

## Sheep as biological control agents against St. John's wort (*Hypericum perforatum* L.): factors affecting hypericin variation and hypericin tolerance

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**Summary** The seasonal variation in hypericin production by two biotypes of *Hypericum perforatum* was determined on field grown plants. It varied from <100 ppm to almost 5000 ppm. The relative hypericin production potential of 12 Australian biotypes of *H. perforatum* was assessed using glass house grown plants. At full flowering they varied between 1212 and 2767 ppm. Hypericin production suppression in an *Hypericum* (broad leaved biotype) pasture, following heavy grazing during spring and early summer, was assessed. By early January protected plants contained hypericin at 1400 ppm, but regrowth on grazed plants contained only 350 ppm. Hypericin tolerance was appraised in three wool types of Merino sheep and two breeds of cattle (Angus and Hereford). Only animals exposed to direct sunlight were affected by ingested hypericin. At 3 mg hypericin per kg body weight 26.5 % of wool protected Merino sheep were found to be intolerant, compared with 94% of shorn sheep. Tolerance varied between different wool types, with superfine sheep being the most tolerant. Cattle were found to be more tolerant to hypericin than previously suspected. Hypericin tolerance appears to be more strongly associated with skin light protection than with a difference in hypericin metabolism and excretion rate. Suggestions for the design of a humane grazing management strategy for *Hypericum* control are outlined.

**Keywords** Hypericin, *Hypericum*, biotype, Merino sheep, wool type, grazing.

### INTRODUCTION

St. John's wort (*Hypericum perforatum*) is an exotic perennial weed that infests large areas of non-arable agricultural land throughout southern Australia. It contains a mixture of potentially poisonous pigments collectively called hypericin. Grazing can be a very effective means of controlling weeds. Sheep are very effective, largely non-selective and non-seasonal defoliators of plants, and sheep can be found in all southern Australian agricultural districts. This combination of factors makes them an ideal choice as biological control agents for weed control in agricultural landscapes. Potentially poisonous weeds such as *Hypericum* present an additional challenge. However, by investigating when a plant might become

poisonous, and under what circumstances an animal may be poisoned by it, it is possible to design relatively safe but effective grazing management strategies for the control of potentially poisonous weeds such as *Hypericum*.

Over many centuries livestock have inadvertently grazed *Hypericum* infested pastures and unfortunately many have been poisoned. Anecdotal evidence has been accumulated in this way to suggest differences in *Hypericum* tolerance between sheep, goats and cattle, but science-based assessments are rare. Sheep and goats have generally been preferred to cattle for weed control, but combinations of either with cattle have sometimes been found to be more effective. Fully pigmented sheep have traditionally been regarded as less susceptible to *Hypericum* poisoning than non-pigmented or partially pigmented animals. Because the Australian sheep and wool industry is based upon the use of non-pigmented breeds of sheep, pigmented sheep are rarely chosen for use against *Hypericum* infestations. The following paper addresses factors affecting the rise and fall of hypericin production in the plant, together with factors affecting the ability of sheep and cattle to tolerate hypericin. It considers a *Hypericum* control strategy based on sheep, but includes a supporting role for cattle.

### MATERIALS AND METHODS

**Hypericin plant determinations** Hypericin levels in *Hypericum* plant material were determined using the method described in Southwell and Bourke (2001). Extractions were carried out on 2 g dry weight samples of plant. The plant was air dried at room temperature in light protected containers. Seasonal hypericin variations were determined on field grown plant material, protected from animal, machine, or chemical defoliation. Two *Hypericum* biotypes, commonly referred to as Tuena narrow-leaved and Orange broad-leaved, were sampled every three weeks over two consecutive years. On each occasion a bulked sample was taken from the green soft growth present on 50 randomly selected plants.

**Australian *Hypericum* biotypes** Hypericin variations were determined on glass house grown plant

material. The potted plants were harvested at the full flowering stage. On the basis of previous genetic studies (Mayo unpublished data), 12 biotypes representative of the full spectrum of *Hypericum* genotypes identified throughout Australia, were used. *Hypericum* hypericin production suppression was examined by the repeated high stocking rate grazing of a 2 ha natural infestation of *Hypericum* (biotype Orange broad leaf), during the spring and early summer of one year, using Merino wethers. At the end of this period, the pasture was left ungrazed for 21 days, then both the *Hypericum* re-growth in the grazed area and the *Hypericum* in an adjacent ungrazed protected area were sampled and hypericin content determined.

**Hypericin animal tolerance studies** Hypericin tolerance in Merino sheep (n = 67) and Hereford (n = 18) and Angus (n = 18) cattle, was assessed by the oral administration of measured amounts of finely milled, air dried, *Hypericum* plant material of known hypericin content. Only minimum toxic (or threshold) dose rates were administered. The hyperthermia response, in sunlight exposed animals, to circulating blood levels of hypericin, was used as the earliest and least stressful clinical indicator of *Hypericum* poisoning (Bourke 2000). Additional confirmatory clinical signs anticipated were agitation, rubbing of the head and face against fixed objects, mild diarrhoea and mild depression. Accordingly animals were given a single dose of *Hypericum* on day 1 and then exposed to direct sunlight on days 2 to 5. Their rectal temperature and clinical appearance was recorded at 9 a.m. and 2 p.m. on each of these days. Animals experiencing a body temperature rise in excess of 40°C on one or more days, together with the above signs, were deemed intolerant to the dose of *Hypericum* administered.

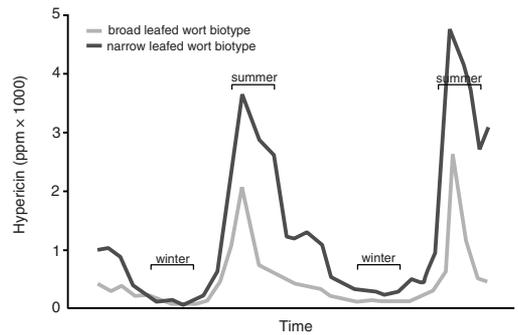
RESULTS

**Plant factors affecting hypericin variation** The hypericin content of two biotypes of *Hypericum*, growing under field conditions, and recorded over two consecutive years, are presented in Figure 1. The following mean hypericin values were demonstrated. From mid-September to late December the hypericin levels increased from 73 to 2438 ppm for the broad leaved type and from 186 to 4092 ppm for the narrow leaved type. From early January to mid March they fell from 983 to 439, and from 3455 to 2160 ppm respectively. From late March to early September they continued to fall from 416 to 73, and from 1144 to 186 ppm respectively. The lower rainfall year resulted in lower hypericin production levels than those observed for the higher rainfall year. Peak hypericin production in the year of above average rainfall (409 mm above

annual) was 20% higher than that for the year of below average rainfall (248 mm below annual).

The hypericin determination levels for 12 Australian biotypes of *Hypericum* are presented in Table 1. The comparison is based upon stage of growth equivalence (full flowering stage), and because the plants were potted and glass house cultivated the hypericin values are not necessarily indicative of maximum hypericin production potential but rather comparative relative hypericin production potential.

The 12 biotypes represent the genetic spectrum for *Hypericum* in Australia and demonstrate well the large variation that exists in hypericin production potential with different biotypes. The two field strains of *Hypericum* used, Tuena narrow leaf and Orange broad leaf, equated respectively with the highest and the lowest ends of the plant's hypericin production range.



**Figure 1.** Three-weekly variations in hypericin production by two biotypes of St. John's wort over two consecutive years. The two sites sampled were naturally occurring field growing infestations.

**Table 1.** The relative hypericin production capacity of 12 Australian biotypes of *H. perforatum*. Plants were glass house grown and harvested at full flowering.

<i>Hypericum perforatum</i> biotype	Hypericin content dry basis (ppm)
Nelson broad leaf (Vic)	1212
Turon river broad leaf (NSW)	1294
Orange broad leaf (NSW)	1373
Wyangla dam (NSW)	1640
Bundaleer Forest (SA)	1792
Scott Creek (SA)	1818
Avenal (Vic)	1984
Cowra (NSW)	2131
Grenfell (NSW)	2139
Cassilis-Mudgee short/inter (NSW)	2325
Mudgee tall narrow leaf (NSW)	2439
Tuena narrow leaf (NSW)	2767

Hypericin production was directly associated with flower stalk development and presumably affords the plant an advantage during flowering and seeding. Three growth stages were recognisable. From mid-September to late December the upright flower stalks grow rapidly and flowering occurs. From early January to mid-March seed capsules form. From late March to early September the mature senescent flower stalks turn brown, concurrently prostrate, non-flowering, green and fresh, new growth develops at the base of the plant. This latter growth stage is deceptive because at first the plant appears to be dead, however the basal growth stems are quite palatable to livestock, much lower in hypericin, and probably make a significant contribution to the plants over-winter survival. Heavy grazing during this stage, and during early spring flower stem development, may significantly depress the weed's ability to re-establish itself the following season and this should allow competing vegetation to progressively outgrow the *Hypericum*.

In the 2 ha grazing experiment it was observed that when the new season flowering stems appeared in spring, continued heavy grazing significantly suppressed their rate of growth. This process also suppressed the weed's hypericin production. This in turn enabled the sheep to graze it without ill effects through to early summer. By early January, whereas the protected *Hypericum* had a hypericin level of 1400 ppm, the regrowth post-grazing *Hypericum* was only at 350 ppm.

The more the weed infestation is reduced each year, the earlier in the season safe grazing can commence and the later it can continue.

**Animal factors affecting hypericin tolerance** This study has shown that it is only after ingested hypericin has been absorbed into the blood stream, and become photo-energised in the cutaneous circulation, that it becomes physiologically active. In an animal with circulating hypericin, less than 5 h of continuous direct sunlight was all that was required to produce a clinical effect. Animals that had stopped ingesting *Hypericum* continued to be at risk of poisoning for another four days, presumably because of the difficulty that ruminants have in metabolising and excreting hypericin. The most obvious sign reported in cases of *Hypericum* poisoning is photosensitisation of unprotected areas of skin, but other clinical signs also manifest themselves. In the present study these included: restlessness, pawing of the ground, head shaking, pruritus with associated head rubbing, followed by swelling and inflammation of the forehead, ears, lips, face and around the eyes. In field cases, rubbing of these itchy, erythematous areas of skin on fixed objects can result in raw weeping wounds, and

eventually, scab formation. Concurrently affected animals developed hyperthermia and depression. Associated with this hyperthermia animals displayed excessive panting, salivation, respiratory distress, tachycardia and weakness, leading to recumbency. In field cases that are shade deprived, this hyperthermia can become so severe as to be lethal. Some animals in the present study developed an unusual nervous sign, which consisted of a transient, intermittent, moderately severe, hind limb paresis. Inappetence or anorexia, photophobia, and diarrhoea were also encountered. Sometimes observers describe bizarre 'nervous signs' in field outbreaks of *Hypericum* poisoning, but these are in fact bizarre behavioural responses to the intense skin irritation experienced with this condition. Affected animals should be taken off *Hypericum* and moved into full shade, ideally inside a shed. They will need to remain shade protected for at least four to seven days.

The threshold dose rate for an adverse clinical effect from a single days ingestion of hypericin, in sun exposed, white, wool protected, Merino sheep, was between 2 and 3 mg hypericin per kg body weight per day (Bourke 2000, 2002a). This would equate with a 100% *Hypericum* diet, where the *Hypericum* contained about 150 ppm hypericin, ingested during the high sunlight intensity period of early Summer. Higher plant hypericin levels would be tolerated during the low sunlight intensity period of winter, and under pasture conditions where *Hypericum* constituted less than 100% of the dry matter ingested each day. Thus safe grazing of broad leaved *Hypericum* by Merino sheep should be possible from early May to mid October, and of narrow leaved *Hypericum* from early July to mid September.

At 3 mg hypericin per kg body weight approximately 26.5% of sheep were found to display intolerance. With a similar group of recently shorn sheep 94% were found to display intolerance (Bourke 2002a). In the wool protected group the percentages of intolerant animals, on a wool type basis were, superfine 14%, fine 28.5% and medium 33.3%. Conversely, in the recently shorn group the percentage of intolerant animals in all three groups approached 100%. The difference in performance of different wool types probably reflects the greater crimp frequency and fibre density of the finer types contributing to a tighter fleece with more skin surface light protection capacity (Bourke 2002a).

The experiments with cattle demonstrated that they are more tolerant of *Hypericum* than previously thought (Bourke 2002b). Two earlier studies using Hereford cross cattle concluded respectively, that sheep were four times more tolerant of *Hypericum* than cattle (Marsh and Clawson 1930), and that the

minimum toxic dose of hypericin in cattle was between 0.37 and 0.62 mg kg<sup>-1</sup> body weight (Araya and Ford 1981). However in the present study all 36 head of cattle tested were found to tolerate an oral *Hypericum* dose equivalent to an hypericin dose of 1.5 mg kg<sup>-1</sup> body weight. These cattle were not challenged at a higher dose rate. The two earlier studies contained sheep feeding inconsistencies and hypericin assay errors respectively. However the amount of plant required to intoxicate cattle in both studies appears to have been consistent and correct. From what we now know about hypericin levels in *Hypericum*, by using more accurate chemical analyses and a greater understanding of biotype and growth stage differences, it is likely that the minimum toxic hypericin dose for Hereford cross cattle is somewhere between 6 and 9 mg kg<sup>-1</sup> body weight. This would suggest Hereford cross cattle are at least two to three times more tolerant of *Hypericum* than white wool protected Merino sheep. Likewise, fully pigmented cattle may be as much as four to six times more tolerant.

The present study indicates an animal's hypericin tolerance is more directly associated with the amount of skin surface light protection it has, rather than any large difference in its hypericin metabolisation rate capacity, relative to its peers or another animal species. Consequently hypericin tolerance will be greater with pigmented versus non-pigmented skin, thicker and tougher versus thinner and softer skin, wool covered versus hair covered skin, tight wool covered versus more open wool covered skin, and longer wool covered versus shorter wool covered skin. Anecdotal generalisations, for example that goats are more tolerant of *Hypericum* than sheep, may not necessarily stand up to scientific scrutiny. A white, coarse haired, Saanen doe for example may be less tolerant of hypericin than a white, superfine, wool protected, Merino wether, and a fully pigmented Angus steer may possibly be more tolerant than either of these.

The softer thinner skin of young, weaner sheep, or sucking lambs, together with their relatively lighter fleece cover, will reduce their hypericin tolerance. In addition, sucking lambs would be ingesting hypericin from two sources, the *Hypericum* in the pasture and the hypericin in their mothers milk. In pregnant ewes it is very likely photo-activated hypericin from the mothers blood circulation will pass into her foetus and intoxicate it, and that this is the reason for the poor reproductive performance encountered with sheep on *Hypericum* infested pastures.

When following grazing management guidelines are recommended for *Hypericum* control. With large, dense, infestations start the grazing process by putting a fully pigmented breed of cattle onto the infestation,

to knock it down and open it up. Cattle can be used earlier than the nominated safe grazing period for sheep, because they are more hypericin tolerant. Eventually remove the cattle and introduce the sheep, although less hypericin tolerant than cattle, sheep can exert far greater grazing pressure on the *Hypericum*. Use superfine wool type, adult, Merino wethers (or dry, non-pregnant ewes). These animals must have at least four months of protective wool growth cover. The stocking rate used should be as high as practicable, several shorter periods of grazing using high stocking rates will be more effective than one long period using a lower stocking rate. If possible, fence off heavy wort infestations to reduce the size of each area to be grazed to less than 100 ha and thereby facilitate repeated heavy grazing. The retention of as many shade trees as possible, on an *Hypericum* infested paddock, will enable sheep to minimise their exposure to direct sunlight during daylight hours, and indirectly encourage them to preferentially graze during the night. By following these guidelines each year, *Hypericum* infestations can be gradually brought under control and distress to the animals grazing them minimised.

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