Aeroponics: An Alternative Production System for High-Value Root Crops

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Abstract

An aeroponic system was developed for the production of root crops used in the herbal and phytopharmaceutical industries. The variability in the phytochemical quality of botanical products precludes the ability to administer uniform dosing in clinical studies. Aeroponic systems allow the producer to precisely control root zone nutrient and water regimes and environmental conditions, as well as have complete access to the roots throughout the life of the crop. This control promises a more uniform harvest. An A-frame aeroponic system was designed to maximize root yields and permit free access to the roots for monitoring. Burdock (*Arctium lappa* L.) plants were grown in aeroponics with controls grown in a greenhouse soilless potting mix for ten weeks in a research greenhouse in Tucson, Arizona. The plants were harvested and the dry weights of aerial parts and roots were determined, as well as the chlorogenic acid concentration in the dried roots. Chlorogenic acid is a caffeoylquinic acid derivative known to have antioxidant activity. The biomass yields of the aerial parts were significantly higher in the aeroponically grown plants compared to the controls. The root biomass yields showed no significant difference between treatments. The chlorogenic acid concentrations were also not significantly different, however the plant-to-plant variability was significantly lower in the aeroponically grown plants, suggesting the potential for more consistent phytochemical yields using this production technique.

INTRODUCTION

The natural products and phytopharmaceutical industries are facing some formidable challenges by regulatory agencies around the world. The issues of safety, sustainability, and supply of raw materials permeate discussions of production of medicinal and aromatic crops (Craker, 1999). Variability in the quality of the raw materials is a recognized problem. Most approaches to quality control have centered on various methods of standardizing the herbal extracts and selection of marker compounds (Brinker, 1999). However, using chemistry techniques alone to standardize botanical preparations ignores one of the major causes of the variability: the horticultural production of the raw materials. Improving the uniformity of the raw material before it is harvested is one step in improving the consistency of the final product (Simon, 1999). This is the focus of the research presented here.

Many of the popular products on the market today are produced from root material, and unlike vegetable root crops, herb roots tend to be fibrous, very deep rooted, slow-growing, or otherwise difficult to harvest from the soil. Positive identification of root material is another problem, and accidental adulteration with roots from other species can have serious consequences. Burdock tea has been implicated in two separate cases of atropine poisoning (Bryson, 1978; Bryson et al., 1978; Rhoads et al., 1984). Although never determined unequivocally, it is likely that roots from toxic nightshade (*Atropa belladonna*) were mixed in with the burdock root (Bisset, 1994). The risk of poisoning from accidental adulteration by roots of other plants can be eliminated in controlled

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environment agriculture (greenhouse culture) and hydroponic systems, which are inherently weed-free.

Aeroponics is a system of hydroponics in which the roots of the plants are suspended in a closed chamber and a nutrient solution is sprayed from below. A distribution system of pipes, spray nozzles, a pump and timer distributes the spray from a nutrient solution storage tank. Because of the easy access to the roots, aeroponics has been used as a research tool since the 1940s, with work done using vegetable crops in the 1970s and 1980s (Jensen and Collins, 1985; Nir, 1981).

A fast-growing plant with a large root biomass having phytochemical compounds of interest was selected for this research to serve as a model crop for testing the new aeroponic design. Great burdock (*Arctium lappa* L., Asteraceae) was selected because it meets those criteria, is sold as an herbal dietary supplement in the United States, and has a long history of use in Europe and Asia. Root from two closely related species, *A. minus* and *A. lappa*, are used for a wide variety of purposes around the world. The dried root is found in over 30 medicinal products currently on the British market (Phillipson and Anderson, 1984), mainly in anti-inflammatory preparations and as an herbal treatment of rheumatic disorders. The root is prepared as a fresh vegetable in Japan, where over 140,000 metric tons are consumed annually (New Zealand Institute for Crop and Food Research Ltd, 1996). In the United States, burdock root sells for US $41.22 kg^{-1} (dried, cut and sifted) (Frontier, 1999) and is marketed by most of the leading herbal dietary supplement companies. It is used as a popular "blood purifier" or "detoxifier," and for various ailments and complaints of the gastrointestinal track (Gruenwald et al., 2000). Burdock root is one ingredient in both Flor-Essence™ and Essiac™ herbal mixtures, which are popular nutritional supplements used for chronic conditions, particularly cancer. Combined sales of Essiac™ and Flor-Essence™ products was estimated to be over US $15 million in 1998 (Tamayo et al., 2000). Aqueous extracts of burdock root have shown promising antioxidant (Duh, 1998) and anti-inflammatory activity (Lin et al., 1996). Presently, there is no generally accepted method of standardizing or measuring quality of burdock root, although some limited studies on the phytochemistry of the plant have been done recently (Maruta et al., 1995). In this research, chlorogenic acid was chosen as a marker compound to estimate the concentration of bioactive principles in dried burdock root.

MATERIALS AND METHODS

As an experimental crop, burdock was grown in two horticultural systems in a controlled environment greenhouse. Burdock plants grown in the aeroponic system were compared to plants grown in a typical artificial greenhouse soil-less mix (AGSM) in a study of statistical design. The experimental treatments were aeroponic and AGSM culture. Measured outcomes were root and aerial biomass and phytochemical yields. To estimate the phytochemical quality of the roots, the concentration of chlorogenic acid was measured by High Performance Liquid Chromatography (HPLC) as a marker compound in root extracts from each treatment.

Burdock seeds were obtained from Horizon Herbs LLC, in Williams, Oregon (*A. lappa,* Lot #1428, packed for 2000). As is often the case in the herbal products industry, certified seed was not available for this genus. There were no voucher specimens available to verify the seed, therefore six plants were planted in large (100 L) containers in AGSM and grown to maturity for positive identification. *Arctium* spp. are facultative biennials that can take several years to flower (Kenkel and Graham, 1994). The reference plants flowered in June, 2002 and were identified as *A. lappa* by staff in the Herbarium at the University of Arizona. Voucher specimens were deposited at the University of Arizona Herbarium (ARIZ). There is some discussion in the literature of possible hybridization of *Arctium* species. Rollo et al. (1985) describe two possible hybrids between *A. lappa* and *A. minus* found in Ontario, Canada. Gross et al. (1980) discuss the variability observed within the *A. minus* species in Europe. It is the opinion of the lead author of this paper that the research plants were quite possibly a natural hybrid of *A.*

lappa and *A. minus*, based on inflorescence (long peduncle length) and leaf characteristics (hollow petioles in cross-section).

Seeds for both experimental treatments were germinated in a mistbed in 2.5 cm rock wool cubes. Plants were seeded on September 2, 2000. Nine days after seeding, a random one half of the plants were transplanted into 5 cm pots filled with AGSM for the controls. The remaining plants were transplanted into 7.6 cm rock wool cubes for the aeroponic treatments. The seedlings were again transplanted into the experimental treatments 24 days after seeding.

The experiment was laid out in a single-factor "split-plot" design, with two replicates of aeroponically-grown plants and two replicates of AGSM-grown plants. Each replicate contained 30 plants, for a total of 120 plants.

The experiment was conducted at the Campus Agricultural Center of the University of Arizona in Tucson, Arizona. The greenhouse facility included a 6 m x 15 m climate-controlled polycarbonate covered greenhouse oriented north-south. The blocking of the experiment attempted to consider both the difference in air temperatures (the north end, near the cooling pads being cooler than the south end, near the exhaust fans), and the differences in light (the east side, which is gutter-connected to another greenhouse, received less light than the west side, which is an end-wall). The greenhouses were cooled by an automated fan-and-pad system, and heated by a standard natural gas hot air heater.

The AGSM controls were constructed of 86 cm tall sections of 20 cm diameter polyvinyl chloride (PVC) pipe standing on end. These pipes, open on each end, were supported by concrete blocks that held the pipes securely in place while spacing the plants 41 cm on center, for a density of 6.2 plants m^{-2}. Each pipe was lined with a black plastic bag and filled with a typical greenhouse potting mix of Sunshine #1^{TM} (peat moss and perlite amended with dolomitic lime and a nutrient starter charge) mixed 2:1 with sterilized sand. The volume of AGSM in each pipe was approximately 30 L.

The classic A-frame aeroponic growing chamber historically used for lettuce production (Jensen and Collins, 1985) was re-designed with the addition of a vertical knee-wall below the inclined panels of the A-frame. Nutrient solution was recirculated by means of an external reservoir and pump. This design addressed some problems described by earlier researchers (Repetto et al., 1994) by elevating the plants so all roots were suspended in the spray zone, rather than permitting some roots to grown horizontally in the recirculating nutrient solution, which is more like a nutrient film technique (NFT) system. In addition, a three-dimensional placement of the spray nozzles was included and the number of spray nozzles was increased in order to improve uniformity of the spray on the roots of all plants.

The aeroponic unit was built using 3.2 cm diameter PVC plastic pipe. Its rectangular base dimensions were 2.4 m long, 1.7 m wide, and 0.6 m tall. This base supported two plant-growing surfaces mounted on top of the base, creating an A-frame that reached 1.5 m at the peak. The entire surface of the A-frame structure was covered with a white-on-black co-extruded polyethylene film to prevent leakage of light into, and nutrient solution spray out from, the root zone.

Planting density in both treatments was similar to that recommended for the field production of burdock at 41 cm on center plant-to-plant (New Zealand Institute for Crop & Food Research Ltd, 1996). Both the AGSM and aeroponic treatments were irrigated with a basic hydroponic nutrient solution modified from Resh (1998). This ensured comparable nutrient levels across both treatments. The pH was adjusted to 5.5-6.2 and the electrical conductivity (EC) was maintained at 2.8 mS cm^{-1}.

The plants were harvested from the AGSM controls on Nov 13 and 14, 2000 and from the aeroponic treatments on Nov 14 and 15, 2000. The aerial parts (leaves and petioles) of each plant from both treatments were air-dried. The roots of the plants from the AGSM were removed from the medium carefully and rinsed well with tap water. The roots of the plants from the aeroponic units were also rinsed with tap water to remove any fertilizer salts. Roots from both treatments were then allowed to air-dry one hour before being cut into pieces and frozen. The frozen roots were shipped in coolers with dry ice by

overnight air to Eclectic Institute, Inc. in Sandy, Oregon for lyophilization. The roots were hand cut (while frozen) to 1 cm, then dried in a Pinwald Stokes freeze dryer (model 480) for a minimum of 32 hours. The dried root material was weighed, then ground through a 40 mesh screen using a Wiley mill.

A random sample of one half of the experimental plants was sent to Nutritional Laboratories, International (NLI) for extraction and analysis. Since certified reference samples of burdock root were not available, four commercial products of burdock root in powdered form were purchased from a local health food store in Tucson, Arizona for use as reference materials. All samples were extracted by sonicating 500 mg of the dry, ground root in 25 ml of 50 % ethanol for 20 minutes. Longer sonication times were investigated, but no significant increases in extractive concentrations were observed. After sonication, samples were centrifuged for 10 minutes, and a 2 ml aliquot of the supernatant was filtered with a 45 µm PTFE syringe filter for HPLC. The extracts were run on an Alliance Waters 2690 Separations Module with a Waters 996 Photodiode Array Detector. The column used was a Phenomenex, Luna 5 μ C18(2), 250 mm x 4.6 mm. The operating temperature was 35 C^{o}. The wavelength monitored was 320 nm. A flow rate of 1.5 ml min^{-1} was used to run a gradient program ranging from 100 % Solvent A (0.05M NaH_2PO_4 adjusted to pH 2.9) to 100 % Solvent B (1 % 0.1N H_3PO_4 in Acetonitrile). The total run time was 15 minutes per sample. Chromatographic analysis was performed using Waters Millennium[32] Chromatography Manager software. Major peaks seen in the HPLC data for the reference samples had spectral characteristics of cinnamic acid derivatives and their esters. For this reason, 320 nm was used as a wavelength for 2-D chromatograms, since this wavelength coincides with a maximum in the spectra. The peak with a retention time of approximately 5.8 minutes corresponded to chlorogenic acid, by retention time and spectral profile. This was confirmed by comparison with a reference standard of chlorogenic acid purchased from Sigma Chemical Company.

Statistical analysis was performed using Microsoft Excel software. Means and standard deviations were calculated on biomass and phytochemical yield data, and differences between means were determined by two-tailed Student's t-test. Differences between the variances for chlorogenic acid concentration were tested using an F-test.

RESULTS AND DISCUSSION

A highly significant difference (P<0.00001) was found between the biomass yield (measured as dry weight) of the aerial parts of the aeroponically-grown plants compared to the biomass yield (measured as dry weight) of the aerial parts of the plants grown in AGSM, with the aeroponically grown plants being greater. The average dry weights of aerial parts were 45.6 g (±13.5g) and 30.6 g (±6.7 g) per plant in the aeroponic and AGSM treatments, respectively. There were no significant differences (P=0.31 and 0.75, respectively) between the replicates within the treatments, indicating that the aerial parts of plants growing on the west side of the house (which received more light) were not significantly different from those harvested from the east side of the house (which was partially shaded). These data indicate that aeroponics may have an increased yield advantage making it a promising production technique for any leaf crops having a market value high enough to cover the costs associated with the greenhouse and aeroponic equipment.

There were no significant differences (P=0.88) in the harvestable root biomass yields between the aeroponic and AGSM-grown plants. The means of the dry weights of harvestable roots from the two treatments were 20.0 g (±7.3 g) and 20.2 g (±5.4 g) per plant for aeroponic and AGSM-grown plants, respectively. There were, however, significant differences (P<0.05) between the replicates within the treatments, indicating that the roots of plants growing on the west side of the house, which received more light, were significantly larger than those growing on the east side of the house, which was partially shaded [21.7 g (±7.4 g) and 21.7 g (±5.8 g) for aeroponically-grown and control plants, respectively on the west side versus 18.4 g (±6.7 g) and 18.6 g (±4.5 g) for aeroponically-grown and control plants on the east side]. This is interesting since no

significant differences (P>0.05) between replicates were seen for the aerial biomass yields.

The concentrations of chlorogenic acid in the dry roots of plants from both the aeroponic and AGSM treatments were very low, but measurable. This appeared to have been a factor of the young age of the plants at the time of harvest (ten weeks from seeding). The mean concentration of chlorogenic acid in all the experimental samples was 0.032 mg g^{-1} of dry root. The reference samples contained much higher concentrations of chlorogenic acid: 0.32, 0.49, 1.75 and 2.94 mg g^{-1} dry weight for each of the four samples purchased from local stores. This variation is consistent with results published by Wang et al. (2001) for thirteen samples purchased at markets or cultivated in Japan. Those researchers found chlorogenic acid concentrations to vary between 1.35 and 4.75 mg g^{-1} dry weight.

A subsequent project using the same aeroponic units and seed source was undertaken to determine if a longer growing period would affect the biomass yields and phytochemical quality of the roots (Pagliarulo and Hayden, 2002). Fifteen *A. lappa* plants were grown for six months in the aeroponic system and the concentration of chlorogenic acid averaged 1.61 ±0.61 mg g^{-1} dry weight, which is comparable to the chlorogenic acid concentrations of burdock root samples purchased from the local market as well as that reported by Wang et al. (2001). No AGSM controls were grown in the second study, so it was not possible to evaluate the phytochemical variability across treatments.

In the original study, there were no significant differences (P=0.98) between the concentrations of chlorogenic acid in the roots of the aeroponically grown plants compared to the roots of plants grown in AGSM. The mean values were 32.6 ±5.7 µg g^{-1} and 32.7 ±12.5 µg g^{-1} (based on dry weight of root) in aeroponically-grown and AGSM plants, respectively. There were also no significant differences (P>0.05) between the two replicates within each treatment.

It is very interesting that the plant-to-plant variation of the chlorogenic acid concentration was significantly lower (P<0.01) in the roots of aeroponically-grown plants compared to those of the AGSM plants in the original ten week study (5.7 and 12.5 µg g^{-1}, respectively, F=7.38). The 95 % confidence interval for the population variance for aeroponically-grown plants was 20.9-59.6, whereas the 95 % confidence interval for the population variance for AGSM-grown plants is 98.8-282.1. Since the two confidence intervals do not overlap, it may be inferred that the population variances are significantly different, leading to the conclusion that the concentration of chlorogenic acid in roots may be more consistent in aeroponically-grown plants in this study.

CONCLUSIONS

Aeroponics appeared to be a highly feasible method for the production of both aerial parts and roots as raw materials for the herbal dietary supplement and phyto-pharmaceutical industries. In the controlled environment of the greenhouse, where growing seasons can be extended and all root material is easily accessible for harvest, biomass yields may be increased for some crops that have been historically difficult to grow and harvest using conventional horticulture in the field. Furthermore, using aeroponics, planting densities can be increased since plant-to-plant competition for nutrients and water is essentially eliminated. The A-frame design permits higher density planting per unit of floor area than conventional growing methods. The higher biomass yield of aerial parts from the aeroponic treatment indicated that this production technique should not be limited to root crops, but should be considered for other types of crops as well.

Although the absolute concentrations of chlorogenic acid in both treatments proved to be very low when compared with commercial samples, that is probably a result of immaturity. However, the concentrations were measurable, and no differences were found between the two treatments. It was very interesting that the consistency of the chlorogenic acid concentration appeared to be higher in the aeroponically grown plants, indicating that aeroponics may be a valuable production method for crops requiring phytochemical consistency to facilitate standardization and dosing of the final products.

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