

## OESTROGENIC SUBTERRANEAN CLOVER GUIDE

**IDENTIFICATION AND REMEDIATION** 

### OESTROGENIC SUBTERRANEAN CLOVER GUIDE IDENTIFICATION AND REMEDIATION

### Important disclaimer

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#### Authors

Kevin Foster, Megan Ryan and Daniel Kidd; The University of Western Australia, 2022.

### Design and illustrations

Ooid Scientific and Chloe Joyce. www.ooidscientific.com and www.chloejoyceillustration.com Photographs

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Department of Primary Industries and Regional Development



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## HOW TO USE THIS GUIDE

This pictorial guide is designed to assist with the identification and remediation of **oestrogenic** subclover pastures in Australia.

Five cultivars of subclover have been highlighted in this guide due to their high levels of the oestrogenic compound **formononetin** in leaf tissue. These cultivars are Dwalganup, Geraldton, Yarloop, Dinninup and Tallarook. These are arranged in order of **time to first flowering.** 

The primary identification tools in this guide are photographs and illustrations. The text is organised around a general description, followed by highlights of key features to aid correct identification. Photographs of each subclover cultivar are shown in order of growth stage during the season. It is important to note the leaf marks often fade mid flowering onwards and other characters such as leaf shape change from winter to spring. Leaf marks are best observed from about the fourth leaf stage onward. The colour of the **stipules** and **calyx** is most apparent when exposed to sunlight, thus in a dense pasture sward the pigmentation will be weaker. **Calyx** colour remains fairly constant during most of the flowering period and is an excellent aid in identification of cultivars.



To further aid with identification, the five oestrogenic subclover cultivars are compared to those subclovers that are similar in appearance but considered safe to graze due to a much lower formononetin content. Side-by-side comparisons of key plant characters during the season are provided to aid in distinguishing the cultivars.

The second half of this guide provides decision-making tools to help you evaluate whether you have a significant problem with oestrogenic subclover in your pasture. Guidance is provided on how to perform a paddock assessment and the best time to do so. Information is also provided on the effect of oestrogenic subclover on sheep health. However, always consult your local veterinarian to rule out other causes.

**Bolded text** throughout the guide is defined in the glossary at the end of the guide.

## INTRODUCTION

#### A background on oestrogenic subterranean clovers in southern Australian pastures

Subterranean clover (subclover, *Trifolium subterraneum* L.) is an annual pasture legume that provides high quality feed for livestock. It is estimated to be sown over 30 million hectares across southern Australia, with cultivars developed for the low, medium and high rainfall zones. Unfortunately, some older cultivars contain high levels of **phytoestrogen** which can affect the reproductive system of sheep and cause other health issues. This became a significant issue for sheep producers from the 1940s as new land was cleared and sheep grazed subclover-dominant pastures. There was a collection of livestock issues observed and these were grouped together under the umbrella term of "**clover disease**".

By the 1960s, the **oestrogenic** compound responsible for clover disease was identified as **formononetin**, which is largely present in green leaves. Leaf tissue testing identified that four cultivars of subclover subspecies *subterraneum* (Dinninup, Dwalganup, Geraldton, Tallarook) and one cultivar from subspecies *yanninicum* (Yarloop) were high in formononetin and these were removed from commercial production. Plant breeders responded by screening for new subclover selections with lower (safer) levels of formononetin and by the 1990s the issue was presumed to have been resolved.



However, the oestrogenic cultivars have proven highly adaptable and persistent, with a combination of high hard seed levels, prolific seed production and excellent **burr** burial. In addition, high levels of formononetin may reduce animal preference when green (relative to other cultivars). Thus, these cultivars increasingly dominate in semi-permanent and permanent pastures. In addition, these high oestrogen cultivars may be present in pastures which have been renovated with new cultivars. This is because they are competitive and continue to produce seed. The issue has received little attention since the 1990s prompting a recent response to re-educate and extend this message to livestock producers, consultants and veterinarians.

### SUBCLOVER CHARACTERS FOR IDENTIFICATION



### SUBCLOVER LEAF MARKING PATTERNS

### GREEN CRESCENT OR BAND

### WHITE OR PALE GREEN ARMS







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## DWALGANUP

### **KEY FEATURES**:

Trifolium subterraneum (ssp. subterraneum). Introduced commercially in 1934 and widely distributed, particularly in Western Australia. Similar appearance in many ways to Dalkeith (see page 19). Can be distinguished from Dalkeith by lack of leaflet indentation, presence of a **flush** pattern in winter and high formononetin content. Leaf is grey-green in colour and hairy on both sides. **Stipules** green with reddish veins.







RUNNFR

Hairy

## **△** GERALDTON

### **KEY FEATURES**:

Trifolium subterraneum (ssp. subterraneum). Naturalised strain released commercially in 1959 and sown extensively in low rainfall, cereal and sheep districts. Similar to Nungarin (see page 20) but can be distinguished by its distinctly spaced leaflets and by its thinner leaf band. The **stipules** are green with red veins, the **petioles** are relatively short and often with red pigmentation.





## **△** GERALDTON

#### LEAF MARK:



### **KEY FEATURES**:

Trifolium subterraneum (ssp. yanninicum). A white-seeded cultivar that grows well in winter waterlogged soils. Seedling can be very erect and the **hypocotyl** can be green or have a faint brown pigmentation. Whole leaflet can show red pigmentation in winter. Strong reddish-purple **stipule** pigmentation. Runner, **peduncles**, **petioles** and leaf upper surfaces are hairless. **Burr** often contains only 1-3 seeds.



VI-3 seeds. Unifoliate leaf Winter leaf WINTER WIN

CALYX:



Unifoliate leaf

### **KEY FEATURES**:

Trifolium subterraneum (ssp. subterraneum). Broadly adapted cultivar that can become dominant in pastures due to prolific seed set. Very good **burr** burial and high levels of hard seed. Leaflets have little to no indentation or flecking. In winter, strong tendency to produce a distinctive brown flush along the midrib. **Peduncle** very hairy especially at end near base of florets. Spring leaf similar to York, Goulburn and Woogenellup but can be distinguished by leaf shape, stem, leaf hairiness and formononetin content (see page 21).

WINTER

Winter leaf

Spring leaf



Runner

SPRING

Flower

### LEAF MARK:

**Unifoliate leaf**: very rounded with slight indent and often brown flush along midrib.

Trifoliate leaf: large wide pale green crescent and weak arms which can be difficult to observe early and late in the season. In winter, has a distinctive brown flush pattern and thin red line surrounding leaf mark, coming to a "sharp peak" at the top of the leaflet. Moderately broad leaves with little indentation. Little to no flecking. The arms can also be difficult to see later in the season and the crescent appears more flattened once the brown flush disappears.



### **KEY FEATURES**:

Trifolium subterraneum (ssp. subterraneum). Naturalised strain released in Tasmania in 1935. Leaflets reasonably broad. Moderately strong **anthocyanin** flecking and flush. Similar to Junee but can be distinguished by the hairiness of its runners and **peduncles** (see page 24). Very late maturity, flowering around mid October. **Stipules** and **calyx** are green. Limited plantings in Western Australia.





#### I FAF MARK Unifoliate leaf: sometimes with pale CALYX: green central dot and white arms Green calyx with extending to edge of leaf. no pigmentation Trifoliate leaf: crescent with white arms which tend to fade in spring. During winter there is a well-marked pale green central crescent with white arms which often forms a fringe of brown around the crescent and a chocolate area below it. Slight indentation of leaflets. RUNNFRS: Hairy STIPULE: Green

### OTHER SUBCLOVER CULTIVARS WITH POTENTIAL TO ADVERSELY IMPACT SHEEP

The following cultivars are not widely sown and/or not generally associated with **clover disease**. However, there is a risk that under adverse environmental conditions, such as severe soil nutrient deficiency (particularly that of phosphorus and sulphur) and waterlogging, formononetin may increase to levels considered unsafe for sheep.



#### 

### ssp. subterraneum

Mid-season cultivar. Low to moderate formononetin. Leaf mark is a large triangular C<sup>3.4</sup> crescent and occasionally very narrow arms. Calyx green and moderate to strong **anthocyanin** leaf flecking.



### ESPERANCE

ssp. subterraneum

Mid–late season cultivar. Low to moderate formononetin. C<sup>1</sup> leaf mark but often not apparent. Moderate leaf **anthocyanin** flecking and occasionally midrib flush. Deep reddish/purple pigmentation along whole **calyx**.



#### WINTER SPRING HOWARD ssp. subterraneum Mid-season cultivar. High formononetin. Not sown in

formononetin. Not sown in Western Australia. C<sup>1</sup>A<sup>2</sup> leaf mark. Arms very white and **calyx** and **stipules** are green. Strong purplish brown flush in basal area below leaf mark early in the season.







### METEORA

ssp. yanninicum

Late season cultivar. Formononetin higher than desirable. C<sup>4</sup> leaf mark which becomes flatter during the season to look more like a B<sup>1</sup> band. Moderately strong brown flush along the midrib. Hairless runners, **petioles**, **peduncles** and upper leaf surface. Calyx are green. Cream to amber seeds.



### TRIKKALA ssp. yanninicum

Mid season cultivar. Not generally associated with **clover disease** but recent testing found formononetin higher than desirable. C<sup>2</sup>A<sup>1-2</sup> leaf mark. Strong tendency to produce a brown flush often covering the entire basal area below leaf mark. Hairless runners and slightly hairy **peduncles** and upper leaf surface. Little to no flecking and green **calyx**. Cream to amber seeds.



#### WINTER SPR WOOGENELLUP ssp. subterraneum

Mid season cultivar. Generally considered low risk to sheep but can be a problem early in the season especially if under stress. Leaf mark  $C^2A^2$  with heart shaped leaflets with strong indentation. Arms pale green. Later in the season crescent becomes flattened to look more like a  $B^2$  band. **Calyx** tube green and stems smooth. **Stipules** often have strong red pigmentation.

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### DISTINGUISHING BETWEEN OESTROGENIC AND NON-OESTROGENIC CULTIVARS













LEAF WOOGENELLUP DINNINUP GOULBURN  $\wedge$ YORK Hairier leaves than Crescent Crescent Dinninup smaller and becomes flattened stronger arms **FLOWER** DINNINUP WOOGENELLUP GOULBURN YORK  $\triangle$ Very hairy penduncle 1/4 -1/2 Slightly hairy -penduncle Most of calyx calyx tube No calyx pigment tube red red 22











# THE EFFECT OF OESTROGENIC SUBCLOVER ON SHEEP

There are a number of sheep fertility and health issues that occur as a result of grazing oestrogenic subclover pastures. The most common sign of flock fertility issues is a greater than expected proportion of dry ewes at scanning, increase in prolapse, still births, **dystocia** and/or reduced lambing percentages. Oestrogenic pastures can cause two distinct infertility conditions in ewes; these being temporary and permanent infertility.

### TEMPORARY INFERTILITY

Temporary infertility occurs when ewes graze on green oestrogenic subclover pastures just before or during joining causing a reduced ovulation rate and a temporary reduction in fertility. Ewes usually recover after several weeks once moved onto safer pastures. Temporary infertility may also occur in the absence of any visible signs, particularly in Merino ewes. However, observing more dry ewes in the flock than would be expected would indicate a problem. Temporary infertility may be less common in when sheep are joined on dry pasture which has lost most or all of its oestrogenicity.



Image: © State of Victoria (Department of Primary Industries)





### PERMANENT INFERTILITY (Clover disease)

Ewes grazed for several seasons on oestrogenic subclover may suffer permanent infertility which becomes progressively worse with continued exposure. The reproductive tract of the ewe is permanently impaired and does not allow for the successful movement of sperm to the egg. This can occur even when ewes are moved onto dry or non-oestrogenic pastures. Permanent infertility can occur in the absence of any obvious clinical signs.







Uterine prolaspe

### DYSTOCIA

A difficult or prolonged birth caused by uterine inertia resulting in the birth of weak or dead lambs as well as significant stress to the ewe, or death.

### UTERINE PROLAPSE

Where the uterus effectively turns inside out and protrudes from the rear of the ewe (see image to left). The extent of the prolapse can vary but the ewe normally dies or must be euthanised.

### FALSE BLADDER

Can occur in wethers and is a result of an enlarged **bulbourethral gland** causing swelling below the anus. Commonly associated with urinary obstructions.





## LACTATION IN MAIDEN EWES AND WETHERS

The development of udders and lactation among non-bearing or maiden ewes and wethers.

### EWE AND LAMB MORTALITY

Although ewe and lamb mortality can be a common occurrence on any pasture, there is often a higher incidence for livestock grazing oestrogenic pastures.

### EFFECTS ON RAMS

There is currently no scientific evidence of fertility issues associated with rams grazing oestrogenic subclover pastures. However investigations are continuing.

### EFFECTS ON OTHER RUMINANTS

Little research has been conducted in Australia on the effect of oestrogenic subclovers on other ruminants. However, there are reports from overseas of temporary infertility of livestock from the feeding of red clover silage.



Photo: Getty Images.

### HOW TO DO A PADDOCK ASSESSMENT FOR OESTROGENIC SUBCLOVER

### WHEN AND HOW TO ASSESS YOUR PADDOCK ...

Depending on the cultivars present in your paddocks you can likely distinguish between them early in the season at the **unifoliate** or early **trifoliate** leaf stages. The best time to identify cultivars by leaf mark is mid winter as leaf markings and the coloured pigmentation or flush and flecking is the most visible due to cold temperatures. Leaf marks often fade or change considerably into late spring, which makes identification by leaf mark alone more difficult. However, the presence of flowers, stipules and runners in spring can also aid identification. Note that pigmentation of the **calyx** in some cultivars is often only apparent when exposed to sunlight and may not be fully expressed in a dense sward.





### PASTURE COMPOSITION

If oestrogenic subclover has been identified and comprises >20% of your pasture composition then it may have a detrimental effect on the fertility or health of your sheep. Here are two useful techniques to help you gauge your pasture composition:

### QUADRAT SAMPLING

This method involves the use of small **quadrats** (e.g.  $0.25 \times 0.25$  m) to sample the paddock. These quadrats can be made from PVC pipes and elbows. Walk a measured line across your paddock and randomly place your quadrat on the line. Cut the sample inside the quadrat at ground level. Separate the sample into their various pasture components (i.e. grasses, weeds and subclover), identify cultivars and oven-dry. You will need at least 10 assessments, with more required if the pasture composition is highly variable. Like components are then weighed and the results from each quadrat combined. This can give a very accurate representation of the pasture composition, but is time consuming.



Separate into like components, identify the cultivar, dry and weigh.



### ROD POINT TECHNIQUE

- Take a 400 mm long rod and drop it at least 50 random spots across your pasture by starting at a corner of a paddock and walking diagonally across it.
- 2. Each time the rod is dropped, record the plant that is touching or close to each end of the rod. It can be as simple as "clover", "other legume", "broadleaf weed" and "grass".
- 3. Once the plant is recorded, identify the nearest subclover at each end of the rod using this guide to help you.
- 4. Calculate the proportion of each pasture component in the paddock (see next page).





### CALCULATING PASTURE COMPOSITION

(A) Throw the rod and record the nearest plant touching each end of the rod (B) as either grass (C), broadleaf weed (D) or clover (E). At each end of the rod also identify the nearest clover and record in column (F), this gives the relative abundance of each clover type.

ROD THROW (A)	ROD POINT (B)	GRASS (C)	BROADLEAF WEED (D)	CLOVER (E)	CLOVER ID (F)
1	1			$\checkmark$	Dalkeith
I	2	$\checkmark$			Dinninup 🛆
2	3		$\checkmark$		Yarloop 🛆
	4			$\checkmark$	Dalkeith
3	5			$\checkmark$	Dinninup 🛆
3	6		$\checkmark$		Dalkeith
4	7	$\checkmark$			Dalkeith
	8	$\checkmark$			Dinninup 🛆
		3	2	3	



## CALCULATION FOR PASTURE COMPOSITION

Add up all columns in table. Calculate proportion of grass, broadleaf and clover in the pasture by:

% Grass = C/B = 3/8 = 37.5%% Broadleaf = D/B = 2/8 = 25%% Clover = E/B = 3/8 = 37.5%

The pasture contains 37.5% grasses, 25% broadleaf and 37.5% clover.

## COMPOSITION OF SUBCLOVER CULTIVARS

Add up the number of times each clover cultivar occurs in column (F)

• Dalkeith = 4, Dinninup = 3, Yarloop = 1

Divide each cultivar total by the total number of observations taken in the paddock (B)

- Dalkeith = 4/8 = 50%
- Dinninup = 3/8 = 37.5%
- Yarloop = 1/8 = 12.5%

The percentage composition of the subclover cultivars is 50% Dalkeith, 37.5% Dinninup and 12.5% Yarloop.



## CALCULATION OF THE OESTROGENIC SUBCLOVER % IN THE PASTURE

% Oestrogenic subclovers of pasture = (percentage of oestrogenic clovers x total subclover in pasture)/100

% Oestrogenic subclovers of pasture = ((37.5% (Dinninup) +12.5% (Yarloop)) X 37.5% (total clover))/100 = **18.8**%

## PADDOCK RANKING: SAFE

Percent oestrogenic subclover in the paddock: 0 – 20 % Safe 20 – 40 % Moderate 40 – 100% Potent

Modified from Little and Frensham (1993). A rod-point technique for estimating botanical composition of pastures. *Australian Journal of Experimental Agriculture* 33, 871-875.



### HOW TO MANAGE A PASTURE PADDOCK IF YOU SUSPECT A PROBLEM

### MANAGE YOUR SHEEP ROTATIONS

- Do not allow maiden ewes or lambs to graze oestrogenic pastures.
- Graze terminal lambs on high risk pastures.

- Reduce the length of grazing for wethers and older ewes, but monitor for health issues.
- Avoid grazing ewes on oestrogenic subclovers, including germinating pastures just before or at the time of mating as this can cause temporary infertility.





### MANAGE YOUR FEEDBASE

• Dilute oestrogenic subclover intake by promoting the growth of other pasture species including weeds.

• Don't grass clean, this will likely increase the sheep intake of oestrogenic subclover.

• Sow competitive species such as ryegrass or oats to dilute the subclover content.

• Provide alternative feed sources.



Oats crop. Image: © State of Western Australia (Department of Primary Industries and Regional Development, WA)



### MANAGE YOUR SOIL NUTRITION

Ensure soil nutrition is adequate as severe deficiencies of nutrients such as phosphorus and sulphur may increase the formononetin content in green leaf tissue.



Image: Getty Images.

Image:  $\ensuremath{\mathbb{C}}$  State of Western Australia (Department of Primary Industries and Regional Development, WA)





### PLANT DEVELOPMENT AND OESTROGENIC CONTENT

Subclover pastures are oestrogenic while they are green. However, formononetin levels in leaf tissue often peak prior to flowering and then decrease as the plant dies. Avoid grazing subclover pastures that are waterlogged as this increases the formononetin levels in the green leaves. Grazing oestrogenic subclover 4 – 6 weeks after the pasture naturally dries off is currently considered safe. However, silage or green hay that is dried quickly may maintain high levels of formononetin.





#### **RENOVATE YOUR PASTURES**

• Significantly reducing the oestrogenic subclover seed bank will improve the successful establishment of new pasture cultivars when renovated. Where possible, crop the paddock for several years and produce a dense leafy canopy to smother the subclover. Importantly, apply appropriate broadleaf herbicides in-crop to prevent the oestrogenic subclover setting seed.

• When sowing a new subclover cultivar consider a double knockdown to control background subclover and then sowing the new variety at a high rate (15-20 kg per ha) to ensure it can out-compete any remaining oestrogenic cultivars. • Do not move sheep from dry oestrogenic pastures straight onto stubble as undigested subclover seed will contaminate the paddock.

• Maximise seed set of your new subclover in year one to allow it to regenerate into a dense sward the following year and thus out-compete any remaining oestrogenic subclover.

• Choose a cultivar to suit your soil type and rainfall zone. New subclover cultivars often have superior vigour and disease tolerance to older cultivars.





• If re-sowing subclover, use certified seed to ensure you are getting the correct cultivar and it has a high level of genetic purity. There is the possibility of reintroducing oestrogenic subclover as a contaminant in uncertified seed.

• Apply the latest **rhizobia strain** at sowing to improve **nitrogen fixation** and productivity, particularly in older paddocks where **rhizobia strains** have been lost or become ineffective.

- Consider this an opportunity to address low soil pH or nutritional deficiencies with lime and fertilisers.
- Be sure to monitor new pastures over time as oestrogenic subclovers are often less preferred by sheep in spring and can their population density can increase in dominance.



Effective rhizobium nodulation on subclover plant. Photo: M. Ryan.



## GLOSSARY

Anthocyanin: red, purple, blue or black pigments that can appear in plant tissue.

**Bulbourethral gland**: a small gland located posterior and lateral to the urethra at the base of the penis of most male mammals.

Burr: a seed or dry fruit that has hooks or teeth.

**Calyx**: the collective name for sepals (which are the base support of a flower). The calyx is the outermost whorl of a flower.

**Clover disease**: a term used to describe the complex of sheep fertility and health issues associated with grazing sheep on oestrogenic subclover pastures.

**Corolla**: The petal of a flower considered as a group. For subclover the colour pattern of the petal is either pure white or white with pink veins.

**Dystocia**: a difficult or prolonged birth caused by uterine inertia resulting in the birth of weak or dead lambs and significant stress to the ewe or even death of the ewe.

**Flush**: purple or brown anthocyanin pigmentation pattern that in some cultivars tends to surround the central leaf mark or extend from the midrib.

**Formononetin**: a phytoestrogen found in a number of plants such as red clover and subclover. This is the major compound identified as responsible for clover disease.

Hypocotyl: the stem of a germinating seedling.



**Oestrogen**: a general term for female steroid sex hormones that are secreted by the ovary and responsible for typical female sexual characteristics.

Peduncle: elongated stem of a flower.

Petiole: the stalk that attaches the leaf blade to the stem.

**Phytoestrogen**: a plant-derived non-steroidal plant compound that has structural similarity to estradiol (an oestrogen) and can cause estrogenic and/or antiestrogenic effects.

**Quadrat**: A quadrat is a frame, traditionally square, used in ecology to isolate a standard unit of area for study of the distribution of an item over a large area.

**Rhizobia**: Rhizobia are bacteria inside the root nodules of legumes that fix nitrogen from the atmosphere to organic forms.

**Stipule**: an outgrowth borne on either side (sometimes just one side) of the base of a leafstalk (the petiole).

Time to first flowering: from early May sowing in Perth and is averaged over the seasons. Trifoliate: a leaf with three leaflets.

**Truncate base**: leaf base or leaf apex appearing to terminate abruptly, as if by cutting off. **Unifoliate**: a leaf with a single leaflet (often referred to as a spade leaf).

