

# Mediating mutualisms: farm management practices and evolutionary changes in symbiont co-operation

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## Summary

1. Root symbionts (rhizobia and arbuscular mycorrhizae) are often assumed to increase agricultural productivity consistently. However, rhizobial and mycorrhizal strains vary in effectiveness, resulting in symbiotic associations that range from parasitic to mutualistic.

2. The extent to which different farming practices mediate evolutionary changes along this continuum of symbiont effectiveness is rarely discussed. However, evolutionary theory suggests that (i) fertilizer use will favour parasitism unless host-plants impose sanctions against less-effective mutualists; (ii) tillage will have contrasting effects because it decreases within-plant symbiont relatedness but also decreases the risk that mutualism will benefit future competitors; (iii) crop rotation can act as a selective agent against dominating symbiont genotypes; and (iv) rhizobial inoculation adds beneficial strains to the soil but may increase the frequency of mixed nodules that allow parasitic strains to escape host sanctions.

3. However, the existing empirical data are inadequate to test our predictions thoroughly. Changes in species composition have been documented as a result of management practices, but evolutionary changes in symbiont effectiveness are difficult to detect. Therefore, a major aim of this study was to stimulate research that will assesses directly changes in symbiotic effectiveness as a function of management practices.

*Key-words:* arbuscular mycorrhizae, crop rotations, inoculation, parasitism, rhizobia, symbiosis, tillage.

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## Introduction

Studies of mutualisms in agriculture have traditionally focused on the potential of nitrogen ( $N_2$ )-fixing bacteria (rhizobia) and arbuscular mycorrhizae (AM) to improve crop yields. At least 70 million metric tons of nitrogen per year are produced by legume symbioses (Brockwell, Bottomley & Thies 1995), often contributing 100–300 kg N ha<sup>-1</sup> year<sup>-1</sup> (Burns & Hardy 1975; Paul 1988; Peoples, Herridge & Ladha 1995). Arbuscular mycorrhizae are considered valuable components in most agricultural systems due to their role in plant nutrition and soil health (Bethlenfalvai & Linderman 1992). For decades, crop scientists have attempted to maximize agricultural productivity by improving the effectiveness of these symbiotic relationships

(Roughly, Blowes & Herridge 1976; McLoughlin, Hearn & Alt 1990). However, manipulation to increase field-scale nitrogen fixation and phosphorous transfer continues to be met with limited success (Hamel 1996; van Kessel & Hartley 2000).

Symbiotic effectiveness describes the benefits to the host derived from the symbiotic association. The more effective a symbiont, the more benefits it provides to its host. Different genotypes of rhizobia and mycorrhizae vary in the benefit that they provide their host-plant (Thrall, Burdon & Woods 2000). For example: (i) in a study of the effectiveness of association between native rhizobia and *Acacia* species, Burdon *et al.* (1999) found that some rhizobial strains resulted in plants only 10% the size of those inoculated with the best strains; (ii) Denton *et al.* (2000) surveyed 61 Australian pastures and found that in 60% of these sites, the mean level of nitrogen fixation was less than half that of reference strains; (iii) of 150 mycorrhizae isolates tested on cassava *Manihot esculenta*, 40% were shown to be either ineffective or have marginal effectiveness (Howeler,

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Sieverding & Saif 1987). The effectiveness of some strains of mycorrhizae may depend on host genotype or soil conditions (Krishna *et al.* 1985; Manske 1989). However, symbiotic effectiveness should not be confused with the host-specificity of a strain. We define effectiveness as the benefit derived from the symbiont after the relationship has been formed. Specificity refers to the ability to form the relationship, not the benefits derived (Chanway, Turkington & Holl 1991).

The evolutionary dynamics underlying the relative success and abundance of strains with various mutualistic/parasitic tendencies is rarely discussed in agronomic studies. However, this is a fundamentally important topic, as different agricultural practices may favour more (or less) parasitic strains, leading to: (i) a physiological change in the effectiveness of rhizobia or mycorrhizae; or (ii) an evolutionary change in relative abundance of these strains. If we understand how agricultural practices affect the relative Darwinian fitness of different strains, then we could employ agronomic practices that would favour an increase in the relative abundance of the better strains. Practical applications of this information will depend on balancing long-term benefits derived from better mutualists with any short-term costs.

In recent years, understanding how selection pressures favour the evolution of mutualistic and parasitic tendencies has been the focus of much work by evolutionary biologists and ecologists. A large body of theory has been developed aiming to predict the factors that influence shifts in symbiotic functioning. These include very general models on the evolution of mutualists/parasites (Axelrod & Hamilton 1981; Bull & Rice 1991; Frank 1994, 1995, 1996, 1998; Ebert & Herre 1996; Doebeli & Knowlton 1998; Hochberg *et al.* 2000; van Baalen & Jansen 2001) as well as models developed for mycorrhizae and rhizobia (Jimenez & Casadesus 1989; Olivieri & Frank 1994; Simms & Bever 1998; Parker 1999; Bever & Simms 2000; West *et al.* 2002; West *et al.* 2002b). A crucial point in all these models is the recognition that symbionts face trade-offs between using or storing resources for their own reproduction vs. providing resources to their host. Symbionts that sacrifice their own fitness in favour of that of the host will be displaced by symbionts that maximize their own inclusive fitness. Therefore, mutualism will persist or increase only when some factor favours symbionts that contribute to their host-plants. Host-plants that provide less resources to or completely discontinue interactions with symbionts found to be less effective (termed host-plant sanctions; Denison 2000) could be one such factor.

Our major aim in this study was to demonstrate that the predictions of evolutionary theory can be applied to agricultural systems. We show how existing theoretical models can be used to predict how symbiotic effectiveness will be selected to increase or decrease under certain agricultural practices. Specifically, we considered the consequences of (i) fertilization regimes,

(ii) tillage management, (iii) crop rotations and (iv) symbiont soil inoculation. We argue that specific agronomic practices may increase the reproductive success (proliferation) of parasitic rhizobia/mycorrhizae strains relative to more mutualistic/beneficial strains, and so lead to a long-term deterioration in the composition and functioning of the symbiont communities.

Our second aim was to examine the extent to which existing data on rhizobia and mycorrhizae can be used to test our theoretical predictions.

### Variation in resources and productivity: effects of increased fertilizer use

High productivity, within environmental and economic constraints, is the ultimate goal of modern agricultural systems. Global utilization of N fertilizer has increased sevenfold since 1960 (Peoples, Herridge & Ladha 1995); P fertilization use is forecasted to increase anywhere from 1.6-fold to 3.4-fold by 2050 (Tilman *et al.* 2001). It is well appreciated that in the short term, high fertilizer use will decrease the benefits derived from root symbionts. These effects are due to physiological rather than evolutionary change, and they do not necessarily involve decreases in the symbiont's N or P contribution per unit C invested by the host-plant, i.e. costs might decrease proportionally. For instance, addition of N fertilizer can lead to lower production of nodules in legumes (Rubio Arias *et al.* 1999; Vargas, Mendes & Hungria 2000) as well as lower N<sub>2</sub> fixation rates per nodule (Denison & Harter 1995). Mycorrhizal formation may be radically reduced in high P soils (Hayman 1975; Jensen & Jakobsen 1980; Hicks & Loynachan 1987).

However, we argue that there are a number of reasons why high resource availability may also favour a long-term (evolutionary) change in symbiont effectiveness. Changes in effectiveness could result from the following.

Increasing nutrient availability to plants can lead to a decrease in resource allocation to the roots (e.g. fewer nodules are initiated by legumes and less root area is colonized by mycorrhizae under high nutrient availability; see the references above). As opportunities for mycorrhizal infection decrease under high fertilization regimes, competition for these opportunities increases, favouring the proliferation of more aggressive strains (Johnson *et al.* 1992; Scullion, Eason & Scott 1998). It has been suggested that strains characterized by increased aggression towards conspecifics in high fertility soils are unlikely to be good mutualists (Johnson 1993; Graham, Drouillard & Hodge 1996; Johnson, Graham & Smith 1997). Investment in antibiotic production, for example, can come at the expense of symbiotic performance, as seen with rhizobia (Goel, Sindhu & Dadarwal 1999).

Increases in soil resource availability mean that resources available to symbionts from their host (e.g. photosynthate) are less dependent upon the symbiont's contribution. Models developed specifically

for rhizobia, which could also be applied to mycorrhizae, suggest that, in the absence of host sanctions, this decreased dependence on symbiont contribution would select for rhizobia with a lower level of N<sub>2</sub> fixation (West *et al.* 2002). This is because nutrients acquired via symbionts have a smaller effect on plant photosynthesis rates and therefore the overall level of circulating resources available to the symbionts.

However, at least in rhizobia, if host-plants preferentially supply more resources to nodules containing more beneficial mutualists, as has been suggested (Udvardi & Kahn 1993), this could reduce, eliminate or possibly even reverse the effects of fertilizer on the evolution of microsymbionts. Conclusive evidence for such plant sanctions is still lacking, although there is a strong theoretical argument that they are essential to the evolutionary stability of mutualism within multiply infected hosts (Denison 2000; West *et al.* 2002).

By reviewing existing studies that focus on the effects of long-term fertilizer application, we can begin to assess the validity of these predictions. Recent studies have shown that application of fertilizer can lead to long-term changes in mycorrhizal functioning, although it is difficult to determine if this is due to evolutionary changes within a species, by displacement of one species with another or by both. Johnson (1993) studied changes in mycorrhizal communities after an 8-year period in fertilized and unfertilized plots and found (i) test plants colonized by mycorrhizae from the fertilized treatment were smaller with reduced inflorescences; (ii) roots inoculated with mycorrhizae from the fertilizer treatments were dominated by vesicles (resource storage structures, suggested to be indicative of more parasitic behaviour) rather than arbuscules (nutrient transfer structures); and (iii) mycorrhizal species composition between the two treatments was significantly different. The last result (iii) is in agreement with other studies that have found shifts in mycorrhizal species composition but not necessarily evolutionary change within a species (Thomson, Robson & Abbott 1992; Gryndler & Lipavsky 1995). It is unclear if the species-composition data of Johnson (1993) also predict evolutionary trends. More details of the fungal community dynamics are needed to assess whether this species shift involved interactions between mycorrhizal effectiveness and soil fertility, or whether the less effective species happened, coincidentally, to have a competitive advantage in high P soils. Changes in the abundance of fungal structures in the fertilized and unfertilized plots suggest a shift but not necessarily an evolutionary change. Adaptation to high P fertilization within mycorrhizal populations has been documented (Cooper 1978; Jasper, Robson & Abbott 1979; but see Porter, Abbott & Robson 1978) but under much extended periods of time (48 years and 26 years).

The rate of evolution of mycorrhizae in response to P fertilizer would depend on both the degree of genetic variability and the strength of the selection pressure (i.e. effects of soil fertility and its importance relative to

other factors that influence fitness). Kahiluoto, Ketoja & Vestburg (2000) studied the impact of 23 years of cumulative P fertilization on mycorrhizal infectivity and effectiveness using inoculum (whole soil) derived from plots under different P fertilization histories to run bioassays of mycorrhizal communities. Their results were consistent with the hypothesis that cumulative fertilization decreases mycorrhizal effectiveness in flax, even under moderate amounts (45 kg P ha<sup>-1</sup> year<sup>-1</sup>) of annual P fertilizer. However, as whole soil inoculum was used these results do not exclude the possibility of plant growth differences due to other soil micro-organisms. Similarly, Scullion, Eason & Scott (1998) found evidence that inocula (spores only) derived from soil under organic management regimes (with lower available P) were more effective in increasing plant growth than inocula derived from conventional farms (varying with season, time since cultivation). However, this trend disappeared when the conventional and organic farm soils had comparable levels of P fertility (Scullion, Eason & Scott 1998).

To our knowledge, no studies have directly documented the effects of long-term fertilizer use on the evolutionary stability of rhizobial mutualism. In comparing communities practising traditional agriculture (low levels of soluble N) and high input agriculture (high levels of soluble N), Souza *et al.* (1997) found that high input agricultural practices modified the genetic structure and decreased nodule activity (as measured by colour of nodules; red usually indicates active fixation) of rhizobia in the field. However, comparisons were made *in situ* and could be attributed to various environmental conditions (including short-term physiological effects of soil N), cropping patterns, herbicide use, etc., rather than evolutionary changes in rhizobia. In a general survey of Australian pastures, Gibson *et al.* (1975) found no correlation between mean effectiveness of rhizobial isolates and annual rate of fertilizer application. Similarly, symbiotic effectiveness of rhizobial populations (whole soil inoculum) derived from 18 different pastures was not related to total soil N (Quigley *et al.* 1997).

The trends from these limited data suggest that cumulative fertilizer use may have no detectable effect on the evolution of rhizobial effectiveness. Three possible justifications for this may exist: (i) the interaction between host sanctions and soil N may result in no soil N effect on the evolution of mutualism, as suggested by West *et al.* (2002); (ii) gene flow from repeated introduction of effective strains might have overpowered natural selection; and (iii) there is simply a lack of studies that have systematically compared symbiotic effectiveness of rhizobial isolates over N fertilization gradients *ex situ*.

### Spatial structuring: effects of tillage regimes

Spatial structuring is an important component of natural ecosystems as it may contribute to selection

pressures mediating pathogenicity (Frank 1998). In natural ecosystems, bacterial and fungal dispersal may be limited by soil structure (Wadisirisuk *et al.* 1989; Chanway, Turkington & Holl 1991; Bever *et al.* 1996; Bever & Simms 2000). In contrast, agricultural systems, many of which are characterized by frequent tillage and soil disturbance, lack strong spatial structuring in the soil. We suggest that tillage may modify selection pressures for hosts and symbionts in two opposing ways.

First, tillage may decrease relatedness between symbionts within a plant (more distinct lineages per plant) and also decrease relatedness between symbionts in the plant and symbiont conspecifics in nearby soil. Reducing relatedness at both these scales increases the potential for competition, which would favour more parasitic strains (Frank 1994, 1996). For example, in rhizobia the benefits of increased N<sub>2</sub> fixation through increased plant growth (higher circulating resources and/or root exudates) would be shared with unrelated non-mutualists leading to a reduction in any 'kin selected' advantage of N<sub>2</sub> fixation (Bever & Simms 2000). This would select for lower levels of N<sub>2</sub> fixing. Similarly, tillage could lead to decreased relatedness between mycorrhizal strains both within and between plants by decreasing the spatial structuring of the soil, decreasing kin selection for mutualism.

Second, tillage may decrease competition between related strains that had infected the same host-plants by dispersing them to different areas (West *et al.* 2002). Competition between relatives reduces the kin-selected benefit of N<sub>2</sub> fixing as described above: put simply, helping relatives is less beneficial if their fitness increase comes at a cost to yourself or your other relatives (Taylor 1992; Queller 1994; West *et al.* 2001; West, Pen & Griffin 2002; Griffin & West 2002). Consequently, tillage may also select for higher levels of N<sub>2</sub> fixation in rhizobia or increased nutrient transfer in mycorrhizae.

The net effect of these two opposing forces will be difficult to predict. None the less, they clearly emphasize that soil tillage may have long-term consequences for symbiont performance.

In six out of eight relevant studies, conventional tillage led to a decrease in N<sub>2</sub> fixation (or in parameters plausibly linked to N<sub>2</sub> fixation) compared with systems with conservation tillage (Rennie *et al.* 1988; Hughes & Herridge 1989; Wheatley, Macleod & Jessop 1995; Dalal *et al.* 1997; Matus *et al.* 1997; Höflich *et al.* 1999; but see Doughton, Vallis & Saffigna 1993; Horn *et al.* 1996). For example, Höflich *et al.* (1999) showed a significant increase of leghaemoglobin and nodule number per plant under a conservation tillage treatment, relative to an otherwise identical system with conventional tillage. Rennie *et al.* (1988) found superior yield and N<sub>2</sub> fixation of soybeans in zero-till plots grown in paddy rice rotations. Unfortunately, these and other studies are *in situ* comparisons of N<sub>2</sub> fixation and the differences found are not necessarily due to evolutionary changes in the rhizobia. However, in the one

case where symbiotic performance was directly tested in the glasshouse, rhizobial isolates derived from zero-till plots of soybean/wheat and soybean/wheat/maize rotations showed the highest rates of N<sub>2</sub> fixation (Ferreira *et al.* 2000).

To understand the effects of tillage on rhizobial evolution, studies are also needed to assess genetic structuring of rhizobial communities as related to spatial structuring imposed by tillage regime. Studies of both naturalized and agriculturally derived rhizobial populations suggest that there is relatively low genetic differentiation among populations but high variability within plants (Souza *et al.* 1994; Hagen & Hamrick 1996; Silva, Eguiarte & Souza 1999). Tilled and zero-till systems provide ideal models for studying the consequences of how spatial structuring modifies competitive interactions. This has been the subject of much theoretical work, although there is a lack of empirical data (West *et al.* 2001).

Correlations have also been found between increased physical disturbance in soil and decreased mycorrhizal functioning (Evans & Miller 1990; Galvez *et al.* 2001). Data are still needed to confirm that these are a result of evolutionary modifications in mycorrhizal effectiveness due to various tillage schemes. Douds *et al.* (1995) present data to support suggestions that tillage, like fertilization regimes, may exert selection pressures on mycorrhizal communities, resulting in shifts in community composition, as some species may cope with disturbance better than others. The question is whether there is any consistent relationship between disturbance tolerance and greater or lesser mutualism. If so, then selection within species could also occur. Tillage was shown to decrease mycorrhizal diversity and result in a competitive dominance by only three species in cultivated Argentinean fields (Menéndez, Scervino & Godeas 2001). Relationships between soil disturbance and mycorrhizal communities characterized by more vigorous but less mutualistic species have been hypothesized (Hamel 1996) but, to our knowledge, no empirical data have been published.

### Temporal structuring: crop rotations

In agricultural systems, the same plant genotype may be planted each year, usually independent of any variation in reproductive success among genotypes. A cultivar (or species) that performs poorly may be abandoned, but differences in individual fitness of crop plants will have no effect on the genetic composition of next year's crop, except when farmers save their own seed for planting.

Current theory suggests that mutualisms can be viewed as reciprocal exploitations in which some net benefit is provided to each partner (Herre *et al.* 1999). In natural systems and on farms that save their own seed, we would expect constant co-evolution between host and symbiont, with selection favouring (i) plants that are able to avoid less effective symbionts and (ii)

symbionts that obtain more from their hosts while paying a lower cost for these benefits. To some extent, evolutionary changes in the plant host may constrain evolution of decreased effectiveness in microbial symbiont populations. Agricultural systems that replace plants each year with the same genotype may lead to situations where the microbial symbionts will continue to evolve but the host-plant does not (termed one-sided co-evolution).

Continuous monocultures not utilizing crop rotation will tend to speed up the evolution of micro-symbiont adaptations (mutualistic or parasitic) to particular hosts; host genotype is replanted each year, regardless of changes in the effectiveness of the symbiont. Root symbionts under these management conditions are predicted to evolve measures to counter plant strategies that were once effective in enforcing co-operation by microbial symbionts, perhaps leading to a decrease in effectiveness over time. We predict that crop rotation may change symbiont evolution in ways that may differ between mycorrhizae and rhizobia.

Mycorrhizal fungi will encounter different host-plants in successive years, thereby increasing the diversity of plant selection pressures. For mycorrhizal fungi there may be trade-offs in the ability to overexploit different hosts, as suggested by evidence for host specificity in arbuscular mycorrhizae (Harley & Smith 1983; Sanders & Fitter 1992; Kiers *et al.* 2000).

If legumes are rotated with non-legumes, data suggest that there are selection pressures favouring rhizobia that survive for longer in the soil without a host (Triplett, Albrecht & Oplinger 1993), perhaps leading to a shift towards a saprophytic lifestyle, but not necessarily driving changes towards parasitism or mutualism. However, when the same legume species is replanted each year (soybean monocultures), changes are more likely to occur along the parasitism–mutualism continuum. Local changes in mutualistic functioning of rhizobia to annual hosts in natural ecosystems have been demonstrated (Parker 1995).

Therefore, if host-plants employ a variety of strategies for controlling microsymbionts, greater host diversity in either space (mixed cropping or greater genetic diversity within a crop species) or time (crop rotation) may reduce selection for host-specific adaptations that allow symbionts to overcome possible plant sanctions.

Studies of the effectiveness of root symbionts have suggested that continuous monocultures may select for decreased mutualistic functioning, relative to crop rotation. Johnson *et al.* (1992) evaluated the symbiotic effectiveness of the mycorrhizal communities by running reciprocal transplants in plots that had been in either continuous corn or soybean for the preceding 5 years. Spore populations of mycorrhizae derived from plots of continuous corn were negatively correlated with corn yields but positively correlated with yields of soybeans. This mainly appeared to involve a species shift, rather than evolutionary change within a species.

Reciprocal patterns were found in the soybean plots, although the statistical correlation was not as strong. These results suggest a certain degree of host specificity between crops and mycorrhizal species resulting in negative correlations between continued host presence and effectiveness on that host. Spore abundance was negatively correlated with crop yield (Johnson *et al.* 1992). This is in contrast to various studies that have suggested abundant sporulation is an indicator of a successful mutualism (Koomen, Grace & Hayman 1987; Brundrett 1991; Bever *et al.* 1996).

The association of the Glomales mycorrhizal fungi *Glomus macrocarpum* on tobacco *Nicotiana tabacum* illustrates a particularly extreme case of mutualistic modification (Johnson, Graham & Smith 1997). This mycorrhizal species causes tobacco stunt; increased root colonization is correlated with increased disease severity (Modjo & Hendrix 1986). Increased tobacco yields have been correlated with changes in mycorrhizal communities when crops are in rotation with fescue (Hendrix, Jones & Nesmith 1992). This suggests that crop rotation may act as a strong selective agent by preventing particular mycorrhizal strains (in this case *G. macrocarpum*) from dominating the soil profile. However, whether crop rotation simply prevents the build-up of detrimental symbionts or actually favours the increase of mutualistic symbionts warrants further investigation. Both of these cases could be seen as instances of the ecological control of soil-borne disease by crop rotation. However, they are also consistent with the hypothesis that consistent presence of the same host could favour the evolution of parasitism even in species that are often mutualistic.

### Symbiont competition: crop inoculation

Inoculation of legumes with introduced rhizobial strains is standard agronomic practice in the majority of legume cropping systems; mycorrhizal inoculation with highly effective strains has been suggested as a nutrient management strategy (Brockwell, Bottomley & Thies 1995; Hamel 1996). In general, inoculation is considered beneficial in that it introduces symbiotic genes into the soil (see the discussion below). However, we suggest that inoculation simultaneously increases the degree of symbiont competition and this may, in turn, modify host benefits derived from the relationship (for a related discussion on competition effects on parasite virulence see Read & Taylor 2001). It is well established that roots of crop plants can be colonized simultaneously by more than one strain of mycorrhizae but, due to the lack of empirical information on host effects of mycorrhizal inoculum strain competition, we will focus on the effects of rhizobial inoculation. Specifically, we predict the following.

High rhizobial inoculum densities may result in increased incidences of nodules containing more than one strain of rhizobia (Lindemann, Schmidt & Ham 1974). If mixed nodules undercut nodule-level host

sanctions hypothesized to constrain the evolution of rhizobial parasitism, high inoculum densities could increase the absolute reproductive success of less effective rhizobia (Denison 2000; West, Pen & Griffin 2002; West *et al.* 2002). The direct effects of introducing highly effective rhizobia could swamp this evolutionary effect in the short term, increasing the average effectiveness of rhizobia in nodules in the months following inoculation. But if the introduced strain survives poorly in soil after release from nodules, the evolutionary effects of increasing the frequency of mixed nodules could lead to adverse long-term changes on rhizobial effectiveness.

Although competition between rhizobial strains in the rhizosphere, particularly between introduced and indigenous strains, has been well-documented (Ham, Cardwell & Johnson 1971; Roughly, Blowes & Herridge 1976; Ames-Gottfred & Christie 1989; Gibson *et al.* 1990; Thies, Singleton & Bohlool 1991), competition studies between strains within a nodule remain relatively unexplored. This may be the result of the general assumption that nodules only contain one strain of rhizobia. However, accumulating evidence suggests that when plants are exposed to high densities of inoculum, nodules can be infected by more than one strain of rhizobia (Lindemann, Schmidt & Ham 1974; Rolfe & Gresshoff 1980; Trinick, Rhodes & Galbraith 1983; Demezas & Bottomley 1986; Nambiar, Anjaiah & Srinivasa Rao 1987; Stuurman *et al.* 2000).

Few studies have determined the frequency of mixed nodules in the field, but the mere existence of mixed nodules raises important questions. Do the high inoculum densities promoted by agronomic inoculation practices contribute to an increase of mixed infections in the field? How do mixed infections affect symbiotic functioning in the short and long term? Is this effect greater than the gene flow effect? Under field conditions, 5–59% of nodules tested contained more than one strain when inoculation rates ranged from  $10^6$  cells/seed to  $10^8$  cells  $m^{-1}$  (Diatloff & Brockwell 1976; May & Bohlool 1983; Moawad & Schmidt 1987; McLoughlin, Hearn & Alt 1990). It has been suggested that occupancy is largely based on inoculum population sizes and various environmental controls (Renwick & Jones 1986). However, whether it is inoculum population sizes or total soil population sizes that determine double occupancy rates is unknown. Inoculation could increase rhizobium populations around some parts of the roots even if there is little effect of inoculation on total rhizobial numbers in fields where the same legume has been grown previously. Legumes may employ mechanisms to limit the degree of doubly infected nodules, analogous to mechanisms that limit the number of wasps *Agaoninae* sp. per fig fruit in *Ficus* sp. (Herre 1989), but these could perhaps be overwhelmed by high inoculation rates.

Even if doubly infected nodules prove to be commonplace in both natural ecosystems and inoculated

fields, can we infer that interstrain competition leads to a decrease in effectiveness? Interstrain competition has been documented in a commercial multistrain inoculum in which low  $N_2$  fixation rates were found, even though the mixture contained a superior  $N_2$  fixing strain (Bromfield & Jones 1980; Rennie & Dubetz 1984; Danso & Owiredu 1988). Caldwell (1969) found increased nodule weight when plants were inoculated with two strain combinations in comparison with three-strain or single-strain inoculation, but the largest plant fresh weight was found under single-strain inoculation, suggesting a negative correlation between net host benefit and nodule fresh weight.

An assessment of the short-term advantages to inoculation vs. the longer-term evolutionary consequences of inoculum-induced symbiont competition is needed. Because inoculum strains are generally more effective than indigenous strains, mixed nodules may have higher  $N_2$  fixation rates than singly infected nodules containing only indigenous strains. It has also been suggested that rhizobial inoculants may lead to the lateral transfer of chromosomal symbiotic genes to non-symbiotic (saprophytic) rhizobia in the soil (Sullivan *et al.* 1995), as well as lateral transfer of *sym* plasmids (Laguerre, Bardin & Amarger 1993; Sprent 1994). Segovia *et al.* (1991) found that for every one symbiotic *Rhizobium leguminosarum* isolate found, there was an average of 40 non-symbiotic strains present in a Mexican bean field. Transfer of either chromosomal or plasmid genes may lead to an increased frequency of symbiotic isolates with enhanced adaptive traits to local biotic and abiotic conditions (Sullivan *et al.* 1995). Wernegreen, Harding & Riley (1997) suggest that this type of transfer may be a result of selection pressures influenced by (i) introduction of plants in a monoculture, (ii) high inoculation densities, and/or (iii) lack of selection acting on host-plants for symbiotic compatibility with indigenous strains. However, plasmids may also be lost or undergo rearrangement leading to deterioration of acquired symbiotic capacity (Soberón-Chávez *et al.* 1986). Such instability of symbiotic functioning was noted in a *R. leguminosarum* bv. *trifolii* population introduced into five field sites in Australia; significant differences in effectiveness were found in 11 of the 19 strain  $\times$  site combinations (Gibson *et al.* 1990). Similarly, 12 years after introduction, Diatloff (1976) found a decrease in symbiotic effectiveness of 68 of 80 cultures from the field compared with their stock cultures; six isolates were more effective. Ultimately, crop plants that preferentially enhance reproduction and release of more mutualistic strains (from among the various strains that infect each plant) into the soil may be more useful than inoculation with elite strains.

## Conclusion

If agronomic practices are driving symbioses towards increased parasitism, modifying the practices is a

daunting task. In examining the above-mentioned agricultural practices (fertilization regimes, tillage management, crop rotations and symbiont soil inoculation), it is clear that the short-term costs of altering certain parameters (e.g. reducing fertilizer use) to promote long-term increased efficiency of agronomic symbioses could lead to well-founded resistance. However, steps such as increasing the breadth of breeding programmes to include selection for effective symbioses under both high- and low-fertilization regimes could prove an effective tool for symbiont management. For instance, Manske (1989) compared yields of 22 land races of wheat to 22 high-yielding varieties of wheat when inoculated with mycorrhizae in low P soil and found a higher increase in average yields of the land races compared with the yield increases for high yielding varieties. In high P soils, mycorrhizal inoculation caused a greater yield depression in the high-yielding varieties than in the land races, resulting in slightly greater total yield of the land race varieties (Johnson & Pflieger 1992b). It has been demonstrated that modern wheat breeding practices tend to reduce mycorrhizal dependence, as wheat cultivars released before 1950 consistently show increased dependence on mycorrhizae compared with those released subsequently (Hetrick, Wilson & Cox 1993). Data suggest that the host-plant's ability to form effective symbioses is a heritable trait that may be selected for or against in plant breeding programmes (Kesava Rao, Tilak & Arunachalam 1990).

To conclude, there is much interest in the increased utilization of agronomic symbioses in agricultural systems. We suggest that these attempts will be met with greater success through a consideration of how natural selection shapes mutualistic interactions. Specifically, to what extent (and why?) do different agricultural practices select for more beneficial or parasitic symbionts? Once this is understood it may be practical to modify some agricultural practices to reduce selection for more parasitic symbionts (as we suggest may currently be widespread), and even specifically to farm for more effective symbionts. Increasing our ability to exploit microbial mutualisms may be an integral step in moving towards more sustainable systems of agricultural production. Our major conclusion is that there is a lack of data directly testing our predictions, especially from experimental studies. We hope that this paper will stimulate such research.

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