Australian Government
Department of Health
Office of the Gene Technology Regulator

The Biology of *Trifolium repens* L. (White Clover)

Photo: Mary-Anne Lattimore, NSW Agriculture, Yanco

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This document provides an overview of baseline biological information relevant to risk assessment of genetically modified forms of the species that may be released into the Australian environment.

This document is an update of Version 3 (November 2020).

For information on the Australian Government Office of the Gene Technology Regulator visit the OGTR Web page.

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ABBREVIATIONS USED IN THIS DOCUMENT

ABARES	Australian Bureau of Agriculture and Resource Economics and Sciences
ABS	Australian Bureau of Statistics
ACIAR	Australian Centre for International Agricultural Research
APVMA	Australian Pesticides and Veterinary Medicines Authority
ASA	Australian Seeds Authority
ASF	Australian Seed Federation
DM	Dry matter
DNA	Deoxyribonucleic acid
DAFWA	Department of Agriculture and Fisheries Western Australia
GM	Genetically modified
GMO	Genetically modified organism
GRDC	Grains Research & Development Corporation
ha	Hectare
HCN	Hydrogen cyanide
m	Metres
n	Haploid number of chromosomes
NSW	New South Wales
NSW DPI	New South Wales Department of Primary Industries
NVT	National Variety Trials
OECD	Organisation for Economic Co-operation and Development
OGTR	Office of the Gene Technology Regulator
QDAF	Queensland Department of Agriculture and Fisheries
Qld	Queensland
SA	South Australia
spp.	Species
Tas.	Tasmania
USA	United States of America
USDA	United States Department of Agriculture
USDA-GRIN	USDA Germplasm Resources Information Network
Vic.	Victoria
WA	Western Australia

GLOSSARY

Polyploid	Cells or organisms containing more than two paired (homologous) sets of chromosomes. Polyploids (see below) are labelled according to the number of chromosome sets in the nucleus, with the letter x used to represent the number of chromosomes in a single set. Thus a diploid would have 2x chromosomes, a tetraploid 4x and so on.
Diploid	An organism made up of cells containing two sets of chromosomes (2x). Most species whose cells have nuclei (eukaryotes) are diploid, meaning they have two sets of chromosomes - one set inherited from each parent.
Tetraploid	An organism made up of cells containing four sets of chromosomes (4x).
Allotetraploid	A hybrid that has a chromosome set 4 times that of a haploid organism (one with a single set of chromosomes). Both chromosome sets of each parents are present in gametes. (Source: https://www.biologyonline.com/dictionary/allotetraploid)
Homologous	Having the same structure, relation, or relative position, or evolution (homo - the same). May have a similar, but not the same function.
Homologous chromosomes	Chromosomes with the same or allelic genes with genetic loci usually arranged in the same order (Source: https://www.merriam-webster.com/dictionary/homologous).
Phylogenetics	The study of the evolutionary history and relationships among individuals or groups of organisms.
Progenitor	An ancestor or parent of an organism.
Interspecific	Existing, arising or occurring between species.
Primordia (plural)	Organs or tissues in their earliest recognizable stage of development. Root primordia are those from which roots develop.
F_1, F_2…	F_1 are the (hybrid) offspring (generation) resulting from a cross between two parent individuals. If F_1 hybrids are crossed, the resulting offspring are F_2 generation.

PREAMBLE

This document addresses the biology of *Trifolium repens* L. (referred to as white clover) with particular reference to the Australian environment, cultivation and use. Information included relates to the taxonomy and origin of white clover, general descriptions of its morphology, reproductive biology, biochemistry, and biotic and abiotic interactions. This document also addresses the potential for white clover to transfer genes via pollen and seed movement and for weediness. It should be noted that due to the large number of white clover cultivars as well as the highly heterozygous nature of white clover populations, which results in many genotypes, it has been necessary to generalise much of the information provided in this document and exceptions may be common. The purpose of this document is to provide baseline information about the parent organism for use in risk assessments of genetically modified (GM) *Trifolium repens* L. that may be released into the Australian environment.

White clover is the most important pasture legume in many temperate parts of the world and in Australia, it is the main legume grown in perennial pastures in cool temperate, summer rainfall zones (Archer and Robinson, 1989). White clover is mostly grown in conjunction with grasses, and is used for grazing, pasture hay and ground cover in horticultural situations, where it supplies a rich source of proteins and minerals to grazing livestock, and fixes large amounts of nitrogen in pastures, thereby improving soil fertility and reducing the need for fertilisers. While white clover is regarded as a perennial plant, it is able to behave as an annual in warm climates or under moisture-stressed conditions. The plant is capable of both asexual (or vegetative) reproduction through the generation of stolons, and sexual reproduction through seed production and dispersal.

SECTION 1 TAXONOMY

White clover is also commonly known as Dutch clover, white trefoil, creeping trifolium, ladino clover or honeysuckle clover (Shaw, 1906). The genus name for clover derives from the Latin tres, "three", and folium, "leaf", so called from the characteristic form of the leaf, which has three leaflets (trifoliate); hence the popular name trefoil. The specific name repens refers to the creeping and rooting stems.

White clover, *Trifolium repens* L., is one of the most agronomically important species in the genus *Trifolium*. The United States Department of Agriculture, Agricultural Research Service, National Plant Germplasm System (USDA GRIN) lists over 350 accepted members of the genus *Trifolium* including approximately 250 species and a further 100 subspecies, varieties and forms (UDSA GRIN Taxonomy database; accessed July 2020). *Trifolium* belongs to the tribe Trifolieae of the subfamily Papilionoideae (also known as Faboideae) of the family Fabaceae (Australian Plant Name Index), although older taxonomic classifications placed the *Trifolium* genus in the family Leguminosae (Williams, 1987b).

Molecular phylogeny analysis of the *Trifolium* genus confirmed the monophyly of *Trifolium* but at the same time proposed a new infrageneric classification of the genus (Ellison et al., 2006). This study indicated that only 22 of the *Trifolium* species are polyploid and that five or six are of putative hybrid origin. They confirmed the ancestral chromosome number as 2n=16. *T. occidentale* and *T. pallescens* were identified as the likely progenitors of *T. repens* within a new section *Trifoliastrum*. More recent work following on from the work of Ellison and colleagues (2006) also strongly suggests that these species are the most likely progenitors (Williams et al., 2012), although there is some inconsistency in the evidence available and it is unlikely that *T. repens* is a result of a simple hybrid between two ancestors (Williams, 2014).

White clover is a natural tetraploid with a chromosome number of 2n=4x=32 (Voisey et al., 1994; Abberton, 2007). Due to the addition of divergent genomes, inheritance in allopolyploids (which includes white clover) is disomic; i.e. pairing behaviour during meiosis is similar to that of nonhomologous pairs of chromosomes in diploids (Voisey et al., 1994). Within the *Trifolium* genus there is variation in the chromosome number and ploidy (see Table 1 for some examples).

Table 1: A selection of *Trifolium* species with their chromosome number, ploidy, compatibility and perenniality[1]

Species	Chromosome number	Ploidy	Compatibility	Annual/Perennial
T. repens	32	4x	Self-incompatible	Perennial
T. pratense	14	2x	Self-incompatible	Biannual/perennial
T. nigrescens	16	2x	Self-compatible	Annual
T. ambiguum	16	2x, 4x, 6x	Self-incompatible	Perennial
T. occidentale	16	2x	Self-compatible	Perennial
T. hybridum	16	2x, 4x	Self-incompatible	Annual
T. pallidum	16	2x	Self-incompatible	Annual
T. pallescens	16	2x	Self-incompatible	Perennial
T. alpestre	16	2x	Self-incompatible	Perennial
T. alexandrinum	16	2x	Self-incompatible	Perennial

[1] Adapted from Abberton (2007).

Additionally, white clover has a well-developed genetic gametophytic self-incompatibility mechanism with only a small proportion of plants in a population being quite strongly self-compatible (Thomas, 1987a). In essence, this means that autogamous self-pollination is infrequent in white clover. The self-incompatibility system is based on the multiple oppositional alleles of the S locus, which means that incompatibility occurs when the S allele in the pollen is the same type as that present in the style (Williams, 1987a). However, white clover also shows frequent pseudo-self-incompatibility (the breakdown of the S allele system). Therefore, self-incompatibility can be somewhat dependent on genotype and environmental conditions such as temperature, resulting in a limited degree of self-pollination (Thomas, 1987a).

White clover cultivars are classified arbitrarily by the size of the leaves, as small, intermediate (medium) and large.

Predominant out-breeding and disomic inheritance means that white clover populations are composed of a heterogeneous mixture of highly heterozygous individuals. This results in high levels of genetic variation both within and between populations (Voisey et al., 1994). By having high variability, white clover is more adaptable to competitive microenvironments. This is an important attribute as white clover does not naturally cross with other *Trifolium* species (See Section 9.2) and therefore cannot gain genetic variation by forming hybrids.

SECTION 2 ORIGIN AND CULTIVATION

2.1 Centre of diversity and domestication

The genus *Trifolium* is thought to have originated in the Mediterranean in the early Miocene period, 16-23 million years ago (Ellison et al., 2006). Members of the genus are native to most temperate and subtropical regions of the world, with the exception of south-east Asia and Australasia (Zohary and Heller, 1984, as cited by Ellison et al., 2006). White clover originated 15-28 thousand years ago (Griffiths et al., 2019) from the Mediterranean region of Europe and was spread through Europe and western Asia with migrating animals before recorded history. Domestication of white clover occurred 400 years ago in the Netherlands and it migrated along with European settlers to various continents on which it is now considered naturalised (Zeven, 1991; Lane et al., 1997). The main white clover growing areas in the world are in the temperate regions of western Europe, the United States, New Zealand and South America (Mather et al., 1996).

It is generally accepted that European settlers accidentally introduced white clover into Australia in the late 18th century and deliberate sowing of Dutch and English strains of white clover occurred soon after with the establishment of pastures (Lane et al., 1997). It is now considered a naturalised pasture legume in the coastal regions and tablelands of eastern Australia and was present in 7.8 million hectares of pasture in Australia in the 1990s (Hill and Donald, 1998). More information is given in section 2.3.

Many other *Trifolium* species are also widely distributed throughout temperate and subtropical parts of Australia (Atlas of Living Australia). Approximately 38 *Trifolium* species are naturalised in Australia (Flora of Victoria) and about 17 *Trifolium* species are sown in pastures in Australia (Nichols et al., 2012). Subterranean clover (*T. subterraneum*) is the most common clover species in Australian pastures and is present on more than 10 million hectares (Drew et al., 2014).

2.2 Commercial uses

White clover is typically grown in a mixed sward with grasses. It is used for grazing, pasture hay and ground cover in horticultural situations (NSW DPI). It is valuable in the dairy, meat and wool industries, as it significantly improves the productivity of pastures (Ayres et al., 2000). For example, in Victoria, the average stocking rate on white clover/perennial ryegrass pastures is 18 dry sheep equivalents per hectare (DSE/ha), while the stocking rate on subterranean clover/perennial ryegrass pastures is 12 DSE/ha and the stocking rate on unimproved native pastures is 3 DSE/ha (Donald, 2012). A density of greater than 25% ground cover is commonly sought by pastoralists (Garrett and Chu, 1997).

In 2015, it was estimated that New Zealand white clover seed production, which supplied approximately 50% of the world market, was worth $NZ 20 million (MPI, 2015).

There are many advantages to using white clover in pastures. It has a high nutritive value because it supplies a rich source of proteins and minerals, and has high voluntary intake by grazing animals (Heuzé et al., 2019). Furthermore, it is adaptable to a wide range of soil and environmental conditions and combines well with many perennial grasses. White clover is valuable in providing high quality feed in mixed pastures, especially in spring, although it may not perform as well during summer as its shallow roots leave it vulnerable to moisture stress (Moore et al., 2006; Dairy Australia, 2012; NSW DPI, 2020c). However, it has dual regenerative capabilities via stolons and seed bank (discussed in Section 4.1) that enable it to recover from die-back during dry summers (Moore et al., 2006).

A highly desirable attribute of white clover in pasture systems is its ability to fix atmospheric nitrogen. This attribute improves the nitrogen content of soil, reducing the need for fertilisers. Nitrogen fixation arises through a symbiotic relationship between white clover and the bacterium *Rhizobium leguminosarum* biovar *trifolii*, resulting in the formation of nodules, which allow the rhizobia to fix atmospheric nitrogen. Environmental factors such as soil pH, temperature, soil nutrient status and water stress can influence rhizobia infection, nodulation, and nitrogen fixation processes (Drew et al., 2014). Presence of compatible rhizobia is essential for white clover establishment on nitrogen-deficient soils (Lowther and Kerr, 2011). Rhizobia compatible with clover are common in Australian pasture soils, and most perennial clover seed sold in Australia is also pre-inoculated with rhizobia (Drew et al., 2014).

White clover has been investigated for its potential in phytomanagement of metal-polluted soils. Metals such as cadmium, zinc and lead were shown to be taken up and subsequently accumulated in the roots of the plant. White clover has higher tolerance to metal-induced oxidative stresses than perennial ryegrass grown in the same soils (Bidar et al., 2007).

White clover is a valuable plant for honey production in a number of regions worldwide, including Australia, New Zealand, Britain, northern parts of the United States, Canada, and parts of Europe (Howes, 2007). For example, it has been stated that a greater quantity of honey is obtained from white clover than from any other individual plant (Howes, 2007). In south-east Australia, white clover flowers are attractive to honey bees and an important source of nectar and pollen for commercial honey production (Somerville, 2001; Birtchnell and Gibson, 2008). In years with good white clover growth, Australian beekeepers situate their hives in white clover growing areas in spring to breed bees and collect the high quality clover honey (Stace, 1996). The pollen of white clover is a good source of nutrition for bees, having a crude protein content of 22.5% to 25.9% (Stace, 1996; Somerville, 2001).

There are also some disadvantages to growing white clover. For example, white clover has poor drought and heat tolerance, and therefore is reliant on good summer rainfall or irrigation. Additionally, soils with medium to high fertility containing phosphorus and sulphur are needed for good yields and careful grazing management is required (Moore et al., 2006; NSW DPI, 2020c). Although white clover improves the nitrogen content of soil, the nitrogen fixation rates of Australian pastures are generally low due to low pasture clover content, and if supplementary nitrogen fertiliser is applied this further depresses nitrogen fixation (Unkovich, 2012). Furthermore, white clover is adversely affected by several pests and diseases (See Section 7.2) and contains some toxic and anti-nutritional compounds (See Section 5.2).

2.3 Cultivation in Australia

White clover is grown in regions with rainfall greater than 750 mm, or under irrigation (NSW DPI, 2020c). The first cultivar - Grasslands Huia - was registered in Australia in 1935 (Register of Australian herbage plant cultivars, accessed 15 July 2020). A detailed summary of the history and cultivation of white clover in Australia is available in Nichols et al. (2012).

As mentioned above, white clover is predominantly grown in conjunction with grasses in pastures. The types of companion grasses commonly used include perennial ryegrass (*Lolium perenne*), phalaris (*Phalaris aquatica*), cocksfoot (*Dactylis glomerata*), fescue (*Festuca arundinacea* Schreb.), paspalum (*Paspalum dilatatum*), Italian ryegrass (*Lolium multiflorum*) and kikuyu (*Pennisetum clandestinum*), although exact combinations are dependent on the area in Australia in which the pasture is grown (Betts and Ayres, 2004; Moore et al., 2006; NSW DPI, 2020c). In a survey of grazing land across Australia in 2011, the main white clover pasture mix reported containing white clover was with perennial ryegrass, which accounted for 0.3 % of the total grazing area for NSW, 8.7% for Vic. and 17.9% for Tasmania. In Tasmania, cocksfoot/white clover mixed pasture accounted for 4% of the total grazing area (Donald, 2012).

In its database, the Australian Seed Federation lists 34 varieties of white clover, of which 17 are registered for production under the OECD seed scheme (Australian Seed Federation, 2020). Different cultivars are recommended for different areas and conditions, with NSW DPI listing over 20 varieties, which are categorised as large, medium/large, medium or small leaf varieties (NSW DPI). Pasture mixtures containing white clover will also vary significantly across different regions (NSW DPI, 2008).

2.3.1 Commercial propagation

White clover seed is predominantly produced in South Australia and Victoria. The Australian Seeds Authority recorded production of 3946 tonnes of white clover seed in 2005-2006 under all certification schemes, approximately 65% of which was of the cultivar Haifa (ASA, 2007). Approximately 82% of this seed was produced under the OECD seed certification scheme, which is used to comply with export requirements.

Two classes of seed are certified under the OECD scheme - basic and certified (OECD, 2008). Basic seed is used for production of certified seed, and has the most stringent production requirements to maintain varietal purity. Certified seed is used for production of further certified seed or for pasture planting. A number of requirements are stipulated for the production of such seed and are administered in each country by the National Designated Authority, which for Australia is the Australian Seeds Authority (ASA, 2011). Other seed certification schemes exist in Australia. For example, the South Australian Seed Certification Scheme has requirements that mirror OECD standards for white clover production (Smith and Baxter, 2002). Requirements (ASA, 2011; Seed Services Australia, 2013; ASA, 2018) for *T. repens* are as follows:

Field and crop requirements:

- Seed crops can only be grown in fields that have not produced any other crop of the same species within three years.
- Presence of off-types and contaminating species are limited to one plant per 30 m^2 for basic seed and one plant per 10 m^2 for certified seed.

- Isolation distances for fields of up to 2 ha are 200 m for basic seed and 100 m for certified seed; for fields of greater than 2 ha, the isolation distances are 100 m for basic seed and 50 m for certified seed.
- Basic seed production may occur in a field for up to two years, which certified seed production may occur for up to four years.

Seed standards:

- Seed purity (by mass) 99.0% for basic, 97.0% for certified seed.
- Minimum germination 60.0% (by count) for basic and certified seed.
- Maximum other seeds (by mass) 0.2% for basic and 2.0% for certified seed (For all *Trifolium* spp. the maximum number of seeds of other *Trifolium* spp. is 200/kg).

Globally, approximately 220 varieties of white clover are included on the OECD seed scheme list of varieties eligible for seed certification (OECD, 2020).

Weed infestation in commercial white clover seed production areas can cause a significant drop in profit. The lack of options for weed control remains one of the main limiting factors for achieving high seed yield and cuts into profits as the costs of weed control can be up to 25% of the annual production costs (Riffkin et al., 2005). Control of weeds is discussed further in Section 7.1.

2.3.2 Scale of cultivation in Australia

White clover is part of the legume-based pastures that are important in Australia to support the grazing industry due to its contribution to feed quality. It may also help conserve water resources, sustain soil fertility and provide crop protection in farming systems when it is used in crop rotations (Ayres and Lane, 2007).

While white clover is grown in large areas of pasture in Australia - estimates vary between over five million hectares (Drew et al., 2014) and 7.8 million hectares (Hill and Donald, 1998) – it is rarely grown as a monoculture pasture. Agronomists recommend a mixture of 30% white clover with 70% grass in pastures (NSW DPI, 2020c), however the actual white clover content in dairy pastures in Australia is usually less than 20% (Unkovich, 2012). In 2011, the area of pasture covered by white clover was calculated as approximately 600,000 ha across NSW, Vic., Tas. and WA (Donald, 2012).

The white clover zone extends from south-eastern Queensland along the coast and adjacent tablelands of New South Wales and Victoria to Mt Gambier in South Australia as well as irrigated regions of south-west Western Australia and high rainfall areas of the Australian Alps and most of Tasmania (Jahufer et al., 2001; Nichols et al., 2012). The maps presented in Appendix A show the distribution of white clover in Australia during the late 1990s (Figure A1) and the areas of Australia that white clover could potentially inhabit (Figure A2) (Hill and Donald, 1998). Figure A3 shows the proportion of white clover pastures in agricultural zones across NSW, Vic. and Tas. in 2011 (Donald, 2012).

White clover seed production is based predominantly in western Victoria and south-eastern South Australia. These regions produce about 90% of the white clover seed in Australia as a result of the availability of ground water for irrigation and land that has previously been free of white clover seed.

Production of certified white clover seed in Australia varies substantially across production years. In the five years to 30 September 2019, total certified white clover seed production was 4444 t[1], with yearly production figures ranging from 452 t to 1134 t. On average, white clover accounted for 29% (range 14 % to 45%) of total certified seed production of *Trifolium* species. By comparison, total production of certified *T. subterraneum* (subterranean clover) seed was 5510 t (35%, also variable from year to year), and total certified *T. resupinatum* (Persian clover) seed production was 2771 t (18%) (ASA, 2020).

[1] Sum of production figures from individual reporting years from 2014/15 to 2018/19.

2.3.3 Cultivation practices

White clover requires particular soil conditions to ensure good seedling establishment and to maintain high production. White clover performs well on soils with medium to high fertility containing good levels of phosphorus, sulphur, potassium and molybdenum. It prefers soils that have a pH above 4.5 and have good water-holding capacity but are well draining (Frame, 2003; Betts and Ayres, 2004). White clover pastures often require fertilisers to maintain productivity. The type of white clover cultivars planted depends on the final use (e.g. pasture or seed production), and environmental conditions such as pest and disease pressures, soil type and temperature.

White clover can be established a number of ways as a pasture component. White clover-grass mixtures can be seeded onto freshly prepared soil. Alternatively, white clover can be seeded into existing grass pastures by either surface sowing, direct drilling (sod seeding) or partial cultivation (Frame, 2003). Such over-sowing into grass swards is important if the white clover population has declined due to drought or overgrazing. The highest yields of white clover are in spring and summer, with mixed swards producing peak yields later in the season compared to clover monocultures (Frame, 2003).

Various white clover sowing rates are reported, depending upon variety and the type of planting: 2-5 kg/ha for production of a dense sward; 0.5-3 kg/ha in pasture mixtures; 3-5 kg/ha for seed production (Betts and Ayres, 2004; Seed Technology and Marketing Pty Ltd, 2007a, b, c). White clover is sown in a fine firm seed bed to optimise seed-soil contact and covered with up to 15 mm of firm soil. Seed is often pre-treated, for example with systemic pesticides, trace elements and rhizobia as a cost effective way to optimise establishment of the emerging seedlings (PGG Seeds, 2008).

During establishment in a pasture, white clover performs best under rotational grazing cycles of 1-2 days of grazing every 20-30 days. Established clover can be grazed continuously during the winter, spring and early summer when it is actively growing. White clover should not be grazed until the plant is 15-20 cm tall and not grazed below 5 cm tall. Grass is best kept at below 12 cm so as not to compete with white clover for light (Seed Technology and Marketing Pty Ltd, 2007b; PGG Seeds, 2008).

White clover is grown in horticultural and viticultural situations as a cover crop, often in conjunction with ryegrass. It provides breeding areas for beneficial insect species, improves soil structure, improves soil fertility through the supply of nitrogen and suppresses summer weeds (Pocock and Panagiotopoulos, 2003; Madge, 2007).

White clover has also been investigated for use in intercropping in organic or low input farming systems, where a groundcover crop, often a legume, is used in conjunction with a grain crop. The legume is thought to provide nitrogen to the cereal crop, reduce nutrient leaching by providing permanent soil cover and to reduce pests and diseases (Thorsted et al., 2002). Increased crop yields with white clover have been seen in oats (Thorsted et al., 2002) and rice (Cho et al., 2003), whereas more variable results have been seen in wheat (White and Scott, 1991; Thorsted et al., 2002; Thorsted et al., 2006; Hiltbrunner et al., 2007) and rye (White and Scott, 1991).

2.4 Crop Improvement

Breeding of new cultivars in Australia has concentrated on reducing sensitivity to summer moisture stress and has led to the release of cultivars to address this issue (see, for example, Ayres and Lane, 2007). White clover breeding programmes in the temperate regions of Australasia are focusing on improving root systems for phosphate uptake, pest tolerance and accessing genetic variation from white clover-related species (Jahufer et al., 2012). Recent varieties show improvement in cool season activity and dry matter production through winter and early spring, increased stolon density, increased persistence and improvement in year round performance (PGG Wrightson Seeds)(Ford et al., 2015)[2].

[2] A list of commercially-available cultivars is available in Table 4.

2.4.1 Breeding

A number of varieties are being promoted for their use in different farming regions. Furthermore, the creation of interspecific hybrids opens up the opportunity to generate clover varieties with advantageous traits.

Williams (2014) grouped closely-related species, into primary, secondary, tertiary, quaternary and marginal gene pools for white clover, based on their ability or potential to cross with *T. repens* and produce fertile hybrids. There are no other species in the primary gene pool, so although *T. repens* is an almost obligate outcrosser, natural outcrossing occurs with other individuals and other cultivars of the same species. Species in the secondary and tertiary gene pools are those that have been crossed with *T. repens*, while the quaternary and marginal pools include species which have not been crossed but may be used to introduce genetic material into the *T. repens* gene pool.

The secondary gene pool includes species which produce partly fertile hybrids from crosses with *T. repens*, without embryo recue. The tertiary gene pool contains species which have been crossed with *T. repens* to provide partly fertile hybrids, with the aid of embryo rescue. The quaternary gene pool includes species that have not yet been crossed with *T. repens*, but can be crossed with other species in the white clover complex and as such could potentially contribute to the white clover genepool via a 'bridge' species. Species in the marginal pool have either formed infertile hybrids or shown some signs of forming hybrid embryos with *T. repens*. Table 2 shows the species included in each gene pool.

Table 2: Grouping of species in gene pools for *T. repens*[1]

Primary	Secondary	Tertiary	Quaternary	Marginal
T. repens	2x *T. occidentale* 4x *T. occidentale*	*T. uniflorum*	2x *T. ambiguum*	*T. montanum*
	T. nigrescens ssp. *nigrescens*	*T. isthmocarpum*	6x *T. ambiguum*	*T. hybridum*
	T. nigrescens ssp. *petrisavii*	4x *T. ambiguum*	*T. thalii*	*T. argutum*
	T. nigrescens ssp. *meneghinianum*		*T. pallescens*	*T. semipilosum*

[1] Source: Williams (2014)

Table 3 outlines some of the desirable traits of related clover species that could potentially be used to create new *T. repens* hybrids.

Table 3: Advantageous traits in related species[1]

Desirable trait absent in *T. repens*	Putative hybrid parent species containing desirable trait
Deeper roots	*T. ambiguum*, *T. uniflorum*
Presence of rhizomes	*T. ambiguum*
Faster seedling development	*T. thalli*
Profuse flowering	*T. nigrescens*
Drought tolerance	*T. ambiguum*, *T. uniflorum, T. occidentale*
Salt and drought tolerance	*T. occidentale*
Heat tolerance	*T. nigrescens*
Nematode resistance	*T. nigrescens*, *T. ambiguum*
Virus resistance	*T. ambiguum*

[1] Sources: Adapted from Williams et al. (2007) and Abberton (2007).

2.4.2 Genetic modification

White clover has been genetically modified for several traits including *White clover mosaic virus* resistance (Voisey et al., 1994), *Alfalfa mosaic virus* (AMV) resistance (Emmerling et al., 2004; Panter et al., 2012), insect resistance (Dudas et al., 1998), altered nutritional quality (Christiansen et al., 2000), delayed leaf senescence (Lewis et al., 2013) and nutrient efficiency (Woodfield et al., 2019). However, there are currently no commercial approvals of GM white clover globally.

SECTION 3 MORPHOLOGY

3.1 Plant morphology

White clover is a prostrate legume and tends to be a short-lived perennial but can behave as an annual under moisture-stressed conditions (Hutchinson et al., 1995).

The basic structural component of a mature white clover plant is the stolon (an above ground creeping stem with roots at the nodes). The stolon consists of a series of internodes separated by nodes. Each node bears a trifoliolate leaf, two root primordia and, during vegetative growth, an axillary bud which is capable of growing into a lateral stolon (Figure 1). If the node comes into contact with moist soil, adventitious roots may form from the root primordia closest to the ground (Thomas, 1987b). The primary root is a shallow tap root, with most roots (> 80%) present in the top 10-20 cm, however, white clover roots may also be found at depths of 1-1.5 m (Caradus, 1990; Smoliak et al., 2008).

A 10-week-old seedling consists of a central primary axis of stem and root from which several secondary stolons can grow forming a branched network radiating from the initial seedling. When the primary root dies off, the lateral stolons are nutritionally supported by adventitious roots and become independent plants. The development and establishment of new stolons from the tips of the plant is continuous and older tissue gradually dies off (Thomas, 1987b).

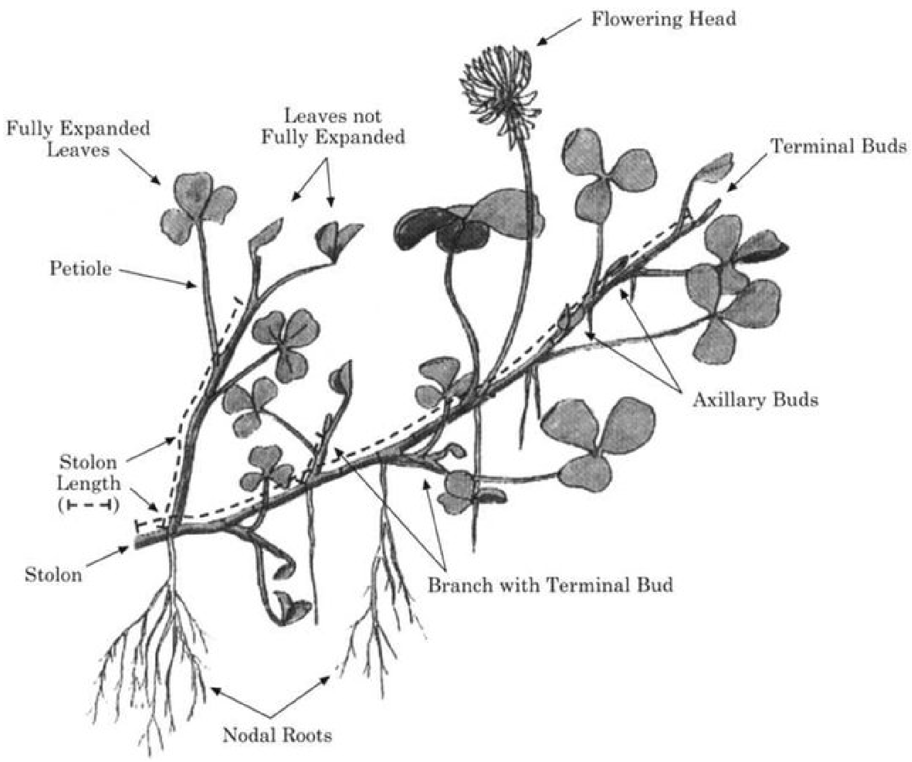

Figure 1. White clover showing the plant organs measured during winter. Image as presented in Wachendorf et al. (2001) and as modified from Thomas (1987b).

Leaf size is highly dependent on type and cultivar of white clover as well as environment, ranging from very small (leaflets less than 1 cm long) in prostrate short petioled types to large (leaflets more than 2 cm) in the more erect, longer petioled types. Table 4 lists the cultivars available in Australia based on their leaf size. The trifoliate leaves on stolons are arranged alternately and tend to be attached to the side of the stem relative to the surface of the ground. Leaflets are generally elliptical, egg-shaped or heart-shaped with minute serrate margins and are uniformly green or patterned with whitish V-shaped marks and/or purplish colourations (Thomas, 1987b).

Table 4. Varieties of white clover available in Australia based on their leaf size[1]

Leaf size	Varieties/brand
Large	Braidwood, El Lucero, Emerald, Excel Ladino, Grasslands Kopu II, Grasslands Legacy, Grasslands Mainstay, Haifa, Osceola, Quest, RD 19 Taree Ladino, Super Haifa, SuperHaifa II, Super Ladino, Tamar, Tamarmax, Tillman II, Trifol sweet, Waverley, Will Ladino, Winter White II
Large-Medium	Beast, Grasslands Bounty, Grasslands NuSiral, Grasslands Pitau, Grasslands Sustain, Grasslands Trophy, Grasslands Tribute
Medium	Canterbury, Grasslands Challenge, Grasslands Demand, Grasslands Huia, Grasslands Pitau, Irrigation, Mink, Quartz, Storm, SuperHuia, Weka
Small	Grasslands Nomad, Grasslands Prestige, Grasslands Tahora, Prop, Star

[1] Sources: Reed (2008); NSW DPI (2020c), Stephen Pasture Seeds (2020), Barenbrug and PGG Wrightson Seeds (websites accessed October 2020).

3.2 Reproductive morphology

Flowers are produced from active apical buds. Each bud can contain up to seven developing leaves but not necessarily with an associating flower head. There may be two to three leaves between flowers (FAR, 2005). Inflorescences are globular racemes and are supported by long peduncles. Each inflorescence consists of 20-40 florets, which are white and commonly tinged with pink. Three to four seeds per pod can be produced after pollination. The seeds are smooth, heart-shaped and range in colour from bright yellow to yellowish brown, and darken with age (Frame, 2003).

SECTION 4 DEVELOPMENT

4.1 Reproduction

White clover possesses two complementary mechanisms of reproduction: regeneration by seedling recruitment and vegetative perennation through the stolon system (Lane et al., 2000). The primary adoption of either one of these mechanisms depends on the influence of the environment on stolon survival. For example, in the cool temperate regions of the UK, reproduction of white clover from seed is rare, and stolon growth is the main means of reproduction. In subtropical environments, stolon survival is less common and instead white clover persists through seedling regeneration (Archer and Robinson, 1989). There is an inverse relationship between stolon branching (and hence persistence) and flowering vigour. Therefore, profusely flowering white clover tends to have fewer stolons and has the potential to produce a large number of seeds (Thomas, 1987a). The persistence of white clover from one growing season to the next is influenced by the strength of its flowering (Thomas, 1987a).

4.1.1 Asexual reproduction

White clover is capable of vegetative reproduction through the generation of stolons. The stolon has two nodal root buds from which roots grow when the buds come into contact with moist soil. Further branching occurs at the lateral buds. Each growing tip has an apical bud from which leaves, flowers and lateral buds form. When the older stolons die the nodal roots support the new growing tips (FAR, 2005).

4.1.2 Sexual reproduction

White clover is capable of sexual reproduction through flowering and seed production when environmental conditions are not favourable for vegetative reproduction. Unfavourable environmental conditions that encourage sexual reproduction include drought, cultivation or overgrazing (Archer and Robinson, 1989). As a result, sexual reproduction is an important survival strategy for white clover (Archer and Robinson, 1989).

Many factors influence flowering, including genotype, photoperiod, temperature, nutrition, grazing management and the amount of moisture in the soil (Lane et al., 2000). Flower development requires the appropriate amount of light and light intensity; abortion of flower formation can occur when days are short and light intensity is low. White clover is generally regarded as a long-day plant (LDP), with inflorescence development also strongly influenced by low temperatures. However, the relative importance of these two factors depends on the geographical origin of cultivars. For example, plants with high latitude origin are more sensitive to long-days, while plants of low latitude origin are more sensitive to low temperatures (Thomas, 1987a).

The rate of flower emergence is dependent on the rate of leaf emergence from the apical bud (Thomas, 1987a). Under normal conditions, the time of full flower emergence from the appearance of the first leaf is about nine weeks, with one leaf emerging per week (FAR, 2005). White clover produces flowers from the axillary buds at the nodes, with each flower being made up of 20-150 florets (Lane et al., 2000). Each flower head can produce between 100 and 200 ovoid-truncate seeds (Riffkin et al., 2005).

4.2 Pollination and pollen dispersal

As white clover is predominantly self-incompatible (see Section 1 for details), cross-pollination is essential for significant seed set. White clover pollen is not easily dispersed by wind and any airborne pollen does not result in effective pollination. Therefore, insect pollinators are normally required to transfer pollen between individual clover plants. In Australia, the honeybee (*Apis mellifera*) is the most important pollinator of white clover. A study in western Victoria recorded that 88% of insect visits to white clover flowers were made by honeybees, with the next most common visitors being native bees (*Lasioglossum spp*., 4.3%) and blowflies (Calliphoridae, 3.5%) (Goodman and Williams, 1994). The same study found that excluding insect pollinators from white clover resulted in a 30-fold reduction in seed set. Diptera (flies) and Lepidoptera (butterflies and moths) may visit flowers but are not effective pollinators, reviewed by Harris (1987).

Bees are attracted to the nectar produced by the flower (FAR, 2005) and temperatures below 18°C reduce the nectar flow. Thus, attraction and subsequent pollination via bees are reduced in cooler temperatures. Nectar flow and pollen viability are also reduced in periods of water stress. However, a certain amount of stress can reduce vegetative bulk, which in turn may encourage an increase in bee activity leading to increased seed set (FAR, 2005).

Self-pollen is always present by the time a pollinator visits the flower (possibly up to 50% saturation of the stigma) but self-incompatibility normally prevents self-fertilisation. Additionally, self-pollen that has not been deposited by bees may have inadequate contact with the stigma for pollen germination and fertilisation to occur. Seed set can be increased by artificially pollinating florets by hand pollination or rubbing the flower heads together to promote a degree of selfing, as mechanical damage has been shown to be important in stimulating pollen germination (Harris, 1987). There is evidence that bees improve the efficiency of pollen deposits and several visits by bees to a floret maximises pollen tube access to ovules (over 90%) (Rodet et al., 1998). In a report where inflorescences had an average of 54.9 florets, an average of 22.4 florets contained seeds, which represented 86% of florets that had been probed by a bee (Michaelson-Yeates et al., 1997).

Although honey bees can travel up to 10 km and distances of 2.5 km are regularly recorded (Beekman and Ratnieks, 2000), when there is an abundant nectar source, the forage area is a lot smaller (Williams, 2001).

In a study of honeybee foraging on white clover, 60.9% of flower heads visited were within 10 cm of the previous head visited and only 13.6% were beyond 25 m (Weaver, 1965). It has been reported that when a bee visits a pollen donor, that particular pollen is deposited on the next 15 to 20 inflorescences visited and then sporadically up to the 50th inflorescence (Marshall et al., 1999). Bees have been reported to visit an average of 2.5-3.5 white clover florets per inflorescence (Weaver, 1965; Michaelson-Yeates et al., 1997) and generally the pollen most effective in fertilisation comes from the last pollen donor visited by the bee (Michaelson-Yeates et al., 1997).

Glover (2002) reviewed factors contributing to gene flow in Australian crops, and listed several factors contributing to the likelihood of gene flow. After considering that white clover is outcrossing and bee-pollinated, important factors contributing to cross-pollination between two flowering white clover plants include: the separation distance and relative plot sizes of donor and receptor plants; environmental factors affecting bee activity; availability and attractiveness to bees of other flowers; factors contributing to successful pollination such as presence of competing pollen and pollen viability. Pollen biology is an important component in the assessment of potential for gene flow, and in the evaluation of a need for and the type(s) of pollen confinement strategies such as buffer rows or isolation distances.

There are a number of reports of outcrossing rates for white clover in the scientific literature. Any differences are likely to be due to the cultivars used, experimental design, differences in the size of the pollen source and pollen sink and their spatial arrangement, local topology and environmental conditions (Eastham and Sweet, 2002). Pollination rates of 50% were measured between two adjacent blocks of white clover plants at 0.75 m, dropping to approximately 10% at 4.5 m apart (Marshall et al., 1999). Clifford et al. (1996) studied gene flow in white clover over two seasons, reporting that 0.7% and 1.3% of seed was produced by cross-pollination at 2 m from a pollen source. At 10 m, these figures had dropped to 0.02% and 0.04%. Another study on white clover similarly reported outcrossing rates of 0.68% and 0.02% at 2 and 10 m, respectively (Woodfield et al., 1995). These authors concluded that an isolation distance of 100 m is adequate to minimise gene flow. In Australia, white clover plants exhibiting a red leafmark phenotypic trait were used to study gene flow to recipient plants without leaf markings (De Lucas et al., 2012). When the red leafmark trait was used as the pollen donor plot without competition from other white clover plants, more than 70% of progeny had red leafmarks at close distances (2-6 m) from the donor plot, dropping to less than 30% at 150 and 200 m. When competitive white clover plants were present, ~50% red leafmark progeny were observed closest to the pollen donor plot (2-4 m), decreasing to less than ~10% at 20 m and stabilising to less than 5% between 40 - 200 m (De Lucas et al., 2012).

In a study examining manual pollination of *T. repens* with pollen from another *T. repens* plant, or with pollen from other *Trifolium* species, it was shown that when the pollen was from *T. repens*, pollen germination was rapid and within 30 minutes nearly all pollen had germinated. Pollen tubes grew rapidly, with initial fertilisation occurring within eight hours, and all ovules were fertilised within 24 hours (Chen and Gibson, 1972).

Pollen viability is thought to be high. Long term viability of white clover pollen when carried by bees is unknown, although the pollen remains viable for at least 60 minutes in a sucrose solution (Erith 1924 as cited in (Thomas, 1987a).

Separation distances are used for commercial seed production to maintain varietal purity. In the USA, general clover standards for fields of less than 2 ha require an isolation distance of 274 m for foundation seed (maximum 0.1% contamination with other varieties permitted), 137 m for registered seed (maximum 0.25% contamination with other varieties permitted) and 50 m for certified seed (maximum 1.0% contamination with other varieties permitted) (Association of Official Seed Certifying Agencies, 2003). In Europe, an isolation distance of 200 m is required (for fields of 2 ha or less) for basic legume seed, or 100 m for certified seed, with half these distances applicable to fields greater than 2 ha (OECD, 2018). OECD standards for legumes are consistent with South Australian white clover standards (Smith and Baxter, 2002) and the New Zealand seed standards for seed field production (Ministry for Primary Industries New Zealand, 2014).

4.3 Seed development and seed dispersal

White clover flowers have between 21 and 104 florets per flower head. Based on the number of ovules present, each floret should be able to produce six seeds. In commercial seed crops in New Zealand, florets produce between zero and seven seeds, with an average of approximately three seeds (Goodwin, 2012). Seed development from pollination to full ripening takes 26 ± 5 days. Seed first starts becoming viable at about day 10 with approximately 90% of seed viable by day 15 (Harris, 1987). The seeds are contained within tiny pods (4-6 mm long and up to 2 mm wide) and remain hidden inside the old flower parts (Weeds of Australia, 2016). The seed is shed once maturity is reached (Harris, 1987). Under permanent pasture, seeds are confined largely to the top five centimetres of soil (Hyde and Suckling, 1953).

Components that affect seed yield include the number of flower heads per unit area, the number of florets per flower head, number of seeds per floret, and the weight of ripe seeds. Each of these components are dependent on environmental and developmental factors and genotype (Thomas, 1987a; Marshall, 1994). For example, cold temperatures at flowering increase pollen sterility, high temperatures increase bee foraging and hence increase pollination, increased ovule age at pollination decreases seed set, long day length decreases flower initiation and close grazing during spring minimises stolon shading and can lead to high seed production (Thomas, 1987a; Jakobsen and Martens, 1994). In general, seed set decreases with decreasing temperatures. Interestingly, seed set is not influenced by pollen age (up to 5 days after anthesis[3]) (Jakobsen and Martens, 1994) and it appears that failure of high seed yield is not a result of inadequate pollination or the self-incompatibility system (Cowan et al., 2000).

White clover seeds can be dispersed long distances by human activities and through the digestive tract of birds and grazing animals. Alternatively, short distance dispersal may occur by dehiscence, stock trampling, worms, ants, and to a small extent by wind. Seeds can remain viable after passing through the digestive tracts of sheep, cattle and goats several days after consumption (Suckling, 1952; Yamada and Kawaguchi, 1971, 1972). Viable white clover seed can also be recovered from birds such as sparrows, pigeons, pheasants and rooks (Krach, 1959), and it is eaten by species including crimson and Adelaide rosellas (*Platycercus elegans*) and galahs (*Elophus roseicapilla* syn. *Cacatua roseicapilla*) (Tracey et al., 2007). However, white clover seed is thought to be relatively unattractive to birds due to its very small size. Ingestion of white clover seeds by earthworms does occur and viable seed has been found in worm casts (McRill and Sagar, 1973). Distribution by common Australian animals and birds has not been extensively studied. However, cattle and ants have been shown to carry white clover seeds in Australian pastures (Campbell, 1966; Reed, 2008).

4.4 Seed dormancy and longevity

4.4.1 Dormancy and germination

Mature white clover seed can be either ‘soft’ (i.e. permeable to water and able to germinate readily) or ‘hard’ (impermeable to water leading to delayed germination); the ratio is dependent on the conditions under which they ripen. If the seed ripens under dry conditions then nearly all the seed has the dormant hard characteristic, whereas under moist conditions the seed will immediately be able to germinate (Harris, 1987; D'Hondt et al., 2010). A study by Hyde (1954) evaluated seed ripening under five different levels of relative humidity (RH). Seeds that ripened at 70% RH were all soft, whereas at 30% RH most were hard, indicating a high negative correlation between moisture content of the seed and duration of impermeability (Hyde, 1954). In general, most naturally reseeded white clover seed is hard (Harris, 1987). Under very humid conditions, vivipary[4] has been observed in white clover, where seed germinates on the dried flower heads with little or no dormancy period (Majumdar et al., 2004; D'Hondt et al., 2010). In field

[3] The flowering period of a plant, from the opening of the flower bud.

[4] Is the phenomenon that involves seeds germinating prematurely while they are still inside or attached to the parent plant or fruit.

studies in New Zealand, 'almost all' hard seeds collected from soil germinated readily once scarified[5], leading the authors to the conclusion that legume seeds rarely lose viability while they remain as hard seeds in soil (Hyde and Suckling, 1953). A more recent study also noted that higher numbers of seeds per head were correlated with higher proportions of dormant seeds in white clover (D'Hondt et al., 2010).

The breaking of seed dormancy is again dependent on the cultivar (adaptability) and environment, and scarification may be required for some white clovers (Harris, 1987). Germination of seed occurs in winter and spring and timing is dependent on environmental conditions (Archer and Robinson, 1989). Ideally, scarification, pre-exposure to low temperatures (less than 15°C) and adequate levels of moisture will ensure maximum germination of white clover seeds. The maximum growth rate of white clover plants occurs at about 24°C but is very similar between 18°C and 30°C (Harris, 1987).

4.4.2 Seed banks and persistence

Longevity of white clover seed is highly dependent on the level of hard seed and is influenced by levels of aeration and temperature. Under New Zealand conditions, a five year break from white clover cultivation coupled with either annual cultivation or herbicide treatment is successfully employed in 90% of cases when white clover cultivars are changed for seed production, reducing the volunteer plants to less than 1 per 10 m^2 (Clifford et al., 1990; Clifford et al., 1996). One study in the USA found that 2% of white clover seed was viable for up to 16 years (or possibly 21 years with scarification of hard seeds) after burial at a depth of 42 inches (approximately 107 cm). However, this observation was for seeds that were buried in pots and germination of retrieved seeds was tested in sterilised soils in a greenhouse (Toole and Brown, 1946), rather than under field conditions. In general, viability is retained for longer at low temperatures. Without active control, viability of hard seed in various undisturbed soil conditions has been shown to be reduced to 1% after 20 years (Lewis, 1973) and there are rare reports of white clover seed being viable for longer than 20 years (Harris, 1987).

In a study of established mixed pasture containing white clover in sub-tropical area of south-east Qld., soil seed numbers were recorded at approximately 3000 m^{-2} in a drier site and 6000 m^{-2} in a wetter site. However, seedling emergence at the experimental sites was lower with a range from 0.5 to 770 seedlings m^{-2} (Jones, 1982).

4.5 Vegetative growth and stolon development

As discussed in Sections 3.1 and 4.1.1, white clover is capable of vegetative reproduction through the generation of stolons and stolon growth and survival has been shown to be vital as a regenerative strategy for white clover in determining the persistence of white clover in pastures (Hutchinson et al., 1995). The stolon consists of nodes and internodes. A single trifoliate leaf is produced from each node, along with a lateral bud and two nodal root buds (FAR, 2005).

White clover has three distinct development stages. The first is a small, slow growing, compact rosette 'seedling' phase. In the seedling phase, which lasts for up to three months after germination, a few stolon branches are produced. The taproot also elongates in preparation of the second phase, which lasts up to 18 months from sowing and is characterised by rapid stolon elongation and active branching with nodal roots. It is at the second stage when white clover produces its greatest amount of herbage per unit area. The final stage starts one to two years after sowing, and leads to the mature clonal set of plantlets. In this stage, the taproot dies and stolons become solely reliant on the nodal root network (Widdup and Barrett, 2011).

Stolon growth ensures that the white clover plant persists as a perennial. As discussed earlier, a branched network of stolons radiates out from the primary plant and each stolon establishes itself as an independent plant (Thomas, 1987b). Vegetative spread by stolon formation has led to patches of clonal white clover up to 2 m wide in sand dunes in Scotland (Harberd, 1963), although this spread does depend on the availability

[5] Scarification in botany involves weakening, opening, or otherwise altering the coat of a seed to encourage germination.

of areas of bare ground (Clatworthy, 1960 as cited by Harris, 1987). Drops in soil moisture lead to a decline in white clover mass but once soil moisture conditions improve in cooler months, surviving stolons resume growth. Maximum growth potential of white clover is only achieved by stolon survival, as seedling growth is slow and variable (Archer and Robinson, 1989). White clover differs from red clover and alfalfa (*Medicago sativa*) in that it is capable of continuous leaf generation (OECD, 2005).

Branching of stolons occurs at the lateral buds. The apical bud at the growing tip of the stolon is where the leaves, flowers and lateral buds form. More branches produce more growing tips thus more laterals, leaves and subsequently flowers. Branching from the lateral buds is stimulated by the formation of nodal roots and light. Conversely, shading and a lack of nodal development reduce branching (FAR, 2005).

The cultivar characteristics determine the height, leaf and flower size of the plant. Large apical buds also produce larger leaves, usually earlier in the growing season. Towards the end of the growing season, leaf and flower size decreases (FAR, 2005).

4.5.1 Root development

White clover has a stoloniferous habit, the taproot develops from the seedling primary root and the nodal roots develop from nodes on the stolons (see Figure 1). Its root systems are weakly taprooted when compared to alfalfa, strawberry clover (*T. frugiferum*) or Caucasian clover (*T. ambiguum*), but are more taprooted than other *Trifolium* species, such as *T. dubium*, reviewed in Caradus (1990). White clover does not have a dense fibrous root system as found in many grass species. The depth of white clover roots is considered to be shallow, with > 80% of roots in the top 10-20 cm of soil, reviewed in Caradus (1990). The seedling taproot can persist for 12-18 months (Caradus, 1990).

SECTION 5 BIOCHEMISTRY

5.1 Toxins

White clover is not a pathogen and is not capable of causing disease in humans, animals or plants. However, white clover can potentially be toxic to grazing animals if ingested in large quantities or under particular situations because of the presence of toxic and anti-nutritional compounds.

5.1.1 Bloat

Some legumes including white clover can cause bloat in ruminants. Bloat occurs if foams containing gases form and stabilise in the rumen during microbial fermentation leading to respiratory and circulatory malfunction. Ingestion of foliage containing high levels of starch and carbohydrates may promote bloat, and saponins, colloidal particles, and soluble proteins present in white clover may all play a role in bloat (Hart, 1987; Lane et al., 2000).

5.1.2 Cyanogenesis

Cyanogenesis is the release of hydrogen cyanide from damaged plant tissue. White clover is polymorphic for cyanogenesis and therefore populations can be composed of both acyanogenic and cyanogenic individuals. White clover plants that are cyanogenic contain the cyanogenic glucosides laminarin and lotaustralin and the enzyme linamarase which is responsible for hydrolysing cyanogenic glucosides and releasing hydrogen cyanide (Hart, 1987). The two components are separated in intact tissue and do not mix until tissue is damaged (Hughes, 1991). The cyanogenic phenotype is dependent on two complimentary dominant genes; one which determines the production of cyanogenic glucosides and the other which determines the production of linamarase (Li) (Collinge and Hughes, 1984; Hart, 1987). The *Ac/ac* forms of the *Ac* gene are related to the presence/absence polymorphism for the P450 protein which is the first step in the biosynthesis of cyanogenic glucosides, while the *Li/li* forms of the *Li* gene are related to the presence/absence of linamarase precursor (Collinge and Hughes, 1984; Olsen et al., 2007; Olsen et al., 2008; Kooyers and Olsen, 2012). Plants with the *Ac/Li* form are cyanogenic phenotypes, while *Ac/li*, *ac/Li* or *ac/li* plants are acyanogenic (Kooyers and Olsen, 2012). Levels of cyanogenic activity differ among cyanogenic plants and, although this variation does have a heritable basis, it also can be influenced by other factors such as light intensity, plant maturity, temperature, moisture stress and phosphorous application (Vickery et al., 1987).

The level of the glucosides in the foliage may be sufficiently high for grazing animals to suffer from direct cyanide poisoning but the risk of this is low. However, cyanide metabolites interfere with selenium metabolism, which in turn causes problems in ruminants. Highly cyanogenic white clover has been implicated in nutritional myopathy, a muscle wasting condition of young rapidly growing animals, from exposure to highly cyanogenic forage during pregnancy (Lehmann et al., 1991; Gutzwiller, 1993). High levels of cyanogenesis may also increase the risk of goitre formation in ruminants such as sheep depending on body iodine reserves (Gutzwiller, 1993). Work aimed at determining whether cyanogenic white clover was implicated in equine grass sickness was unable to make a conclusive link between white clover in pastures and horse disease (McGorum et al., 2012). However, it is recommended that only white clover with levels of less than 700 µg hydrogen cyanide (HCN)/g dry matter (DM) be used to ensure limited disruption to selenium metabolism (Lehmann et al., 1991).

The white clover plants benefit from limited levels of cyanogenesis by having increased resistance to insect herbivory and increased seedling establishment (Pederson and Brink, 1998). White clover grown in increasing levels of soil salinity showed higher levels of cyanogenic glucosides and higher HCN potential, as well as reduced herbivory by invertebrates (Ballhorn and Elias, 2014).

5.2 Allergens

The pollen and foliage of white clover are described as suspected allergens in unsubstantiated reports found during web searches, and specific white clover pollen allergy tests are reportedly available internationally. However, a search of medical literature for references to white clover allergenicity (PubMed, October 2020) found only one report of a single subject showing an eczematous skin reaction to patch testing with white clover leaves (Jovanovic et al., 2003).

5.3 Other undesirable phytochemicals

Levels of phytoestrogens, including isoflavones (genistein, daidzein, formonontein, biochanin-A) and coumesterol produced by other clovers are associated with reproductive problems in female cattle and sheep (Adams, 1998), but there have been only sporadic reports of these conditions being linked to white clover (Wright, 1960). In white clover, levels of phytoestrogens are low (0.01%-0.06% of dry matter). Formononetin comprised 90-95% of the total estrogenic isoflavones, with small amounts of genistein detected (Saloniemi et al., 1995). Coumestrol, an oestrogenic flavonoid, is present in white clover and can interfere with reproduction or have a growth enhancing effect on foetuses. However in this study, coumestrol levels in white clover were shown to be less than 10 ppm (or 0.001%) and although an increase in rat uterine weight was observed, no link was found between these levels of coumestrol and increased uterus weight in rats and the cause of this increase could not be linked to a specific biochemical compound (Saloniemi et al., 1995). Most white clover cultivars have little oestrogenic activity, although fungal infection has been shown to increase levels of coumestans and produce detectable effects in mice (Wong et al., 1971). However, it should be noted that the effects of phytoestrogens on animal production vary markedly depending on the specific compound and the animal species, as well as a number of other factors. These effects have been observed and managed in animal production systems for many years.

White clover may affect the germination and survival of other plants due to allelopathic effects. A number of compounds can move into the surrounding soils, either through secretion by the roots or by leaching from plant material (Macfarlane et al., 1982a, b; Carlsen and Fomsgaard, 2008; Carlsen et al., 2012; Weston and Mathesius, 2013). This may be regarded as a detrimental effect when it limits the growth of other desired species, but may also be part of the effectiveness of white clover in suppressing weeds when used as a cover crop.

5.4 Beneficial phytochemicals

White clover is high in protein and minerals (Table 5). It contains 22-28% crude protein, 2.7-3.3% crude fat, 9.4-11.9% ash, 6.6-7% lignin and a crude fibre content of 15.7-21.1% (OECD, 2005). It is deemed more digestible than other temperate forage legumes and therefore the ingested nutrients can be used more efficiently. Tannins accumulate in the flowers of white clover but not in the leaves or stolons (OECD, 2005). As animal forage, white clover functions optimally as a 10-20% component when grown in conjunction with other grasses (OECD, 2005).

Table 5. White clover mineral composition[1]

Chemical constituent	Content range (per kg DM)
Phosphorus	1.9-5.1 g
Potassium	15.4-38.0 g
Magnesium	1.5-4.8 g
Sulphur	2.1-4.3 g
Calcium	12.0-23.1 g
Sodium	0.5-3.9 g
Chlorine	3.0-15.6 g
Iron	102-470 mg
Molybdenum	1.3-14.2 mg
Manganese	40-87 mg
Copper	5.4-10.0 mg
Zinc	22-32 mg
Boron	26-50 mg
Cobalt	0.10-0.38 mg
Iodine	0.14-0.44 mg
Selenium	0.005-153 mg

[1] Sources: OECD (2005), Frame and Newbould (1986).

SECTION 6 ABIOTIC INTERACTIONS

6.1 Abiotic stress limiting growth

6.1.1 Nutrient stress

White clover requires good levels of nitrogen, sulphur, potassium and molybdenum especially when grown with other grasses. High levels of nitrogen can inhibit white clover growth (PGG Seeds, 2008).

6.1.2 Temperature

The soil temperature should ideally be above 10°C, with the maximum growth of white clover occurring between 18 and 30°C. High temperatures in the first months of growth can also limit establishment. Sensitivity to temperature can be somewhat cultivar dependent (PGG Seeds, 2008). In cooler climates, white clover tends to exist as a perennial/biannual while in the warmer climates it exists as an annual.

6.1.3 Water

White clover is intolerant to long periods of waterlogging (PGG Seeds, 2008). Due to its shallow root system, leading to an inability to access moisture deeper in the soil, white clover is also sensitive to drought (Dairy Australia, 2012). In Australia, optimal white clover growth requires a minimum of 750 mm rainfall (NSW DPI, 2020c). Unlimited water supply can lead to excess leaf development resulting in a shading canopy. This, in turn, can lead to ovule abortion, a late maturing crop and infection by fungal diseases. Tolerance to drought depends somewhat on the cultivar and on whether the crop is grown as fodder or for seed production. Severe water deficit affects vegetative and reproductive growth/processes, but moderate water deficit enhances some (Bissuel-Belaygue et al., 2002a, b). However, inadequate water will result in a reduction in the number of stolons produced per plant as well as phytomers (structural subunit made up of a leaf, an internode, a node and an axillary bud) per stolon (Bissuel-Belaygue et al., 2002a).

6.1.4 Other tolerances

Grazing/cutting can be detrimental to white clover although some cultivars (e.g. Tribute) can be more persistent under hard grazing. White clover does not grow well in shady conditions and, although it can adapt to shade, the adaptation is more successful if it occurs gradually. Moderate to hot fires will kill or severely thin white clover, and as white clover does not bury its seed, the recovery after fire can be patchy and depends on the growth stage of the plant (NSW DPI, 2020a).

White clover seeds are small and have low reserves for sustaining seed development, and sowing too deeply or in compacted soil can have adverse effects on germination and establishment (Murray and Clements, 1993). White clover does not tolerate soil that is too acidic or too alkaline. Soil acidity influences availability of several nutrients and decreases survival of *Rhizobium* bacteria, which are necessary for nitrogen fixation (UGA Extension, 2016). White clover performs optimally in soils with a pH range of 5.6-6.5 (in water) (PGG Seeds, 2008).

6.1.5 Residual herbicides

Residual activity from herbicides (broad-leaf activity) can pose problems to white clover seedlings. 2,4-D (2,4-Dichlorophenoxyacetic acid) has only a short residual activity (two to three weeks) and should not pose many problems, but dicamba has a 120 day residual activity and picloram has residual activity up to one year after spraying (UGA Extension, 2016).

SECTION 7 BIOTIC INTERACTIONS

7.1 Weeds

Weed infestations in commercial white clover production have resulted in the identification of six major problem areas. These include: (i) a reduction in seed yield through competition with weeds for water, nutrient and space availability; (ii) contamination of seed resulting in rejection from certification; (iii) rejection from overseas markets; (iv) cost associated with herbicide application; (v) possible development of resistance to the herbicides; and (vi) chemical and herbicide use, potentially leading to environmental and social problems (Riffkin et al., 2005).

Grower groups have identified four major weed species that are problematic in the clover seed production industry (Riffkin et al., 2005) and these are discussed below.

Annual ryegrass (*Lolium rigidum*) has been found to be particularly difficult to control in 4-5 year old stands of clover. Effective control methods include grazing regimes and application of herbicides such as trifluralin, metolachlor, glyphosate, imazethapyr, carbetamide and clethodim.

Sowthistle (*Sonchus oleraceus*) is a late season germinating weed and is a problem in 2-3 year old stands, especially since it overlaps with white clover flowering, making herbicide or control through grazing difficult. The infestations are worst in bare patches, header rows and on heavy soils. Some effective herbicides include simazine, paraquat, diquat, 2,4-D ester and diflufenican (Riffkin et al., 2005).

Maltese cockspur (*Centaurea melitensis*) is a problem mainly in the first year of clover crops. It favours cultivation and heavier soil types. Late germination results in an overlap in flowering with clover, resulting in contamination of clover seed and subsequent reduction of marketability. MCPA (2-methyl-4-chlorophenoxyacetic acid), glyphosate and clopyralid are some of the herbicides used as a control method.

Jersey cudweed (*Pseudognaphalium luteoalbum*), once established, can be very aggressive and has the ability to destroy a white clover stand by the third year. It initially establishes in bare patches on loam and heavier soils. It can also block machinery at the time of harvest. Paraquat, metribuzin, glyphosate and imazethapyr are some of the herbicides used to control the weed (Riffkin et al., 2005).

In general, white clover is not affected by herbicide treatments as they are usually pre-emergence treatments, however high rates of simazine and glyphosate can lead to a reduction in yield (Riffkin et al., 2005). Grower groups have been advised not to use glufosinate in white clover seed crops as it significantly reduces seed yield. The effectiveness of herbicide treatment varies with the farming practices, climatic factors and the presence of resistant weed populations (Riffkin et al., 2005). The effect of individual herbicides on white clover may also vary depending on the rate of application and the white clover plant growth stage at which it is applied (Adami et al., 2017).

The most comprehensive information about herbicides currently approved for use in controlling weeds in white clover crops or pastures in Australia can be obtained from the APVMA PubCRIS database.

7.2 Pests and pathogens

7.2.1 Vertebrate pests

As discussed in Section 4.3, white clover seed is eaten by a range of birds. Kangaroos, rabbits and possums are pests known to decrease yield of improved pastures and can be assumed to feed on white clover, although this is not specifically documented in the scientific literature.

7.2.2 Invertebrate pests

The range of invertebrate pests in Australia varies according to location and whether the white clover is used for seed production or grazing. Some important pests that affect the establishment of white clover in Australia include red-legged mite (*Halotydeus destructor*), blue oat mite (*Penthaleus major*), lucerne flea (*Sminthurus viridis*), corbies, pasture web worms and related caterpillars (*Oncopera spp.*), blackheaded pasture cockchafer (*Aphodius tasmaniae*), pink cutworm (*Agrotis munda*) and reticulated slug (*Deroceras reticulatum*). The main pests affecting white clover seed-crops include native budworm (*Helicoverpa punctigera*), clover casebearer (*Coleophora frischella*) and bluegreen aphid (*Acyrthosiphon kondoi*) (Berg, 1993). Another pest that requires control and close monitoring is the pea aphid (*Acyrthosiphon pisum*) (Barenbrug, 2020).

There is little information published on the economic impact of invertebrate pests on white clover productivity. The red-legged mite and lucerne flea may have the greatest impact on white clover productivity especially in the establishment of pastures in Victoria (Berg, 1993). Short-lived insecticides such as organophosphate are the main chemicals used to control invertebrate pests in white clover (Berg, 1993), and pre-planting insecticide treatment of seeds can assist establishment and reduce plant losses due to attack of insects such as the red legged earth mite (PGG Seeds, 2008).

Nematodes may significantly reduce white clover performance by reducing root growth and nitrogen fixation, and by stunting both leaf and stolon growth (Lane et al., 2000). Examples of nematodes found in white clover swards in the subtropics of Australia include the root-knot nematode (*Meloidogyne spp.*) the clover cyst nematode (*Heterodera trifolii*) and a free-living nematode (*Helicotylenchus dihystera*) (Zahid et al., 2001). Other nematodes that attack white clover include the stem nematode (*Ditylenchus dipsaci*), and root lesion nematodes. The cultivar Will Ladino has been developed to resist stem nematodes (PGG Seeds, 2008).

7.2.3 Pathogens

Fungal disease is associated with damage to taproots and stolons and can lead to the subsequent death of these structures. The fungus *Sclerotinia trifoliorium* causes clover rot and can have a great impact on white clover productivity. Outbreaks occur most years although the incidence and severity of the disease varies from year to year. Other minor fungal diseases include grey mould, which is caused by the fungus *Botrytis cinerea* and wart disease, which is caused by the fungus *Physoderma trifolii* (Clarke, 1999b). Fungi that cause leaf spot diseases in white clover include *Leptosphaerulina trifolii* (Pepper spot), *Pseudopeziza trifolii* (Common leaf spot), *Cymadothea trifolii* (Black/Sooty spot), *Stemphylium spp*. (Stemphylium leaf spot), *Stagonospora spp*. (Stagonospora leaf spot), *Peronospora trifoliorium* (Downy mildew) and *Erysiphe trifolii* (Powdery mildew) but all rarely cause significant losses (Clarke, 1999c).

7.2.4 Viral diseases

The presence of White clover mosaic virus (WCMV), Alfalfa mosaic virus (AMV) and Clover yellow vein virus (CYVV) in clover is widespread throughout Australia and all have an impact on white clover productivity. AMV can cause severe losses, with reports of up to 60% damage to pasture legumes (Garrett, 1991). AMV and CYVV are transmitted by aphids only, whereas WCMV is not aphid- or seed-borne but is readily spread by machinery (Garrett, 1991). Incidences of viral infection in stands older than two years are commonly 20% or more (Clarke, 1999a), although in one study, infection of white clover by AMV and WCMV exceeded 90% (Norton and Johnstone, 1998) and in another greater than 86% infection by AMV was found (McKirdy and Jones, 1995). Glasshouse studies have shown dry mass losses of up to 24% due to the presence of WCMV (Clarke, 1999a) and up to 60% due to AMV (Kalla et al., 2001). In the field, AMV, CYVV and WCMV may reduce white clover pasture production by up to 30% through reduced foliage yield and quality, reduced nitrogen fixing capacity and reduced vegetative persistence (Kalla et al., 2001). Virus control generally involves an integrated approach using methods such as grazing management to reduce the availability of infected material and to allow healthy plants to out-compete infected plants, reducing stock or machinery presence to control contact-borne viruses, not replanting new pastures near infected areas, sowing of mixed swards containing non-host species, and controlling insect vectors (Jones, 2013).

Other viruses known to infect white clover in Australia include Bean common mosaic virus, Clover yellow mosaic virus, Peanut stunt mosaic virus, Subterranean clover red leaf virus and Rugose leaf curl virus, with many other viruses also being detected in white clover (Garrett, 1991). Most viruses that affect white clover, are predominantly present in pastures, whereas CYVV is also present in natural environments (Godfree et al., 2004). It is estimated that the agronomic impact of viruses on white clover equates to a loss of $30 million annually to the Australian diary industry (Garrett, 1991).

7.3 Symbionts

White clover is known to form mutualistic associations with nitrogen-fixing bacteria and arbuscular mycorrhizal fungi (AMF). These soil microorganisms have the capacity to benefit plants by reducing the effects of environmental stresses, including drought (Dong et al., 2008; Xiao-Qing et al., 2017).

7.3.1 Rhizobia

Like all legumes, white clover forms a symbiotic relationship through its root hairs with soil bacterium from *Rhizobium* species, which initiates nodule formation. Through nitrogen-fixation, *Rhizobium* makes atmospheric nitrogen available to the plant and also to subsequent crops. Fixation occurs in root nodules and the bacteria utilises the sugars produced by the plant.

In Australia, commercial inoculants for white clover belong to Group B, which contain *Rhizobium leguminosarum bv. trifolii* and are specific to perennial clovers and most annual clovers (GRDC, 2013). *Rhizobium leguminosarum bv. trifolii* do not naturally occur in Australia (Atlas of Living Australia, accessed 24 September 2020).

Soil properties, such as pH, environmental conditions and the presence of compatible hosts influence the proliferation and persistence of rhizobia in a paddock (Drew et al., 2014). Under severe phosphorus deficiencies, nodulation of roots inoculated by rhizobia does not occur (Almeida et al., 2000). Inoculation is recommended every time a leguminous crop is sown, as rhizobial populations can decline in soil over time and rhizobial communities can become less effective at fixing nitrogen, compared with commercial strains (GRDC, 2013).

7.3.2 Arbuscular mycorrhizal fungi

White clover can utilise root colonisation with arbuscular mycorrhizal fungi (AMF), which facilitate the extraction of nitrogen, phosphorus, potassium, and copper from the soil (Xiao-Qing et al., 2017).

7.3.3 Rhizobia and Arbuscular mycorrhizal fungi

In China, single or dual inoculation with AMF (*Funneliformis mosseae*, *Paraglomus occultum*, and *Rhizophagus intraradices*) and rhizobia (*Rhizobium trifolii*) were evaluated on white clover growth (Xie et al., 2020). AMF inoculation significantly ($P < 0.05$) increased root nodule number by 117–173%, and addition of rhizobia stimulated mycorrhizal growth. Compared to non-inoculated controls, single AMF or rhizobia treatment increased shoot growth and root biomass by 36-281% and 16-36%, respectively. Dual inoculation of rhizobia and AMF (*P. occultum* or *R. intraradices*) further increased shoot growth and root biomass. Additionally, leaf and root nitrogen content, root total soluble protein content, root nitrogenase activity, and amino acid concentrations were significantly increased by single or dual inoculation. Moreover, dual inoculations of AMF and rhizobia has a greater effect than single corresponding AMF or rhizobia inoculations (Xie et al., 2020).

SECTION 8 WEEDINESS

There are a number of attributes that may contribute to making a plant weedy. White clover has some of these weedy characteristics, such as a proportion of seeds being hard and able to persist for many years in the soil, high seed output and long distance seed dispersal by animals. Other characteristics of white clover that increase its potential to persist in the environment include its ability to regenerate by either seedling recruitment or by vegetative perennation through the stolon system, profuse flowering throughout spring and summer providing prolonged opportunity for pollen and seed dispersal, and its highly heterozygous nature due to outcrossing, which allows rapid adaptation (See Section 1.5). However, the degree to which these attributes may lead to successful establishment and persistence is highly dependent on environmental conditions as well as genotype.

As discussed in Section 2.3, optimum growth conditions for white clover include an annual rainfall of above 750 mm, a soil pH of > 4.5, sufficient levels of phosphorous, potassium, and sulphur in the soil, medium to well drained soils, and temperatures between 18 and 30°C (Harris, 1987; Lane et al., 2000; Betts and Ayres, 2004).

8.1 Weediness status on a global scale

Weeds are plants that spread and persist outside their natural geographic range or intended growing areas such as farms or gardens. Weediness in Australia is often correlated with weediness of the plant, or a close relative, elsewhere in the world (Panetta, 1993; Pheloung et al., 1999). The likelihood of weediness is increased by repeated intentional introductions of plants outside their natural geographic range that increase the opportunity for plants to establish and spread into new environments (e.g. escapes of commonly used garden plants) (Groves et al., 2005).

The most comprehensive compilation of the world's weed flora is produced by Randall (2017). Most of the information contained in this book has been sourced from Australia and North American countries but also includes numerous naturalised floras from many other countries. Seventy one *Trifolium* species are listed in this book and are categorised as naturalised, weeds and/or environmental weeds and many are found in a number of different countries highlighting the successful spread and establishment of the genus *Trifolium* in many countries. Randall gives a number of descriptors for the weed status of white clover in various countries. These include naturalised (self-sustaining and spreading populations with no human assistance), weed (normally an economic weed), introduced (purposely planted and therefore desirable in certain situations), garden escape (species originating from gardens), and environmental weed (invades native ecosystems).

8.2 Weediness status in Australia

White clover is a valuable pasture species in Australia. It is not declared or considered a noxious weed by any State government. However, it is widely naturalised in south-east Australia, and is a common weed of lawns, parks, gardens, roadsides, waste areas, disturbed sites, riparian vegetation, grasslands, open woodlands and alpine vegetation (Weeds of Australia, 2016).

Persistence, as well as productivity and seed production, of white clover is limited by a number of factors in Australia as outlined by Ayres and Reed (1993). These factors include: limited growth in cold winters; low tolerance to summer moisture stress, local ecotypes more persistent than introduced ecotypes; poor performance in soils with low fertility, high acidity or poor drainage; performance affected by competition with companion grasses and close grazing; and yield and persistence affected by pests and diseases. Summer moisture stress is the primary environmental constraint limiting the persistence and agronomic performance of white clover in Australia (Jahufer et al., 2002). In a study carried out over 30 years, moisture stress caused by low rainfall and/or high temperature, and high stocking rate were determined to have significant detrimental effects on white clover persistence in pastures located in the Northern Tablelands of New South Wales, Australia (Hutchinson et al., 1995).

In summary, a complex interaction between the genotype/phenotype of the white clover, climatic conditions and the physical environment will determine if the white clover can be successful in establishing itself and persisting in a given area.

8.3 Weediness in agricultural ecosystems

Ratings have been given on a State/Territory level for white clover as a weed in agricultural ecosystems and there is a large variation in the ratings (Groves et al., 2003). The State with the highest rating is Queensland, which rates white clover as 5, meaning it is known to be a major problem at 4 or more locations within the State. This is due to its presence as a weed in turf. A rating of 3 is given by New South Wales, Victoria and Western Australia, which means white clover is naturalised and known to be a minor problem warranting control at 4 or more locations within a State or Territory, with Western Australia actively controlling populations within some parts of the State. White clover has a rating of 1 in South Australia, which means it is naturalised and may be a minor problem but is not considered important enough to warrant control at any location. White clover is noted to be present in Tasmania, where it is not described as an agricultural weed.

In the US, white clover has been identified as a weed in apple orchards both because it competes with the trees for nutrients and water and also because its flowers are an unwanted attractant of bees at times when orchards are sprayed with insecticides that could harm bees (MacRae et al., 2005).

8.4 Weediness in natural ecosystems

A rating for white clover has been given for its status as a naturalised non-native species in natural ecosystems in each State and Territory of Australia, on the same rating scale as weeds of agricultural ecosystems described above. Under the categories for assessing the status of naturalised non-native species in natural ecosystems, the highest rating given to white clover is 4, which means it is naturalised and known to be a major problem at 3 or fewer locations within a State or Territory (Groves et al., 2003).

As shown in Appendix A, Figure A1, white clover is distributed in many temperate areas of Australia. These areas include potentially sensitive ecosystems such as woodlands and grasslands of montane and sub-alpine regions, of which some may be areas of national environmental significance and therefore included in the Environment Protection and Biodiversity Conservation Act (1999). However, to date there is limited information on the impact of white clover on native flora and fauna. Potentially, white clover may spread even further into new areas (See Appendix A, Figure A2). White clover tends to establish in areas that have been disturbed and where there is minimal competition from other plant species, such as roadsides and freshly excavated areas (Godfree et al., 2004).

In lowland grasslands of Victoria, white clover is common along roadsides, but tends to be restricted to the shoulder of the road and does not enter the adjacent native grassland verge (Garrett and Chu, 1997). In the Bogong high plains, white clover is found in clearings but only at low densities of less than 1% ground cover in moist flushes on ridges, slopes and streamside flats. It has been found around recently disturbed areas around ski lodges. It does not establish under closed shrub canopies (Garrett and Chu, 1997).

White clover is common in south-eastern Australian subalpine grassland communities and present to a lesser degree in woodland communities (Godfree et al., 2004). In mesic grassland communities, white clover constitutes over 40% of total herb cover. Although white clover does not dominate the invaded communities (Godfree et al., 2006; Godfree et al., 2007), it is considered a significant environmental weed in south-eastern Australia, mostly due to high adaptability. Where it is present in semi-natural native plant communities at a relatively high abundance it competes for growing space with native analogues and may even exclude native species altogether by forming a mat. This is especially the case along creek lines or in mesic grasslands (Godfree et al., 2006).

A recent report on environmental weeds in Victoria, rated white clover as a high risk, with the ranking determined by scoring weedy species for impact, potential spread to other areas, invasiveness, rate of dispersal, and the range of susceptible habitat(s) it could impact (White et al., 2018). An earlier NSW study ranked 340 weeds that impact on or pose a threat to biodiversity, based on spatial threat, native species impact, invasive ability, number of native species potentially at risk and habitat type invaded. Regional assessments were used as the basis for a statewide risk score, used to determine weed management priorities. White clover was assessed as having a medium risk, and was not noted as posing a threat to a species at risk (Downey et al., 2010).

8.5 Control measures

White clover can be described as hard to control because of its persistence by stolon proliferation (PennState Extension, 2020) and presence in the soil seed bank, it can even 're-appear' in pastures after severe droughts (Dairy Australia, 2012). Control measures can focus on reducing the competitiveness of white clover relative to grasses, for example by heavy grazing or close mowing. In high nitrogen soils, white clover is not as competitive compared to soils where its nitrogen-fixing ability gives it an advantage. Hence, application of nitrogen fertiliser has also been suggested as a control measure. Where white clover is a weed in native ecosystems, spot application of herbicides and hand pulling are standard control measures. 2,4-D, dicamba and MCPA are known to be effective on white clover (Griffin et al., 1984; Rolston, 1987; Riffkin et al., 2005). The most comprehensive information about herbicides currently approved for controlling white clover as a weed in Australia can be obtained from the APVMA PubCRIS database. White clover must also be controlled in clover seed production areas where the cultivar in production is being changed. In this situation, legume seed certification rules require a break in cultivation of at least three years combined with monitoring subsequent white clover stands for off-type volunteer plants (OECD, 2018). Clifford et al. (1990) found that in New Zealand conditions, a five year cropping break combined with either annual cultivation to encourage germination or herbicide treatment to kill volunteer plants was necessary to pass contamination requirements in the following white clover seed crop. An increased rate of successful certification of seed crops has been observed after adoption of these practices as industry standards in New Zealand (Clifford et al., 1990).

8.6 Weed risk assessment of white clover

The weed risk potential of white clover has been assessed (Appendix B) using methodology based on the Australia/New Zealand Standards HB 294:2006 National Post-Border Weed Risk Management Protocol (Standards Australia et al., 2006). The National Post-Border Weed Risk Management Protocol evaluates weediness by relating the likelihood of risk to the feasibility of control methods for weeds (Auld, 2012). The Protocol has been used as the basis for several weed management systems, for example, the South Australian weed risk management guide (Virtue, 2008). These properties relate to invasiveness, impacts and potential distribution. The distribution of wheat is driven by economics, as well as factors such as climate and soil suitability.

In summary, as a volunteer (rather than a pasture species) white clover is considered to:

- have a medium ability to establish amongst existing plants
- have a low tolerance to average weed management practices in cropping land use and medium tolerance in intensive land uses
- have a short time to seeding (less than one year)
- have a high annual seed production and a high ability for volunteers to establish in any land use
- reproduce by vegetative means
- be unlikely to undergo long distance spread by natural means
- be commonly spread long distance by people from dryland and irrigated cropping areas, as well as from intensive land uses
- have a limited ability to reduce the establishment or yield of desired plants
- have a low ability to reduce the quality of products or services obtained from all land use areas
- have no potential to restrict the physical movement of people, animals, vehicles, machinery and/or water
- have a low potential to negatively affect the health of animals and/or people
- have low potential to act as a reservoir for a range of pests and pathogens
- have a low effect upon soil nutrients, salinity, stability and the water table.

This is consistent with previous assessments of white clover in Australia summarised in Section 8.2, and provides a baseline for the assessment of GM white clover.

SECTION 9 POTENTIAL FOR VERTICAL GENE TRANSFER

The possibility of genes transferring from *T. repens* L. to other organisms is addressed below. Potentially, genes could be transferred to: (1) domestically cultivated white clover and naturalised white clover populations; (2) other cultivated and naturalised *Trifolium* species; (3) other plant genera; and (4) other organisms. With particular regard to the possibility of gene transfer to other plants, potential barriers must be overcome before gene flow can occur successfully. Pre-zygotic barriers include differences in floral phenology, different pollen vectors and different mating systems such as stigmatic or stylar incompatibility systems. *Post-zygotic* barriers include genetic incompatibility at meiosis, selective abortion, lack of hybrid fitness and sterile or unfit backcross progeny. Even where pre-zygotic and post-zygotic barriers do not exist, physical barriers created by geographic separation can still limit gene transfer to other plants.

Successful gene transfer requires that three criteria be satisfied. The plant populations must: (1) overlap spatially; (2) overlap temporally (including flowering duration within a year and flowering time within a day); and (3) be sufficiently close biologically that the resulting hybrids are fertile, facilitating introgression into a new population (den Nijs et al., 2004).

9.1 Intraspecific crossing

Cross-pollination of one *T. repens* plant with another mediated via an insect vector is the most likely means by which white clover genes could be dispersed in the environment. In Australia, the honeybee (*Apis mellifera*) is the predominant pollinator of white clover.

As discussed above in Section 1, white clover has a well-developed genetic gametophytic self-incompatibility mechanism with only a small proportion of plants in a population being quite strongly self-compatible and therefore white clover is considered an obligate out-breeder. As a result of the high degree of crossing that occurs between individual plants, white clover populations are composed of a heterogeneous mixture of highly heterozygous individuals (Voisey et al., 1994).

Viable seed and fertile progeny would be produced from cross-pollination of white clover plants, irrespective of whether the white clover plants are in the semi-cultivated environment of a pasture and whether they are present in other areas such as roadsides or native grasslands.

Gene flow between white clover populations is limited by geographic distance as determined by bee foraging ranges. As discussed in Section 4.2, when abundant nectar is available, the foraging range of bees is quite small and successful cross-pollination occurs only over short distances. However, if food is scarce then bees may travel many kilometres increasing the likelihood of long distance cross-pollination.

9.2 Natural interspecific crossing

The species with which *T. repens* is most likely to hybridise and exchange genes are those belonging to the genus *Trifolium*. *Trifolium* species are widely distributed throughout more temperate and subtropical parts of Australia and many clover species are commonly sown in pastures, including subterranean clover (*T. subterraneum*), arrowleaf clover (*T. vesiculosum*), red clover (*T. pratense*), persian clover (*T. resupinatum*) and strawberry clover (*T. fragiferum*) (Agriculture and Food Western Australia, 2018; NSW DPI, 2020b; Tasmanian Institute of Agriculture, 2020). A number of other species are also planted including balansa clvoer (*T. michelianum*), eastern star clover (*T. dasyurum*), bladder clover (*T. spumosum*) (Agriculture and Food Western Australia, 2018), gland clover (*T. glanduliferum*) (Agriculture and Food Western Australia, 2018; NSW DPI), berseem clover (*T. alexandrinum*), crimson clover (*T. incarnatum*), Kenya clover(*T. semipilosum*), purple clover (*T. purpureum*), rose clover (*T. hirtum*) (NSW DPI, 2020b), Caucasian clover (*T. ambiguum*), tallish clover (*T. tumens*) and alsike clover (*T. hybridum*) (Donald, 2012; NSW DPI, 2020b; Tasmanian Institute of Agriculture, 2020).

Trifolium species with similar karyotypes to *T. repens* have the greatest chance of forming hybrids (Chen and Gibson, 1972). Karyotypic differences such as different chromosome numbers or dissimilar linear arrangement of genes in homologous chromosomes, limit gene transfer to other species of *Trifolium*, and therefore crosses generally only occur in nature between different *T. repens* cultivars/genotypes (Chen and Gibson, 1971; Williams, 1987b). The cross-incompatibility of *T. repens* with related species is due to both pre- and post-fertilisation barriers. Failure to form viable seeds from interspecific crosses may be attributed to the inability of the pollen to germinate on a foreign stigma, the inability of the pollen tubes to grow normally in a foreign style, failure of fertilisation, or seed abortion. It has been shown that pollen tube germination normally occurs in crosses between *T. repens* and many other *Trifolium* species. However, pollen tube growth is slow, and swelling, bursting, coiling and/or misdirected growth is a common occurrence (Chen and Gibson, 1972). Success in producing hybrids can also be dependent on which species acts as the pollen donor as well as which cultivars are used (see 9.4 for examples).

One species that has been studied as a possible source of genetic material to introgress desirable attributes for white clover, such as increased flowering, heat tolerance and pest resistance is *T. nigrescens* (see Table 3), which is included in the secondary gene pool for white clover (Williams, 2014). This species is present in Australia (Australia's Virtual Herbarium, accessed 28 September 2020). However, due to the difficulties of white clover forming viable, competitive hybrids with this species it is extremely unlikely that they would occur in nature.

9.3 Natural intergeneric crossing

White clover is known to hybridise only with other plants and other cultivars of its own species, no natural intergeneric crossing has been documented.

9.4 Artificial interspecific and intergeneric crossing

Based on their ability to cross with *T. repens*, species have been grouped into secondary and tertiary genepools for *T. repens*, while other have been grouped into a quaternary or marginal genepool based on potential for use in introducing genetic material into the *T. repens* genepool, although unlikely to be able to be crossed directly with *T. repens* (Williams, 2014) – see Section 2.4.1 for more detail. *T. repens* has successfully been crossed with *T. nigrescens*, *T. uniflorum*, *T. occidentale*, *T. isthmocarpum*, *T. argutum*, and

T. ambiguum in experimental situations (reviewed by Williams, 1987b). All of these, except *T. argutum*, are grouped in the secondary or tertiary gene pools for *T. repens* (Williams, 2014). However, most hybrids were recovered through tissue culture, although many were sterile or showed abnormal development as discussed below. Of these species, only *T. nigrescens* (ball clover) is able to produce hybrids with white clover following hybridisation without further intervention, albeit with some difficulties; many hybrids that formed had sterile pollen, had chlorophyll deficiencies or produce non-viable seeds (Chen and Gibson, 1972; Williams, 1987b; Marshall et al., 1995). The rate of successful hybridisation is greatest when *T. repens* is used as the female parent plant (Hovin, 1962). The formation of hybrids is also highly dependent on the cultivar of *T. nigrescens* used in the cross (Hovin, 1962).

There have been numerous unsuccessful attempts to hybridise *T. repens* with many other *Trifolium* species, even when embryo rescue methods were used. These include species commonly found in Australia such as *T. arvense* (hare's foot clover), *T. fragiferum* (strawberry clover), *T. hirtum* (rose clover), *T. scabrum* (rough clover) and *T. subterraneum* (subterranean clover). Embryo culture and colchicine induced chromosome doubling was required to produce fertile hybrids (Hussain et al., 1997).

Hybrids of white clover and *T. ambiguum* (Caucasian or Kura clover) have been produced by embryo culture (Williams and Verry, 1981; Meredith et al., 1995) and subsequent backcrosses of the hybrids to the parent species have been successful (Anderson et al., 1991; Abberton et al., 2002).

White clover has been artificially crossed with *T. occidentale* (Western clover), most successfully when an induced tetraploid of *T. occidentale* was used (Gibson and Reinhart, 1969). In another study, initial *T. repens* x *T. occidentale* hybrids produced using embryo culture gave rise to fertile F2 progeny (Pederson and McLaughlin, 1989).

Pollination of *T. repens* by *T. uniflorum* (one flower clover) has been found to result in poor fertilisation rates and high rates of seed failure (Chen and Gibson, 1971). However, abnormal and fertile hybrids can be rescued using embryo culture techniques (Pandey et al., 1987).

Of these species, *T. uniflorum* and *T. ambiguum* have both been recorded in Australia (Atlas of Living Australia; accessed July 2020) with *T. ambiguum* being a relatively common clover in pastures of NSW (Hackney and Dear, 2007). However, due to the difficulties of forming hybrids with these species, it is extremely unlikely that *T. repens* would form viable competitive hybrids with other *Trifolium* species in nature.

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APPENDIX A DISTRIBUTION OF WHITE CLOVER IN AUSTRALIA - MAPS

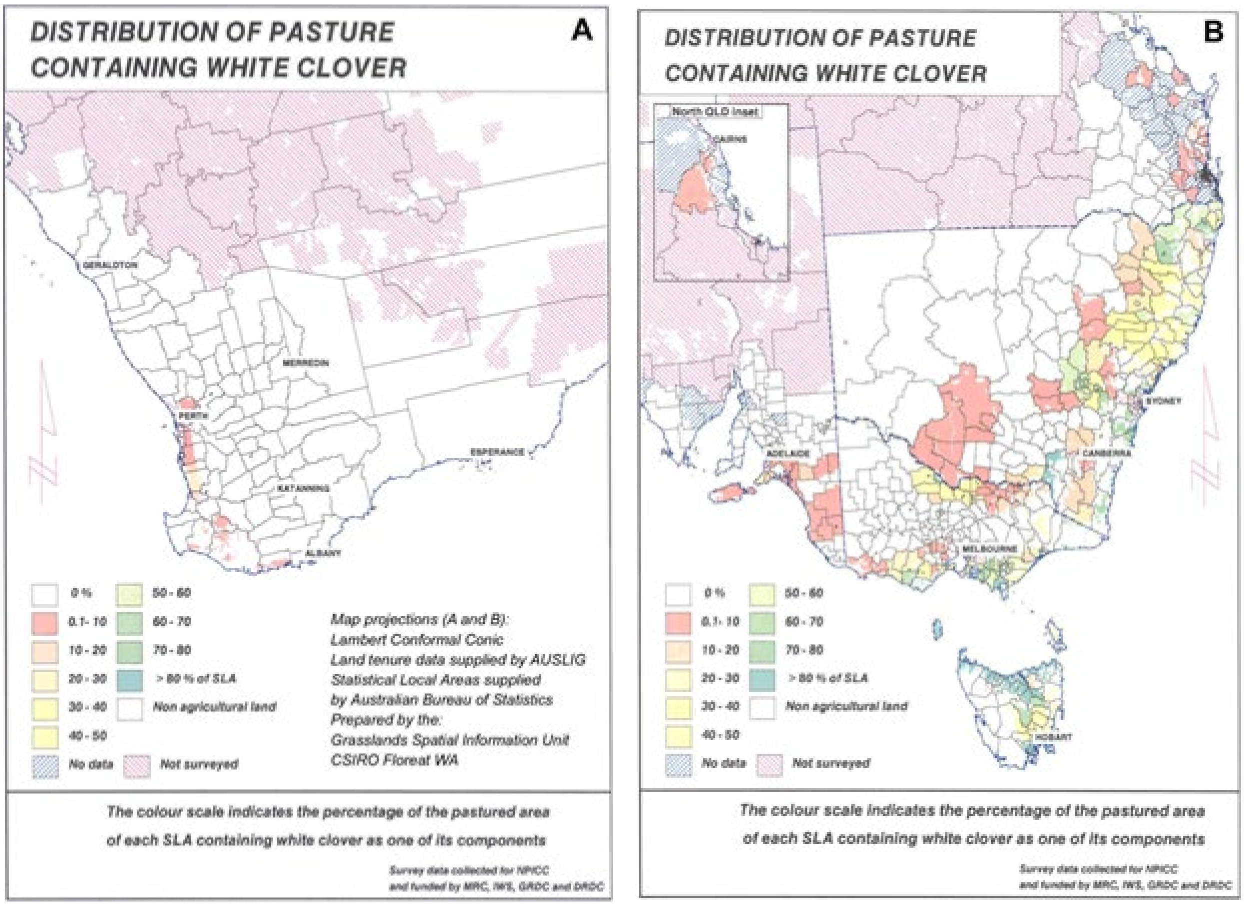

Figure A1. Distribution of white clover in Australian pastures. Source: Australian Temperate Pastures Database (Hill and Donald, 1998).

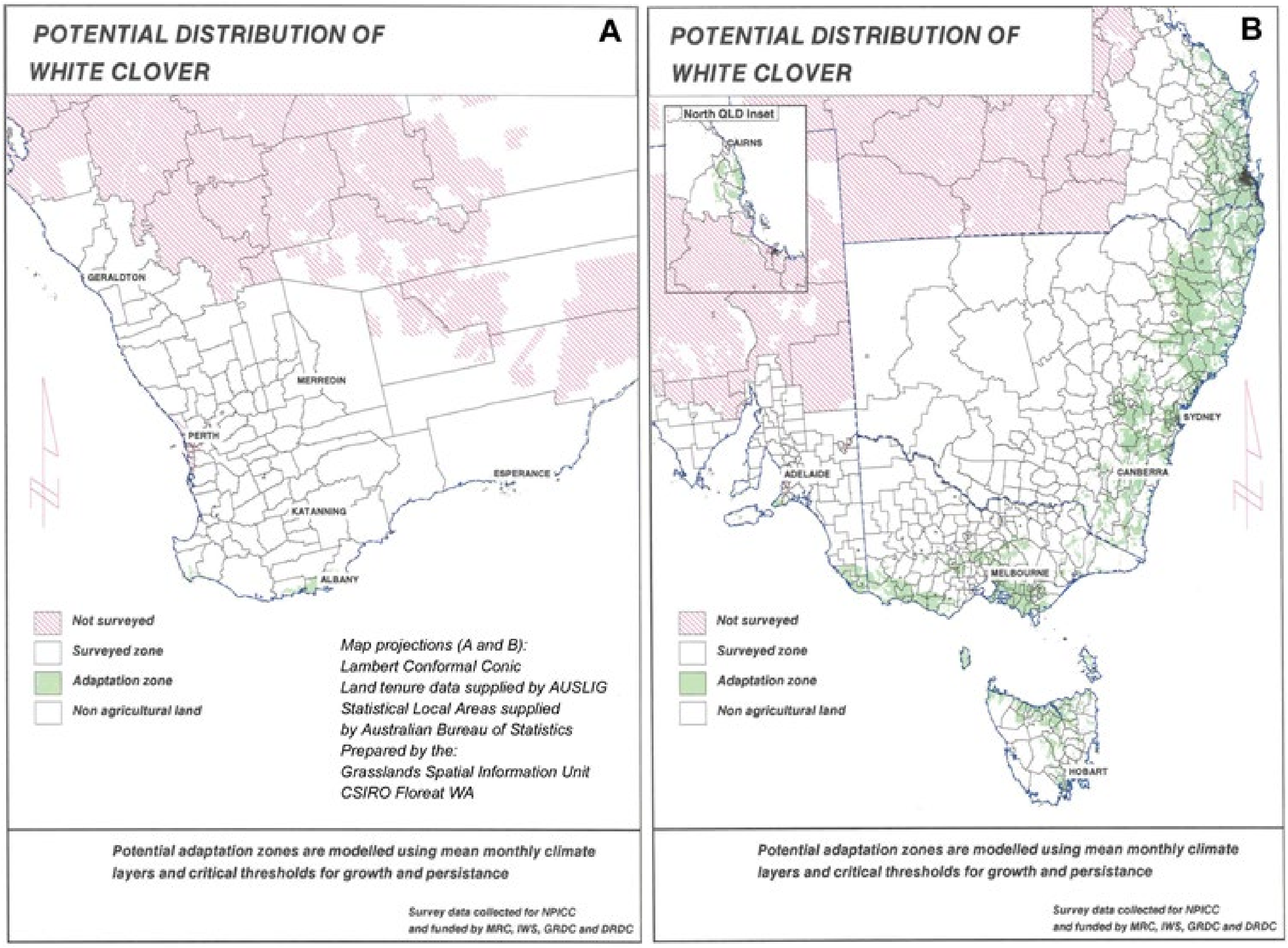

Figure A2. Potential distribution of white clover in Australian pastures. Source: Australian Temperate Pastures Database (Hill and Donald, 1998).

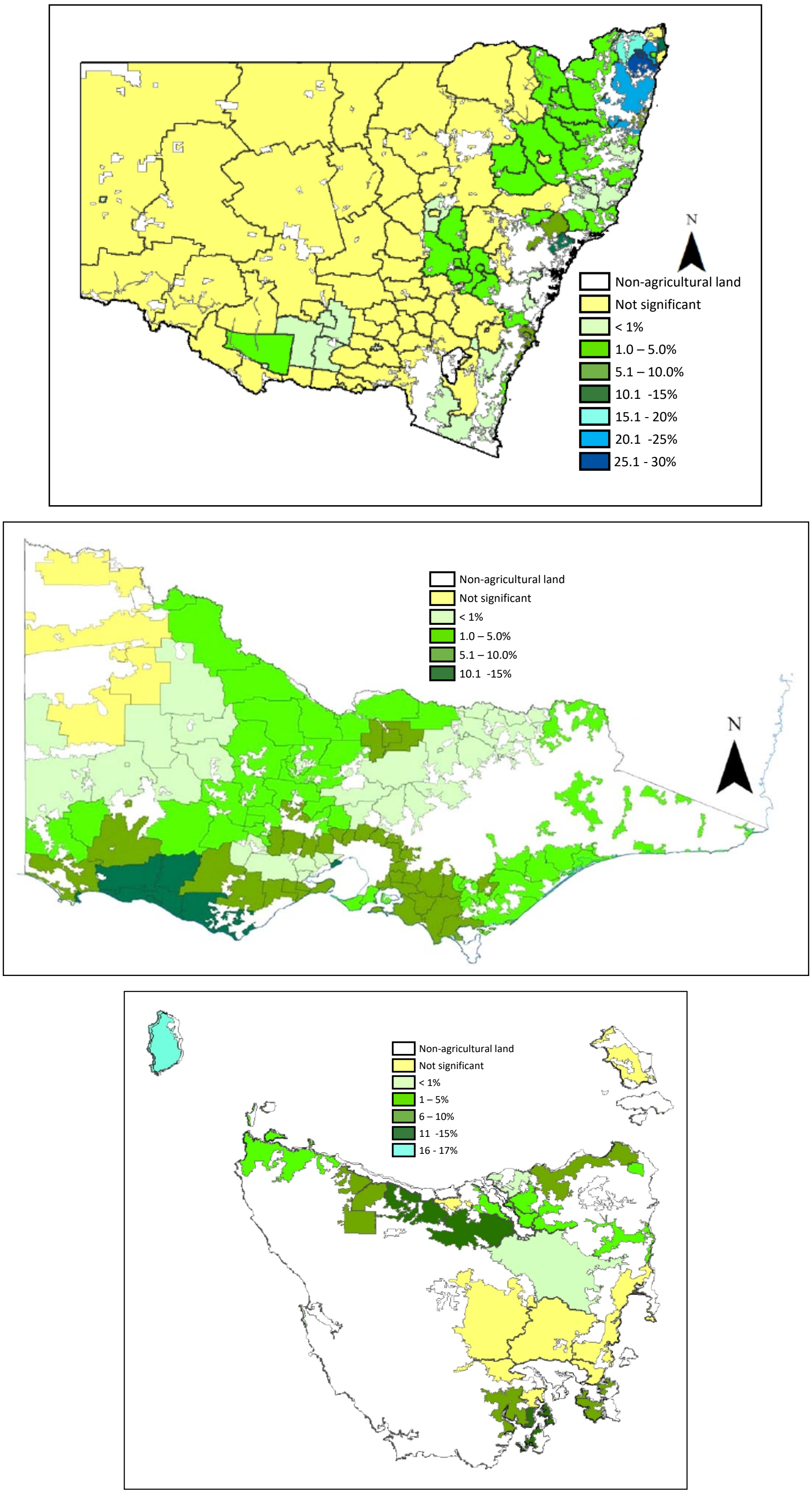

Figure A3: Distribution of white clover pasture in NSW, Victoria and Tasmania. Source: (Donald, 2012). Note: Keys for percantage white clover coverage are given with each state separately. Map scales vary from state to state.

Appendix B Weed Risk Assessment of White Clover

Species: *Trifolium repens* L. (white clover)

Relevant land uses (based on ALUM classification[6]):

1. Conservation and natural environments (Class 1.1 – Nature conservation)
2. Production from dryland agriculture (Class 3.2 – Grazing modified pastures: pasture legumes (3.2.2) and pasture legume/grass mixtures (3.2.3))
3. Production from irrigated agriculture (Class 4.2 – Grazing irrigated modified pastures: pasture irrigated legumes (4.2.2) and irrigated pasture legume/grass mixtures (4.2.3))
4. Intensive uses (Class 5.4 – Residential and farm infrastructure)

Background: In Australia, white clover occurs in a wide range of environments, as deliberate plantings and volunteer populations. It is cultivated in dryland or irrigated cropping areas as a pasture legume for grazing or pasture hay, or as ground cover for horticulture. It is common and widespread throughout the southern and eastern parts of Australia. It is commonly naturalised in Victoria, the Australian Capital Territory, eastern New South Wales, Tasmania, south-eastern South Australia and south-eastern Queensland, and less common in south-western Western Australia (Weeds of Australia, 2016). White clover becomes a weed when its range of growth extends beyond the boundaries of areas of deliberate plantings, which is facilitated by both its seed and its stoloniferous growth habit.

Weeds are usually characterised by one or more of a number of traits, such as rapid growth to flowering, high seed output, and tolerance of a range environmental conditions. Further, they may cause one or more harms to human health, safety and/or the environment. Although white clover has some traits associated with weeds, it is not considered as an invasive weed in Australia because it is not recorded in the Australian government's *Weeds of National Significance* list and the *National Environmental Alert List* on the Department of Agriculture, Water and the Environment website (accessed August 2020), or the *Noxious Weed List for Australian States and Territories* (Invasive Plants and Animals Committee, 2015). In Australia, Groves et al. (2003) consider white clover to be a category 4[7] weed of natural ecosystems, and a category 5[8] weed of agricultural ecosystems mainly due to its presence as a weed in turf in Qld while a category 3[9] or less rating is given in other States. Globally, the weed risk rating for white clover is extreme (Randall, 2017). In NSW it has been rated as a medium risk environmental weed (Downey et al., 2010) and in Victoria as a high risk environmental weed (White et al., 2018).

The Weed Risk Assessment (WRA) methodology is adapted from the Australian/New Zealand Standards HB 294:2006 National Post-Border Weed Risk Management Protocol (Virtue et al., 2006). The questions and ratings used in this assessment are based on the South Australian Weed Risk Management Guide

[6] ALUM refers to the Australian Land Use and Management classification system version 8 published October 2016 (ABARES, 2016).

[7] Category 4 weeds are characterised as naturalised and known to be a major problem at 3 or fewer locations within a State or Territory.

[8] Category 5 weeds are characterised as naturalised and known to be a major problem at 4 or more locations within a State or Territory.

[9] Category 3 weeds are characterised as naturalised and known to be a minor problem warranting control at 4 or more locations within a State or Territory.

(Virtue, 2008). Questions 1–5 relate to the invasiveness of white clover and questions 6-11 relate to the impact of white clover on relevant land use area. Unless cited, information in this appendix is sourced from the main document, *The Biology of* Trifolium repens L. (*White clover*) v3 (OGTR 2020).

This WRA is for **non-GM white clover volunteers**. Reference made to white clover as a cultivated pasture species is only to inform its assessment as a volunteer. It is consistent with previous assessments of white clover in Australia described in Section 8 and provides a baseline for the assessment of GM white clover crops.

Invasiveness questions	White clover
1. What is white clover's ability to establish amongst existing plants?	**Rating: Medium in all relevant land uses** White clover is able to grow in soil usually considered infertile due to its ability to fix nitrogen through nodulation by rhizobia but grows best under agricultural conditions with irrigation and application of fertilisers to maintain productivity. In pastures, white clover is predominantly grown in conjunction with grasses, such as perennial ryegrass. White clover is able to establish through seedlings and the growth of stolons. In situations of heavy grazing or mowing, the plant is not able to set seed so the primary mode of growth is vegetative via stolons. White clover tends to establish in areas that have been disturbed and where there is minimal competition from other plant species. It is common in grasslands and present to a lesser degree in woodlands. Although white clover usually does not dominate the invaded grassland communities, it can still be a significant environmental weed due to its high adaptability. When under favourable conditions, it can compete for growing space with native plants and may even exclude native species by forming a mat.
2. What is white clover's tolerance to average weed management practices in the land use?	**Rating: Low – Medium in all relevant land uses** White clover is susceptible to several broad spectrum and broadleaf herbicides that are used for the control of a range of weeds in agricultural production areas, in residential gardens and in open urban and recreational spaces. In agricultural land uses, white clover has low tolerance of primary cultural methods of weed control, in particular the establishment of and management for dense plant populations of the target crop or pasture. White clover is relatively tolerant of land cultivation, which may be a weed control practice in many land uses. Cultivation may destroy a portion of established plants but in suitable conditions, fragments of stolons may be able to re-establish. In recreational, residential or roadside land uses, physical methods of weed control such as mowing or slashing will not control white clover. There may be instances where white clover is a 'welcome' volunteer in these land uses.

Invasiveness questions	**White clover**
3. Reproductive ability of white clover in the land use:	
3a. What is the time to seeding in the land uses?	**Rating: < 1 year** White clover is perennial. However, when environmental conditions are not favourable for vegetative reproduction such as drought and high temperature, it can develop to maturity in one growing season. Thus, seed is produced in less than one year.
3b. What is the annual seed production in the land use per square metre?	**Rating: High in all relevant land use areas** As a perennial, white clover has indeterminate growth from stolon tips that form either secondary stolons or flowers and therefore can have seed production over several years. Seed production varies among different white clover cultivars. Soil water content, herbage removal time, and stand age are also primary agronomic factors that can affect seed yield. A study in the USA showed that the mean seed yield from six white clover cultivars in the first and second seed production years are 402 kg/ha (40.2 g/m^2) and 275 kg/ha (27.5 g/m^2), respectively (Medeiros and Steiner, 2000). Considering that the white clover's 1000-seed weight is around 0.7 g, this means greater than 57,000 seeds/m^2 and 39,000 seeds/ m^2 in an agricultural setting. Reduction of seed yield in the second year has been shown to be correlated with increase of vegetative growth over reproductive development. Taking into account lower plant densities in volunteer populations where vegetative growth might be encouraged, white clover is expected to produce less seeds than that in agricultural settings but would exceed the guidance value of >1000 seeds/m^2 for high annual seed production.
3c. Can white clover reproduce vegetatively?	White clover can reproduce by vegetative propagation. It multiplies rapidly by sending out stolons, or root offshoots that grows from the mother plant to create new plant. It generally has a prostrate, stoloniferous growth habit.

Invasiveness questions	**White clover**
4. Long distance seed dispersal (more than 100 m) by natural means in land uses:	
4a. Are viable plant parts dispersed by flying animals (birds and bats)?	**Rating: Occasional in all relevant land uses** Viable white clover seed can be recovered from birds such as sparrows, pigeons, pheasants and rooks. The dispersal of viable segments of white clover by flying animals has not been reported in Australia or overseas. Further, the success of dispersal would depend on stolon segments being transferred to a conducive location to take root, which requires adequate moisture availability and soil contact.
4b. Are viable plant parts dispersed by land based animals?	**Rating: Occasional in all relevant land uses** Transport of seeds or vegetative propagules in the hooves or other body parts of land-based animals is possible, yet not likely as these propagules have no particular features such as burrs, barbs or sticky surfaces that would attach to fur or feet of animals. Also, dispersal by vegetative propagule would depend on stolon segments being transferred to a conducive location to take root, which requires adequate moisture availability and soil contact. Some dispersal via the digestive system of wild land-based animals is expected, given that white clover seeds are known to survive digestion in livestock for several days after consumption. In addition, seed-hoarding rodents or marsupials might transfer seeds over relatively short distances. However, there are no reported incidences of this in Australia. Short-distance transport of viable white clover seed by ants has been observed, but this is unlikely to travel long distance.
4c. Are viable plant parts dispersed by water?	**Rating: Occasional in all relevant land uses** The most likely avenue of dispersal of viable plant parts by water is the transportation of stolon fragments from pasture production areas in close proximity to waterways that supply and drain irrigation water. The morphology of white clover seeds may facilitate transport in water, but there are no direct reports. The seeds are light and numerous. Concern about white clover as a weed in riparian zones in the USA (White, 2013) seems to confirm its dispersal by water.

Invasiveness questions	White clover
4d. Are viable parts dispersed by wind?	**Rating: Unlikely in all relevant land uses** Dispersal of white clover seeds by wind is possible, but unlikely over long distances. White clover seed pods develop very close to the ground, reducing the likelihood of wind dispersal. Further, no physical characteristics aiding wind dispersal are apparent on white clover seeds. Stolon fragments that could form new plants are unlikely to be dispersed by wind over long distances.
5. Long distance seed dispersal (more than 100 m) by human means in land uses:	
5a. How likely is deliberate spread via people	**Rating: Common in all relevant land uses** White clover has been spread deliberately throughout the world by humans as a species for pasture production, lawns in gardens and public areas, and ground cover in horticulture. It is also deliberately spread throughout Australia, and the world, as certified seed for sowing in widely distributed pastures.
5b. How likely is accidental spread via people, machinery and vehicles?	**Rating: Occasional - common in all relevant land uses** Accidental dispersal could be associated with the disposal of garden waste, or transfer of ground cover and topsoil from building sites. Small viable sprigs of the plant may have the opportunity to transfer to non-target areas on the tyres and wheels of vehicles and machinery. The success of dispersal would depend on sprigs being transferred to a conducive location to take root, which requires adequate moisture availability and soil contact. Spillage during seed transport is a major source of volunteer populations along transport routes, and in agricultural supply centres.
5c. How likely is spread via contaminated produce?	**Rating: Occasional in all relevant land use areas** Seeds and viable stolon segments of white clover could feasibly be a contaminant of landscaping materials (soil, sand, gravel and mulch) given these materials are sourced and stored in areas conducive to the growth of white clover.

Invasiveness questions	**White clover**
	If white clover is harvested for hay or fodder, long distance dispersal via hay and forage containing white clover seeds may occur from cropping areas to areas for supplementation feeding of livestock, particularly during drought.
5d. How likely is spread via domestic/farm animals?	**Rating: Common in all relevant land uses** Fragments of plants could be spread in mud on animal hooves as animals are moved from one paddock to another, to a feedlot, or to other farms. This pathway is limited as white clover seed and viable stolon segment have no particular features such as burrs, barbs or sticky surfaces that would attach to fur or feet of animals. However, white clover seedpods are more easily entangled in animal coats, especially sheep wool. Vegetative dispersal would depend on stolon segments being transferred to an appropriate location to take root, which requires adequate moisture availability and soil contact. White clover biomass and seeds are consumed widely as fodder by ruminants. Whilst the optimum fodder quality occurs during early growth before seed has set, grazing mature seed-bearing pastures to stubble is a widespread land management practise. Dispersal of viable white clover seed has been observed by endozoochory, and a small proportion of white clover seed can be viable after digestion by ruminants so this likely forms a common pattern of dispersal.

Impact Questions	**White clover**
6. Does white clover reduce the establishment of desired plants?	**Rating: Reduces establishment by < 10% reduction in all relevant land uses** Once established, white clover forms a thick dense sward that can reduce the establishment of, and for some species displace, other plants. This may be a desirable trait in pasture; however it inhibits the establishment of desirable species outside its intended range. It is most problematic in the sub-alpine regions of south-eastern Australia, and in some areas it threatens endangered species and native plant communities (Weeds of Australia, 2016). White clover is allelopathic, reducing the establishment of some plant species in the close vicinity.

Impact Questions	White clover
7. Does white clover reduce the yield or amount of desired plants?	**Rating: Reduces yield/amount by <10% reduction in all relevant land uses** White clover may reduce the yield of desired vegetation when established. It may be vigorous and can out-compete native or other desirable vegetation due to its ability to fix atmospheric nitrogen when nutrition is a limiting factor. It establishes rapidly and is especially competitive in disturbed land. Nonetheless, commonly used weed management practices would lead to low density of white clover volunteers in relevant land uses, and would cause a moderate to low reduction in yield of desired vegetation. In home and public gardens, runners of volunteer white clover may encroach on bare soils and prevent the growth and coverage expected of desired plants. White clover is also used as ground cover in plantations, suggesting that it does not reduce the yield of plants that have taller canopies and deeper root systems than white clover.
8. Does white clover reduce the quality of products or services obtained from the land use?	**Rating: Low in all relevant land uses** In general, white clover is a desirable species for forage due to its vigour and nutritional qualities and for ground cover in horticulture. However, the establishment of volunteer white populations could reduce the quality of products and services in some land uses. In turf cultivation, it may enter sod production areas and reduce product quality. This is the main reason why white clover is considered a category 5 weed in Qld. In pasture areas, white clover volunteer populations may displace more preferred pasture species, or lead to allelopathic reduction in yield of desired pasture species. White clover volunteers may contaminate seed harvests in other similar crops, however effective management of white clover volunteer populations limits this possibility. On land used for nature conservation, white clover may marginally reduce biodiversity.
9. What is the potential of white clover to restrict the physical movement of people, animals, vehicles, machinery and/or water?	**Rating: None in all relevant land uses** White clover is a low growing plant, and does not restrict the physical movement of people, animals, vehicles, machinery, and water even when densely planted in an agricultural setting. Volunteer populations, being less densely grown than agricultural crops would not restrict the movement of

Impact Questions	**White clover**
	people, animals, vehicles or machinery. Dense swards that have had the opportunity to develop in seasonal channels or waterways may marginally slow the flow of water, but white clover would not survive in permanently or frequently inundated waterways.
10. What is the potential of white clover to negatively affect the health of animals and/or people?	**Rating: Low in all relevant land uses** White clover is not known to cause diseases in humans and animals and it does not obviously cause any allergic reactions in humans through its pollen or foliage. However, white clover can potentially be toxic to grazing animals if ingested in large quantities due to cyanide poisoning following cyanogenesis. It may also cause bloat in ruminants, similar to some other legumes.
11. Major positive and negative effects of white clover on environmental health in each relevant land use:	
11a. Does white clover provide food and/or shelter for pathogens, pests and/or diseases in the land use?	**Rating: Minor or no effect in all land uses** A range of pests and pathogens are reported to affect white clover. Volunteer white clover populations, being outside active management, might be expected to harbour pathogens and pests that could affect pastures containing white clover, other crops or other adventitious targets. White clover can be infected by or harbour a number of viruses including WCMV, AMV, CYVV and some minor viruses. Although these viruses mainly affect white clover pasture production, they may also affect other closely related plant species. The vast majority of white clover is grown in pastures, so it might be expected that the risk of pathogens and pests spread from volunteer populations is outweighed by the risk of pathogen spread from minimally-managed pastures.
11b. Does white clover change the fire regime in the land use?	**Rating: Minor or no effect in all relevant land uses** Ruderal or volunteer white clover is not known to increase fire risk in any of the land uses it may occur. Primarily its low and evergreen growing habit reduces fire risk.

Impact Questions	**White clover**
11c. Does white clover change the nutrient levels in the land use?	**Rating: Rating: Minor or no effect in all relevant land uses** When seed is inoculated with *Rhizobium* at time of planting, white clover can fix atmospheric nitrogen, increasing the nitrogen content of field soil for the subsequent crop. Ruderal white clover plants germinate from uninoculated seeds, so nodulation and nitrogen fixation is expected to be reduced and thus the effects on the nutrient levels in nature reserves would be limited.
11d. Does the species affect the degree of soil salinity in the land use?	**Rating: Minor or no effect in all relevant land uses** As a ruderal weed, volunteer white clover may have a small positive effect on soil salinity, i.e. reducing the extent of percolation of water and salt transport through the soil profile by active transpiration in an otherwise non-vegetated area.
11e. Does the species affect the soil stability in the land use?	**Rating: Minor or no effect in all relevant land uses** White clover is commonly used as ground cover (living mulch) in horticultural situations. As a volunteer weed, the presence of white clover may have a positive effect in stabilising light-textured or sandy soils.
11f. Does the species affect the soil water table in the land use	**Rating: Minor or no effect in all relevant land uses** White clover has a shallow root system and would not be expected to affect the soil water table by depletion of water from the soil. As a ruderal weed, volunteer white clover may have a small positive effect on the soil water table, i.e. by reducing the extent of percolation of water through the soil profile by active transpiration in an otherwise non-vegetated area.
11g. Does the species alter the structure of nature conservation by adding a new strata level?	**Rating: Minor or no effect in all relevant land uses** The morphology of the plant is similar to other small legume species that are widespread in Australia and therefore white clover would not be expected to add a new strata level to nature conservation.