CHAPTER 8

RHIZOBIUM MANAGEMENT AND NITROGEN FIXATION

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Abstract: Through fixing their own nitrogen, growing lentils offers a substantial saving in the need to use fossil fuels to generate fixed nitrogen for agricultural production. Much of the nitrogen fixed by the lentil crop may then be available for subsequent crops in the rotation as crop residues break down. Estimates for the contribution of N to soils by lentils are generally in the order of $20 \, \text{kg N ha}^{-1} \, \text{yr}^{-1}$. However, the level of N fixed by lentils varies considerably, spatially and temporally in response to a host of environmental and ecological factors. Lentils require effective infection by *Rhizobium leguminosarum* in order to fix nitrogen. This infection process could fail due to a number of reasons including a lack of or inappropriate strains of rhizobia, failure of the plant to invest in the symbiosis, or through altered metabolism. While conditions that suit better growth of the lentil crop will normally enhance the nitrogen fixation of the crop there may also be specific situations in which the fixation process is more sensitive and fixation limits the growth of the crop

1. INTRODUCTION

Although the earth's atmosphere is 80% di-nitrogen (N_2) gas, nitrogen availability often limits agricultural production as this form of nitrogen cannot be utilised by plants. Plants require their nitrogen in a fixed form (e.g. ammonia, nitrate or organic compounds) primarily for use in synthesising proteins and nucleic acids. This fixed nitrogen limitation on global productivity is likely to increase with increasing global demand for food. Nitrogen fixation occurs both biologically and non-biologically. Burns and Hardy (1975) gave an early estimate of global (biological and non biological) fixed nitrogen of about $175 \, \text{Tg N yr}^{-1}$ which has been updated and increased by Cleveland et al (1999) who used over100 pre-existing published estimates of BNF to generate global-level estimates of biological N fixation. Their

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best estimate of nitrogen fixation by biological ecosystems is 195 Tg N yr^{-1}, with a range of 100–290 Tg N yr^{-1}. This compares with 82 Tg N yr^{-1} from N fertilizer production in 1998 (primarily by the Haber-Bosch process and up from 3.5 Tg N yr^{-1} in 1950; Mosier, 2002). There are also lesser non biological inputs from combustion and lightning (Nesbitt et al., 2000).

Biological nitrogen fixation depends on bacterial enzymic reduction of N_2 via nitrogenase. This reduction can occur through the actions of free living, associative or symbiotic bacteria. The reduction to ammonia requires large amounts of energy and reductant. At least 16 molecules of ATP are consumed during the reduction of each molecule of di-nitrogen. However, reduction of NO_3^- by plants also requires energy (or direct use of photosynthetic electrons) at a similar level (Atkins, 1982). Legume root nodules consist of a symbiotic association between bacteria and plants and are a major source of fixed N for crops. Together with other crop systems (e.g. rice) this source of fixed N resulting from human crop production activities provides about 40 Tg N yr^{-1} equivalent to half of the total N applied annually as industrially produced fertilizers (Vitousek et al., 1997). They also point out that both biological and non biological anthropogenic (i.e. resulting from human activity) N fixation has been increasing. While this has greatly increased global food productivity it is also associated with increased environmental damage. The high cost and negative environmental impacts of artificial nitrogen fertilizers gives N fixing legume crops (e.g. lentils) a competitive advantage of being independent of soil nitrogen. Through fixing their own nitrogen, growing lentils offers a substantial saving in the need to use fossil fuels to generate fixed nitrogen for agricultural production. Much of the nitrogen fixed by the lentil crop may then be available for subsequent crops in the rotation as crop residues break down. Estimates for the contribution of N to soils by lentils are generally in the order of 20 kg N ha^{-1} yr^{-1} (Peel, 1998). However, the levels of N fixed by legumes (including lentils) varies considerably, spatially and temporally in response to a host of environmental and ecological factors (e.g. soil N level, water, P, pathogens etc.; Vitousek et al., 2002). For example, lentil plant residues after the harvest of seed varied from 1.0 T/ha to 5.8 T/ha across 3 years and 4 sowing times in Australia (Materne 2003).

2.   PHYSIOLOGY OF N FIXATION IN LENTIL

Lentils form a symbiosis with *Rhizobium leguminosarum* (Humphrey et al., 2001) in which the bacteria are enclosed within root nodules in a specialized form known as bacteroids. Formation of this symbiosis is host specific through the interaction of both plant and bacterial genes (Begum et al., 2001, Smit et al., 1992). The complex structure of nodules exists to enable the functioning of nitrogenase enzymes that are highly sensitive to oxygen damage and the rhizobia which are obligate aerobes. In nodules, internal oxygen is regulated to 3–30 nM and oxygen diffusion is facilitated through intercellular air spaces and an oxygen binding haemoprotein (leghaemoglobin). The bacteroids are enclosed within a host-derived peribacteroid membrane that regulates flows between the bacteroids and their environment. The

plant provides organic acids as a carbon source for the bacteria and the bacteroids export fixed N in the form of ammonia. The lentil nodules then modify the ammonia to export amides to the above ground plant parts in the xylem (Chopra et al., 2002). Detailed energy balances have been carried out for a number of symbioses but not lentil (Atkins, 1984). Rates of fixation by the bacteria are regulated by access to oxygen which may be controlled by the plant and subject to influences from a range of external factors (McNeil et al., 1984). These processes have not been well studied in lentil, however, processes appear to be similar to those in other amide exporting *Rhizobium leguminosarum* infected grain legume species (Begum et al., 2001). The structure and morphology of lentil nodules has been well documented in a light and electron microscopy study by Haswell et al., (2001) where nodules were examined every 1–2 weeks throughout the lifecycle of greenhouse grown plants.

3.   TOTAL FIELD NITROGEN FIXATION

Peoples et al., (1995) summarised publications for % total plant nitrogen gained from fixation (%Ndfa) and total nitrogen from fixation for a range of crops, trees and pastures. They provide a range of values for lentil of 39–87% and 10–192 kg N ha^{-1}. While estimates are not as numerous for lentil as for some other grain legume crops (eg peas) it is possible to get a general consensus of likely N fixation outcomes for lentils (Table 1). Generally %Ndfa is high and similar to other species under

Table 1. Examples of trials from different regions around the world determining lentil fixation percentages and absolute rates either alone or relative to other pulses

Source	location	Lentil		Situation & plant parts analysed		Other pulses*	
		%N from fixation	Total fixed kg N ha^{-1}			%N from fixation	Total fixed kg N ha^{-1}
Peoples et al., 2001	SE wheat belt Australia	79	90	D#	Above ground	75–89	128–160
Shah et al., 2003	NWFP Pakistan	73	68	D	Above ground	75	112
Shah et al., 2002	Peshawa Pakistan	82–96	42–91	D	Above ground	–	–
Rennie & Dubetz, 1986	Alberta Canada	67	84	I	Above ground	79–85	176–216
van Kessel, 1994	Saskatchewan Canada	92	127	I	Above ground	–	–
Moawad et al., 1998	Egypt	53	127	I	Above ground	–	–

D = Dryland, I = Irrigated,
* these differed among experiments but included, mung, lupin, chickpea, pea, faba bean.

both dryland and rainfed conditions in eastern and western hemispheres. However, total N fixed tends to be lower than from other pulse crops in all situations and is likely to reflect the generally lower total biomass yields of lentils compared to other pulses. A survey by Unkovich and Pate (2000) of the quantities of N_2 fixed concluded that the principal grain crop legumes were ranked in the following descending order: soyabean, lupin, field pea, faba bean, common bean (*Phaseolus vulgaris*), lentil and chickpea but total nitrogen fixation will still be dependent on where and how well the crop is grown, and in species comparative studies the relative adaptation of species to the local testing environment. Of particular note also, however, is the paucity of experiments that have also included the below ground fixed N in their calculations. While often referred to, it is generally not measured and there do not seem to be any good values for lentils in the literature. Peoples et al., (2001) assumed an increase in total plant N of 50% (that is 33% of total plant N is below ground at harvest) if below ground N is included across a range of temperate pulses. Use of this figure would substantially increase the rates of N fixed ha^{-1}. Pate et al., (1979) give an estimate of 38% of N in below ground parts of a 70 day old lupin plant. Hood et al., (1990) give a very similar estimate of 37% of total plant N is either lost to the soil or held in roots and nodules in a chickpea plant at harvest. Khan et al., (2002) using four grain legumes, not including lentils, generally produced estimates of between 30 and 50% of total N held in the roots at harvest. This below ground plant N will greatly increase the calculated carryover of N to the next crop assuming 50–80% of the above ground N is removed in harvested product.

Using averaged data from table 1 and FAOSTAT data for 2004 it is possible to make some calculations of global lentil N fixation and carry over to subsequent crops. FAOSTAT indicated a global harvested area of 3.200 million ha of lentils, global production of 3.7 million tonnes and thus average yields of about 1.16 tonnes ha^{-1}. Use of these data and an average from the dryland experiments reported in table 1 of approximately 73 kg N fixed ha^{-1} yr^{-1} or 110 kg N fixed ha^{-1}yr^{-1}including below ground parts, means lentils fix in the vicinity of 0.23 to 0.35 Tg N yr^{-1} with and without below ground N included. However, with an average removal of approximately 65 kg N ha^{-1} yr^{-1} in harvested grain, global carryover of additional fixed N to following crops is relatively low at 8 kg N fixed ha^{-1} yr^{-1} or 45 kg N fixed ha^{-1}yr^{-1} with and without below ground N included. This total net N balance of fixed N ha^{-1}yr^{-1} returned by lentils is relatively low compared to the values of 92–126 kg fixed N ha^{-1}yr^{-1} reported for pea and lupin by Peoples et al., (2001). However, it is consistent with the ranking for fixation among grain legume crops reported by Unkovich and Pate (2000).

4.   NITROGEN ROTATION BENEFITS

Failure to account for below ground fixed nitrogen can partly explain many claims in the literature (e.g. Buddenhagen, 1990) that harvested grain legume crops are likely to have little if any net return to the cropping system. Incorporating the below

ground fixed N estimates a general expectation of nitrogen carryover benefits for a wheat crop following a harvested lentil crop is possible using the total net N balance of 45 kg fixed N ha^{-1}yr^{-1} returned by lentils reported above. It is also important to account for multiyear effects of the nitrogen carryover such as Strong et al., (1986) found when growing wheat for two years after a range of cereal and grain legume crops. In the first year after lentil rather than cereal crops they had an increased yield of 510 kg ha^{-1}(50% increase) with 19 additional kg of N in the wheat crop (105% increase). In the second year there was also a benefit to the wheat crop of 500 kg ha^{-1}(19% increase) with 12 additional kg of N in the wheat crop (27% increase). Soil N analyses allowed them to attribute the additional benefits of the rotation system almost entirely to different levels of available nitrogen. Strong et al., (1986) found a first year yield increase of 19% for wheat following oilseeds and a second year increase of 5%. Taken over the two seasons this indicated the non fixed nitrogen benefit of a broadleaf crop in the rotation accounted for 1/3rd of the total benefit. Similarly, in a more recent series of rotation trials in Canada across 3 years and 2 locations, Miller et al., (2003) found yield increases of 19% for wheat following mustard and 29% (605 kg ha^{-1}) for wheat following lentil with little difference in mean grain protein ($< 1\%$ averaged) among the treatments. Expectations would be much higher using lentil as a green manure crop where the full 110 kg N fixed ha^{-1}yr^{-1} would be returned to the soil. Of course these numbers would be modified by the particular yield and fixation levels of individual lentil crops as well as the rate of breakdown of the stubble, degree of N limitation of the following crop and the presence of other limiting factors in the following crop such as water stress or disease. For example Kirkegaard et al., (2004) reported residual water amounts of 59 mm following wheat against 74 mm following lentils. The benefits of the extra water could easily be confused as a nitrogen benefit. Equally situations have been reported where there is no yield or economic benefit from including lentils in a cereal rotation (e.g. for lentil wheat rotation in Jordan by Badarneh, 2005). In such circumstances other limiting factors may need to be overcome before the benefits of fixed N is realised. Research by ICARDA (1980) in Syria found a 70% increase in wheat yields following lentils but only if the lentils were fertilized with 50 kg P ha^{-1}. In many areas fixed N is supplemented with N fertilisers to achieve an optimum yield, and in these circumstances N is not limiting if the target yield is not reached, primarily due to low water availability, disease or abiotic stresses. It is thus worth looking more generally to see how well this prediction for a nitrogen based rotation response with lentils/cereal is borne out by experimental results.

   Numerous experiments around the world have reported responses of following cereals after lentils rather than 0 N treated cereals. Prakash et al., (2002) reported a 23.4% increase in rice yields (460 kg ha^{-1}yr^{-1}) following lentil rather than wheat in a rabi crop grown in India. Such increases have not been confined to the developing world with Guy and Gareau, (1998) producing a 7% (200 kg ha^{-1}yr^{-1}) increase in Idaho, USA. This crop was of interest because the comparison crop to lentil in the rotation was mustard which would have been expected to give other rotational

benefits exclusive of the N effect. Thus the yield increase is likely to be more directly attributable to N rather than other compounding effects. Miller et al., (2003) also found in Canada that adjusting fertilizer rates for additional N input when comparing mustard and lentil prior to wheat in a rotation effectively eliminated the additional benefit of lentils. Shah et al., (2003) reported 49% gains in maize and sorghum yields in NWFP, Pakistan following lentils. Yau et al., (2003) reported 44% increases in barley yields in Lebanon. Miller et al., (2002) reported 21% increases in wheat yield in Canada. Importantly they also found a 5 kg N ha^{-1}increase in soil N of the lentil stubble in the 0–120 cm depths prior to sowing the wheat as well as an 8% increase in grain protein. This allowed them to directly link yield increases with increased N availability. An even higher increase in soil N (40 kg ha^{-1}) following lentil has been reported from Thailand (Patwary et al., 1989). In other experiments in Canada wheat lentil rotations were found to be the most profitable (including increased wheat protein levels) as well as having a 19% reduction in CO_2 emissions (primarily due to reduced N fertilizer inputs) making the option the most sustainable of those trialled in the experiments (Zentner, 2002). These data also indicate there are other potential benefits in rotation systems from the N fixation including reduced fertilizer costs, increased wheat quality and reduced greenhouse gas emissions.

Other, in crop, differences may also confound the nitrogen benefit. The general health of the crop and productivity may increase or decrease growth and thereby alter the level of fixation and benefits of the lentil nitrogen fixation in the rotation. A comparison of the dryland (mean of 73 kg N fixed ha^{-1}yr^{-1}) and irrigated (mean of 113 kg N fixed ha^{-1}yr^{-1}) fixation rates in table 1 indicates a substantially greater fixation in the irrigated crops (60 kg N fixed ha^{-1}yr^{-1} if below ground fixation is included).

There is a need however to be reasonable in the expectations of benefits from nitrogen fixed by lentils. As Unkovich and Pate (2000) have indicated lentils are relatively low on the scale compared with most other grain legume crops. However, compared to many pasture systems lentils are very low. Peoples et al., (2001) found on average 30% to 80% higher levels of N fixation in southern Australian vetch, lucerne and subterranean clover pastures when compared to lentils. In southern Lebanon Yau et al., (2003) found vetch in a rotation had almost twice the benefit of lentils on barley production. Looking globally Peoples et al., (1995) found reports of a maximum of 192 kg N fixed ha^{-1}yr^{-1}for lentil compared with maximum annual pasture fixation rates of 386 (lucerne), 373 (red clover), 380 (desmodium) and 291 kg N fixed ha^{-1}yr^{-1} (white clover). Vigil and Nielsen (1998) compared the economic value of replacing a clean fallow with a lentil green manure crop in a water limited environment in Colorado, USA. They found yield reductions due to water limitations of up to 1050 kg ha^{-1}. Their conclusion was thus, "At current fertilizer costs, legume N (in this system) was too expensive to be considered a reasonable alternative to chemical fertilizer". Pikul et al., (1997) also point to another potential problem with green manuring lentils. They found yield for spring wheat grown in Montana USA, was 25% less following a lentil green manuring treatment. This was in spite of no differences in water availability in the treatments

and no differences in most soil parameters. The exception was soil NO_3^- N was 35% less in green manured treatments in spite of the potential for a 66% increase due to the green manure. They concluded slow breakdown of the residues was preventing the N from becoming available. This situation is quite different from the data of Strong et al., (1986) in near tropical Queensland and Evans et al., (2003) in the SW slopes of NSW, Australia where breakdown in the intervening season is high. Evans et al., (2003) found wheat yield increases of up to 930 kg ha^{-1} in the first season after a green manure and 370 kg ha^{-1} in the second season. While lentils were not in their experiments they achieved wheat yield increases following grain pea of up to 520 kg ha^{-1}. The slow release of N resulting in greater available N late in the season can also be beneficial for increasing protein concentration in cereal grain. As an alternative, late applications of N fertilisers have also been used in cereals to increase protein (Woodard and Bly, 1998).

5. RHIZOBIAL LIMITATIONS ON LENTIL N FIXATION

Lentils require effective infection by *Rhizobium leguminosarum* (Humphrey et al., 2001) in order to fix nitrogen. This infection process could fail due to a number of reasons including; no or few rhizobia in the soil, inability of the rhizobia to survive in the soil due to intrinsic limitations, inappropriate strains of rhizobia in the soil, efficient rhizobia being out competed by less efficient/effective soil rhizobia, failure of the plant to invest in the symbiosis either through lack (eg early in the seedling stage) of time or resources, or through altered metabolism (e.g. towards nitrate reduction in soils high in available nitrogen). In any of these situations fixation may be reduced and the grain yield and or rotational value of the lentil will be reduced.

Attempts to overcome rhizobial limitations of fixation have been conducted for a considerable period (e.g. McNeil et al., 1981) and have typically been of the following form. Potential inoculating bacteria are selected for increased growth and performance, relative to uninoculated controls, of target legume lines or species in sterile media with or without additional limitations (e.g. acidity). The performance of the bacteria (survival, growth e.g. Slattery and Pearce, 2002) may also be tested in soils or media with intrinsic limitations such as high pH or high salinity. Responses to inoculation are then attributed to the nitrogen fixation capacity of the inoculating bacteria. Typically these bacteria are then also screened in the field for real effects on lentil production by addition of the inoculating bacteria (with or without other inputs such as *Azospirillum* { El-Komy and Wahab, 1998} or N and P {Shah et al., 2000}) to field plots. Yield and various fixation parameters (e.g. nodule mass/ number or various methods for directly measuring fixation{McNeil, 1982b, Tewari et al., 2004, Hardarson and Danso, 1993}) are then measured and increases attributed to fixation. Care needs to be taken in interpreting these results for a number of reasons. Firstly, strong agreement between the greenhouse and field experiments are not always evident (Slattery and Pearce, 2002). Second, many of the negative results may not be reported. Additionally, however, several other observations are

needed to gain good interpretation of these trials. These are; the level of rhizobia initially in the soil (Slattery and Pearce, 2002), the percentage occupancy of nodules by the inoculating bacteria (McNeil et al., 1983) and the survival of the inoculating bacteria in the soil (Slattery and Coventry, 1999).

Many soils will not have grown lentil crops previously or recently in many production areas. Particularly if the soils also suffer from a constraint such as acidity, waterlogging or high salinity *Rhizobium leguminosarum* populations may be low and there may be significant responses in the field to inoculation with appropriate strains. In low pH (4.4−5.0) soils in the field in Victoria Slattery and Pearce, (2002) identified low populations of *Rhizobium leguminosarum* and using acidity tolerant bacteria as inoculants achieved nodule number increases of 8 fold, grain yield increases of almost 50% and 25% increases dry matter for 13 test strains relative to uninoculated field controls. The three parameters were reasonably well correlated. However, some strains were very ineffective and gave no or negative responses. Dilworth et al., (2001) have reviewed the selection of rhizobia for acid soil tolerance. Rai and Singh (1999) selected salt tolerant rhizobia and found them successful in increasing yield in salt tolerant lentil genotypes on sodic soils. Similarly Shah et al. (2000) found in a Pakistan soil low in N, P and rhizobia that lentil yield increases of 77% (393 kg ha^{-1}) were achieved via inoculation. They also found 71–95% occupancy of the nodules by the inoculant bacteria confirming the direct effect of the inoculation. In a rhizobium deficient soil in Chile Herrera and Longeri (1985) achieved 85 fold increases in nodulation, plant dry weight increases of 341%, seed yield increased by 114% from 530 kg ha^{-1} to 1150 kg ha^{-1} and seed protein yield by 132%. In situations where soil rhizobia are more common yield increases are often not as great. Khurana and Sharma (1995) evaluated several lentil cultivar by strain treatments in the Punjab in India. They found interactions among the treatments with an average yield response to inoculation of 16%, however, they do not provide data on the nodule occupancy by strains. In other trials in India Dhingra et al., (1988) only found significant yield increases in 3 out of 5 years. In South Eastern Australia no increase in yield was found after inoculating lentils grown on fertile, alkaline soils that had a long history of host crops and naturally high levels of rhizobia (Slattery and Pearce, 2002).

The interaction between the competitive ability of the inoculant strains in the soil and their ability to enhance yields is well demonstrated by Moawad et al., (1998) in trials in Egypt. They found less than 10% of field isolates to be high in their effectiveness. In field trials they obtained an average of 17% occupancy by inoculant lines with a non significant average 5% yield increase. This represents a 2.9% increase in yield for each 10% increase in nodule occupancy. However, their data for berseem clover for which the field isolates were equally ineffective showed the potential of inoculation. They achieved an average of 66% occupancy by inoculant strains and an average 38% increase in yield. This represents a 5.8% increase in yield for each 10% increase in nodule occupancy. Shah et al., (1996) also found correlations in Pakistan between nodule occupancy and response to inoculation. They found higher native populations of *Rhizobium leguminosarum* in soils

which had recently or frequently grown lentils. Kumar and Chandra (2005) found differences in application methods resulted in different levels of nodule occupancy. When nodule occupancy by the introduced strains was high they also found significant yield increases. In a meta analysis of soybean field inoculation trials which had determined nodule occupancy done up to 1984 (McNeil, pers. comm. NifTAL, Hawaii, 1984) there was a significant regression between nodule occupancy by inoculant bacteria and yield. For each 10% increase in nodule occupancy by the inoculant strain (USDA 110) soybean yields increased by 1%. The data from lentil for nodule occupancy would seem to support a similar, but potentially higher, level of response.

A number of authors have also looked at improving fixation by combined addition of rhizobia and mycorrhiza or soil bacteria. The latter in an attempt to increase P availability which is in high demand by legume crops. Badr El-Din and Moawad (1988) found a synergistic response to combined inoculation in a soil low in both mycorrhiza and rhizobia consistent with responses of N fixation for improved P availability. However, successful commercial scale establishment of mycorrhiza on plants in the field is extremely difficult. El-Komy and Wahab, (1998) found increases in nodulation, yield and fixation for lentil simultaneously inoculated with *Azospirillum* and rhizobia. At this stage there do not appear to be commercial applications of these dual inoculation technologies, however, there may be some applications in the future if they prove reliable in achieving infection and/ or high populations of both inoculants in the field.

The general indications from these experiments are therefore that responses to inoculation are common in lentils but that a primary limitation is the ability to out compete existing, less efficient, bacteria in the soil. It is thus only when suitable efficient bacteria can achieve high levels of nodule occupancy that responses are achieved.

6.   OTHER ENVIRONMENTAL LIMITATIONS ON N FIXATION

While it has already been stressed that conditions that suit better growth of the lentil crop will normally enhance the nitrogen fixation of the crop (e.g. irrigation in Table 1) there may also be specific situations in which the fixation process is more sensitive and fixation limits the growth or %Ndfa of the crop. Some of these that have been suggested are: high soil available N, micronutrient limitations (B, Mb, Co, Fe), Phosphorous limitation, limited N availability at specific stages, and water limitations (either drought or waterlogging). However, in a fixation limited crop, growth will also be limited and it is difficult to separate the specific effects on fixation from effects on growth limitation. Numerous studies exist in which various combinations of minor and micro nutrients have been added to lentils in deficient soils and responses have occurred (e.g. Sinha, 1994), however, these will be dealt with elsewhere. There are, however, some situations where it is specifically possible to separate the effects. For example, significant levels of Mo and Co are only required by the fixation system, and deficiencies would inhibit N fixation

without affecting growth in soils where N was non limiting (Lucinski et al., 2002) or where a transient stress destroys N fixation capability (nodule death) and the plant either cannot re-establish nodules (eg a near senescent plant {McNeil and LaRue, 1984} or must significantly reinvest in their structure). Alternatively small amounts of added N have been suggested in the form of starter N to assist in establishing nodules, or as leaf applied N to delay the onset of nodule senescence (McNeil and LaRue, 1984).

6.1.   Micronutrient Limitations

Co, Mo, and Fe are all involved in N fixation and may be required specifically by the fixation process in greater amounts than needed by the plant. Cu and Mn are involved in nitrogen metabolism. Mo limitations on legumes has been widely observed and generally overcome in southern Australian soils (Donald and Prescott, 1975). Specific responses (11% yield increase) to Mo seed application by lentils have also been observed (Golubev and Lugovskikh, 1974) and fertilisation is generally needed to overcome the problem. More recently work by Gahoonia et al., (2005) has indicated that lentil lines exist with different root morphologies that may be better able to scavenge micronutrients from the soil and give 10–20% yield increases. Lentils have been shown to respond to Co in pot experiments (Sarada and Polasa, 1992) but levels needed are very low 0.15 ppm. Lentil genotypes have been shown to differ in iron absorption and translocation and fixation has responded positively to greater absorption (Rai et al., 1984). However, evidence does not exist for a specific fixation dependant Fe effect in lentil.

6.2.   Phosphorus Limitations

Phosphorus has been suggested to be more limiting for N fixers, particularly agricultural grain legumes and pasture species, than other species (Vitousek, 2002, Robson, 1983). Thus it seems reasonable that P limitation may lead to reductions in fixation greater than simply reductions in plant growth. Badr El-Din and Moawad, (1988) have shown responses of fixation to mycorrhizal inoculation presumably due to enhanced P uptake. There are numerous reports of yield increases and N fixation stimulation with application of P to lentils (e.g. ICARDA, 1980, Shah et al., 2000). Badarneh (1995) reported that %Ndfa increased in lentils grown under water stress when P was added. This suggests that the P was specifically benefiting the fixation process. It is clear that lentils need P for both fixation and growth and probably that the effects on fixation are direct as well as by reduction of overall plant growth.

6.2.1.   Nitrogen interactions

Research on the use of starter nitrogen (small amounts at planting to establish the fixation process) have generally found advantages in greenhouse experiments but no or little advantage in the field (Turay et al., 1991). This is consistent with

most reports for other grain legume crops. McNeil and LaRue, (1984) reported that small doses of applied nitrogen could inhibit fixation and lead to loss of fixation capacity and yield reductions or stimulate fixation when applied to leaves late in the plant life cycle and stimulate yield in soybeans. Lentils like all other legumes have their fixation rates suppressed by available soil nitrogen (Bremer et al., 1988). The ecological basis for this is simply to prevent surrounding competing vegetation gaining access to the N and hence suppressing legume growth. Nitrate use and N fixation are energetically similar in their requirements. However, when nitrate is directly assimilated in the leaf it may directly access photosynthetic electrons and thus require less carbohydrates. In addition if the Rhizobium are inefficient (e.g. Hup- strains) energy costs of fixation may increase (Atkins, 1982) relative to nitrate assimilation. Typically fertilization of lentils with nitrogen does not make any yield benefits (Dutta, 1985, Islam and Afandi, 1980) provided the crop is adequately inoculated either artificially or naturally. However, the %Ndfa may fall substantially under elevated soil N. Selection with or without mutation may produce symbioses effectively uncoupled from nitrate inhibition (Carroll et al., 1985) though this has not yet been done for lentils. Minor gains are possible by selecting rhizobia which perform better in the presence of elevated soil nitrate (Saxena et al., 1996).

6.2.2.   *Salinity and sodicity interactions*

Maher et al., (2003) have demonstrated it is possible to select salt tolerant lentils and that their growth and fixation is enhanced. Rai and Singh (1999) found inter-actions exist between rhizobium strain, lentil cultivar, yield and nitrogen fixation in lentil. They suggested that the salt stress was directly affecting the perfor-mance of the symbiosis and a combined selection for salt tolerant genotypes and bacteria could increase lentil yields (up to 28% above salt tolerant genotypes alone, substantially more relative to salt intolerant genotypes) fixation and fixation enzyme activities.

6.2.3.   *Water availability*

Potentially waterlogging can cause reductions in fixation and nodulation and if severe enough lead to loss of nodules. McNeil and Slatter (pers comm.. Nepal 2003) observed severe loss of nodules after a waterlogging event in some, but not all, lentil lines growing in a field trial in Nepal. This suggests the possibility that part of the effect of waterlogging on lentils could be the effect on N fixation and the subsequent energy cost of re-establishing nodules. Drought stress may also act in this way. Athar (1998) demonstrated differences in rhizobium survival in droughted soils in Pakistan and this would interact with nodulation and fixation under those conditions. Badarneh (1995) found that %Ndfa was lower under drought conditions than well watered conditions suggesting more adverse effects of drought on lentil fixation than total productivity. Bremer et al., (1988) also found using ^{15}N approaches that the proportion of N assimilated from the atmosphere declined with increasing soil nitrate levels and increasing drought stress. This may mean that the

symbiosis was preferentially sensitive to drought stress or may simply be consistent with the concept that soil N is preferentially used first. Thus under drought stress or any other stress the reduced growth means it takes longer to overcome the soil available N limitation on nitrogen fixation.

6.3.   Potential to Breed for Increased N Fixation

The concept of breeding both rhizobia and grain legumes (e.g. by reducing fixation sensitivity to soil N, of rhizobia {McNeil, 1982a} or soybean plants {Carroll et al., 1985}) for increased nitrogen fixation both to increase pulse crop yields and increase the rotation benefits described above has a long history with a breeding program in place in CIAT in the early 1980's (Graham, 1981). The area has been well reviewed by Herridge and Danso (1995). Their conclusion was that only a relatively few cultivars have been released with a specifically improved capacity for nitrogen fixation. For lentil, which are typically grown on low N soils with high %Ndfa (Table 1) it is likely that selection for plant growth (and indirectly plant yield), particularly under adverse conditions, will produce better adapted, higher yielding, higher fixing plants. This is particularly the case when selecting for tolerance to conditions that specifically limit N fixation more than overall plant growth (e.g. soil N level, available P) indicated in section 6 above. Hobson et al., (2004) have produced substantial increases in lentil photosynthesis rate and green matter accumulation, and thus N fixation using boron tolerant lines in high boron soils.

It would be expected that increasing the total levels of biomass through agronomy or breeding would also increase levels of N fixation. In lentil, earlier sowing increased total biomass and seed yield in Australia but improvements in disease resistance are necessary to enable early sowing without risk of yield and quality losses (Materne 2003). High biomass lentil varieties have also been released from ICARDA for the developing world (Sarker, et al., 2004). However an increase in harvest index will continue to be a focus of breeding programs to increase seed yield and thus result in a greater removal of fixed N from the system. Lentil can effectively convert biomass to seed, achieving harvest indices of up to 50% (Materne 2003).

Levels of nitrogenase activity have been shown to decrease less rapidly under soil sodicity in an improved (PL-406) lentil line than in a local cultivar (Singh et al., 1993) and this has been suggested as a reason for the superiority of Pl-406. However, the cultivar was not specifically bred for increased N fixation. A second breeding option is the specific selection for N fixation by plants under defined conditions. This has been done in soybeans for tolerance of high soil N (e.g. Carroll et al., 1985) as well as selections for high nodulation in chickpeas (Rao et al., 1993). However, there does not appear to be any successful releases of lentil lines bred in this manner. A third option is, the selection for, and better inclusion in the symbiosis, of more efficient (e.g Hup-, see Atkins, 1982), and more effective

rhizobia. There have been limited gains also in this area which are covered in the earlier section (5) on rhizobia.

7.   CONCLUSIONS

Lentils occupy a minor position in global N fixation relative to other major crops such as soybean and world N fertilizer production. They generally have fixation rates towards the lower end of those achieved by pasture and grain legume species. However, where lentils are grown for profitability in their own right their N fixation can have significant beneficial effects on their own yield and that of non N fixing rotation crops. Lentil fixation is generally highest under conditions that promote good growth of the crop in total. However, in situations where field populations of rhizobia are low or where inoculant bacteria can achieve high nodule occupancies they can achieve significant responses to inoculation. There are also specific environmental conditions which are not favourable to fixation that can limit the %Ndfa and total fixation by the crop. While economic data indicate that lentil fixed N is not a commercially competitive fertilizer in most cropping situations, when N carryover from lentil crops results as a by-product of growing the crop it can have significant advantages, especially in improving cereal quality. Thus when growing lentils as a crop it is worthwhile managing the crop to achieve maximum fixation and thereby maximum total productivity and profitability.

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