10 Red Clover in Cropping Systems

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Abstract
Red clover has played an important role as a supplier of reactive nitrogen to cropping systems in European agriculture for hundreds of years. Today, it is mostly valued for its good nutritional properties for ruminants, and for reducing the need for nitrogen fertilizer inputs. Red clover is a short-lived perennial capable of producing dry matter yields in the range of 9–18 t/ha/year, but the yield declines sharply after the first 2 harvest years. It forms an efficient symbiosis with *rhizobium* and can fix in excess of 350 kg/ha of nitrogen, most of which is transferred to the harvested biomass. Red clover is rich in protein and minerals, and contains unique compounds that improve nitrogen use efficiency at farm level and that improve the quality of animal products for human consumption with respect to fatty acid profiles, compared with white clover or lucerne (alfalfa). Red clover is usually grown mixed with grasses. It should be sown in the first half of the growing season and is easy to establish. It thrives in most soils but does not tolerate very acid or wet soils. Systematic breeding has been carried out for more than 100 years, and the main focus of breeding programmes is to increase crop persistence through improved disease resistance and winter hardiness.

Introduction

Worldwide, red clover (*Trifolium pratense* L.) is the second most important sown forage legume after lucerne (alfalfa; *Medicago sativa* L.) in terms of seed sales and in the number of cultivars available (Boller *et al.*, 2010). As indicated by seed sales (Table 10.1), it is an important component of short-term leys, particularly in northern and Eastern Europe and North America. Red clover is indigenous to Europe, the Near East, North Africa and central Asia (Boller *et al.*, 2010), and has a long history of cultivation in Europe. It was domesticated and cultivated in

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Table 10.1. Certified red clover seed sales in Europe in 2010. (Data from European Seed Certification Agencies Association, 2014.)

Country	Quantities (t)	Country	Quantities (t)
Austria	74	Lithuania	0
Belgium	4	Luxembourg	0
Croatia	7	The Netherlands	0
Czech Republic	456	Poland	0
Denmark	349	Romania	6
Estonia	66	Serbia	67
Finland	49	Slovakia	13
France	2,342	Spain	7
Germany	797	Sweden	388
Hungary	20	Switzerland	60
Italy	199	UK	3
Latvia	60		

southern Spain in the 3rd and 4th centuries. By the middle of the 16th century red clover was grown in the Netherlands and by the 17th century across most of Europe (Kjærgaard, 1995). The species played an important role in transforming the increasingly unsustainable production systems that prevailed in Europe in the 16th and 17th centuries and provided the basis for a substantial increase in agricultural production as it became part of new cropping systems (Kjærgaard, 1995). The major system change was the extension of the crop rotation from a 3- to a 4-year system, and the replacement of the fallow year with the cultivation of red clover, which improved soil fertility. As a result of this system change, wheat yields in Europe more than doubled (Grigg, 1992).

Red clover is a diploid and out-crossing species that is pollinated by bumblebees (*Bombus* spp.) and honeybees (*Apis mellifera* L.). The ease of establishment, high seedling vigour, rapid growth, high forage quality and excellent soil improvement characteristics are important advantages. It is a temperate crop adapted to a wide range of soil and environmental conditions (Frame *et al*., 1998). Another important feature of red clover is the reduced rate of decline in digestibility with advancing maturity in comparison with grasses. Thus, mixing red clover with grasses increases the time span within which a highly digestible crop suitable for feeding high-yielding dairy cows can be harvested (Rinne and Nykänen, 2000; Dewhurst *et al*., 2009).

Red clover is most commonly used as silage for winter feeding of ruminants. It can be included in grazed swards, but this will decrease its production potential (Frankow-Lindberg, 1985) and it is not as well suited to grazing as white clover. In addition to forage use, red clover can be cultivated for green manuring. It can either be sown on its own when the nitrogen fertilization effect is utilized by the non-N_2 fixing plant growing after clover, or undersown in a non-N_2 fixing plant. Globally, the use of red clover has decreased since the 1950s due to the access to cheap nitrogen fertilizers, but increasing prices of these and a stronger emphasis on home-grown protein in recent years have rekindled interest in this legume.

Botany

Morphology

Red clover is a short-lived perennial species with peak production during the 2–3 first harvest years (Frame *et al.*, 1998). It has an upright growth habit that makes it most useful for mowing. The plant forms a rosette, and the regrowth after the first cut is produced from axillary buds formed at the base of the plant. It has a deep taproot and is moderately tolerant of drought conditions.

Red clover is a quantitative long-day species, but the response differs between populations. There are early, medium and late types depending on its flowering response to the day length. Early red clover types (i.e. those least responsive to day length) produce more than one generation of axillary buds, so can withstand several cuts per year. Late types (i.e. those most responsive to day length) produce only one new generation of axillary buds per year, so can tolerate only one cut per year. The early types are suitable for southern latitudes and the later for northern latitudes. In North America, the types are termed medium or double-cut, and mammoth or single-cut types, respectively (Boller *et al.*, 2010).

Plant breeding

In 1742, Carl von Linné noted in Sweden that 'Spanish clover, which is the same species as our native red clover, is bigger in size, but not very persistent. Further, it does not reseed itself as our native species does' (Osvald, 1962). Selection among native plant material for persistent and productive material therefore formed the basis for the breeding of well-adapted red clover cultivars (Boller *et al.*, 2010). Before red clover was the subject of targeted breeding, locally adapted populations, termed landraces, were developed by harvesting and re-sowing seed within a restricted area (Boller *et al.*, 2010). Swiss landraces are genetically distinct from Swiss natural populations (Hermann *et al.*, 2005), which indicates that the cultivated landraces were valued by farmers and that care was taken to maintain their traits.

Breeding based on landraces began as early as 1910 in Sweden (Sjödin, 1986). The most important characteristics bred for then related to resistance to nematodes and *Sclerotinia trifoliorum*. These traits as well as general persistence and winter hardiness continued to be the focus of efforts (Sjödin, 1986), not just in Sweden, but globally (Boller *et al.*, 2010). Systematic red clover breeding in other parts of the world began after World War II (Boller *et al.*, 2010), and in many countries more persistent and disease-resistant cultivars were produced compared to the landraces. The most widely used breeding methods are recurrent mass selection and maternal line selection (Boller *et al.*, 2010).

The development of tetraploid cultivars began in the 1940s. A common way to produce these is by colchicine treatment of young seedlings (Boller *et al.*, 2010) which then are used for intercrossing. Tetraploid plants are larger than diploids, with improved disease resistance and persistence, but seed production has proved to be a challenge (Sjödin, 1986; Boller *et al.*, 2010).

Currently, there are 267 cultivars of red clover on the Organisation for Economic Co-operation and Development (OECD) list of cultivars eligible for seed certification (OECD, 2013). Seed production in Europe occurs mainly in France and Germany, but can be carried out as far north as central Sweden. Since red clover is an out-crossing species, genetic drift may occur within a short time span in environmentally challenging environments (Collins *et al.*, 2012). This means that care is needed to retain the properties of a cultivar over time when it is multiplied.

Agronomy

Seed mixtures

Red clover is sown mostly in mixtures with grasses as companion species. This practice increases total yield and protects against weeds and plant diseases (Frankow-Lindberg *et al.*, 2009a). In addition, the risk of nitrogen leaching from mixed grass–clover swards is less than in pure red clover (Frankow-Lindberg and Dahlin, 2013). The recommended proportion of red clover seed in the seeding mixture varies depending on the main production objective of the crop. Where maximum protein yield is the objective, the stand should be dominated by clover. To achieve this, 12–15 kg/ha red clover seed and 4–5 kg/ha grass seed (Frame *et al.*, 1998) is recommended. In Sweden and Finland, the recommendation for a more general-purpose mixture has for a long time been 5–7 kg/ha red clover seed sown with 15 kg/ha grass seed (Frankow-Lindberg, 1990). The regulation of red clover content in such a mixed crop by varying seeding rates is rather limited once the rate exceeds 6 kg/ha. It depends to a much greater extent on management factors such as nitrogen application and harvesting regime (Frankow-Lindberg, 1989). Lower rates of red clover seed (and a higher rate of grass seed) are now common where the application of quite high rates of nitrogen fertilizer has become standard practice. To improve and stabilize the legume content (Frankow-Lindberg *et al.*, 2009b), and thereby the longevity of the crop, commercial seeding mixtures in Sweden now often contain both red and white clover seeds.

An important aspect of mixed swards is the contribution of biologically fixed nitrogen, which decreases the fertilizer nitrogen needed to achieve a specified dry matter yield (Nyfeler *et al.*, 2009). This is evident from the poor response to nitrogen application in red clover-dominated swards (Fig. 10.1). However, since it is a short-lived species, red clover content declines with time irrespective of initial seeding and nitrogen application rates (Frankow-Lindberg, 1989). In practical farming, the amount of nitrogen applied is therefore usually increased with time as the content of red clover in the crop decreases. The response of a clover–grass mixed sward to nitrogen fertilizer is strongest in the spring harvest. A recent field study of mixtures of grass and red or white clover showed that there was virtually no effect of applying nitrogen to the regrowth (Frankow-Lindberg unpublished results, Fig. 10.2). Unfortunately, it is very difficult to properly estimate the clover content in early spring when it is time for nitrogen application, and thus adjust rates to the actual content.

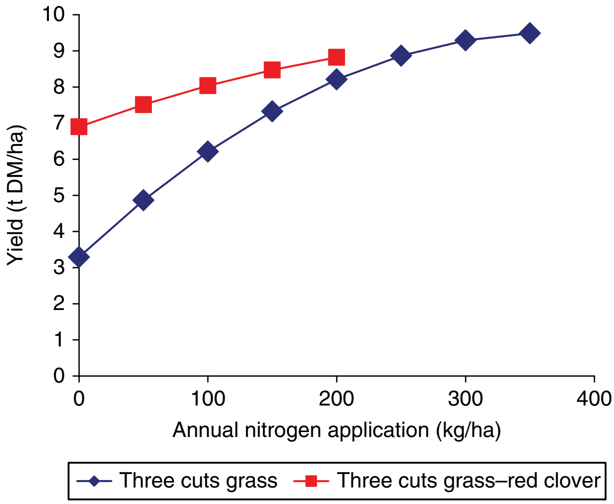

Fig. 10.1. The effect of nitrogen application on the dry matter (DM) yield of a mixed grass–red clover sward and a pure grass sward harvested three times. (Data from Kornher, 1982.)

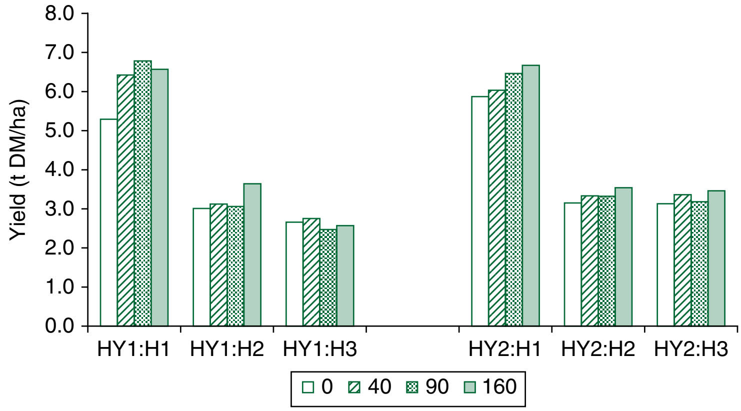

Fig. 10.2. The effect of nitrogen application on the dry matter (DM) yield of a mixed grass–red and white clover sward harvested three times. The treatments were: no nitrogen applied (0), 40 kg/ha nitrogen applied in spring (40), 90 kg/ha nitrogen applied in spring (90) and 90 kg/ha nitrogen applied in spring followed by 35 kg/ha nitrogen applied to each regrowth (160). HY1 and HY2 denote the first and the second harvest years, respectively. H1, H2 and H3 denote the respective harvests within each harvest year. The experiment was established 1 year before harvests began.

Timothy (*Phleum pratense* L.) is the traditional companion species due to its relatively low competitive ability. In the most northern regions where red clover is cultivated, this is the only grass species that is suitable. In southern Sweden, meadow fescue (*Festuca pratensis* Huds.) is often included, as well as small amounts of perennial ryegrass (*Lolium perenne* L.) and hybrid ryegrasses. In the UK, the recommended companion grasses are Italian ryegrass (*Lolium multiflorum* L.) or hybrid ryegrasses. Other species used in the USA are cocksfoot (*Dactylis glomerata* L.), tall fescue (*Festuca arundinacea* Schreb.), smooth bromegrass (*Bromus inermis* Leyss.) and reed canary grass (*Phalaris arundinacea* L.) in bi-species mixtures.

Red clover should be sown from early to mid-season at a depth of 10–15 mm in order to establish well before the winter (Frame *et al.*, 1998). Undersowing in a grain or silage (whole-crop) cereal nurse crop reduces weed pressure. Provided the cereal is harvested early and the straw removed, this practice does not impair the yield of the established ley crop. However, with such undersowing, a harvest in the establishment year is not possible. Slot-seeding into an existing sward can be successful, but competition from the existing swards must be controlled along the slots (Komárek *et al.*, 2010). Red clover does not thrive in very wet or acid soils, but is otherwise not demanding in relation to soil conditions. Ideally, soil pH should be in the range 6.0–6.5 for optimum crop development and root nodule formation (Frame *et al.*, 1998). With a dry matter yield of 10 t/ha (pure clover crop), uptake of phosphorus and potassium is approximately 30 kg/ha and 250 kg/ha respectively. This needs to be replenished. Regular fertilization with sulfur may also be required.

The number of cuts that may be taken per year, and the yields obtained, depend on climate. Where high-quality feed is required, three to four cuts are common in southern Sweden, and in countries further south up to five cuts per year can be taken. In northern Sweden and Finland the number of cuts varies from one to three depending on the latitude. Maximum dry matter yield (first harvest year, Europe) is in the range of 9–18 t/ha without nitrogen fertilizer (Frame, 2005). Yields decrease sharply after the second harvest year.

Fixation and Transfer of Nitrogen to Companion Species

Nitrogen acquisition through biological nitrogen fixation is high in red clover. Nitrogen fixation above 350 kg/ha/year in the above-ground biomass has been reported (Carlsson and Huss-Danell, 2003). An estimate of the total amount of biologically fixed nitrogen by red clover (above and below ground) is approximately 50 kg/t dry matter harvested when it is grown in mixtures with grasses (Frankow-Lindberg, 2003). Around 80% of the nitrogen in the stand comes from biological nitrogen fixation when red clover is grown in mixtures with grasses (Carlsson and Huss-Danell, 2003). The presence of grasses increases the quantity of nitrogen fixed by red clover by providing a sink for the nitrogen fixed, while high application rates of nitrogen fertilizer decrease this directly by suppressing the proportion of fixed nitrogen in the plant (Nyfeler *et al.*, 2011) and indirectly through reducing the proportion of clover in the sward (Nykänen *et al.*, 2008). Few studies have estimated the amounts of biologically fixed nitrogen present in the stubble and the root system. In one study from northern Sweden (with two harvests taken), between 25% and 60% of the total amount of nitrogen fixed was found below harvesting height, in the respective cuts (Huss-Danell *et al.*, 2007). It was concluded then that the fixed nitrogen found in non-harvested plant parts is 40% of the amounts found in the harvested plant material.

There is usually no need to inoculate red clover seeds with *rhizobium* at sowing since most European soils contain species that readily colonize and form an efficient symbiosis with it (Frame *et al.*, 1998). Nitrogen fixed by red clover eventually becomes available to companion species, most likely through *in situ* decomposition of clover tissue deposited above and below ground (Dahlin and Stenberg, 2010a). The transfer of fixed nitrogen from red clover to a non-fixing species is less than from white clover (*Trifolium repens* L.) (Høgh-Jensen and Schjoerring, 2000) but more than in lucerne (*M. sativa* L.) (Pirhofer-Waltzl *et al.*, 2012; Frankow-Lindberg and Dahlin, 2013). The amount of nitrogen transferred increases over time, and transfers of up to 68 kg/ha have been recorded (Høgh-Jensen and Schjoerring, 2000; Dahlin and Stenberg, 2010b).

As red clover is a short-lived species, it is often grown in short-term leys in crop rotations where cereals are included, providing residual effects of the ley crop on the following crops. Lindén (2008), summarizing a large number of conventional farming field trials in Sweden, stated that the main residual effect of red clover-based leys is a nitrogen effect. The residual effect of these crops reduces the fertilizer nitrogen requirement of the following cereal crop by 30–40 kg/ha. The effect is largest in the cereal crops grown directly after the ley crop. This contributes to a yield benefit of 0.7–1.0 t/ha for winter wheat grain compared with yields after a barley pre-crop. This pre-crop effect is still noticeable in the second cereal crop with a benefit of 0.25–0.75 t/ha for winter wheat (Lindén, 2008). When red clover was intercropped (undersown) with winter wheat and ploughed in after the wheat harvest, it provided both weed control and nitrogen to the following barley crop without causing a yield penalty to the covering winter wheat crop (Bergkvist *et al.*, 2011) (Fig. 10.3).

Fig. 10.3. Red clover as a cover crop undersown in winter wheat. The yield of winter wheat was unaffected by the presence of red clover, while the yield of the following barley crop was increased by the ploughed-under red clover. (Photo credit: Göran Bergkvist.)

Feeding Quality

Red clover provides a high-quality fodder not only for ruminants (Dewhurst *et al.*, 2009), but also for pigs (Reverter *et al.*, 1999) and fish (Turan, 2006). Red clover is rich in protein and minerals, and intake rate by ruminants is high. This is due to its cell structure, which differs from grass in that it breaks down more easily in the rumen and thus passes through the rumen more rapidly (Dewhurst *et al.*, 2009). On average, the daily intake of dry matter from red clover-based diets are 1.2 kg higher than from grass diets, and the daily milk yield is increased by an average of 1.5 kg (Steinshamn, 2010). Further, in contrast to white clover, red clover contains the enzyme polyphenol oxidase (PPO), which provides the forage with beneficial properties such as reducing emissions of nitrogen to the environment (Parveen *et al.*, 2010) as well as improved milk and meat quality. PPO produces quinones that bind to proteins, which in turn reduces protein degradability during silage making. As a result, the feeding quality of the silage is improved, and nitrogen losses from the silage through effluents during storage are reduced. PPO also reduces the protein degradability in the rumen (Parveen *et al.*, 2010), which improves the nitrogen use efficiency of ruminants fed red clover compared with those fed with white clover (Dewhurst *et al.*, 2009). PPO is also thought to be involved in the reduction of rumen biohydrogenation of polyunsaturated fatty acids (Van Ranst *et al.*, 2011). This results in higher levels of the n-3 fatty acid α-linolenic acid in milk from dairy cows fed red clover silage than from cows fed grass-based diets (Dewhurst *et al.*, 2009). This has positive effects on consumer health. Meat from cattle consuming red clover-rich forage also has a more beneficial fatty acid profile with respect to consumer health than cattle consuming all-grass or white clover-rich forage (Dewhurst *et al.*, 2009). Another feature of milk from dairy cows fed on red clover is the high concentrations of the isoflavone equol, which may confer potential positive health effects for consumers similar to those observed in human populations where soy products are included in the diets, for example reduced rates of cardiovascular diseases (Tham *et al.*, 1998). A drawback of the presence of isoflavones (particularly formononetin) in red clover forage is their oestrogen-like effects within animals, because this is thought to impair the fertility of sheep (Dewhurst *et al.*, 2009). However, a recent study where diets fed to ewes consisted of either red clover or grass silage found no such effect (Mustonen *et al.*, 2014). Red clover contains more magnesium and calcium in relation to potassium compared with grasses, which is beneficial for the health of cows. The phosphorus concentration is low in relation to calcium, which has to be taken into consideration when the cows are in the late phase of pregnancy. Low phosphorus in cows' diets prior to calving can increase the incidence of postparturient hypocalcemia (milk fever).

Forage from red clover can be a part of pig diets. Increasing forage feeding has been found to reduce the apparent digestibility of the diet compared with a traditional cereal-based diet (Andersson and Lindberg, 1997). However, the apparent ileal digestibility of the crude protein is not impaired (Reverter *et al.*, 1999), which shows that red clover is a good protein source also for monogastric animals.

Conclusions

Red clover can successfully be grown across Europe in areas not characterized by drought. It is most suitable for the production of preserved winter feed for cattle from short-term leys. The aftermath may be grazed, but this will penalize total yield. The yield potential of pure stands or mixed stands with grass is high (and higher than that from white clover) without any nitrogen applications. The full exploitation of red clovers' nitrogen fixation in rotational leys would considerably reduce the carbon footprint from the production of forage. However, more reliable predictors of red clover performance in mixed swards are needed to induce non-organic farmers to omit or strongly reduce nitrogen fertilization to such swards.

The quality of the forage produced from red clover-based leys is excellent, provided that the harvest is made at early phenological stages and the crop is well preserved. Forage rich in red clover is suitable for feeding high-yielding dairy cows, and the presence of PPO in red clover additionally provides the milk with, for human consumption, better nutritional fat qualities compared with forage made from white clover or grass. At the moment, such quality differences are not acknowledged by the market.

Red clover grown in crop rotations reduces the need of nitrogen fertilizer in following cereal crops and increases their yields. It may be intercropped with a cereal without any yield penalties of the main crop. Such practice has the potential to provide much of the nitrogen fixed by red clover to the following crop, but weather and soil conditions may modify the efficiency of this transfer. The lack of cheap herbicides for weed control in cereals intercropped with red clover is one bottleneck for the uptake of this practice by non-organic farmers. Further, if mineral nitrogen fertilizer is applied to boost cereal yield, the growth of red clover will be uncertain and the money spent to purchase red clover could be lost.

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