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Chapter 1.2

LOTUS-RELATED SPECIES AND THEIR AGRONOMIC IMPORTANCE

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More than 180 species within the genus Lotus occur worldwide. Four have been domesticated and improved through selection and plant breeding: Lotus corniculatus, L. uliginosus, L. glaber and L. subbiflorus. Since the model legume L. japonicus is related taxonomically to these species, knowledge can be transferred to the agronomical arena. The slow progress observed in Lotus cultivar improvements to date could be explained by the polyploid nature of some of these species, a feature not present in L. japonicus. This chapter reviews briefly the taxonomical relationships among these species. Secondly, it illustrates how Lotus species are currently used to improve pastures for which other forage legume species are not suitable. Finally, it touches on beneficial microorganism-plant interactions and the benefits of using Lotus species as animal fodder.

INTRODUCTION

One of the principal protein sources of the human diet comes from animal origin. Beef and sheep meat production is based on natural, cultivated pastures and feedlot system with nutrient supplement. Cultivated pastures can be composed of a single cultivated species or a mixture of forage species. These pastures include grass species as rye grass, cocks-foot, oat, and *Festuca* sp. Other important botany components in these pastures are legume species such as trefoils, lucerne, and Lotus sp.

An alternative generalised technology to improve the production and the nutritive value of natural grasslands is legume surface over sowing with phosphate fertilisation. In the natural improved grassland from South America it has been observed that the introduction of legume pastures promotes sustainable development

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of native vegetation and a higher productive level (Berreta *et al*., 2000). Forage legumes play an important role in this beneficial effect through their contribution to the nitrogen content of soil derived from nitrogen fixation, and the phosphorous availability generated by the fertilisation of the natural grasslands.

There is a potential use of Lotus species related to their ability to grow in acid soils or soils with low fertility and their tolerance to aluminium, manganese and sodium chloride (Blumenthal and McGraw, 1999). *Lotus corniculatus* is also used as bioremediator in boron and selenium contaminated soils (Banuelos *et al*., 1992) and its use for other contamination problems must be considered.

Besides its agronomical and environmental attributes, there is a great deal of interest in Lotus because some species are extremely amenable to tissue culture, in particular *Lotus corniculatus* L., and *L. japonicus* (Regel) K. Larsen (Webb *et al*., 1990; Handberg and Stougaard, 1992; Jiang and Gresshoff, 1997; Aoki *et al*., 2002). In spite of the fact that *L. japonicus* is not used as forage legume, this species is a good model legume in which a wealth of genetic, biochemical and molecular biology studies have been directed to elucidate bacteria-plant symbiosis, mycorrhizal interactions and nitrogen metabolism (Parniske, 2000; Udvardi, 2000; Orea *et al*., 2002). More studies should be carried out in *L. japonicus* plants in order to elucidate existing problems in the Lotus agriculturally used species, and in this way improve their field performance.

DISTRIBUTION AND SOWING

The genus *Lotus* includes more than 180 species that are found world wide except in very cold regions and the low land tropical areas of Southeast Asia and South and Central America. Different species of Lotus are currently used to improve pastures and hay quality where other forage legume species are not suitable (Papadopulous and Kelman, 1999).

There are four species of Lotus that have been domesticated and improved by selection and plant breeding: *L. corniculatus* (birds foot trefoil), *L. uliginosus* Schkuhr. (greater lotus), *L. glaber* Mill. (narrow-leaf trefoil) and *L. subbiflorus* Lagasca (hairy birdsfoot trefoil). This last species is the only annual Lotus with agricultural importance and is principally sown in South America (Asuaga, 1994a). *L. glaber* and *L. uliginosus* were denoted for many years as *L. tenuis* and *L. pedunculatus,* respectively.

The natural distribution of agronomical important Lotus species is mainly in the Mediterranean basin (Europe and North Africa). *L. corniculatus* is undoubtedly the species considered to have the greatest agricultural importance and the widest distribution. The other species like *L. glaber*, *L. uliginosus*, *L. subbiflorus* and *L. japonicus* have earned recognition for their intrinsic value. There are more than 175 other Lotus species about which we know little yet. The high number of Lotus species provides the genus with wide genetic diversity resulting in adaptations to different environmental conditions. This genetic diversity is the key in genetic breeding programs for various important agronomic characters.

The principal world regions where Lotus species are sown are South America, North America, and Europe, with 1.85, 1.39 and 1.38 million hectares respectively. Ten countries sow the 95 % of Lotus species in the world and more than 90% of this area is planted with *L. corniculatus* (Figure 1). Within other agronomic Lotus species, in Argentina *L. glaber* is sown in the Salado region (Vignolio *et al*, 1994) and in USA mainly in western and northeastern states (Blumenthal and McGraw, 1999). *L. uliginosus* is sown in New Zealand and coastal southeast Australia. In Uruguay, 50,000 ha of *L. subbiflorus* are sown annually which makes this country the main user of this species for pastures improvement (Asuaga, 1994a).

Figure 1. Countries with more than 100 thousand hectares sown with Lotus species. Under the name of the country, the sowing area in thousands of hectares is indicated.

BOTANICAL CHARACTERISTICS AND TAXONOMY

The genus Lotus comprises both annual and perennial species adapted to a wide range of ecological habitats. The agronomical species and also *L. japonicus*, are included in the subgenus *Edentolotus*, characterised for its non dentate style, section *Xantolotus*, composed with legume terete and laterally compressed yellow flowers (Arambarri, 1999; Izaguirre and Beyhaut, 1998).

Plants have erect or decumbent stems and pentafoliate leaves with two of the leaflets at the petiole base resembling stipules (Figure 2). Leaves are green to grey-green. Inflorescences with eight flowers (four for *L. subbiflorus*) are umbel-like cymes at the end of long axillary branches. All the agricultural important Lotus species mentioned above are self-sterile plants and must cross-pollinate mainly by insects. *L. japonicus* has the same morphological characteristics but the auto-compatible plants are self-pollinated.

The seedpods are about 2 cm long, contain 20 seeds, change from green to brown as they ripen and become coiled at dehiscence. Lotus species have seeds with round to oval shape, *L. corniculatus* and *L. glaber* present greenish to dark brown seeds as does *L. japonicus*, and *L. uliginosus* and *L. subbiflorus* present yellowish-brown to dark brown seeds.

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Key to identifying *L. japonicus* and commercial Lotus species

1. Perennial plants: glabrous and subglabrous. Banner 8-14 mm long, wings 7-12 mm long, keel 7-12 mm long, style 4-7 mm long, straight pods 15-40 mm long.
    a. Stems solid, sometimes hollow just at the base. Rhizomatous and arhizomatous plants.
        i. Leaflets of long upper leaves 2-4 times longer than wide, flowers 10-17 mm long.
            1. Stems green…………………………*L. corniculatus*
            2. Stems with dark pigmentation…………*L. japonicus*
        ii. Leaflets of long upper leaves 3-5 times longer than wide, flowers 7-10 mm long……………………...………..*L. glaber*
    b. Stems hollow, rhizomatous plants…………………….…..…*L. uliginosus*
2. Annual plants: pubescent. Banner 6 mm long, wings 5 mm long, keel 6 mm long, style 4 mm long, straight pods 9-14 mm long……………………………………………...……...…….*L. subbiflorus*

ADAPTATIONS TO ENVIRONMENTAL CONDITIONS

The agronomical Lotus species are best adapted to temperate and humid environments. *L. corniculatus* winter hardiness depends on cultivar origin. North American and European cultivars are most tolerant to low temperatures but are adversely affected by severe winters with fluctuating temperatures (Beuselinck and Grant, 1995). *L. glaber* is less winter hardy than *L. corniculatus*. However, *L. glaber* proved to be tolerant to low temperatures and ice-sheeting of water in central-east Argentina (Vignolio *et al*., 1999). *L. corniculatus* is more tolerant to low winter temperatures than *L. uliginosus*

Lotus species are adapted to infertile and acidic soils; they can be regarded as pioneer forage legumes and are moderately tolerant to soil salinity. Among the agronomical Lotus species, *L. uliginosus* presents more tolerance to acidic soil followed by *L. corniculatus*, independent of pH values. In soils with high aluminium concentration *L. uliginosus* and especially cv 'Maku' a tetraploid cultivar, shows more tolerance than *L. corniculatus* (Wheeler *et al*., 1992). *L. corniculatus* and *L. uliginosus* agronomic performance respond to improve soil pH, drainage, and fertility. Application of phosphorous and potassium on infertile soils enhances forage yield, seed yield and winter hardiness (Russelle *et al*., 1991). *L. uliginosus* also has a high tolerance to manganese (Wheeler and Dodd, l995). Different Lotus species and cultivars exhibit different degree of tolerance to edaphic conditions such as low pH and aluminium soil content, but every species are more tolerant to these factors than lucerne or trefoils (Wheeler *et al*., 1992).

In flooded soils, *L corniculatus* showed the best performance followed by *L glaber* and *L. uliginosus*. *L. corniculatus* tolerates poor soil drainage and is more tolerant to flooding than lucerne, though not highly tolerant. *L. glaber* also tolerates poor soil drainage and swampy conditions. This species has colonised the flooding pampa grasslands in central-east Argentina (Vignolio *et al*., 1999). Besides, *L. uliginosus* tolerates periods of water logging better than most forage legumes on account of root suberisation, production of adventitious roots, thickening of submerged stems and transpiration of high quantities of water (Shiferaw *et al.*, l992)

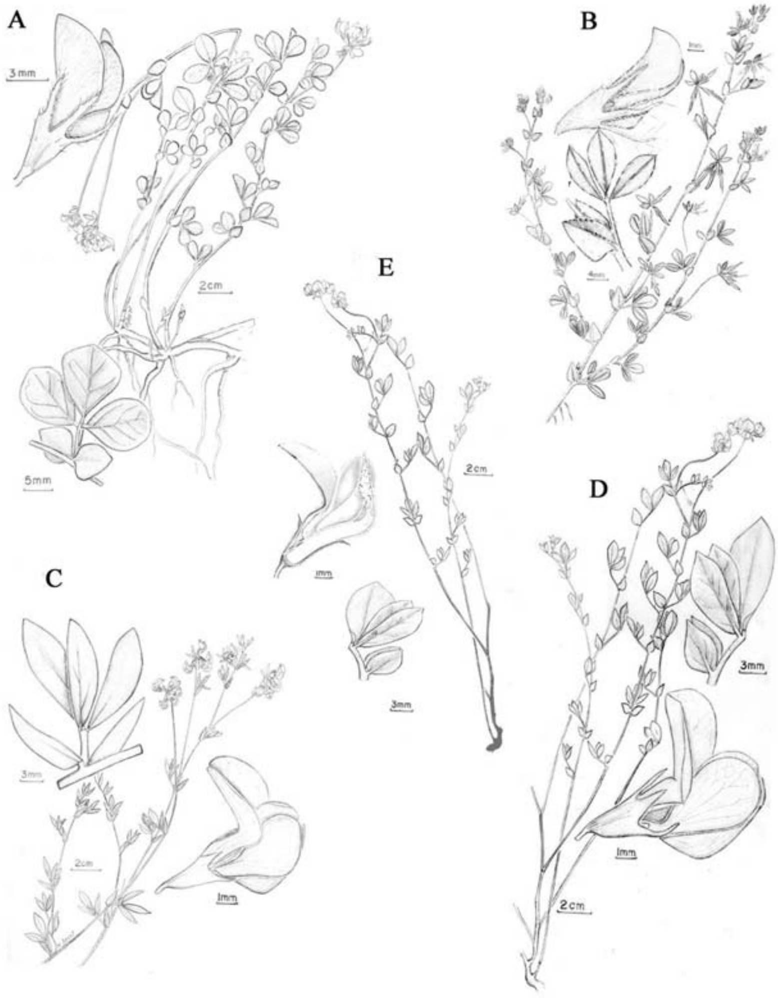

Figure 2. Botanical drawing of agriculturally important Lotus species and L. japonicus. (A) L. uliginosus;(B) L. subbiflorus; (C) L. glaber; (D) L. corniculatus (reproduced with permission from Izaguirre and Beyhaut, 1998. Las leguminosas del Uruguay y regiones vecinas Ed. Hemisferio Sur); and (E) L. japonicus.

Other edaphic factor that affects legume productivity-survival in the pasture is water soil content. *L. corniculatus* is more tolerant to dry soils than both *L. glaber* and *L. uliginosus*, although *L. corniculatus* is not as well adapted to high temperature or drought as *Medicago sativa* (Blumenthal and McGraw, 1999, Peterson *et al.*, l992). On the other hand, *L. glaber* is moderately drought tolerant because of its shallow rooting and therefore can withstand high summer temperatures. *L. uliginosus* is low tolerant to drought, much less than *L. corniculatus*. *L. subbiflorus* is an annual species with a very short cycle finished at beginning of summer, so the plants tolerance to the dry season has not yet been evaluated.

L. uliginosus has certain shade tolerance and is sown as ground cover in pine (*Pinus radiata*) plantations in New Zealand (Gadgil *et al*., l986). Moreover, *L. glaber* and *L. corniculatus* are intolerant to shading from other plants, particularly during establishment.

SEEDLING ESTABLISHMENT AND REGROWTH

Lotus species show poor seedling vigour and therefore a clean seedbed is essential for a good establishment. Because of their slow establishment ability, Lotus species does not compete well with broad-leaved weeds or aggressive grass weeds. The land for Lotus establishment must be well laboured. A uniform and firm seedbed is required since the small seeds need shallow sowing.

Lotus seeds are normally drilled or broadcasted in spring after conventional seedbed cultivations. Broadcasting, surface over sowing and direct drilling (sod seeding) have been used on marginal and rough land in north-east North America (Kunelius *et al*., l982) and New Zealand (Lowther *et al*., l996)

Spring is the best moment to sow Lotus species. If they are sown in autumn, sufficient time is needed for plants to develop before the onset of winter cold. Inoculated seeds of *L. corniculatus* and *L. glaber* are sown at 6-l0 kg/ha (over seeding 3-5kg/ha) for monocultures and at 3-5 kg/ha when sown with grasses. *L uliginosus* diploid cultivars are sown at 1-3 kg/ha and tetraploids at 1-5 kg/ha. On the other hand, *L. subbiflorus* sowing densities are 3-5 kg/ha (Asuaga, 1994).

Once established, *L. corniculatus* and *L. uliginosus* showed vigour and good growth rhythm and were capable of vigorous growth when well fertilised, but *L. glaber* showed moderate growth. Growth assays carried out in controlled conditions in our laboratory showed that the growth rate of *L. corniculatus* and *L. uliginosus* was higher than that of *L. glaber* and *L. japonicus. L. subbiflorus* had the lowest growth rate.

Lotus species grow slowly during early spring and have growth peaks in summer. In South America, they grow during early spring and have the growth peak in late spring (Asuaga, 1994b). *L corniculatus* growth in spring is directly proportional to the build-up of storage carbohydrates in the roots (Alison and Hoveland, l989).

L. glaber shows a moderate vigour of regrowth after defoliation, but it is more tolerant to intensive grazing than *L. corniculatus*. Regrowth of this last species depends on enough axillary buds and photosynthesising foliage being left after defoliation. This can be done by ensuring that adequate sites for regrowth remain after cutting crops for hay or silage, or by controlling the severity of defoliation when grazing. Prostrate cultivars of *L. corniculatus* cv ‘INIA-Draco®’ showed ability of more vigorous regrowth than erect types since more of their foliage escape defoliation. *L. corniculatus* growth varies from prostrate to erect with numerous stems arising from a basal, well-developed crown and branches arising from leaf axils. Primary growth originates from the crown but regrowth originates from buds formed at nodes on the stubble left after defoliation. Another Lotus species with good regrowth is *L. subbiflorus* because it has a prostrate habit that avoids an intensive defoliation.

Cultivars of Lotus species with an erect growth habit are the best suited for hay or silage cropping, essentially *L. uliginosus* and *L. corniculatus* cultivars. Two or three cuts of *L. corniculatus* can usually be taken depending on the duration of the growing season and yields are optimal at 6-week cutting intervals (Hoveland and Fales, l985).

BENEFICIAL PLANT-MICROBE INTERACTIONS

Lotus species are nodulated by both *Mesorhizobium loti* and *Bradyrhizobium* sp. (*Lotus*), usually with different levels of effectiveness. Rhizobial inoculation of seed by effective, specific strains *M. loti* (Díaz et al., 1995; Monza *et al.*, 1997) are needed when sowing, particularly on land where *L. corniculatus* and *L. glaber* has not been cultivated before. A similar management is needed with *L. uliginosus* and *L. subbiflorus*, but inoculating in this case with specific strains of *Bradyrhizobium* sp. (*Lotus*) when native rhizobia species are not present in soils. However, *M. loti* NZP2037 is a particular strain that can form effective root nodules in *L. uliginosus*, *L. glaber*, *L. corniculatus* (Vance *et al.*, 1987), and *L. subbiflorus* with different nitrogenase activities (Gonnet and Díaz, 2000). When *L. subbiflorus* was sown in soil with a history of *L. corniculatus*, ineffective root nodules without bacteroids were formed (Figure 3). Flavolans (condensed-tannins) accumulation resulting in red pigmentation of these nodules was observed (Monza *et al.*, 1992). After plant defoliation, root nodule senescence was induced and a new nodule generation was developed during plant regrowth (Vance *et al.*, l982).

In grass-dominant, grazed tall fescue / *L. glaber* swards on an alkaline hydromorphic soil in the Flooding Pampa area, Argentina, *L. glaber* in symbiosis fixed 27-42 kg N_2/ha annually (assessed by the nitrogenase activity method) while in similar tall fescue / white clover swards, white clover fixed 14-59 kg N_2/ha (Refi and Escuder, 1998). *L. uliginosus* in grass / *L. uliginosus* stand in which the legume constituted 22% of the total, fertilised with lime and phosphate, fixed 35 kg N_2/ha (Laidlaw, 1981). Amounts of 40 to 60 kg N_2/ha were cited for an improved upland pasture in Scotland (Wedderburn, l980). However, near twice nitrogen fixed was reported for inoculated *L. corniculatus* in North America (Heichel *et al.*, l985; Farnham and George, l994; Danso *et al.*, 1991). When grown in association with grass, most of the nitrogen in *L. corniculatus* was derived from nitrogen fixation. In contrast, the grass competitively took up soil nitrogen, and benefited by the transfer of fixed nitrogen by the legume (Farnham and George, l994).

Roots of Lotus species are infected by arbuscular micorrhizae and several results have shown that non-mycorrhizal Lotus plants required more phosphorous to obtain the same yield than mycorrhizal plants in a phosphorous deficient soil (Mendoza and Pagani, 1997). *L. corniculatus* mycorrhizal plants were more efficient in phosphorous utilization than *L. glaber* mycorrhizal plants and produced larger yields of shoot tissue per unit of phosphorous in a low available phosphorous soil (Mendoza, 2001).

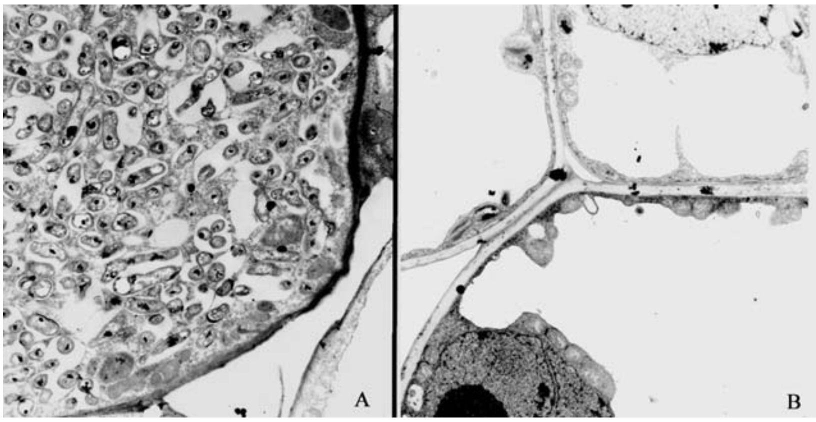

Figure 3. Transmission electron micrograph of nodular cells (3976X). (A) Effective nodule induced by M. loti strain BM1-5 in the roots of L. corniculatus. The cytosolic nodular cell containing the symbiosomes with bacteroids inside can be seen. (B) Ineffective nodule induced by the same strain in the roots of L. subbiflorus. A cell with a big vacuole and peripheral mitochondria can be seen (Monza et al., 1992).

ANIMAL NUTRITION AND BLOATING

Lotus species are highly acceptable forage either at vegetative stage for grazing or as conserved hay or silage. The feeding value is largely determined by the stage of growth at the time of utilisation since feeding value falls with increasing maturity and associated steminess. In *L. corniculatus* one-third of its dry matter accumulates at the late vegetative stage, and more than half the maximum amount of nutrients accumulates by then. Leaves generally have a higher mineral content than shoots, especially after the start of flowering (McGraw *et al*., 1986). The rate of forage digestibility decline is greater in late than in early season (Buxton et al., l985). The overall forage quality under drought conditions is better for Lotus than for lucerne because of its higher leaf:stem ratio, delayed maturity and improved quality of every plant fraction (Peterson *et al*., l992). The types and combination of essential amino acids in *L. corniculatus* are optimal for the production of high quality animal products (Waghorn *et al*., l990).

One of the most attractive characteristics of Lotus species is the presence of condensed tannins (CT) in the forage that prevents bloating in ruminants. This nutritional advantage is due to the tannins-plant proteins conjugation in the rumen. This reaction protects the protein diet from degradation to ammonium, thus allowing more protein to be utilised in the small intestine (Waghorn *et al*., l987; Jean-Blain, 1998). Voluntary intake and rumen digestion are adversely affected if there is a high concentration of CT in the forage fed (Barry and Duncan, l984). The nutritional value of CT depends upon their concentration in the forage and levels of 20-40 g CT/kg DM are thought to be beneficial (Barry, l989). A study with *L. uliginosus*, *L. glaber* and *L. corniculatus* showed that *L. uliginosus* had very high CT levels and concluded that the CT concentrations of this last species were high enough to be detrimental to protein availability and palatability for grazing animals. *L. corniculatus* has moderately low CT, while the CT levels in *L. glaber* may be too low to benefit protein utilisation. (Gebrehiwot *et al*., 2002). The tannin contents are different in the leaves, flowers, and stem and can vary as consequence of environmental factors, like drought (Carter *et al*., 1999; Gebrehiwot *et al*., 2002).

BREEDING OF LOTUS SPECIES

Among the positive attributes of forage Lotus for pasture use we can find: they have high nutritive value but do not induce bloat when direct-grazed, they can persist through reseeding or rhizomes and they tolerate infertile, acidic, drought or wet soil conditions. The improvements of Lotus species intend to make them more persistent, reliable, and productive. A great deal of genetic variation exists in Lotus species for further genetic improvement. Variation for agronomic and herbage quality characteristics including plant decumbency, winter injury, herbage dry mass, and *in vitro* digestible dry matter exists among different accessions.

Improving persistence is a complex task because plant death or loss of stands results from the combined effects of disease susceptibility, adverse environment, low reproduction rate, and limited genetic variation. The persistence of a Lotus stand can vary depending upon the amount of disease, the level of natural reseeding, and the type of cultivar. Characters of specific interest to improve the persistence of Lotus species include rhizomatous growth habit, disease resistance, condensed tannins, and hydrocyanide. The transfer of rhizomatous growth from the putative *L. corniculatus* obtained from Morocco into domesticated *L. corniculatus* with the purpose of increasing long-term persistence has been developed and evaluation studies are carried out in USA in cooperation with INIA-Uruguay

In Australia, the improvement of *L. uliginosus* pays attention to optimizing the levels of condensed tannins to provide bloat protection. In Argentina, selection among naturalized ecotypes of *L. glaber* has been the basis of cultivar development for alkaline and saline soils of the Pampas.

The breeding of cultivars with improved disease resistance, vigour, ability to reseed or to spread by rhizomes should make these Lotus species more reliable and productive. *L. japonicus* is related taxonomically with agronomical Lotus species and the derived knowledge of this model legume will be transferred to agronomical Lotus. The slow progress observed in Lotus cultivar improvement could be explained by cross pollination of species and the polyploidy nature of some of them. These features are not present *in L. japonicus*.

To determinate the relation between the species and *L. corniculatus* origin will be one of the tasks to attain species improvement. Biochemical and genetic evidences indicate that *L. japonicus, L. glaber,* and *L. uliginosus* together with *L. alpinus* would be the ancestors of *L. corniculatus*.

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