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Seasonal variation of biomass and bioactive alkaloid content of goldenseal, *Hydrastis canadensis*

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ABSTRACT

Seasonal variations in biomass and alkaloid contents of goldenseal (*Hydrastis canadensis*) were investigated. Five-year-old plants gave 5× the yield of roots and rhizomes of two-year-old plants, and summer growth gave significant increases in root biomass but not rhizomes. Berberine contents of roots plus rhizomes did not vary significantly and were >3.4% in all samples. Hydrastine contents of 5 y roots plus rhizomes showed significant seasonal variation. These variations were due to significant changes in the hydrastine contents of the roots (1.3–1.9%), but not the rhizomes (2.2–2.8%). Goldenseal leaves plus stems had lower contents of hydrastine (0.4–0.8%) and berberine (1.0–1.5%).

1. Introduction

Goldenseal (*Hydrastis canadensis* L., Ranunculaceae) is a valuable medicinal herb native to woodlands of north eastern America [1]. Echinacea combined with goldenseal is one of the top-selling botanical dietary supplements in the US, with 2008 sales around US$7 million [2]. Goldenseal has antimicrobial properties [3, 4], but also inhibits human cytochrome P450 3A, leading to potential herb–drug interactions [5, 6]. The roots and rhizomes are the parts of goldenseal which are in the official monographs [7, 8] and over-harvesting of these from wild populations has resulted in goldenseal being listed under Appendix II of the Convention on International Trade in Endangered Species [9]. Increased cultivation is needed to meet the market demand, and harvesting leaves and stems of goldenseal has been suggested as a way to sustain wild populations [10].

The two major alkaloids in goldenseal roots and rhizomes are hydrastine and berberine (Fig. 1) [1], with at least 2% hydrastine and 2.5% berberine required by the US Pharmacopoeia (USP) [7], and at least 2.5% hydrastine and 3% berberine by the European Pharmacopoeia (EP) [8]. Lower levels of these alkaloids occur in the stems and leaves [11, 12]. Hydrastine is only known from goldenseal, whereas berberine occurs in a range of plants [13].

Cultivated goldenseal is usually harvested three to five years after planting rhizome pieces or five to seven years after planting seeds [14]. An early recommendation was to harvest goldenseal in summer, with spring harvesting considered inferior [15]. One current recommendation is to harvest the roots and rhizomes in autumn after the top growth has died [16]. Where leaves are traded, the recommendation is to harvest them in early autumn when they are green [14]. Early research concluded that the level of alkaloids varied seasonally [15] and more recently it has been stated that maximum alkaloid concentrations occur in the

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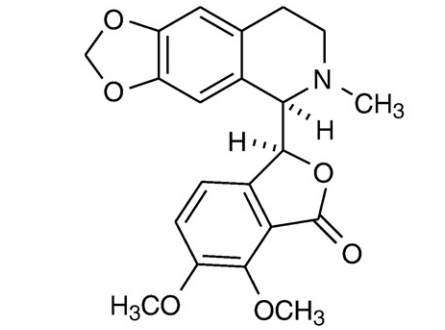
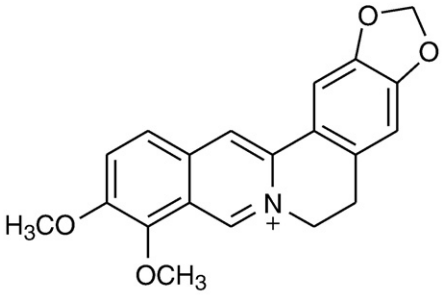


Hydrastine (**1**)



Berberine (**2**)

Fig. 1. Structures of the main alkaloids in goldenseal.

autumn [17]. It has been suggested that contemporary data are needed on the effect of harvest time on quantity and quality of goldenseal [1], but there is a 1931 study which found: "In general the alkaloidal content increases with the age of the root system. Monthly assays indicated that the alkaloidal content of the rhizome is practically const. from [winter] to [summer]. It then declines until [autumn], after which it rises until [winter]. The assay curve for the root, in general, follows that of the rhizome." [18].

We now report results from field trials on the seasonal variation of biomass yields and individual alkaloid contents of different parts of goldenseal plants.

2. Materials and methods

2.1. Plant material

Dormant goldenseal rhizomes were imported into New Zealand from McZand Herbal, Inc., California, USA in 1993, with subsequent planting stock increased by division at Ruakura Research Centre, Hamilton (latitude 37° 52′ S). A voucher specimen has been kept (Invermay number 820).

2.2. Five-year-old plants

Rooted rhizome cuttings were planted 0.5 × 0.5 m apart in fertilised soil in October 1996 at Hamilton, with the plants kept weed free by sawdust mulch, non-residual herbicides and hand weeding. From 1996 to 1999, 55% shade was provided by shade cloth, but from 1999 this was increased to 87%, based on our finding in a parallel study that this higher level of shade gave greater growth [19]. The plants were healthy and vigorous and by 2001 they had spread laterally to fill the inter-row spaces. Three individual plants were harvested, one each taken at random from rows in replicated blocks, in December 2001 (early summer), February (late summer), May (autumn) and August 2002 (winter). A 0.5 × 0.5 m sample was taken of the above-ground stems and leaves and the roots and rhizomes to a depth of 250 mm around each plant. At the May sampling, the plant top growth was dead and this was fully decayed by the August sampling.

2.3. Two-year-old plants

Rooted rhizome cuttings were planted at 0.25 × 0.3 m spacing in September 1999 at Mosgiel (45° 51′ S) in raised beds formed with fertile soil and fine bark mixed into the top 10 cm. The plants were grown under 90% shade cover (chosen to match as closely as possible the shade level for the five-year-old plants) with regular watering under weed-free conditions. They established and grew well into healthy, disease-free plants. Three individual plants were harvested at random from replicated blocks in December 2001, March 2002 and August 2002.

2.4. Sample preparation and chemical analyses

Plants were washed and separated into root, rhizome, stem and leaf parts. The bottom 50 mm of the stem was sampled separately to take account of the below- and above-ground portions of the stem. Plant parts were dried at 40 °C, weighed to measure the dry matter yields, then ground and stored at −18 °C for chemical analyses. The main alkaloids and quinic acid derivatives of goldenseal were quantified by our previously reported HPLC method [11]. The dry weight of the harvested roots and rhizomes and the alkaloid analyses were used to calculate the mean weighed concentrations of hydrastine and berberine in mixed root and rhizome samples.

2.5. Statistics

Analyses of variance were carried out on the yield and concentration data from each plant component for each site to compare mean values from the harvests, using GenStat version 12 (VSN International Ltd, 2009).

3. Results and discussion

Rooted rhizome cuttings were grown at two sites in New Zealand, and harvested after five years' growth at one site and two years' growth at the other. The plant part biomass yields are given in Table 1. These different plant parts were analysed for their contents of alkaloids and quinic acid derivatives by HPLC [11] (full results in Supporting information). Table 2 gives the contents of hydrastine and berberine in the separated roots and rhizomes, and the calculated contents for combined roots plus rhizomes, and leaves plus stems.

The five-year-old plants gave about four times higher total plant biomass yields than the two-year-old plants (Table 1), with most of this biomass in the roots and rhizomes. This supports the recommendation that cultivated goldenseal should be harvested three to five years after planting rhizome pieces [14]. The yield of root biomass per plant increased significantly during summer growth (Table 1). Rhizome yields