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Symbiotic nitrogen fixation and distribution of *Spartocytisus supranubius* on Las Cañadas, Tenerife

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ABSTRACT: Visual inspection, acetylene tests for nitrogenase activity and microbiological assay of soil samples showed that bushes of *Spartocytisus supranubius* were nodulated effectively by *Rhizobium* at three sites on Las Cañadas, Tenerife. A sixteen - fold increase in the total nitrogen content of surface soil from under bushes, compared to that of soil between bushes, suggests that symbiotic nitrogen fixation in *Spartocytisus* makes a substantial contribution to the nitrogen budget of these high altitude ecosystems. Bushes in two populations, with densities of 210 and 560 plants ha^{-1}, were shown to have regular dispersion patterns, typical of desert shrub communities in areas of low rainfall. Allelopathy was eliminated as a cause of plant dispersion, which was probably a result of intraspecific competition for water and of herbivore grazing pressures on seedling.
Key words: *Spartocytisus*, nitrogen, fixation, distribution, Las Cañadas

RESUMEN: La inspección visual, los tests del acetileno para la actividad de la nitrogenasa y el ensayo microbiológico de muestras del suelo mostraron que los matorrales de *Spartocytisus supranubius* estaban nodulados de forma efectiva por *Rhizobium* en tres puntos de La Cañadas, Tenerife. El incremento de 16 veces el contenido total de nitrógeno de la superficie del suelo bajo el matorral en relación con el suelo situado entre el matorral, sugiere que la fijación de nitrógeno simbionte en *Spartocytisus* contribuye sustancialmente al presupuesto de nitrógeno en ecosistemas de grandes altitudes. Dos poblaciones de matorral, con densidades de 210 y 560 plantas por ha^{-1} mostraron un modelo de dispersión regular, típico de comunidades de matorral de desierto en áreas de baja pluviosidad. La alelopatía fue eliminada como una causa de la dispersión de las plantas puesto que era probablemente un resultado de la competición intraespecífica por el agua y de la presión de los herbivoros.
Palabras clave: *Spartocytisus*, nitrógeno, fijación, distribución, Las Cañadas.

INTRODUCTION

Spartocytisus supranubius (L.) Webb and Berth. is the dominant leguminous shrub of the open scrub community which characterises the vegetation of Las Cañadas, Tenerife (Fig. 1) at altitudes of approximately 1900 to 2500 m (BRAMWELL & BRAMWELL,

1974). It is abundant in the north and western approaches to Las Cañadas National Park but is of more occasional occurrence in the drier, south and east. The volcanic rocks of Las Cañadas give rise to neutral to alkaline red, lithosol, skeletal soils which are characteristically low in nitrogen.

Biological nitrogen fixation by *Rhizobium* within the root nodules of plants of the papilionoid genus *Cytisus* is an important factor for their success as pioneer species, along with morphological adaptations to water and temperature stress such as reduced leaves, green, photosynthetic stems and a deep rooting habit. The colonisation of the soils of Las Cañadas, with their low nitrogen content, and the subsequent dominance of the vegetation of this area by *Spartocytisus supranubius* would be facilitated by symbiotic nitrogen fixation in this species. Over a period of time, the decay of litter and root material from the shrub should enhance the nitrogen content of the soil in which it grows. The occurrence of symbiotic nitrogen fixation in brooms at high altitudes has not been studied and the question whether the volcanic soils of Las Cañadas harbour rhizobia which can nodulate *Spartocytisus* effectively is examined here.

Although competition from other plant species is low in the harsh environment where *Spartocytisus* grows (DICKSON et al., 1987), intraspecific competition is to be expected and this may contribute to the dispersion pattern shown in Las Cañadas. A regular spatial pattern of dispersion has been noted frequently as a feature of the sparse, shrub vegetation of desert areas, which may be the result of particular physiological or environmental interactions. Possible causes are competition for water by the roots of adjacent plants at sites of low rainfall, competition for minerals, allelopathic interactions between bushes, and seed and / or seedling predation by rodents and other small animals (SILVERTOWN, 1985; WENT, 1955; WOODELL et al., 1969). The second part of this paper examines the dispersion of *Spartocytisus* in two areas of Las Cañadas.

MATERIAL AND METHOD

Location of study areas: Two areas were studied, the first in December 1984 and the second in December 1985 and again in December 1986. Site 1 was located at an

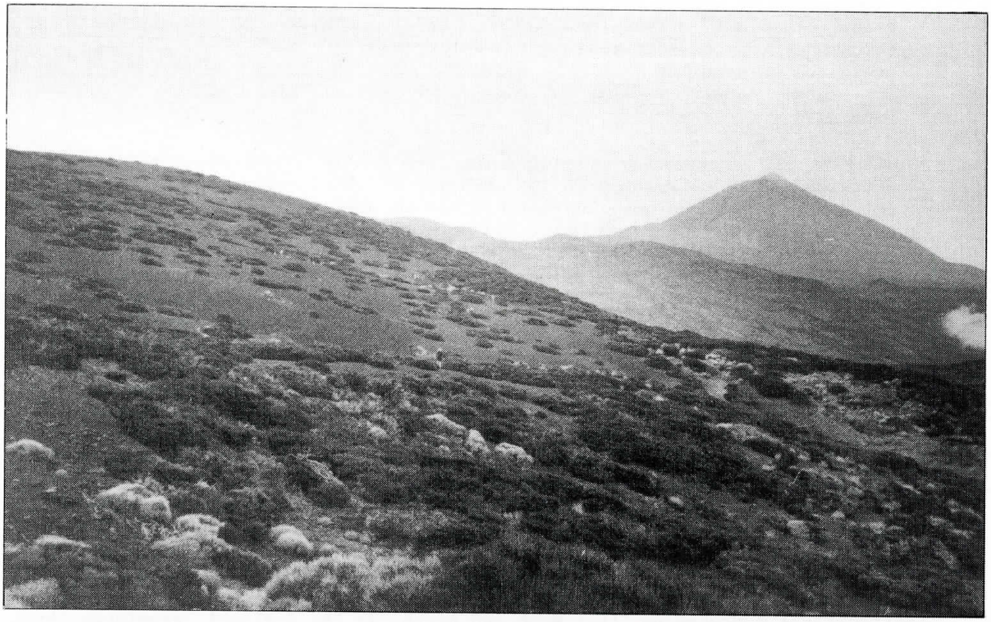

FIG. 1: The population of *Spartocytisus supranubius*, studied at Site 2 at Las Cañadas.

altitude of 2100 m in the western caldera of Las Cañadas, south of but adjacent to route C824 and about 94 m west of its junction with C823. Site 2 was at an altitude of 2000 m, east of but adjacent to C824 and approximately 6 km north west of the junction leading to Observatorio de Izaña. At both sites, *Spartocytisus supranubius* was dominant together with some *Scrophularia glabrata* and *Pterocephalus lasiospermus*.

Estimation of nodulation and nitrogenase activity: Nodulation of *Spartocytisus* in the field was determined by visual inspection of plant roots excavated at site 2 and at an additional site near Portillo de la Villa, just out with the National Park boundary. Acetylene reduction tests for nitrogenase activity were carried out on nodulated roots dug from site 2 and from the site near Portillo de la Villa in Decembre 1982. The nodulated roots were incubated at soil temperature for 1h in 30 ml vials with air containing 10 % acetylene, generated from calcium carbide. Sub-samples of gas were then collected in "Vacutainers" (Becton and Dickinson Ltd., Meylan, France) for transport back to the U.K. and analysis by gas chromatography (McNABB & GEIST, 1979).

Collection and analysis of soil samples: Samples for microbiological analysis were collected from the top 10 cm of the soil beneath three bushes at site 2. The samples were mixed and about 1 kg sealed in a plastic bag which was kept at ambient temperature during transport to the U.K. (Importation licence IP / MISC / 66 / 1985). Microbiological assessment of the soil was commenced 40 h after collection. The soil was placed in trays which were sown with seed of *Cytisus scoparius* L., collected near Glasgow. The trays were isolated from other plants in a heated glasshouse, lit by daylight supplemented with four 60w fluorescent lights ("Warm White", Osram) to give a 16 h light period, and were watered as required with distilled water. Two control trays, with *Cytisus* seed sown in Perlite ("Silvaperl", Harrogate, England) and supplied with Crone"s mineral nitrogen - free nutrients (DIXON & WHEELER, 1983), were placed ar random among the trays of soil. These remained unnodulated during the experiment. Bacteria that did not take up Congo Red and which formed effective root nodules following inoculation of seedlings of *Cytisus scoparius*, were isolated as described by VINCENT (1970) from nodules that formed on *Spartocytisus* seedlings which developed following germination of seed present in the soil sample.

Soil samples to be analysed for total nitrogen were collected at site 2 with a 5 cm diameter corer, to a depth of 10 cm beneath the litter layer of three visually similar *Spartocytisus* bushes. Samples were taken from the centre of the bush and then at 1 m intervals to a distance of 5 m from the centre. For two of the bushes, a further sample was taken 7 m from their centres, at a position equidistant between neighbouring bushes. Each sample was sealed in a separate polythene bag and mixed and 4 h later a sample was transferred into a 100 ml sealed bottle for transit. Sub - samples remained at ambient temperature for 30 h and then were refrigerated at 2 °C. Prior to analysis, the samples were allowed to come to room temperature and then were ground in a mortar and pestle. Small stones were sieved from the soil and three weighed, replicate portions from each of the ground samples were taken for total nitrogen analysis. Samples were digested with concentrated sulphuric acid containing 30 g^{-1} salicylic acid with a mercuric oxide, copper sulphate, potassium sulphate catalyst and the ammonium produced determined by semi - micro Kjeldahl analysis (WHEELER et al., 1987). A further three replicates from each of the samples were dried at 80 °C to constant weight to permit comparison of the nitrogen content of samples on a dry weight basis.

Population dispersion pattern: The "nearest neigbour analysis" method of CLARK & EVANS (1954) was used to detect departure from randomness in the distribution of individual bushes of *Spartocytisus*. At each site, points were located at random. The distance between the centre of the *Spartocytisus* bush nearest to this point and the centre of its closest neighbour was measured. Fifty such measurements were made. The density of the *Spartocytisus* population was estimated within thirty randomly located 20 x 20 m quadrats at site 1 and within forty 10 x 10 m quadrats at site 2 (the population density at site 2 was perceived by eye to be greater than at site 1).

RESULTS AND DISCUSSION

Nodulation and nitrogen fixation: Quantification of the nodulation of *Spartocytisus* was not attempted because of the extensive destructive sampling that

would have been required to obtain meaningful results. Most of the bushes inspected, and particularly young individuals, bore nodules somewhere on their root systems. These were elongate, multi-lobed structures of indeterminate growth, similar to the perennial nodules of *Cytisus scoparius* (PATE, 1961). Positive tests for nitrogenase activity were obtained on all of ten samples tested, rates of acetylene reduction varying from 0.09 to 7.1 µmole C_2H_4 g^{-1} dry weight nodules h^{-1}. Clearly, therefore, the rhizobia present in the soils of Las Cañadas are fully effective for nodulation of *Spartocytisus* even at altitudes in excess of 2000 m. This conclusion was confirmed by tests on soil carried out in the greenhouse in Glasgow. Effective nodules, that were pink in cross section and that reduced acetylene at rates in excess of 8 µmole C_2H_4 g^{-1} dry weight nodules h^{-1}, were formed both on surface sterilised seed of *Cytisus scoparius* that was sown in the soil and on seedlings of *Spartocytisus supranubius* that developed from dormant seed in the soil sample.

The question arises whether nitrogen fixation in *Spartocytisus* dominated ecosystems makes a significant contribution to the nitrogen budget of the area. From the assay of nitrogenase activity and of the accretion of nitrogen in *Cytisus scoparius* dominated areas in Oregon and in Scotland, it was estimated that symbiotic nitrogen fixation contributed between 10 and 36 kg N ha^{-1} to these ecoystems (WHEELER et al., 1987). The known history of these experimental sites showed that the plants were no older than six years by completion of the study. The age of the *Spartocytisus* bushes at Las Cañadas is not known but their size suggests an age greatly in excess of six years. Over a period of years, mineralisation of litter and root material, enriched with fixed nitrogen, is likely to result in measurable increases in soil nitrogen.

Data indicating the degree to which the nitrogen content of the top soil layer is increased by the presence of *Spartocytisus* are shown in Fig. 2. The average

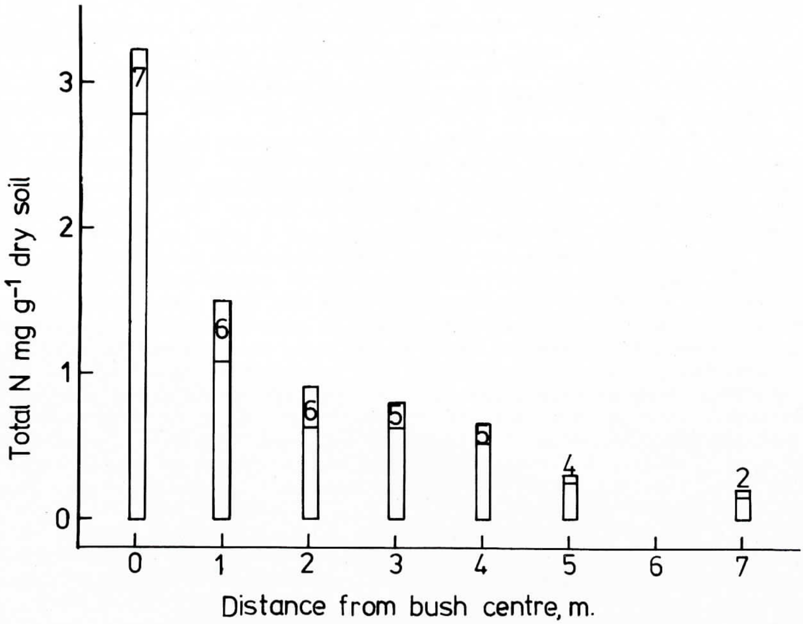

FIG. 2: The effect of *Spartocytisus supranubius* on soil nitrogen content. Soil from beneath the litter layer was collected to a depth of 10 cm under, or adjacent to, three bushes. The mean distance ± standard error of the bush edge from the centre was 1.42 ± 0.208 m. Samples were collected from the centre and at 1 m intervals along a transect from the centre of each bush. At least one sample was collected at each location from each bush, except that at 7 m samples were taken adjacent to two bushes only. Bar inserts indicate the standard error of the mean N content. The inserted numerals indicate the number of samples analysed at each location.

results obtained from the analysis of samples from under three bushes showed that the nitrogen content of litter-free surface soil increased almost two-fold from the edge to the centre of the bush and about 16-fold from areas located outwith at least 5 m from the edge of the bush. While these date are strongly indicative of a role of symbiotic nitrogen fixation in increasing the soil nitrogen content, it is not possible to estimate the actual contribution which nitrogen fixation may make. Not all of the extra soil nitrogen under the bushes will have originated from fixation, for the deep rooting habit of *Spartocytisus* will have recycled to the surface mineral nitrogen from deep in the soil profile. Uptake of mineral nitrogen by mycorrhizal roots can also increase the availability to the shrub of soil nitrogen which may then be recycled to the upper soil as the litter decays. The occurrence of mycorrhizas in *Spartocytisus* has not been studied. On the other hand, nitrogen will have been lost from the surface soils by leaching, by denitrification and by uptake to support plant growth so that the data presented are an underestimate of the input of nitrogen to this soil layer. It would be surprising if a notable proportion of the nitrogen present did not originate from fixation.

Population distribution: Visual inspection of the two sites selected for study and of other *Spartocytisus* dominated areas suggests that the shrub adopts a disperse distribution. Experimental evidence supporting this view was obtained from statistical analysis of the populations at the two study sites. Values for R, the ratio of the experimentally determined mean distance between adjacent shrub centres in a population to the calculated mean distance if individuals in the populations are randomly distributed, were 1.80 and 1.78 for Sites 1 and 2, respectively (Table I). Both these ratios are well in excess of unity, the ratio which would be given by a population in which the distribution of individuals is completely random. Under conditions of maximum aggregation R=0 and under conditions of maximum spacing R=2.15 (CLARK & EVANS, 1954). The values for R determined here provide clear evidence for a regularity of dispersion of *Spartocytisus* at both the study sites in Las Cañadas.

This distribution pattern could be the result of factors such as intraspecific competition for water or mineral resources, allelopathy or grazing pressures. Allelopathy seems unlikely as a cause of the disperse distribution for seed of *Spartocytisus* germinated in soil collected from beneath bushes, while the percent germination in similar soil of seed of the related *Cytisus scoparius* was not significantly different from controls germinated in John Innes potting compost. Additionally, germination of seed of *Cytisus* was not affected by watering with filtered leachate from *Spartocytisus* obtained by shaking equal weights of shoots and distilled water at room temperature for 1 h.

	Population density (r) n° plants m^{-2} ± S.E.	Mean of distance (r) between adjacent shrub centres m.		
		Experimental ± S.E. (n = 50)	Calculated for a random population	
		$rA = \frac{r}{n}$	$rE = \frac{1}{2/r}$	$R = \frac{rA}{rE}$
Site 1.	0.021 ± 0.0022 (n = 30)	6.20 ± 0.446	3.45	1.80
Site 2.	0.056 + 0.0147 (n = 40)	3.75 ± 0.159	2.11	1.78

Table I: Dispersion of *Spartocytisus supranubius* in two populations growing in Las Cañadas. Differences between the experimental (rA) and the calculated (rE) means of distance between adjacent shrub centres, carried out according to Clarck & Evans (1954), were statistically significant ($p < 0.001$) at both sites.

Although not studied specifically, it is possible that both intraspecific competition for water and herbivory may contribute to the development of the disperse distribution pattern. Regular dispersion patterns have been observed for the desert creosote bush (*Larrea divaricata*) when rainfall is low, while aggregation is more common at sites of high rainfall (WOODELL et al., 1969). There is low or no rainfall on Las Cañadas for several months each year so that competition between bushes for the available water could contribute to the development of the observed dispersion pattern. This possibility is supported by the higher density of *Spartocytisus* at Site 2 compared with Site 1 within the caldera, where precipitation is lower. Few small individuals were found in any of the *Spartocytisus* dominated areas. This could be in part due to competition for water, for a number of young plants were observed between the older bushes in the caldera in the relatively wet winter of 1984/85. However, there were abundante rabbit droppings in the study area and it is probable that many young seedling are eliminated by grazing by herbivores.

It is apparent that further study of the ecophysiology of *Spartocytisus* is required to identify and where appropiate to quantify those characteristics that contribute to its growth and successful colonisation of the harsh, high altitude environment of Las Cañadas. Further comparison with low altitude brooms such as *Cytisus scoparius* would be rewarding and consideration should be given to the possibility of planting *Spartocytisus* for land stabilisation and reclamation at high altitudes in areas out with the Canary Islands.

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