Research reports

Variation and varietal determination in *Hypericum perforatum* L. (St. John's wort) in New South Wales

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Summary

Since 1929 it has been accepted that the only variety of the apomictic species, *Hypericum perforatum* L. (St. John's wort) in Australia is the narrow leaved var. *angustifolium* DC. To test this assumption specimens were collected in 1985-6 from various locations in New South Wales, grown for four years in a common environment at Bathurst and examined for differences in morphology, cytology, chemical content, protein electrophoresis and germination characteristics. The specimens had a range of leaf sizes from what is accepted as broad (mean 12 × 24 mm) to what is accepted as narrow (mean 8 × 28 mm). Differences between broad and narrow leaved plants could not be recognized using protein electrophoresis but broad leaved specimens were shorter, earlier flowering, had larger capsules, thicker stems, lower levels of hypericin and fewer pellucid glands in the leaves than narrow leaved specimens. There were no differences in chromosome number, all forms having 2n = 32. It is concluded that all forms collected in New South Wales, though they differ in leaf width, other morphological characters and chemical content are best considered as belonging to the same variable taxon, *H. perforatum*.

Introduction

Specimens of *H. perforatum* (St. John's wort) collected in 1929 in New South Wales, Victoria and South Australia were classified as the narrow leaved var. *angustifolium* DC. (Calvert 1930, 1932) at the Royal Botanic Gardens, Kew (B. Mathew personal communication 1983). As a result it has been accepted that var. *angustifolium* is the only variety of this species present in the large areas infested in Australia (Campbell 1977, Shepherd 1983). However, more recently, differences noted between plants of *H. perforatum* (Campbell 1987) suggested that other named varieties may be present. If there are a number of varieties in N.S.W. it could point to differences in susceptibility to herbicides or biological control agents. The reaction of the biocontrol agents *Aphis chloris* (Koch) and *Aculus hyperici* (Liro) to the different forms of *H. perforatum* in N.S.W. is presently being ascertained at the Division of Entomology, CSIRO, Canberra (P. Jupp personal communication 1992).

In Europe, varietal names have sometimes been given to forms of *H. perforatum* with broad leaves (var. *perforatum*, in northern Europe), narrow leaves (var. *angustifolium*, in southern Europe) and small, but not necessarily narrow, leaves (var. *microphyllum* DC.) (Robson 1967, 1968, 1977). Intergrading forms have also been observed. To investigate whether these varieties occur in N.S.W., specimens were collected from various locations, grown in a common environment at Bathurst, and observed over time.

Materials and methods

Plants were raised from seeds collected at Orange (1 and 2), Coolah, Captains Flat, Adelong, Mudgee (short and tall) and Tuena, N.S.W. Initially, observations were made on morphological characters of growing plants (as distinct from dried herbarium specimens) to assess whether different varieties or ecotypes exist in N.S.W. Later the accessions were examined for differences in cytology, chemical (hypericin) content, protein electrophoresis and germination characteristics.

Results and Discussion

Morphology

Observations between 1985 and 1988 (Campbell 1987) revealed the possible existence of three groups: broad leaved (Orange 1 and 2) with broad leaves (often reflexed vertically on main stem), early flowering, short plants, thick light green stems and large capsules; narrow leaved (Captains Flat, Adelong, Mudgee tall, Coolah, Tuena) with narrow leaves (not reflexed), late flowering, tall plants, thin often pink stems and small capsules; and intermediate (Mudgee short) (Table 1).

Broad leaves were 10 to 14 mm wide × 23 to 25 mm long and narrow leaves 7 to 9.9 mm wide × 26 to 30 mm long (Table 1). The leaves measured were from the central six nodes on the main stem because the coefficient of variation of their measurement was lower (6.1%) than that of leaves from the central 10 nodes on the main stem (8.4%), or that of the third leaf on branches (9.4% for 6 and 11.7% for 10 leaves). During the four years of observations morphological differences between broad and narrow leaved specimens remained consistent, the most reliable diagnostic character being leaf width with a coefficient of variation of 5.0% for broad and 9.25% for narrow.

Leaf length and width measurements have not been recorded for Europe, the

Table 1. Characteristics of broad, intermediate and narrow leaved forms of *Hypericum perforatum*.

Characteristics	Broad	Intermediate	Narrow
Leaf width[A] (mm)	12	9	9
Leaf length[A] (mm)	24	26	28
Flowers open[A] (%)	86	78	28
Height[A] (cm)	62	73	88
Stem diameter[A] (mm)	5.9	4.2	4.6
Capsule size[A] (mm)	7.8 × 2.1	6.3 × 1.8	6.6 × 1.9
Hypericin content (ppm)	425	–	1295
Pellucid glands in top leaves (no. mm^{-2})	1.7	4.8	5.7
Chromosome number (2n)	32	32	32

[A] In early December 1985 to 1988

distinction between varieties being based on leaf proportions (length:width), broad being 2:1 and narrow up to 6:1 (N.K.B. Robson personal communication 1986), which confirms our classification of broad (1.9:1 to 2.0:1), but ratios for our narrow-leaved forms have never been recorded above 4:1.

Inspection of overseas collections at herbaria in Sydney, Melbourne and Adelaide revealed 22, two and six specimens respectively that we would class as broad leaved, i.e., with dried leaves > 10 mm wide; two specimens, one from Belgium and another from Netherlands had leaves 20 mm wide. Sydney and Adelaide herbaria each had a var. *microphyllum* specimen, from Belgium and Italy respectively, with leaves on the main stem 5 mm wide × 17 mm long. All Australian specimens at the above herbaria were narrow leaved.

Measurements of other morphological characteristics of broad, intermediate and narrow leaved forms of *H. perforatum* were made in early December 1985 to 1988 (Campbell 1987) and are presented in Table 1. Some of the narrow leaved specimens could be further distinguished. For example, the young buds, top bud bearing stems and sepals of Mudgee tall were strongly maroon coloured. Other specimens that had some maroon colour were Adelong (bud bearing stems) and Tuena (sepals).

Although flower size has been used to distinguish between varieties in Europe and Asia (large flowers in var. *perforatum* and sometimes smaller flowers in var. *angustifolium*, Robson 1967) it was not useful here as flowers on all specimens were approximately the same size. Parsons (1973) mentions the diameter of the flowers of Australian *H. perforatum* vary from 19.1 to 25.4 mm, which is almost completely out of the range given by Fröhlich (1911) for var. *angustifolium* (under the designation ssp. *angustifolium*) of 15 to 20 mm. Measurements of diameter of flowers in the collection from NSW varied from 23–30 mm well out of the range of Fröhlich's var. *angustifolium*.

Germination

Hesse (1924) found KNO_3 (10 m mole L^{-1}) could wholly or partially substitute for light in breaking dormancy in seeds of a northern European, presumably broad leaved, variety of *H. perforatum*; 57% germinating in 20 days at 17/22°C in KNO_3, compared to 28% for seeds germinating in water. The experiment was conducted in darkness with one month old seeds.

To test whether broad (Orange 1), intermediate (Mudgee short) and narrow leaved (Tuena) forms collected in N.S.W. reacted in the same way, one month old seeds were germinated in light (8 μE M^{-2} S^{-1}) and dark with and without KNO_3 (1 m mole L^{-1}) + gibberellic acid (0.14 m mole L^{-1}). The temperatures imposed were 18/25°C and germination was recorded for 111 days.

Germination of all forms responded to KNO_3 + gibberellic acid in both light and dark (Table 2) indicating that our forms react to chemical germination stimulators in the same way as the variety used in Hesse's 1924 experiment (Campbell 1985). Additionally, it appears that the use of these germination stimulators does not provide a method of distinguishing between broad and narrow leaved forms in N.S.W.

Electrophoresis/Isoelectric focusing (IEF)

Examination of the leaves of seven specimens including broad and narrow, failed to give reproducible, easily discernable differences using the following techniques:

- SDS-PAGE Laemmli System in reducing/non reducing conditions: Protein
- SDS-PAGE Jovin System in reducing/non reducing conditions: Protein
- PAGE with Triton X-100 in reducing/non reducing conditions: Protein + Enzyme IEF (pH 3-10) : Protein + Enzyme

Several different extraction methods were tried with each system but to no avail. Problems encountered were: low overall protein content; high proportion of Fraction 1 protein (RUBP carboxylase) to total protein; high oil content that led to blurring of bands; low enzyme levels; and streakiness without mercaptoethanol.

There were some indications that differences may be obtained on IEF using protein staining but new extraction, purification and concentration techniques will be needed. Investigation of seeds may provide more scope although preliminary analysis of seeds on SDS-PAGE revealed few differences on protein staining.

Table 2. Effect of light and KNO_3 + gibberellic acid (GA) on germination of three forms of *Hypericum perforatum* in 111 days.

Light/dark	KNO_3 + GA	Narrow leaved (Tuena)	Intermediate (Mudgee short)	Broad leaved (Orange 1)
Light	+	61 b[A]	78 ab	85 a
	-	28 c	31 c	32 c
Dark	+	61 b	28 c	59 b
	-	1 d	0 d	0 d

[A] Values not followed by a common letter differ significantly ($P < 0.05$)

Chemical composition

Examination of the level of hypericin in leaves showed that the broad leaved variety Orange 2 contained less hypericin (425 ppm) than two narrow leaved specimens (Mudgee tall 1160 ppm; Tuena 1430 ppm; Southwell and Campbell 1991). These levels agreed with visual observation of fewer pellucid glands in the top leaves of the broad leaved variety (1.7 mm^{-2}) than in the top leaves of narrow leaved specimens (Mudgee tall 4.8; Tuena 5.7 mm^{-2}). Leaves at the top of the main stem were used because they had more glands (5.0 mm^{-2}) than lower leaves (0.9 mm^{-2}) on the three accessions. For the variety Orange 2, the concentration of hypericin declined in order: flowers 2248, capsules 771, top leaves 438, lower leaves 354, side stems 115 and main stem 40 ppm. The petals contained only dark glands (on their margins, Campbell and Delfosse 1984) whereas leaves had dark and pellucid glands (in the ratio 1:8.4) indicating that hypericin, or an analogue that converts to hypericin, are contained in both glands. The high level of hypericin in the petals confirms the observation by Dodd (1920) that *H. perforatum* is more toxic if ingested at flowering than at other stages.

Cytology

Cytological examination of eight different accessions, including broad, intermediate and narrow leaved, revealed that all had chromosome counts of 2n = 32 and 16 bivalents during metaphase 1 of meiosis. This is the same chromosome number as the broad leaved var. *perforatum* from northern Europe (N.K.B. Robson personal communication 1983) and for nearly all chromosome counts of *H. perforatum* made in Europe and elsewhere (Darlington and Wylie 1955, Moore 1973, Goldblatt 1981). Strid and Franzén (1981) recorded 2n = 16 for var. *angustifolium* from Greece. Another count of 2n = 16 has also been recorded for Greece (Papanicolaou, 1984). Examination of the Strid and Franzén voucher specimen (on loan from the Copenhagen herbarium) revealed a leaf size on the main stem of 7 × 19 mm. The Papanicolaou specimen was not available but another specimen from the same location, also on loan from the Copenhagen herbarium, was almost identical to the Strid and Franzén specimen. We believe that both these specimens are possibly *H. perforatum* var. *microphyllum* based on their similarity to a var. *microphyllum* specimen from Italy located at the Adelaide herbarium. Thus it may well be that var. *perforatum* and var. *angustifolium* have 2n = 32 chromosomes and var. *microphyllum* has 16.

We conclude that varying morphological characteristics and chemical composition of the Australian material studied, simply represents a variable taxon and

Figure 1. Differences in height and time of flowering between broad leaved (Orange 1 'Duntry') and narrow leaved (Mudgee tall) accessions at Bathurst on December 6, 1986.

that it is best to call our plants *H. perforatum* and not to attempt any classification at varietal level following Robson (1977) and Jessop and Toclken (1986). *H. perforatum* is a well known pseudogamous apomict (Noack 1939, Gustaffson 1946, 1947 a,b, Robson 1968) in which, although a male parent does not contribute to the embryonic tissue, pollination is necessary for the successful agamospermous development of the seed (Stace 1980). *H. perforatum* may be taken to be a relatively small and simple agamic complex (Stebbins 1950) in which a polymorphic number of forms have arisen, through, for example, hybridization and allopolyploidy between the original sexual ancestors of the complex or through chromosomal and genetic changes within the apomictic clones themselves. This does not preclude variation in response to herbicides or bio-control agents amongst the polymorphic forms.

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