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# *Trifolium repens*

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## Scientific Name

*Trifolium repens* L.

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## Synonyms

*Lotodes repens* Kuntze, *Trifolium limonium* Phil., *Trifolium repens* f. *riparia* Hauman, *Trifolium repens* L. ssp. *giganteum* (Lagr.-Fossat) Ponert, *Trifolium repens* L. var. *giganteum* Lagr.-Fossat, *Trifolium stipitatum* Clos

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## Family

Fabaceae, also placed in Papilionaceae

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## Common/English Names

Honeysuckle Clover, Ladino Clover, Lodi Clover, Dutch Clover, Dutch White Clover, White Clover, White Sweet Clover

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## Vernacular Names

**Brazil:** Trevo Branco (Portuguese)

**Chinese:** Bai Che Zhou Cao, Bai Hua San Ye Cao, Bai San Ye Cao, San Xiao Cao

**Czech:** Jetel Plazivý, Jetel Plazivý Bílý, Jetelovec Plazivý

**Danish:** Hvid Kløver

**Dutch:** Schapebloem, Witte Klaver

**Eastonian:** Valge Ristik

**Esperanto:** Trifolio Blanka

**Finnish:** Valkoapila

**French:** Trèfle Blanc, Trèfle De Hollande, Trèfle Rampant

**Gaelic:** Seamair Bhán

**German:** Kriech-Klee, Kriechender Klee, Kriechender Weiss-Klee, Lämmer-Klee, Weiss-Klee, Weisser Wiesen-Klee

**Hebrew:** Tiltan Zochel

**Hungarian:** Fehér Here, Kúszó Here

**Icelandic:** Hvítmári

**India:** Safed Tipatiya Ghaas (Hindi)

**Indonesia:** Semanggi Landi (Java)

**Italian:** Trifoglio Bianco, Trifoglio Ladino, Trifoglio Rampicante

**Japanese:** Oranda Genge, Shiro Kurooba, Shiro Tsume Kusa

**Norwegian:** Hvitkløver, Kvitkløver, Okseblom, Smære, Småkløver

**Polish:** Koniczyna Biała

**Portuguese:** Trevo-Branco, Trevo-Coroa-De-Rei, Trevo-Da-Holanda, Trevo-Ladino, Trevo-Rasteiro

**Russian:** Klever Belyj, Klever Polzuchii

**Slovaščina:** Bela Detelja, Detelja Plazeča, Plazeča Detelja

**Slovincina:** Ďatelina Plazivá

**Spanish:** Trébol Amargo, Trébol Blanco, Trébol Rastrero

**Swedish:** Hvitklöfver, Krypklöver, Vitklöver, Vitvåpling

**Taiwan:** Shu Cao

**Thai:** Thua Clover

**Turkish:** Aküçgöl, Yonca, Yonca Beyaz

**Vietnamese:** Chè Ba, Cỏ Ba Lá Hà Lan, Cỏ Ba Lá Hoa Trắng

**Welsh:** Meillion Gwyn

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## Origin/Distribution

White clover is native to Mediterranean Europe, North Africa and West Asia and has been used as a pasture legume in both Europe and the British Isles for centuries. It is also cultivated as a pasture crop in many cool temperate and subtropical parts of the world such as North America, southern Latin America, South Africa, Australasia, China and Japan. It has naturalized in many of these areas.

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## Agroecology

White clover grows in turfgrass, crops and landscapes. It is also found in a wide range of different soil type environments but less vigorously on acid, poorly drained or shallow, drought-prone soils especially soils with toxic levels of exchangeable aluminium and manganese. It thrives on moisture-retentive but free-draining soils with adequate soil pH, 5.8–6.0 on mineral soils and 5.5–5.8 on peaty soils. Optimum temperature for growth is 20–25 °C. It tolerates moderate but not severe drought.

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## Edible Plant Parts and Uses

Young leaves and flower heads are eaten (Cribb and Cribb 1976; Launert 1981; Facciola 1990; Schofield 2003). Clovers are a valuable survival food: they are high in protein, are widespread, are abundant and are eaten boiled or cooked as potherb, in soups and also used in salads. The dried leaves impart a vanilla flavour to cakes and other confectionery. Young flower heads can be used in salads. Dried flower heads and seedpods can also be ground into nutritious flour and mixed with other foods such as rice. Dried flower heads also can be steeped in hot water for a healthy, tasty

tea-like infusion. Roots are also edible cooked (Kunkel 1984; Schofield 2003). The high quercetin concentration and soyasaponin occurrence make the seeds of some *Trifolium* species (*T. repens*, *T. pratense*) a potential source of health beneficial phytochemicals for use in human nutrition (Sabudak and Guler 2009).

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## Botany

Glabrous perennial, low growing, herbaceous plant, rooting at nodes with trifoliate leaves arranged alternately. Leaflets broadly ovate or circular, rounded or retuse at the tip and usually with whitish leaf markings on the upper mid surface (Plates 1 and 2). Stipules pale and translucent with a short point and broad at the base. Leaf sizes vary from very small in the prostrate, short-



**Plate 1** White Clover pasture crop



**Plate 2** Close view of leaves and inflorescence

petiolate types to large in the longer-petiolate, more erect cultivar types. Stolons initiated from leaf axils form a branched network radiating from an initially tap-rooted seedling. Inflorescences are globular racemes, 15–25 mm across, with 20–40 florets at the end of long peduncles originating from leaf axils on the stolons (Plates 1 and 2). Flowers scented; calyx 2–6 mm, 10-nerved with unequal teeth; corolla white often tinged pink, becoming deflexed with age with 8–13 mm long vexillum. Fruit linear, 3–4-seeded legume. Seeds cordate with a smooth surface, bright yellow to yellowish brown, becoming darker with age.

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## Nutritive/Medicinal Properties

### Plant/Shoot Phytochemicals

White clover had been reported to be high in protein and minerals (Anonymous 2005; Frame and Newbould 1986; Frame et al. 1998), containing 22–28 % crude protein, 2.7–3.3 % crude fat, 9.4–11.9 % ash, 6.6–7 % lignin and a crude fibre content of 15.7–21.1 % (Anonymous 2005). White clover was reported to contain per kg dry matter: 26.6–5.3 g N, 1.9–4.7 g P, 15.4–38.0 g K, 12.0–23.1 g Ca, 1.4–2.9 g Mg, 2.4–3.6 g S, 0.5–4.6 g Na, 3.4–25.6 g Cl, 102–448 mg Fe, 40–87 mg Mn, 22–32 mg Zn, 5.4–9.7 mg Cu, 0.10–0.38 mg Co, 0.14–0.44 mg I, 1.3–14.2 mg Mo, 0.005–153 mg Se and 26–50 mg B (Frame and Newbould 1986). As animal forage, white clover contributes optimally as a 10–20 % component when grown in conjunction with other grasses (Anonymous 2005). It was found to be more digestible than other temperate forage legumes and therefore the ingested nutrients could be utilized more efficiently.

Healthy and stressed white clover plants contained many types of secondary metabolite such as flavonols, flavones, condensed tannins, isoflavones, isoflavanones, pterocarpans, coumestans, cyanogenic glucosides and saponins in various plant tissues (Carlsen and Fomsgaard 2008). Many of these bioactive compounds from white clover could be exploited for suppressing weeds

and soil-borne diseases. The oil yield for *T. repens* was small 0.021 % (weight/fresh weight basis) (Tava et al. 2009). Several classes of compounds were found in the oil, including alcohols, aldehydes, ketones, terpenes, esters, hydrocarbons, phenolics and acids.

HE strains of white clover in New Zealand were reported to contain the cyanogenic glucosides lotaustralin and linamarin, the glucosides of methylethylketone cyanhydrin and acetone cyanohydrin, respectively (Butler and Butler 1960). The isoflavones genistein, biochanin A and formononetin were found in glycosidic forms in white clover and insignificant amount of coumestrol (Francis et al. 1967). The cyanogenic glucosides linamarin and lotaustralin were isolated as a mixture from *Trifolium repens* (Maher and Hughes 1971).  $\alpha$ -Hydroxy-methylbutyronitrile- $\beta$ -D-glucoside was found in white clover (Hughes and Conn 1976). Linamarin and lotaustralin were identified in *T. repens* cv. Armenia in concentrations of 1.29 and 3.13 mg/g of dry matter, respectively (Stochmal and Oleszek 1994). The linamarin/lotaustralin ratio ranged from 0.4 to 0.8 and was inversely correlated with the total cyanogen content; cultivars with higher cyanogen level had a lower linamarin/lotaustralin ratio (Stochmal and Oleszek 1997). At temperatures below 15 °C all varieties contained the highest cyanogen content; an increase in temperature during the summer time resulted in a drastic decrease in cyanogen synthesis.

In leaves, petioles, roots and nodules of white clover, pinitol (3-O-methyl-chiro-inositol) was found to be the predominant sugar, with sucrose present in lower amount (Davis and Nordin 1983). Significant amounts of  $\alpha$ -methyl glucoside and  $\beta$ -methyl glucoside, linamarin, glucose and fructose were found in the leaves and petioles. In the nodules, glucose was rarely present at detectable levels. Malonic acid did not appear to be present in unusually high concentrations in either leaves or nodules.

From white clover plant, saponins soyasapogenols A, B and C were isolated (Walter et al. 1955). From the whole plant of white clover, five triterpenoid saponins, designated cloversaponins I–V, were isolated together with four known

soyapoinins,  $\beta$ -D-glucuronopyranosylsoyasapogenol B, soyasaponin I, soyasaponin II, azukisaponin II as their methyl esters and astragaloside VIII (Sakamoto et al. 1992).

Phytoestrogens isolated from ladino clover included genistein, biochanin A, formononetin and most significantly coumestrol (Bickhoff et al. 1957, 1958, 1960, 1962; Guggolz et al. 1961). Sachse (1974) reported five estrogenic isoflavones—biochanin A, formononetin, pratensein, genistein, daidzein—and the estrogenic coumarin derivative coumestrol in 32 white clover varieties. Other phenolic compounds isolated from ladino clover included 3',4',7-trihydroxyflavone (Livingston and Bickhoff 1964), daphnoretin (2-hydroxy-6-methoxy-3-(2-oxochromen-7-yl)oxochromen-7-one) (Livingston et al. 1964a), trifoliol (3,7-dihydroxy-9-methoxy-6H-benzofuro(3,2,-c)-1-benzopyran-6-one) (Livingston et al. 1964b; Bickhoff et al. 1965a) and 7,4'-dihydroxyflavone (Bickhoff et al. 1965b). An acetylated isoflavone, genistein 7-(2''-p-coumaroylglucoside), genistein (Saxena and Jain 1986), 2''-O-acetylated formononetin and formononetin (Saxena and Jain 1989) were identified in *Trifolium repens*. Saloniemi et al. (1993) found the following isoflavones daidzein, formononetin, genistein and biochanin A in white clover varieties.

Flavones 4',5,6,7,8-pentahydroxy-3-methoxyflavone and 5,6,7,8-tetrahydroxy-3-methoxyflavone, as well as two flavones 3,7-dihydroxy-4'-methoxyflavone and 5,6,7,8-tetrahydroxy-4'-methoxyflavone, were isolated from white clover shoots (Ponce et al. 2004). The known quercetin, rhamnetin, acacetin, 7-hydroxy-4'-methoxyflavonol; 3,5,6,7,8-pentahydroxy-4'-methoxyflavone; 2',3',4',5',6'-pentahydroxy-chalcone; 6-hydroxykaempferol; 4',5,6,7,8-pentahydroxyflavone; and 3,4'-dimethoxykaempferol were also obtained.

Two bicoumarins, named repensin A and B, were isolated from *Trifolium repens* and their structures established as 7-methoxy-7',8'-dihydroxy-8,6'-bicoumarinyl and 7,5'-dihydroxy-3,6'-bicoumarinyl, respectively (Zhan et al. 2003). Shoots of *T. repens* were found to contain cyanogenic glycosides, mostly linamarin ( $\alpha$ -hydroxyisobutyronitrile- $\beta$ -D-glucopyranoside)

and a smaller proportion of lotaustralin (2-hydroxy-2-methylbutyronitrile- $\beta$ -D-glucopyranoside) that breaks down to release toxic hydrogen cyanide when damaged (Gleadow et al. 2009).

In all tissues of clover seedlings not infected by the stem nematode, *Ditylenchus dipsaci*, isoflavonoids occurred predominantly as their glycosidic conjugates, in the order roots > meristems > leaves with formononetin-7-O-glucoside-6''-O-malonate (FGM) and medicarpin-3-O-glucoside-6''-O-malonate (MGM) as the major metabolites (Cook et al. 1995). The conjugates accumulated with age in all tissues and no differences were observed between the isoflavonoid content of healthy susceptible and resistant plants. Infection with either *D. dipsaci* race (oat and clover) elicited the accumulation of medicarpin, MGM and FGM in the meristems to a similar degree in resistant and susceptible seedlings. However, formononetin accumulated only in the infected meristems of the resistant plants. Johnson et al. (2005) found that different flavonoids increased in concentration in plants without rhizobial nodules than in plants with active or inactive nodules. The concentration of 4',7-dihydroxyflavone was higher in plants without rhizobial nodules than in plants with active or inactive nodules. The content of formononetin was higher in roots with active rhizobial nodules than in inactive nodules and roots alone. Flavonol contents of white clover seeds (2.8–2,000 mg/g), leaves (<2–1,700 mg/g) and total above-ground material (20–2,210 mg/g) were higher than in roots (n.d.–208 mg/g) and flowers (66–481 mg/g) (Carlsen et al. 2008).

### Leaf Phytochemicals

L-pipecolinic acid (piperidine-2-carboxylic acid) was isolated from the leaves (Morrison 1953) and a leaf protease (Brady 1961). Four coumestans detected in white clover infected with various foliar pathogens and were identified as coumestrol, 12-O-methylcoumestrol, trifoliol and 7,10,12-trihydroxycoumestan (repensol) (Wong and Latch 1971a; 1971b); other phenolic compounds found in diseased white clover included isorhamnetin; 4',7-dihydroxyflavonol;

kaempferol; quercetin; 4',7-dihydroxyflavone; 3',4'',7-trihydroxyflavone; geraldone; luteolin; formononetin; daphnoretin; and a trihydroxycoumestan (Wong and Latch 1971b). White clover contained the phytoalexin medicarpin (Gustine 1981). Isoflavonoids medicarpin and demethylmedicarpin were detected in the droplet of white clover leaflets inoculated with *Monilia fructicola*; several minor isoflavonoids detected in the extracts were identified as formononetin (7-hydroxy-4'-methoxyisoflavone), 2'-hydroxyformononetin and vestitone (7,2'-dihydroxy-4'-methoxyisoflavanone) (Woodward 1981). The following isoflavonoid phytoalexins were detected in fungus-inoculated leaflets: 7,2',4'-trihydroxyisoflavanone, vestitone, medicarpin, demethylmedicarpin, variabilin, sativan, vestitol, genistein, formononetin, glycitein and daidzein (Ingham 1978, 1982).

In white clover, decreased foliar protein coincided with an increased number of protease isoforms (Kingston-Smith et al. 2003). Major flavonoids in the leaves were found to be derivatives of quercetin and kaempferol (Hofmann et al. 2000). Total leaf flavonoids in *Trifolium repens* were found to be low only 1 mg/g compared to 50–65 mg/g for *T. dubium* and *Lotus corniculatus* and up to 15 mg/g for *T. pratense* (de Rijke et al. 2004). In *T. pratense* and *T. repens*, the main constituents were flavonoid glucoside-(di)malonates, while *T. dubium* and *L. corniculatus* mainly contained flavonoid (di)glycosides.

### Leaf and Flower Phytochemicals

A total of 12 flavonoids, pterocarpan and methyl caffeate were isolated from leaves and flowers and identified as quercetin 3-*O*-(6''- $\alpha$ -rhamnopyranosyl-2''- $\beta$ -xylopyranosyl)- $\beta$ -galactopyranoside (1), kaempferol 3-*O*-(6''- $\alpha$ -rhamnopyranosyl-2''- $\beta$ -xylopyranosyl)- $\beta$ -galactopyranoside (2), kaempferol 3-*O*-(2''-6''- $\alpha$ -dirhamnopyranosyl)- $\beta$ -galactopyranoside, mauritianin(3), quercetin 3-*O*-(2''- $\beta$ -xylopyranosyl)- $\beta$ -galactopyranoside (4), kaempferol 3-*O*-(2''- $\beta$ -xylopyranosyl)- $\beta$ -galactopyranoside (5), kaempferol 3-*O*- $\beta$ -(6''-*O*-acetyl)-galactopyranoside (6),

quercetin 3-*O*- $\beta$ -(6''-*O*-acetyl)-galactopyranoside (7), trifolin (8), hyperoside (9), myricetin 3-*O*- $\beta$ -galactopyranoside (10), quercetin (11), ononin (12), medicarpin 3-*O*- $\beta$ -glucopyranoside (13) and methyl caffeate (14) (Kicel and Wolbiś 2012b).

### Flower Phytochemicals

More than 50 compounds were identified in the essential oil samples of red and white Austrian clover flowers (Buchbauer et al. 1996). The main constituents (concentration >2 %) were maltol (8.2 %), linalool (4.2 %), 1-phenylethyl alcohol (3.2 %), phenol (2.9 %), phenylethyl acetate (2.7 %), acetophenone (2.4 %) and (*Z*)-3-hexenyl acetate (2.2 %) for red clover flowers and maltol (5.3 %), linalool (3.8 %), phenol (3.6 %), phenylethyl acetate (3.3 %) and 2-phenylethyl alcohol (2.8 %) for white clover flowers.

Major flavonoids in white clover flowers were identified as glycosidic derivatives of quercetin and myricetin (Foo et al. 2000; Schittko et al. 1999). Three main flavonoid components of flower extracts, quercetin-3-*O*-galactoside, its 6''-*O*-acetyl derivative and myricetin-3-*O*-galactoside, were isolated from the flowers (Schittko et al. 1999). White clover flowers were found to contain an abundance of phenolics, namely, quercetin myricetin, *cis-p*-coumaric acid 4-*O*- $\beta$ -D-glucopyranoside; *trans-p*-coumaric acid 4-*O*- $\beta$ -D-glucopyranoside; the 3-*O*- $\beta$ -D-galactopyranosides of myricetin, quercetin and kaempferol together with two new derivatives, namely, myricetin 3-*O*-(6''-acetyl)- $\beta$ -D-galactopyranoside and kaempferol 3-*O*-(6''-acetyl)- $\beta$ -D-galactopyranoside (Foo et al. 2000). Gallocatechin, epigallocatechin, gallocatechin-(4 $\alpha$ -8)-epigallocatechin and their corresponding prodelphinidin polymers were also present. The following coumarins umbelliferone, scopoletin, repensin B, daphnoretin and daphnorin (daphnoretin 7-*O*- $\beta$ -D-glucoside) were isolated from the flowers (Kicel and Wolbiś 2012a). Coumarins, bicoumol (7,7'-dihydroxy-6,8'-bicomariny) and umbelliferone (7-hydroxycoumarin) were found in ladino clover (Spencer et al. 1967). Floral tissues in white clover plants were found to produce both proanthocyanidins and antho-

cyanins (Abeynayake et al. 2012). The white clover floral prodelphinidins were found to consist of terminal units with nearly equal proportions of epigallocatechin (52 %) and galocatechin (48 %) and extender units showing epigallocatechin (56 %) and galocatechin (39 %) (Sivakumaran et al. 2004). Tannins were found to accumulate in the flowers of white clover but not in the leaves or stolons (Anonymous 2005).

### Seed Phytochemicals

Aqueous extracts of white clover seeds were found to contain myricetin, which was not toxic to *Rhizobium trifolii*, and condensed tannins (prodelphinidins) which were separated into three fractions each of which was toxic to *R. trifolii* (Young and Paterson 1980). The three tannin fractions, one of which had molecular weight of 6,000–12,000 and the other 2,6000–18,000, produced delphinidin on hydrolysis. A natural substance, 2,3-dihydroxy-2,4-cyclopentadien-1-one, was isolated from the seeds along with kaempferol, quercetin and myricetin (Nakatani et al. 1989). The flavonol quercetin and saponins astragaloside VIII, soyasaponin I 22-*O*-glucoside and soyasaponin I 22-*O*-diglucoside were found in the seeds (Oleszek and Stochmal 2002). Main flavonoids found in the seeds were quercetin, myricetin and kaempferol (Prati et al. 2007).

Germinated white clover seeds were found to contain oligosaccharides and galactomannans and enzymes, 5  $\alpha$ -galactosidases I–V (Williams et al. 1978), galactomannans, manno-oligosaccharides and 2  $\beta$ -mannanases I and II (Villarroya et al. 1978).

### Root Phytochemicals

Quercetin, acacetin and rhamnetin accumulated in roots of arbuscular mycorrhizal fungus *Glomus intraradices*-inoculated plants, whereas they were not detected in non-inoculated plants. Two isoflavonoids isolated from clover roots grown under phosphate stress were characterized as formononetin (7-hydroxy,4'-methoxy

isoflavone) and biochanin A (5,7-dihydroxy,4'-methoxy isoflavone) (Nair et al. 1991). At 5 ppm, both compounds stimulated hyphal growth in-vitro and root colonization of an undescribed vesicular-arbuscular mycorrhiza, a *Glomus* sp. The following flavonoids were found in white clover root exudates which acted as signal compounds for nodulation by *Rhizobium* bacteria: 7,4'-dihydroxyflavone, geraldone and 4'-hydroxy-7-methoxyflavone (Redmond et al. 1968). Quercetin, acacetin and rhamnetin accumulated in roots of mycorrhizal inoculated plants, whereas they were not detected in non-inoculated plants (Ponce et al. 2004). Eight flavonoids kaempferol, medicarpin, demethylmedicarpin coumestrol, daidzein, formononetin, genistein and biochanin A were found in the roots (Carlsen et al. 2008). Only plants colonized with the arbuscular mycorrhizal fungus, *Glomus claroideum*, showed detectable concentrations of either coumestrol or kaempferol (cultivar dependant). Only the concentrations of formononetin and daidzein increased in clover roots in response to infection with *Pythium ultimum*. White clover contained the flavonoid aglycones, formononetin, medicarpin and kaempferol and glycosides kaempferol-Rha-Xyl-Gal and quercetin-Xyl-Gal which it released into the soil (Carlsen et al. 2012).

### Antioxidant Activity

*T. repens* flower and leaf extracts showed antioxidant activity towards DPPH radical with EC<sub>50</sub> values ranging from 72.3 to 179.3  $\mu$ g/ml, respectively (Kicel and Wolbiś 2013). Significant linear correlations were found between antioxidant potentials of flowers and leaves and total phenolic and flavonoid contents determined ( $R^2$  in the range of 0.97–0.99). The flowers were the richest source of phenolics ranging from 28.7 to 38.8 mg GAE/g and flavonoids, calculated for hyperoside, up to 20 mg HP/g, which hydrolyzed mainly to flavonols (the quercetin level greater than 6 mg/g). *T. repens* was found to be poor in isoflavones; similar quantities of Ca 0.2 mg/g were detected in the flowers and leaves.

Phenolic compounds from leaves and flowers, quercetin 3-*O*-(2''- $\beta$ -xylopyranosyl)- $\beta$ -galactopyranoside (4), quercetin 3-*O*- $\beta$ -(6''-*O*-acetyl)-galactopyranoside (7), hyperoside (9), myricetin 3-*O*- $\beta$ -galactopyranoside (10) and quercetin (11), were found to have potent antioxidant effect against DPPH, but the most effective were compounds 9, 10 and 11 (EC<sub>50</sub> values in the range 7.51–9.52  $\mu$ M) (Kicel and Wolbiś 2012b).

### Estrogenic Activity

Estrogenic compound coumestrol together with other related compounds, coumestans, had been found in white clover (Bickoff et al. 1969; Wong and Latch 1971a); however their normal levels in plants were very low and estrogenically insignificant. Wong et al. (1971) demonstrated estrogenic activity in diseased white clover samples when fed to mice and sheep. Potencies were in parallel to concentration of coumestans. Phytoestrogens can cause infertility in sheep and cattle (Adams 1995). Cows and ewes fed estrogenic forage may suffer impaired ovarian function, often accompanied by reduced conception rates and increased embryonic loss. In cows, clinical signs resembled those associated with cystic ovaries. The infertility was found to be temporary, reverting to normal within 1 month after abstinence from the oestrogenic feed. However, ewes exposed to oestrogen for prolonged periods may suffer a second form of infertility that was permanent, caused by developmental actions of oestrogen during adult life. There have been sporadic reports of estrogenic problems in animals grazed on white clover (*Trifolium repens*). If affected by foliar disease, white clover may produce higher levels of oestrogenic coumestans including coumestrol, 9-*O*-methyl-coumestrol, trifoliol and repensol (Wong and Latch 1971a; Wong et al. 1971). The estrogenic isoflavone contents in white clover varieties were generally low, consisting mainly of formononetin (90–95 %) and genistein (5–10 %), and their contents did not differ between varieties (Nykänen-Kurki et al. 1993). Saloniemi et al. (1993) also reported that white clover varieties contained very small

quantities of estrogenic isoflavones and coumestrol, and they did not explain the increased weight of the immature rat uterus observed in the biological studies. Some coumestrol was found in the autumn. The oestrogenic effect of white clover on rat uterus was found to be positive, the uterine weight of control rats averaged 21 mg, in test groups from 29 to 66 mg. Bickoff et al. (1969) showed coumestrol to be active in mice and sheep, while some poly-hydroxycoumestans were active in mice. Most growth studies indicated that coumestrol had no effect on the rate of growth of either cattle or sheep. However, some evidence existed which suggested that coumestrol may have beneficial effects on carcass quality in lambs.

### Traditional Medicinal Uses

The plant is regarded as antirheumatic, antiscrophulatic, depurative, detergent and tonic and a tincture of the leaves is applied as an ointment for gout (Duke and Ayensu 1985). An infusion of the plant has been used in the treatment of coughs, colds, fevers and leucorrhoea, and flower infusion has been used as an eyewash (Moerman 1998). In Turkish folk medicine, for example, some *Trifolium* species including *T. pratense* and *T. repens* are used for their expectorant, analgesic, antiseptic properties and also to treat rheumatic aches (Sabudak and Guler 2009).

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### Other Uses

White clover is highly important in the dairy, meat and wool industries, significantly improving yields of these products. It is one of the best quality forage legume and the most important one in grazed pastures of moist temperate regions. White clover is suited to both continuous stocking and rotational grazing systems. It provides highly acceptable and nutritious forage to livestock whether as silage, hay or when grazed at a leafy stage. White clover is protein and mineral rich and retains a high digestibility for animals. Physical, chemical and anatomical features contribute to superior intake of white clover compared with grass.

White clover makes a good green manure crop especially with mixture of *Lolium perenne* as well as a good ground cover in sunny locations. It is also an important honey crop for beekeeping.

The isoflavonoids, formononetin and biochanin A, identified from clover roots, stimulated colonization and growth of white clover, while several other flavonoid compounds were inactive when tested at concentrations of 5 mg/l (Siqueira et al. 1991). The stimulatory effects of these isoflavonoids on plant growth were mediated by vesicular-arbuscular mycorrhizal fungi.

## Comments

Refer also to notes under *Trifolium pratense*, red clover, which share numerous similar bioactive phytochemicals imparting many pharmacological effects.

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