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Review paper

Biologically active secondary metabolites in white clover (*Trifolium repens* L.) – a review focusing on contents in the plant, plant–pest interactions and transformation

Sandra C. K. Carlsen and Inge S. Fomsgaard

University of Aarhus, Faculty of Agricultural Sciences, Department of Integrated Pest Management, Research Centre Flakkebjerg, DK-4200 Slagelse

Summary. To exploit biologically active compounds from white clover (*Trifolium repens* L.) for suppressing weeds and soil-borne diseases, either as isolated products (biopesticides) or through cultivars with enhanced production of these compounds, the biologically active compounds must be identified, plant content measured, and their fate in soil known. The present review summarizes the published knowledge needed for such exploitation; providing essential information on structure and concentration of flavonols, flavones, condensed tannins, isoflavones, isoflavanones, pterocarpans, coumestans, cyanogenic glucosides, and saponins in healthy and stressed white clover plants. Various stresses and particular cultivars affect the concentrations of several of the compounds. Information on biological effects and the degradation/transformation of these compounds in plants or by microorganisms is available. There is no information on the degradation pathway in soil, the mechanisms of exudation and leaching of compounds from plants, and soil sorption properties of the compounds. The clover soil fatigue problem is increasing in grasslands and causes problems especially in organic farming. Research efforts focused on biological elements of clover soil fatigue have not explained it, and the influence of secondary metabolites has not been investigated. There are few investigations into the interaction between beneficial fungi/fungal-diseases and the occurrence of biologically active secondary metabolites in white clover plants. Such studies are critical to better understand beneficial fungi and pathogens.

Key words: Secondary metabolites – white clover – *Trifolium repens* – content – plant–pest interactions – degradation

Correspondence to: Inge S. Fomsgaard,
e-mail: Inge.Fomsgaard@agrsci.dk

Introduction

Legumes can improve cereal growth in crop rotations by supplying the soil with nitrogen derived from symbiotic fixation (Foo et al., 2000). Legumes also improve soil physical properties and reduce soil erosion (Inderjit and Keating, 1999); the beneficial soil-sanitation effects of legumes in rotations are widely acknowledged in agricultural practice.

While rotation is an absolute prerequisite in organic farming, it is playing an important role even in conventional farming in preventing weeds, pests, and diseases. A reduction in weed pressure and diseases in cereals following legume crops has been reported in numerous cases (Abdin et al., 2000; Clayton et al., 1997; Doyle et al., 1988; Hiltbrunner et al., 2007; Ohno et al., 2000; Reeves et al., 1984; Stevenson and van Kessel, 1996), and secondary metabolites have been assumed to play an important role. White clover is highly used as cover crop and source of green manure in Northern Europe, and the use of white clover as cover crop for winter wheat (*Triticum aestivum* L.) showed the highest reduction in various weeds in the field compared to other legumes but at the same time had a negative effect on growth and yield of winter wheat (Hiltbrunner et al., 2007). The negative impact on winter wheat may be reduced by a different management strategy and compensated for by a high production of biomass of white clover, which can be used for feed for cattle (Hiltbrunner et al., 2007). However, farmers have occasionally observed unexplained oversowing failure of grasses on clover-dominant swards, even when soil nitrogen, climatic conditions, and management practices seem adequate (Macfarlane et al., 1982). Whether this depressive effect is due to secondary metabolites (allelochemicals) released from clover is unknown. The self-inhibitory effect that white clover (*Trifolium repens* L.) can have may be relevant to clover soil fatigue, believed to explain the reduced growth of white clover crops in Denmark. This has occurred especially in

organic farming, with total crop failure in some instances (Søegaard et al., 2004). Grasslands are a vital resource in agriculture, and clover soil fatigue is becoming more frequent when new clover–grass mixed swards are established after ploughing-under older clover–grass fields. In some cases clover growth has completely failed, leaving only grass. Research has focused on biological elements of this phenomenon and no satisfactory explanation has been determined (Søegaard et al., 2004).

To exploit biologically active compounds from white clover for suppressing weeds and soil-borne diseases, either as isolated products (so-called biopesticides) or through cultivars with enhanced production of these compounds, then the compounds must be identified, the amounts in plants determined, and the fate in soil known in detail. The present review summarizes current knowledge.

Biological effects of white clover plants or plant extracts

Allelopathic effects in clover plant extracts or whole clover plants are often studied without assigning the observations to specific compounds; such results are summarized in Table 1. White clover can depress growth of grasses and dicotyledons, including itself and other legumes (Grant and Sallans, 1964; Newman and Rovira, 1975; Scott, 1975). Macfarlane et al. (1982) observed that the allelopathic effect of white clover dominated at germination and initial seedling establishment stages and was slowly lost or replaced by mineralization-promoting effects on the mature plant. Some cultivars have antibiotic effects (Hale and Mathers, 1977), and seed toxins have been found to affect rhizobia survival on the seed and thereby the establishment of oversown white clover (Hale and Mathers, 1977; Hale et al., 1979). White clover incorporated into the soil can also reduce the number of nematode (*Pratylenchus penetrans*) populations (Widmer and Abawi, 1998). White clover also has estrogenic properties in mice, rats, sheep, and cattle (Table 1). Estrogenic activity can increase with fungal infection (Wong et al., 1971), growth stage, location, climatic conditions, and date of harvest (Bickoff et al., 1960a). Virus infection in white clover may increase estrogenic activity in mice (Bickoff et al., 1960a).

Due to its allelopathic effects on both weeds and other cultivars, Breland (1996) suggested that spring sowing of white clover should be delayed 3–4 weeks after fresh cover-crop material is mixed into the seedbed to avoid inhibition of the newly sown crop.

Secondary metabolites in white clover

Some secondary metabolites in white clover have documented biological effects and are suspected of causing many of the effects described in section 2 above. These compounds include flavonoids/isoflavonoids, cyanogenic glucosides, and saponins – all are widely distributed in plant species (Harborne and Williams, 2000; Oleszek and Marston, 2000) and divided into several subclasses (Table 2; Aoki et al., 2000). The defence compounds of white clover may also include several other chemical classes of compounds, some undiscovered, or compounds like for instance small amino acids and antifungal macromolecules such as proteins, but these are normally not categorized as secondary metabolites and therefore falls out of the scope of present review. Several important compounds (Table 3), their content in plants (Table 4), their biological effects (Tables 2 and 5, and sections 4–11), and their degradation (Figs. 1–5) are summarized in this review. Plant flavonoids are generally sugar conjugates (Duke, 1986); the first step in their degradation is the release of aglucones (Bickoff, 1968; Price and Fenwick, 1985), but further soil degradation has been little investigated. Some degradation pathways involve anaerobic bacteria not usually present in soil. However, these pathways have been included in the present review, as the same degradation products could be formed in the soil as a result of the action of other bacteria or fungi.

Flavonols

Flavonols in white clover

The flavonol contents of white clover (Table 3) vary significantly among cultivars (Hofmann et al., 2000). Flavonol contents of white clover seeds (2.8–2000 μg/g), leaves (<2–1700 μg/g), and total above-ground material (20–2210 μg/g) are higher than in roots (n.d.–208 μg/g) and flowers (66–481 μg/g) (Table 4), but in no experiments were contents determined in different parts of the same plant. Consequently, any conclusions on the flavonol distribution between plant parts should be cautious. The three flavonols quercetin, kaempferol, and myricetin were the main flavonoids in seeds of a single cultivar (Prati et al., 2007), major flavonoids were derivatives of quercetin and kaempferol in leaves of white clover plants (Hofmann et al., 2000), and major flavonoids in flowers of white clover were identified as glycosidic derivatives of quercetin and myricetin (Foo et al., 2000; Schittko et al., 1999). There is little knowledge of flavonol content variations in white clover in relation to stress; some evidence indicates increases with plant exposure to UV-B light or to drought (Hofmann et al., 2000, 2003). Plant flavonol concentrations do not increase with fungal infections (Carlsen et al., 2008; Wong and Latch, 1971b), and there is no consistent influence of inoculation by arbuscular mycorrhizal (AM) fungi, with some increases and other decreases (Carlsen et al., 2008; Ponce et al., 2004).

Table 1 Biological effects of white clover plants or plant extracts

Article	White clover plant material	Target	Type of effect	Effect	No effect
Bennet et al., 1967	Swards of cultivars Victorian Irrigation, Grasslands Huia and Ladino were grazed	Sheep	Estrogenic (uterine weight response of ovariectomized ewes)	+ (Cultivar Victorian Irrigation variable activity)	+ (Cultivar Grasslands Huia and Ladino)
Bickoff et al., 1960a	Above-ground material of 6 clones as well as cultivar Ladino clover	Mice	Estrogenic (increase in uterine weight)	+ (Ladino clover, the clones had variable activity)	+ (Some of the clones in some of the instances)
Bickoff et al., 1969	Above-ground material	Cattle	Estrogenic	+	–
Cheng et al., 1953	Extract of above-ground material of cultivar Ladino clover	Mice	Estrogenic (increase in uterine weight)	-	+
Grant and Sallans, 1964	Aqueous extracts of above-ground material of cultivar Ladino clover	4 grasses and 4 legumes, including white clover.	Inhibition of germination, shoot growth and root length	++ (3 grasses and 3 legumes)	+ (Timothy and birdsfoot trefoil)
Grant and Sallans, 1964	Aqueous extracts of roots of cultivar Ladino clover	4 grasses and 4 legumes, including white clover.	Inhibition of germination, shoot growth and root length	+ (Red canary grass)	+ (3 grasses and 4 legumes)
Hale and Mathers, 1977	Aqueous extracts of seed of cultivar Grasslands Huia	24 cultures of 15 species of bacteria and an actinomycete	Antibiotic	++ (All but two *Rhizobium* isolates)	+ (Other genera tested)
Hiltbrunner et al., 2007	White clover as cover crop in the field	Monocotyledonous, dicotyledonous, spring-germinating and annual weeds.	Density	+++	–
Newman and Rovira, 1975	Leachate	4 grasses and 4 dicotyledonous species including white clover	Inhibition of shoot growth	++ (Mean inhibitory effect one of the largest)	–
Saloniemi et al., 1993	Above-ground material of cultivars Jögeva 4, Sandra, Tammisto and Undrom	Rats	Estrogenic (increase in uterine weight)	++ (Clearly positive)	–
Scott, 1975	Aqueous extracts of shoot material	19 legumes and grasses	Inhibition of germination	++ (Among the most depressive materials)	–
Widmer and Abawi, 1998	Whole plants incorporated into the soil	Nematode (*Pratylenchus penetrans*)	Population	++	–
Wong et al., 1971	Frozen fungal infected aerial parts	Sheep	Estrogenic (changes in teat length of Romney wethers)	+ (Infected with *Leptosphaerulina trifolii*)	– (Infected with *Uromyces trifolii*)
Wong et al., 1971	Fungal infected leaves and aerial parts, freeze dried or extracted with alcohol and petroleum ether as well as uninfected leaves, extracted the same way.	Mice	Estrogenic (uterine weight and vaginal smears)	+++/++/+ (Infected with *Pseudomonas trifolii* / *Uromyces trifolii* / *Leptosphaerulina trifolii*, alcohol extract)	– (Infected with *Uromyces trifolii* and *Leptosphaerulina trifolii*, freeze dried)

Table 2 Structure and functionality/effects of classes and subclasses[a] of white clover secondary metabolites

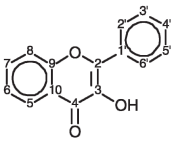
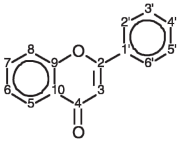
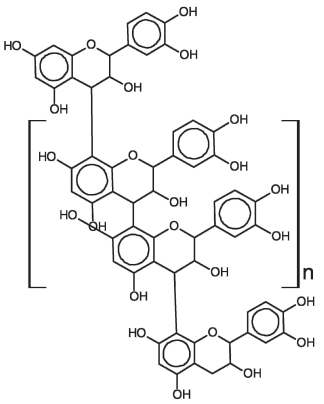
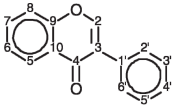
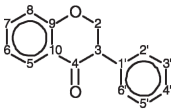
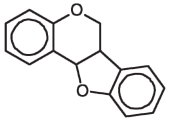
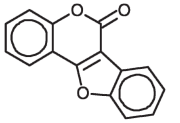
Class	Subclass	Compounds	Functionality / effects	Structure
Flavonoids	Flavonols	4′,7-Dihydroxyflavonol 7-Hydroxy-4′-methoxyflavonol Kaempferol Quercetin 6-Hydroxykaempferol Rhamnetin Isorhamnetin Myricetin 5,6,7,8-Tetrahydroxy-4'-methoxyflavonol	Toxic to insects (Elliger et al., 1980; Levin, 1976; Todd et al., 1971), fungi (Weidenbörner and Jha, 1994) and plants (Duke, 1986). Stimulate nitrogen-fixing association with Rhizobium (Edwards and Parry 1994; Novikova, 1994).	
	Flavones	4″,7-Dihydroxyflavone 3′,4′,7-Trihydroxyflavone Geraldone Acacetin Luteolin 4′,5,6,7,8-Pentahydroxyflavone 5,6,7,8-Tetrahydroxy-4'-methoxyflavone	Toxic to insects (Elliger et al., 1980), animals (Demole, 1962), bacteria (Van Etten and Pueppke, 1976), fungi (Weidenbörner and Jha, 1994) and plants (Duke, 1986). Stimulate nitrogen-fixing association with Rhizobium (Edwards and Parry 1994).	
	Condensed tannins	Prodelphinidin including gallocatechin-(4α-8)-epigallocatechin	Toxic to bacteria (Fottrell et al., 1964; Hale and Mathers, 1977; Mila and Scalbert, 1994; Scalbert, 1991; Young and Paterson, 1980), fungi (Levin, 1976; Masterson, 1965; Scalbert, 1991), insects (Levin, 1976), plants (Duke, 1986) and yeasts (Scalbert, 1991). Feeding barriers to phytophagous insects (Harborne, 1994) and grazing animals (Harborne, 1994; Singleton and Kratzer, 1973). Reduce feeding because of bitter taste (Jones et al., 1976).	
Isoflavonoids	Isoflavones	Daidzein Pseudobaptigenin Formononetin Genistein 7,2′,4′-Trihydroxyisoflavone 2′-Hydroxyformononetin Biochanin A Glycitein Calycosin Pratensein	Toxic to fungi (Ingham, 1982; Van Etten, 1976; Virtanen and Hietala, 1958; Weidenbörner et al., 1990) and bacteria (Cruickshank, 1963; Van Etten and Pueppke, 1976). Phytoestrogens (Pettersson et al., 1984; Saloniemi et al., 1993; Shutt and Braden, 1968; Shutt and Cox, 1972). Stimulate colonization by an arbuscular mycorrhizal fungus (Siqueira et al., 1991a).	

Table 2 (Continued)

Class	Subclass	Compounds	Functionality / effects	Structure
	Isoflavanones	7,2′,4′-Trihydroxyisoflavanone Vestitone	Fungitoxic (Denny and Van Etten, 1981; Ingham, 1982).	
	Pterocarpans	Demethylmedicarpin Medicarpin	Fungitoxic (Denny and Van Etten, 1981; Ingham, 1982) and phytotoxic (Gregory and Edwards, 1994)	
	Coumestans	Coumestrol 9-O-Methylcoumestrol Repensol Trifoliol Daphnoretin BV bBV	Some toxicity to fungi (Van Etten, 1976) and bacteria (Keen and Kennedy, 1974). Phytoestrogens (Bickoff et al., 1960b, 1962, 1969; Livingston et al., 1964b; Saba et al. 1974; Wong and Latch, 1971b; Wong et al., 1971).	
Cyanogenic glucosides		Linamarin Lotaustralin	Toxic to fungi (Levin, 1976), herbivores (Hughes, 1991; Stochmal and Oleszek, 1997) including molluscs (Angseesing, 1974; Angseesing and Angseesing, 1973; Grawford-Sidebotham, 1972; Horrill and Richards, 1986; Raffaelli and Mordue, 1990), insects (Ellsbury et al., 1992; Raffaelli and Mordue, 1990) and mammals (Coop and Blakley, 1949; Lehmann et al., 1990; Moran, 1954; Vickery et al., 1987; Viette et al., 2000).	
Saponins		Cloversaponin I-V methyl ester Soyasaponin I Soyasaponin I methyl ester Soyasaponin II methyl ester Azukisaponin II methyl ester Astragaloside VIII	Toxic to fungi (Wolters, 1968), insects (Horber et al., 1974), molluscs (Agarwal and Rastogi, 1974; Mølgaard et al., 2000) and ruminants (Agarwal and Rastogi, 1974). Health-promoting activities (Rao and Gurfinkel, 2000).	

[a] Chemicals are divided into classes and subclasses according to Aoki et al. (2000).

Table 3 Common- and systematic names, molar mass, cas no. , and structural- and molecular formula of aglyconic compounds from white clover

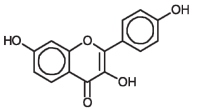
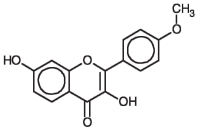
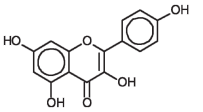
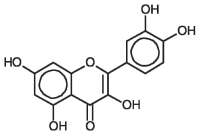
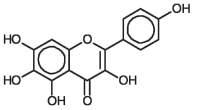
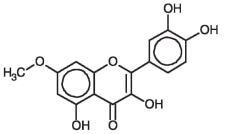
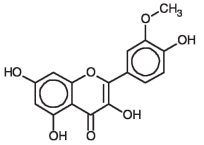
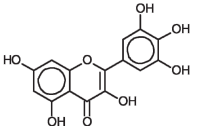
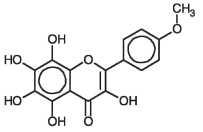
Group	Common name	Systematic name	Mass (g/mol)	CAS no.	Structural formula	Molecular formula	Ref
FLAVONOLS	4′,7-Dihydroxy-flavonol	3,4′,7-Trihydroxyflavone	270			$C_{15}H_{10}O_5$	Wong and Latch, 1971b
	7-Hydroxy-4′-methoxyflavonol	3,7-Dihydroxy-4′-methoxyflavone	284			$C_{16}H_{12}O_5$	Ponce et al., 2004
	Kaempferol	3,4′,5,7-Tetrahydroxyflavone	286	520-18-3		$C_{15}H_{10}O_6$	Carlsen et al., 2008; Foo et al., 2000; Hofmann et al., 2000, 2003; Nakatani et al., 1989; Prati et al., 2007; Wong and Latch, 1971b
	Quercetin	3,3′,4′,5,7-Pentahydroxyflavone	302	117-39-5		$C_{15}H_{10}O_7$	Foo et al., 2000; Hofmann et al., 2000, 2003; Masterson, 1965; Nakatani et al., 1989; Oleszek and Stochmal, 2002; Ponce et al., 2004; Prati et al., 2007; Wong and Latch, 1971b
	6-Hydroxy-kaempferol	3,4′5,6,7-Pentahydroxyflavone	302	4324-55-4		$C_{15}H_{10}O_7$	Ponce et al., 2004
	Rhamnetin	3,3′,4′,5-Tetrahydroxy-7-methoxyflavone	316	90-19-7		$C_{16}H_{12}O_7$	Ponce et al., 2004
	Isorhamnetin	3,4’,5,7-Tetrahydroxy-3′-methoxyflavone	316	480-19-3		$C_{16}H_{12}O_7$	Wong and Latch, 1971b
	Myricetin	3,3’,4’,5,5’,7-Hexahydroxyflavone	318	529-44-2		$C_{15}H_{10}O_8$	Foo et al., 2000; Fottrell et al., 1964; Masterson, 1965; Nakatani et al., 1989; Prati et al., 2007
	5,6,7,8-Tetrahydroxy-4’-methoxyflavonol	3,5,6,7,8-Pentahydroxy-4’-methoxyflavone	332			$C_{16}H_{13}O_8$	Ponce et al., 2004

Table 3 (Continued)

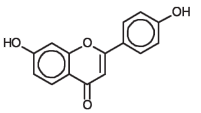
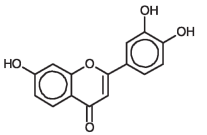
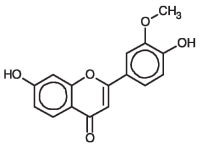
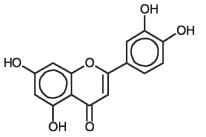
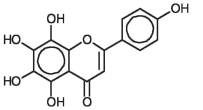
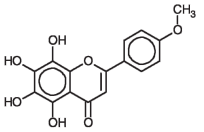
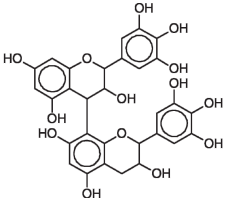
Group	Common name	Systematic name	Mass (g/mol)	CAS no.	Structural formula	Molecular formula	Ref
FLAVONES		4′,7-Dihydroxyflavone	254	2196-14-7		$C_{15}H_{10}O_4$	Bickoff et al., 1965; Johnson et al., 2005; Wong and Latch, 1971b
		3′,4′′,7-Trihydroxyflavone	270			$C_{15}H_{10}O_5$	Livingston and Bickoff, 1964; Wong and Latch, 1971b
	Geraldone	4′,7-Dihydroxy-3′′-methoxyflavone	284	21583-32-4		$C_{16}H_{12}O_5$	Wong and Latch, 1971b
	Acacetin	5,7-Dihydroxy-4′-methoxyflavone	284	480-44-4		$C_{16}H_{12}O_5$	Ponce et al., 2004
	Luteolin	3′,4′,5,7-Tetrahydroxyflavone	286	491-70-3		$C_{15}H_{10}O_6$	Wong and Latch, 1971b
		4′,5,6,7,8-Pentahydroxyflavone	302			$C_{15}H_{10}O_7$	Ponce et al., 2004
		5,6,7,8-Tetrahydroxy-4′-methoxyflavone	316			$C_{16}H_{12}O_7$	Ponce et al., 2004
CONDENSED TANNINS	Prodelphinidin		610			$C_{30}H_{26}O_{14}$	Foo et al., 2000; Jones et al., 1976; Meagher et al., 2006; Young and Paterson, 1980

Table 3 (Continued)

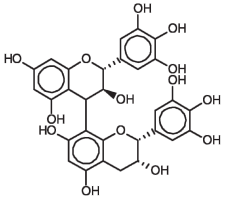
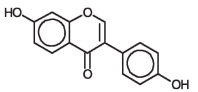
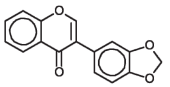
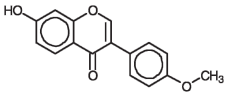
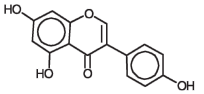
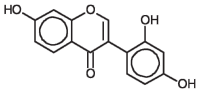
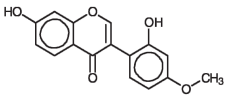
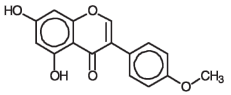
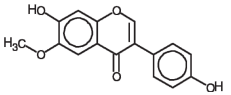
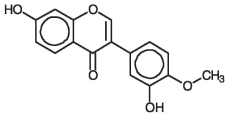
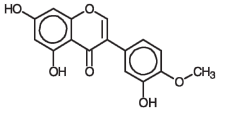
Group	Common name	Systematic name	Mass (g/mol)	CAS no.	Structural formula	Molecular formula	Ref
	Gallocatechin-(4α-8)-epigallocatechin		610			$C_{30}H_{26}O_{14}$	Foo et al., 2000
ISOFLAVONES	Daidzein	4′,7-Dihydroxyisoflavone	254	486-66-8		$C_{15}H_{10}O_4$	Carlsen et al., 2008; Saloniemi et al., 1993; Vetter, 1995; Wu et al., 2003
	Pseudobaptigenin	3-(1,3-Benzodioxol-5-yl)-7-hydroxy-[1]benzopyran-3-one	266			$C_{16}H_{10}O_4$	Wu et al., 2003
	Formononetin	7-Hydroxy-4′-methoxyisoflavone	268	485-72-3		$C_{16}H_{12}O_4$	Bennet et al., 1967; Bickoff et al., 1965; Carlsen et al., 2008; Cook et al., 1995; Johnson et al., 2005; Jurzysta et al., 1988; Saba et al., 1974; Sachse, 1974; Saloniemi et al., 1993; Saxena and Jain, 1989; Vetter, 1995; Wong and Latch, 1971b; Woodward, 1981b; Wu et al., 2003
	Genistein	4′,5,7-Trihydroxyisoflavone	270	446-72-0		$C_{15}H_{10}O_5$	Bennet et al., 1967; Carlsen et al., 2008; Jurzysta et al., 1988; Saloniemi et al., 1993; Saxena and Jain, 1986; Vetter, 1995; Wu et al., 2003
		7,2′,4′-Trihydroxyisoflavone	270			$C_{15}H_{10}O_5$	Woodward, 1981b
	2′-Hydroxy-formononetin	7,2′-Dihydroxy-4′-methoxyisoflavone	284	1890-99-9		$C_{16}H_{12}O_5$	Woodward, 1981a,b
	Biochanin A	5,7-Dihydroxy-4'-methoxyisoflavone	284	491-80-5		$C_{16}H_{12}O_5$	Bennet et al., 1967; Carlsen et al., 2008; Saloniemi et al., 1993; Vetter, 1995
	Glycitein	6-Methoxy-4′,5,7-trihydroxyisoflavone	284			$C_{16}H_{12}O_5$	Wu et al., 2003
	Calycosin	5′7-Dihydroxy-4′-methoxyisoflavone	284	20575-57-9		$C_{16}H_{12}O_5$	Wu et al., 2003
	Pratensein	5,5′,7-Trihydroxy-4′-methoxyisoflavone	300	2284-31-3		$C_{16}H_{12}O_6$	Wu et al., 2003

Table 3 (Continued)

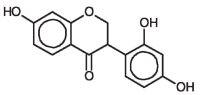
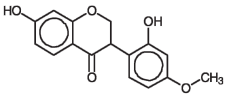
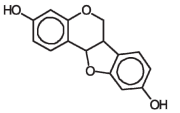
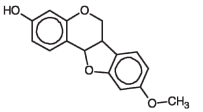
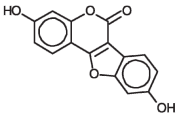
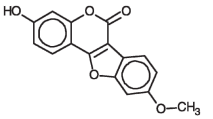
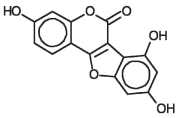
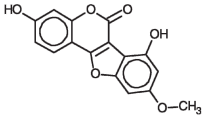
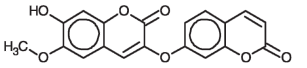
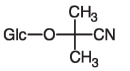
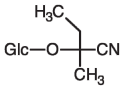
Group	Common name	Systematic name	Mass (g/mol)	CAS no.	Structural formula	Molecular formula	Ref
ISOFLAVANONES		2′,4′,7-Trihydroxyisoflavanone	272			$C_{15}H_{12}O_5$	Woodward, 1981b
	Vestitone	2′,7-Dihydroxy-4′-methoxyisoflavanone	286	57462-46-1		$C_{16}H_{14}O_5$	Woodward, 1981b
PTEROCARPANS	Demethylmedicarpin	3,9-Dihydroxypterocarpan	256			$C_{15}H_{12}O_4$	Woodward, 1981b
	Medicarpin	3-Hydroxy-9-methoxypterocarpan	270	33983-40-3 32383-76-9		$C_{16}H_{14}O$	Carlsen et al., 2008; Cruickshank et al., 1974; Cook et al., 1995; Ingham, 1978; Woodward, 1981b
COUMESTANS	Coumestrol	3,9-Dihydroxycoumestan	268	479-13-0		$C_{15}H_8O_5$	Bennet et al., 1967; Bickoff et al., 1958, 1960a, 1965; Carlsen et al., 2008; Francis et al., 1967; Price and Fenwick, 1985; Saba et al., 1974; Sachse, 1974; Saloniemi et al., 1993; Wong and Latch, 1971a, 1971b
	9-O-Methylcoumestrol	3-Hydroxy-9-methoxycoumestan	282	1690-62-6		$C_{16}H_{10}O_5$	Wong and Latch, 1971a, b
	Repensol	3,7,9-Trihydroxycoumestan	284	33280-69-2		$C_{15}H_8O_6$	Wong and Latch, 1971a, b
	Trifoliol	3,7-Dihydroxy-9-methoxycoumestan	298	1857-26-7		$C_{16}H_{10}O_6$	Livingston et al., 1964b; Wong and Latch, 1971a, 1971b
	Daphnoretin	2-Hydroxy-6-methoxy-3-(2-oxochromen-7-yl)oxychromen-7-one	352	2034-69-7		$C_{19}H_{12}O_7$	Bickoff et al., 1965; Livingston et al., 1964a; Wong and Latch, 1971b
	BV	A trihydroxymethoxycoumestan					Wong and Latch, 1971b; Wong et al., 1971
	bBV	A trihydroxycoumestan					Wong and Latch, 1971b
CYANOGENIC GLYCOSIDES*	Linamarin	α-Hydroxyisobutyronitrile-β-D-glucoside	247	554-35-8		$C_{10}H_{17}NO_6$	Butler and Butler, 1960; Hughes, 1991; Hughes and Conn, 1976; Maher and Hughes, 1971; Stochmal and Oleszek, 1994, 1995, 1997
	Lotaustralin	α-Hydroxy-α-methylbutyronitrile-β-D-glucoside	261	534-67-8		$C_{11}H_{19}NO_6$	Butler and Butler, 1960; Hughes, 1991; Hughes and Conn, 1976; Maher and Hughes, 1971; Stochmal and Oleszek, 1994, 1995, 1997

Table 3 (Continued)

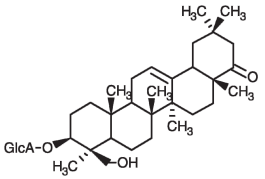
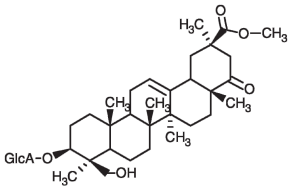
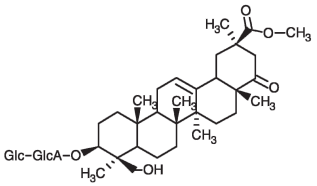
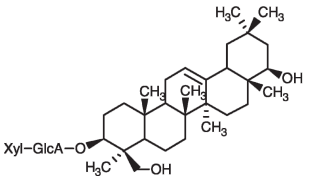
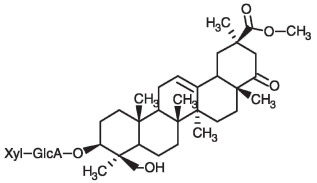
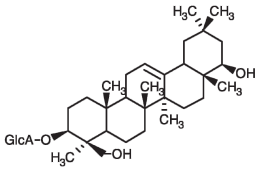
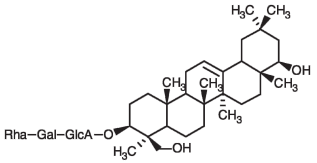
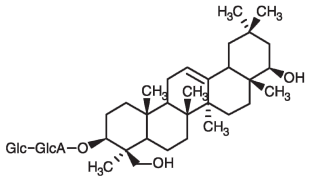
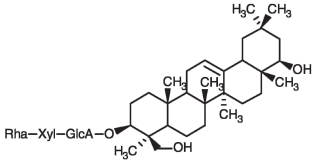
Group	Common name	Systematic name	Mass (g/mol)	CAS no.	Structural formula	Molecular formula	Ref
SAPONINS*	Cloversaponin I	3-*O*-β-D-Glucuronosylsoyasapogenol E	632			$C_{36}H_{56}O_9$	Sakamoto et al., 1992
	Cloversaponin II	3-*O*-β-D-Glucuronosylmelilotigenin	676			$C_{37}H_{56}O_{11}$	Sakamoto et al., 1992
	Cloversaponin III	3-*O*-[β-D-Glucosyl-(1→2)-β-D-glucuronosyl]-melilotigenin	838			$C_{43}H_{66}O_{16}$	Sakamoto et al., 1992
	Cloversaponin IV	3-*O*-[β-D-Xylosyl-(1→2)-β-D-glucuronosyl]-soyasapogenol B	766			$C_{41}H_{66}O_{13}$	Sakamoto et al., 1992
	Cloversaponin V	3-*O*-[β-D-Xylosyl-(1→2)-β-D-glucuronosyl]melilotigenin	808			$C_{42}H_{64}O_{15}$	Sakamoto et al., 1992
		3-O-β-D-Glucuronosylsoyasapogenol B	634			$C_{36}H_{58}$	Sakamoto et al., 1992

Table 3 (Continued)

Group	Common name	Systematic name	Mass (g/mol)	CAS no.	Structural formula	Molecular formula	Ref
	Soyasaponin I	3-*O*-[α-L-Rhamnosyl-β-D-galactosyl-β-D-glucuronosyl]-soyasapogenol B	942	51330-27-9		$C_{48}H_{78}O_{18}$	Oleszek and Stochmal, 2002; Sakamoto et al., 1992
	Azukisaponin II	3-*O*-[β-D-Glucosyl-(1→2)-β-D-glucuronosyl]-soyasapogenol B	796			$C_{42}H_{68}O_{1}$	Sakamoto et al. 1992
	Astragaloside VIII	3-*O*-[α-L-Rhamnosyl-(1→2)-β-D-xylosyl-(1→2)-β-D-glucuronosyl]-soyasapogenol B	912	86361-64-0		$C_{47}H_{76}O_{17}$	Oleszek and Stochmal, 2002; Sakamoto et al., 1992

* Abbreviations: Glc = glucose, GlcA = glucuronic acid, Gal = galactose, Rha = rhamnose

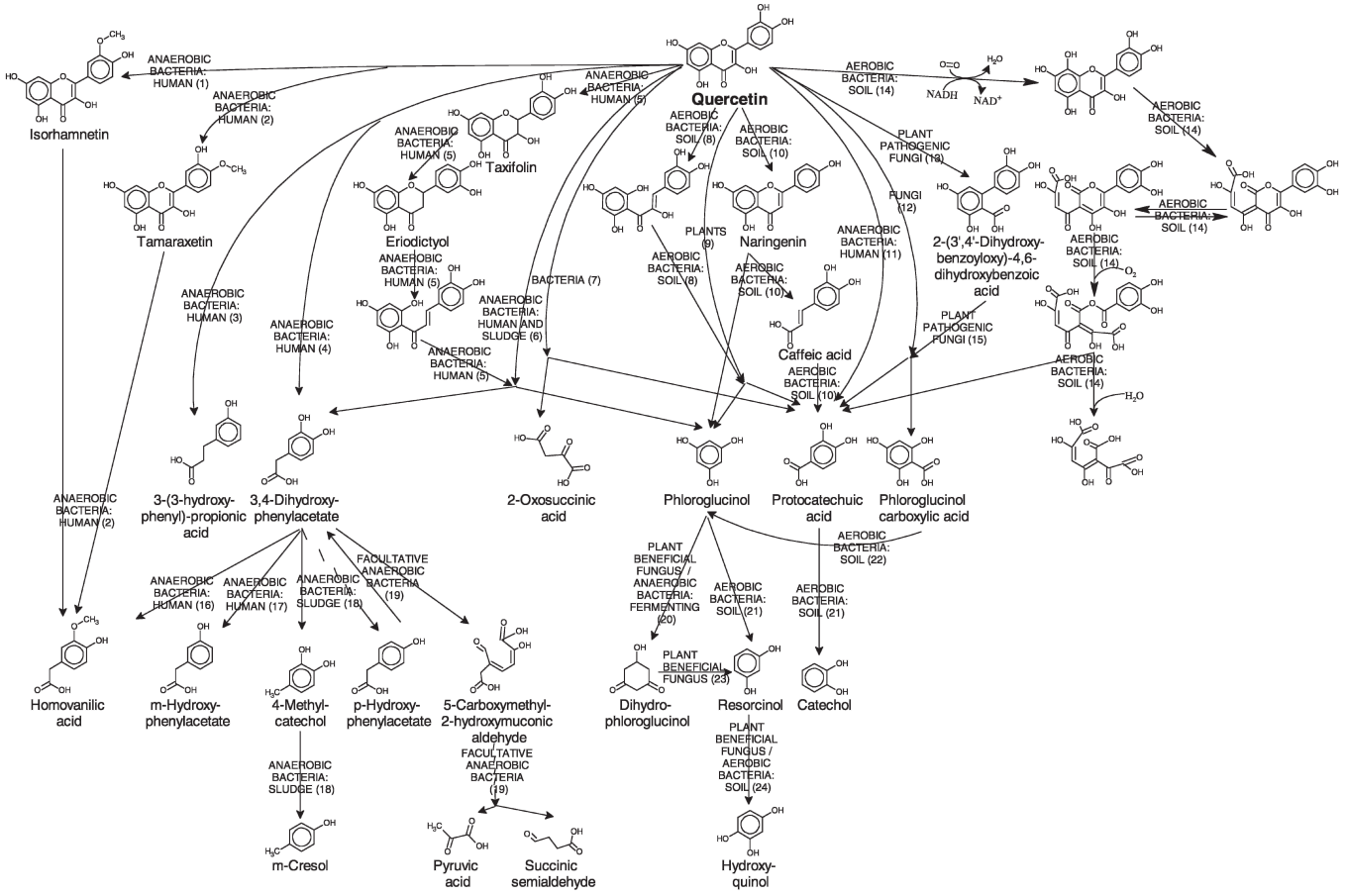


Fig. 1. Degradation of quercetin inside the plant, by fungi and by bacteria. **1:** Bacteria from liver/kidney (Olthof et al., 2003) and colon (Rechner et al., 2002). **2:** Bacteria from colon (Rechner et al., 2002). **3:** Bacteria from colon (Rechner et al., 2004). **4:** Bacteria from fecal bacteria (Aura et al., 2002) and colon (Olthof et al., 2003; Rechner et al., 2004). **5:** *Eubacterium ramulus* (Schneider and Blaut, 2000; Schneider et al., 1999). **6:** Bacteria from intestine (Winter et al., 1989), *Eubacterium oxidoreducens* sp. nov. (Krumholz and Bryant 1986) and bacteria from digested municipal sludge (Herrmann et al., 2001). **7:** Bacteria (Siqueira et al., 1991b). **8:** *Rhizobia leguminosarum* bv. phaseoli (Rao and Cooper, 1994). **9:** Plants (Siqueira et al. 1991b). **10:** *Pseudomonas putida* (Pillai and Swarup, 2002). **11:** Bacteria from colon (Rechner et al., 2004). **12:** Fungi (Siqueira et al., 1991b). **13:** *Aspergillus flavus* and *Aspergillus niger* (Simpson et al., 1960; 1962; Westlake et al., 1959). **14:** *P. putida* (Schultz et al., 1974). **15:** *A. flavus* and *A. niger* (Simpson et al., 1960; Westlake et al., 1959). **16:** Bacteria from gut (Konishi, 2005) and liver/kidney (Olthof et al., 2003). **17:** Bacteria from colon (Olthof et al., 2003; Rechner et al., 2004), fecal bacteria (Aura et al., 2002) and gut bacteria (Konishi, 2005). **18:** Bacteria from digested municipal sludge (Herrmann et al., 2001). **19:** *Escherichia coli* W (Prieto et al., 1996). **20:** *Pelobacter acidigallici* (Brune and Schink, 1992) and *Penicillium simplicissimum* (Patel et al., 1990). **21:** *Pseudomonas solanaceraum* and *Rhizobium japonicum* (William et al. 1986). **22:** *P. solanaceraum* and *R. japonicum* (William et al. 1986). **23:** *P. simplicissimum* (Patel et al., 1990). **24:** *P. simplicissimum* (Patel et al., 1990) and *P. solanaceraum, R. japonicum* (William et al. 1986).

Degradation of flavonols

Degradation of quercetin has been studied extensively (Fig. 1). In vitro studies indicate co-metabolic anaerobic degradation of quercetin (Schneider and Blaut, 2000; Schneider et al., 1999) and kaempferol (Schneider and Blaut, 2000) by *Eubacterium ramulus*, a dominant bacterium in the human intestinal tract (Schneider et al., 1999). Degradation of quercetin is relatively fast: in human fecal suspensions bacteria degraded 60 % of 2 mM quercetin-3-glucoside in 6 h and 100 % in 24 h (Schneider et al., 1999); *Pseudomonas putida,* a plant growth-promoting rhizobacteria, aerobically degraded 98 % in 60 h (Pillai and Swarup, 2002).

Degradation of other flavonols may be similar to that of quercetin. Schneider and Blaut (2000) suggested that the degradation pathway of flavonols by a human intestinal bacterium could be more general, and Winter et al. (1989) found that quercetin and kaempferol were degraded similarly by human intestinal bacteria.

Biological activity of flavonols and their degradation products

Flavonols are toxic to insects, fungi, bacteria and plants (Table 5). Rhamnetin inhibits many AM fungal parameters (Scervino et al., 2005), while quercetin, myricetin and keampferol all stimulates the growth of AM fungi (Bé-

Table 4 Contents of secondary metabolites found in healthy and stressed white clover

Compound	Stressed		Healthy		No. of cultivars investigated	Ref
	Content (μg/g dry wt)	Condition	Content (μg/g dry wt)	Condition		
FLAVONOLS	**1245**^{V}	well-watered, UV-B treatment	**559**^{V}	well-watered, no treatment	4 + 3 ecotypes + 2 breeding lines	Hofmann et al., 2003
	1700^{V}	droughted, UV-B treatment	**477**^{V}	droughted, no treatment	4 + 3 ecotypes + 2 breeding lines	Hofmann et al., 2003
			1840^{I}		1	Oleszek and Stochmal, 2002
	810-2210^{VIII}	UV-B treatment	**290-960**^{VIII}	no treatment	4 + 3 ecotypes + 2 breeding lines	Hofmann et al., 2000
4′,7-Dihydroxy-flavonol						No records
7-Hydroxy-4′-methoxyflavonol	**84.8**^{III}	no treatment	**not found**^{III}	AM-treatment	1	Ponce et al., 2004
Kaempferol	**n.d.-33.5**^{III}	no treatment with or without infection or infected AM-treated	**n.d.-26.2**^{III}	AM-treatment	2	Carlsen et al., 2008
	474^{V}	well-watered, UV-B treatment	**297**^{V}	well-watered, no treatment	4 + 3 ecotypes + 2 breeding lines	Hofmann et al., 2003
	651^{V}	droughted, UV-B treatment	**352**^{V}	droughted, no treatment	4 + 3 ecotypes + 2 breeding lines	Hofmann et al., 2003
			62^{VI,d}		1 experimental selection	Foo et al., 2000
	230-930^{VIII}	UV-B treatment	**80-580**^{VIII}	no treatment	4 + 3 ecotypes + 2 breeding lines	Hofmann et al., 2000
			2.8^{I}		1	Nakatani et al., 1989
	<2–119^{V}	glasshouse, diseased	**139**^{V}	glasshouse, healthy	1	Wong and Latch, 1971b
Quercetin	**<3**^{III}	no treatment with or without infection or infected AM-treated	**<3**^{III}	AM-treated	2	Carlsen et al., 2008
	not found^{III}	no treatment	**208**^{III}	AM-treatment	1	Ponce et al., 2004
	771^{V}	well-watered, UV-B treatment	**262**^{V}	well-watered, no treatment	4 + 3 ecotypes + 2 breeding lines	Hofmann et al., 2003
	1049^{V}	droughted, UV-B treatment	**125**^{V}	droughted, no treatment	4 + 3 ecotypes + 2 breeding lines	Hofmann et al., 2003
			1160^{I}		1	Oleszek and Stockmal, 2002
			450^{VI,d}		1 experimental selection	Foo et al., 2000
	490-1280^{VIII}	UV-B treatment	**20-620**^{VIII}	no treatment	4 + 3 ecotypes + 2 breeding lines	Hofmann et al., 2000
			17^{I}		1	Nakatani et al., 1989
	<2–21^{V}	glasshouse, diseased	**14**^{V}	glasshouse, healthy	1	Wong and Latch, 1971b
6-Hydroxykaempferol	**75.8**^{III}	no treatment	**not found**^{III}	AM-treatment	1	Ponce et al., 2004
Rhamnetin	**not found**^{III}	no treatment	**179**^{III}	AM-treatment	1	Ponce et al., 2004
Isorhamnetin						No records
Myricetin			**137**^{VI,d}		1 experimental selection	Foo et al., 2000
			5^{I}		1	Nakatani et al., 1989
			2000^{I}		1	Fottrell et al., 1964
5,6,7,8-Tetrahydroxy-4'-methoxyflavonol	**69.7**^{III}	no treatment	**167**^{III}	AM-treatment	1	Ponce et al., 2004
FLAVONES						
4′,7-Dihydroxyflavone	**843**^{III,c}	without rhizobial root nodules	**326**^{III,c}	with active rhizobial root nodules	1	Johnson et al., 2005

Table 4 (Continued)

Compound	Stressed		Healthy		No. of cultivars investigated	Ref
	Content (μg/g dry wt)	Condition	Content (μg/g dry wt)	Condition		
	441[III,c]	with inactive rhizobial root nodules			1	Johnson et al., 2005
	3–14[V]	glasshouse, diseased	**< 2**[V]	glasshouse, healthy	1	Wong and Latch, 1971b
	9–55[V]	field, diseased	**< 2**[V]	field, healthy	1	Wong and Latch, 1971b
3′,4′,7-Trihydroxyflavone	**<2–23**[V]	glasshouse, diseased	**< 2**[V]	glasshouse, healthy	1	Wong and Latch, 1971b
	7-40[V]	field, diseased	**< 2**[V]	field, healthy	1	Wong and Latch, 1971b
Geraldone	**<2–15**[V]	glasshouse, diseased	**< 2**[V]	glasshouse, healthy	1	Wong and Latch, 1971b
	7–28[V]	field, diseased	**< 2**[V]	field, healthy	1	Wong and Latch, 1971b
Acacetin	**not found**[III]	no treatment	**250**[III]	AM-treatment	1	Ponce et al., 2004
Luteolin						No records
4′,5,6,7,8-Pentahydroxyflavone	**not found**[III]	no treatment	**44.4**[III]	AM-treatment	1	Ponce et al., 2004
5,6,7,8-Tetrahydroxy-4′-methoxyflavone	**130**[III]	no treatment	**not found**[III]	AM-treatment	1	Ponce et al., 2004
CONDENSED TANNINS			**6,000-94,000**[VI]	spring/ summer/ fall	1 + 1 experimental selection	Burggraaf et al., 2006
			12,000[VI]	spring	1	Meagher et al., 2006
	13,000–50,000[VI]	early spring / late summer / autumn, healthy	**30,000-79,000**[VI]	late spring/ early summer, healthy	1 + 1 experimental selection	Burggraaf et al., 2003
			0-12,100[VIII,b]		1 + 1 experimental selection	Burggraaf et al., 2003
			n.d.–600[V]	healthy		Li et al., 1996
	18,700–44,400[VI]	autumn	**26,000-46,300**[VI]	spring/ early summer	1	Stockdale and Dellow, 1995
	0–4000[VIII,b]	autumn	**1600-11,600**[VIII,b]	spring/ early summer	1	Stockdale and Dellow, 1995
			33,000[VI]	summer	1	Stockdale, 1994
			60,000[VI,e]	summer	1	Stockdale, 1994
Prodelphinidin			**2400**[VI]		1 experimental selection	Foo et al., 2000
Gallocatechin-(4α-8)-epigallocatechin			**48**[VI]		1 experimental selection	Foo et al., 2000
ISOFLAVONES			**253**[III]	summer, field	1	Wu et al., 2003
			354[IV]	summer, field	1	Wu et al., 2003
			327[V]	summer, field	1	Wu et al., 2003
			213[VI]	summer, field	1	Wu et al., 2003
			119[IV]		1	Vetter, 1995
			27[V]		1	Vetter, 1995
			94[VI]		1	Vetter, 1995
			100–600[VIII]	summer, field	4	Saloniemi et al., 1993
			500[VII]		1	Jurzysta et al., 1988
			< 2500[V]		2	Francis et al., 1967
Daidzein	**0.7-62.1**[III]	no treatment with or without infection or infected AM-treated	**0.3-40.6**[III]	AM-treatment	2	Carlsen et al., 2008
			6[III]	summer, field	1	Wu et al., 2003

Table 4 (Continued)

Compound	Stressed		Healthy		No. of cultivars investigated	Ref
	Content (μg/g dry wt)	Condition	Content (μg/g dry wt)	Condition		
			4[IV]	summer, field	1	Wu et al., 2003
			5[V]	summer, field	1	Wu et al., 2003
			1[VI]	summer, field	1	Wu et al., 2003
			9[IV]		1	Vetter, 1995
			5[V]		1	Vetter, 1995
			6[VI]		1	Vetter, 1995
			< 1–10[VIII]	summer, field	4	Saloniemi et al., 1993
Pseudobaptigenin			**42**[III]	summer, field	1	Wu et al., 2003
			nd[VIII]	summer, field	1	Wu et al., 2003
Formononetin	**658-1987**[III]	no treatment with or without infection or infected AM-treated	**669-1134**[III]	AM-treated	2	Carlsen et al., 2008
	557[III,c]	without rhizobial root nodules	**4920**[III,c]	with active rhizobial root nodules	1	Johnson et al., 2005
	191[III,c]	with inactive rhizobial root nodules			1	Johnson et al., 2005
			900[V,i]		1	Rijke et al., 2004
			nd[III, VIII]	summer, field	1	Wu et al., 2003
	67-92[III,c]	infected with stem nematode	**17-94**[III,c]	healthy	2 populations	Cook et al., 1995
	5-49[IV,c]	infected with stem nematode	**4-11**[IV,c]	healthy	2 populations	Cook et al., 1995
	5-8[V,c]	infected with stem nematode	**4-8**[V,c]	healthy	2 populations	Cook et al., 1995
			85[IV]		1	Vetter, 1995
			7[V]		1	Vetter, 1995
			52[VI]		1	Vetter, 1995
			90–570[VIII]	summer, field	4	Saloniemi et al., 1993
	3600[III]	under phosphate stress			1	Nair et al., 1991
			200[VII]		1	Jurzysta et al., 1988
	0.080 μg/ml[V,g]	infected with fungi			1	Woodward, 1981b
	88–420[VII]	May – October, infected with fungi			1	Saba et al., 1974
	154–365[VII]	November – December, heavily infected with fungi			1	Saba et al., 1974
			220–920[VIII]		32	Sachse, 1974
	44–192[V]	glasshouse, diseased	**6**[V]	glasshouse, healthy	1	Wong and Latch, 1971b
			0–800[VIII]		3	Bennet et al., 1967
Genistein	**3.5-14.4**[III]	no treatment with or without infection or infected AM-treated	**4.1-11.9**[III]	AM-treatment	2	Carlsen et al., 2008
			nd[III, VIII]	summer, field	1	Wu et al., 2003
			20[IV]		1	Vetter, 1995
			5[V]		1	Vetter, 1995
			23[VI]		1	Vetter, 1995
			5–60[VIII]	summer, field	4	Saloniemi et al., 1993
			300[VII]		1	Jurzysta et al., 1988
			0–200[VIII]		3	Bennet et al., 1967
7,2',4'-Trihydroxyisoflavone	**<0.001 μg/ml**[V,g]	infected with fungi			1	Woodward, 1981b
2'-Hydroxyformononetin	**0.071 μg/ml**[V,g]	infected with fungi			1	Woodward, 1981b
Biochanin A	**2.7-7.5**[III]	no treatment with or without infection or infected AM-treated	**2.4-5.5**[III]	AM-treatment	2	Carlsen et al., 2008
			nd[III, VIII]	summer, field	1	Wu et al., 2003
			5[IV]		1	Vetter, 1995

Table 4 (Continued)

Compound	Stressed		Healthy		No. of cultivars investigated	Ref
	Content (μg/g dry wt)	Condition	Content (μg/g dry wt)	Condition		
			10^{V}		1	Vetter, 1995
			13^{VI}		1	Vetter, 1995
			< **1–60**^{VIII}	summer, field	4	Saloniemi et al., 1993
	2800^{III}	under phosphate stress			1	Nair et al., 1991
			0–600^{VIII}		3	Bennet et al., 1967
Glycitein			**150**^{III}	summer, field	1	Wu et al., 2003
			282^{IV}	summer, field	1	Wu et al., 2003
			241^{V}	summer, field	1	Wu et al., 2003
			40^{VI}	summer, field	1	Wu et al., 2003
Calycosin			**55**^{III}	summer, field	1	Wu et al., 2003
			69^{IV}	summer, field	1	Wu et al., 2003
			82^{V}	summer, field	1	Wu et al., 2003
			171^{VI}	summer, field	1	Wu et al., 2003
Pratensein			**n.d.**$^{III, VIII}$	summer, field	1	Wu et al., 2003
ISOFLAVANONES						
7,2′,4′-Trihydroxyisoflavanone	<**0.001 μg/ml**^{V,g}	infected with fungi			1	Woodward, 1981b
Vestitone	**0.103 μg/ml**^{V,g}	infected with fungi			1	Woodward, 1981b
PTEROCARPANS						
Demethylmedicarpin	**2.050 μg/ml**^{V,g}	infected with fungi			1	Woodward, 1981b
Medicarpin	**16.1-84.3**^{III}	no treatment with or without infection or infected AM-treated	**17.0-76.6**^{III}	AM-treatment	2	Carlsen et al., 2008
	4-12^{III,c}	infected with stem nematode	**1-8**^{III,c}	healthy	2 populations	Cook et al., 1995
	6-50^{IV,c}	infected with stem nematode	**6-18**^{IV,c}	healthy	2 populations	Cook et al., 1995
	11-35^{V,c}	infected with stem nematode	**9-32**^{V,c}	healthy	2 populations	Cook et al., 1995
	10.81 μg/ml^{V,g}	infected with fungi			1	Woodward, 1981b
	89–313 μg/ml^{V,g}	infected with fungi			6	Ingham, 1978
	2630^{V,c}	infected with fungi			6	Ingham, 1978
	6.1 μg/ml^{V,g} ≈ **233.3**^{V,c}	high temperature, infected with fungi			1	Cruickshank et al., 1974
	3.6 μg/ml^{V,g} ≈ **137.7**^{V,c}	low temperature, infected with fungi			1	Cruickshank et al., 1974
	4.8 μg/ml^{V,g} ≈ **183.6**^{V,c}	long day, infected with fungi			1	Cruickshank et al., 1974
	3.3 μg/ml^{V,g} ≈ **126.2**^{V,c}	short day, infected with fungi			1	Cruickshank et al., 1974
COUMESTANS	> **2**^{V}	diseased			1	Wong and Latch, 1971a
Coumestrol			< **1–8.9**^{VIII}		4	Saloniemi et al., 1993
	<**5–19.3**^{VII}	May – October, infected with fungi			1	Saba et al., 1974
	13.6–52.5^{VII}	November – December, heavily infected with fungi			1	Saba et al., 1974
			< **10**^{VIII}		32	Sachse, 1974
	5-74^{V,a}	diseased			1	Wong and Latch, 1971b
	3–11^{V}	glasshouse, diseased	< **2**^{V}	glasshouse, healthy	1	Wong and Latch, 1971b
	14–64^{V}	field, diseased	< **2**^{V}	field, healthy	1	Wong and Latch, 1971b

Table 4 (Continued)

Compound	Stressed		Healthy		No. of cultivars investigated	Ref
	Content (μg/g dry wt)	Condition	Content (μg/g dry wt)	Condition		
			0–10^{VIII}		3	Bennet et al., 1967
			12^{V}	field, healthy, fertilized	2	Francis et al., 1967
	14–170^{VIII,f}	with virus	**0–122**^{VIII,f}	virus-free	1 + 6 clones	Bickoff et al., 1960a
9-*O*-Methylcoumestrol	**4-25**^{V,a}	diseased			1	Wong and Latch, 1971b
	<5–29^{V}	glasshouse, diseased	**< 2**^{V}	glasshouse, healthy	1	Wong and Latch, 1971b
	9–56^{V}	field, diseased	**3**^{V}	field, healthy	1	Wong and Latch, 1971b
Repensol	**4-165**^{V,a}	diseased			1	Wong and Latch, 1971b
	2–9^{V}	glasshouse, diseased	**< 2**^{V}	glasshouse, healthy	1	Wong and Latch, 1971b
	16–166^{V}	field, diseased	**< 2**^{V}	field, healthy	1	Wong and Latch, 1971b
Trifoliol	**7-82**^{V,a}	diseased			1	Wong and Latch, 1971b
	6–16^{V}	glasshouse, diseased	**< 2**^{V}	glasshouse, healthy	1	Wong and Latch, 1971b
	24–147^{V}	field, diseased	**< 2**^{V}	field, healthy	1	Wong and Latch, 1971b
Daphnoretin						No records
BV						No records
bBV						No records
CYANOGENIC GLUCOSIDES (measured as potential of release of HCN)			**53–2242 HCN**^{V}		7 + 3 natural populations	Tava and Annicchiarico, 2000
			58-2205 HCN^{VIII}		6	Viette et al., 2000
	0-1204 HCN^{VII}	spring and autumn	**0-267 HCN**^{VII}	summer	8	Stochmal and Oleszek, 1997
			15-1440 HCN^{VIII}		24	Lehmann et al., 1995
			n.d.-745 HCN^{VIII}	spring	8	Stochmal and Oleszek, 1995
			600 HCN^{VIII}		1	Stochmal and Oleszek, 1994
			19–2405 HCN		41	Lehmann et al., 1990
	24-640 HCN^{VIII}	unfertilized	**6-287 HCN**^{VIII}	fertilized	16	Wheeler and Vickery, 1989
	505 HCN^{VIII}	low light intensity	**220 HCN**^{VIII}	high light intensity	1	Vickery et al., 1987
	408 HCN^{VIII}	low temperature	**343 HCN**^{VIII}	high temperature	1	Vickery et al., 1987
	382 HCN^{VIII}	unfertilized	**343 HCN**^{VIII}	fertilized	1	Vickery et al., 1987
			900-1230 HCN^{VIII,c}		2	Horrill and Richards, 1986
			16–1760 HCN^{V,c}		20 collections	Butler, 1965

Table 4 (Continued)

Compound	Stressed		Healthy		No. of cultivars investigated	Ref
	Content (μg/g dry wt)	Condition	Content (μg/g dry wt)	Condition		
			400 HCN[VIII,c]		1	Coop and Blakley, 1949
Linamarin			**n.d.–2534**[VIII]	spring	8	Stochmal and Oleszek, 1995
			1290[VIII]		1	Stochmal and Oleszek, 1994
Lotaustralin			**n.d.–4844** [VIII]	spring	8	Stochmal and Oleszek, 1995
			3130 [VIII]		1	Stochmal and Oleszek, 1994
SAPONINS			**3160**[I]		1	Oleszek and Stochmal, 2002
Cloversaponin I			**1.5**[IX,h]		1	Sakamoto et al., 1992
Cloversaponin II			**2.4**[IX,h]		1	Sakamoto et al., 1992
Cloversaponin III			**1.72**[IX,h]		1	Sakamoto et al., 1992
Cloversaponin IV			**20**[IX,h]		1	Sakamoto et al., 1992
Cloversaponin V			**11.06**[IX,h]		1	Sakamoto et al., 1992
3-O-β-D-Glucuronosylsoyasapogenol B			**1.5**[IX,h]		1	Sakamoto et al., 1992
Soyasaponin I			**1120**[I]		1	Oleszek and Stochmal, 2002
Mixture of soyasaponin I and astragaloside VIII			**74**[IX,h]		1	Sakamoto et al., 1992
Soyasaponin I 22-*O*-glucoside			**< 50**[I]		1	Oleszek and Stochmal, 2002
Soyasaponin I 22-*O*-diglucoside			**1180**[I]		1	Oleszek and Stochmal, 2002
Azukisaponin II			**12.53**[IX,h]		1	Sakamoto et al., 1992
Astragaloside VIII			**860**[I]		1	Oleszek and Stochmal, 2002

[I] In seeds. [II] In shoots. [III] In roots. [IV] In stem. [V] In leaves. [VI] In flowers. [VII] In leaves and stem. [VIII] In above-ground material. [IX] In whole plant.
[a] Concentrated in the lesion areas, actual coumestan concentrations being about fourfold those reported.
[b] Assuming that there are neglible condensed tannins in other plant parts.
[c] The content was determined in fresh weight, but has been calculated in dry weight, assuming a water content of 80%.
[d] Calculated amount of aglucone from glucopyranosides.
[e] In flower petals.
[f] Apparent coumestrol content determined from estrogenic activity in mice.
[g] In inoculation fluid from leaves.
[h] Contents given for methyl esters and recalculated without the methyl ester portion
[i] Formononetin detected as one glucoside-malonate and two glucoside-dimalonates
n.d.: Not detected

card et al., 1992; Scervino et al., 2005). With the exception of plants, it seems that the activity of myricetin is higher than that of quercetin. In general, the biological activity of quercetin seems to be higher than that of kaempferol (Table 5).

Quercetin is highly toxic to plants, but has a variable activity towards fungi, and has very low antibacterial activity (Table 5). Its fungal-degradation product, protocatechuic acid, is toxic to insects (Todd et al., 1971) and has in vitro activity against fungi at levels of ED_{50} >50 to >1000 μg/mL, while phloroglucinol (a fungal and bacterial degradation product) has ED_{50} >1000 μg/mL (Fawcett and Spencer, 1967). At low concentrations, quercetin and its derivatives stimulate plant nodule formation by the beneficial fungi *Rhizobium trifolii* and N_2-fixing activity, while at high concentrations the plant growth and development of the plant–rhizobia symbiosis are inhibited (Novikova, 1994). Quercetin and its glucoside have the potential to stimulate the growth of *Rhizobium* spp. but not of bacterial or fungal pathogens (Edwards and Parry, 1994). Quercetin has many health-promoting effects, indicated by the number of hits (163 published from 2001 to May 2007) when a search is made in the bibliographic database "Food Science and Technology Abstracts" via OVID of "quercetin and health".

Myricetin is highly toxic towards fungi and bacteria at high concentrations, but shows no activity at lower concentrations (Table 5). Nakatani et al. (1989) observed no activity against bacteria or fungi at 100 μg/mL (≈ 100 μg/g), > 50 times the highest reported content of myricetin in healthy white clover plants (Table 4); it is not known whether the content in stressed plants exceeds this level.

Flavones

Flavones in white clover

There are few records of flavone content of white clover (Table 3), and flavones have only been quantified in roots (n.d.–843 μg/g) and leaves (<2–55 μg/g) (Table 4) with no concurrent measurements in different parts of the same plants. Production of flavones is higher when white clover is grown in fields compared to glasshouses, or when they are infected with a pathogenic fungus (Wong and Latch, 1971b). Inoculation with an AM fungus can increase or decrease flavone concentration (Ponce et al., 2004), while the concentration of 4',7-dihydroxyflavone is higher in plants without rhizobial nodules than in plants with active or inactive nodules (Johnson et al., 2005).

Degradation and biological effects of flavones

Like the flavonols, there is evidence of co-metabolic degradation of flavones when glucose is required for degradation of luteolin by *Eubacterium ramulus* in the human intestinal tract (Schneider and Blaut, 2000).

Flavones are toxic to insects (Elliger et al., 1980), bacteria (Van Etten and Pueppke, 1976), fungi (Weidenbörner and Jha, 1994), plants (Duke, 1986), and animals including mice, rats, guinea pigs and rabbits (Demole, 1962). Fungicidal activity is usually reduced if one or more hydroxyl or methoxy group(s) are introduced (Weidenbörner and Jha, 1994).

Like some of the flavonols, luteolin can stimulate the growth of *Rhizobium* spp. and AM fungi, while not stimulating the growth of bacterial or fungal pathogens (Bécard et al., 1992; Edwards and Parry, 1994; Scervino et al., 2005b; Table 5). Luteolin is toxic to the insect *Heliothis zea* (Elliger et al., 1980). In contrast, acacetin shows an inhibitory effect on many AM fungal parameters (Scervino et al., 2005a; Table 5), but shows no insecticidal behaviour (Elliger et al., 1980).

Condensed tannins

Condensed tannins in white clover

Condensed tannins (or proanthocyanidins) are present in white clover seeds (Fottrell et al., 1964; Masterson, 1965; Young and Paterson, 1980), flowers (0.048–94 mg/g) (Burggraaf et al., 2003, 2006; Foo et al., 2000; Hart, 1987; Jones et al., 1976; Meagher et al., 2006; Stockdale, 1994; Stockdale and Dellow, 1995) and leaves (n.d.–0.6 mg/g) (Li et al., 1996). Comparisons within experiments show higher contents in flowers (13–79 mg/g) than in total above-ground material (0–12.1 mg/g) (Burggraaf et al., 2003; Stockdale and Dellow, 1995; Table 4) and higher in petals (60 mg/g) than in entire flowers (33 mg/g) (Stockdale, 1994).

Contents in flowers vary seasonally, being highest when warm (Burggraaf et al., 2003; Stockdale and Dellow, 1995); and with developmental stage, being higher in flowers in full bloom compared to green buds or senescent flowers (Burggraaf et al., 2006) (Table 4).

There has been little research on tannins in white clover. Few have been identified, and their exact distribution is not known (Table 3). The molecular weight distribution is 6000–18000 g/mol from seeds (Young and Paterson, 1980) and 8500–9100 g/mol from flowers (Jones et al., 1976). Prodelphinidins are present in seeds (Young and Paterson, 1980) and flowers (Foo et al., 2000; Jones et al., 1976; Meagher et al., 2006; Sivakumaran et al., 2004). In flowers, the terminal units of prodelphinidins contain similar amounts of epigallocathin and gallocatechin (Foo et al., 2000; Meagher et al., 2006; Sivakumaran et al., 2004); there is more epigallocatechin than gallocatechin in the extender units (Meagher et al., 2006; Sivakumaran et al., 2004).

Table 5 Biological effects of white clover secondary metabolites on plants, fungi and bacteria arranged after decreasing effects within each main target.

Target	Compound	Conc. in test	Type of effect	Target species	Effect (Inbibited by %)*	No effect*	Article
Plants	Coumestrol	30 and 100 μM	ATP formation in mitochondria	Cucumber (*Cucumis sativus* L.) hypocotyls and pea (*Pisum sativum*) roots	+++ (Cucumber: 30 μM (49) and 100 μM (73), pea: 100 μM (69))	–	Stenlid, 1970
	Biochanin A	2.5-20 mg/l ≈ 9-70 μM	Shoot fresh weight	White clover (*Trifolium repens* L.)	–	+ (Stimulated at 5 mg/l)	Siqueira et al., 1991
		30 and 100 μM	ATP formation in mitochondria	Cucumber hypocotyls	+++ (30 μM (40) and 100 μM (68))	–	Stenlid, 1970
	Quercetin	30 and 100 ppm	Shoot growth	Thale cress (*Arabidopsis thaliana*)	+++ (70)	–	Parvez et al., 2004
		1000 μM	Germination	Cress (*Lepidium sativum* L.), radish (*Raphanus sativus* L.) and soybean (*Glycine max* L.)	+++ (Cress (90), radish (38), soybean (86))	–	Paszowski and Kremer, 1988
		1000 μM	Radicle length	Cress, radish and soybean	+++ (Cress (34), radish (77), soybean (83))	–	Paszowski and Kremer, 1988
		5 and 50 μM	Protoplasmic streaming	Oat (*Avena sativa*) root hairs	+++ (50 μM)	+ (5 μM promoted)	Popovici and Reznik, 1976
		30 and 100 μM	ATP formation in mitochondria	Cucumber hypocotyls	+++ (30 μM (16) and 100 μM (85))	–	Stenlid, 1970
	Kaempferol	1-1000 μM	Mitochondrial phosphorylation (State 3 respiration)	Corn (*Zea mays* L.)	++ (50 μM (40-80))	–	Koeppe and Miller, 1974
		5 and 50 μM	Protoplasmic streaming	Oat root hairs	++ (50 μM)	+ (5 μM promoted)	Popovici and Reznik, 1976
		30 and 100 μM	ATP formation in mitochondria	Cucumber hypocotyls and roots, maize (*Zea mays* L.) coleoptiles and pea roots	+++ (Cucumber hypocotyls: 30 μM (18) and 100 μM (81), cucumber roots: 100 μM (86), maize: 100 μM (77), pea: 100 μM (70))	-	Stenlid, 1970
	Rhamnetin	30 and 100 μM	ATP formation in mitochondria	Cucumber hypocotyls	++ (100 μM (60)	–	Stenlid, 1970
	Genistein	30 and 100 μM	ATP formation in mitochondria	Cucumber hypocotyls	++ (30 μM (9) and 100 μM (47)	–	Stenlid, 1970
	Myricetin	1000 μM	Germination	Cress, radish and soybean	++ (Cress (82), radish (50), soybean (34))	–	Paszowski and Kremer, 1988
		1000 μM	Radicle length	Cress, radish and soybean	++ (Cress (46), radish (76), soybean (57))	–	Paszowski and Kremer, 1988
		5 and 50 μM	Protoplasmic streaming	Oat root hairs	++ (50 μM)	+ (5 μM promoted)	Popovici and Reznik, 1976
	Formononetin	2.5-20 mg/l ≈ 9-75 μM	Shoot fresh weight	White clover	-	+ (Stimulated at 5-20 mg/l)	Siqueira et al., 1991
	Soyasaponin I	100-500 ppm ≈ 105-531 μM	Germination, growth of seedling roots and shoots	Wheat (*Triticum aestivum* L. var. Boja)	–	+ (Positive linear correlation between conc. and seedling shoot and root length)	Oleszek, 1993

Table 5 (Continued)

Target	Compound	Conc. in test	Type of effect	Target species	Effect (Inbibited by %)*	No effect*	Article
	Saponins (From red clover roots equal or very similar to those from white clover top)	0-1000 ppm	Seedling growth	Winter wheat (*T. aestivum* L.)	–	+	Oleszek and Jurzysta, 1986
Fungi	Biochanin A	10 μM ≈ 3 μg/g	Hyphal growth and formation of auxilary cells	*Gigaspora margarita* (Arbuscular mycorrhizal (AM) fungus)	+++ (90)	–	Becard et al., 1992
		≤ 30 μg/g	Radial growth	*Nectria haematococca* MP I isolate T-145 (phytoalexin sensitive fungus) and MP VI isolate T-30 (pisatin tolerant fungus)	++ (20 μg/g as effective as 24 μg/g medicarpin towards isolate T-145)	+ (Isolate T-30)	Denny and VanEtten, 1981
		500 μg/g soil	Total number of microorganisms	Soil fungi	–	+ (Prolonged stimulation of growth)	Ozan et al., 1997
		2.5-20 mg/l ≈ 2.5-20 μg/g	Mycorrhizal colonization and nodule number	*Glomus intraradices*	–	+ (Stimulated at 5-10 mg/l)	Siqueira et al., 1991
		50-800 μM ≈ 14-229 μg/g	Mycelial growth	*Rhizoctonia solani, Sclerotium rolfsii*	++ (*R. solani*: 200 μM(50), 800 μM(80)) +++ (*S. rolfsii*: 50 μM(65), 200 μM(57), 800 μM(91))	–	Weidenbörner et al., 1990
	Medicarpin	24-270 μg/g	Radial growth	*N. haematococca* MP I isolate T-145 and MP VI isolate T-30	++ (Isolate T-145)	(+) (Isolate T-30: increase in lag period at 270 μg/g)	Denny and VanEtten, 1981
		12-82 μg/g	Dry weight increase	*N. haematococca* MP I isolate T-145 and MP VI isolate T-30	+++ (Isolate T-145: Almost completely inhibited at 36 μg/g)	+ (Isolate T-30)	Denny and VanEtten, 1981
		11-90 μg/g	Spore germination	*N. haematococca* MP I isolate T-145 and MP VI isolate T-30	+++ (Isolate T-145: Severely affected at conc. > 24 μg/g) ++ (Isolate T-30: Affected at conc. > 54 μg/g)	–	Denny and VanEtten, 1981
		No information	Mycelial growth, germe-tube growth and spore germination	Fungi normally non-pathogenic or weakly pathogenic on legumes	+++ (ED_{50} < 50 μg/ml ≈ < 50 μg/g)	–	Ingham, 1982
		70 μg/ml ≈ 70 μg/g	Mycelial growth	10 isolates of *N. haematococca* MP VI	+ (9 isolates (4-29))	+ (Isolate T390)	Miao and VanEtten, 1992
		100 μM ≈ 27 μg/g	Radial growth	*Aphanomyces euteiches, Fusarium solani* f. sp. *cucurbitae*	+ (*A. euteiches* (26-31)) ++ (*F. solani* (65-78))	–	VanEtten, 1976
	Rhamnetin	0.5-8 μM ≈ 0.16-2.5 μg/g	Spore germination	AM fungi: *Gigaspora rosea, G. margarita, Glomus mosseae, G. intraradices*	–	+	Scervino et al., 2005a

Table 5 (Continued)

Target	Compound	Conc. in test	Type of effect	Target species	Effect (Inbibited by %)*	No effect*	Article
		0.5-8 μM ≈ 0.16-2.5 μg/g	Hyphal length / hyphal branches	*G. rosea, G. margarita, G. mosseae, G. intraradices*	++ (0.5 – 8 μM: *G. rosea* (60/80), 2 and 8 μM: *G. margarita* (70/80), 8 μM: *G. mosseae* (60/60), *G. intraradices* (70/55))	+ (0.5 μM: *G. margarita*, 0.5, 2 μM: *G. mosseae, G. intraradices*)	Scervino et al., 2005a
		0.5-8 μM ≈ 0.14-2.3 μg/g	Cluster of auxiliary cells or secondary spores	*G. rosea, G. margarita, G. mosseae*	+++ (8 μM: *G. rosea* (80), *G. margarita* (85), *G. mosseae* (85))	+ (0.5, 2 μM: *G. rosea, G. margarita*, *G. mosseae*)	Scervino et al., 2005a
	Acacetin	0.5-8 μM ≈ 0.14-2.3 μg/g	Spore germination	*G. rosea, G. margarita, G. mosseae, G. intraradices*	–	+	Scervino et al., 2005a
		0.5-8 μM ≈ 0.14-2.3 μg/g	Hyphal length / hyphal branches	*G. rosea, G. margarita, G. mosseae, G. intraradices*	++ (0.5 – 8 μM: *G. rosea* (60/80); 2, 8 μM: *G. margarita* (60/75); 8 μM: *G. mosseae* (60/75), *G. intraradices* (70/70))	+ (0.5 μM: *G. margarita*; 0.5, 2 μM: *G. mosseae, G. intraradices*)	Scervino et al., 2005a
		0.5-8 μM ≈ 0.14-2.3 μg/g	Cluster of auxiliary cells or secondary spores	*G. rosea, G. margarita, G. mosseae*	+++ (8 μM: *G. rosea* (80), *G. margarita* (85)) ++ (8 μM: *G. mosseae* (65))	+ (0.5, 2 μM: *G. rosea, G. margarita*, *G. mosseae*)	Scervino et al., 2005a
	Myricetin	10 μM ≈ 3 μg/g	Hyphal growth and formation of auxilary cells	*G. margarita*	-	+ (Stimulated)	Becard et al., 1992
		1 mM ≈ 320 μg/g	Mycelial growth	Two potential seed pathogens (*Aspergillus niger* and *Fusarium* sp.) and three seed-benificial antibiotic-producing fungi (*Gliocladium roseum*, *Penicillium diversum* and *Trichoderma viride*)	+++ (*A. niger* (89) and *Fusarium* sp. (39))	+ (*G. roseum*, *P. diversum* and *T. viride*)	Paszowski and Kremer, 1988
		1 mM ≈ 320 μg/g	Sporulation	*A. niger, Fusarium* sp., *G. roseum*, *P. diversum* and *T. viride*	+++ (No sporulation of any of the fungi)	–	Paszowski and Kremer, 1988
		100 μg/ml ≈ 100 μg/g	Growth inhibition	*Mucor mucedo, Rhizopus chinensis, A. niger*	–	+	Nakatani et al., 1989
	Quercetin	10 μM ≈ 3 μg/g	Hyphal growth and formation of auxilary cells	*G. margarita*	–	+ (Stimulated)	Becard et al., 1992
		100-2000 μg/mL ≈ 100-2000 μg/g	Growth inhibition	*Candida tropicalis*	+ (2000 μg/g low activity compared to antifungal drug)	+ (100 μg/g)	Ghazal et al., 1992
		0-1 mM ≈ 0-300 μg/g	Mycelial growth	*Fusarium culmorum*	–	+ (1 mM: No effect alone, 0.25-1 mM: toxic effect of copper reversed)	Park et al., 1998
		No information	Colonial germination	*Neurospora crassa*	–	+	Parvez et al., 2004

Table 5 (Continued)

Target	Compound	Conc. in test	Type of effect	Target species	Effect (Inbibited by %)*	No effect*	Article
		1 mM ≈ 300 μg/g	Mycelial growth	*A. niger, Fusarium* sp., *G. roseum*, *P. diversum* and *T. viride*	++ (*A. niger* (53) and *Fusarium* sp. (50))	+ (*G. roseum*, *P. diversum* and *T. viride*)	Paszowski and Kremer, 1988
		1 mM ≈ 300 μg/g	Sporulation	*A. niger, Fusarium* sp., *G. roseum*, *P. diversum* and *T. viride*	++ (No sporulation of *P. diversum*, sporulation of *A. niger* and *Fusarium* sp. < control)	+ (Promoted *G. roseum* and *T. viride*)	Paszowski and Kremer, 1988
		50 and 500 ppm ≈ 50 and 500 μg/g	Growth inhibition	*Pythium irregulare*, *Alternaria alternata*, *R. solani*	+++ (*P. irregulare*: 50 ppm (46-50), 500 ppm (100), *R. solani*: 50 ppm (47-55), 500 ppm (100)) + (*A. alternata*: 50 ppm (0-5), 500 ppm (26-67))	–	Nemec, 1976
		0.5-8 μM ≈ 0.15-2.4 μg/g	Spore germination	*G. rosea, G. margarita, G. mosseae, G. intraradices*	–	+ (2 and 8 μM: stimulated *G. rosea* and *G. margarita*)	Scervino et al., 2005a
		0.5-8 μM ≈ 0.15-2.4 μg/g	Hyphal length and hyphal branches	*G. rosea, G. margarita, G. mosseae, G. intraradices*	++ (8 μM: hyphal branches of *G. margarita* (70))	+ (2 μM: stimulated *G. rosea* and *G. margarita*)	Scervino et al., 2005a
		0.5-8 μM ≈ 0.15-2.4 μg/g	Cluster of auxiliary cells and secondary spores	*G. rosea, G. margarita, G. mosseae*	–	+ (Stimulated *G. rosea* (0.5-8 μM), *G. margarita* (0.5 μM))	Scervino et al., 2005a
	Vestitone	108 μg/g	Spore germination	*N. haematococca* MP VI isolate T-30	+ (< half the effect of medicarpin)	–	Denny and VanEtten, 1981
		No information	Mycelial growth, germe-tube growth and spore germination	Fungi normally non-pathogenic or weakly pathogenic on legumes	+++ (or ++) (ED_{50} < 50 μg/ml (or 50-100 μg/ml) ≈ < 50 μg/g (or 50-100 μg/g))	–	Ingham, 1982
	Demethylmedicarpin	No information	Mycelial growth, germe-tube growth and spore germination	Fungi normally non-pathogenic or weakly pathogenic on legumes	++ (ED_{50} = 50-100 μg/ml ≈ 50-100 μg/g)	–	Ingham, 1982
	Genistein	No information	Mycelial growth, germe-tube growth and spore germination	Fungi normally non-pathogenic or weakly pathogenic on legumes	++ (or +) (ED_{50} = 50-100 μg/ml (or > 100 μg/ml) ≈ 50-100 μg/g (or > 100 μg/g))	–	Ingham, 1982
		50-800 μM ≈ 14-216 μg/g	Mycelial growth	*R. solani, S. rolfsii*	+ (*R. solani*: 200 μM(34)) ++ (*S. rolfsii*: 50 μM(45), 200 μM(65), 800 μM(67))	–	Weidenbörner et al., 1990
	Kaempferol	10 μM ≈ 3 μg/g	Hyphal growth and formation of auxilary cells	*G. margarita*	–	+ (Stimulated hyphal growth)	Becard et al., 1992
		0.5 and 2 μM ≈ 0.15 and 0.6 μg/g	Spore germination, hyphal length, hyphal branching, secondary spores	*G. mosseae, G. intraradices*	–	+	Scervino et al., 2005b

Table 5 (Continued)

Target	Compound	Conc. in test	Type of effect	Target species	Effect (Inbibited by %)*	No effect*	Article
		0.5 and 2 μM ≈ 0.15 and 0.6 μg/g	Hyphal length, hyphal branching, clusters of auxiliary cells	*G. rosea, G. margarita*	–	+ (Stimulated hyphal length and clusters of auxiliary cells)	Scervino et al., 2005b
		0.5 and 2 μM ≈ 0.15 and 0.6 μg/g	Spore germination	*G. rosea, G. margarita*	++ (*G. margarita* (45))	+ (Stimulated *G. rosea*)	Scervino et al., 2005b
	5,6,7,8-Tetra-hydroxy-4'-methoxyflavone	0.5-8 μM ≈ 0.15-2.4 μg/g	Spore germination	*G. rosea, G. margarita, G. mosseae, G. intraradices*	–	+ (Stimulated *G. rosea* and *G. margarita*)	Scervino et al., 2005a
		0.5-8 μM ≈ 0.15-2.4 μg/g	Hyphal length and hyphal branches, cluster of auxiliary cells or secondary spores	*G. rosea, G. margarita, G. mosseae, G. intraradices*	+ (Hyphal length at 2 and 8 μM: *G. margarita* (50))	+ (Stimulated *G. rosea* (0.5-8 μM), *G. margarita* (0.5 μM))	Scervino et al., 2005a
	6-Hydroxykaempferol	0.5-8 μM ≈ 0.15-2.4 μg/g	Spore germination	*G. rosea, G. margarita, G. mosseae, G. intraradices*	–	+ (Stimulated *G. rosea* and *G. margarita*)	Scervino et al., 2005a
		0.5-8 μM ≈ 0.15-2.4 μg/g	Hyphal length, hyphal branches, cluster of auxiliary cells or secondary spores	*G. rosea, G. margarita, G. mosseae, G. intraradices*	+ (Hyphal length at 2 and 8 μM: *G. margarita* (50))	+ (Stimulated *G. rosea* (0.5-8 μM), *G. margarita* (0.5 μM))	Scervino et al., 2005a
	Daidzein	No information	Mycelial growth, germe-tube growth and spore germination	Fungi normally non-pathogenic or weakly pathogenic on legumes	+ (ED_{50} > 100 μg/ml ≈ 100 μg/g)	–	Ingham, 1982
		50, 200, 800 μM ≈ 13-214 μg/g	Mycelial growth	*Aspergillus ochraceus, Penecillium digitatum, F. culmorum*	+ (*F. culmorum* (15.6-21.9))	+ (*A. ochraceus, P. digitatum*)	Krämer et al., 1984
		800 μM ≈ 214 μg/g	Dry weight	*A. ochraceus, P. digitatum, F. culmorum*	+ (*F. culmorum* (23))	+ (*A. ochraceus, P. digitatum*)	Krämer et al., 1984
	Glycitein	No information	Mycelial growth, germe-tube growth and spore germination	Fungi normally non-pathogenic or weakly pathogenic on legumes	+ (ED_{50} > 100 μg/ml ≈ 100 μg/g)	–	Ingham, 1982
		50, 200, 800 μM ≈ 13-214 μg/g	Mycelial growth	*A. ochraceus, P. digitatum, F. culmorum*	+ (*A. ochraceus* at 50 μM (9))	+ (*A. ochraceus* at 200 and 800 μM; Stimulated *P. digitatum* and *F. culmorum* at 200 and 800 μM)	Krämer et al., 1984
		800 μM ≈ 214 μg/g	Dry weight	*A. ochraceus, P. digitatum, F. culmorum*	–	+ (Stimulated *P. digitatum* and *F. culmorum*)	Krämer et al., 1984
	Formononetin	No information	Mycelial growth, germe-tube growth and spore germination	Fungi normally non-pathogenic or weakly pathogenic on legumes	+ (ED_{50} > 100 μg/ml ≈ 100 μg/g)	–	Ingham, 1982
		50, 200, 800 μM ≈ 13-214 μg/g	Mycelial growth	*A. ochraceus, P. digitatum, F. culmorum*	–	+ (Stimulated *P. digitatum* at 800 μM)	Krämer et al., 1984
		800 μM ≈ 214 μg/g	Dry weight	*A. ochraceus, P. digitatum, F. culmorum*	–	+	Krämer et al., 1984

Table 5 (Continued)

Target	Compound	Conc. in test	Type of effect	Target species	Effect (Inbibited by %)*	No effect*	Article
		500 µg/g soil	Total number of microorganisms	Soil fungi	–	+	Ozan et al., 1997
		2.5-20 mg/l ≈ 2.5-20 µg/g	Mycorrhizal colonization and nodule number	*G. intraradices*	–	+ (Stimulated at 5-10 mg/l)	Siqueira et al., 1991
		100 µM ≈ 27 µg/g	Radial growth	*A. euteiches, F. solani* f. sp. *cucurbitae*	+ (*A. euteiches* (17) and *F. solani* (18))	–	VanEtten, 1976
	Coumestrol	up to 1000 µg/g	Spore germination	*Venturia ineaqualis* and *Phytophthora infestans*	–	+	Bickoff et al., 1969
		up to 1000 µg/g	Mycelial growth	*R. solani*, *Phythium ultimum* and *Monilinia fructicola*	–	+	Bickoff et al., 1969
		No information	Mycelial growth, germe-tube growth and spore germination	Fungi normally non-pathogenic or weakly pathogenic on legumes	+ (ED_{50} > 100 µg/ml ≈ 100 µg/g)	-	Ingham, 1982
		100 µM ≈ 27 µg/g	Radial growth	*A. euteiches, F. solani* f. sp. *cucurbitae*		+	VanEtten, 1976
	Trifoliol	up to 1000 µg/g	Spore germination	*V. ineaqualis* and *P. infestans*	–	+	Bickoff et al., 1969
		up to 1000 µg/g	Mycelial growth	*R. solani*, *P. ultimum* and *M. fructicola*	–	+	Bickoff et al., 1969
	9-O-Methyl-coumestrol	100 µM ≈ 28 µg/g	Radial growth	*A. euteiches, F. solani* f. sp. *cucurbitae*		+	VanEtten, 1976
	7-Hydroxy-4'-methoxyflavonol	0.5-8 µM ≈ 0.15-2.4 µg/g	Spore germination, hyphal length, hyphal braches, cluster of auxiliary cells, secondary spores	*G. rosea, G. margarita, G. mosseae, G. intraradices*	–	+	Scervino et al., 2005a
	Red clover root saponins = very similar or equal to white clover top saponins	up to 10 mg × 100 cm^3	Growth inhibition	*T. viride*	–	+	Oleszek and Jurzysta, 1986
	Isorhamnetin	0.5 and 2 µM ≈ 0.15 and 0.6 µg/g	Spore germination, hyphal length, hyphal branching, clusters of auxiliary cells or secondary spores	*G. mosseae, G. intraradices, G. rosea, G. margarita*	–	+ (Stimulated hyphal length and clusters of auxiliary cells of *Gigaspora* spp.)	Scervino et al., 2005b
	Luteolin	10 µM ≈ 3 µg/g	Hyphal growth and formation of auxilary cells	*G. margarita*	-	+ (Stimulated formation of cells)	Becard et al., 1992
		0.5 and 2 µM ≈ 0.15 and 0.6 µg/g	Spore germination, hyphal length, hyphal branching, clusters of auxiliary cells or secondary spores	*G. mosseae, G. intraradices, G. rosea, G. margarita*	-	+ (Stimulated hyphal length of *Glomus* spp. and all factors of *Gigaspora* spp.)	Scervino et al., 2005b
Bacteria	Myricetin	2000 µg/g	Antibiotic activity	*Rhizobium leguminosarum*	+++	–	Fottrell et al., 1964

Table 5 (Continued)

Target	Compound	Conc. in test	Type of effect	Target species	Effect (Inbibited by %)*	No effect*	Article
		Rel. high conc. - extracted and concentrated from white clover seeds	Antibiotic activity	*R. leguminosarum* and *Rhizobium trifolii*	+++ (Toxicity proportional to conc.)	–	Masterson, 1965
		100 µg/ml ≈ 100 µg/g	Growth inhibition	*Staphylococcus aureus, Bacillus subtilis, Escherichia coli, Pseudomonas auruginosa*	–	+	Nakatani et al., 1989
		Up to 3.18 mg/ml ≈ 3180 µg/g	Inhibition zone	*R. trifolii*	+ (Finely dispersed suspension at 3.18 mg/ml)	+ (Saturated aqueous solution)	Young and Paterson, 1980
	Condensed tannins extracted from white clover seeds	No information	Antibiotic activity	*R. leguminosarum*	+++	–	Fottrell et al., 1964
		5 µg/ml ≈ 5 µg/g	Inhibition zone	*R. trifolii*	+++	–	Young and Paterson, 1980
	Coumestrol	15-50 ppm ≈ 15-50 µg/g	Colony development	*Pseudomonas glycinea*	++	–	Keen and Kennedy, 1974
	Quercetin	Trace	Antibiotic activity	*R. leguminosarum*	(+) (very low activity)	–	Fottrell et al., 1964
		100-2000 µg/mL ≈ 100-2000 µg/g	Growth inhibition	*S. aureus, Enterobacter aerogenes, E. coli*	+ (2000 µg/g low activity compared to antibacterial drugs)	+ (100 µg/g)	Ghazal et al., 1992
		Lower conc. than myricetin - extracted and concentrated from white clover seeds	Antibiotic activity	*R. leguminosarum* and *R. trifolii*	+ (Toxicity much lower than myricetin)	–	Masterson, 1965
		10^{-7} M ≈ 0.03 µg/g	Nitrogen fixing activity	*R. trifolii*	–	+ (Stimulated nodule formation)	Novikova, 1994
	Biochanin A	500 µg/g soil	Total number of microorganisms	Soil bacteria	–	+ (Stimulated growth)	Ozan et al., 1997
	Formononetin	500 µg/g soil	Total number of microorganisms	Soil bacteria	–	+ (Stimulated growth more than biochanin A)	Ozan et al., 1997
Insects, nematodes, slugs	Soyasaponin I	10 ppm in diet ≈ 10 µg/g	Development and growth, pupal development and reproduction	Insect (moth): *Spodoptera littoralis*	+++ (Increased: days to pupation (16), larval mortality (20), pupal instar (13). Decreased: food consumed (32), pupal weight (26), survival to adults (43), eggs/female (20), egg hatchability (23), progeny per female (39), population growth (62))	–	Adel et al., 2000
		125, 250, 500 µg/ml ≈ 125, 250, 500 µg/g	Mortality	Nematode: *Xiphinema index*	+++ (48 h: 250 µg/ml (52), 500 µg/ml (81))	+ (125 µg/ml not significant effect)	Argentieri et al., 2007

Table 5 (Continued)

Target	Compound	Conc. in test	Type of effect	Target species	Effect (Inbibited by %)*	No effect*	Article
	Myricetin	2-10 mmol/kg wet wt of artificial diet ≈ 636-3180 μg/g wet wt	Larval growth	Insect (moth): *Heliothis zea*	++ (ED_{50} = 3.1 mmol/kg diet ≈ 986 μg/g wet wt)	–	Elliger et al., 1980
	Quercetin	2-10 mmol/kg wet wt of artificial diet ≈ 604-3020 μg/g wet wt	Larval growth	*H. zea*	++ (ED_{50} = 3.5 mmol/kg diet ≈ 1057 μg/g wet wt)	–	Elliger et al., 1980
		187 and 375 μM ≈ 56-113 μg/g incorporated into diet	Growth and reproduction	Insect (aphid): Biotype B greenbugs	++ (Growth: 187 μM(20), 375 μM(40)) +++ (Reproduction: 187 μM(50-55), 375 μM(80-100))	–	Todd et al., 1971
	Luteolin	2-10 mmol/kg wet wt of artificial diet ≈ 572-2860 μg/g wet wt	Larval growth	*H. zea*	++ (ED_{50} = 5.4 mmol/kg diet ≈ 1544 μg/g wet wt)	–	Elliger et al., 1980
	Cyanogenic glycosides	180-246 μg/g white clover fresh wt	Preferential eating	Slug: *Arion hortensis*	+ (Seedlings at 16-35 days age with > 180 μg/g fresh wt)	+ (5 days old seedlings: 180 μg/g fresh wt)	Horrill and Richards, 1986
	Acacetin	2-10 mmol/kg wet wt of artificial diet ≈ 568-2840 μg/g wet wt	Larval growth	*H. zea*	–	+	Elliger et al., 1980
	Kaempferol	2-10 mmol/kg wet wt of artificial diet ≈ 572-2860 μg/g wet wt	Larval growth	*H. zea*	–	+	Elliger et al., 1980

* Number of + corresponds to relative toxicity judged from data in the investigations by the authors of present review. + Corresponds to relatively low toxicity, while +++ correponds to relatively high toxicity towards the specific target.

Degradation of condensed tannins

Condensed tannins do not break down readily under physiological conditions; if treated drastically, they usually produce less soluble polymeric "phlobaphenes" or flavonoid monomers (Haslam, 1966). There are few data on microbial degradation pathways of tannins (Scalbert, 1991).

Biological effects of flavan-3-ols and condensed tannins

Condensed tannins are toxic to bacteria (Fottrell et al., 1964; Hale and Mathers, 1977; Mila and Scalbert, 1994; Scalbert, 1991; Young and Paterson, 1980; Table 5), fungi (Levin, 1976; Masterson, 1965; Scalbert, 1991), insects (Levin, 1976), plants (Duke, 1986) and yeasts (Scalbert, 1991). They constitute significant feeding barriers to phytophagous insects (Harborne, 1994) and grazing mammals (Harborne, 1994; Singleton and Kratzer, 1973). The minimum inhibitory concentration of tannins varies according to microorganisms, between 0.012 g/L and 1 g/L (≈0.012–1 mg/g) for bacteria and between 0.5 g/L and 10–20 g/L (≈0.5–20 mg/g) for fungi (Scalbert, 1991). Differences in toxicity to groups of microorganisms could be a result of their ability to degrade tannins or secrete polymers that combine with tannins (Scalbert, 1991).

Prodelphinidin is very important in toxicity of white clover seed tannins to *Rhizobium trifolii* bacteria (Young and Paterson, 1980) and the astringency of the white clover plant (Jones et al., 1976). However, most investigations have been on raw tannin extracts (Scalbert, 1991), and little is known of the effects of tannin structure on toxicity.

Tannins inhibit plant growth and seed germination, but they have relatively non-specific binding to proteins and low efficacy, indicating that they are not suitable herbicides (Duke, 1986).

Isoflavones and isoflavanones

Isoflavones/isoflavanones in white clover

Isoflavones have been quantified in white clover roots (n.d.–4920 μg/g), stems (4–354 μg/g), leaves (4–900 μg/g), flowers (1–213 μg/g), leaves and stems (88–500 μg/g) and total above-ground material (n.d.–920 μg/g) (Table 4). However, there is no pattern in isoflavone distribution, either as single compounds or total contents among the different plant parts (Cook et al., 1995; Vetter, 1995; Wu et al., 2003; Table 4). There are some large differences between cultivars (Bennet et al., 1967; Carlsen et al., 2008; Sachse, 1974), while some show no differences (Saloniemi et al., 1993). The formononetin content can differ between resistant and susceptible populations (Cook et al., 1995).

Contents in healthy white clover plants are low (Francis et al., 1967; Saloniemi et al., 1993; Shutt, 1976; Wong and Latch, 1971b). When plants are infected with pathogenic fungi the formononetin concentration can increase markedly at infection sites in leaves (Wong and Latch, 1971b), stems (Cook et al., 1995) and roots (Carlsen et al., 2008), while there is no relationship between plant contents and degree of infection of a fungal disease (*Leptosphaerulina trifolii*) (Saba et al., 1974). The content of formononetin is higher in roots with active rhizobial nodules than in inactive nodules and roots alone (Johnson et al., 2005), while formononetin and daidzein contents decreases with AM fungal inoculation (Carlsen et al., 2008).

The proportions of different isoflavones may change during infection; formononetin (90–95%) and genistein (5–10%) are the main isoflavones in healthy white clover (Saloniemi et al., 1993). In plants infected with fungi, both formononetin and 2'-hydroxyformononetin were present in almost equal amounts (Woodward, 1981b).

There are no records of isoflavanoid contents in healthy plants or cultivar differences.

Degradation of isoflavones and isoflavanones

The degradation of isoflavones and isoflavanones is complex, with isoflavones and isoflavanones formed from each other (Figs. 2– 4). Biochanin A, genistein, formononetin, daidzein, and vestitone are the isoflavones/isoflavanones whose degradation has been the most studied. Vestitone degradation is highly coupled with medicarpin and is therefore presented with pterocarpan degradation (Fig. 4). Formononetin and biochanin A are stable in sterile soil during a 15 day period, but are degraded in soil (40% and 80%, respectively) and even faster in soil planted with corn seedlings (95% and 100%, respectively), with the degradation of biochanin A being much faster than that of formononetin (Ozan et al., 1997).

Biological effects of isoflavones and isoflavanones and their degradation products

Isoflavone phytoalexins have antimicrobial properties (Cruickshank, 1963; Van Etten and Pueppke, 1976), and biochanin A and genistein show phytoalexinic behaviour (Stenlid, 1970; Weidenbörner et al., 1990). Many isoflavones show antifungal properties (Ingham, 1982; Van Etten, 1976; Virtanen and Hietala, 1958; Weidenbörner et al., 1990) and estrogenic effects in ruminants (Bennet et al., 1967; Pettersson et al., 1984; Saloniemi et al., 1993; Shutt and Braden, 1968; Shutt and Cox, 1972). The isoflavones formononetin and biochanin A stimulate colonization by one AM fungus (*Glomus intraradices)* and white clover growth (Siqueira et al., 1991a), while biochanin A inhibit the growth of another AM fungus (*Gigaspora margarita*) (Bécard et al., 1992).

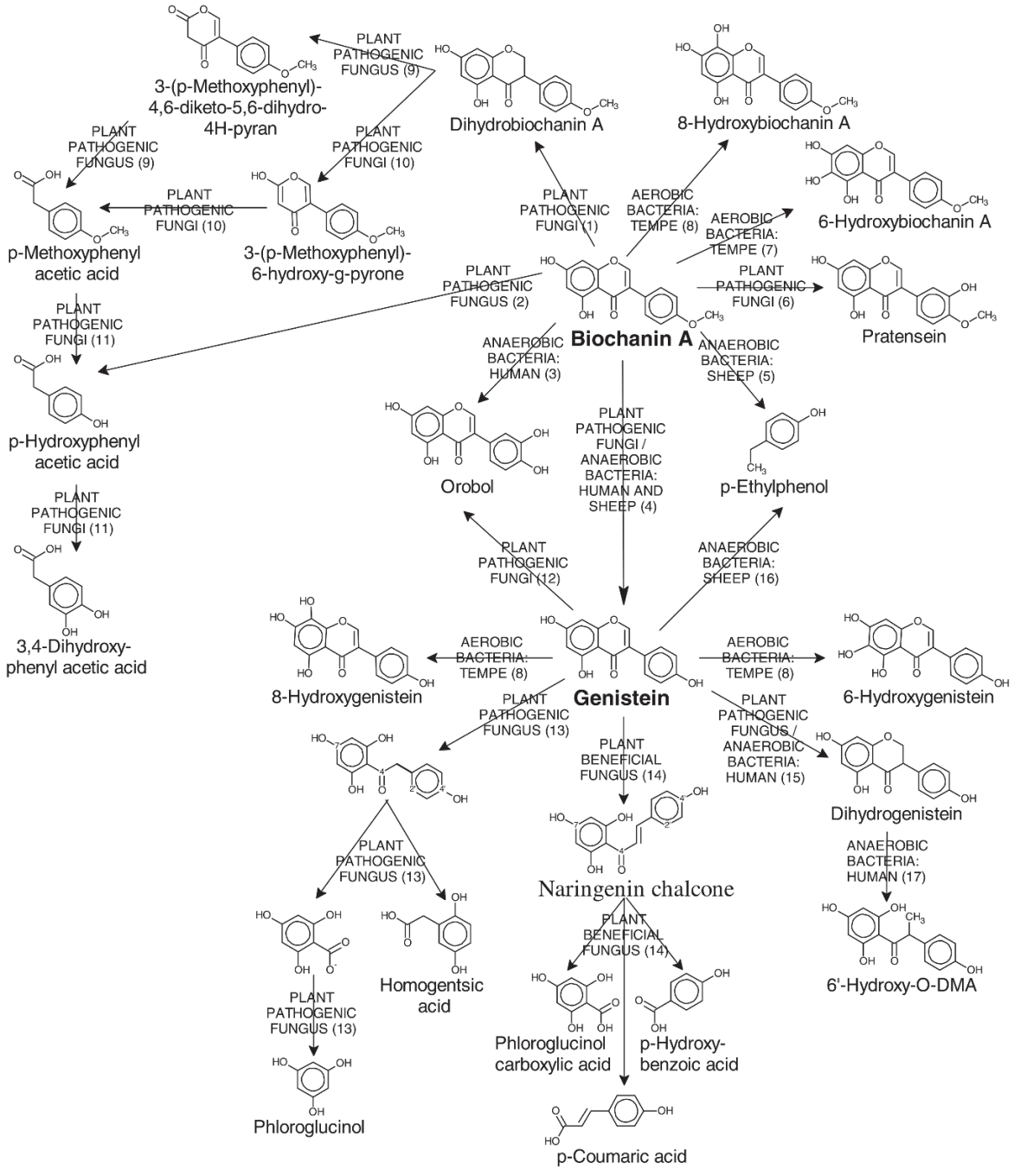


Fig. 2. Degradation of biochanin A and genistein by fungi and by bacteria. **1:** *Fusarium javanicum* (Schlieper et al., 1984) and *Nectria haematococca* isolates (Willeke et al., 1983). **2:** Very small amounts by *Ascochyta rabiei* (Kraft and Barz, 1985). **3:** Human liver microsomes (Tolleson et al., 2002). **4:** Metabolised in sheep (Batterham et al., 1965; 1971; Braden et al., 1967), in rumen fluid from sheep (Nilsson et al., 1967), human faces (*Eubacterium limosum*) (Hur and Rafii, 2000), human liver microsomes (Tolleson et al., 2002), by 16 *Fusarium* species (Barz et al., 1976), *Armillaria mellea* Vahl (Kuhn) (Curir et al., 1994) and *Ascochyta rabiei* (minor metabolite) (Kraft and Barz, 1985). **5:** Metabolised in sheep (Batterham et al., 1965, 1971; Braden et al., 1967). **6:** *Ascochyta rabiei* (major metabolite) (Kraft and Barz, 1985), *Fusarium oxysporum* f.sp. *lin* (very slowly), *Fusarium oxysporum* f.sp. *lycopersici* (almost quantitatively) (Weltring et al., 1982). **7:** Tempe-derived bacterial strain I (gram-positive short rod cells, *Micrococcus* or *Arthrobacter* specie) (Klus and Barz, 1998). **8:** Tempe-derived bacterial strains I and III (gram-positive short rod and coccoid cells, *Micrococcus* or *Arthrobacter* species) (very low amounts of 8-hydroxygenistein) (Klus and Barz, 1998). **9:** *Fusarium javanicum* (Schlieper et al., 1984). **10:** *Nectria haematococca* isolates (Willeke et al., 1983). **11:** *Fusarium javanicum* (Schlieper et al., 1984) and *Nectria haematococca* isolates (Willeke et al., 1983). **12:** 16 *Fusarium* species (Barz et al., 1976) and *Ascochyta rabiei* (Kraft and Barz, 1985). **13:** *Armillaria mellea* Vahl (Kuhn) (Curir et al., 1994). **14:** *Rhizobia fredii* (Rao and Cooper, 1994). **15:** Human gut bacteria (Joannou et al., 1995) and *Ascochyta rabiei* (Kraft and Barz, 1985). **16:** Metabolised in sheep (Batterham et al., 1971; Braden et al., 1967). **17:** Gut bacteria (Joannou et al., 1995).

Biochanin A, genistein, formononetin, daidzein and glycitein are antifungal (Table 5). Biochanin A and its fungal degradation product, dihydrobiochanin A, are more fungitoxic than genistein and its fungal and bacterial degradation product, dihydrogenistein (Weidenbörner et al., 1990), and genistein is more toxic than formononetin and daidzein (Ingham, 1982). When one looks at the C-4' position in genistein and biochanin A, the presence of a methoxy group (in biochanin A) instead of a hydroxyl group (in genistein) gives biochanin A a higher activity (Weidenbörner et al., 1990). Equol and methylequol (anaerobic bacterial degradation products of formononetin and daidzein) also shows some antifungal activities depending on fungal species and concentration of the compounds (Krämer et al., 1984). The isoflavanone vestitone is fungitoxic, although less active than medicarpin (Denny and Van Etten, 1981; Ingham, 1982). As concluded by Krämer et al. (1984) and is shown in Table 5, the fungitoxic activity of isoflavones and isoflavanones is highly dependent on the individual fungi, the specific compound and their concentrations.

Rumen degradation of formononetin and daidzein produces estrogenically active equol (Saloniemi et al., 1993; Shutt and Braden, 1968) that is probably responsible for estrogenic effects in sheep known as 'clover disease'. Genistein and biochanin A are inactive in ruminants as they are degraded to inactive paraethylphenol (Pettersson et al., 1984; Shutt and Braden, 1968). However, there were no relationships between effects on sheep grazing white clover and contents of formononetin, genistein or coumestrol (Bennet et al., 1967). In monogastric animals such as mice and rats, the estrogenic effects of isoflavonoids (Bickoff et al., 1962; Bradbury and White, 1954; Jurzysta et al., 1988; Saba et al., 1974; Saloniemi et al., 1993) and their degradation product equol (Medlock et al., 1995) are more moderate than for ruminants.

Pterocarpans

Pterocarpans and their degradation products in white clover

Medicarpin and demethylmedicarpin are difficult to synthesize or isolate in a purified form (Oleszek, W., personal communication), and thereby quantify. Few records exist on the levels (Table 4) of the pterocarpans (Table 3) in white clover. In two populations of healthy white clover, medicarpin occurred in the following order: leaves (9–32 μg/g) > stems (6–18 μg/g) > roots (1–8 μg/g) (Cook et al., 1995). The concentration increased in diseased plants at the site of fungal infection (Cook et al., 1995), and with temperature and day length in which infected plants were grown prior to fungal infection, but was unaffected by light intensity (Cruickshank et al., 1974). The contents of medicarpin and its fungal degradation product vestitol varied among cultivars infected with a fungal pathogen (Ingham, 1978). In white clover infected with fungi, medicarpin and demethylmedicarpin levels were 135 and 27 times higher, respectively, than those of the measured isoflavones and isoflavanones (Woodward, 1981b). The influence of inoculation with AM fungi on medicarpin contents in roots of two white clover cultivars was highly variable and depended on fungal isolate and plant cultivar (Carlsen et al., 2008).

Degradation of pterocarpans

Medicarpin degradation is mainly studied using fungi (Fig. 4). The degradation of vestitone is highly coupled to that of medicarpin and so is presented with the pterocarpans.

Biological effects of the pterocarpans and their degradation products

Medicarpin is antifungal (Table 5) as are several of its degradation products following plant metabolization (Ingham, 1982; Van Etten, 1976; Weidenbörner et al., 1990). For example, the following have antifungal properties: sativan (Ingham, 1982; Van Etten, 1976; Weidenbörner et al., 1990), variabilin (Ingham, 1982), and vestitol (Van Etten, 1976); vestitol is less active than sativan (Van Etten, 1976). Medicarpin is known to be phytotoxic to alfalfa (*Medicago sativa*) while the autotoxic effects of sativan and variabilin are unknown, and further metabolism of medicarpin could produce compounds that, while still fungitoxic, are less phytotoxic (Gregory and Edwards, 1994). Fungi can metabolize medicarpin into less fungitoxic compounds (Denny and Van Etten, 1981; Ingham, 1982), with vestitone (Denny and Van Etten, 1981; Ingham, 1982), 1a-OH-medicarpin (Denny and Van Etten, 1981; Miao and Van Etten, 1992a), 6a-OH-medicarpin (Denny and Van Etten, 1981; Miao and Van Etten, 1992a), and demethylmedicarpin (Ingham, 1982) all less toxic than medicarpin.

Coumestans

Coumestans in white clover

Coumestan contents in white clover (Table 3) have been determined in leaves (<2–166 μg/g), leaves and stems (<5–52.5 μg/g), total above-ground material (0–170 μg/g) and roots (n.d.-97.0 μg/g) but not in seeds or flowers (Table 4). Coumestrol content is lower in immature than in mature plants (Price and Fenwick, 1985) and varies among cultivars (Bennet et al., 1967; Carlsen et al., 2008; Saloniemi et al., 1993). In healthy white clover plants the levels (Table 4) of most of the compounds (Table 3), including coumestrol (Francis et al., 1967), are very low (Wong and Latch, 1971b). When infected with pathogens,

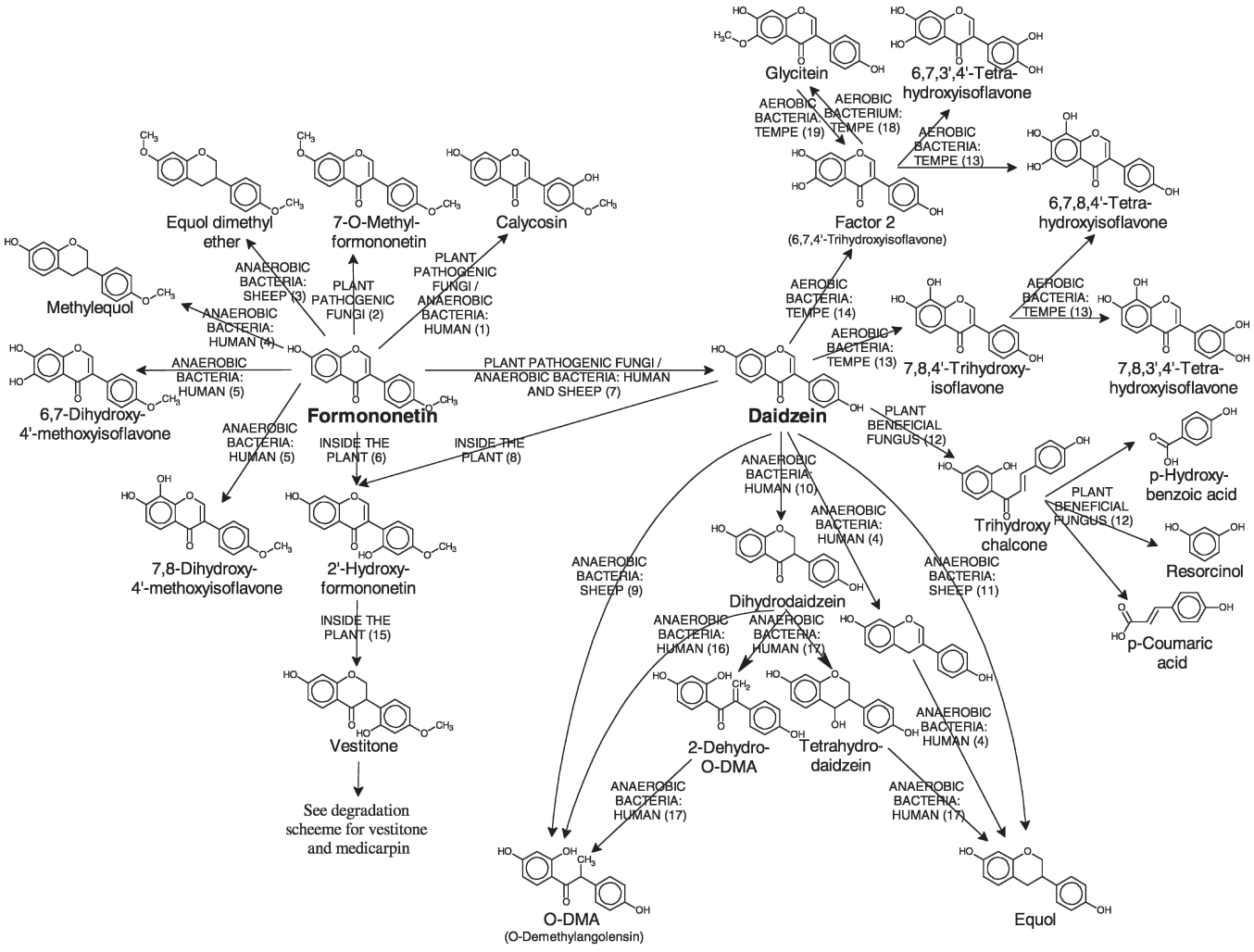


Fig. 3. Degradation of daidzein and formononetin inside the plant, by fungi and by bacteria. **1:** Human liver microsomes (Tolleson et al., 2002) and *Fusarium avenaceum* (Weltring et al., 1982). **2:** *Fusarium proliferatum* (Weltring et al., 1982). **3:** Metabolism in rumen of sheep (Braden et al., 1967). **4:** Human ruminal bacteria (Adlercreutz et al., 1987). **5:** Human liver microsomes (Tolleson et al., 2002). **6:** Enzymatic (isoflavone 2'-hydroxylase) conversion in *Lotus japonicus* (Shimada et al., 2000) and in white clover inoculated with *Monilinia fructicola* (Woodward, 1981b). **7:** Metabolism in rumen of sheep (Batterham et al., 1965, 1971; Braden et al., 1967; Nilsson et al., 1967) and in humans (Adlercreutz et al., 1987), hydrolysed in humans by cytochrome P450 1B1 (Roberts et al., 2002) and metabolised by *Fusarium proliferatum* (Weltring et al., 1982). **8:** Enzymatic (isoflavone 2'-hydroxylase) conversion in *Lotus japonicus* (Shimada et al., 2000). **9:** Metabolism in sheep (Batterham et al., 1971). **10:** Ruminal (Adlercreutz et al., 1987) and gut bacteria (Joannou et al., 1995). **11:** Metabolism in rumen of sheep (Batterham et al., 1965, 1971; Nilsson et al., 1967). **12:** *Rhizobia* sp. strain NGR234) (Rao and Cooper, 1994). **13:** Tempe-derived bacteria (*Micrococcus* or *Arthrobacter* species) (Klus and Barz, 1995). **14:** Tempe-derived bacteria (*Micrococcus* or *Arthrobacter* species (Klus and Barz, 1995) and *Microbacterium aborescens* (Klus et al., 1993). **15:** enzymatic (isoflavone reductase) conversion in *Lotus japonicus* (Shimada et al., 2000) and in white clover inoculated with *Monilinia fructicola* (Woodward, 1981b). **16:** Metabolism in humans (Adlercreutz et al., 1987). **17:** Human gut bacteria (Joannou et al., 1995). **18:** *Microbacterium aborescens* (Klus et al., 1993). **19:** Tempe-derived bacteria (*Brevibacterium epidermidis* and *Micrococcus* or *Arthrobacter* species (Klus and Barz, 1995), *Brevibacterium epidermidis* and *Micrococcus luteus* (Klus et al., 1993).

the contents increases markedly in the lesion areas (Saloniemi et al., 1995; Wong and Latch, 1971a, 1971b). The coumestrol content can increase with disease severity (Saba et al., 1974). The coumestan contents are lower in glasshouse-infected samples than in the field (Wong and Latch, 1971b). Higher contents of coumestrol in two cultivars of white clover were detected in plants inoculated with the AM fungus *Glomus claroideum* as compared to non-AM plants, while inoculation with *Glomus mosseae* and/or infection with *Pythium ultimum* had no significant influence on contents (Carlsen et al., 2008).

Biological effects of coumestans

Coumestans apparently lack antifungal activity (Bickoff et al., 1969; Perrin and Cruickshank, 1969; Van Etten, 1976; Table 5), while coumestrol has some antibacterial

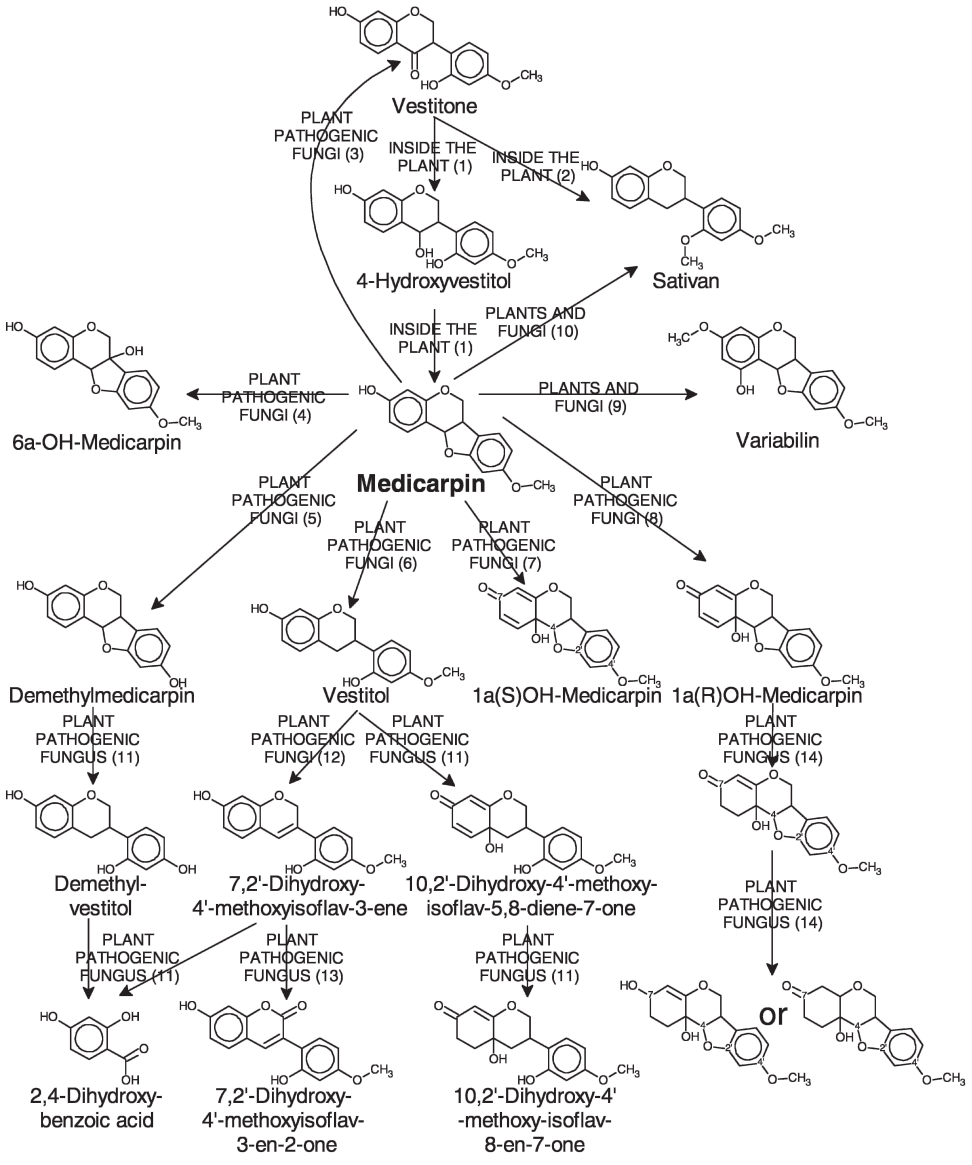


Fig. 4. Degradation of vestitone and medicarpin inside the plant and by fungi. **1:** In white clover inoculated with *Monilinia fructicola* (Woodward, 1981b). **2:** Enzymatic conversion in *Lotus japonicus* (Shimada et al., 2000). **3:** *Nectria haematococca* isolate T-30 (Denny and Van Etten, 1981), isolates of *Fusarium solani* (Denny and Van Etten, 1982), *Nectria haematococca* MP VI (Miao and Van Etten, 1992b) and *Colletotrichum trifolii* (Soby et al., 1996). **4:** *Cercospora arachidicola* (Edwards and Strange, 1991), *Colletotrichum trifolii, C. dematium, C. destructivum, Verticillium albo-atrum, Cercospora medicaginis* (Soby et al., 1996) and *Monilinia fructicola* (Wint.) Honey (Woodward, 1981b). **5:** *Nectria haematococca* isolate T-145 (Denny and Van Etten, 1981), isolates of *Fusarium solani* (Denny and Van Etten, 1982), *Ascochyta rabiei* (Kraft et al., 1987), *Nectria haematococca* MP VI (Miao and Van Etten, 1992b), *Colletotrichum trifolii, C. dematium* and *C. destructivum* (Soby et al., 1996). **6:** *Ascochyta rabiei* (Kraft et al., 1987), *Stemphylium alfalfae* (Soby et al., 1996), *Stemphylium botrosum* (Van Etten and Pueppke, 1976) and *Fusarium oxysporum* f.sp. *lycopersici* (Weltring et al., 1983). **7:** *Nectria haematococca* isolate T-30 (Denny and Van Etten, 1981), isolates of *Fusarium solani* (Denny and Van Etten, 1982), *Nectria haematococca* MP VI (Miao and Van Etten, 1992b), *Colletotrichum trifolii, Leptosphaerulina briosiana, Verticillium albo-atrum* and *Fusarium oxysporum* f. sp. *medicaginis* (Soby et al., 1996). **8:** *Nectria haematococca* isolate T-30 (Denny and Van Etten, 1981), isolates of *Fusarium solani* (Denny and Van Etten, 1982) and *Cercospora medicaginis* (Soby et al., 1996). **9:** Fungi (Edwards et al., 1994) and by plants analogous to pathogenic fungi (Gregory and Edwards, 1994). **10:** By plants analogous to pathogenic fungi (Gregory and Edwards, 1994). **11:** *Ascochyta rabiei* (Kraft et al., 1987). **12:** *Ascochyta rabiei* (Kraft et al., 1987) and *Fusarium oxysporum* f.sp. *lycopersici* (Weltring et al., 1983). **13:** *Fusarium oxysporum* f.sp. *lycopersici* (Weltring et al., 1983). **14:** *Cercospora medicaginis* (Soby et al., 1996).

(Keen and Kennedy, 1974) and phytotoxic activity (Stenlid, 1970).

Coumestans are generally estrogenic (Bickoff et al., 1960b, 1962, 1969; Livingston et al., 1964b; Saba et al., 1974; Wong and Latch, 1971b; Wong et al., 1971), but there are contrasting results on the estrogenic properties of coumestrol in white clover (Bickoff et al., 1960a, 1962; Medlock et al., 1995; Saba et al., 1974). It is the predominant phytoestrogen in white clover (cv. Ladino) (Bickoff et al., 1960a), and is more active than several of

the isoflavones (Bickoff et al., 1962). Others found that coumestrol only accounted for a small part of the observed estrogenic activity in mice (Saba et al., 1974), that the activities of coumestrol and repensol are comparable in mice (Livingston et al., 1964b), or that coumestrol is weakly estrogenic in rats (Medlock et al., 1995). The minimum coumestan level in white clover that will cause sheep fertility disorders is 20–50 μg/g (Wong et al., 1971), similar to content in diseased plants (Table 4). 9-O-methylcoumestrol (Bickoff et al., 1960b) and trifoliol (Livingston et al., 1964b) are relatively inactive in mice. The potency of diseased white clover in sheep (Wong et al., 1971) and mice (Saba et al., 1974; Wong et al., 1971) has shown a relationship to coumestan levels, while no similar relationship was found for coumestrol in grazing sheep (Bennet et al., 1967).

Cyanogenic glucosides

Cyanogenic glucosides in white clover

There is high variation in cyanoglucoside contents (Table 4) among cultivars (Butler, 1965; Corkill, 1943; Lehmann et al., 1990; Stochmal and Oleszek, 1995, 1997; Tava and Annicchiarico, 2000; Vickery et al., 1987; Viette et al., 2000; Wheeler and Vickery, 1989). Cyanogenic glucosides have been detected in leaves (16–2242 μg/g), leaves combined with stems (0–1204 μg/g) and total above-ground material (n.d.–4844 μg/g) (Table 4), there is no data for roots or flowers, and the distribution between plant parts is unknown.

White clover is polymorphic and two unlinked loci (*AC/ac* and *Li/li*) determine the presence of cyanogenic glucosides and an enzyme (linamarase), respectively (Corkill, 1943; Jones, 1972); thus the cyanogenic property in varieties is genetically determined (Hughes, 1991; Hughes and Conn, 1976; Jones, 1972). Within populations, the variation is highly significant ($P < 0.001$) (Tava and Annicchiarico, 2000). The frequency of cyanogenic individuals within populations is related to latitude (Daday, 1965), altitude (Araújo, 1976) and drought (Foulds and Grime, 1972; Raffaelli and Mordue, 1990); there are less cyanogenic individuals with lower temperature (Daday, 1965), dry conditions (Foulds and Grime, 1972; Raffaelli and Mordue, 1990), and high altitude (Araújo, 1976). For a more complete review of cyanogenic polymorphism in white clover, see Hughes (1991).

In white clover, two related glycosides occur as native substrates: linamarin and lotaustralin (Table 3). The linamarin/lotaustralin ratio differs between white clover cultivars (0.25–1.0; Butler, 1965; Daday, 1965; Maher and Hughes, 1971; Stochmal and Oleszek, 1997). In white clover linamarin and lotaustralin always occurs together (Jones, 1972); high-cyanogen varieties preferably synthesize lotaustralin over linamarin (Stochmal and Oleszek, 1997), and thus have a lower linamarin/lotaustralin ratio.

In contrast to cyanoglucosides, determined by modifying genes, cyanogen synthesis in white clover is dependent on a number of external factors, including temperature, season, water availability, light intensity, P fertilization and altitude (Stochmal and Oleszek, 1997; Vickery et al., 1987; Viette et al., 2000; Wheeler and Vickery, 1989). When white clover is stressed, due to periods of low temperature ($< 15^{\circ}$C) (Stochmal and Oleszek, 1997) or water stress (Vickery et al., 1987), the plant produces greater amounts of HCN. By contrast, when plants are provided with good growth conditions – increased light intensity (Vickery et al., 1987), higher temperature (Vickery et al., 1987; Stochmal and Oleszek, 1997), P fertilizer application (Vickery et al., 1987; Wheeler and Vickery, 1989), and/or a low grazing hazard (Stochmal and Oleszek, 1997), cyanogen synthesis is reduced.

Cyanogen synthesis varies with plant age; increasing with seedling age (by 37%, day 5–35) (Horrill and Richards, 1986), but decreasing with mature plant age (by 90%, day 19–159) (Vickery et al., 1987). The greater synthesis in seedlings than in mature plants probably results from the fact that the cyanogenic defence mechanism is more important for seedlings, as the fitness of mature plants will be only marginally reduced if mature plants are attacked by herbivores (Crawford-Sidebotham, 1972).

Production of HCN is not influenced by fungal infection (Angseesing and Angseesing, 1973).

Degradation of cyanogenic glucosides

The cyanogenic glucosides and the enzyme linamarase are produced during shoot growth (location not known), while the components of cyanogenesis are synthesized during leaf development and then stored in the mature leaf (Hughes, 1991). In plants containing glucosides and linamarase, damage to the leaf (Raffaelli and Mordue, 1990) by insect attack or animal ingestion (Tava and Annicchiarico, 2000), brings the enzyme into contact with glucosides and releases cyanide (Fig. 5) (Butler and Butler, 1960; Hughes and Conn, 1976; Raffaelli and Mordue, 1990). The hydrolysis is very rapid, and most glucosides are broken down in several minutes (Tapper and Reay, 1973).

Biological effects of cyanogenic glucosides

Cyanogenesis in white clover is effective against fungi (Levin, 1976), herbivores (Hughes, 1991; Stochmal and Oleszek, 1997) including slugs and snails (Angseesing, 1974; Angseesing and Angseesing, 1973; Crawford-Sidebotham, 1972; Horrill and Richards, 1986; Raffaelli and Mordue, 1990), weevils (Ellsbury et al., 1992; Raffaelli and Mordue, 1990) and mammals (sheep, cattle, water voles) (Coop and Blakley, 1949; Lehmann et al., 1990; Moran, 1954; Vickery et al., 1987; Viette et al.,

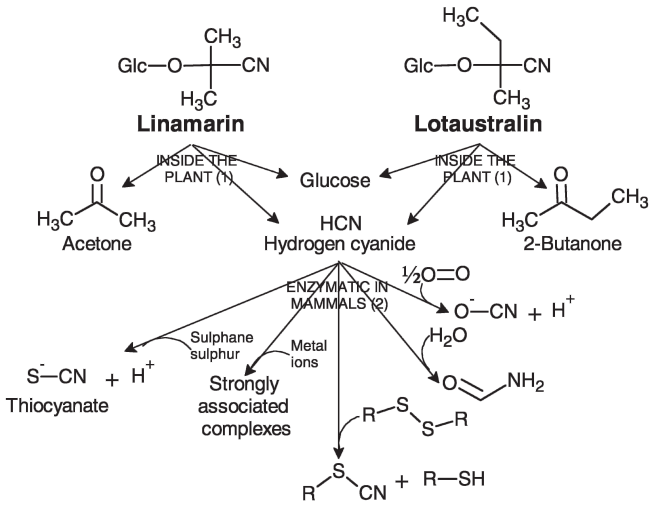


Fig. 5. Degradation of cyanogenic glucosides in plants and mammals. **1:** Enzymatic with linamarase (Maher and Hughes, 1971), is hydrolysed in damaged tissue by linamarase (Hughes and Conn, 1976). **2:** Enzymatic detoxification in mammals (Westley, 1988).

2000). The suppressing effect of sudan grass (*Sorghum vulgare var. sudanense*) on nematode (*Pratylenchus penetrans*) populations has been linked to its content of cyanogenic glucosides (Widmer, 2000; Widmer and Abawi, 1998; 2002) and therefore the supressing effect of white clover on the same nematodes (Abawi and Ludwig, 1995) may also be correlated to its content of cyanogenic glucosides. Some fungi have adapted to host cyanide production; *Stemphylium loti* converts HCN to non-toxic formamide (Ingham, 1973). Ruminants (e.g. sheep and cattle) are more susceptible to HCN poisoning than monogastrics (e.g. rats and mice) (Kingsbury, 1964; Moran, 1954) due to differences in degradation and absorption (Coop and Blakley, 1949; Couch, 1932; Dykstra, 1952; Kingsbury, 1964).

Molluscs, insects and some mammals will eat cyanogenic white clover when given no alternative, but prefer to eat the non-cyanogenic form (Angseesing, 1974; Angseesing and Angseesing, 1973; Ennos, 1981b; Horrill and Richards 1986; Raffaelli and Mordue 1990; Viette et al., 2000).

In Switzerland, white clover varieties that release >370 μg/g of HCN are not recommended (Lehmann et al., 1990), while Coop and Blackley (1950) considered 700 μg/g dry weight of HCN in leaves as a safe limit for new clover strains in general in New Zealand .

Saponins

Saponin in white clover

There is limited information on saponin contents (Table 4) in white clover (Table 3) and it is not possible to draw conclusions about factors influencing plant levels. Saponins have been detected in seeds (<50–3160 μg/g; Oleszek and Stochmal, 2002) of one, and in whole plants (1.5–75 μg/g; Sakamoto et al., 1992) of another cultivar.

Degradation of saponins

Molluscicidal saponins of berries from the Endod plant (*Phytolacca dodecandra* L'Herit) are quickly degraded in aerobic aqueous solutions (50 % in 16 h, 100 % in 10 d), but are stable for a long time when bacterial growth is prevented (Mølgaard et al., 2000). Degradation of white clover saponins has not been investigated.

Biological effects of saponins

Saponins are toxic to fungi (Wolters, 1968), insects (Horber et al., 1974; Adel et al., 2000), molluscs (Agarwal and Rastogi, 1974; Mølgaard et al., 2000), nematodes (Argentieri et al., 2007) and ruminants (Agarwal and Rastogi, 1974), but can have health-promoting activities (Rao and Gurfinkel, 2000). Soyasaponin I is toxic to the plant-parasitic nematode *Xiphinema index* (Argentieri et al., 2007), causes prolongation of the larval and pupal stages, retards growth, increase mortality, and reduce fecundity and fertility of the insect *Spodoptera littoralis* (Adel et al., 2000), but stimulates the growth of wheat (*Triticum aestivum* L.) (Oleszek, 1993). Of 19 saponins and saponin fractions originating from various plant species, all were active in different degrees towards 15 species of fungi, mostly plant pathogens (Wolters, 1968). Acid saponins from alfalfa are strongly toxic to potato leafhopper larvae (*Empoasca fabae* (Harris)) and pea aphids (*Acyrthosiphon pisum* (Harris)) (Horber et al., 1974), and water-extracted saponins from the Endod plant berries are lethal to snails (Mølgaard et al., 2000). Six of the nine described saponins from white clover contains the saponin aglycones soyasapogenol B and soyasapogenol E, both of which are much less active (almost inactive) towards the insect *Spodoptera littoralis* than soyasaponin I (Adel et al., 2000). This is in contrast to the other secondary metabolites described in this review, which are more active in their aglyconic form.

Discussion

To exploit biologically active compounds from white clover for suppressing weeds and soil-borne diseases, either as isolated products (i.e. biopesticides) or through the use of cultivars with enhanced production, their fate in soil must be understood; from release of compounds to soil transformations, that may be influenced by the compounds' sorption properties. To optimize clover production without harming grazing animals, knowledge is needed of secondary metabolite contents in plant parts that depend on genetics or on plant interactions with beneficial/harmful microorganisms or other ecological stresses.

Release of secondary metabolites from white clover

The release of these compounds from white clover into the soil has not been studied in living plants or decaying plant material. Flavonoids are exuded from roots of soybean (*Glycine max* L. Merr.) (Pueppke et al., 1998) and knapweed (Baldwin 2003), and from seeds of alfalfa (*Medicago sativa* L.) (Hartwig and Phillips, 1990). It is therefore likely that white clover secondary metabolites will also be exuded from white clover present in soil after incorporation of plant material or during growth.

Transformation pathways of secondary metabolites from white clover in soil

The transformation pathways summarized in this review occur inside the plant or under influence of bacteria and fungi. Degradation by microorganisms in literature is mainly anaerobic (occurring in the guts of animals and humans), and it is important to investigate aerobic degradation of flavonoids (Pillai and Swarup, 2002), as they are highly reactive under these conditions. The soil environment is mainly aerobic, and the microbial environment is very complex, but anaerobic micro-sites exist in soil, and anaerobic and aerobic pathways can produce the same compounds. There have been degradation studies of these compounds using isolated fungi, but these have proved to be useful for soil degradation studies on other groups of natural defense compounds (Etzerodt et al., 2006; Fomsgaard, 2006; Fomsgaard et al., 2004, 2006; Gents et al., 2005; Understrup et al., 2005). Information about possible degradation products obtained from these studies is a useful basis to understand soil degradation of white clover natural defense compounds.

Sorption properties of secondary metabolites from white clover

The sorption properties of compounds are critical to their fate in soil, if they are sorbed strongly, they may not be available to soil microorganisms. A search in "Web of Science" from 1945 up to now, on sorption properties in soil of the parent agluconic compounds described in the review, returned no records. Incorporation of cyanogenic glucosides from white clover in sandy and loamy soil resulted in leaching of cyanogenic glycosides or toxic cyanide species to a depth of 1 m corresponding to 0.9–3.2 % of the amount applied (Bjarnholt et al., 2008). A large part of this leachate exeeded the EU treshold for drinking water and US treshold for cyanide chronic ecotoxicity in fresh water (30 % and 85 % of leachate, respectively) (Bjarnholt et al., 2008). This result highlights the need for fundamental studies on the transformation of natural defense chemicals of white clover in soil, performed under realistic conditions.

Biological activity of secondary metabolites from white clover versus concentrations in the plant

If the compounds discussed in the present review are released in substantial amounts and not sorbed strongly into the soil, the information in this review can help to address the use of white clover for its anti- pest, plant, fungi, and bacteria capabilities, in place of man-made compounds.

Flavonols can be present in the plants in relatively high concentrations (Table 4) and their toxic effects on insects, fungi, and plants – especially the case of quercetin – suggests a useful biopesticide role. However, the degradation of quercetin is relatively fast and even though some of the degradation products have insecticidal and fungicidal properties, there is little information on the soil degradation pathways and the time that pests should be exposed to the flavonols for biopesticidal effects.

The potential content of flavones in white clover are much lower than the flavonols (Table 4) but the biological effects are the same, and they may contribute to observed effects on insects, fungi or plants – but not as key compounds.

The tannin content of white clover is high compared to other secondary metabolites (Table 4), but the toxicity to microorganisms is correspondingly low (Scalbert, 1991). The minimum inhibitory concentration of tannins is 0.012–1 g/L (≈0.012–1 mg/g) for bacteria, and 0.5–20 g/L (≈0.5–20 mg/g) for fungi (Scalbert, 1991), which suggests that white clover can be toxic to some bacteria and fungi, as tannin content of healthy plants is 0–79 mg/g (Table 4).

Isoflavonoids, many fungitoxic, are formed in infected plant tissue and contents can be high compared to flavones. These compounds may therefore be important as a defense against pathogens.

Not much is known about pterocarpan levels in white clover; medicarpin is fungitoxic, and more importantly so are several of its degradation products. They could be very important in the defense against fungi, as the effect does not necessarily disappear as soon as they are released into soil.

The content of coumestans in white clover is low (<170 μg/g, Table 4) compared to the other groups of compounds, and biological activity is variable and it is unlikely that they are of importance in the defense mechanisms of white clover.

Cyanogenic glucosides are toxic to herbivores and some fungi. Stochmal and Oleszek (1997) suggested that white clover cannot be considered as a low-cyanogenic substitute for red clover. In earlier times white clover had low cyanogenic levels, as all varieties in eastern European countries were bred from local populations, which were low in cyanogenic compounds. Since that time, breeders have been developing better, pest resistant varieties with higher growth rates, and often use breeding lines containing genes responsible for high levels of cyanogens (Ennos 1981a; Stochmal and Oleszek, 1997). As previously mentioned, Swiss varieties that release more than 370 μg/g of HCN are not recommended (Lehmann et al.,

1990), and Coop and Blakley (1950) considered HCN of 700 μg/g dry weight as a safe limit for new clover strains. These levels are well exceeded in some white clover varieties under some environmental conditions (see Table 4).

Knowledge of saponin contents in white clover is limited, but some are present in comparable amounts with the other groups of compounds in the review. Their wide effects on fungi, insects, molluscs and ruminants, suggest that these compounds also could be important in plant defense against pathogens.

External factors affecting the contents of secondary metabolites in white clover plants

The synthesis of many secondary metabolites in white clover plants is highly dependent on external factors, including weather (sunlight and temperature), infection with pathogenic fungi, symbiosis with rhizobium or AM fungi, and P and water content of the soil (Table 4).

The synthesis of the pterocarpan medicarpin (Cruickshank et al., 1974) increases with day length, and flavonol contents increase when plants are exposed to UV-B light (Hofmann et al., 2000, 2003). By contrast, synthesis of cyanogenic glucosides (Vickery et al., 1987) decreases at higher light intensity. Higher temperatures lead to greater production of condensed tannins (Burggraaf et al., 2003; Stockdale and Dellow, 1995) and medicarpin (Cruickshank et al., 1974), but lower production of cyanogenic glucosides (Stochmal and Oleszek, 1997; Vickery et al., 1987). This information is difficult to use in practice, as weather cannot be controlled. However, it highlights the importance of investigations on seasonal variations in contents of secondary metabolites in white clover, as were done with the cyanogenic glucosides (Stochmal and Oleszek, 1997), and that comparing contents over different experiments/environmental conditions should be done with care.

Infection with pathogenic fungi often increases synthesis of secondary metabolites, including flavones (Wong and Latch, 1971b), the isoflavone formononetin (Carlsen et al., 2008; Cook et al., 1995; Wong and Latch, 1971b), the pterocarpan medicarpin (Cook et al., 1995), and coumestans (Saloniemi et al., 1995; Wong and Latch, 1971a, 1971b), the coumestrol content even increasing with the severity of disease (Saba et al., 1974). This increased production could, considering the antifungal properties of many of the secondary metabolites, be explained by induction of a plant defense system, including production of defense compounds.

While some studies were performed under biological stress – with diseased plants, investigations into the correlation between growth of beneficial fungi on white clover roots and the concentrations of biologically active secondary metabolites are almost absent from literature. If fungal diseases affect concentrations, beneficial fungi could have similar – and important – effects. Ponce et al. (2004) found that different flavonoids accumulated in roots of white clover inoculated with an AM fungus (*Glomus intraradices*), compared with roots of non-inoculated plants, and Johnson et al. (2005) found that different flavonoids increased in concentration in plants without rhizobial nodules than in plants with active or inactive nodules. This change in the composition of the phytoalexins in the host could be very important in the interaction between beneficial fungi and fungal diseases. Wyss et al. (1991) suggested that increased resistance of soybean (*Glycine max*) to a soil-borne pathogen (*Rhizoctonia solani*) was due to an increased production of phytoalexins by the host in response to colonization by an AM fungus (*Glomus mosseae*). Sundaresan et al. (1993) found a three times higher concentration of daidzein in AM (*Glomus fasciculatum*) cowpea (*Vigna unguiculata*) compared with non-mycorrhizal plants; they suggested that the reduced colonization and disease severity by a soil-borne pathogen (*Fusarium oxysporum*) in plants with a pre-established mycorrhizal association could be a result of production of phytoalexins. Carlsen et al. (2008) studied the effects of two AM fungi (*Glomus mosseae and G. claroideum*) and a pathogenic fungus (*Pythium ultimum*) on the production of eight flavonoids in roots of two white clover cultivars, and found that the flavonoid production varied, not only due to presence of AM fungi and *P. ultimum*, but also depending on fungal isolate and plant cultivar. Combined studies in which the growth of both beneficial fungi and pathogens are followed and the concentration levels of secondary metabolites are measured in white clover are necessary to understand these interactions.

Factors that farmers can more easily influence are the P status of the soil, with application decreasing contents of cyanogenic glucosides (Vickery et al., 1987; Wheeler and Vickery, 1989) and the water status of the soil, with drought increasing contents of flavonols (Hofmann et al., 2003) and cyanogenic glucosides (Vickery et al., 1987). The production of flavones and coumestans increased when white clover was grown in fields instead of glasshouses (Wong and Latch, 1971b), possibly due to higher levels of UV radiation, lower temperatures and different moisture conditions.

Contents of secondary metabolites in different cultivars of white clover

Concentration levels of some of the secondary metabolites vary substantially among white clover cultivars. This has been reported for flavonols (Carlsen et al., 2008; Hofmann et al., 2000), isoflavones (Bennet et al., 1967; Carlsen et al., 2008; Sachse, 1974), medicarpin (Carlsen et al., 2008; Ingham, 1978), coumestrol (Bennet et al., 1967; Carlsen et al., 2008; Saloniemi et al., 1993) and cyanogenic glucosides (Butler, 1965; Lehmann et al., 1990; Stochmal and Oleszek, 1995, 1997; Tava and Annicchiarico, 2000; Viette et al., 2000; Wheeler and Vickery, 1989).

In spite of large variations, the choice of white clover variety is almost never made for production of biologically active secondary metabolites – allelochemicals – even though this could be essential for the interaction with the soil environment. It is important to know the concentrations of a range of secondary metabolites, as a significant effect of these compounds is more likely when they work in combination with each other, and not as a single defense mechanism. Some secondary metabolites would be desirable to have in high concentrations (e.g. flavonoids), and others at low concentrations (e.g. cyanogenic glucosides). However, research has been almost exclusively limited to the difference in cyanogenic glucoside content between cultivars and there has been little research on the other compounds.

The research of Bennet et al. (1967) indicated that content of isoflavones and the coumestan coumestrol followed the same trend in three different cultivars, but this possible relationship needs to be confirmed and the relationship between the contents of other groups of secondary metabolites remains to be investigated.

Contents of secondary metabolites in different parts of white clover plants

The majority of quantifications of the compounds have been in whole plants or single plant parts. Cultivars and environmental conditions varied among the experiments, and both are factors expected to have large influences on contents in the plant. Thus contents in different plant parts determined in different experiments cannot be directly compared. The location in the plant of most of the compounds is not known. Contents determined from older studies of secondary metabolites in white clover plants can be used for approximate levels, but should not be used as exact values.

Clover soil fatigue

As regards clover soil fatigue, the autotoxicity of white clover (probably caused by secondary metabolites) is an obvious hypothesis, taking earlier research into account (Table 1). However, Søegaard et al. (2004) concluded that the clover cyst nematode was probably the primary reason for clover soil fatigue, that pathogenic fungi could play a role, and that chemical compounds could not have a direct effect in the clover-fatigued soil. An indirect effect of allelochemicals was suggested as a trigger for the early and synchronous hatching of clover cyst nematodes in the clover-fatigued soil. This trigger was proposed to be degradation products from newly ploughed clover. However, they only considered water extractable chemicals, and less extractable compounds may have a more direct role in clover soil fatigue.

Allelopathic compounds from other plants in the clover–grass mixture could also affect white clover or the clover cyst nematode. The leaf extracts of tall fescue (*Festuca arundinacea* Schreb.), commonly grown with white clover in pastures of southeastern USA, had a toxic effect on germination and/or root growth of many of the 40 white clover genotypes tested (Pederson, 1985). However, Hoveland (1964) did not find reductions in germination or radicle length of white clover tested by tall fescue extracts, but did find a reduction with extracts from four other grasses, and this was suggested as a factor in frequently poor clover stands. Root competition between perennial ryegrass (*Lolium perenne* L.) and white clover has been investigated, and proved to be disadvantageous for the clover (Kooistra, 1964), suggesting that the root competition could be one of the causes of clover soil fatigue.

The possible effects on the clover fatigue phenomenon of biologically active secondary metabolites or their soil transformation products should be a focus of future research.

Conclusion

The studies which were included in this review provide essential information on the structure and concentration of a variety of secondary metabolites of white clover. Important information on the biological effects of a number of these compounds and on the degradation/transformation of these compounds inside the plant, by fungi or anaerobic bacteria, was available. However, it is clear from the results of this review that substantial information is missing if the potential of white clover for suppressing weeds and diseases is to be better exploited, without causing negative effects in the soil environment. If the increasingly important clover fatigue problems (Søegaard et al., 2004) are to be solved, additional research is necessary.

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