

# Drying Temperature and Developmental Stage at Harvest Influence the Parthenolide Content of Feverfew Leaves and Stems

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## Abstract

Feverfew (*Tanacetum parthenium*) plants produced during 2000 and 2001 on raised beds and black plastic mulch in four replicated plots were harvested at four stages of development beginning at the early vegetative stage when plants were approximately 40 cm tall and stems were succulent. At approximately 14 day intervals harvest was repeated until the plants had begun to flower. Harvested tissues were dried with forced air at either 40, 60, 70, 80, or 90 C° until the moisture content was in the range of 8 to 10 %. After drying, leaves and stems were separated and parthenolide was extracted and analyzed by high performance liquid chromatography. Drying temperature influenced the amount of parthenolide recovered from dried tissues. Leaves dried at 40 C° had a mean parthenolide content of 0.43 % (dry wt). This decreased linearly with increasing drying temperature, with approximately 25 % less parthenolide following drying at either 80 or 90 C°. Stems typically had approximately one-tenth the amount of parthenolide found in leaves. Harvest date significantly influenced the parthenolide content of leaf, with higher parthenolide in later harvests. Biomass production increased as plants matured, therefore the amount of parthenolide produced per ha increased. Recommendations derived from this research were extended to commercial growers. The results of a pilot project with 8 growers are reviewed.

## INTRODUCTION

Feverfew [*Tanacetum parthenium* (L.) Schultz Bip., Asteraceae] historically was valued for its use to control pain and fever. In recent years studies have been focused on its utility for the treatment of migraine headache. Some clinical trials provide evidence that feverfew administered as a prophylactic treatment can reduce the frequency and severity of migraine pain and lessen the degree of associated nausea and vomiting. The active principle in feverfew believed to have medicinal properties is parthenolide, a sesquiterpene lactone (Johnson, et al., 1985; Murphy, et al., 1988). Some horticultural studies have been published on feverfew (Hendricks, et al., 1997), but limited information is available about horticultural practices, e.g. production and postharvest management protocols, that maximize the parthenolide content of feverfew. Our objectives were: to identify the most appropriate developmental stage for feverfew harvest; to determine the drying temperature that maximizes the preservation of parthenolide in feverfew tissues, and; to examine the feasibility for implementing our recommendations in commercial tobacco handling systems.

## MATERIALS AND METHODS

### Plant Production

Seeds of feverfew obtained from Richter's Seed Co. (Ontario, Canada) were planted in size 288 trays, cell size 2 X 2 cm top diameter X 3.8 cm depth (TLC Polyform, Inc., Atlanta, GA) with Metro Mix 300 (Scotts-Sierra, Marysville, OH) media. They were fertilized at each watering with 15-0-15 (Miracle Gro) mixed to 50 ppm nitrogen. After 14 weeks, transplants were moved to the field and planted on raised beds, 0.95 m width on 1.8 m centers, covered with black plastic mulch with one drip irrigation tape placed down the center of the bed. A pre-plant application of 10-10-10 fertilizer was applied

under the mulch at a rate of 500 kg/ha. Each bed had two rows of plants spaced 30 cm apart with 15 cm between the plants. Weed control was done manually. Irrigation was applied twice weekly. Four rows, each approximately 50 m long, were planted.

### **Harvesting and Drying Experiments**

Initial experiments were conducted during the fall of 2000 with summer-grown feverfew. The work was repeated on a larger scale with plants grown through the winter for harvest in the spring of 2001. Plants were harvested by hand using a sharp knife to cut about 8 to 10 cm above ground. For each harvest, an area of 2 m length within each row was selected at random and flagged to represent a replication for the each particular harvest and drying treatment, thus there were four replicates of each treatment at each sampling date. The fresh weight of each replicate varied from approximately 3 to 5 kg.

For the first harvest, plants were approximately 40 cm tall with no flowers and succulent stems. With each ensuing harvest, plants were maturing with progressive stem toughening. Initiation of flowering had occurred by the third harvest. With the final harvest, plants were estimated to be at approximately 10 % of full bloom, based on observations of some plants that were left in the field to continue growing through full development. Harvested plants were placed in plastic field lugs (45 cm X 60 cm X 22 cm deep) that had 250 holes of 1.0 cm diameter evenly distributed across the bottom and sides to facilitate air flow during forced-air drying.

The forced-air dryer had originally been designed for research with tobacco curing. The dryer chamber was 80 cm wide X 150 cm long X 120 cm tall and had the capacity to dry up to approximately 40 kg of herb when it was placed in field lugs and properly stacked to ensure uniform air flow. Heat was provided by electrical heat strips with sufficient capacity to maintain an air temperature of up to 90 C. A single speed fan circulated air via bottom delivery.

Only one forced-air dryer was available for drying studies so it was not possible to harvest for all drying temperature treatments on the same day. The following dates were recorded for each set of harvests in 2001: 1) Mar 6-10; 2) March 19-23; 3) April 5-16, and; 4) April 20-26. Field lugs with samples were transported directly to the drying area after harvest, weighed, and placed in the forced air dryer.

The experiment was a randomized complete block design with four replications including three factors (leaf or stem tissue, harvest time, and temperature). All data were subjected to ANOVA and if F-test was significant at  $P=0.05$ , LSDs were calculated.

### **Laboratory Analyses**

Dried samples of feverfew tissue were passed through a mill (Tector Cyclotec Model 1093 Sample Mill, Sweden) one time. A sample (2.5g) of the crude, milled powders was placed into a clean, dry 100 ml volumetric flask to which 60 ml methanol was added. An analogous portion was used for determination of moisture content (Sartorius Model MA-30, Edgewood, NY) and discarded.

The sample for analysis was sonicated (Branson Model 3510R-MT, Danbury, CT) for 10 min. with water added to the sample level. Flasks then were placed on a wrist action shaker (Burrell Model 75, Pittsburg, PA) for 30 min., then diluted to volume with methanol and mixed well. Approximately 80 ml was decanted into a centrifuge tube and spun (IEC Clinical Centrifuge, Needham Hts., MA) at 2500 rpm for 10 min. The supernatant was filtered (Whatman GF/C Glass Fiber Filters, England ) under suction and an aliquot transferred to an injection vial.

Parthenolide content of the samples was analyzed on a Shimadzu LC-10AT liquid chromatograph equipped with a SCL-10A system controller, a DGU-14A degasser, a SPD-10A diode array detector, and a SIL-10AD auto injector, and a Waters Nova-Pak C18 (Milford, MA) column with matching guard column. Purified standards of parthenolide were supplied by Triarco Industries (Patterson, NJ). The mobile phase was 40 % (v:v) acetonitrile in water, filtered and degassed. The flow rate was 1ml/min., injection vol. 20 ul, detector wavelength 215 nm, and run time 10 min. Data (peak areas)

were processed on an AST Bravo Computer equipped with Shimadzu 4.2 software. Parthenolide content was expressed as a percentage of the sample dry wt.

### **Pilot Project with Tobacco Growers**

In 2001, eight tobacco growers in the Coastal Plain and Piedmont regions of South Carolina each produced 0.5 ha of two feverfew types, common and golden (Richter's Seed Co., Ontario, Canada) in the spring for harvest in the summer. One of the growers was a certified organic producer and all growers were instructed to use no pesticides. For a second planting in the fall of 2001, five growers participated and produced only the common type. This crop over-wintered and was harvested in the spring of 2002. For field production, growers generally implemented the same protocol described earlier. After the first cutting, some growers allowed the plants to continue to grow for a second cutting. Recommendations for handling the cut feverfew were provided to growers based on results obtained from research trials described in this paper. Growers were instructed to dry the product in tobacco curing chambers set at 60 C<sup>o</sup> to a moisture content of 8 %. In 2001, the dried herb was baled in a commercial tobacco warehouse and stored at ambient conditions in same. In 2002, the dried herb was boxed and stored at 16 C with 20 % relative humidity in a controlled environment. Parthenolide content was evaluated as described.

## **RESULTS AND DISCUSSION**

### **Rate of Drying**

Prediction of drying times under the environmental conditions that prevail in the South Carolina coastal area was a tenuous task. Ambient relative humidity may range from below 40 % to near saturation. Neither commercial tobacco curing chambers nor our research chamber were equipped with equipment to dehumidify fresh air that is introduced into the chamber, thus the prevailing weather conditions affected the rate of drying. In general, drying at 40 C required 4 to 6 days to reach 8 % moisture content in the herb. Raising the temperature to 60 C decreased the drying time to approximately 36-48 hours. At 70 C less than 24 hours was required and at 80 or 90 C the herb usually dried within 8 to 10 hours. Clearly there are dramatic advantages for productivity, e.g. kg dried per day, by increasing the drying temperature. However the influence of heat on the quality of the herb must be considered.

### **Influence of Drying Temperature on Parthenolide Content of Leaves and Stems**

Feverfew drying tests conducted in 2000 and 2001 provided similar results and only data from 2001 are presented here.

As plants matured, parthenolide content (% dry wt.) increased significantly in leaf tissue and decreased significantly in stem tissue (Fig. 1). When data from all drying temperatures were pooled by harvest, from the first harvest until the last the parthenolide content in leaf increased from 0.330 % to 0.385 % but in stems it decreased from 0.121 % to 0.026 % (Fig. 1). In all studies, stems typically contained only 10 to 20 % of the parthenolide measured in leaves (Fig. 1,2 and 3).

Increasing drying temperature caused a significant decrease in parthenolide (% dry wt.) in leaf tissue (Fig. 2). There was an apparent, but insignificant, decrease in stems. Pooling data from all harvests by drying temperatures revealed an almost linear decrease in the parthenolide content in leaf tissue from 0.429 % at 40 C to 0.304 % at 90 C. In stem tissue, the decrease was from 0.058 % at 40 C to 0.038 % at 90 C (Fig. 2).

Fig. 3 illustrates the effect of drying temperature on parthenolide in leaf and stem tissue for each individual harvest.

### **Pilot Project with Tobacco Growers**

In 2001, feverfew was harvested from all 8 commercial farms. All farmers were provided recommendations for harvesting and drying but were left to their own initiative to implement the recommendations. Results varied from excellent to unacceptable. One

certified organic grower carefully followed recommended protocol and yielded almost 400 kg of dried herb. Parthenolide content (% dry wt.) of common feverfew was ~ 0.4 % and that of golden feverfew was ~0.8 %. Although the parthenolide content of golden was much higher than that of common feverfew from all farms, the plants of the golden type were much smaller and the yield of herb was dramatically lower than that of the common type (data not shown).

Some growers attempted to dry the herb in forced air without applied heat. In these cases the development of mold was apparent. Herb was collected from all farms and taken to a commercial tobacco warehouse for baling. The bales (approximately 175 kg each) were stored in a non-climate controlled warehouse. Organically grown herb was not baled, but was ground to powders in a commercial facility (Triarco Industries, Green Pond, SC) and currently is stored at 20 C for evaluation of parthenolide stability over long term storage.

Attempts to market the 2001 herb were precluded by four quality concerns that all relate to the implementation of Good Agricultural Practices (GAPs) and Good Manufacturing Practices (GMPs). The first deficiency was excessive ash content (> 11 % dry wt.) resulting from sand and soil that contaminated the plants during production and harvesting. The second concern was an excess of foreign material, specifically weeds (> 1 % dry wt.), in the dried product. The third problem was the development of mold in the baled product. Tobacco typically is baled with approximately 18 % moisture which was excessive for feverfew. Finally, tobacco beetles infested the baled product in high numbers. Some companies have zero tolerance for live insects in medicinal herb. The parthenolide content, which ranged from approximately 0.25 to 0.80 % dry wt, was acceptable to a number of the companies that were contacted. Parthenolide content of baled feverfew declined during the 8 month storage period (data not shown) and the rate of decline under varying storage conditions is still under investigation.

For feverfew harvested during the spring, 2002 season, greater attention was given to the implementation of GMPs. Only three of the five participating farms were harvested because two growers planted too late in the fall and the crop suffered severe cold damage which stunted the plants. The first author participated in cutting and handling at all three farms. All weeds were removed from the rows by hand prior to cutting. During cutting, any herb that fell onto the soil was not collected. Dryers were carefully monitored and herb was dried to 8 % moisture, then collected and placed in sealed boxes for climate-controlled storage. Parthenolide content was ~0.4 % dry wt. At the time of preparation of this report, the herb remains in controlled environment storage for further evaluation.

## **CONCLUSION**

Leaf tissue consistently contained significantly more parthenolide than stem tissue. In commercial production systems, it would be advantageous to separate leaf from stem after drying in order to provide consumers with a higher quality product.

Succulent stems contain significantly more parthenolide than stems that are more mature and tougher. If dried feverfew herb is to be marketed with leaf and stem tissue both, it appears to be advantageous to harvest the crop about three weeks prior to flowering when the stems contain higher parthenolide. This recommendation, however, needs further refinement.

Increasing drying temperatures causes a decrease in parthenolide in leaves and stems both. For maximum parthenolide content, we must conclude that the lower drying temperatures are advantageous. However, drying bulk amounts of herb on a large scale may necessitate elevated temperature in order to shorten drying time and thus increase productivity. This is a compromise that will need to be agreed upon by the producer and the buyer of the herb.

Implementation of our harvesting and handling recommendations was generally unsatisfactory in a pilot project on commercial farms. Considerable attention to GAPs and GMPs will be required in order for commercial tobacco farmers to adopt the production of feverfew or other medicinal plants as a supplemental crop.

### Literature Cited

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### Figures

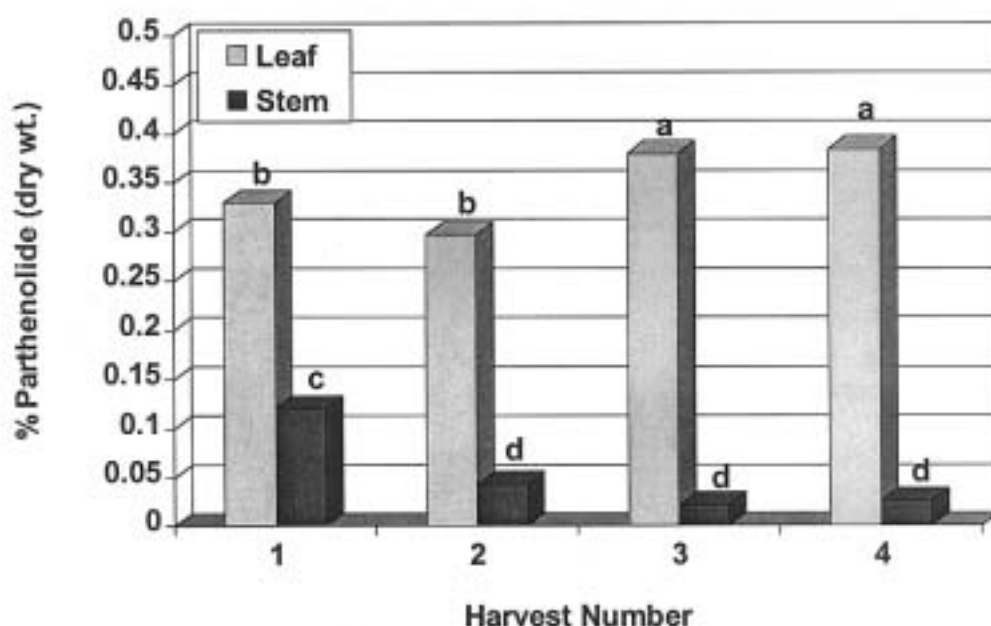


Fig. 1. Data from all drying temperatures were pooled to show the influence of harvest no., e.g. stage of development, on the parthenolide (% dry wt.) content of leaf and stem tissue from feverfew. Mean separation by LSD at  $P=0.05$ .

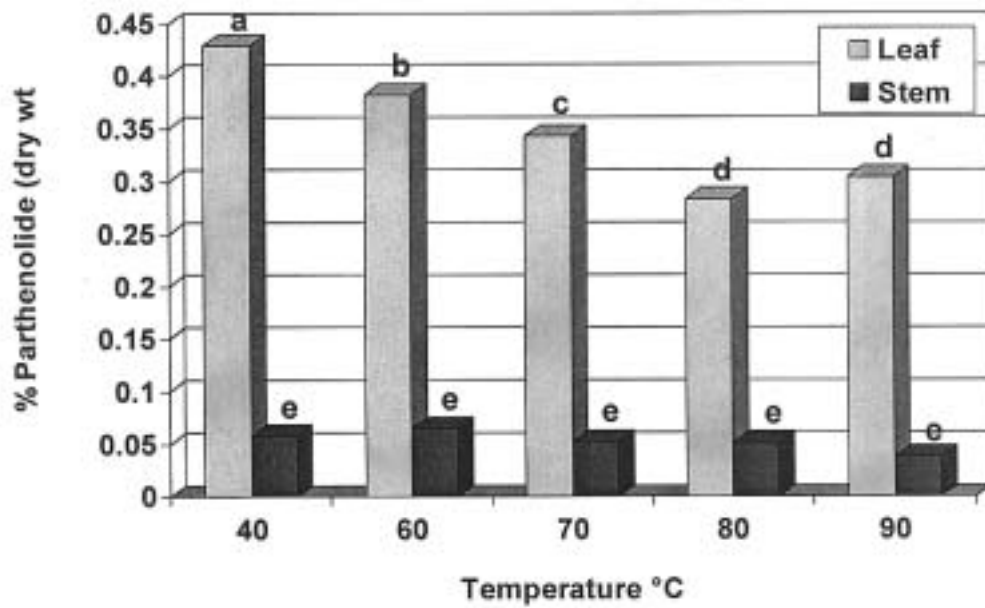


Fig. 2. Data from all four harvests were pooled and plotted to show influence of drying temperature on the parthenolide (% dry wt.) content of leaf and stem tissue of feverfew. Mean separation by LSD at P=0.05.

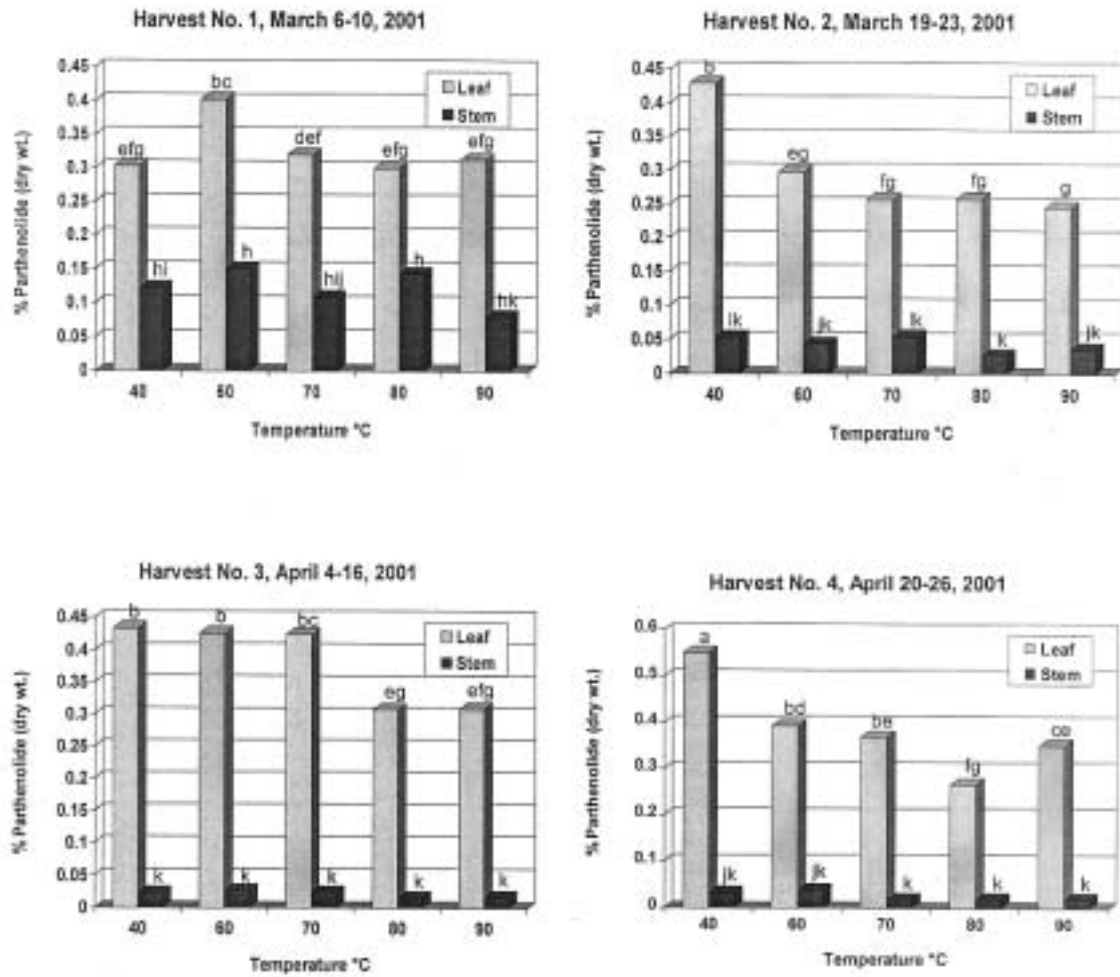


Fig. 3. The effect of drying temperature on parthenolide (% dry wt.) content of leaf and stem tissue for each harvest date. Mean separation by LSD at P=0.05.