

A Century of Clover Breeding Developments in the United States

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ABSTRACT

The perennial clovers, red (*Trifolium pratense* L.) and white clover (*T. repens* L.), have been important legumes for livestock feeding in the United States during the past century, particularly in the eastern United States. Recently, sustainable agriculture, organic farming, integrated crop–livestock systems, and high nitrogen prices have stimulated interest in forage legumes. This paper describes the present status of clover improvement programs—their objectives, successes, short falls, and challenges for the future. One goal of most clover breeding programs is increased persistence (longevity of individual plants) because of its association with general adaptability and yield. Persistence, which limits life of clovers to about three to four years, is largely governed by programmed senescence but can be influenced somewhat by breeding for general adaptation to the area of use. New, more-adapted red clover cultivars have been bred in Wisconsin for use in the northern states, in Kentucky for the middle states, and in Florida for the southern states. For white clover, researchers in several states in the southeast have cooperated in the development of improved cultivars. The unique qualities of these improved species along with high seedling vigor, ease of establishment, and general competitiveness ensure that red and white clover will continue to be the major forage legumes of the United States and justify continued breeding efforts.

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Abbreviations: 2,4-D, 2,4-dichlorophenoxy acetic acid; AMV, alfalfa mosaic virus; BYMV, bean yellow mosaic virus; CYVV, clover yellow vein virus; PSV, peanut stunt virus; SRVR, Southern regional virus resistant.

THE PERENNIAL CLOVERS (*Trifolium* spp.), especially red (*T. pratense* L.) and white clover (*T. repens* L.), have been important livestock feed for the past century in the United States, but usage has declined in the last five decades. Although several factors are involved in this decline, a primary factor has been increased use of fertilizer resulting from low nitrogen prices. Kjaergaard (2003) explained the situation as follows: Clover was first domesticated around the year 1000, spread throughout Europe, and was the chief provider of atmospheric nitrogen for cereals needed to feed an expanding population. In the thirteenth and fourteenth centuries, nitrogen starvation of crops became increasingly acute, almost a matter of life or death. Kjaergaard suggested that the unrivaled mortality due to the Black Death (1347–1352) occurred because it affected a population already weakened by undernourishment as a result of a nitrogen-deficient agriculture. At the close of the seventeenth century, clover became widespread throughout northern Europe. The effects of clover on the nitrogen revolution were not limited to grain fertilization. Clover is excellent feed for cattle (*Bos* spp.) and therefore helped spur a dramatic increase in the cattle population. Kjaergaard went on to suggest a relationship between the advent of clover and the decline of malaria in northern Europe. The scenario is as follows: The most common mosquito (*Anopheles atroparvus*) in that area prefers cattle to humans, and the drastic rise in the number of domestic animals

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led to a decline in the number of humans being bitten. Inasmuch as cattle do not develop malaria, the mosquito's life cycle was severed, leading to its virtual elimination in northern Europe. Clover had many other indirect consequences, including an increase in milk and honey production. Another was transformation of the landscape. The white and red clover fields in blossom transformed the countryside into a wonderful flower garden that has been romantically depicted by poets, painters, and composers. Clover probably had a greater influence on civilization that did the potato (*Solanum tuberosum* L.) (Piper, 1924). In fact, without clover, potatoes could not have thrived.

The defining year for the decline of clover was 1909, when German chemist Fritz Haber developed the process of extracting nitrogen through ammonia synthesis (McNeill, 2000). By World War II, industrial production of nitrogen expanded dramatically, and its use for fertilizer at a low price led to the disappearance of clover in many areas. The landscape became dominated by monoculture and lost its picturesque charm. Clover in Europe became confined to botanical gardens, awaiting the day when the enormous energy costs and deleterious effects of industrially produced nitrogen on the environment and climate were no longer acceptable.

A similar decline in the use of clovers has occurred in other countries. Seed production of red clover in most countries except Canada peaked about 1950 (Taylor and Quesenberry, 1996). A factor other than increased nitrogen use contributing to the decline in the United States, included a shift to alternative legumes such as alfalfa (*Medicago sativa* L.). Cattle were no longer fattened on the land, and the landscape, particularly in the Corn Belt, shifted to a monoculture of soybeans [*Glycine max* (L.) Merr.] or corn (*Zea mays* L.). The decoupling of livestock from the land lessened the need for forages such as red clover (Naylor et al., 2005). More recently, the use of row crops has intensified due to interest in fuel production, that is, ethanol and biodiesel. Another factor influencing the decline in use of the clovers was the shift of seed production to the western United States, particularly Oregon. Because seed yields are higher in the West, fewer hectares are needed to sow the crop. Much of this production is in the Willamette Valley, where more grass and legume seeds are produced than in the rest of the world combined (Wong, 2005). In the 1950s, the National Foundation Seed Project was initiated to rapidly increase seeds of eastern-adapted U.S. cultivars. The pilot cultivar in this program was 'Kenland' red clover. Later, however, this function was taken over by private seed companies that have exclusive rights to cultivars, and the Foundation Seed Project was no longer needed. Another factor contributing to the reduction of clovers in mixed pastures has been the use of 2,4-dichlorophenoxy acetic acid (2,4-D) for broadleaf weed control. Although usage of the clovers in the Corn Belt has

declined, clover seed is still used for pasture renovation in the midsouth. Cattle gains and reproduction rates have been shown to be greater when clovers are included in the mixture, especially for ameliorating the effect of the endophytes in grasses. Often, red and white clover seeds are sown together for this purpose.

Some recent evidence indicates a resurgence of interest in forage legumes, including clovers, because of increasing energy costs and deteriorating environmental effects from the use of synthetic nitrogen. The possibility of reducing feed input costs and reliance on protein concentrates are also factors. Interest in organic crops that do not pollute water supplies through nutrient runoff and soil erosion also bodes well for greater use of forage legumes. As a consequence, clover breeding programs are beginning to be reconsidered after a long period of neglect.

The objective of this paper is to describe the status in the United States of breeding efforts with the perennial clovers in the last century. I deal primarily with red, white, and Kura clover (*T. ambiguum* Bieb.) and include past breeding efforts, accomplishments, short falls, as well as suggest research for the future with possible avenues of approach. Particular emphasis will be given to applications for U.S. agriculture, but attention will be drawn to research from other countries that may have the potential to impact U.S. breeding programs. An attempt will be made to update previous reviews (Pederson, 1995; Taylor and Quesenberry, 1996; Taylor, 1987; Taylor and Smith, 1995, 1998).

BREEDING OBJECTIVES

Most clover breeding programs have given primary emphasis to persistence (individual plant longevity) because of its association with general adaptability and yield. To improve persistence, it has often been necessary to augment disease and insect resistance. On the other hand, breeding for improved adaptability has often led to greater insect and disease resistance. Other objectives have involved changes in morphological characters such as pubescence, grazing types, and stolon characteristics in the case of white clover.

Sources of Genetic Variation

Clovers in the United States were introduced from Europe, either accidentally or from hay or hayloft sweepings. Over time, farmers maintained their seeds and in the process developed "farmer strains" that were well adapted locally. Eventually, however, it became desirable to develop more widely adapted cultivars from these farmer strains and to distribute them widely. Generally, most early cultivars were developed by mass selection. This led to early clover cultivars such as Kenland red clover and 'Louisiana S-1', an intermediate type of white clover. These early cultivars turned out to be very productive over wide areas. In fact,

Kenland, released about 1947, is still being grown and has significant certified seed hectareage.

Other sources of genetic variation for the clovers include mutagenesis, ionizing radiation (Taylor et al., 1961), and somaclonal variation (Bagley and Taylor, 1987). None of these methods have been particularly useful for the cross-pollinated clovers. The inability to effectively inbreed by selfing means that no suitable method exists as in self-pollinated crops for uncovering changes in recessive genes. Also, many of the induced changes lead to infertility.

Breeding Procedures

Mass selection with control of pollination has been a highly effective method for clover breeders. Usually, seeds are sown in an environment similar to that where the cultivar is to be grown. Epiphytotics of diseases, insects, nematodes, or other pathogens that are common to the area may be encouraged by the use of coverings, cages, or artificial inoculation. Seed is usually harvested from only intercrops of selected plants. Mass selection may not be effective for characteristics with low heritability.

Recurrent phenotypic selection as used for clovers is very similar to cyclic mass selection. The main difference is that seeds of half-sib families are maintained individually rather than bulked. Parent plants are not progeny tested similar to mass selection, which suggests that the method is most effective for simply inherited characters. Many variations of the process are possible (Taylor and Quesenberry, 1996). It is worth emphasizing, however, that performance of individual spaced plants may not be typical of performance in a sward. Cultivars that have been developed by this method include 'Freedom!' red clover, bred for absence of pubescence (Taylor and Collins, 2003), and 'Pilgrim' white clover, a ladino type, released in 1953 (Hollowell, 1962).

The advantage of the polycross method is that breeding materials are progeny tested, and thus the method is suitable for selection of complex characters. However, a major disadvantage is the length of time required and the expense involved for each cycle of selection. For that reason, it has not been used much in clover breeding. 'Kenstar', a 10-clone synthetic (Taylor and Anderson, 1974b), is the only red clover cultivar of this type bred in the United States to my knowledge. Synthetic cultivars have been developed in other legumes, of which 'Regal' a five-clone white clover synthetic is an example (Johnson et al., 1970).

Strain building is similar to the polycross method except that parents are maintained by seed populations rather than by clones. Populations are developed and maintained separately, and after testing, the most superior populations are combined to form a synthetic. This method is less laborious than the polycross method using

clonal parents and the materials may be tested as broadcast plots typical of normal seeding conditions. No spaced plant tests are needed. Smith and Maxwell (1973) used this method successfully in the development of 'Arlington' and other cultivars of red clover in Wisconsin. A disadvantage is that intrapopulation crossing is not prevented and genetic gains are likely to be based only on additive genetic variance.

The backcross procedure was used in Kentucky to incorporate resistance to bean yellow mosaic virus (BYMV) into the Kenstar cultivar (Taylor et al., 1986), which has been released as 'Kenton'. Backcrosses to each of the 10 clonal parents of Kenstar resulted in inbreeding that was overcome by developing 10 corresponding populations that were single crossed to form five populations that later were combined for the synthetic. The primary disadvantages of the procedure are the excessive length of time necessary to make the backcrosses and the necessity of overcoming inbreeding depression.

Single- and double-cross hybrids have been produced in red clover using homozygous S-alleles to control crossing. Such hybrids exploit both general and specific combining ability that have been shown in red clover (Anderson et al., 1974). A disadvantage of the method, as diagrammed by Taylor and Quesenberry (1996), is that parental clones must be inbred to isolate homozygous S-alleles. Unfortunately, the double-cross hybrids that were developed did not exceed the Kenstar cultivar in yield or persistence (Taylor and Anderson, 1974a; Anderson et al., 1972). Hybrid red clover has great potential, but the expense of isolating high-combining ability parents and seed production must be offset by economic benefit. It is doubtful that clover breeding programs will be funded at levels sufficiently large enough to isolate high combining ability inbred parents. Other methods of exploiting heterosis in forage crops have been delineated by Brummer (1999).

Tetraploid red clover ($2n = 28$) that exceeds diploid clover in several respects has been produced in Europe (Sjodin and Ellerstrom, 1986) but has not been a success in the United States. Although tetraploid flowers and seeds are larger and forage yields and persistence are greater, seed yields are lower than in their diploid counterparts. Chromosome doubling may be produced in three ways: use of colchicine, use of nitrous oxide, and by gametic nonreduction (Taylor and Quesenberry, 1996). The data indicate that any one or a combination of methods may be used to develop tetraploids. The reason tetraploids are successful in Europe but not in the United States is not understood but may be related to the length of time breeding programs have been underway. Tetraploid experimental strains have been produced in Florida, Kentucky, and Wisconsin, and one or more of these may be released if seed-yield deficiencies can be overcome. Incidentally, research

showed for the first time that the triploid block in red clover is weak. When tetraploids are crossed with diploids, triploids are occasionally produced (Taylor and Wiseman, 1987). Backcrosses of triploids to diploids produced the complete trisomic series (Taylor and Chen, 1988).

Adding diversity by bringing genes from perennial species to white and red clover via hybridization has received considerable attention. The genus *Trifolium* consists of about 230 species, of which seeds of more than 200 species are maintained at the University of Kentucky in Lexington and at the National Plant Germplasm System located at Pullman, WA, for the perennials and Griffin, GA, for the annuals.

In white clover, Chen and Gibson (1970, 1971, 1972) studied crossability of related species and chromosome pairing in hybrids. Their research showed close affinity of white clover with *T. nigrescens*, *T. occidentale*, and *T. uniflorum* and somewhat less affinity with *T. ambiguum*. They hypothesized that white clover originated as a doubled version of *T. occidentale*. See Cleveland (1985) for a review and diagram of putative relatives of white clover based on ease of crossing and reproductive data. The most extensive interspecific hybridization program with white clover was initiated by Williams (1978) and Williams and Verry (1981) for the cross of *T. repens* × *T. ambiguum*, continued by Anderson et al. (1991) in Kentucky, Abberton et al. (1998) in Wales, and Hussain and Williams (1997) in New Zealand. Apparently, it is quite difficult to obtain transgressive segregates necessary for the expression of the rhizomatous character of *T. ambiguum* in the white clover background. A further challenge is the low fertility in the backcrosses. The cross of *T. repens* with *T. nigrescens* has been further investigated Hussain et al., (1997) and Marshall et al. (1998), but no cultivars have been released from any hybrid combination involving *T. repens*. The research by Ellison et al. (2006) involving phylogenetic analyses based on nuclear ribosomal DNA internally transcribed spacer and chloroplast intron sequences suggests that *T. occidentale* and *T. pallescens* are the likely parents of *T. repens*.

Success in interspecific hybridization of red clover likewise has been limited. Cleveland (1985) has summarized the results of interspecific crosses with red clover and its close relatives. Taylor et al. (1963) produced the first hybrid, *T. pratense* ($2n = 14$) × *T. diffusum* ($2n = 16$), a sterile annual. Doubling the chromosomes of both parents and remaking the cross resulted in a completely fertile hybrid ($2n = 30$), which, unfortunately, remained annual. To obtain perenniality, red clover was crossed with *T. sarosiense* Hazsl. ($2n = 48$) (Phillips et al., 1982). However, the offspring were completely sterile, and chromosome doubling did not increase fertility. Isobe et al. (2002) made four backcrosses of a *T. pratense* × *T. medium* hybrid and obtained adequate fertility to select for increased persis-

tence. The research of Ellison et al. (2006) confirmed that the close relatives of red clover are *T. diffusum*, *T. pallidum*, and *T. andricum*, and the more distant relatives include *T. sarosiense* and *T. medium*, all of which is in very close agreement with the crossability data. In summary, no interspecific hybrids have been released as cultivars in the *Trifolium* genus primarily because of sterility problems.

Despite great promise, molecular breeding has been used very little in the United States, and no cultivars have been developed. Undoubtedly, a decline in the number and funding of breeding programs has limited progress primarily to the development of transgenic techniques. In red clover, Beach and Smith (1979) worked out details for plant regeneration. However, plant regeneration in red clover is genotype dependent (Myers et al., 1989). Quesenberry and Smith (1993) used recurrent selection for increasing regeneration efficiency, resulting in release of the germplasm NewRC (Smith and Quesenberry, 1995). Apparently, the more northern cultivars are more easily regenerated than southern cultivars. Quesenberry et al. (1996) went on to use this material to transform red clover for neomycin phosphotransferase II using *Agrobacterium*. Sullivan and Quesenberry (2006) described this protocol in detail. Using this protocol, Sullivan et al. (2004) and Sullivan and Rierson (2004) succeeded in silencing one of the polyphenol oxidase genes in red clover. They obtained four plants that exhibit little or no postharvest browning as a result of this gene deactivation. In white clover, plant regeneration is apparently less difficult than in red clover. Schmidt et al. (2004) showed that phenotypic recurrent selection is effective for increased transgene expression. Woodfield and Brummer (2000) detailed the potential of molecular techniques, including marker-assisted selection and the impact of genomics for forage legumes. They cautioned that the potential of molecular breeding cannot be achieved without accompanying strong conventional breeding programs. Genomics investigations of red and white clover are quite limited in the United States. Structural analyses of the genome of red clover is underway in Japan (Sato et al., 2005), and Spangenberg et al. (2001) has summarized the molecular breeding of forage plants including white clover in Australia. It is expected that mapping the genomes of the model legumes, *Lotus japonicus*, and *Medicago truncatula*, ultimately will provide insights into the molecular genetics of red and white clover.

BREEDING PROGRESS

The least-expensive and most-efficient method of pest control is through the development of resistant varieties. Disease resistance may increase stand longevity or at least assurance of stand. One of the first diseases to be controlled in this manner was southern anthracnose of red clover caused by *Colletotrichum trifolii* B. & E. Kenland

was the first cultivar bred for resistance. Now practically all cultivars in the southern part of the clover belt (mid-Illinois southward) are resistant, and the disease has not been important since the early 1950s. In a similar manner, northern anthracnose caused by the fungus *Kabatella caulivora* (Kirch.) Karak. has been eliminated from red clover fields in the northern part of the clover belt (Smith and Maxwell, 1973). Resistance to the disease, controlled by two or three dominant genes, is present in the cultivars Lakeland, Arlington, Marathon, and Kenton. Summer stolon rot caused by *Macrophomina phaseolina* (Tassi) Goidanich frequently causes death of white clover plants that have been stressed by other factors (Pratt et al., 1998). Resistance to this pathogen was found in adapted white clover, especially Brown Loam Syn. 2 (Pederson et al., 2000).

Powdery mildew caused by *Erysiphe polygoni* D.C. em Salm. occurs frequently on red clover and occasionally on white and Kura clover. Infected leaves turn yellow, wither, and die, thereby reducing forage yield and quality. Inasmuch as several races of the fungus occur, and resistance is dominant, about four or five generations of selection are necessary to obtain a high level of resistance. Resistant cultivars include Arlington and Marathon, developed in Wisconsin, and FreedomMR, developed in Kentucky. Recently, cultivars, such as Kenland, that showed moderate levels of mildew infection have become almost 100% susceptible. The strongly resistant cultivars, such as Marathon, have still maintained most, if not all, of their resistance, suggesting that no changes in races have occurred but that possibly the existing races have developed increased aggressiveness. The reason for this is unknown, but climatic change is a possible explanation.

Crown and root rots are caused by several fungal pathogens. Probably the most important crown rot on both red and white clover is caused by *Sclerotinia trifoliorum* Erikss. Some diploid and tetraploid red clover cultivars, mostly in Europe, have limited resistance to this disease, but breeding has been difficult. The Kenland cultivar of red clover apparently has a limited amount of tolerance to the disease as a result of natural selection. Root rots of red and white clover have been attributed to many different fungi, but the most important are probably those of the genus *Fusarium*. Resistance breeding has been attempted frequently using various techniques (Taylor and Quesenberry, 1996). One method is to select roots of red clover for low incidence of rot after one or two seasons in a field. However, Pederson et al. (1980) indicated that such selection tended to reduce root diameter, and Taylor (unpublished data) observed a similar relationship. This subject is reviewed in more detail in the section on persistence, below.

Leaf spot diseases of red clover, mostly caused by *Stemphlium sarcinaeforme* (Cav.) Wiltshire (target spot),

have been controlled by breeding by the USDA Agricultural Research Service at the University of Wisconsin (Smith and Kretschmer, 1989). Four cycles of phenotypic selection increased resistance by about 24%, about 6% per cycle. Some resistant materials probably have been included in northern U.S. cultivars. Rust caused by *Uromyces trifolii* var. *fallens* produces pustules on leaves, stems, and petioles of red clover and may be particularly severe in the northeastern states. No resistant cultivars have been developed, even though simply inherited resistance is probably available.

Breeding also efficiently controls virus diseases of clovers. Although 30 or more viruses have been reported to infect clovers, only a few are of sufficient importance to justify breeding for resistance. In red clover, the cultivars Kenway from Kentucky and Marathon from Wisconsin have been bred for resistance to BYMV. Strain 204-1 is by far the most important strain of BYMV in Kentucky (Taylor et al., 1986). Two types of reactions occur in the Kenland cultivar; some plants are mottled, whereas other plants become necrotic. The necrotic reaction is controlled by a single factor (N) that is dominant to the mottling (n) reaction. Two types of necrosis, local and systemic, also occur. In the hypersensitive response, necrotic spots localized on the leaves are controlled by the gene (H) that is dominant over necrosis (h). Another type of resistance that shows no symptoms is controlled by a single factor, R, dominant over susceptibility and apparently is epistatic to the hypersensitive factor. For white clover mosaic virus, tolerance in red clover is apparently controlled by polygenes (Martin, 1989), and for red clover vein mosaic virus, resistance to a single isolate is controlled by a dominant gene (Kahn et al., 1978). White clover is adversely affected by peanut stunt virus (PSV), clover yellow vein virus (CYVV), and alfalfa mosaic virus (AMV). Another source of resistance to viruses has been located in the *T. repens* × *T. occidentale* hybrid (Pederson and McLaughlin, 1989). Southern regional virus resistant (SRVR) germplasm (but no cultivars) has been released that possesses resistance to these viruses (Pederson and McLaughlin, 1994; McLaughlin and Pederson, 2000). Pederson and McLaughlin concluded that breeding procedures using additive genetic effects would be the most effective in improving PSV, CYVV, and possibly AMV resistance in white clover. Crossing 40 plants of the intermediate type with 40 plants of SRVR developed the cultivar Patriot. However, no claims for virus resistance were made in the registration of Patriot (Bouton et al., 2005). In the southeastern United States, interest in resistance to viruses in red and white clover ranked sufficiently high to justify formation of Regional Project S-228, Forage Legume Viruses: Identification and Genetic Resistance for Improved Productivity (see Edwardson and Christie, 1986, for a project report). As a part of that project,

near identical experiments were initiated with red and white clover in Kentucky and Mississippi by Taylor et al. (1995) to quantify the yield benefits from incorporation of virus-resistant clover germplasm into grass legume systems. The experiments compared spaced plants with broadcast plots with and without companion grasses. With both red and white clover, little or no virus was detected in broadcast plots even though a high incidence was found in adjacent spaced plants. Also, virus incidence was lower in plots with a grass companion crop than those without grass. The final results indicated that yield and persistence data from plants infected before or even after transplanting should not be used to estimate losses that would occur under broadcast conditions typical of normal field operations. Moreover, the percentage of plants infected in farmer fields may not correlate with yield losses due to the compensating ability of noninfected plants; and since both red and white clover parent plants persist only about 3 yr, non-seed-borne viruses such as BYMV and PSV will be eliminated from the seed crop and new volunteer seedlings. These data emphasize the need to conduct evaluations under broadcast conditions typical of farmer operations.

Several nematode species have been reported to attack red clover (Taylor and Quesenberry, 1996). The most important of these in the United States are four species of the root-knot group (*Meloidogyne* spp.) that seriously affect red and white clover particularly in the southern United States (McGlohon and Baxter, 1958). Other than cultural control, the most feasible method of lessening the effect of nematode infestation is by breeding. Quesenberry et al. (1989) conducted five cycles of selection for resistance to root-knot nematodes that have held up under field conditions. This research has culminated in the release of the 'Cherokee' and 'Southern Belle' red clover cultivars.

Although more than 95% of 171 white clover accessions tested were moderately to highly susceptible to all root-knot species, sufficient variability for resistance breeding was available (Kouame et al., 1998). Resistant plants were selected (Windham and Pederson, 1991, 1992) that resulted in release of a germplasm, MSNR4 (Pederson and Windham, 1995). Pederson and Windham (1989) concluded that *Trifolium nigrescens* Viv. may be a valuable source of resistance to southern root-knot nematodes. However, no white clover cultivars with root-knot nematode resistance have been released.

A considerable number of insect pests attack red and white clover, but none have been deemed of sufficient importance to develop major breeding programs for resistance. Although most if not all insects may be controlled by insecticides and parasites, host plant resistance may be the most economical method. Gorz et al. (1979) released a strain of red clover resistant to the pea aphid (*Acyrtosiphon pisum* Harris) that lowered the reproductive rate and

increased development time for the first instar larvae compared with susceptible cultivars. Cyanogenesis in white clover is apparently a defense mechanism against insect attack. Ellsbury et al. (1992) showed that a cyanogenic cultivar, Louisiana S-1, was least damaged among cultivars exposed to attack by the alfalfa weevil [*Hypera postica* (Gyllenhal)] and the clover head weevil [*Hypera meles* (F.)]. Cyanogenic frequency was greater in white clover plants collected at low altitudes and at sites with high winter temperatures, lower summer precipitation, greater spring cloudiness, and less snow cover (Pederson et al., 1996).

The potato leafhopper (*Empoasca fabae* Harris), a major pest of alfalfa, also attacks red, white, and Kura clover. The latter two species are particularly susceptible apparently because they lack the pubescence possessed by U.S. cultivars of red clover. However, other undetermined factors are involved in the resistance of American red clovers, inasmuch as the cultivar Freedom!, bred for reduced pubescence, is more resistant than comparable glabrous European cultivars (Taylor and Collins, 2003). Root-feeding insects, including the clover root curculio (*Sitona hispidulus* F.), which attacks both red and white clover, and the red clover root borer (*Hylastinus obscurus* Marsham), are thought to be involved in root rot complexes (Leath and Byers, 1973). However, root rot occurs in areas where the insects are not present, and the question of relation to persistence of clovers remains unanswered (see section below on persistence).

Breeding for changes in morphological characteristics of red clover includes increased stem length (Bowley et al., 1984) and increased number of multiple-parted heads (Taylor et al., 1985). Six cycles of selection dramatically increased plant height. However, over cycles, stem number declined, and persistence decreased compared with the check cultivar. Six cycles of selection likewise increased multiple head parts in red clover. Red clover heads usually are single parted with up to 125 sessile flowers. The occurrence of two flower heads tightly compressed together is common, and triple heads are rare. Over the six cycles of selection, parts of the largest head increased, but heads per plant declined. Even though seeds per head increased, it was not enough to offset the reduction in number of heads. In later generations of selection, the production of multiple-parted heads was related to fasciation of stems. In summary, the effectiveness of recurrent selection and the genetic plasticity of red clover were again demonstrated, but it was extremely difficult to change one morphological character without incurring undesired morphological changes in other characters.

Another character that has been changed in red clover is pubescence of stems and petioles (Taylor and Collins, 2003). Five cycles of selection, conducted to reduce pubescence associated with slow drying and dusty hay, resulted in the release of the cultivar Freedom! No

adverse effects associated with the reduction in pubescence have been observed, other than slightly less potato leafhopper resistance. However, inasmuch as pubescence tends to be intensified by natural selection, generations of seed increase of Freedom! will need to be monitored.

Another morphological character of considerable interest is grazing type. In 1993 a stoloniferous Tasmanian red clover cultivar, Astred, developed from Portugal introductions, was described by Smith and Bishop (1993) as being more productive and longer lived under grazing than other Australian cultivars. This cultivar is not well adapted in the United States, being highly susceptible to the potato leafhopper, and lower yielding under a three- or four-cut system. Dr. Margot Forde collected a similar type in Spain. She described the type as extremely prostrate, not very productive, but appearing persistent, and in wet pastures strongly rooting from nodes, forming patches up to a meter across (Forde, personal communication, 1989). These germplasms have been used in cultivar development in New Zealand (Rumball et al., 2003) but only to a limited extent in the United States. Several commercial cultivars of red clover have been selected for grazing tolerance, but whether these will live longer than standard cultivars remains to be seen.

White clover, being stoloniferous, is adapted for pastures. Cultivars of the intermediate type, mostly naturalized populations, are more grazing tolerant and higher seed yielding than the ladino or large type (Brink et al., 1999; Pederson et al., 1999). This is important because the original crowns of white clover persist only about two years. Stand maintenance depends on stolons and volunteer seedlings from the soil. Stands, then, may consist of three types of plants: originals, stolons, and volunteer seedlings. The old cultivar Louisiana S-1 and new cultivars such as Durana and Patriot (Bouton et al., 2005) are of this type. The latter two cultivars were specifically selected for persistence under grazing.

Taylor et al. (1989) examined the tolerance of 2,4-D in red clover. Four cycles of recurrent selection increased levels of 2,4-D tolerance by approximately 35%. The authors concluded that several more cycles of selection would be necessary to obtain tolerance levels high enough for use in production systems.

Genetic investigations of yield in the clovers have mostly involved disease and insect resistance and persistence. Woodfield and Caradus (1994) evaluated the genetic improvement of a worldwide group of white clover cultivars under New Zealand conditions. Using spaced plants, they found a 6% gain in dry weight per decade and comparable gains in percentage clover and mean stolon number. Seven U.S. cultivars included in the New Zealand study did not indicate appreciable genetic gain in yield except compared with Louisiana S-1, released in the 1930s. This yield difference was likely associated with the introduc-

tion of the large or ladino type into the U.S. breeding programs. Evaluation under U.S. conditions and inclusion of newer cultivars in such tests might produce different results. Pederson et al. (1999) showed that white clover originating in the United States had 49 to 53% greater plant spread and 79 to 222% greater dry matter yield than New Zealand cultivars.

Estimates of genetic gain for red clover are difficult to obtain, in part because of the lack of viable seeds of older cultivars. However, gains resulting from breeding red clover in the United States have undoubtedly occurred. Smith (1996) cited progressive improvement in performance of red clover resulting from four decades of breeding for persistence and disease resistance. Cultivars and experimentals that have been improved include Lakeland, Arlington, Marathon, and a Wisconsin synthetic.

Although red clover is generally considered to be high-quality forage, several antiquity factors including bloat, isoflavones, and slaframine deserve consideration. One solution to the bloat problem would be to increase tannin levels. Although 11 species of *Trifolium* have been shown to contain varying levels of tannin (Fay and Dale, 1984), none will hybridize with red clover. Genetic transfer of tannins will likely be possible only with improved understanding of tannin biosynthesis and with improved genetic transfer techniques. Forage management such as dilution with grass in the pasture is often used to prevent bloat by both red and white clover. Isoflavones, especially formononetin, have been shown to cause infertility in sheep (*Ovis*) but generally do not adversely affect larger animals. Little research has been conducted on breeding for lower isoflavone content in the United States. Slaframine is a piperidine alkaloid metabolite produced when animals consume red clover contaminated with the fungus *Rhizoctonia leguminicola* Gough & Elliott, the causal agent of black spot disease (Essig, 1985). Animals consuming hay contaminated with this fungus may exhibit excessive salivation. No genetic resistance to the fungus has been found.

Among quality factors exhibited by red clover, lowered protein degradability results in more efficient digestibility than in forages such as alfalfa (Broderick et al., 1994, 2001). They also demonstrated genetic variation among red clover introductions for rumen degradability and suggested that breeding could improve protein utilization in ruminants. Red clover contains high levels of polyphenol oxidase activity and o-diphenol substrates. These result in the postharvest browning reaction associated with decreased protein degradation during ensiling.

PERSISTENCE OF CLOVER

Persistence of clovers, defined as the length of life of individual clover plants, has been of special interest to breeders because of its relation to yield. Many factors have been

investigated as causes of short-lived stands both in red and white clover. In the southern United States, southern anthracnose of red clover severely depleted stands. This problem, as indicated above, was largely solved with the development of the resistant cultivar Kenland. Similarly, in northern sections of the United States, northern anthracnose was eliminated with the development of resistant cultivars. Resistance to diseases, insects, and viruses and concurrent selection for general adaptation have maintained the persistence in the United States to about three to four seasons of production. However, it is doubtful that potential production was increased, that is, above the persistence of susceptible red clover in the absence of the diseases.

Another factor considered to influence persistence is seed production in the year of sowing. Smith (1957) and Choo (1984) found that red clover plants that did not flower in the year of sowing persisted longer than those that produced seed. However, no heritability studies on this character were conducted. Data from Kentucky indicate that if seed-producing plants are allowed to develop strong rosettes by removing top growth in the fall, carbohydrate reserves are restored before the onset of short days and low temperatures, and most plants survive. Further, seed production only on third-year stands has been justified on the assumption that the practice would select for persistence. However, data from experiments on genetic shifts indicated no positive results from the practice (Taylor et al., 1979, 1991). These experiments showed that seed lots increased outside the area of adaptation did decline in persistence, apparently associated with shifts to earlier-flowering, nonpersistent types.

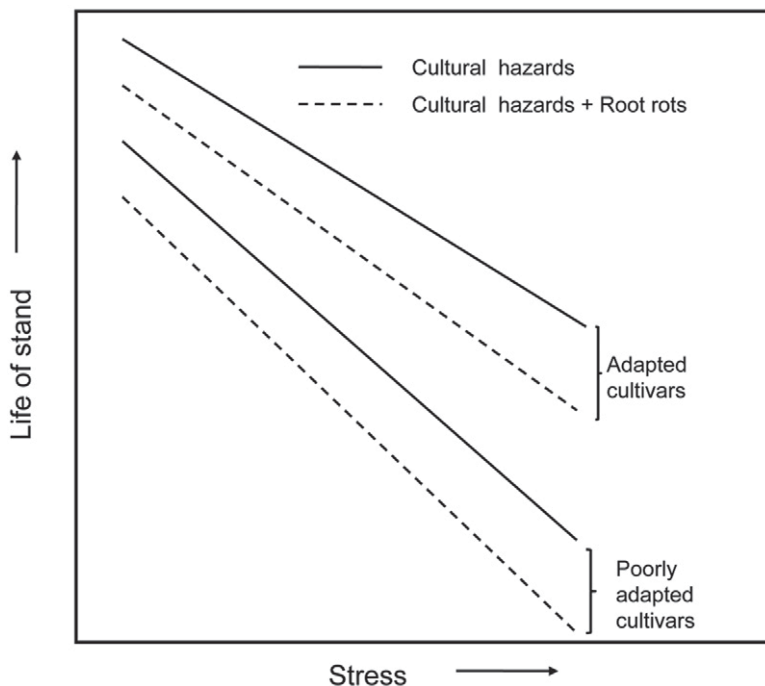


Figure 1. Impact of cultural hazards and root rots on longevity of red clover stands.

Somewhat later, a factor thought to be associated with lack of persistence in red clover was “internal breakdown,” a progressive disintegration of the crown that ultimately led to death of the plant (Cressman, 1967). This breakdown was originally thought to be related to persistence but was later shown to be physiogenic in nature.

The presence of a host of secondary bacteria and fungi complicated the issue leading to the so-called root rot complex. Two cycles of recurrent phenotypic selection for fibrous root system by Smith (1989), although perhaps eliminating the problem of internal breakdown by reducing crown size, did not increase persistence.

Two types of reproductive systems, delineated by Molisch (1938) and Thomas et al. (2000), are typified by white and red clover. White clover may be classified as having the open system in which death of plant parts do not occur simultaneously due to vegetative propagation by stolons. In some environments, white clover stands may persist indefinitely by vegetative propagation of stolons. Apparently, at some time during its evolutionary life history, white clover escaped the annual or biennial habit by producing stolons, as was suggested by Hollowell (1966) and Forde et al. (1989). However, perenniality likely was offset by somewhat lower vegetative yield. Red clover, in contrast, is primarily a plant of the closed reproductive system. As the plant ages, deterioration of the taproot begins even without pathogens (internal breakdown), and later, saprophytic or weakly pathogenic organisms invade. Subsequently, loss of the tap root results in the death of the whole plant. Interestingly, however, some red clover types possess the ability to develop adventitious roots (Montpetit and Coulman, 1991) and stolons (Smith and Bishop, 1993). Strong development of either one of these systems would change red clover to a plant of the open system.

The life cycle of the clovers may, within limits, be increased by selection for greater adaptability, which will delay senescence or increase persistence. The relationship of environmental factors and stand life is illustrated in Fig. 1 (modified from Taylor and Smith, 1977).

As root-rotting organisms build up in the soil or if the crop is exposed to poor management or other hazards, persistence will be shortened. Eventually, regardless of all factors, with the onset of senescence, death of the plant will occur. Therefore, persistence within life-cycle constraints is the result of interactions between the adaptation of the plant and its stress load. Stress load may be defined as any factor, whether physiogenic or pathogenic, that contributes to less-than-luxuriant growth. A well-adapted plant with little stress load would be expected to be strongly persistent for a time but

would eventually succumb due to senescence. On the other hand, a poorly adapted plant with the same or even a lower stress load would be weakened and die earlier. It should be noted that open-system populations consist of plants in various stages of senescence and rejuvenation so that the stand is maintained. Selection for resistance to one or another of the root-rotting agents such as the fungi *Fusarium* and *Leptodiscis*, the clover root borer (*Hylastinus obscurus* Marsham), and the clover root curculio (*Sitona hispidulus Fabricius*) may have limited success but ultimately is doomed to failure because other weakly pathogenic agents will still invade, and persistence will not be increased beyond the usual life span of the plant.

One possible means of increasing the life span of clover species would be greater concentration on breeding for general adaptation. Red clover in particular has shown a considerable amount of hidden or latent variability. Classic cases are the multiple-head and long-stem characters. Because persistence is probably much less heritable than these morphological characters, selection on half-sib family means in microplots may be an effective procedure. Means by which persistence could be evaluated earlier, such as by adjusting stress load or by marker-assisted selection, should be considered. Another method that may be effective for red clover would be changing the plant to an open system of reproduction similar to white clover if the problem of reduction in yield could be overcome. Examining the effect of genes for the stoloniferous habit at the tetraploid level, in interspecific hybrids, and by genetic transformation offers intriguing possibilities.

In summary, persistence of clover plants is primarily controlled by natural life spans. Control of biological hazards by breeding to achieve potential life spans is a worthwhile goal. However, increasing longevity probably will necessitate genetic restructuring of these clover species.

KURA CLOVER (*TRIFOLIUM AMBIGUUM* BIEB.)

Kura clover is quite distinct from red and white clover, and breeding goals for it are radically different. This species was introduced into the United States in 1944 and was hailed as the long-sought legume that would persist under grazing conditions with grasses. However, *Rhizobium* species necessary for nitrogen fixation were not introduced until 1954. The obvious advantages of Kura clover are its long-term persistence and its ability to withstand grazing without depletion of stand. It is also resistant to most virus, foliar, stem, and root diseases and nematodes that seriously affect red and white clover (Pederson and Windham, 1989; Pederson and McLaughlin, 1989).

It has now become apparent that the species possesses some serious deficiencies. First and foremost, it is difficult to establish even though seeds and seedlings are about the same size as red clover. Shortly after germination, when

the seedlings are 10 to 15 cm tall, top growth is inhibited to develop the beginnings of an extensive root system. Stress at this stage from drought or competition of any sort may result in loss of stands. This means that the species cannot be sod seeded or with a companion crop unless a grass and weed-inhibiting herbicide is used to reduce competition. Once the extensive but shallow root system is developed, the plant will maintain itself perennially, but lack of summer moisture in the southern United States probably will limit its use to the northern United States. The plant partitions much of its growth to the roots at the expense of shoots; consequently, yields may be low even in the northern United States, where the species is best adapted. Finally, unlike most cultivated species of legumes, the plant will not flower in the year of sowing. This tends to limit production and may be a factor in the lack of aftermath recovery in subsequent seasons. In addition, being a succulent legume with little or no tannin content, it is likely to cause bloat.

Breeding Procedures

Kura clover was first introduced into Australia by F.W. Hely, who developed cultivars at three ploidy levels, diploid, tetraploid, and hexaploid (Anonymous, 1987). The hexaploid form turned out to be the most vigorous. A hexaploid cultivar, Endura, developed by a private company, Wrightson Seeds (New Zealand), was introduced into the United States from New Zealand. The only cultivar of Kura released in the United States is 'Rhizo', developed by the USDA Soil Conservation Service in cooperation with the University of Kentucky (Henry and Taylor, 1989). This was a seed increase of PI 325489 after several generations of natural selection at Quicksand, KY. Phenotypic recurrent selection programs have been conducted at Minnesota, Wisconsin, and Kentucky.

Breeding Progress

DeHaan et al. (2001) conducted three cycles of divergent selection for shoot/root ratio and a control selection for large plant biomass under greenhouse conditions. As evaluated under field conditions, the selection program was effective for an increase in shoot yield and reduced shoot/root ratio. Selection for high shoot/root ratio did not affect seedling size in the field. The authors concluded that greenhouse selection for seedling size is an effective means of increasing seedling vigor in Kura clover. Smith (1995) used two cycles of selection to increase seed size in diploid, tetraploid, and hexaploid germplasm. Seed size in the hexaploid population was increased by 27%, and total forage yield in the second year was increased by 1.05 Mg ha⁻¹. Taylor and Cornelius (1994) selected plants for first-season flowering. The rationale was that first-season blooming may be associated with greater seedling and aftermath vigor. After six cycles of selection, the

first-cycle program led to increased flowering in the first and second season as well but also led to progressive reduction in forage and seed yields. The yield reductions may have been associated with inbreeding depression or detrimental effects of first-season flowering.

In summary, despite its outstanding persistence, much more breeding is likely necessary to ensure widespread farmer usage of Kura clover.

CONCLUSIONS

As we have seen, there has been a decline in the use of clovers in the United States and, consequently, in the number of breeding programs. This decline started with the development of nitrogen synthesis by the Haber–Bosch process and continued in the United States due to major changes in U.S. agriculture. A shift to other legumes, primarily alfalfa, was accompanied by decoupling of livestock from the land in the midwestern states. Instead of the rotation of corn, small grains, and red clover, the primary rotation now is corn followed by soybeans or continuous corn or soybeans. Such monoculture has been encouraged by government policies as well. Corn, and the other feed grains, wheat (*Triticum aestivum* L.), and soybeans, plus cotton (*Gossypium* L.), receive three-fourths of all crop subsidies (Parr et al., 1990). Consequences of this decline have been recently highlighted in the 2005 symposium “Integrated Crop–Livestock Systems for Profit and Sustainability” (Russelle and Franzuebbers, 2007). As a part of that symposium, Sulc and Tracy (2007) suggest that integration of crops and livestock on the same land base offers tremendous potential to diversify farm ecosystems while being economically competitive and more environmentally compatible than specialized production systems. Nutrients are removed from farms as crop products, and more fertilizers must be purchased to make up the difference. Denitrification also becomes more of a problem because of the lack of red clover and other legumes to serve as a nitrogen sink. Leaching, particularly of nitrogen, is prevalent (Elliot et al., 1987; Scott and Burt, 1987). Erosion from soil under monoculture has been adequately documented (Hargrove and Frye, 1987). Water-use efficiency and quality are likewise detrimentally affected. A more-dispersed pattern of livestock production is a necessary step to reduce the often-severe regional water-quality problem that results when local supply of manure exceeds the capacity of cropland to use the nutrients in manure efficiently. Insect, disease, and weed problems also become more difficult to control under monoculture systems (Luna and House, 1990; Byers and Stromberg, 1987).

Systems such as sustainable agriculture, conservation tillage, minimum tillage, regenerative agriculture, and organic farming are receiving more recognition. However, government programs will have to be modi-

fied to promote these practices. Today’s clovers are much improved over early common types and can help to attain the goals of these systems. More-persistent cultivars have been developed that possess greater adaptation to areas of use. All major diseases and nematodes are under control by cultivars bred for resistance. Although clover use in rotations has declined, its use to provide a higher quality of pasture and hays has increased. Cultivars, such as ‘Astrid’ red clover and Durana and Patriot white clover, are beginning to be developed for use under grazing conditions. Also, interspecific hybridization programs hold promise for incorporating greater perenniality into white clover from *T. ambiguum* and into red clover from *T. medium*. Further, the movement of clover seed production to specialized areas has had positive effects. More seed of improved cultivars and less seed of common types are becoming available.

Molecular breeding, although holding much promise, faces many challenges relating to the lack of acceptance of transgenic crop plants in many parts of the world. Socioeconomic impacts due to control of seed supplies by multinational seed companies and consequent effects on sustainable agriculture are all factors that must be addressed. Economics undoubtedly will govern whether these multinational seed companies will move their transformation capabilities to the clovers. Important characteristics such as yield and persistence are controlled by many genes and are less amenable to the usual transformation procedures. Marker-assisted selection and genomics add much to our knowledge base and are important plant-breeding tools; they are expected to maximize efforts to breed more and better clovers. Challenges remain, however. These include greater persistence, elimination of antiquity factors, higher yields of both forage and seed especially of tetraploid red clover, increased fertility of interspecific hybrids, and adapting to climatic change.

New legumes such as Kura clover are being grown. However, the introduction of new crops faces a long road to general acceptance by the farming community that is familiar with red and white clover. The unique qualities of high-seeding vigor, ease of establishment, and general competitiveness ensure that these clovers will continue to be major legumes in the forage area of the United States and thus are worthy of continued breeding efforts.

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