left: Puke, Pania, Tere, Small Sieve Freezer)

Differences in seed size are most likely to have resulted from genetic factors, as irrigation generally influences this factor less than other components (Stoker, 1937, 1977; Martin and Tabley, 1981). Partridge seed is normally small, but the overall very poor performance of this cultivar is a result of sowing at a time which is unsuitable for the full development of its normal yield potential.

The very late development of Partridge was further shown by the low harvest indices (Figure 5.7, Appendix 5.2). In contrast, the very early maturing cultivar Tere had a harvest index of 58 per cent which equalled Huka and was significantly greater than other cultivars. Hocking (1982) showed that severe water stress significantly reduced the ability of blue lupin (*L. angustifolius*) to redistribute nutrients from senescing leaflets. In Canterbury, irrigation increased blue lupin yields, but reduced the proportion of crop dry matter that was seed (Herbert, 1977). Harvest index for another lupin species, *L. albus*, was less than 0.22 when grown in a wetter than usual season in Canterbury (Herbert, 1977). Thus there are two processes which influence harvest indices. Severe drought which causes premature senescence will reduce HI and NHI, and irrigation may also reduce HI and NHI by greatly increasing biomass production without a similar increase in seed yield. Partridge became senescent soon after flowering began, because of the drought which significantly reduced its harvest indices. Tere, however, stored 85 per cent of its above-ground nitrogen in seeds, possibly because of the early flowering which would allow greater plant development before drought stress reduced growth in other cultivars.

Nitrogen harvest index has implications for the fertility building role of peas in crop rotations. Farmers are not paid on protein yield in seed, but on seed dry matter. Thus, from a purely economic point of view, a crop which produces a large seed yield with much of the plant nitrogen remaining in residues will give good gross margins. These

high protein residues could then be used for stock feed after harvest or as hay with subsequent return of nitrogen to the soil. Seed protein levels are important in peas when the seed is used for human or animal nutrition, and seed protein measurements could be incorporated in a pricing structure. In this respect, Huka and Rovar had the lowest seed protein levels with highest seed yields.

5.5 CONCLUSIONS

The eight pea cultivars studied showed that considerable variability exists in seasonal profiles of nitrogen fixation. This variability may be utilised in breeding programmes to increase the reliance of plants on atmospheric nitrogen. If payouts to farmers are based on protein yield instead of seed dry matter, the objective of increased fixation may be more rapidly realised. At present, field peas appear to rely less on soil nitrogen than do garden pea cultivars, and where soil fertility maintenance is important these cultivars should be chosen.

Soil nitrogen availability in particular, and inadequate soil moisture, appeared to limit nitrogen fixation more than inadequate supplies of photosynthate to nodules. Although the cause of the very early peak in nitrogen fixation of Partridge was not conclusively ascertained, the slow growth rate and therefore reduced demand for nitrogen suggest that soil nitrogen was more than adequate for this cultivar, after an initial soil nitrogen shortage was overcome by increased nitrogen fixation. Self-shading of lower leaves may have reduced photosynthate supply to nodules, but this shading became prominent after the peak was reached, and thus did not cause the peak. The seed yield of only 96 g m^{-2} for Partridge showed that this very late maturing cultivar must be autumn- or winter-sown to achieve more economic yields. Seed size of Partridge was much smaller than other cultivars and yield of this cultivar may always be

limited because of this characteristic. Of the cultivars tested, Huka was the best as it yielded most seed (360 g m^{-2}).

CHAPTER 6

DIURNAL VARIATION IN NITROGEN-FIXING ACTIVITY IN PEAS

6.1 INTRODUCTION

The validity of the acetylene reduction assay to measure total nitrogen fixation has been frequently questioned (Bergersen, 1970; Raper and Patterson, 1972; Goh *et al.*, 1978; Witty, 1979). A few one-hour assays over the growth of a crop are not likely to reflect the total nitrogen fixation, because of fluctuations in diurnal nitrogen fixation rates (Goh *et al.*, 1978). Assessment of the influence of diurnal variation in calculations of nitrogen fixed by white clover in New Zealand by Carran, Rumball, Tough, Brock and Crush (1982) showed generally small diurnal variation in activity, with considerable variability between assays. Although nitrogen-fixing activity was generally lower at night than during the day, night-time increases in activity were observed.

Although these diurnal fluctuations in fixation rates make it difficult to estimate daily totals of nitrogen fixation, they do allow field assessment of the factors which limit fixation. A "typical" diurnal profile of a daytime peak in di-nitrogen fixation rate, followed by a decline throughout the night, is generally taken to indicate that di-nitrogen fixation relies on current photosynthesis (Hardy and Havelka, 1976; Minchin *et al.*, 1981). Hardy and Havelka (1976) considered that inadequate photosynthate supply frequently limits nitrogen fixation in grain legumes, and particularly in soybeans. The supply of photosynthate is important during seed growth when nodules and seeds compete for available carbohydrate (Sinclair and de Wit, 1975). Lipid and protein contents

of soybean seeds are higher than those of peas (Sinclair and de Wit, 1975; Hill *et al.*, 1977), and thus the competition for photosynthate between nodules and seeds will be greater for soybeans than for peas. In the tropics where soybeans are frequently grown (FAO Production Yearbook, 1982), the net photosynthate available for nitrogen fixation may not be adequate as day length is limited to approximately 12 hours, and warm night temperatures increase respiratory losses. In contrast, peas grown in Canterbury experience summer days of high insolation with day-lengths of up to 15.5 hours, and with night temperatures frequently less than 15^{o}C. These conditions are known to favour dry matter production and nitrogen fixation of this species (Roponen *et al.*, 1970; Minchin and Pate, 1974). Thus the conclusion reached by Hardy and Havelka (1976) of inadequate photosynthate supply limiting nitrogen fixation, may be valid for soybeans grown in tropical climates, but may not be valid for peas in Canterbury.

Diurnal changes in temperature may have a considerable effect on the daily pattern of nitrogen fixation. In soybeans, Gibson (1976) showed the positive effect of increased acetylene reduction assay temperatures on nitrogen fixation, although the three temperatures at which plants had been grown modified the responses. Although the effect of incubation temperature *per se* in peas has not been assessed, peas are known to have a broadly based temperature optimum between 10 and 30^{o}C (Pate, 1977a), but nitrogen fixation may be reduced by temperatures above 22^{o}C (Wheeler and Lawrie, 1976). Constant temperatures (18^{o}C) also reduced nitrogen fixation, whereas a cool night (12^{o}C) and warm day (18^{o}C) enhanced fixation (Minchin and Pate, 1974). Temperatures in Canterbury during north-westerly (fohn) winds frequently exceed 20^{o}C and so nitrogen fixation during these winds may be reduced as a direct result of heat stress and not as a result of diurnal cycles.

The aims of this study were:

i) To assess variation in acetylene reduction activity during three diurnal cycles.

ii) To identify discrepancies between estimates of daily fixation based on either a mean of nine assays or estimates from individual assays.

iii) To relate diurnal cycles of nitrogen fixation in peas to both the changing demands made by developing reproductive structures and to temperature changes. This aim was subjective, as growth changes were confounded by environmental conditions.

6.2 METHODS

Trial plots for this study formed part of the Crop Research Division pea cultivar trial at DSIR, Lincoln. Two year pasture preceded the trial.

6.2.1 Crop Establishment

Maple peas cv. Whero were sown on 30 September 1981, at a rate designed to give 100 plants m^{-2}. The soil, a Templeton silt loam, received 250 kg ha^{-1} of serpentine reverted superphosphate (4.5 per cent citric acid soluble phosphorus and 16 per cent calcium) prior to crop establishment. Plots measured 1.5 m x 10 m and were replicated 4 times.

6.2.2 Acetylene Reduction Assays

Diurnal changes in acetylene reduction were measured at three hourly intervals from 1700 h (New Zealand Standard Time), over three 24 hour cycles; cycle 1 at bud formation (26/11/81), cycle 2 at first flower (5/12/81), and cycle 3 at pod fill (19/12/81). Departures

from the three hourly intervals at cycles 1 and 3 are plotted in Figure 6.1. Dates were chosen to coincide with stable weather conditions, with wind from the north-east. This wind is the prevailing wind at Lincoln, and is characterised by relative humidities above 80 per cent (Christchurch Meteorological Service, pers. comm.).

At each assay, plants from 0.1 m^2 were dug, whole root systems counted, and incubated as described previously (Appendix I). Temperatures in the incubation pit, and at 5 and 10 cm depth in the soil under the pea canopy, were recorded at the start, and in the pit at the end of each incubation period. Before each assay, jars were stored in the shade where their temperatures would have been close to the screen air temperature. Incubation temperatures were calculated from the mean of screen air and pit temperatures, to allow for the time-lag between the start of assay and equilibration of root temperature with pit temperature.

At the first cycle, plant tops only were retained for dry matter determination, but in subsequent cycles, roots were washed and nodules greater than 1 mm were removed. Oven dry weights of roots and nodules were recorded separately. Nodule dry weight per plant was used as a covariate when calculating ethylene production (Appendix 6. 1).

At cycles 1 and 3, gravimetric soil moistures were determined to 20 cm. On 14 December 1981, during pod filling, approximately 25 mm water were applied by spray irrigation.

6.3 RESULTS

6.3.1 Weather

The conditions prevailing through most of this trial are shown in Table 6.1, from data obtained from the DSIR Meteorological Station at Lincoln.

Table 6.1: Wind direction and photosynthetically active radiation two days prior to and including the periods of assay.

Days prior to start of 24 h cycles	Wind direction			Photosynthetically active radiation		
	Cycle			Cycle		
	1	2	3	1	2	3
2	NE	NW	NE	10.5	9.8	6.5
1	NE	NE	S	12.3	11.8	8.6
Period of Assay	NE	SE	S	12.7	8.8	9.1
	NW	NE*	NE	11.7	9.9	13.7

*North-westerly wind developed in the afternoon.

Ryu (1978) obtained soil water potential data for a Templeton silt loan three kilometres from this trial site. He measured water contents of 0.39 and 0.12 cm^3 water cm^{-3} soil in the top 20 cm, at -0.2 bars and -15 bars respectively. From a curve calculated from Ryu's data (Appendix 6.2), and from the gravimetric soil moistures obtained in this experiment, the soil water potential (0 - 20 cm) was -7 bars at the start of this trial (25/11/81), and at the completion (5/12/81), three days after spray irrigation, the water potential was -9 bars. No rain fell between cycles one and two, and 9 mm fell between cycles two and three. Evaporation between irrigation applied on 14/12/81 and cycle 3 (19/12/81) was 21 mm, measured at the DSIR Meteorological Station, with an American Class A pan. During cycle 2, plants wilted during the day. Moisture was noted on the plants during the night of each assay, although meteorological data showed that no dew fall occurred. Humidity and incubation temperatures are given in Table 6.2. Northwesterly wind was accompanied by a relative humidity of 21 per cent (Table 6.2).

Table 6.2: Humidity and incubation temperatures during three diurnal cycles.

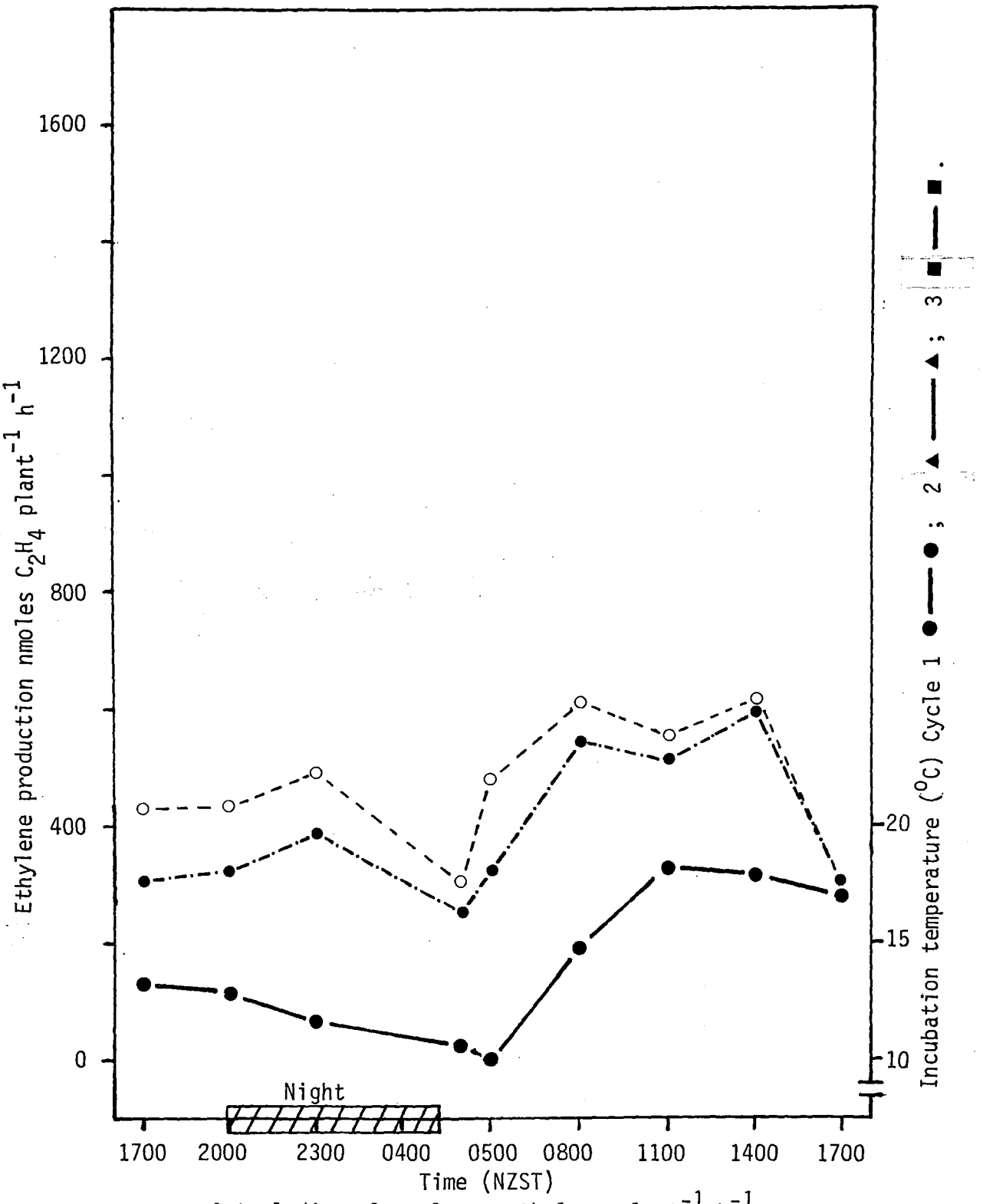
Time NZST	Relative humidity			Incubation temperature		
	Cycle			Cycle		
	1	2	3	1	2	3
1700	60	72	56	13	14	15
2000	75	79	70	13	14	14
2300	80	80	72	12	13	13
0200	84	82	73	11	13	13
0500	89	81	74	10	13	12
0800	54	57	58	15	15	13
1100	50	48	45	18	15	15
1400	57	21	48	18	20	16
1700	53	21	56	17	19	15

6.3.2 Crop Growth

Between bud formation and flowering, pea top dry matter increased at 23 g m^{-2} day^{-1} and in the second interval, tops increased at 15 g m^{-2} day^{-1}. Vine length and nodes increased from initial values of 68 cm and 15 nodes respectively to 116 and 23 at the end of the trial period.

6.3.3 Data Analysis

At each cycle differences were observed between the rates of fixation measured at 1700 h for the start and at 1700 h for the completion of the 24 h periods (Figure 6.1). These differences were caused by environmental changes (e.g., increasing drought) upon which the diurnal cycles were superimposed. To show clearly the diurnal cycles, the initial rate at 1700 h was adjusted to equal the final rate. Assays



— — — Actual diurnal cycles; ethylene plant^{-1} h^{-1}.

—·—·— Diurnal cycle after initial 1700 h assays adjusted to equal final assays. Assays inbetween were adjusted by a percentage which depended on their position relative to the final 1700 h assay.

Figure 6.1: Influence of time of assay on ethylene production at each cycle, and the effect of allowance for growth change over the twenty-four hour interval of each cycle.

in between were adjusted by a percentage which depended on their position relative to the final 1700 h assay. The initial diurnal trends remained after these corrections were made (Figure 6.1).

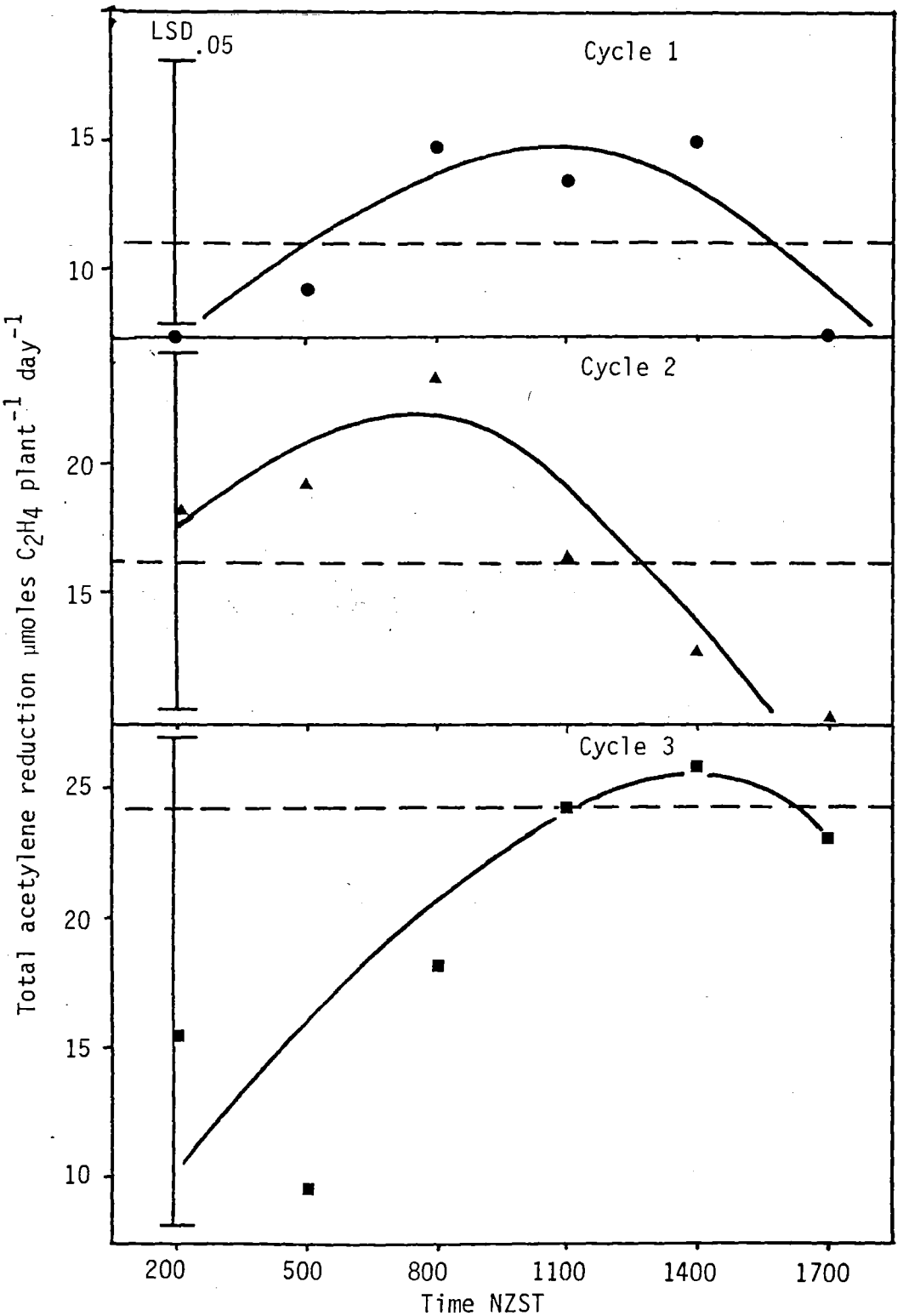
6.3.4 Effect of Diurnal Cycles on Calculations of Total Nitrogen Fixation

Figure 6.2 shows total ethylene production per plant per day calculated from individual daytime assays and from the mean of all assays at each cycle. Assays at 1100 or 1400 h gave better estimates of the overall mean than other assays. Data for Figure 6.2 used the initial ethylene production values, with no adjustments for environmental changes.

6.3.5 Nitrogen Fixation

At bud formation, ethylene per plant per hour showed no obvious diurnal trends (Figure 6.1), but nitrogen fixation at flowering increased during the night and reached a peak at 0800 h (Figure 6.1). Soil moisture level (0 - 20 cm) was very low as no rain fell between bud formation and flowering. By pod fill, which occurred after the irrigation on 14/12/81, nitrogen fixation was reduced between 2300 and 1100 hours (Figure 6.1).

The relationship of ethylene production to nodule dry weight (Figures 6.3, 6.4) at cycles 2 and 3 showed that ethylene production increased with nodule weight. Separation of the relationship into night and day, at cycle 2, did not increase the coefficient of determination, but in cycle 3 (Figure 6.4), less ethylene was produced during the night than during the day. Two plants with nodule weights greater than 40 mg plant^{-1} markedly influenced the night-time regression line.



Mean of all assays at each cycle.
Cycles 1, 2, 3 respectively; curves drawn by eye.

Figure 6.2: Effect of time of assay on the calculation of daily totals of acetylene reduction, at each cycle.

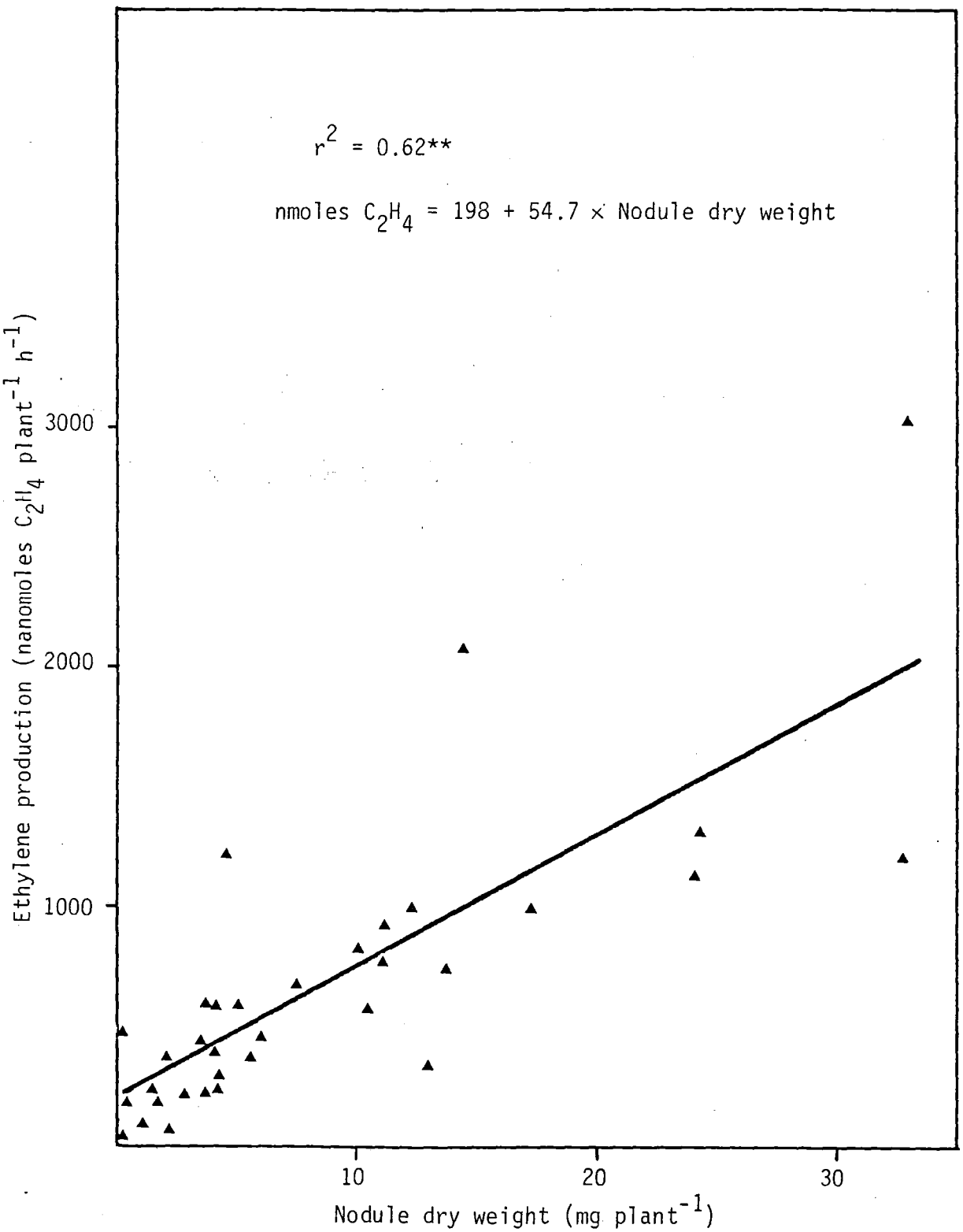


Figure 6.3: Effect of nodule weight on ethylene production at cycle 2.

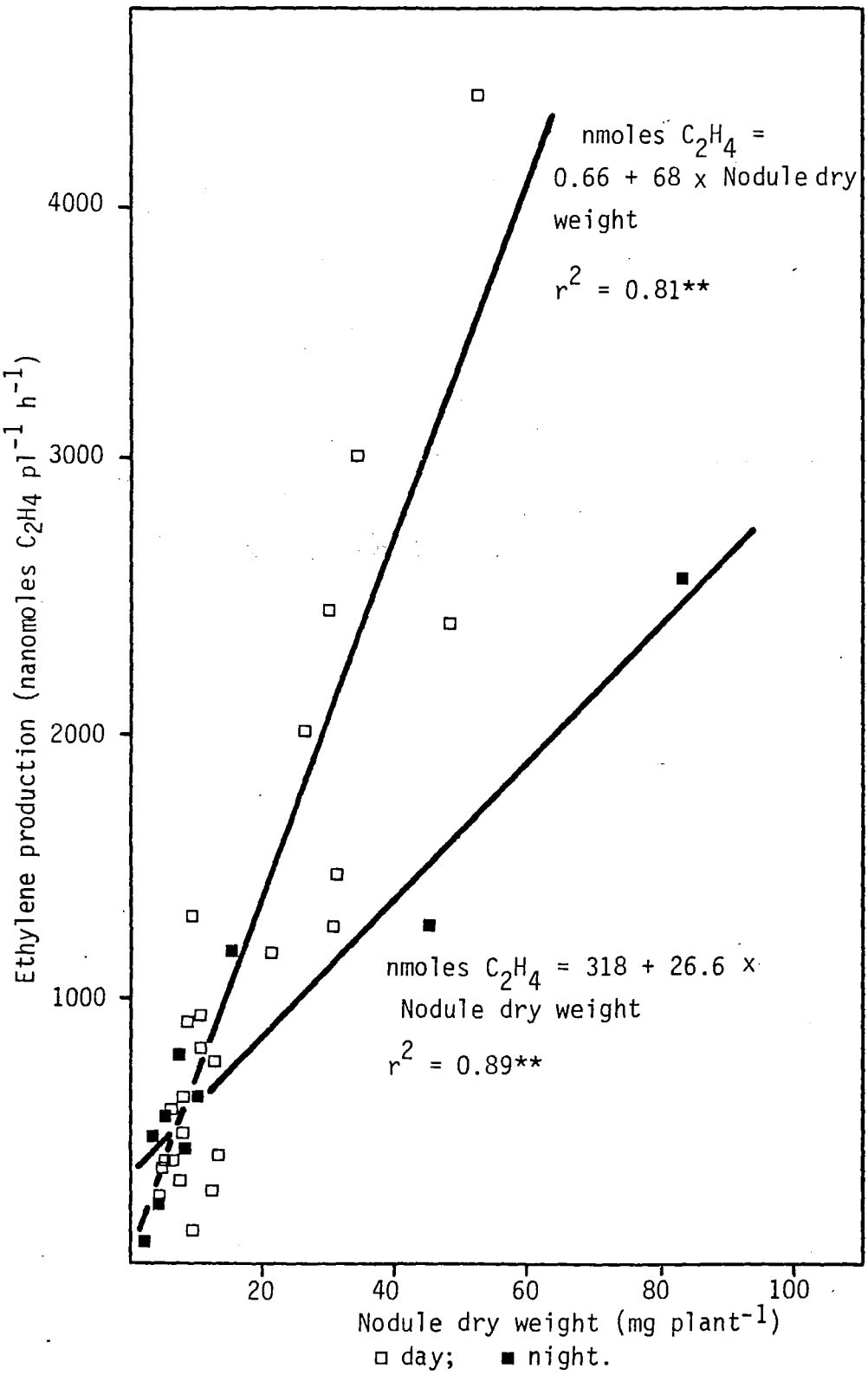


Figure 6.4: Effect of nodule dry weight on ethylene production during both day and night at cycle 3.

At cycle 2, an increase in incubation temperature did not result in increased nitrogen fixation. Northwesterly wind, however, was associated with depressed fixation. At cycle 3, for each one degree change in incubation temperature, ethylene production was altered by 160 nmoles C_2H_4 $plant^{-1}$ h^{-1} (Figure 6.5). Incubation temperature dropped four degrees between day and night and thus from the regression equation, nitrogen fixation may have been depressed by 640 nmoles C_2H_4 $plant^{-1}$ h^{-1}.

6.4 DISCUSSION

6.4.1 Calculation of Total Nitrogen Fixation

The large discrepancies frequently noted in the calculation of total nitrogen fixation highlights the caution needed when using a single assay per day. Assays between 1100 - 1400 hours may give the most accurate indication of daily nitrogen fixation when only one assay per day is possible. Weather patterns influence the optimum time of assay, but more detailed studies are needed to elucidate these effects. In particular, northwesterly winds occurring during periods of water-stress have a marked influence on diurnal cycles of nitrogen fixation (Figure 6.5). The usefulness of acetylene reduction as a measure of the effect of agronomic treatments on nitrogen fixation is not altered by these results, unless individual diurnal cycles are altered by particular treatments. Differing cycles may occur in trials incorporating irrigation treatments. When plants are water-stressed, northwesterly wind may alter the diurnal cycles in a manner which contrasts with cycles from fully irrigated plots.

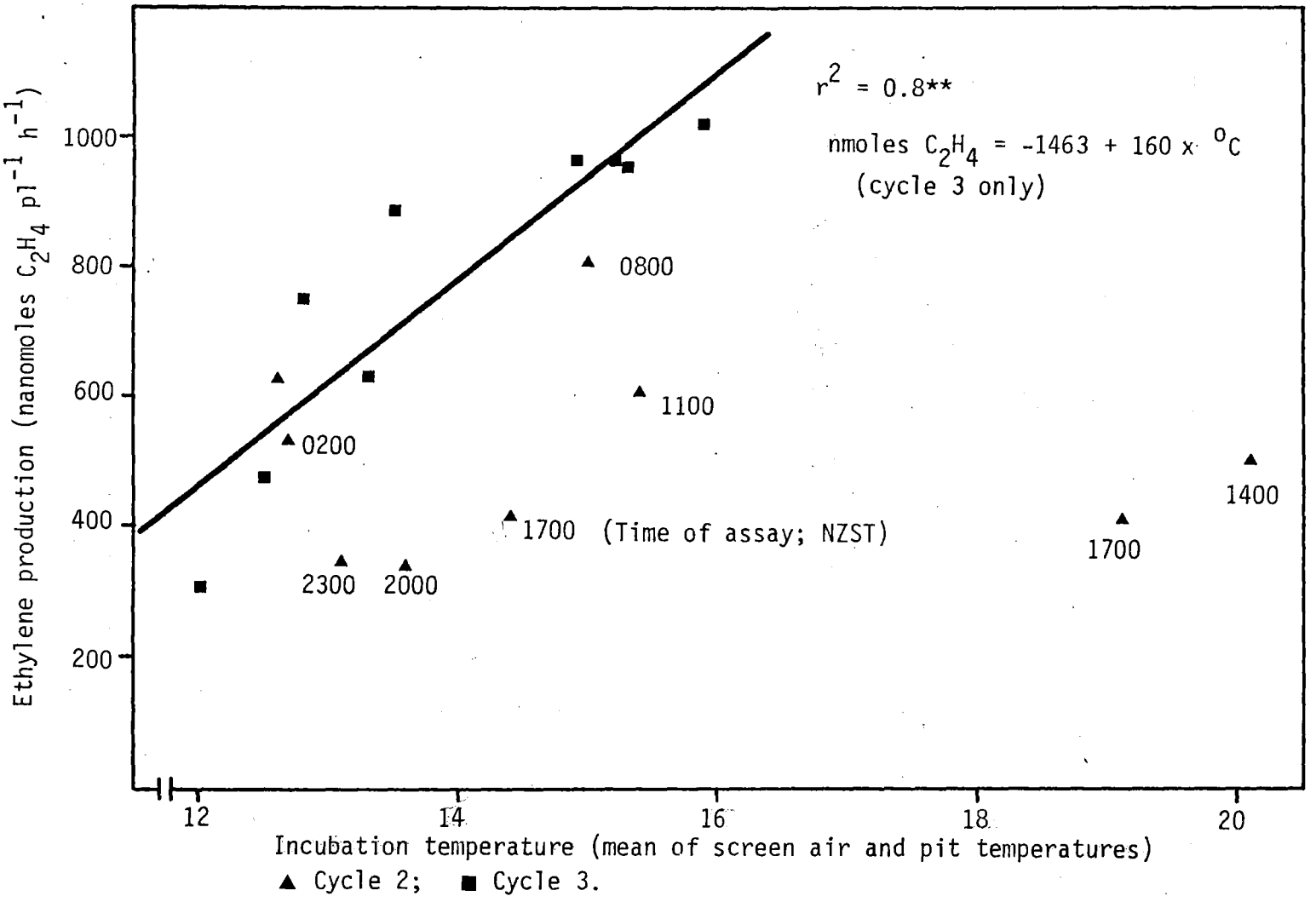


Figure 6.5: Effect of incubation temperature on ethylene production at cycles 2 and 3.

6.4.2 Growth Rates and Nitrogen Fixation at the Three Cycles

The rapid growth of peas over the experimental period indicated that roots must have obtained adequate moisture from below 20 cm. Rates of acetylene reduction, however, were lower than those frequently reported for field-grown peas (Chen and Phillips, 1977; Rhodes, 1980), although peas grown in the field with adequate soil nitrate (Dean and Clark, 1980) showed similar rates. High levels of available soil nitrate reduce nitrogen fixation in grain legumes (Mahon and Child, 1979; Dean and Clark, 1980) and as this trial was preceded by two years of pasture, mineral nitrogen levels are likely to have been high enough to depress nitrogen fixation. At the levels of nitrogenase activity observed in this trial, the carbohydrate demand of the nodules would be less than that of plants with more active nodules. In peas (Minchin and Pate, 1973) and soybeans (Hardy *et al.*, 1968), the level of nitrogen fixation has been shown to increase as plant demand for nitrogen increases during reproductive growth. An overall increase in nitrogen fixation was observed in this trial also (Figure 6.1), but at cycles 2 and 3, nitrogenase activity was declining during the 24 hour periods.

Although diurnal cycles cannot be compared directly because of the confounding effect of growth differences and varying weather conditions at each assay, some qualitative comparisons can be made.

Bud formation: The lack of a clear diurnal cycle at bud formation suggested that soil moisture and carbohydrate were adequate for fixation at the level observed throughout the 24 hour period. Sheikholeslam *et al.* (1980) showed that peas grown under insolation levels of 10 MJ m^{-2} day^{-1} stored photosynthate in root nodules. Photosynthetically active radiation was 12.7 MJ m^{-2} day^{-1} at cycle 1 and thus enough carbohydrate storage may have occurred in these pea plants to enable continued fixation during the night.

Flowering: Diurnal cycles of nitrogen fixation frequently show increased activity during the day and reduced activity during the night (Hardy *et al.*, 1968; Bergersen, 1970; Mague and Burris, 1972; Minchin and Pate, 1974; Ayanaba and Lawson, 1977). Insufficient carbohydrate supply to the nodules during the night is generally accepted as the cause of the night-time depression. At cycle 2, carbohydrate supply was adequate for night-time fixation, as nodule activity increased at this time. Thus other environmental influences, discussed below, must have limited the day-time fixation, and allowed the increase at night. Although plants were actively growing, soil moisture between 0 - 20 cm was depleted as there had been no rain for 15 days. Water loss from transpiration exceeded uptake, indicated by the wilted plants in the afternoon of cycle 2. The dry soil surrounding the sampled nodules may have directly affected nodule function. Nodules require an adequate water supply to export the products of nitrogen fixation. Minchin and Pate (1973) showed that approximately half of the water supply of nodules may be drawn from the phloem when plants are adequately watered, but when plants are water stressed, nodules rely more heavily on water supplied by the xylem flow (Sprent, 1972c). As nodule structure is designed for efficient gas exchange (Sprent, 1976a), water loss from nodule surfaces results in desiccation, when other sources of water are inadequate (Sprent, 1972c).

Minchin and Pate (1975) showed that drought stress in peas reduced total nitrogen in the tops without a similar reduction in root nitrogen. Thus, nitrogen transport from the roots appeared to have been reduced in their experiments. If drought during flowering was sufficient to shut stomata and thus reduce total water flow through the plant, nitrogen fixation products could have accumulated in the nodules with subsequent reductions in nitrogen fixation. Growth rates, however, were high during this trial so that gas exchange, water loss and therefore water movement must have continued. Thus nitrogen build-up in the nod-

ules *per se*, is unlikely to have caused the diurnal cycle observed at flowering. During drought, inadequate supplies of photosynthate may limit nitrogen fixation, but with the rapid growth rate observed, photosynthesis must have continued and thus supplies of photosynthate are not likely to have limited nitrogen fixation. The increased rate of nitrogen fixation during the night also suggests that stored photosynthate was more than adequate for nitrogen fixation.

Thus it appears that the reduced fixation from 0800 hours was caused by desiccation of nodules and direct water loss during the afternoon caused by the dry soil atmosphere and inadequate supplies of water via the xylem or phloem. Direct measurements of nodule turgidity during diurnal cycles at various levels of soil moisture would allow the cause of the depression in nitrogen fixation to be further evaluated. At the second cycle, nitrogen fixation was reduced in the late afternoon when northwesterly wind blew. Low relative humidity and high air temperatures coupled with the dry soil surrounding the sampled nodules may have been responsible for the decline. Sprent (1971b) suggested that desiccation of nodules near the soil surface may result in reduced oxygen uptake and thus nitrogen fixation. Although the time between digging of plants and incubation was kept as short as possible, the low humidity and warm temperatures may have rapidly desiccated nodules. Assays of plants *in situ* would overcome this problem.

Pod Fill: At pod fill nitrogen fixation was limited from approximately 2300 to 1100 hours. Drought stress in the nodules of these plants had been reduced by irrigation, but the marked decline in fixation from the initial rate to that observed 24 hours later (Figure 6.1) could have been caused by a gradual reduction in plant available water, or from inadequate supplies of photosynthate. Pate (1977a) considered that availability of photosynthate to nodules is a most likely pace-setting factor in nitrogen

fixation of peas. Levels of insolation used in the study of diurnal variation (Bergersen, 1970; Day and Dart, 1970; Minchin and Pate, 1974) are often less than those recorded during this experiment. At low insolation, carbohydrate stress due to reduced photosynthesis would operate earlier than in grain legumes growing under optimum insolation. Greater carbohydrate stress would occur in plants grown under short days (near the equator), and warm nights, which may reduce overall photosynthesis and increase carbon losses due to respiration. As nitrogen is translocated from leaves to developing seeds when plants are reproductive, this further reduces photosynthetic efficiency and thus carbohydrate supply to the nodules.

In this experiment, plants with nodule dry weights greater than 40 mg plant^{-1} (Figure 6.4) showed the greatest difference between night and day nitrogen fixation. These plants fixed more nitrogen and therefore used more carbohydrate than plants with nodule dry weights of less than 30 mg plant^{-1}. Lower leaves of all plants were chlorotic, and it is these leaves which supply most of the carbohydrate required by the nodules and roots for efficient nitrogen fixation (Flinn and Pate, 1970; Herridge and Pate, 1977). The reduced photosynthesis of these chlorotic leaves and thus inadequate carbohydrate supply may explain the rapid reduction in nitrogen fixation observed from 1700 to 0500 hours (Figure 6.1).

A direct temperature response may also explain the result obtained at cycle 3 (Figure 6.5). Pate (1977a) considered that peas have a broadly based temperature optimum over the range 10 - 30^{o}C for nitrogen fixation. Peas have been shown to fix more nitrogen and grow better when nights are cool (Roponen *et al.*, 1970; Minchin and Pate, 1974). In Minchin and Pate's studies, carbohydrate supplies in the nodules were used less efficiently when night temperature increased from 12 to 18 degrees. The marked nitrogen fixation sequence which was independ-

ent of temperature, at cycle 2 (Figure 6.5) indicated that temperature was not a major factor limiting nitrogen fixation at that cycle. At cycle 3, however, a close relationship between incubation temperature and nitrogen fixation was noted, a feature also observed by Gibson (1976) in soybeans. The response was not only due to temperature, as nitrogen fixation was reduced more rapidly than temperature between 1700 and 0500 hours. The study of nitrogen fixation responses to temperature in peas merits further study, particularly in Canterbury where high temperatures are frequently associated with low relative humidities.

The last cycle occurred three days after irrigation, and under the previously dry conditions, irrigation would be expected to increase nitrogen fixation. After irrigation, however, the soil dried rapidly and it is not surprising that a decline in nitrogen fixation over the 24 hour period at cycle 3 was observed.

6.5 CONCLUSIONS

The results from the three 24 hour periods which were assayed indicate the caution that is needed when calculating total nitrogen fixation from hourly acetylene reduction rates. From this study, more accurate estimates of daily nitrogen fixation will be obtained from assays between 1100 - 1400 h than at 0800 h.

Although data for only three cycles are available, during the vegetative phase and when water was not limiting, photosynthate supply to the nodules was not the dominant limiting factor for nitrogen fixation. Nitrogen fixation was reduced, however, when transpiration exceeded soil water uptake and plants wilted. During pod fill, at cycle 3, reduced temperature and insufficient carbohydrate supplies appear to have limited nitrogen fixation during the night.

Nitrogen fixation in peas responded positively to increased temperature, except when northwesterly wind blew.

CHAPTER 7

GENERAL DISCUSSION

7.1 PEAS IN CROP ROTATIONS

Two major aspects of nitrogen fixation in peas have been investigated in this study:

1) their effect on soil fertility,

2) factors influencing their nitrogen-fixing activity.

Until the 1970's the influence of grain legumes on subsequent crop growth in New Zealand received little investigation. Grazed white clover/ryegrass pastures were considered to adequately maintain fertility in crop rotations, as demonstrated in Sears' classical experiments (Sears, 1953; Sears *et al.*, 1953; Sears, 1965). The reduction in duration of grazed pasture leads to a decline in soil nitrogen status. Therefore, as cropping intensity rises, soil nitrogen increasingly becomes a constraint to crop yield (Russell, 1973).

Although grain legumes may be able to maintain or restore soil fertility, there have been very few New Zealand studies which assess these effects. In the South Island, studies initiated by Rhodes and White (Experiment 2, Trial 1), followed by a later study on a more fertile soil (Experiment 2, Trial 2) are the only investigations which assess the effect of peas and lupins, harvested at maturity, on subsequent crop growth. Results from Experiment 2 (Trial 1) which showed wheat yield after peas and lupins was 73 per cent greater than after barley, were in very close agreement with those of Hawthorne and Lewis (1980). In their brief report, Hawthorne and Lewis showed barley following lupins yielded 77 per cent more

than barley following barley. They attributed this, at least in part, to enhanced soil nitrogen status after the legume. Earlier Australian studies at the Waite Research Institute over a 50 year period showed that mean annual dry matter production from a wheat/pea rotation was 50 per cent more than from a wheat/fallow rotation. Soil nitrogen was reduced with the pea/wheat rotation but reduced even more with the wheat/fallow rotation (Russell, 1980). Normally most of the nitrogen in grain legumes is removed in their seed. Furthermore, cultivation stimulates mineralisation of organic nitrogen (Russell, 1973) and the increased mineral nitrogen will reduce grain legume reliance on symbiotic fixation (Dean and Clark, 1980; Herridge, 1982c). Thus the nett nitrogen contribution to the soil is lower after grain legumes compared with grazed pastures, or when grain legumes are grazed.

As was expected, on the more fertile soil (Experiment 2, Trial 2), the response in ryegrass production to grain legumes was less than on the infertile soil. Winter-grown ryegrass yield after peas and lupins was respectively 25 and 11 per cent greater than after wheat. With the higher levels of soil nitrogen, plant demand for symbiotic nitrogen decreased and hence nitrogen fixation was reduced. Comparisons cannot be stressed between these trials because of differing season and fertility interactions.

A further trial (Experiment 3) on the same soil as Experiment 2 (Trial 2) produced almost exactly the same response in ryegrass yield, although different cereals were used in the two trials. On that fertile soil, even in the presence of 8 t ha^{-1} of straw incorporated at sowing, there was still 14 ppm N in the surface 20 cm during vegetative growth. The ryegrass yield responses not only supported the hypothesis that grain legumes enhance subsequent crop yield but also demonstrated this across pea cultivars (cv. Huka and Puke). This 28 per cent increase in ryegrass yield after barley, even on a high fertility soil, is large enough to be agronomically significant.

Inclusion of fallow plots would strengthen assessment of absolute fertility changes. In some trials the estimation of changes in soil fertility was limited because of their absence. Simultaneous measurement of nitrogen leaching losses would allow more accurate estimation of nitrogen balances. Soil structural changes were not covered in this thesis because of the difficulty in measuring significant changes in structure after trials lasting only two or three years (Dr C. Ross, Soil Bureau, DSIR, pers. comm.). Despite these limitations, experiments in New Zealand, Australia (Boundy, 1978; Hawthorne and Lewis, 1980; Russell, 1980), India (Ahlawat *et al.*, 1981), Africa (Jones, 1974) and North America (Van Doren, Triplett and Henry, 1976) have clearly shown the benefits to be gained from growing grain legumes in crop rotations.

The principal cause of these differences is the soil nitrogen status (Russell, 1973). Nitrogen fixed by peas may remain in roots and nodules after seed harvest. Above-ground residues may also contain significant quantities of nitrogen (Pate, 1977; Rasmussen and Pumphrey, 1977; Rhodes, 1980; Withers *et al.*, 1981). In Experiment 3 where garden peas cv. Puke peas were harvested at the green pea stage and at maturity, residues contained 6.8 and 2.7 g N m^{-2} respectively. Although the effect of grazing residues after seed harvest has not been assessed for grain legumes, Janson and Knight (1980) demonstrated the increased fertility which resulted after autumn-sown grain legumes were grazed in the spring. On a low fertility soil, subsequent wheat yielded twice as much after peas and tick beans compared with ryegrass/oats greenfeed, subterraneum clover or blue lupins. Lupin performance may have been depressed because sowing date (March 8) was later than optimum (Janson and Knight, 1980).

7.2 INFLUENCE OF WATER ON NITROGEN-FIXING ACTIVITY

Many studies have shown the sensitivity of peas to drought stress and to waterlogging (Chapter 1). Results from Experiments 3 and 4 confirm these responses in terms of yield although responses were less than those reported by Stoker (1973), Anderson and White (1974) and White *et al.* (1982) for vining and dry pea crops in Canterbury. Over 100 mm rainfall during pod filling in Environment 3 reduced the response in that season.

Water stress limits nitrogen translocation from vegetative to reproductive portions in lupins as shown by Hocking (1982). Experiment 4, however, showed that some water stress may increase the nitrogen harvest index, particularly when Maple peas are autumn-sown. Irrigation of autumn-sown peas resulted in a NHI of only 0.29 but drought treatments applied after flowering began results in a NHI of 0.53. Thus for water stress to reduce the NHI, the stress must be severe enough to cause premature senescence. The excessive growth of autumn-sown Partridge, and consequently low harvest index, in Experiment 4 occurred because water was applied before the plant had become fully reproductive. If a crop is to be harvested for grain, then the excess vegetative growth would reduce seed yield and increase harvesting problems. Crops of this nature can develop in wet seasons without irrigation. In these circumstances using the crop for silage or stock feed directly may be the most profitable alternative. Crops sown late in the spring after pea grazing may then benefit from the increased fertility. European plant breeders are presently developing pea lines specifically for stock feed (Potts, 1978).

Nitrogen fixation was expected to increase after irrigation. However, responses were smaller than predicted. In Experiment 3, heavy rain reduced the irrigation response. In Experiment 4, the spring-sown peas did not respond as markedly to irrigation as did the autumn-sown peas, particularly Whero. Maple peas, particularly Partridge in Experiment 5,

reached peak fixation before water stress occurred and before other cultivars reached their peak. Thus, when soil moisture is not a major limit to growth, soil nitrate supply is frequently adequate for pea requirements. This depresses the nitrogen fixation response to irrigation.

7.3 INFLUENCE OF SOIL NITRATE

In all trials nitrogen-fixing activity of peas was low. This is attributed to the ready availability of soil nitrogen. Mineral nitrogen influences on nitrogen-fixing activity in peas are not fully understood. Some pot trials (Virtanen and Hausen, 1952) and controlled environment studies (Oghoghorie and Pate, 1971) have shown peas to be relatively tolerant of nitrate in the growth medium. In Oghoghorie and Pate's solution culture study, 35 ppm N reduced fixation by 40 per cent, while 315 ppm N reduced it by 80 per cent, when compared to peas which were totally reliant on nitrogen fixation. The relative tolerance of peas to nitrogen recorded for solution culture but not for field-grown peas may be a function of the totally different environments and growth media. In Canterbury, field-grown peas have been shown to fix almost all of their nitrogen only when soil nitrogen was very low (< 3 ppm N in surface 60 cm). In those circumstances, however, yield was also depressed due to the poorly structured soil (Rhodes, 1980). In Experiment 3 nitrogen fixation was further reduced by 45 kg N ha^{-1}, applied at nodule formation or flowering. Although some studies have shown that small amounts of nitrogen benefit legume growth and fixation (Oghoghorie and Pate, 1971; Hoglund, 1973) the responses obtained here showed that increased soil nitrogen directly replaced fixation. Nitrogen fixation was also markedly reduced in field studies when soil nitrate levels were similar to those in this thesis (Dean and Clark, 1980; Sosulski and Buchan, 1980).

Shivashankar and Vlassak (1978) showed that nitrogen fixation in soybeans was increased by 345 per cent and yield was increased by 116 per cent (to 269 g m^{-2}) when 4 t ha^{-1} straw was incorporated into the soil prior to drilling. Nitrogen fixation was probably enhanced because of reductions in soil nitrate but changes in this parameter were not measured in their study. Seed yield increases were attributed to increased CO_2 and thus enhanced photosynthesis, resulting from straw decomposition. Similar responses were not obtained in the present studies. In the trial reported here straw had little effect on yield or nitrogen fixation, as soil nitrate levels were not reduced as much as expected. Straw may have increased CO_2 levels in the canopy, but in the windy Canterbury environment possible increases would have been nullified. Increased CO_2 in the lower crop canopy would only benefit yield and nitrogen fixation when photosynthesis limited production.

7.4 INFLUENCE OF CULTIVAR

Nitrogen transfer from vegetative organs to seeds limits that usefulness of grain legumes in crop rotations. Rhodes (1980) showed that lupins have a NHI (nitrogen harvest index) of 0.91 whereas Huka peas were 0.86. In Experiment 5 important differences were observed in the proportion of plant nitrogen transferred to seeds in the eight cultivars studied. The very early maturing cultivar, Tere, had a large NHI of 0.85 whereas Partridge which flowered late had a NHI of only 0.38. Neither of these cultivars yielded as well as Rovar or Huka. These latter cultivars yielded 284 and 360 g m^{-2} and had NHI's of 0.81 and 0.80 respectively. In general, then, lupins may be more efficient than peas at translocating nitrogen into the seed but differences in pea cultivars and time of harvest (green or dry) may be exploited when crop residues are to be used for soil amelioration.

Field peas fixed approximately twice as much nitrogen as did garden peas. El-Sherbeeny *et al.* (1977) have also shown large differences in nitrogen fixation between cultivars of tick beans. Sprent (1979) suggested that the greater fixation observed in climbing cultivars of *Phaseolus vulgaris* compared with bushy types may reflect an unwitting selection against nitrogen-fixing efficiency in the latter. This could occur where breeding programmes are carried out on highly fertile soils where reliance on nitrogen fixation would be minimised. Flowering patterns of the peas in this study did not appear to be correlated with the peak in nitrogen fixation as the indeterminate cultivar Partridge reached peak fixation before flowering.

7.5 INFLUENCE OF CARBOHYDRATE SUPPLY TO NODULES

Many studies, particularly in growth cabinets, have shown that nitrogen fixation in grain legumes is limited by the supply of carbohydrate to nodules (Lowrie and Wheeler, 1973; Hardy and Havelka, 1976; Sheikholeslam *et al.*, 1980). These conclusions have frequently been made for soybeans which have higher demands than peas for carbohydrate during seed filling (Figure 1.1). Peas in the trials reported here did not exceed nitrogen fixation rates of 6 μmoles C_2H_4 plant^{-1} h^{-1}. At these low rates the demand for carbohydrate would be less than for plants with greater nitrogen-fixing activity.

In terms of carbohydrate supply to nodules, the Canterbury environment is well suited to optimising nitrogen fixation with long sunny days and cool nights. The influence of increased insolation has been studied by Sheikholeslam *et al.* (1980) who showed that peas grown at 10 MJ m^{-2} day^{-1} stored 200 per cent more photo-assimilate in the nodules than plants at 6 MJ m^{-2} day^{-1}. They considered that at low light diurnal variation in nitrogen-fixing activity would be greater than where insolation (PAR) was

closer to 10 MJ m^{-2} day^{-1}. Results from Experiment 6 indirectly support this. Nitrogen fixation at flowering increased, rather than decreased, during the night. This strongly suggested that carbohydrate supplied to the nodule during the day did not limit night-time fixation. However, that trial was not designed to assess the influence of carbohydrate supply on nodule function. Peas are noted for their scrambling habit and shading of lower leaves may lead to reduced fixation, particularly in cultivars which lodge readily. Although this has not been assessed in peas, Hardy and Havelka (1976) showed that fixation by lodged soybean was less than one third that of unlodged plants. A future study of diurnal variation could be designed specifically to show the adequacy of carbohydrate supply to nodule function. Plants could be sown at different times to give a range of phenological stages at any one assay. Shade treatments imposed prior to assays would alter the insolation levels. Plants would then need to be assayed at one temperature to minimise the confounding influence of diurnal changes in that parameter. Thus the importance of insolation and phenological stage on nodule performance still requires further assessment in the field.

7.6 MEASUREMENT OF NITROGEN FIXATION

Nitrogen fixing activity of peas in all trials was low. The proportion of total nitrogen from fixation (C_2H_2) was approximately 3 per cent after spring sowing in Experiment 3, and 11 and 9 per cent from autumn and spring sowing (Experiment 4). Cultivar differences ranged from 6 per cent for Pania to 30 per cent for Partridge (Chapter 5). These low values have been attributed to adequate supplies of soil nitrate. Recent evidence for field-grown lupins and soybeans (Herridge, 1982c) supports this, where soybeans fixed only 0.5 g N m^{-2} (2% of plant nitrogen) when sown into a soil containing 18 g N m^{-2} in the surface 120 cm. In that crop 15 g N m^{-2} were

lost in the seed and thus 14.5 g N m^{-2} were lost from the soil. Estimates of nitrogen fixation were based both on acetylene reduction and tissue solute analysis (Herridge, 1982a, b). In the trials reported here, however, acetylene reduction may not have measured all of the fixed nitrogen. This is suggested by the large discrepancy between nitrogen balance estimates (49%) and acetylene reduction (3% of total nitrogen from fixation) in Experiment 3.

Thus before an overall assessment of the factors influencing nitrogen fixation in peas can be made, it is important to evaluate the techniques used to measure nitrogen fixation. As previously discussed (Chapter 1) acetylene reduction is the principal technique used to measure nitrogen-fixing activity. The technique is admirably suited for showing differences between treatments. It is relatively easy, rapid and can record changes in fixation rate over short periods. However, for absolute measurement of nitrogen fixation, the assumptions inherent in the technique limit its usefulness. Hydrogen evolution, which is blocked by acetylene, is assumed to be an insignificant fraction of the nitrogen-fixing activity. Furthermore, relative efficiency is assumed to be constant and unaffected by treatments. Both these assumptions are questionable. The presence of an active uptake hydrogenase that re-metabolises hydrogen produced would also make attempts to measure hydrogen production meaningless (Turner and Gibson, 1980). Bethlenfalvay *et al.* (1978) and Gibson (1978) showed that the amount of hydrogen evolved did not remain constant but varied with both time and treatments that altered photosynthesis. However, if hydrogen evolution was important in this thesis, the calculated levels of nitrogen fixation would be overestimated. In contrast, if the ratio of moles acetylene to moles of nitrogen fixed was as low as 1.5:1 or less as observed for peas (Oghoghorie and Pate, 1971) and soybeans (Herridge, 1982a) then nitrogen fixation estimates could be more than doubled. Here in New Zealand, Rhodes' (1980) estimates of nitrogen fixation from acetylene reduction (which used the theoretical

ratio of 3:1) and nitrogen balance estimate were in close agreement for peas, but differed widely for lupins. It is likely that the ratios used were not constant in experiments described in this thesis. Thus acetylene reduction has been used primarily to show differences in nitrogen-fixing activity between treatments and not for direct estimates of seasonal nitrogen fixation.

A further assumption inherent in the use of acetylene reduction is that the ratio of nodules harvested to those remaining in the soil is the same for all treatments. This is unlikely. In plots where soils were kept artificially dry (Experiment 4), considerable difficulty was experienced in sampling roots and retrieving all nodules. Irrigated treatments (Experiments 3 and 4) and those with straw incorporated (Experiment 3) were considerably easier to sample. In these contrasting conditions the proportion of the total nodules assayed could vary significantly. The difficulty in harvesting all soybean nodules was considered by Herridge (1982b) to be a major reason for the discrepancy between nitrogen fixation estimated by acetylene reduction (45 kg N ha^{-1}) and ureide analysis (203 kg N ha^{-1}). White clover has been shown to fix a greater proportion of its nitrogen lower in the soil profile when drought occurs (Hoglund and Brock, 1978). Therefore, sampling nodulated roots to 20 cm may not only underestimate nitrogen-fixing activity, particularly in dryland plots, but may also underestimate the total nitrogen stored in roots and nodules. Calculations of nitrogen in roots and root dry weight from the top 20 cm only have generally been avoided because of the misleading nature of the results. Researchers in the USA have been developing techniques to sample roots to at least 1 m (Taylor and Klepper, 1978) but apart from the financial and time costs the techniques developed may not be suitable for Canterbury conditions where alluvial gravels in the B horizon impede soil sampling.

In situ assays would help to overcome the problem of sampling all nodules. Thus the development of *in situ*, field-based techniques to measure nitrogen fixation warrants further study, as it would allow assessment of the effect of short-term environmental changes on nitrogen fixation. These have been developed for closed pot systems (Hart, 1976; Mederski and Streeter, 1977) but far less attention has been paid to the difficulties of measuring nitrogen fixation in the field (Mahon and Salminen, 1980). Where low rates of ethylene production occur, direct samples of the soil gas may help to overcome the problem of inadequate resolution in the gas chromatograph, while seals around the base of plants would reduce the volume of air that dilutes the ethylene. Assays of this type, however, would not account for the activity change with soil depth, particularly in droughts (Hoglund and Brock, 1978). A large number of destructive soil cores would be necessary to assess this. These techniques would also reduce sampling error by reducing the number of plants sampled. Furthermore, frequent acetylene reduction assays may be toxic to nodules and any deleterious effects would need to be assessed. *In situ* assays were attempted in Experiment 3. Although preliminary tests in the glasshouse showed that the technique was suitable, it was not successful in the field, primarily because of low rates of fixation.

When using acetylene reduction, accurate estimation of total nitrogen fixation depends on taking many acetylene reduction assays during growth, and assessment of the effect of diurnal variation in nitroge-fixing activity (Hardy *et al.*, 1968; Carran *et al.*, 1982). Plants harvested during acetylene reduction assays were generally divided into root, stem, leaf, pod and peas. This was labour-intensive and limited the number of assays. In future studies, this partitioning may best be done during vegetative growth, at flowering, pod filling and at maturity. The reduced emphasis on plant partitioning would allow more frequent assays, and thus more accurate estimates of nitrogen fixation. The usefulness of measuring nodule weight

and using this as a covariate in estimating ethylene production was shown in Experiment 6. Although more frequent assays and measurement of nodule weight are costly in terms of time, they allow more accurate estimation of treatment effects on nitrogen fixation.

7.7 CONCLUSIONS AND FUTURE STUDY

Peas enhance soil fertility when compared with cereals and this holds for both fertile and infertile soils. Increased soil nitrogen levels after pea crops, however, are unlikely because of their reliance on soil nitrogen and the large amount of nitrogen removed in seeds. Thus soil fertility is likely to be higher after vining pea crops, with a low nitrogen harvest index, than after dry peas particularly when residues are returned.

Nitrogen-fixing activity is depressed by the levels of soil nitrate found in cropping soils. When fertility is low, nitrogen and seed yield may be reduced although fixation will account for most of the plant nitrogen. Irrigation stimulates nitrogen fixation except where soil nitrate levels are sufficiently high to inhibit fixation.

Grain legumes may also enhance fertility by improving soil structure. It is certainly less important than nitrogen, and far more difficult to assess but quantitative investigation of its contribution in enhancing fertility still remains to be done.

The cultivar differences observed in Experiment 5 deserve further attention and cultivar:*Rhizobium* interactions may also lead to enhanced fixation. Identification of a cultivar and *Rhizobium* strain combination which will fix a large proportion of plant nitrogen in a high fertility soil is needed.

ACKNOWLEDGEMENTS

During this project, I have become indebted to many people, and without their assistance it would never have been completed. In particular I would like to thank the following:

Dr J.G.H. White, Professor of Plant Science, Lincoln College, for his supervision, advice and encouragement throughout this study, and for his perceptive and constructive criticism of the draft.

I am grateful to staff and post-graduate students of the Plant Science Department, in particular, Dr J. Gallagher, Mr G. Hill, Dr P. Jarvis and Mr R. Lucas for their stimulating ideas and interest in the project. Dr W. Jermyn and Mr R. Scott of Crop Research Division provided me with seed, and the DSIR mini-viner. Plots for the study reported in Chapter 6 were made available by Dr Jermyn. Mr P. Rhodes of MAF Research, Lincoln, assisted with planning and field assays in Experiment 5. Mr J. Hoglund of Grasslands Division, DSIR, was of particular assistance with the Acetylene Reduction technique and in many discussions and debates relating to analysis and interpretation.

I am grateful to DSIR which funded this study and provided me with a studentship. Lincoln College Research Committee funds are also gratefully acknowledged.

Mr S. McGeorge of Ravensdown Fertiliser Company provided laboratory facilities for the soil nitrate analyses and Mr L. Higgins and Mrs A. McMurdo willingly analysed the samples. Mr B. McKeegan of the Soil Science Department operated the auto-analyser for plant nitrogen determinations. Mr H. Horn and Mr R. McLenaghen also assisted with plant and soil analyses.

Mr B. Love, Mr N. Mountier and Mrs A. Lister bore the brunt of my statistical incompetence. Mr N. Mountier and members of the Computer Centre provided an excellent service which greatly assisted me during data analysis and production of the thesis.

Mr G. Meijer, Mr B. Smith, Mr D. Jack and Mr D. Fowler provided me with excellent and willing technical assistance, both in and out of normal work hours.

Mr Eric Mangin, Dave Cox and Diane Heslop showed me some of the intricacies of photography and produced the plates.

Annette Brome, Nick Fitzgerald and Ronald van Til at various times helped me with acetylene reduction assays and nitrogen analyses.

Elizabeth Burtt and Peter Espie helped with proof reading, useful comments and encouragement. Sandy Hines deciphered and typed much of the initial draft onto the computer, and drew a number of the figures. Mrs Kathy Brown skilfully completed the final typing.

Innumerable friends both here at Lincoln and elsewhere have also contributed to the completion of this project. To those who have laboured, encouraged and prayed - thankyou.

Finally, I am immensely indebted to my family for their support, dedication and love shown in innumerable hours spent dissecting pea plants, grinding samples and proof reading of the thesis.

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Appendix I: Acetylene reduction.

Each root sample from a harvested quadrat was placed in a glass jar of 1 *l* capacity (Agee preserving jars, N.Z. Glass Manufacturing Co.) and was sealed with a gas tight lid fitted with a rubber septum, through which 100 ml of air were withdrawn. The air was immediately replaced with 100 ml of C_2H_2 and the time at which acetylene was injected, noted for each sample. Jars were incubated for one hour in a soil pit, filled with damp sawdust and covered with an asbestos sheet and sawdust filled sack. A thermometer was placed in the soil pit and in adjacent soil to a depth of 10 cm. Temperatures were noted at the start and end of the assay period.

After incubation, jars were withdrawn from the soil pit and gas samples collected in rubber stoppered 10 ml evacuated vials via a double-ended needle. A 0.2 ml sample of gas from each vial was analysed by gas chromatography using a flame ionisation detector and a stainless steel column (6 mm outside diameter and 0.6 m length) packed with Poropak N. An oven temperature of $70^{o}C$, and nitrogen as carrier gas was used at a flow rate of approximately 25 ml min^{-1}. Peak heights of ethylene and acetylene were measured and used to calculate nanomoles C_2H_2 $plant^{-1}$ h^{-1}.

Acetylene was used as an internal standard by using the formula: Corrected C_2H_4 peak height (cm) = assay C_2H_4 peak height (cm) x [C_2H_2 peak height (cm) at 10% concentration x assay C_2H_2 peak height $(cm)^{-1}$].

Corrected ethylene peak heights were then converted to $\log_{10}$ C_2H_4 (nmoles) contained in 0.2 ml gas by the formula:

$\log_{10}$ C_2H_4 (nmoles) = 0.9498 [$\log_{10}$ corrected C_2H_4 peak height (cm)] - 2.14. This formula, derived from the standard ethylene curve was changed after recalibration due to a fire in 1980 to 0.9255 [$\log_{10}$ corrected

C_2H_4 peak height (cm)] - 2.12.

nmoles C_2H_4 jar^{-1} were calculated by multiplying nmoles C_2H_4 1.0 ml^{-1} by the volume of the jar to obtain nmoles C_4H_4 produced per jar over the incubation period. This was divided by the number of plants assayed to give nmoles C_2H_4 $plant^{-1}$ h^{-1}.

An estimate was made of nitrogen fixation (g N m^{-2}) by:

nmoles C_2H_4 $plant^{-1}$ day^{-1} were multiplied by plant density to give nmoles C_2H_4 m^{-2} day^{-1}.

n moles N_2 m^{-2} day^{-1} were calculated by dividing nmoles C_2H_4 $plant^{-1}$ day^{-1} by 3.

moles N_2 were multiplied by 28 to obtain g N fixed m^{-2} day^{-1}.

Appendix II: The determination of nitrate nitrogen in soils using the model 93-07 nitrate electrode.

SUMMARY

The soil sample is stirred with an extractant solution and the nitrate-nitrogen is determined by direct measurement with a specific ion electrode. Care must be taken to keep the soil samples refrigerated to prevent the biological degradation of the nitrate ion.

APPARATUS

Orion model 407A specific ion meter

Model 93-07 nitrate electrode

Orion model 90-02 double junction reference electrode

Magnetic stirrer

METHOD

Weigh out 20 g of the soil sample and place in 150 ml tall form beaker. Add 40 ml (tilt measure) of Orion extractant and magnetic stirring flea. Stir and balance the orion meter using 100 ml of a 5 μg N ml^{-1} standard, to which has been added 1 ml of 15A (ionic strength adjuster; 10 M Potassium fluoride solution). The solution is then checked with 100 ml of a 50 μg N ml^{-1} standard which also contains 1 ml of ISA. The needle should go to 10 on the top scale.

NO_3-N ppm = Top scale reading x standard x ratio of extractant/soil.

Appendix III: Nitrogen analysis of plant material.

Prior to Kjeldahl digestion, ground plant material was oven dried at 70°C overnight and, depending on the nitrogen concentration, duplicates of between 0.150 g and 0.250 g of plant material were weighed into Jay Tee patty pans (McMeeking Manufacturing Ltd, Dunedin), which contained no measurable nitrogen. Weights of 0.250 g of mature stem material and 0.150 g of immature leaf or mature seed were used to give similar nitrogen concentrations in final solutions for auto-analysis. Each digestion run of 40 tubes contained 1 blank, 3 plant standards and 18 duplicated plant samples. To each digestion tube was added 7 ml of concentrated H_2SO_4, 2.8 g ($S_{\bar{x}}$ 0.1) of 1% Se:K_2SO_4 catalyst, a patty pan and plant material. Actual weights of plant material were recorded to 0.0005 g. Digestion proceeded for 1 hour at 100°C and a further 1.5 hours at 380°C, on a hot plate ('Tecator Digestion System 40'). When cool, approximately 95 ml of distilled water were added while tubes were mechanically stirred. They were left to cool again for at least 4 hours before being made up to 100 ml exactly. Samples were run on an autoanalyser using a phenol-hypochlorite method, modified by Weatherburn (1967).

Standards were prepared by the following method:

1) Place analytical grade ammonium sulphate in a desiccator overnight.

2) Weigh out 1.888 g into a 1 *l* volumetric flask and make up to volume with distilled water (= bulk solution).

3) Prepare 14 Tecator digestion tubes (100 ml capacity) by:
   i) adding 7 ml concentrated sulphuric acid (commercial grade contained no measurable nitrogen);
   ii) adding 2.76 g catalyst as above;
   iii) adding one paper cup and digest as normal.

4) When cool, add into all tubes approximately 50 ml of distilled water while stirring solution.

5) Into groups of tubes add 5.0, 7.5, 10.0, 12.5, 15.0, 22.5, 30.0 ml of bulk solution from (2). These solutions when cool and made up to 100 ml exactly will contain 20, 30, 40, 50, 60, 90 and 120 μg N ml^{-1} respectively. Vials for storage should be stoppered and refrigerated.

Each run was corrected to the known nitrogen concentration in the bulk plant standard. Samples were redigested if the standard error of the mean (of the two digests for each sample) was greater than 10 per cent of the mean.

Appendix IV: Orthogonal comparisons.

There are three general approaches to mean separation:

i) the use of least significant differences,

ii) the use of multiple range tests, and

iii) planned F tests.

Planned F tests usually offer the most precise procedure for mean separation (Little and Hills, 1978). An F test with more than one degree of freedom for treatments is the average test of as many independent comparions as there are degrees of freedom. If only one of the comparisons involves a real difference and if this difference should be averaged with differences that are very small, then such a test may not detect the difference of interest (Steel and Torrie, 1960). For this reason, planned meaningful comparisons have been used to separate means. Both references indicate the formal procedure for using orthogonal comparisons.

In Chapter Two, comparisons were planned to test the following differences:

Comparisons	Treatments				
	Manapou barley	Huka peas	Puke peas	Uniharvest lupins	Fallow
i) Barley versus legumes	-3	+1	+1	+1	0
ii) Puke versus Huka	0	-1	+1	0	0
iii) Peas versus lupins	0	-1	-1	+2	0
iv) Cropped versus fallow	-1	-1	-1	-1	+4

The last three comparisons were generally not important and were grouped into deviations in the analysis of variance.

In Chapter 5, comparisons were planned to test the following differences:

Comparison	Treatments							
	Huka	Whero	Partridge	Rovar	Puke	Pania	Tere	SSF
i) Tere versus all other cultivars	+1	+1	+1	+1	+1	+1	-7	+1
ii) Maple versus others, not Tere	-2	+5	+5	-2	-2	-2	0	-2
iii) Whero versus Partridge	0	-1	+1	0	0	0	0	0
iv) Rovar and Huka versus garden cultivars, not Tere	+3	0	0	+3	-2	-2	0	-2
v) Rovar versus Huka	-1	0	0	+1	0	0	0	0
vi) SSF versus Puke and Pania	0	0	0	0	-1	-1	0	+2
vii) Puke versus Pania	0	0	0	0	+1	-1	0	0

The last three comparisons were generally not important and were grouped into deviations in the analysis of variance.

Appendix 4.1 : Soil moistures during growth of autumn and spring peas.

Date	Days after sowing	Treatment	Dry mass water percentage (0-20 cm)	Depth cm	Dry mass water percentage
17. 5.80	10	A W R	27.1	20-60	20.4
9. 9.80	125	A W R	25.3	20-60	19.5
3.10.80	149	A W R	16.6	20-60	14.5
		A W I	34.9	20-60	24.4
30.10.80	176	A W I	19.7	20-60	-
1.11.80	178	A W R	14.5	20-60	12.9
		A W I	31.0	20-60	23.4
	50	S W I	16.7	20-60	16.6
14.11.80	191	A W R	23.5	20-60	14.1
22.11.80	199	A P D	15.0	20-50	12.5
		A P R	21.0	20-50	14.5
27.11.80	204	A W R	23.0	20-40	14.0
		A W I	30.0	20-40	24.5
2.12.80	81	S W D	19.5	20-40	16.1
		S W R	24.0	20-40	18.0
15.12.80	222	A W I	24.0	20-40	19.0
	94	S W D	15.0	20-40	13.0
		S W R	15.5	20-40	13.5
		S W I	23.0	20-40	18.4
17.12.80	224	A P D	9.9	20-40	9.6
		A P N	14.9	20-40	11.8
		A P I	22.1	20-40	16.2
	96	S W I	26.6	20-40	22.2
		S P R	14.5	20-40	12.7
24.12.80	231	A P I	30.8	20-40	23.4
	103	S P I	30.0	20-40	23.0
1. 1.81	239	A P I	27.5	20-40	20.6
	111	S W I	21.9	20-40	17.4
	111	S P I	26.3	20-40	20.2
3. 1.81	113	S W I	32.8	20-40	24.6
5. 1.81	243	A P I	21.6	20-40	18.7
	115	S P I	20.8	20-40	17.7
7. 1.81	245	A P I	29.5	20-40	25.8
	117	S P I	30.0	20-40	23.6
10. 1.81	248	A W R	13.3	20-40	11.6
	248	A W I	15.0	20-40	14.0
	248	A P D	8.6	20-40	9.1
	248	A P R	11.9	20-40	10.5
	248	A P I	23.3	20-40	18.6
	120	S W D	9.8	20-40	10.0
	120	S W R	11.0	20-40	10.0
	120	S W I	24.3	20-40	18.5
	120	S P D	8.6	20-40	11.3
	120	S P R	8.5	20-40	9.0
	120	S P I	23.0	20-40	20.0
19. 1.81	129	S W I	22.0	20-40	20.0
		S P I	21.0	20-40	17.0
22. 1.81	132	S W I	32.0	20-40	26.0
		S P D	8.0	20-40	8.6
		S P R	14.0	20-40	11.0
		S P I	31.0	20-40	25.0
2. 2.81	144	S P R	13.0	20-40	12.0
		S P I	21.0	20-40	17.8
6. 2.81	147	S P I	24.0	20-40	20.0

Appendix 4.2: Influence of cultivar and moisture on nitrogen concentration (% N) of plant components of autumn-sown peas 135, 163 and 188 days after sowing, and the influence of cultivar on nitrogen concentration of plant components of spring-sown peas 38 and 60 days after sowing.

Autumn-sown		Days after sowing										
		135		163				188				
		Root	Whole top	Root	Stem	Leaf	Pod + pea	Root	Stem	Leaf	Pod	Peas
Whero	D			2.37	1.75	4.08	5.37	2.11	1.18	2.72	2.64	4.26
	R	2.59	3.29	2.53	1.62	4.27	5.59	2.33	0.98	2.85	2.96	3.93
	I			2.36	1.65	4.36	4.87	2.85	1.21	3.98	3.07	4.68
Partridge	R	3.01	3.77	2.88	2.04	4.55	-	2.77	1.30	3.82	-	-
$LSD_{.05}$		0. 67	1.48	0.51	0.49	0.78	0.67	0.39**	0.41	0.79*	1.19	0.73
CV		10.7	18.7	12.5	17.2	11.3	7.4	9.7	21.8	14.8	23.9	9.8

Spring-sown		Days after sowing			
		38		60	
		Root	Whole tops	Root	Whole tops
Whero	R	2.09	3.74	3.03	3.85
Partridge	D	2.09	3.88	3.12	3.89
$LSD_{.05}$		0.41	0.52	0.77	1.56
CV %		8.6	4.5	11.1	13.2

Appendix 5.1: Absolute growth rates of pea plants at each harvest.

Cultivar	Days from drilling (g plant^{-1} d^{-1})						
	27	43	57	70	85	104	129
Huka	0.011	0.037	0.080	0.129	0.157	0.086	-0.113
Whero	0.018	0.050	0.099	0.150	0.179	0.112	-0.087
Partridge	0.013	0.034	0.066	0.097	0.0116	0.077	-0.050
Rovar	0.015	0.049	0.108	0.171	0.197	0.071	-0.182
Puke	0.015	0.048	0.103	0.165	0.205	0.133	-0.105
Pania	0.013	0.043	0.098	0.158	0.194	0.096	-0.154
Tere	0.018	0.058	0.121	0.179	0.187	0.058	-0.165
SSF	0.016	0.055	0.120	0.187	0.215	0.088	-0.178
$LSD_{.05}$	0.002	0.006	0.018	0.035	0.059	0.088	00.094
CV	7.6	9.5	12.8	15.7	21.8	64.5	49.9
Orthogonal comparisons, variance ratios:							
Cultivars (7 d.f.)	19.17	13.60	8.68	5.80	2.59	0.68	2.21
1: Tere vs other cultivsrs	32.0	27.3	13.1	4.6	0.1	1.4	1.5
2: Maples vs other cultivars not Tere	9.2	4.8	13.3	14.2	7.9	0.0	8.3
3: Whero vs Partridge	43.8	26.2	12.8	9.5	5.1	0.8	0.7
4: Rovar and Huka vs garden cultivars not Tere	12.3	8.6	4.8	3.2	2.4	1.1	0.0
5: Rovar vs Huka	16.8	14.7	9.3	6.0	2.1	0.1	2.4
6: SSF vs Puke and Pania	12.0	11.3	6.0	2.8	0.4	0.6	1.5
7: Puke vs Pania	8.1	2.3	0.4	0.1	0.2	0.8	1.2

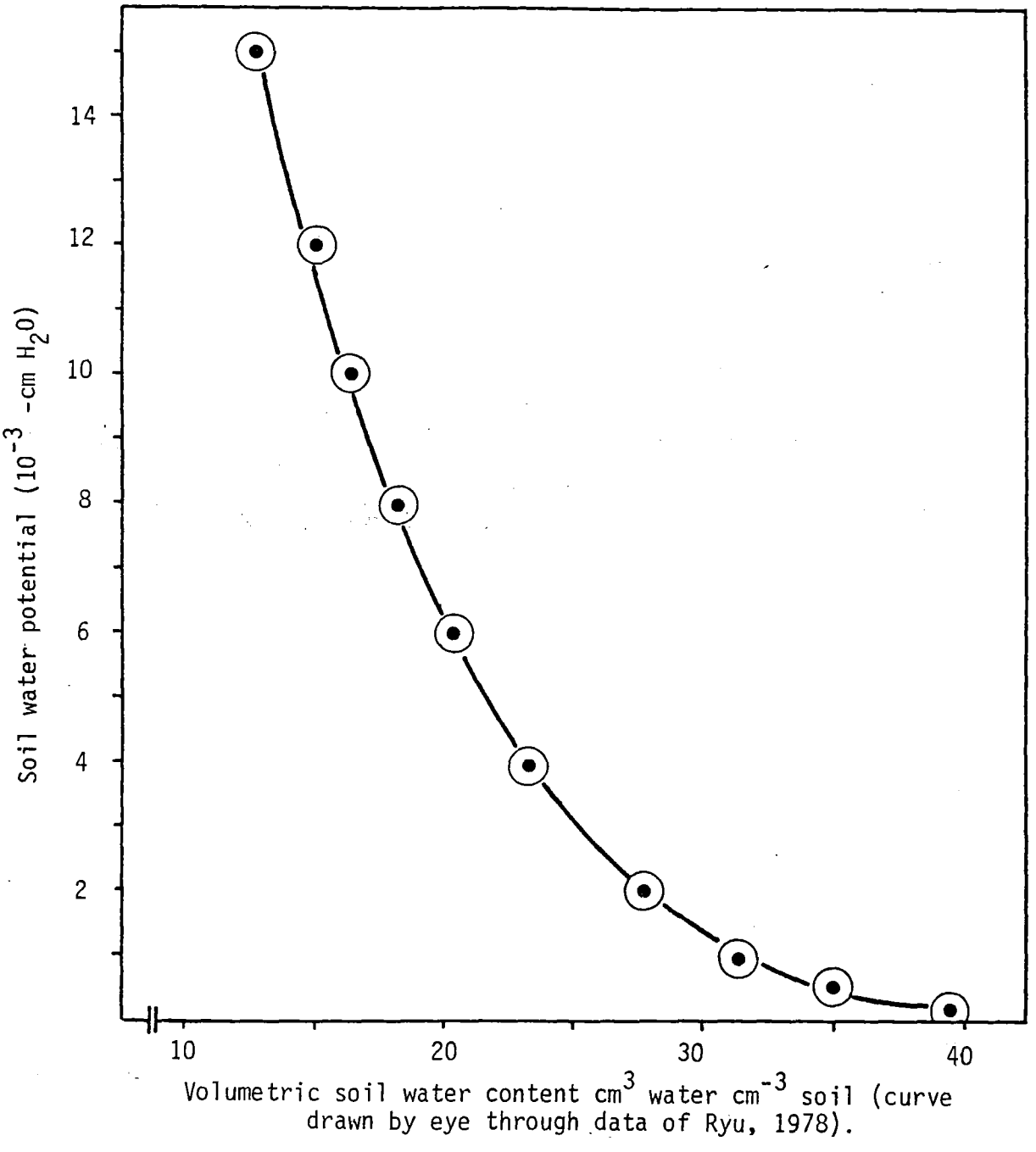
Appendix 5.2: Proportion of dry matter and nitrogen in leaf + stem, pod and pea.

	% of dry matter that is:			% of nitrogen in plant that is:		
	Leaf and stem	Pod	Peas	Leaf and stem	Pod	Peas
Huka	34.8	7.1	58.0	17.4	2.5	80.0
Whero	43.7	7.7	48.7	19.0	2.9	78.1
Partridge	70.7	4.4	24.9	58.7	3.1	38.3
Rovar	35.7	13.0	51.3	15.1	4.0	80.9
Puke	32.2	11.7	56.1	12.9	3.2	83.9
Pania	33.5	11.2	55.3	13.6	3.1	83.3
Tere	27.8	14.6	57.6	10.6	4.6	84.8
SSF	33.4	13.8	52.8	13.2	3.9	82.9
Orthogonal comparisons, variance ratio:						
Cultivars (7 d.f.)	52.9	20.6	40.8	39.4	6.4	41.3
1: Tere vs other cultivars	40.9	30.9	19.3	16.1	21.0	13.3
2: Maples vs other cultivars, not Tere	222.0	62.9	158.0	134.0	3.1	139.6
3: Whero vs Partridge	105.0	8.4	97.5	123.5	0.3	134.2
4: Rovar and Huka vs garden cultivars, not Tere	1.7	8.6	0.0	1.8	0.6	1.7
5-7: Others	0.1	11.1	3.3	0.2	6.6	0.5

Appendix 6.1: The effect of using nodule dry weight per plant as a covariate on ethylene production, at cycles 2 and 3.

Time (NZST)	namomoles C_2H_4 $plant^{-1}$ h^{-1}			
	Cycle 2		Cycle 3	
	Unadjusted means	Means adjusted by covariance	Unadjusted means	Means adjusted by covariance
1700	794	772	1833	1708
2000	618	572	1616	1434
2300	531	528	726*	1121
0200	580	755	589	641
0500	1077	798	966	396
0800	1070	971	534	757
1100	624	682	1112	1012
1400	257	533	1208	1079
1700	479	417	524	961
CV	74.1	58.2	81.4	51.7
VR	1.2	0.81	1.3	2.3
	not significant			

(*0100 h NZST)



Appendix 6.2: Soil water potential for Templeton silt loam soil (0-20 cm).