mother arrival on removal twigs. Of the 699 stem mothers attempting colonisation on the high density tree, 83% arrived over a 3-d period that peaked just after bud burst; on the low density tree, colonisation spanned the same time period but the peak was much reduced. Figure 3*b*, shows that when competitor densities are high, colonising stem mothers rapidly respond to the removal of competitors. The total number of stem mothers removed during seven consecutive days of sampling (535 per 1,000 leaves) was 69% greater than the number of stem mothers found on control leaves (316 per 1,000 leaves) ($\chi^2 = 46.67$, $P < 0.001$). On the tree with a low competitor density, however, the number of stem mothers collected on removal twigs (72 per 1,000 leaves) did not differ significantly from the number of stem mothers found on control leaves (61 per 1,000 leaves) ($\chi^2 = 1.11$, $P > 0.29$). The fact that there was a highly significant difference between treatment and control on the high density tree shows that stem mothers were moving in search of a place to settle and were reacting negatively to the presence of other stem mothers. On the other tree, however, densities of potential competitors were so low that competition for a limited number of gall sites was reduced, enabling all stem mothers to rapidly select leaves and immediately settle. Thus, with high population densities, many stem mothers are displaced through competitive interactions and a floater population is produced. These results are similar to those obtained in other systems as diverse as birds, mammals and fish, where subdominants or floaters moved into vacated spaces[11–17].

Experiments indicate that the floater population suffers much greater mortality than the resident population. Before all stem mothers had permanently settled during colonisation, the positions of 211 stem mothers distributed among 351 leaves of a small branch were recorded. A sticky barrier (Tanglefoot) was placed at the base of the branch to prevent further recruitment. After all stem mothers had permanently settled, their positions were again recorded. Forty stem mothers had abandoned the positions they had held during the first census and attempted gall formation on other leaves. Of these individuals, only 24% survived, while 72% of those that did not move from their original positions survived ($\chi^2 = 21.45$, $P < 0.001$). This experiment has been repeated with another *Pemphigus* species and the results were identical[5]. Such differential mortality could be an important factor in the evolution of territorial behaviour and may account for the observed extended contest of Fig. 2.

The Eriosomatidae, or gall-making aphids of which *Pemphigus* is a member, exhibit a unique reproductive trait in which each stem mother is the sole progeny of a female sexuale[18]. As no other aphid group or known animal lays only one egg throughout their lifespan, this trait must have evolved under rather severe and/or unusual circumstances. I suggest that the advantage large body size confers in territorial interactions may have contributed to the evolution of this trait by favouring the reduction of clutch size and the placement of all resources into a single large egg.

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THOMAS G. WHITHAM

Harold S. Colton Research Center, Museum of Northern Arizona, Flagstaff, Arizona 86001

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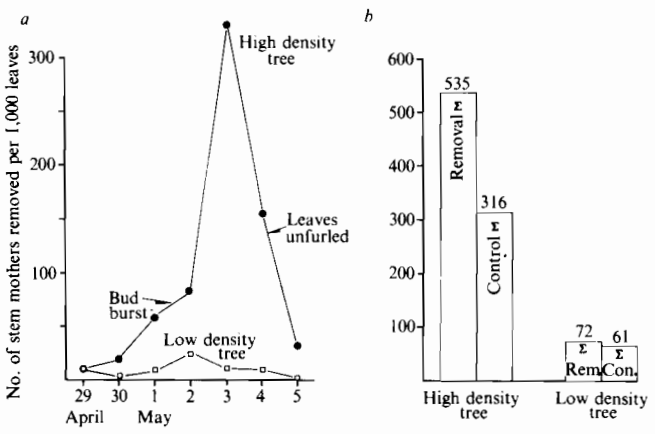

Fig. 3 Results of a removal experiment designed to test the hypothesis that due to territorial interactions occurring during leaf selection, a floater population is produced. Y-shaped twigs on two trees which differed in the density of competing stem mothers were selected. One arm of each twig acted as the control and was sampled only at the end of the experiment. On the other arm stem mothers were removed each day. *a*, Shows the number of stem mothers removed each day from both trees: *b*, compares the removal and control twigs for both trees (see text).

Endomycorrhizal fungi and *Rhizobium* as biological fertilisers for *Medicago sativa* in normal cultivation

LEGUMES can form two types of symbiotic association with microorganisms. One, with *Rhizobium* sp., is involved in the fixation of atmospheric nitrogen; the other, with fungi of the family Endogonaceae that form vesicular-arbuscular (VA) endomycorrhizas, is concerned with the uptake of phosphorus by the plants. Glasshouse experiments have demonstrated that legumes inoculated with both types of microorganism grow and nodulate better, and have higher nitrogenase activity and phosphorus content than plants that are uninoculated or inoculated with either *Rhizobium* or mycorrhizal fungi separately[1–12]. Also, plants with both types of symbiosis may be important as pioneer colonisers of nutrient-deficient habitats[13]. At present, the possibility of field inoculation with mycorrhizas to improve yield, and the subsequent economy in the use of chemical fertilisers, are being considered[14]. Positive responses to VA mycorrhizas are to be expected mainly in soils low in nutrients, particularly phosphate, and where indigenous endophytes are sparse or inefficient[14]. Thus, the use of soil sterilisants to destroy indigenous endophytes has been assayed[15–16]. There are also reports on the effect of VA fungi inoculation in non-sterile soils[17–20]. The effects of endomycorrhizas on legumes in relation to the improvement of hill[19–20] and marginal soils are now being studied[21]. But as far as we know no data for leguminous crops growing on non-sterile arable soils in standard agricultural conditions in temperate regions have been published. We report here that inoculation of *Rhizobium* and *Glomus* improves the growth and nutrition of *Medicago sativa* in normal cultivation on an arable field.

The experiment was carried out on an irrigated calcareous soil (*p*H, 7.8) in a fertile valley ('vega') in Granada province, Spain. Its texture was: 25.2% sand, 30.0% loam and 44.8% clay. The soil contained 1,302 p.p.m. total N, 415 p.p.m. total K,

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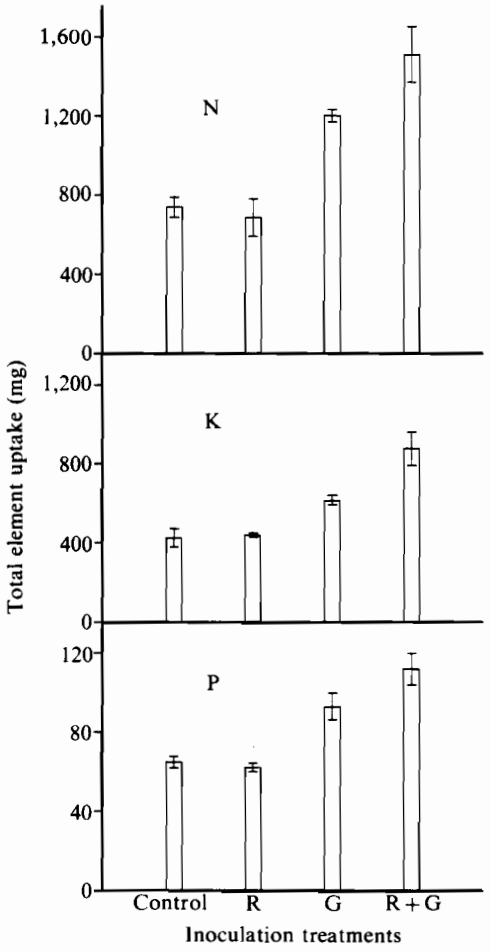

Fig. 1 Effects of *Glomus* (G) and *Rhizobium* (R) on N, P and K uptake by *Medicago sativa* growing in an arable field soil under normal cultivation. Total N, P and K taken up by the plants is calculated from data of shoot dry weights and the percentage of element (mean of four replicates). Standard errors are shown.

611 p.p.m. total P, 9.2 p.p.m. available phosphate[23] and 1.74% organic matter. This field has been intensively cultivated for centuries and two crops per year are harvested. When the present experiment was designed the field had been left fallow for six months. Endogonaceae spores were recovered by the technique of wet sieving and decanting[22]. The number of spores per 100 g soil was: 14±1.4 yellow vacuolate[24] (*Glomus mosseae*[25]), 2±0 laminate[24] (*Glomus macrocarpus*[25]) and 8± 0.7 unidentified. Spore numbers were, therefore, low but fall in the range of about 0.1–5 per g soil, recovered in most cases[26]. The predominance of *Glomus mosseae* spores in this field would justify, from the ecological point of view, the choice of this species as inoculant.

Medicago sativa L. cv. Aragón was the host plant for an endomycorrhizal fungus of the yellow-vacuolate spore type and for *Rhizobium meliloti* 203. Four seedbeds were prepared for plants for the field experiment. These were: uninoculated control (C), *Rhizobium*-inoculated (R), *Glomus*-inoculated (G) and *Rhizobium* + *Glomus* inoculated (R+G). The seedbeds were kept for 20 d in a glasshouse at 19–25°C. At this time, seedlings had a slight VA infection; about 3–5% of their root system. The experimental field was divided into four plots: C, R, G and R+G, to each of which seedlings from the corresponding seedbed were transplanted. Each plot consisted of four replicates with each replicate containing five 1 m^2 microplots. These microplots received 25 groups of three plants. Care was taken in selecting uniform seedlings and allowing some soil from the seedbed to adhere to their roots. Seedlings of R and R+G treatments were reinoculated with *Rhizobium* at transplanting.

Table 1 Effects of *Glomus* and *Rhizobium* on the yield of *Medicago sativa* growing under normal cultivation in an arable field soil

Inoculation treatments	Shoot dry weight (g)
Uninoculated controls	15.18±0.95
Rhizobium	15.94±1.19
Glomus	22.56±1.11
Rhizobium + *Glomus*	32.09±1.77

Mean of four replicates.

Plants were irrigated by the farmers in their usual way, and no chemical fertilisers were applied during the experiment. At harvest, plants which grew during 12 weeks in the five microplots belonging to the same replicate were pooled, dry weights of shoots were recorded and analysed for P, N and K (ref. 27).

Table 1 records plant growth. It is clear that *Glomus* inoculation improved the yield of alfalfa, whereas *Rhizobium* inoculated alone did not. Uninoculated control plants showed nodulation by indigenous rhizobia, however, in *Glomus*-inoculated plants the introduction of *Rhizobium* was effective and plant dry weight was increased by nearly 50% (R+G treatment compared with G treatment). The available P content in the test soil is low; this probably not only determined the response of the plants to *Glomus* inoculation but also that plants not given a *Glomus* inoculum did not respond to *Rhizobium* inoculation. Phosphorus is known to be an essential element for N_2 fixation[8,21] and as it was a limiting factor in this experiment, plant growth and *Rhizobium* activity were not greater than for the uninoculated controls unless the plants were adequately mycorrhizal. *Glomus* inoculation improved total N, P and K uptake and the inoculation with *Rhizobium* was only effective when applied together with *Glomus* (Fig. 1).

The main conclusion from these results is that the mycorrhizal fungi *Glomus mosseae* is an effective 'biological fertiliser' for *Medicago sativa*. Inoculation with *Glomus* not only affected plant growth and nutrition but also enhanced the activity of *Rhizobium meliloti* when it was applied as inoculant. The introduction of *Rhizobium* together with *Glomus* and the subsequent establishment of effective dual symbiotic association with alfalfa was successful, as yield was increased by 211% compared with control. This suggests that it may be possible to reduce the chemical fertiliser inputs to this arable crop. This study may have practical significance for neutral–alkaline soils like the one used in the present investigation. As this soil is under intensive cultivation it has received, and is receiving, large amounts of organic and inorganic additives; as a result, the total P is high but the available P is quite low, probably because the added P fertiliser has become fixed, mostly by the calcium in this high *p*H soil, or by some minerals of the clay fraction[28], which is relatively high in the test soil. The extensive use of fertilisers also accounts for the low number of Endogonaceae propagules[29].

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C. AZCON-G. DE AGUILAR
R. AZCON
J. M. BAREA

Department of Microbiology,
Estación Experimental del Zaidín C.S.I.C.,
Granada, Spain

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Anomalous temperature dependence of the sodium conductance in rabbit nerve compared with frog nerve

THE sodium channel in excitable tissues is an integral component of the membrane, and is intimately associated with the surrounding lipid[1]. Thus, the electrophysiological parameters of the sodium channel might be expected to exhibit a characteristic dependence on temperature, reflecting the marked temperature sensitivity of the physical properties of most lipids. We report here voltage-clamp experiments which show that cooling a rabbit node below the region of about 6 °C does in fact sharply decrease the maximal sodium conductance, and markedly prolongs sodium inactivation. It thus seems that the lipid (or lipid–protein) environment of the sodium channel in the rabbit node undergoes a drastic change below 6 °C.

Single myelinated fibres from rabbit and frog (*Rana pipiens*) sciatic nerves were dissected and voltage-clamped[2]. The nerve chamber was initially allowed to stabilise at room temperature (25 °C). Pre-cooled liquid was then pumped through the brass block enclosing the nerve chamber, gradually cooling the whole preparation to about 0 °C in 15 min. The temperature was measured throughout the experiment by a small thermocouple (50 μm diameter) located about 1 mm beneath the node in the pool containing the node. A single fixed depolarisation to −20 mV (rabbit) or to −5 mV (frog), preceded by a hyperpolarising prepulse to −125 mV to remove sodium inactivation, was used to assay sodium conductance every 0.3–1 °C as the temperature fell from 25 to 0 °C. At the end of the experiment, the temperature dependence of the internodal resistance was measured over the same temperature range and used to correct for consequent changes in current. Typically, the internodal resistance increased progressively, rising by a factor of about 1.3 in both frog and rabbit nerve when the temperature fell by 10 °C. The net ionic current associated with the test pulse in a rabbit node consisted of an inward current superimposed on an outward leak current, potassium current being virtually absent in the mammalian node[2,3]. In frog nerve, tetraethyl-ammonium was added to block potassium current. After subtracting the leak component from the total ionic current, the values of τ_h for both frog and rabbit nodes were determined by fitting the sodium current to the expression:

$$I_{Na} = G_1 e^{-t/\tau_h}(1 - e^{-t/\tau_m})^p$$

with $p = 2$ for rabbit and $p = 3$ for frog.

Figure 1 shows typical sodium current records from a frog and rabbit node of Ranvier at different temperatures. A 5 °C fall in temperature from 23 °C slowed the kinetics of the sodium current in both frog and rabbit nerve by about the same extent and produced a similar decrease in the size of the current (Fig. 1*a*, *b*). However, a 5 °C fall in temperature from 6 °C produced a much bigger decrease in the peak current and a larger prolongation of sodium inactivation in the rabbit than in the frog (Fig. 1*c*, *d*). These effects of cooling were reversible on rewarming.

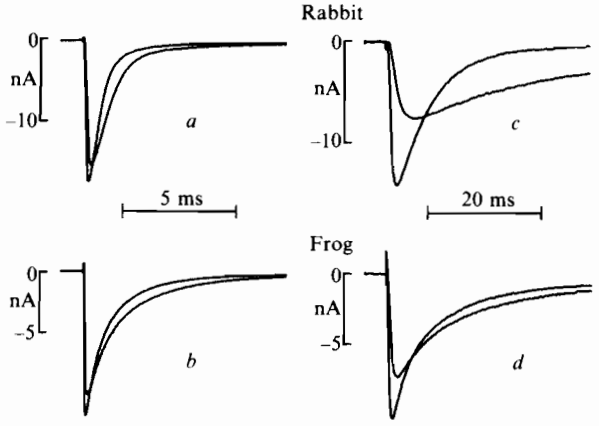

Fig. 1 Sodium currents in a rabbit (*a*, *c*) and frog (*b*, *d*) node. Each pair of currents shows the response at a given temperature (23 or 6 °C) superimposed on one at a temperature 5 °C lower: *a* and *b*, 23–18° C; *c* and *d*, 6–1 °C. In the rabbit E_{Na} was +70 mV at 25 °C and +65 mV at 0 °C; in the frog the corresponding values were +58 and +65 mV, respectively. The sodium current after leak subtraction and correction for temperature-dependent changes in the internodal resistance was calibrated by assuming a value of 10 mΩ for the internodal resistance at 25 °C. Note that the time to peak is increased relatively more in *c* than in *a*.

Figure 2 shows in greater detail the temperature dependence of the maximal sodium conductance, $\bar{g}_{Na}$, and the time constant of inactivation, τ_h, for five rabbit and six frog nodes. The value of $\bar{g}_{Na}$ was determined by extrapolating the decay phase of the sodium current back to the onset of the test pulse at each temperature. Figure 2 shows clearly that the curves for the temperature dependence of these parameters in frog and rabbit nodes have different shapes; in the frog the slope of the curve remains roughly constant over the whole temperature range[4,5], whereas in the rabbit there is a marked change in slope at about 6 °C. Thus, the value of τ_h for rabbit at −20 mV increased by a factor of about 1.73 for a 5 °C drop in temperature from above 15 °C (Q_{10} about 3), whereas below a transition temperature region (about 6 °C) the same drop in temperature increased τ_h 5.7-fold (Q_{10} about 33). Similarly, the values for $\bar{g}_{Na}$, which depended only moderately on temperature above 6 °C (Q_{10} about 1.7), became much more sensitive to temperature below 6 °C, the Q_{10} for the decrease being about 4.7. Because the rising phase of the sodium current was relatively fast, and because relatively few computer sampling points were taken during it, measurements of τ_m were too uncertain for us to determine whether or not a similar discontinuity occurred with this parameter.

Several factors besides a reversible reduction of $\bar{g}_{Na}$ could have caused the marked decrease of sodium current at low temperatures: for example, a reduction in E_{Na} or shifts of the $h_\infty(E)$ and $P_{Na}(E)$ curves with temperature. In the present study, the average values of E_{Na} at 25 °C and 0 °C were both 65 mV for frog nodes, and 65 mV and 60 mV, respectively, for rabbit nodes. A decrease of as much as 10 mV on cooling would have decreased the sodium current associated with the test pulse (at −20 or −5 mV) 1.2-fold at most. Furthermore, although the $h_\infty(E)$ curves in both frog and rabbit were consistently shifted in the hyperpolarising direction (by 5–19 mV) when the temperature was lowered from 25 °C to 0 °C, this shift could not have contributed significantly to the observed decrease in sodium current because a large negative prepulse was used to remove any inactivation. Finally, any shift in the $P_{Na}(E)$ curve was small

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