SOIL AND MULCH EFFECTS ON GINSENOSIDES IN AMERICAN GINSENG PLANTS

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ABSTRACT

Four year old American ginseng plants (*Panax quinquefolium* L.) were grown in control and treated field plots in North Carolina, USA. Soil pH (4.4, 5.5, and 6.5), soil phosphate (19, 89 and 232 ppm) and mulch treatments (wheat straw, pine needle straw, poplar bark, oak bark, pine bark and hardwood leaves) were studied for their effects on total dry weight, total ginsenosides and 5 individual ginsenosides (A1, Rgl, Rd, Re, and Rb2). The leaf and root tissue were analyzed for ginsenosides by high pressure liquid chromatography (HPLC).

The oak and poplar bark mulch treatments appeared to have the best effect upon the growth and production of roots while not significantly decreasing the ginsenoside content of the roots. The oak mulch showed a statistical increase in the ginsenoside content of the leaves.

INTRODUCTION

There has been an ever increasing demand for American ginseng both for use in the USA and for export since the end of World War II. The demand has been met with increased collections of the native plant, so much so that it is now an endangered specie in 8 states and threatened or uncommon in 15 states where it once flourished in the woodlands of eastern United States (1). This has increased the cultivation of the plant which is intensive for large scale production. The plants are grown under artificial shade with seeding rates of 100 lbs per acre giving plant populations of 20-25 per square foot (2,3). Because of its widespread availability and relative ease with which it can be handled, small grain straw is the most commonly used material for mulching the ginseng beds of large scale plantings (4). Sawdust (of unspecified origin) also is used to some extent (2, 4).

In initiating one of the first scientifically based studies, one of us (T. Konsler) evaluated the influence of mulch type as well as the effect of soil pH and phosphate concentrations on root growth and seed production of shade cultivated ginseng. American ginseng plants were grown in field plots in North Carolina, USA. Mulch treatment began within 5 days after seeding with wheat straw, pine needle straw, poplar bark, oak bark, pine bark and hardwood leaves. Monitoring these plants through four years showed a strong influence of the mulch on growth and root production (5). Oak and poplar bark mulches tended to produce the greatest yield of roots and seeds. The oak mulch also tended to increase soil pH and mineral levels when compared to controls (wheat straw). also, a nursery pot study seeded in April, 1978 in North Carolina, USA

compared various soil phosphorus and pH level effects on the growth of American ginseng (6). After two years, these plantsshowed largest root growth in moderately acid soil (pH 5.5) and root weight increased with the soil phosphorus level.

In this study we report the effect of the various treatments upon both the total and individual ginsenosides. Four year old leaf and root samples were analyzed for total ginsenosides and 5 individual ginsenosides were quantitated by HPLC.

EXPERIMENTAL

Plant Material, — Four year old American ginseng (*Panax quinquefolium* L.) root and leaf tissue, grown under cultivation using either different mulching materials (wheat straw, pine needle straw, poplar bark, oak bark, pine bark and harwood leaves) or under different soil pH (4.4, 5.5, and 6.5) or different soil phosphate levels (19, 89 and 232 ppm) were obtained from Dr. Konsler of the North Carolina State University. The plant material was dried in a forced-air oven at 80° to constant weight and ground in a Wiley mill to pass a 40 mesh screen (7).

Extraction *Procedure* (Fig. 1). — The method of N.J. Chung (8) was used with slight modification. Two grams of dried sample are extracted for 2 hours with 15 mls of $CHCL_3$: MeOH:H_2O) (1:2.5:1) by stirring in an erlenmeyer flask. After filtration the residue is re-extracted with the same solvent (5mls) for one hour. The combined extracts are diluted with 5 mls water and separated into two phases. The upper phase (methanol-water) is diluted with 60 mls of water containing 20 grams of NaCl and

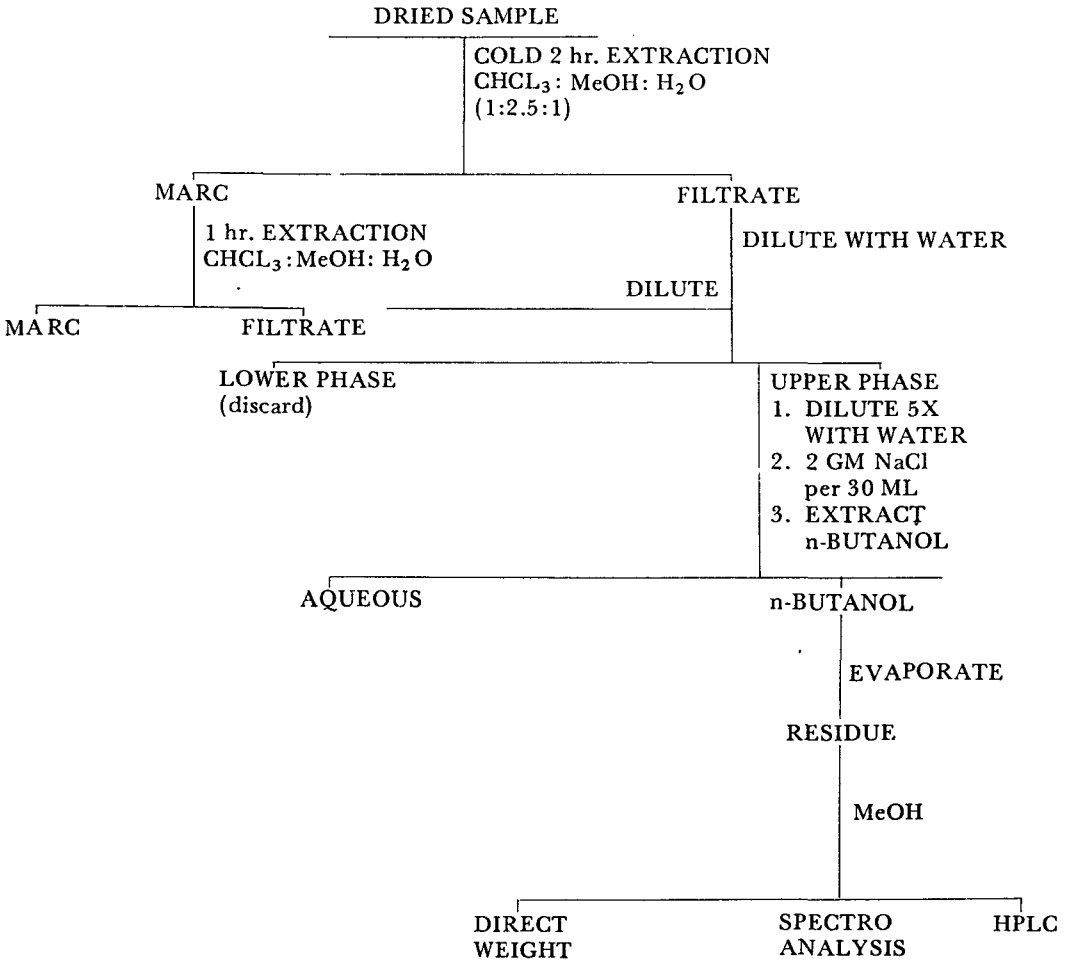

Fig. 1. Extraction of American Ginseng for Ginsenosides

extracted in a separatory funnel against water saturated n-butanol (3x50 mls). Evaporation of the n-butanol to dryness on a rotary evaporator at 60°C gives a residue used for %W/W calculation. The residue is then dissolved in 10 mls of methanol for spectrophotometric and HPLC analyses.

Spectrophotometry. The spectrophotometric method of Hiai *et al.* (9) was used to determine the total ginsenosides present in each sample analyzed. The sample (40ul) is diluted to 0.5 mls with ethanol and reacted at 60°C for 10 minutes with 8% vanillin solution (0.5mls) and 72% sulfuric acid (5mls). The absorbance of the reaction mixture is read at 544nm against a blank. The amount of total ginsenosides is then determined by comparison to a standard plot of concentration versus absorbance.

HPLC Analysis. The method utilizes an HPLC column for carbohydrate analysis purchased from Alltech Associates (Deerfield, IL 60015) with dimensions of 30cm by 4.1mm (ID). The five ginsenosides identified as A1, Rgl, Rd, Re, and Rb2 by direct comparison with reference standards (Generously supplied to us by Dr. Shibata, Tokyo, Japan). The separation is achieved under the following conditions: elution solvent, 20% water in acetonitrile; flow rate, 1.5 mls/min; detector at 205nm; attenuation of 0.5 and chart speed of 0.5cm/min (Fig. 2). Under these conditions A1 had a retention time of 4.2min., Rgl of 5.4min., Rd of 10.4min., Re of 9min. and Rb2 of 22.6min.

RESULTS AND DISCUSSION

The influence of bed mulch on total and individual ginsenoside content of leaf and root tissue is shown in Table 1. This shows that in leaf material, the total ginsenosides are highest in the wheat straw, oak bark and hardwood leaf mulched plants. The individual analysis for the ginsenosides seems to support this for wheat straw and hardwood leaves for all 5 ginsenosides, however oak bark mulched plants showed a decreased amount ginsenosides A1, Rgl and Rb2 when compared to both the wheat straw and

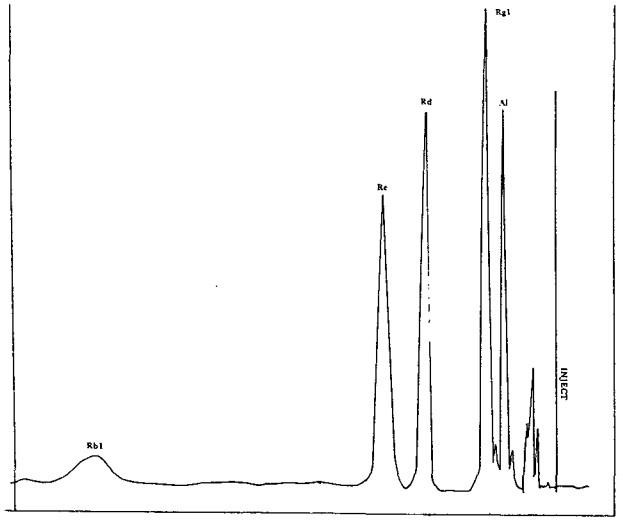

Fig. 2. HPLC chromatogram of a mixture ginsenosides Al, Rgl, Rd, Re and Rbl.
Operating conditions: carbohydrate column; mobile phase, 20% acetonitrile with a flow rate of 1.5ml/min; detector at 205nm.

hardwood mulched plants. On the other hand, root tissue content of ginsenosides, both total and individual showed no significant differences due to the mulch treatments. Comparing this to the results by Konsler (5) who found that oak bark and poplar bark gave the greatest yield of roots and seeds, we see no corresponding decrease or increase in the amount of ginsenosides in the roots. However, the oak bark treated plants do have leaves which contain signficantly higher amounts of ginsenosides than the poplar bark mulched plants.

The influence of soil pH on total and individual ginsenosides content of leaf and root tissue is shown in Table 2. The pH had no significant effect on the total amount of ginsenosides in the leaves although there is a significant increase of ginsenosides Rd, Re and Rb2 at pH 5.5 and an increase in ginsenoside A1 and Rb2 at pH 6.5. In the root samples, there is an increase in total ginsenosides when the plants were grown at pH 5.5 and 6.5. This increase is reflected by an increase only of ginsenoside Re at pH 5.5. This also corresponds very nicely with Konsler (6) who found the largest root growth in moderately acidic soil. These results are interesting in light of Konsler's report of the effect of mulch on pH of the soil. Since oak bark is the only

mulch that changes the pH of the soil from about 4.7 to 5.5, we might expect to see a correlation between the results found with pH 5.5 and oak bark mulch. In fact we do not, because there was no increase in total ginsenosides in the roots of the oak bark mulched plants. Obviously there is much more involved in the effect of mulching than pH.

The influence of soil phosphate concentrations on total and individual ginsenosides of leaf and root tissue is shown in Table 2. In leaf tissue, the total ginsenosides concentration increases with increasing phosphate concentration. This is reflected by increases in ginsenosides Rg_1, Rd and Rb_2. In root tissue, there was no significant effect upon the total ginsenoside concentration by the increase in phosphate. However, ginsenoside A1 increases when phosphate is 232 ppm and ginsenosides Rg_1 and Re decrease with increasing amounts of phosphate. This should be compared with the fact that root weight increases with increasing phosphate concentration (5). Konsler (10) has found that poplar bark, oak bar, pine bark and hardwood leaves all increase the amount of soil phosphate. That fact that there is no correlation with the ginsenoside analysis of root tissue once again says that the effect of mulching is more complicated than just soil phosphate effects.

In conclusion, Konsler (5,6) has shown that oak bark and poplar bark mulches give greater yields of root and seeds and further we have shown no adverse effect on the ginsenoside concentrations by such mulches. The leaves of the oak bark mulched plants contain increased amounts of total ginsenosides whereas the poplar bark mulched plants show a decreased amount of total ginsenosides in the leaves.

Table 1. Bed mulch influence on total and individual ginsenoside content of leaf and root tissue from 4-year-old ginseng plants.

Bed Mulch	Total (W/W%)		Individual (HPLC-W/W%)				
	Dir. wt.	Spectro.	Al	Rgl	Rd	Re	Rb2
			From Leaf Tissue				
Wheat straw	41.0a	3.93a	0.77a	0.57a	0.50a	0.83ab	0.57a
Pine needles	36.0ab	2.77b	0.48cd	0.23b	0.27c	0.66bc	0.40b
Poplar bark/sawdust	32.3bc	2.60b	0.48cd	0.20b	0.33bc	0.73bc	0.30b
Oak bark/sawduct	33.3bc	4.07a	0.57bc	0.20b	0.45ab	1.00a	0.33b
Pine bark/sawdust	24.0 d	3.03b	0.37d	0.23b	0.32bc	0.63c	0.33b
Hardwocbd leaves	28.3 cd	3.97a	0.67ab	0.52a	0.37abc	0.63c	0.32b
LSD (.05)	5.1	0.70	0.12	0.16	0.14	0.18	0.11
			From Root Tissue				
Wheat straw	30.0	2.09	0.73	0.20	0.12	0.47	0.08
Pine needles	31.7	1.67	0.62	0.28	0.08	0.30	0.08
Poplar bark/sawdust	34.7	2.30	0.82	0.20	0.09	0.47	0.08
Oak bark/sawdust	34.3	2.20	0.67	0.18	0.07	0.38	0.08
Pine bark/sawdust	34.7	1.80	0.70	0.22	0.08	0.33	0.08
Hardwood leaves	35.3	1.93	0.73	0.13	0.07	0.37	0.07
LSD (.05)	8.24(NS)	0.57(NS)	0.24(NS)	0.15(NS)	0.03(NS)	0.12(NS)	0.03(NS)

ab . . . Means in a given column of each tissue source followed by a common letter are not significantly (.05) different.

Table 2. Soil pH and phosphate influence on total and individual ginsenoside content of leaf and root tissue from 4-year-old ginseng plants.

Soil pH	Total (W/W%) Dir. Wt.	Spectro.	Individual (HPLC-W/W%) A_1	Rg1	Rd	Re	Rb2
			From Leaf Tissue				
4.4	51.44a	2.92	0.52	0.19b	0.33b	0.54b	0.37b
5.5	38.44b	3.04	0.51	0.21b	0.62a	0.75a	0.46a
6.5	37.67b	2.82	0.60	0.28a	0.37b	0.51b	0.49a
LSD(.05)	7.48	0.45(NS)	0.11(NS)	0.03	0.09	0.10	0.07
			From Root Tissue				
4.4	29.33	1.70b	0.50	0.22	0.06	0.25c	0.029a
5.5	30.11	2.24a	0.52	0.22	0.06	0.31a	0.023b
6.5	27.78	2.09a	0.52	0.21	0.07	0.27b	0.027a
LSD.(.05)	3.28(NS)	0.21	0.05(NS)	0.02(NS)	0.01(NS)	0.02	0.003
Soil Phos (ppm)							
			From Leaf Tissue				
19	45.00	2.27b	0.53	0.19c	0.37b	0.55	0.33b
89	45.22	3.08a	0.56	0.23b	0.46a	0.66	0.50a
232	40.38	3.43a	0 54	0.26a	0.49a	0.58	0.49a
LSD(.05)	7.48(NS)	0.45	0.11(NS)	0.03	0.09	0.11(NS)	0.07
			From Root Tissue				
19	25.11b	1 97	0.50b	0.26a	0.06	0.31a	0.028a
89	31.22a	1.98	0.47b	0.20b	0.06	0.25c	0.022b
232	30.89a	2.09	0.57a	0.18c	0.07	0.27b	0.029a
LSD(.05)	3.28	0.21(NS)	0.05	0.02	0.01(NS)	0.02	0.003

ab . . . Means in a given column followed by the same letter are not significantly (.05) different.

Soldati: Why did you check the content of Rb_2 instead of Rb_1? It is well known that *Panax quinquefolium* contains a small amount of Rb_2 and a large amount of Rb_1.

Zito: The actual choise for ginsenoside testing was dictated by a couple of reasons. One was that we wanted to get a mixture of different ginsenosides such as a mixture of arabinose and one rhamnose. More practical reason is that the samples that we had on hand for analysis was kindly supplied by Dr. Shibata to professor Staba, therefore we didn't have enough samples for the other analysis.

토양과 부초가 미국 인삼 진세노사이드에 미치는 영향

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4년근 미국 인삼을 재료로 미국 North Carolina 소재 인삼포에서 대조군과 부초처리군으로 나누어 재배 시험을 수행하였다.

토양 산도(pH 4.4,5.5,6.5) 토양 인산(19, 89. 232 ppm) 및 부초처리(밀짚, 솔잎, 포프라나무 껍질 소

나무 껍질 등을 상면에 피복) 재배가 개별 진세노사이드 함량에 미치는 영향을 조사했다.

각 시험구에서 재배한 인삼엽과 인삼근 조직을 H-PLC를 이용하여 5가지 진세노사이드(Rg_1, Rd, Re, Rb_2, A_1) 함량을 분석하였다.

각 피복재료중에서 떡갈나무 껍질로 피복 재배한 시험구가 인삼 생육 및 진세노사이드 함량이 전반적으로 좋은 경향을 보였다.

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