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Persistence and regeneration mechanisms in forage legumes

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Summary - Persistence, an important attribute of forage legumes, is influenced by climate, management, pests, and by their interrelations in a given environment. The main mechanisms of germination control, in the reproductive regeneration of annual legumes, are the seed bank dynamics, hardseededness and its breakdown pattern. Seed burial ability, seed dispersal and pod and seed characteristics and their relationship with animal ingestion and excretion are also very important. Persistence of perennial legumes is typically based on the vegetative regeneration even though seed regeneration can be also involved. Persistence strategies and ecological adaptation of perennials are related to one of the following growth characteristics: 1) presence of a deep taproot system and woody crown assuring persistence due to the survival of the original plants; 2) production of rooted stolons which can persist after the original plant has died and 3) plant spread and persistence by the formation of rhizomes. Moreover, other factors can affect the legume persistence such as management, adaptation capacity of species and its simbionts, resistance to virus, bacteria, fungi and insects. Nevertheless the legumes potential has not been fully exploited yet. Indeed new forage sources such as the "alternative legumes" are currently available. Great efforts are being made in breeding for developing persistent pasture legumes.

Key-words: annuals, seed bank, hardseededness, seed dispersal, perennials, vegetative regeneration

Résumé - *La pérennité, attribut essentiel chez les légumineuses fourragères dépend du climat, de la gestion, des maladies et de leur interaction au sein de l'environnement. Les principaux mécanismes de la reproduction sexuée des légumineuse annuelles sont ceux qui contrôlent la germination: la dynamique de la banque de graines, le taux de graines dures et les modalités de levée de la dormance. La capacité d'enterrer ses graines, de les disperser et les caractéristiques de la gousse et des graines par rapport au mécanisme d'ingestion et d'excrétion des animaux sont aussi très importants. La pérennité des légumineuses vivaces est basée sur les mécanismes de reproduction végétative même si certaines se régénèrent également par graines. Trois stratégies d'adaptation ont été développées: 1) présence d'une racine pivotante profonde et d'une structure ligneuse persistante, 2) production de drageons capables de survivre à la mortalité du pied-mère, 3) formation de rhizomes. D'autres facteurs tels que la gestion, la qualité de l'association symbiotique, la résistance aux maladies influent sur la pérennité des légumineuses. Néanmoins, le potentiel des légumineuses n'a pas encore été pleinement exploité et de nouvelles sources de fourrage à partir de légumineuses sont régulièrement proposées. De gros efforts doivent être entrepris pour développer des pâturages durables à base de légumineuses.*

Mots-clés: *légumineuses, banque de graine, graines dures, dispersion, régénération*

Introduction

In absence of persistence, all other measures of success of forage legumes, such as productivity, quality, etc., are meaningless (Carr, 1997). According to Smith (1989), persistence is "the survival of plant material against specific stresses unique to the existing environment". Other definitions include also the important aspect of productivity over time in a given environment, in addition to the maintenance of an adequate number of seedlings (annuals) or viable plants (perennials) at the end of the crop cycle. For example, the persistence of white clover is defined as the ratio between DM yield in the fourth and fifth seasons and DM yield over the entire period of evaluation (Piano and Annichiarico, 1995). In addition the relative legume content in the sward and the ground cover are also considered as stand persistence indices. Forage legumes persistence is influenced by plant genotype, climate, pests, management, ecological situation and especially by their interrelations. Since these factors differ among environments, forage persistence is a complex and highly specific problem. In this paper, some of the main aspects characterising and affecting persistence are discussed with emphasis on the most important annual and perennial forage legumes.

Reproductive regeneration and persistence in Mediterranean annual legumes

Seed constitutes both the start and the end of the life cycle of annual legumes and its production is an obligate persistence mechanism. The key requirements for the persistence of annual legumes are that sufficient seed must be produced and survive under grazing, that breakdown of hardseededness should ensure adequate seedling number at the onset of the following wet season, and that these seedlings survive to maturity and set seed. In addition, a reasonable proportion of seed must survive for more than one year because unsuitable weather conditions (e.g. absence of rainfall, spring frost, etc.) or management interventions may prevent seed set in one or more years. From an ecological point of view, flowering, seed production, seed dormancy, seed dispersal, germination and seedling survival are the key processes determining the adaptation of species to any climatic environment (Cocks, 1997a).

Time to flowering and maturity

In a given environment, the reproductive phase should take place early enough for adequate seed set to occur before the advent of drought. Because of the climatic variability and diversity among environments, the maturity of annual legume species varies greatly throughout the Mediterranean region and even in restricted areas (Piano, 1993). Important aspects of maturity are time from appearance of the first flower to obtainment of viable seed, duration of flowering, rate of inflorescence production and rapidity of seed ripening.

Flowering of *Trifolium subterranean* and annual medics occurs in response to daylength and temperature, but cultivars vary considerably in their response to these factors. In addition grazing can delay floral initiation and flowering. Generally, annual medics flower earlier than subterranean clovers. Flowering time is the most responsive of all reproductive strategies to environmental changes as shown by the differences in behaviour of the same varieties in different environments. For instance, in Sardinia flowering of subterranean clover is delayed compared to Western Australia and the difference in flowering time between the same cultivars (Dwalganup and Seaton Park) is less than 10 days compared with about 30 days in Australia (Piano, 1993). Francis and Gladstones (1974) found that short and fast-flowering strains of *T. subterraneum* produced mature seeds faster than long and slow-flowering strains in Australia. In the Mediterranean basin, ecotypes that flower for a short period have an advantage in marginal areas where the rainy season ends abruptly (Piano, 1993). However with intermittent rainfall in spring and dry period during flowering, seed yield of a short flowering period variety may be reduced, whereas a prolonged flowering variety may flower again under more favourable conditions. According to Piano and Pecetti (1997), within subterranean clover varieties with similar earliness (flowering date), a variety characterised by rapid flowering and seed ripening is favoured in comparison with a longer flowering and seed maturation variety, because the reproductive stage occurs when climatic conditions are more favourable, so allowing higher seed yields. Conversely, in environments with a good soil water content after the vegetative cycle, the moisture favours pathogen and saprophyte attacks on the mature seeds of early varieties, while late maturing varieties are able to escape spring frost damages and to exploit late spring rains. Selection for flowering time has allowed to extend cultivation areas of annual forage legumes.

Earliness in *T. subterraneum* was less important than seedling density at the break of the season for obtaining high forage availability early in autumn (Carroni *et al.*, 1995). On the other hand, some early varieties of subclovers are preferable as cover crops in vineyards, for their fast establishment and shorter life cycle compared to that of the grape vine and also for other alternative uses, such as firebreak lines, etc. (Pardini *et al.*, 1995).

Seed bank

Seed bank is defined as "a reserve of viable seeds present in the soil and on its surface" based on dormancy mechanisms which prevent germination (Russi *et al.*, 1992a). The store of seeds in the soil is increased by seed production and depleted by seed death, germination and predation. There are three sources from which seed enters the bank: (i) parent plants *in situ*, a direct source, (ii) parent plants displaced in time or space so that the seed reaches the soil seed bank by a range of dispersal mechanisms, and (iii) sowing by man to meet particular objectives, even though the natural sources also apply to these sown pastures after they have been established (Pearson and Ison, 1987).

In contrast with annual grasses, Mediterranean annual legumes can have a persistent seed bank where individual seeds may live for several years. In fact, these latter species produce seed in spring and only a variable proportion of that seed germinate in the following autumn. This strategy allows the species to escape drought and high temperature stresses over summer, to survive occasional summer or failed autumn rains and to still maintain seed viability.

The size of a soil seed bank and its changes from year to year reflect inputs from current and past generations of parent plants and also according to rainfall, environment, species, season and management (Loi *et al.* 1999). In grazed grasslands, these changes depend also on the seed fraction consumed by animals. In Mediterranean pastures dominated by annuals, the seed bank has an ecological and agricultural importance because its increase determines an increase of plant density, which strongly affects the pasture productivity and its botanical composition (Russi *et al.*, 1992a; 1992b).

Hardseededness and other mechanisms regulating germination

In order to survive in the soil, seeds must not only remain viable, but also not to germinate. Hardseededness is a physical exogenous dormancy, caused by the impermeability of the seed coat that maintains the quiescence of the embryo by preventing seed imbibition with water. Seed coat impermeability is related to the presence of a suberized thickening surrounding the Malpighian layer; the degree of this thickening is influenced by genotype, conditions during the growing season and dehydration rate of the seed (Patanè and Bradford, 1993; Russi, 1993).

Hardseededness, due to both environmental and genetic factors, is of great ecological significance for legume persistence (Taylor and Ewing, 1988). It is the most important "long term" dormancy mechanism in Mediterranean legumes because it maintains the seed bank from year to year. It may or may not be associated with other forms of dormancy. The level od hardseededness varies widely among species. For instance, in seeds that remained on the soil surface for 2 years, it ranged from about 90% in *Biserrula pelecinus* and *Ornithopus compressus* to about 10% in *T. subterraneum* (Loi *et al.*, 1999) and reached negligible values in *O. sativus* whose seeds are completely soft. Therefore, the natural presence or the use of species mixtures with hard and soft seeds can contribute to legume stability over time.

To assure persistence, the pattern of softening is more important than the initial level of hard seeds. The evolution and pattern of hard seed breakdown of the same Sardinian accessions of *Medicago polymorpha* changed form year to year according to the environmental conditions (Porqueddu *et al.*, 1996).

Dry heat and wide temperature fluctuations play an important role in hard seed breakdown (softening) of species adapted to Mediterranean ecosystems which normally experience summer drought. Temperature mediates natural hard seed breakdown through (i) the heating effect of solar radiation on the surface layers of soils (insolation) coupled with night-time cooling, and (ii) through the brief but intense heating of the soil surface layers caused by manmade or natural fires (Probert, 1992).

According to Taylor (1981), softening of *T. subterraneum* seeds occurs in two stages: a preconditioning period of high temperature before a short period of diurnal alternating temperatures (60/15 °C). Both requirements are met in summer (on soil surface) and as a result most of the seeds become soft by early autumn, when false break risk is still high. In this case, the term "short term hardseededness" is used. Hard seed in buried burrs breaks slow than in surface pods: the softening in subterranean clover may fall dramatically if seed is buried 10 cm (Taylor and Ewing, 1988). Cultivation may also affect seed softening. Taylor (1985) recorded greater emergence of subterranean clover in the year following *minimum* tillage as compared with conventional tillage, although the greater burial of seed by conventional cultivation resulted in better long-term survival of seed.

In contrast with *T. subterraneum,* the softening of *O. compressus* seed was more rapid when buried 2 cm below the soil surface than when left on the soil surface, while in *B. pelecinus* was intermediate (Loi *et al.*, 1999). These three species have different requirements for their seed softening: *T. subterraneum* requires high temperature in the second stage of hard seed breakdown, *O. compressus* requires darkness and *B. pelecinus* requires a high heat input in the first stage.

Seed softening of annual medics requires alternating temperatures in the vicinity of 35/10 °C (Taylor, 1993). Because these temperatures do not normally occur until late autumn, the loss of hardseededness of these species is delayed in comparison to *T. subterraneum.*

In addition to hardseededness, seeds of subterranean clover exhibit: (i) a physiological dormancy due to an immature embryo and (ii) a physiological or embryo dormancy, due to unsuitable ambient temperatures, that prevent germination after sporadic summer rains that would be insufficient to support the growth of seedlings (Quinlivan, 1971) and prior to the formation of an impermeable seedcoat (Fairbrother and Rowe, 1995). In fact, embryo dormant subterranean clover seeds can survive in the soil for 14 days without loss of viability. Embryo dormancy is not considered as important as hardseededness in Mediterranean environments. Inhibition of germination at high temperatures occurs in many clovers (*T. resupinatum*, *T. subterranean*, *T. michelianum*), due to the maintenance of dormancy (Cocks, 1997b), while germination can continue at lower temperatures. Another mechanism is slow germination until effective soil moisture is assured (e.g. *T. clypeatum*).

From an agronomic point of view, it can be useful to overcome seed dormancy with the objective of improving the establishment of desirable legumes from seed in natural pastures. Seed of *M. ciliaris* and *M. rugosa* were exposed to mechanical, physical and chemical treatments to identify the factors which determine their dormancy and the actions required to remove it (Patanè and Bradford, 1993). Hand scarification and ultra-low temperature in liquid nitrogen were the most effective treatments in breaking dormancy. The two species responded differently to the same scarification treatments, due to differences in thickness of the Malpighian cell layer of the seed coat, as revealed by using an electron microscopy.

Seed burial, dispersal and predation

Some species bury their seeds, so limiting the dispersion to the base of the mother plant (basicarpy). The success of *T. subterraneum,* as a pasture species, is linked to its ability to bury pods, escaping the severe overgrazing effects on seed bank size (Rossiter *et al.*, 1985). The burial ability of the same variety may vary according to soil texture and moisture. In general, the better buried burrs produce more viable seeds. Floral peduncles are strong and thick in *T. subterraneum*, whereas they are longer and less vigorous in *T. brachycalycinum*; the latter normally does not bury any burr. Pure lines of *T. subterraneum* and *T.*

brachycalycinum were examined to assess the occurrence and depth of burial, the percentage of buried burrs and the amount of developed burrs 21 days after anthesis. The high differences recorded between species seem related to different burr burial strategies, which are associated to distinct edaphic specializations. *T. subterraneum* has a remarkable burial ability, which is important for the regular development of its reproductive structure, and is common in sandy and friable soil; *T. brachycalycinum* instead is frequent in more compact and clay soils. As the environmental constraints of the origin sites of these populations increased, their burial ability increased (Piano and Pecetti, 1995a). Moreover, an artificial complete prevention of seed burial, by covering the soil with a plastic film, caused a strong reduction of seed yield in both species, the relative effect was slightly smaller in *T. brachycalycinum* (Piano and Pecetti, 1995b). Differences in burial ability within species is also important against insect pests. *Bruchidius trifolii* Motschulsky, a coleopter present in the Mediterranean basin but not in Australia, destroyed from 50 to 100% of *T. brachycalycinum* 'Clare' seeds (not buried) in field during summer (Sulas *et al.*, 1993). This destruction dramatically reduced both the seed bank and, consequently, the legume persistence capacity, despite a satisfactory seed production of 4,500 seeds m^{-2} in the year of sowing. On the contrary, seed damage to buried burrs of *T. yanninicum* 'Trikkala' was negligible.

Amphicarpy is the presence of aerial and underground dimorphic flowers and pods in the same plant (e.g. *Vicia sativa* var. *amphicarpa,* subterranean vetch; *Lathyrus ciliolatus*). Subterranean vetch is common in heavily grazed grasslands of West Asia with 250 mm of rainfall (Christiansen *et al.*, 1996). This species produces both aerial pods, containing 5-7 seeds, that shatter and disperse seeds near the mother plants, and below ground pods buried 1-2 cm under the plant. These buried seeds are protected from grazing, predation, desiccation and fire, being maintained in the same place where they are produced.

Pre-dispersal seed predation by insects can vary with plant type and habitats. For example it ranges from 2-6% in *Vicia sativa* to 39% in *Onobrychis viciaefolia* (Crawley, 1992). In general, artificial seed burial reduces predation rates by insects.

The movement of seeds from a parent plant to the seed bank depends on the pattern of seed release and dispersal. There are three dispersal mechanisms by the mother plant: (i) dispersal by natural elements or by adhesion or attachment to the coat of animal, (ii) ingestion by grazing herbivores, and (iii) dispersal by non-herbivorous predators such as ants. The significance of natural elements in seed dispersal depends on the species (morphological differences), climate and topography of the site. When seed comes into contact with the soil, dispersal can continue with run-on and run-off of water (Pearson and Ison, 1987). Variation in topography creates different microsites for the lodgement of seeds.

Light inflorescences can be dispersed by wind, but this kind of dispersion is less important because the legume seed is dense and usually without appendages such as glumes or bracts of grasses. Spiny pods and seeds of annual legumes can be dispersed by becoming attached to hooves, hides and wool, and by ingestion and subsequent excretion in the *faeces* of animals.

Pod characteristics, seed size and ingestion by ruminants

Legume persistence is affected by both undergrazing and overgrazing. Prior to flowering, grazing management should allow weed control, while after flowering, grazing management should assure the formation of seeds. Therefore, it is important to define a range of grazing pressure by which persistence is guaranteed.

In summer, small pods are less easily selected by grazing sheep than large pods. At the same time, even though the first ones are better incorporated in the soil, they are subjected to greater predation by seed-harvesting ants, which represent an example of non-herbivorous predators, causing losses before seed enters into the seed bank or from the soil seed bank. Vulnerability to grazing is the single most significant weakness in annual medics whose high quality pods, with about 20% of crude protein, represent an important feed resource for the sheep grazing stubble during the summer season (Sitzia and Fois, 1999). The ability to produce large numbers of small, hard seeds, contributes to the ecological success of legumes in natural pasture (Carr, 1997); for pasture persistence, seed number is more important than seed weight, but larger seed species will emerge more vigourly from greater depths. Seeds eaten by livestock are mostly digested. However a certain fraction of seeds ingested by small ruminants is able to escape and is thus returned to the seed bank. The proportion of ingested legume seeds that passes undigested has been related with the animal species, seed size, roughage intake, digestibility of the associated diet, amount of the seed consumed etc. Small seeds are more likely to avoid mastication and rumination, because the smaller the seed size is, the faster the rate of the passage through the digestive tract of animal is. According to Thomson (1990), seeds heavier than 2 *mg* are unlikely to survive in large number, while up to 60 % of seeds lighter than 1 *mg* will survive. Moreover, seed survival after ingestion is lower in sheep, which excrete a lower proportion of ingested seed, than in cattle, but their *faeces* are likely to create most space for germination of seedlings.

Data from Central Italy (Pardini, 1993) have shown that the fate of the seed produced in a *T. subterraneum* sward was as follows: 22-50% were ingested and digested, depending on grazing pressure and animal species; 3-18% were ingested and returned viable to the soil; 14-20% were stocked in the seed bank as hard seed; 33-41% produced seedlings at the following generation and 2-5% were lost. The passage of legume seeds through the digestive tract of cattle and their survival in *faeces* was studied over a 160-hour period after ingestion by Gardener *et al.* (1993). Survival of the legume seeds was largely dependent on the fraction of hard seed in the sample. Wide variations in seed retention time to be excreted were due to specific gravity (density), proportion of hard seeds and seed size. In particular, the digestibility of legume seeds was inversely related to hardseededness.

Small seed size is, at the same time, a potent method of protection from grazing and an efficient mean of seed dispersal by *faeces*. Dissemination by livestock has been suggested from some authors as a way to spread new pasture species over inaccessible areas. Sixteen native legumes belonging to *Trifolium*, *Medicago*, *Hippocrepis* and *Scorpiurus* were compared in northern Syria for their passage through the animal; moreover the role of sheep in transporting legume seeds from one area, where the same legumes were grown, to an un-improved degraded pasture was also studied (Ghassali *et al.*, 1997). *Maximum* seed recovery after ingestion occurred after 72 hours and it resulted 70% for *T. campestre* and *T. tomentosum*, 20% for medic species and 2% for *H. unsiliquosa*. The contribution to the transferred seed by sheep to the un-improved pasture was higher for the small seed species (i.e. *Trifolium*).

Vegetative regeneration and persistence of Mediterranean and temperate perennial legumes

Persistence strategies and ecological adaptation of perennial legumes are related to their development and growth characteristics. Their persistence is typically based on the vegetative regeneration of the original and/or new plant, but regeneration from seed can also occur. Depending on their different strategies, herbaceous perennial legumes can be divided in three classes:

1) Species with a deep taproot system and woody crown whose persistence depends on the survival of the original plants.

Drought tolerance of *Medicago sativa* L. (lucerne) is based on its deep taproot system particularly in mature plants and favourable soil types. The crown is formed by branching at the base of the plant, just above the ground, and represents the main source of regeneration after defoliation. Although lucerne defoliation is followed by a relatively rapid regrowth, the subsequent build-up of carbohydrate reserves is rather slow. Therefore, frequent defoliation results in depletion of root reserves and eventual plant death. Forage production and quality, reserve accumulation and persistence of lucerne are strongly affected by management (Gervais and Giraud, 1987) and by susceptibility to diseases. Usually lucerne is cultivated under irrigation. However, well-watered lucerne may utilise more photoassimilates for herbage growth and less for carbohydrate storage in roots than water-stressed lucerne and thereby could be more susceptible to winter injury with reduction of persistence (Peterson *et al.*, 1992).

Trifolium pratense is used as an alternative to lucerne because of its tolerance to poorly drained, low fertile and acidic soils. It has been characterized as a short-lived perennial which produces its greatest forage yield in the year after establishment. In order to obtain *optimum* persistence and forage yield, *T. pratense* should be harvested 3 times a year, when plants are between the bud and 20% bloom stage (Wiersma *et al.*, 1998).

Light or medium defoliation intensities at the bud or flower phenological stage allow an acceptable stand persistence of *Onobrychis viciaefolia* (Mowrey and Matches, 1991).

The effective coexistence of a double regeneration mechanism occurs in sulla (*Hedysarum coronarium* L.), a short-lived perennial. When sulla plants have not produced seed, due to hay cutting or severe grazing, autumn regeneration will start not only from regrowths of original plants but also from new seedlings, whenever a soil seed bank is present. On the contrary, when sulla plants have produced seeds, the mobilisation of reserves toward the fruits can cause weakening and death of roots, leading to a reduction in regrowth. Moreover, a low seed regeneration can be caused by the high amount of hard seeds produced in the previous spring, if there is a lack of older and softer seed. Experimental results confirm that sulla has a self-reseeding capacity to be exploited in order to improve its scarce plant persistence (Sulas *et al.*, 1999). An appropriate management, aimed at assuring a *minimum* seed set in the year after sowing, seems to be the only effective way of prolonging the short duration of the sulla meadow.

2) Species forming clonal patches of rooted stolons which can persist after the original plant has died.

In *T. repens* (white clover), the stolon is the basic structural unit of the plant; growth of lateral stolons involves adventitious root development which allows these stolons an individual existence when the seminal root system dies. Both the negatively geotropic growth of stolons and the ability of stolon apices to recover from defoliation contribute to the success of white clover as a forage legume. In particular, the capacity to develop independent root systems from each node or a branch favours the performance of white clover in intensively grazed systems, as it enables the species to tolerate and even spread following fragmentation of plants by defoliation or treading. Due to the constant turnover of nodes, white clover is considered a species with a superficial root system and a low root/shoot ratio that affects negatively the persistence and hence usefulness of white clover in summer-dry regions.

However white clover is able to survive during summer drought by maintaining an adequate number of live stolons to regenerate the stand via growth from the stolon tips. In that period large parts of the basal portion of the stolon die, while the terminal end remains alive to produce new growth in the fall. Nevertheless fungal diseases may reduce white clover stolon survival during summer dormancy (Pederson and Pratt, 1995).

Persistence and its relationship with other agronomic traits were studied in landraces, natural populations and varieties of "Ladino" white clover at Lodi (Italy), (Piano and Annichiarico, 1995). Natural populations showed greater persistence than landraces. The persistence of ecotypes was positively associated with stolon density, DM yield in the third year, and internode length, while it was inversely related to seed yield and its components.

Persistence and spread of white clover is also possible via establishment from seed. Where plant canopies are more open, white clover may persist as an annual (Hollowell, 1966). The appearance and survival of *T. repens* seedlings from naturally deposited seed were monitored for 3 years under different management and sites in New Zealand (Chapman, 1987). Only 4.4% of the seedlings survived and formed established, stolon-bearing plants, representing a mean recruitment rate of one seedling per 5.5 m^2 per year. Such a low recruitment indicates that seedling regeneration plays only a minor role in the *T. repens* persistence in that environment. However, the maintenance of genetic diversity within the stolon populations through occasional seedling establishment should be recognized as having an important ecological role.

3) Species that both persist and spread by the formation of rhizomes, or adventitious shoots arising from the root system in addition to the initial crown.

The rhizomatous habit and the creeping rootedness (development of new shoot from horizontal roots) of lucerne are two mechanisms that allow its spreading and increase its persistence under grazing (Pecetti *et al.*, 1995). In fact rhizomatous lucerne is characterised by the emission of rhizomes which allow plant to grow horizontally, tolerate drought and trampling, survive longer and produce more forage under grazing conditions than tap-rooted lucerne. The bigger and deeper crown of rhizomatous lucerne contribute, compared to the conventional one, to a higher tolerance under harsh climatic conditions so increasing plant persistence (Ben Chabaane and Delgado, 1995). Rhizomatous lucerne presents a quite stable protein content and is very interesting for grazing in semi-arid regions, even if its growth and production are lower than those of the conventional one.

Trifolium ambiguum (Kura clover) is a perennial legume with a rhizomatous growth habit that confers an excellent tolerance to grazing. Its crown is similar to that of most perennial clover except for the presence of lateral roots: the rhizomatous root stock may spread as much as 1 m in diameter. Another unusual characteristic of this species is its preponderance in roots (2.74 root-shoot ratio vs 0.16 for *T. repens*). *T. ambiguum* is a relatively new forage legume and, due to its extensive rhizome and root development, has the potential to be a major grazing crop. In fact, most of the commonly used legumes are non persistent under continuous grazing, particularly in periods of heat and drought. So *T. ambiguum* represents a novel legume to overcome problems of persistence due to lack of winterhardiness, disease susceptibility, and/or sensivity to frequent or close defoliation in Northern USA (Taylor and Smith, 1998). From the region of origin, Caucasus, it has been introduced in other temperate part of the world succeeding also in dry regions, such those in Australia. The strongest attribute of *T. ambiguum* is its longevity; in the high rainfall of Southeast Australia it has persisted up to 17 years. The extensive crown and root systems of established stands confer the ability to maintain an adequate concentration of total non-structural carbohydrates under a range of defoliation regimes.

Galega orientalis Lam (Fodder galega) is a quite new fodder crop of temperate regions appreciated for its excellent forage quality for all kinds of domestic animals. Fodder galega is a tap-rooted (up to 60-100 cm) herb, which spreads and propagates also vegetatively with underground stolons (Nommasalu and Meripold, 1996). These stolons spread horizontally in soil up to 10-30 cm forming new above-ground shoots and developing additional roots. Then, the young shoots become independent plants. For these growth characteristics, fodder galega is a relatively drought resistant and very persistent species.

Other factors related with legume persistence and future perspectives

In addition to the factors already mentioned (e.g. tillage and harvest interventions, grazing interaction, etc), other factors can affect legume persistence. The adaptation capacity of forage legumes and its simbionts and their resistance to several diseases and insects are also fundamental for persistence in particular environments. For instance, the ability of rhizobia to persist in the absence of its legume host is crucial for a successful establishment and persistence for pasture systems. The lack of rhizobia or nodulation failure, particularly in new cultivation environments, can reduce legume persistence. Nevertheless, forage legumes have developed several adaptation mechanisms to Mediterranean and temperate climates and their potential has not been fully exploited yet. In fact, new species with forage potential, such as the "alternative legumes" in comparison with subterranean clovers and medics, are being considered. These alternative species have been successfully cultivated Western Australia and New Zealand and could be used in peculiar edaphic and environmental niches. Moreover, some specific species characteristics conferring persistence, such as the deep root system of *Lotus purhsianus*, should be effectively exploited both in conventional and alternative legumes.

Several perennial legumes occur in Mediterranean rangelands, even if, under heavy grazing pressure annuals are dominant (Roggero and Porqueddu, 1998). However they have not been extensively investigated yet. There is a great opportunity to exploit the genetic resources of perennial legumes, particularly of those species that are able to tolerate or escape drought and to associate vegetative propagation with self-reseeding ability. In fact great efforts are been made in breeding for developing persistent pasture legumes. Some example of these efforts are: the selection for creeping types of lucerne to improve tolerance to treading and grazing, for cold resistance, specific flowering time for targeted environments of cultivation to ensure seed yield and regeneration, burr burial ability in *T. subterraneum*, hard seed level at the end of summer, resistance to diseases, etc.

Finally, persistence cannot be taken into consideration *per se* but only in presence of all other success characteristics of forage legumes, and a better knowledge of the mechanisms determining and affecting persistence (e.g. ingestion by ruminants, role of fire, etc.) should be achieved and transferred at farm level to set up an appropriate management of forage legumes.

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