Production and Development of Nutraceuticals as Alternative Crops: Implications for Certification and Branding: Part 1

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<u>Outline of Specific Aims and Evidence:</u>

A. Develop specific guidelines for production of medicinals plants

1. **Results of fertility experiment contrasting bare ground culture and mulch systems on biomass and marker compounds in feverfew and Echinacea purpurea and pallida**

2. **Results of fertility experiment contrasting organic and inorganic fertility on feverfew yield, mineral and parthenolide content**

B. Identify varieties of medicinals with high marker compounds and climatic tolerance

3. **Results of germplasm evaluation of Black Cumin, Echinacea, Feverfew, St. John's Wort, and Valerian on environmental tolerance and suitability for commercial production**

4. **Results of the influence of climate on feverfew marker compounds**

C. Formulate weed control protocols without need for herbicides

5. **Results of the evaluation of clover cover crops as "living mulches" in the production of organic medicinal plants**

D. Establish quality control protocols for harvest and storage of medicinal plants

6. **Report from James Rushing on postharvest handling of feverfew**

E. Implications for certification, branding and marketing of medicinal crops

7. **Impact on marketing strategies for growers**

8. **Impact on marketing strategies for distributors**

A. Develop specific guidelines for production of medicinals plants

1. **Results of fertility experiment contrasting bare ground culture and mulch systems on feverfew and Echinacea purpurea and pallida**
2. **Results of fertility experiment contrasting organic and inorganic fertility on feverfew yield, mineral and parthenolide content**

Goal: The production of medicinal plants is not an easy grower task simply because very little is known about how to best produce these crops to insure highest quality production. With medicinals, two characteristics are required that constitute success. The first is high yields of biomass per acre. With feverfew, this would pertain to foliage since that is the plant part used for extraction of the marker compound, or the chemicals so desired by the dietary supplement industry. With Echinacea, the roots are the product harvested. It is necessary that the correct production practices enhance the production of very high tonnage of biomass per acre. Yet, this is only half the job. The biomass must be highly fortified with marker compounds and without this, the tonnage is worthless to the processor. Presently, most raw material reaching the processor may be poor quality with low levels of marker compounds. Grower guidelines that outline how to enhance biomass and marker compounds are nonexistent for most medicinal crops. Therefore, before any of these crops becomes a commercial endeavor, we have to understand how to grow these crops to insure quality.

The most desirable (high value) commodity sold in the dietary supplement industry are organic herbal supplements. Herbal products are taken by consumers as “natural ways” to enhance health and well-being and organically-grown products are in full synchrony with the philosophy of using herbs—all natural approach to grow the herbs that will naturally improve health. From the horticultural standpoint, we have always approached the production of herbs from both schools of thought—organic and conventional. We are not sure that organic production methods can produce a higher quality product, except to just say they were grown organically. We feel that the chemical analysis of the product is important to document an improved chemical status. To this end, we have conducted organic and conventional production experiments to conclusively determine the superiority of product of each production method. From the growers’ standpoint, production of organic herbs may not always insure greater profits since organic technology may be more labor intensive, especially in controlling weeds. Neither organic or conventional production methodology allows chemical pesticides since nothing is labeled for these very minor, specialty crops.

In the first experiment, our rationale was to compare minimalist approaches to growing medicinal plants that exclude plastic mulch. For a number of years, we had been growing medicinals with drip irrigation and plastic mulch. We hypothesized that since most of these crops are known to grow very well on waste lands in their areas of natural occurrence, plastic mulches may be producing too moist root environments and actually hinder biomass/marker compound yields. It is felt, however, that plastic mulch is a necessity with medicinal crops since no herbicides are available to control weeds.

1. Fertility experiment contrasting bare ground culture and mulch systems on biomass and marker compounds in feverfew and echinacea purpurea and pallida

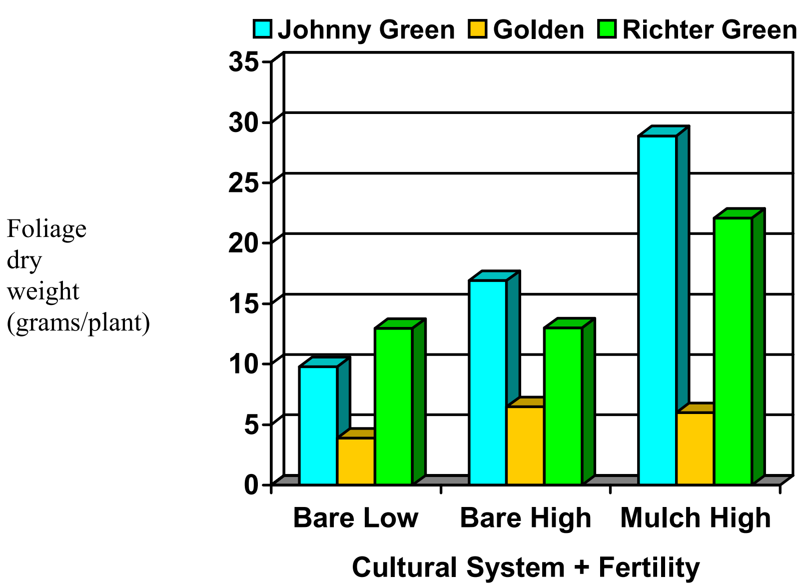
Approach: Echinacea purpurea and pallida and 3 varieties of feverfew were transplanted to the field in March. Individual plots were included that would grow these crops with 3 cultural systems: 1) plastic mulch with high chemical fertility (160 pound nitrogen per acre), 2) bareground with high chemical fertility (160 pound nitrogen per acre) and 3) bareground with low chemical fertility (60 pounds nitrogen per acre). Feverfew foliage was harvested mid-summer and parthenolide (marker compound) determined. Echinacea purpurea and pallida roots were dug in January and cichoric acid and echinacoside analyzed for the crops, respectively.

Results:

Feverfew

Yield and quality of two varieties of green feverfew (Richter's Green and Johnny's Green) and one variety of golden feverfew (Richter's Golden) were contrasted. The first determinant of success is the production of biomass. Figure 1 illustrates that

Figure 1

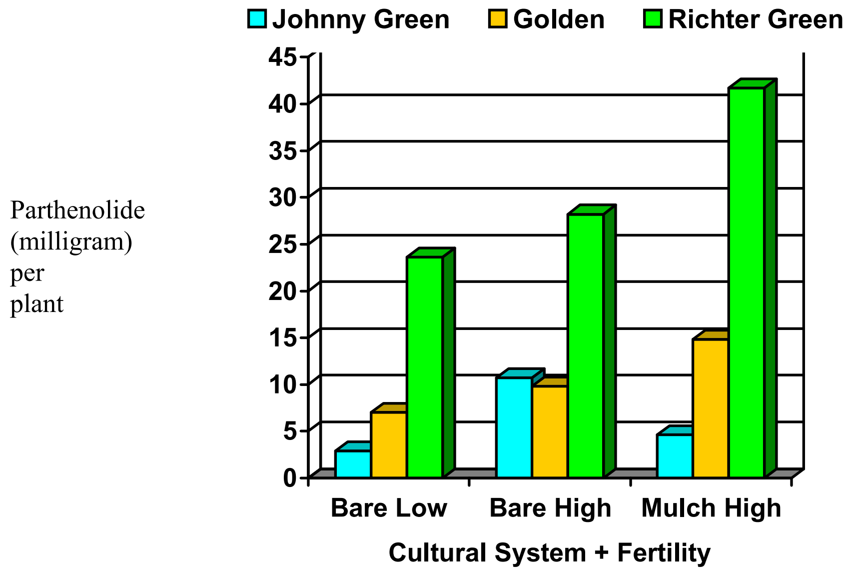


the highest biomass production was with Richter's Green and Johnny's Green varieties grown with high fertility and mulch. Foliage yield of Golden feverfew was unaffected by production system. This data negates the idea that a mulch effect reduces yield as originally hypothesized and, in fact, this mulch effect is very beneficial to increase yield.

Additionally, Golden feverfew has been recommended as the best variety to grow for marker compound, but the biomass production of this dwarf variety renders it a very unsuitable biomass producer.

The second critical need with feverfew is a high concentration of parthenolide. Figure 2 illustrates the differences among varieties and cultural systems.

Figure 2



Johnny's variety was found to contain very little parthenolide and even though this variety produced the highest biomass, the concentration was so low in the foliage that the quality of the yield would be considered unacceptable and very poor. Richter's Green, on the other hand, was adequately fortified with marker compound and when grown with mulch and high fertility, the yield of parthenolide per plant greatly exceeded all other systems. The "ingredient" in the mulch/high fertility system that enhanced yield the most was the mulch since high fertility in bareground plots did not boost parthenolide content to the degree that the mulch/high fertility systems did. **This experiment verifies that the mulch/high fertility system and Richter's Green variety are the best systems evaluated in this study for yield.** We still would like to further increase both biomass and marker compound content to higher levels, but we will abandon bare ground culture, and Golden and Johnny's Green varieties.

Echinacea purpurea

The choice of an appropriate cultural system to grow the roots of both Echinacea species is an important decision. In both crops, the individual root systems weights were highest when grown with high fertility and mulch, very similar to what has been reported for feverfew foliage (Table 1). High fertility alone cannot be assigned the full effect since purpurea and pallida fresh root weights increased 38% and 49%, respectively, with the addition of plastic mulch. Cichoric acid content in E. purpurea was significantly highest with the high fertility/mulch system with marker compound content 34% lower with high fertility/ bareground system and 13% lower with low fertility/bareground system. Echinacoside content in E. pallida was not statistically affected by any of the cultural systems. Since the biomass was highest with the high fertility /mulch system, however, it is obvious that the yield per acre of marker compound would, likewise, be increased because of more root biomass charged with marker compounds that tend to be at the highest content.

Table 1. Effect of cultural system on Echinacea purpurea and pallida root weight and marker compound concentration

Crop	System	Root fresh weight (grams)	Cichoric Acid (%)	Echinacoside (%)
E. purpurea	High fert + bare ground	43.2 b	0.459 b	Na
E. purpurea	Low fert + Bare ground	44.2 b	0.608 ab	Na
E. purpurea	High fert + mulch	69.6 a	0.705 a	Na
E. pallida	High fert + bare ground	29.7 b	Na	1.74 a
E. pallida	Low fert + Bare ground	33.0 b	Na	1.90 a
E. pallida	High fert + mulch	59.1 a	Na	1.80 a

For maximum biomass and marker compound yields in Echinacea purpurea and pallida, cultural systems using high fertility and plastic mulch are recommended.

2. Fertility experiment contrasting organic and inorganic fertility on feverfew yield, mineral and parthenolide content

Approach: Richter's Green feverfew and Richter's common E. purpurea were transplanted in March and fertilized with equivalent amounts of organic fertilizers (bat guano [nitrogen], soft rock phosphate, and greensand [potassium]) and chemical fertilizer (10-10-10) at two rates of 75 (low) and 150 (high) pounds nitrogen per acre. These plots were drip irrigated and mulched with plastic. The feverfew was harvested in July but Echinacea will be harvested in January after dormancy and will not be reported here. Feverfew leaf tissue was analyzed for parthenolide and mineral content (nitrogen, phosphorus, potassium, calcium, magnesium, sodium, sulfur, iron, manganese, copper, zinc, and aluminum). We were interested if biomass and marker compound content differed by fertility regime and also, if any tissue mineral element correlated with parthenolide.

Results:

The yield of feverfew biomass and marker compounds were differentially affected by the type of fertilizer used (Table 2). In the chemical system, biomass and parthenolide were higher with low fertilizer and increasing the fertilizer rate actually reduced biomass and parthenolide by 23% and 18%, respectively.

With organic fertility, biomass yield was statistically similar with high organic fertility versus chemical low yields, yet the actual yield per acre was about 15% less with organic fertility at these treatment levels. Choice of organic fertility rate is important since biomass and parthenolide were reduced 13% and 14%, respectively, if low organic fertility was used versus high organic fertility. Since organic fertility is time-released and not immediately available as are chemical fertilizers, plant growth and parthenolide development may not have proceeded at the same rate as growth induced by chemical fertility.

Table 2. Influence of chemical and organic fertility on feverfew biomass yield and marker compound content.

Fertility System	Fertility Level	Foliage (pounds per acre)	Parthenolide (%)
Chemical	Low	2,476 a	0.602 ab
Chemical	High	1,888 b	0.494 c
Organic	Low	1,837 b	0.538 bc
Organic	High	2,105 ab	0.628 a

Tissue analysis did not show any strong relationships that correlate leaf nutrient status with parthenolide content. However, tissue analysis demonstrated the nutrient status that feverfew attained with the four fertility systems evaluated (Table 3). This information is not available anywhere to my knowledge and makes a contribution to our knowledge. Basically, nutrient content of organic is the same as conventionally grown feverfew.

Table 3. Influence of chemical and organic fertility on feverfew elemental composition.

Element	Chemical Low	Chemical High	Organic Low	Organic High
% N	2.72	2.81	2.81	2.76
% P	0.30	0.31	0.31	0.36
% K	3.75	3.84	3.65	3.76
% Ca	1.30	1.62	1.11	1.12
% Mg	0.50	0.53	0.47	0.51
Na (ppm)	131	114	116	121
% S	0.35	0.40	0.43	0.45
Fe (ppm)	410	343	360	348
Mn (ppm)	100	177	45	54
Cu (ppm)	20.2	16.6	17.0	17.0
Zn (ppm)	47.3	58.9	38.4	46.4
Al (ppm)	380	341	341	344

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If organic production is the goal, then using the higher rate of fertilizer is recommended. If highest yields are desired, without the need for the organic slant, the lower rate of chemical fertilizer is recommended.

B. Identify varieties of medicinals with high marker compounds and climatic tolerance

3. **Results of germplasm evaluation of Black Cumin, Echinacea, Feverfew, St. John's Wort, and Valerian on environmental tolerance and suitability for commercial production**

4. **Results of the influence of climate on feverfew marker compounds**

Goal: If the production of plants useful to the dietary supplement industry is to be successful, the growers' objective must be to provide the industry with the highest quality raw product possible. Also, for South Carolina growers to be most competitive, these high quality variety(s) used must be certified, branded and protected from exploitation by others who are not licensed to use these varieties. At present, there are very few varieties available to use commercially and it is unknown if any are high potency and productive. Part of our efforts were to evaluate the germplasm available and determine superiority. But the greater part of our effort, has been to identify "super" plants within these heterogeneous mixtures that stand out for biomass production and/or production of marker compound. If supers can be found, our goal, therefore, will be to cross and breed these excellent ecotypes and then, in time, produce new cultivars that can be patented and protected. With the highest quality product possible, we feel that the marketing of these new cultivars will entice the dietary supplement industry to want to use the products only our growers can produce.

Identification of super ecotypes is not as easy as just finding a "good" plant and calling it "a new variety". We have found that climate in many cases affects the expression and production of marker compounds in the plants. Any new line of medicinals we think is superior, has to be rigorously screened for performance to insure that these potential new varieties are dependable producers of marker compound and high biomass. It has been our experience that parthenolide in feverfew, for example, may spike in one season and not in the next. These observations indicate that this property of medicinal plants is not a constant, but is illusive. It is known that marker compounds do spike at certain times of the growing season, sometimes in response to stresses from insect and/or disease pressure or climatic stresses, drought, heat, etc. It is therefore imperative in our research to monitor the genesis of marker compounds over times that the plants are harvestable to learn to understand why they react as they do.

Results:

3. **Germplasm evaluation of Black Cumin, Echinacea, Feverfew, St. John's Wort, and Valerian on environmental tolerance and suitability for commercial production**

Approach: The decision to grow certain medicinals plants must first determine if they are suited to our unique tropical summers and subtemperate winters. In spring, we transplanted 13 feverfew lines, 6 echinacea species, 9 varieties of St. John's Wort, 4 varieties of valerian, and 2 seed sources of black cumin. We have found that the crops under study can be now divided into 3 groups. The first is climatically adapted and grown without much interference from the extremes of our climate. Feverfew, by far, thrives in our climate, but some lines excel while others flounder. Next in the hierarchy are Echinacea species, but within that genus, there are differential responses among the species. The most climatically adapted are Echinacea tennesseensis, then E. paradoxa, then E. atrorubens, then E. pallida, and lastly E. purpurea. The most desirable medicinal of all Echinaceas is Echinacea angustifolia, but this weak species does poorly in our climate and without improved, new varieties, this species should not be attempted in coastal South Carolina. The second group of medicinal crops are considered moderate to high risk in that they may grow very well in certain growing conditions, but fail miserably when the climate changes. St. John's Wort and valerian are examples of these crops. Both are highly susceptible to disease which becomes extremely virulent in the heat of summer. If these crops are planted at times that either avoid these rigorous times or the length of time they are exposed is drastically shortened, they may successfully grow to harvest. The last group that has no climatic adaptation includes only black cumin. This crop has been attempted at least 8 times over the years using all known methodology, yet each attempt has failed. Black cumin has been discarded as a potential crop and no future research will be attempted.

Feverfew evaluation

The first indicator of superiority of a feverfew line is its ability to synthesize adequate parthenolide. We found a vast difference in parthenolide concentrations among the lines outlined in Table 4. Of all these, two lines show great potential for future evaluation and search for super ecotypes. USDA Germany 161 is a line contained at the US Germplasm Repository that originally was isolated from a location in Germany. This line has a mean parthenolide content of 0.569 with individuals spiking as high as 0.764. The dietary supplement industry wants raw material with concentrations ranging from 0.5 to 1.0%. Within this line, we have found 16 super ecotypes that excel in parthenolide over the 0.60% level. This line also has good horticultural traits as described in Table 5. The second and last line with high parthenolide content is USDA Mexican 502 which has a lower mean parthenolide content than USDA Germany 161, but it has individual plants that spike at 0.846% parthenolide.

Table 4. Variation in parthenolide among feverfew lines.

Feverfew Line	Mean Parthenolide (%)	Standard deviation	Standard Error	Range	Parthenolide (%)		
					Max	Min	Median
Richters Green	0.320	.124	.0271	.526	.645	.119	.287
Horizon Green	0.348	.086	.0143	.388	.630	.242	.325
Jelitto Green	0.250	.174	.0451	.617	.644	.027	.236
Richter's Golden	0.368	.026	.0105	.077	.409	.332	.365
USDA Italy 902	0.244	.105	.0396	.344	.377	.033	.251
USDA Germany 161	0.569	.139	.0254	.484	.764	.280	.592
Feverfew Line	Mean Parthenolide (%)	Standard deviation	Standard Error	Range	Max	Min	Median

USDA Ukraine 901	0.210	.079	.0279	.244	.364	.120	.201
Chelsea Physic Garden 702	0.129	.075	.0282	.237	.262	.025	.111
Chelsea Physic Garden 703	0.161	.032	.0182	.055	.197	.142	.143
Mexican Green 502	0.449	.172	.0384	.678	.846	.168	.383

We have identified 5 super ecotypes within that line that are worthy of more testing. We have found the following supers in other lines as follows:

Richter's green – 1 super
Horizon green – 1 super
Chelsea Physic Garden (702) – 1 super

Besides parthenolide, we are searching for enormous plant types that will provide extremely high biomass yields per acre. These individuals ideally should contain high parthenolide, but if not, they may be crossed with other high parthenolide supers to yield new varieties with high parthenolide and biomass. Yet, if this is not possible, use of a high biomass feverfew variety would, because of sheer massive yields, give high yields of parthenolide per acre once processed in contrast to a small biomass variety with high parthenolide. We have identified one "super biomass" plant from Chelsea Physic Garden (702) that is an exceptionally enormous plant. This particular plant grew rapidly and covered the bed with very dark green foliage. It did not flower which makes it even more desirable in that flower stems are considered a pollutant and would need to be removed before processing since they add no parthenolide and dilute the potency of the load.

Table 5. outlines the visual observations made concerning each of the feverfew lines evaluated, how they excelled visually, how they failed, and what attributes make them desirable for future research.

Out of the thousands of plants evaluated, we found a handful of extremely promising individual plants with outstanding biomass and parthenolide concentration. The refinement and grooming of new varieties of feverfew will continue into Part II of this research project.

Table 5. Visual observations of growth habit differences among feverfew lines.

Richter's Green - Sporadic growth and flowering habit. Individuals vary from tall to short, wide to narrow. Quite a difference in flowering with most daisy flower types. Plant is yellowish and nutrient deficient looking. Quite a few missing plants, but it has some pretty good biomass production in contrast to others. Have one super plant with high parthenolide but it is very weak. Desirable because: LARGE BIOMASS
Horizon Green - Very small but uniform plant. All plants look identical. No flowering at all, but only about 1 foot in diameter. Not enough biomass to make worthwhile. But lack of flowering is appealing and has not flowered at all this summer. One super parthenolide plant and it is weak. Desirable because: Uniformity and no flowers at all
Jelitto Green - Incredible variability, very tall, wide, lots of stems and flowers. Wide plant and moderate stand loss. Very diverse in growth habit, but has potential with good biomass but too many stems. Desirable because: LARGE BIOMASS BUT DOWNSIDE IS TOO MANY STEMS
USDA Ukraine (901) - Tall plant and lots of variability in growth and flowering and has a lot of stems. Good biomass plant but too variable in plant growth but is big in spite of variability. No supers. Desirable because: LARGE BIOMASS
USDA, Italy (902) - Very weak plant, very small, struggling, not adapted and no potential for biomass with sporadic flowers. Not a single large plant in plot. NO USE TO US
USDA, Germany 160 (903) - Ordinary growth with much flowering and variation in plant growth. No supers found yet and this line looks very different from USDA Germany 161 (904)
USDA, Germany 161 (904) - Exceptional and has at least 16 supers. Moderate in size with sporadic flowering. The leaves have very wide leaflets with pale green color. Not a lot of death and seems to be climatic adapted. I like its looks! This plant has good biomass production and a very pretty plant. Uniformity is good with very similar size and appearance. The flowering is not that profuse and the stems are not that long. Desirable because: HIGH IN PARTHENOLIDE, GOOD BIOMASS, ENVIRONMENTALLY-ADAPTED
Chelsea Physic Garden (702) - Very variable growth and biomass, growth habit. Tends to be small and weak, but there are differences between plants. Have one super plant that is 3 feet in diameter and two feet tall, never flowered, dark green, parthenolide is low but on acre basis it will be productive. The plant has very different leaflets that are very broad with dark green color. Desirable because: SUPER BIOMASS IS OF GREAT INTEREST FOR FUTURE BREEDING
USDA Mexican Green (502) - 5 supers in this line. Some problems with plant death but growth is fairly large, covers most of the bed, 2 feet in diameter, but has a lot of flowers with variable plant growth habit and leaflet pattern. Desirable because: VERY HIGH PARTHENOLIDE BUT SMALL BIOMASS...CROSS WITH LARGE BIOMASS TYPE.
Richter's Golden - Small growth, lime green, 8" in diameter, weak, no flowers, not adapted to climate, no biomass and vigor, need to use 5 golden to equal 1 green for biomass, can be used in breeding program if you could get to flower. Desirable because: High Parthenolide
Chelsea Physic Garden Golden (703) - Too much variability with sporadic flowering, looks like nutrient deficient , lots of death, not adapted, no supers. NO USE TO US.
Chrysanthemum Parthenium Mexico - Weakest feverfew of all, dwarf, all flower and no foliage, 75% dead. NO USE TO US

Echinacea evaluation

Echinacea has been grown at our location for five years in various research studies. In three of five years, the crop has grown excellently and without much interference from climatic and pest stresses. In contrast, in two of the five years, insect pressure has been abnormally extreme and strongly hindering the growth of the Echinacea. This summer our planting of Echinacea was inundated by white fly and sunflower moths, severely retarding the growth of most of the plants. In hindsight, this additional stress was beneficial to make the environment as stressful as possible for selection of the most stress tolerant ecotypes. Table 6 outlines the most obvious visual traits recorded for each Echinacea line. The most vigorous Echinacea purpurea line was Richter's Echinacea purpurea in contrast to the other seed company's' E. purpurea. Echinacea pallida is also used extensively in Echinacea dietary supplements. The best line was Richter's Echinacea pallida which grew more vigorously than the Horizon or the Jelitto lines. It is still unknown if yellow coneflowers, Echinacea paradoxa, contain cichoric acid or echinacoside; if they do, this type of Echinacea is climatically adapted to our region with Jelitto Echinacea paradoxa showing the best growth. **The most climatically adapted Echinacea that actually thrives in our environment is Echinacea tennesseensis, a rare and endangered species common to only Tennessee.** It is purported that this species has very high medicinal activity which we will determine this winter and further into Part II of this project. Within our planting, we have many individuals that are highly vigorous and not phased by insect pestilence. This discovery is one of our most exciting success stories of this work.

Table 6. Visual observations of growth habit differences among Echinacea lines.

Horizon, Echinacea angustifolia, pink, native Tx, Ks, Nb, Wy, Mo. This variety for the most part is not adapted to our climate. Four survivors were moved from the field to the greenhouse. These survivors may have some level of adaptation to observe in the future.
Richter's, Echinacea angustifolia, moved 5 survivors taken from field to the greenhouse. This variety reacted the same as Horizon angustifolia.
Richter's, Echinacea angustifolia, organically grown, moved 5 survivors to greenhouse and reacted the same as other angustifolias.
Horizon, Echinacea pallida, native to Ak, Ok, Nb, Mn. This line is very weak and not adapted. Only about 5% survival by late October from the original planting.
Richter's, Echinacea pallida, good growth with some variability, and about 14 selections that show environmental adaptation.

Jelitto, Echinacea paradoxa, yellow flowers, rare, native to Ozarks. Lot of variability, with some plants very vigorous and some marginal. This line of paradoxa seems most vigorous of all paradoxa with 9 selections showing adaptation.
Richter's, Echinacea paradoxa, lots of variability, a few exhibiting superior growth with much taller, showy flowers and more profuse flowering, 11 selections showing adaptation.
Horizon, Echinacea paradoxa, growth is very good but lots of variability in flowering and size, with a few larger plants, but none really outstanding, 8 selections showing adaptation.
Horizon, Echinacea purpurea, purple-rose, native from Mi to La, lots of variability, 11 selections showing adaptation.
Richter's, Echinacea purpurea, quite vigorous, and this line has the most selections showing adaptation, this line has lots of volunteer seedlings, 19 selections showing adaptation.
Richter's, Echinacea purpurea, organically grown, lots of volunteer seedlings, looks weaker than the previous line grown non-organically, 18 selections showing adaptation.
Richter's, Echinacea purpurea 'White Swan', white flowers, is worthless to us, with about 70% dead, not adapted, 10 plants worthy of further observation for adaptation.
Jelitto, Echinacea purpurea 'White Swan', better stands than Richters' White Swan, but still not real impressive, 22 selections showing adaptation.
Jelitto, Echinacea purpurea 'Magnus', ruby red, lots of variability, most looks weak, 14 selections showing adaptation.
Richter's Echinacea purpurea 'Magnus`, intense red flowers with about the same performance as previous 'Magnus'. Plain Echinacea purpurea may have better vigor, 11 selections showing adaptation.
Jelitto, Echinacea purpurea 'Rubinstern Ruby Star', intense carmine-red, nothing outstanding, probably equivalent to Magnus, 22 selections showing adaptation.
Horizon, Echinacea atrorubens, rare, lavender to dark purple, lots of variability, some differences in leaf habit, some vigorous, and some quite weak, 9 selections showing adaptation.
Horizon, Echinacea tennesseensis, deep purple, rare, high immune, Tennessee native, very unusual, 100% stand, very rare, and thrives here, very tolerant to white fly infestations, looks great, lots of variability, some really big super biomass selections, very promising, marker compounds may be equivalent to Ech. angustifolia, rare and any breeding would be a true innovation, 20 selections with climatic adaptation.

Results

4. Influence of climate on feverfew marker compounds

Approach: Feverfew has traditionally been grown as a spring crop, but we have found that this crop should be considered a winter crop in coastal SC. Feverfew has very high cold tolerance and is not affected by the frosts common in our winters. Ideally, feverfew could be planted in the fall and grow rather large in the winter months with harvest in late spring. The cultural advantage to this system is that insect and weed pressures are at a minimum during this time and production is far easier than in warm weather.

Usually, winter feverfew does not even need irrigation since winter rainfall is timely and evapotranspiration of the crop minimal. We do not know, however, if field planting dates have any superiority for spring biomass and parthenolide yields. It was the goal of this experiment to determine if plantings made on 10/1, 11/1 or 12/1 differed in quality of production. All these plantings were allowed to grow to maximum foliar coverage over the entire beds and then harvested on May 17 and then again after regrowth on July 22. The December 1st planting was abandoned because of a massive infestation of thrips over the winter. Although the December planting was devastated by thrips, the October and November plantings, adjacent to the December planting, were unaffected by the thrips. Apparently, planting dates had a strong influence in the insect's preference for material to feed on. Table 7 indicates that **Johnny's green variety is extremely low in parthenolide although biomass is very high. Johnny's green feverfew should not be used for commercial production in any case.** Richter's green variety, on the other hand, was very productive in biomass and parthenolide, especially in the October 1st planting date in contrast to the November 1st planting. Both yield variables were significantly higher with the October planting. The second harvest made in July was less productive in biomass and parthenolide. Biomass yields between Oct. and Nov. plantings were equivalent in the second harvest, yet parthenolide was still higher with the Oct. planting (although the parthenolide was reduced two-fold in the second harvest). Depending on market, the second harvest of raw material was reduced three-fold in biomass and with low parthenolide content, harvesting twice so soon may not be profitable.

Table 7. Influence of planting date of feverfew yield and parthenolide content.

Planting date	Variety	Biomass (pounds per plot)		Parthenolide (%)	
		Harvest 1	Harvest 2	Harvest 1	Harvest 2
October 1	Richter's green	32.9 ab	10.3 d	0.941 a	0.491 c
November 1	Richter's Green	22.6 c	7.8 d	0.845 b	0.391 d
October 1	Johnny's green	35.3 a	10.3 d	0.166 e	0.105 f
November 1	Johnny's green	30.0 b	10.2 d	0.103 f	0.065 f

Richter's green feverfew should be transplanted October 1st and harvested in May for maximum biomass production and parthenolide content. Plantings made in

November will be less productive in biomass and parthenolide and are inferior to October results.

C. Formulate weed control protocols without need for herbicides

5. Results of the evaluation of clover cover crops as "living mulches" in the production of organic medicinal plants

Goal: Herbicides are not labeled for medicinal crops and whether organically grown or conventionally grown, chemical weed control is not an option until special use permits are issued for herbicide use. Our objective is to determine the best candidates for organic weed control by using living mulches. These crops will be grown in the areas between plastic mulch beds in the field. The candidate crops must be very vigorous to choke out weeds, must be short and tolerate mowing with machinery to keep under control, and must be long-lived. The ideal candidates are clover crops since they also fix nitrogen and can provide natural sources of fertility.

Approach: Fifteen varieties of clover were seeded in the greenhouse Jan. 4th, grown as transplants and planted into field plots Jan. 31st. Five months later, we visually rated the potential of these as living mulches. Table 8 summarizes our evaluations and the criteria we used. **There are a few excellent candidates as a living mulches that are short-lived such as Mt. Barker and Regal ; however, the most stress tolerant and long-lived candidates were Sweet White and Sweet Yellow.** See photos included

Table 8. Potential of clovers as living mulches in organic herb production. Rating determined May 21, 2002, 5 months after transplanting.

Variety	% ground cover	Height (cm)	Weed Control	Vigor	Flowering	Runner	Potential in alleys to suppress weeds	Potential under crop to control weeds	Potential as a dead matt after burn down to control weeds
Dutch White	83	7	fair	declining	some	no	marginal	marginal	poor
Crimson Clover	80	35	good	dying	many	yes	very poor	very poor	good
Regal	95	19	very good	very good	moderate	no	excellent	good	excellent
Mt. Barker	78	5	good	some decline	none	no	excellent	excellent	good
Sweet White	85	13	Fair-good	excellent	none	no	excellent	good	good
Sweet Yellow	90	15	very good	very good	none	no	excellent	excellent	good
New Zealand	90	12	good	fair	moderate	no	good	good	marginal

Nitro	85	15	excellent	dying	many	no	very poor	very poor	good
Berseem	80	38	excellent	declining	many	no	very poor	very poor	good
Overton	17	11	poor	dying	moderate	no	very poor	very poor	very poor
Rose	33	14	poor	dying	many	no	very poor	very poor	very poor
O'Connor	75	7	Fair	good	none	no	excellent	excellent	marginal
Kenland	97	32	Excellent	excellent	some	no	marginal	poor	excellent
Aliske	93	13	very good	excellent	some	no	excellent	good	excellent
Strawberry Salina	48	8	very poor	declining	none	no	poor	poor	very poor

Dutch White - Low growing perennial, dense, and does not tend to spread and competes modestly with weeds. Doesn't show much potential as a living mulch because of sparse stand and short lifespan. Not known to have heat tolerance and better for northern areas. Dead by September.

Crimson Clover - Tall winter annual with plant death by mid-spring. May hold promise as a matting cover after burn down, but short lifespan negates its potential as a living mulch. Utility would be after burn down as a debris mulch.

Regal - Very vigorous, extremely dense, spreads well, drought and heat tolerant. Excellent stands with good ability to compete with weeds and low growth habit, make this one of the best living mulch and debris mulch candidates. Dead by September.

Mt. Barker - Most dense cultivar of all, very compact, and has drought tolerance. Some problems with stand, but extreme prostate growth habit and ability to suppress weeds makes this cultivar a good candidate as a living mulch and debris mulch. Dead by September.

Sweet White - Vigorous, moderate spreader, low growth habit with taller flower stalks. This annual cultivar survived in the field through the summer making it one of two cultivars with good vigor and potential as a living mulch and debris mulch. Still alive by September.

Sweet Yellow - Similar to sweet white, but yellow flowers. This biennial cultivar survived in the field through the summer making it one of two cultivars with that good vigor and potential as a living mulch and debris mulch. Still alive by September.

New Zealand - Nice matting growth, some decline, somewhat invasive. Shows some potential as a living mulch, but not strong as a debris mulch. This perennial cultivar was dead by September.

Nitro - Excellent early growth, thick dense mat, yet 75% dead by late May. Short lifespan of this annual negates its value as a living mulch, but has potential as a debris mulch. Known to have a high water demand. Dead by September.

Berseem - Tall, thick stemmed annual clover, close to senescent, fair to good early growth but declined by late May and died in the heat. Too tall for a living mulch, but has potential as a debris mulch.

Overton - Poor living mulch choice, with poor tolerance to heat and drought. Flowered early and died back with poor vigor, spread and density. Poor stands in the field negate its value. Seems too sensitive to environmental stress. Dead by September.

Hykon Rose -Flowered early, very sparse foliage, without heat and drought tolerance. Poor stands in the field negate its value. Seems too sensitive to environmental stress. No potential. This annual cultivar is known to Thrive in cooler climates.
Strawberry O'Connor - Similar to Mt. Barker. Very dense, compact, low growth habit, appears to be drought tolerant, but meager stands reduce this cultivar's potential as a debris mulch. Dead by September.
Red Kenland - Vigorous, drought tolerant, yet tall with dense growth reduce this short lived perennial cultivar's potential as a living mulch grown under the crop, but has potential in alleys and as a debris mulch. This clover is considered medicinal, yet it was dead by September.
Aliske - Dense somewhat compact, vigorous. This short lived perennial cultivar shows very good potential as a living mulch and debris mulch. Known to need lots of moisture. Dead by September
Strawberry Salina - Poor vigor without much density and doesn't compete well with weeds. Poor stands and growth negate this perennial cultivar as a living mulch. Dead by September.

D. Establish quality control protocols for harvest and storage of medicinal plants

This portion of the work was completed by Dr. James Rushing, post harvest physiologist. Dr. Rushing is on sabbatical with Federal Drug Administration in Washington, DC. Before leaving, Dr. Rushing provided the following summary of the work he completed on feverfew postharvest handling.

6. Report from James Rushing on drying temperature and developmental stage at harvest influence the parthenolide content of feverfew leaves and stems postharvest handling of feverfew

Approach

Feverfew 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. Some horticultural studies have been published, but limited information is available about horticultural practices, e.g. production and postharvest management protocols, that maximize the parthenolide content of feverfew. Our objectives are: 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.

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. 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 and initiation of flowering 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 placed in field lugs and properly stacked to ensure uniform airflow. 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.

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, in the spring for harvest in the summer. 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. Recommendations for handling the cut feverfew were provided to growers based on results obtained from research trials. Growers were instructed to dry the product in tobacco curing chambers set at 60 C 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 material was boxed and stored at 16 C with 20% relative humidity in controlled environment. Parthenolide content was evaluated as described.

Results

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. 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.

Influence of Temperature on Parthenolide Content of Leaves and Stems. Feverfew tests conducted in 2000 and 2001 provided similar results and only data from 2001 are presented here. As plants matured there was a trend for parthenolide content (% dry wt.) to increase in leaf tissue and to decrease in stem tissue (Figure 1). When data from all drying temperatures are pooled by harvest, from the first harvest until the last parthenolide in leaf increased from 0.330% to 0.385% but decreased in stems from 0.121% to 0.026%. In all studies, stems typically contained only 10 to 20% of the parthenolide measured in leaves (Figures 1,2 and 3).

Increasing drying temperature generally caused a decrease in parthenolide (dry wt.) in all tissues. Pooling data from all harvests by drying temperatures reveals 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 (Figure 2).

Figure 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%. 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 (GAPs). 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 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 cold damage 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 storage for further evaluation.

Conclusion Leaf tissue consistently contained substantially 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 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.

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 produce and the buyer of the herb.

Implementation of 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.

E. Implications for certification and branding of medicinal crops

7. Impact on marketing strategies for growers

Variation in the overall quality (marker content, plant identity, microbial and heavy metal concentrations) of the raw plant material used in nutraceutical products continues to be a real concern to the consumer and regulatory agencies. Moreover the lack of standardized plant material has in some cases resulted in product-to-product and in lot-to-lot variation of the same product. The lack of identifiable branded products with a known set of internal standards has had a negative effect on nutraceutical sales. Plant variability is clearly one factor, which has eroded consumer confidence and has prompted other more problematical questions regarding product efficacy. Without question, manufacturers of botanical dietary supplements would greatly benefit by the availability of controlled plant material, which can be used in their products as a marketing advantage.

Producers have capitalized on the need for more uniform plant material by entering into the cultivation of plants that traditionally were wild-crafted, or harvested from native habitats. For example, Chile increased its production and exports of St. John's wort from 1.2 million kg of dried product in 1997 to 4.6 million kg in 1998 with a corresponding increase in value F.O.B. in-country from U.S. $4.6 million to U.S. $ 23.9 million. Many of the new growers in Chile are tobacco producers who recognized the need to diversify into production of an alternative crop, a situation that also exists in the U.S., where medicinal plants could be a new crop opportunity.

As indicated above, product standardization could result in consumer preference and should be considered in nutraceutical marketing strategies. The development of quality-controlled products must, however, begin with the raw material in the field and proceed through post-production handling and processing. Stability of marker compounds (MC) is a concern at all steps in the process. The methodologies used to analyze MC need to be standardized and applied uniformly throughout the industry as well. While adherence to good manufacturing practices is a requirement of the industry, regulations requiring standardization are lacking. Functional foods and botanical dietary supplements currently available in the marketplace usually do not contain information on the label that specifically defines the amount of biologically active ingredient contained in the product. This is a concern for consumers as well as for medical practitioners who must consider the interactions of functional foods with prescription medications.

Significant plant variability now exists in the supply of medicinal botanicals. Part of this is due to varietal differences, growth conditions (e.g. soil, climate, wild crafted or cultivated), and pre- and post-harvest handling procedures. Ideally, scientifically controlled and standardized cultivation practices, like those described in this report, will result in the production of products with the highest levels of desired phytochemical constituents (marker compound) and purity. These quality plant products should be identified (i.e. branded or trademarked) on the "market" as a superior source of raw material for use in reliable nutraceutical products.

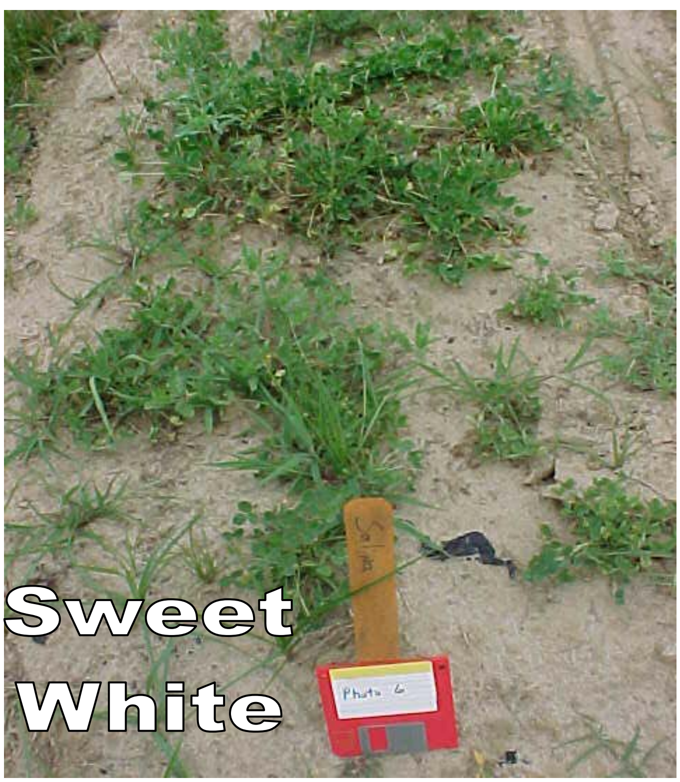
As indicated in this report, we have demonstrated the feasibility of growing selected medicinal plant ecotypes or cultivars in SC with superior market value due to their marker content and growth vigor. The ability to grow these plants under scientifically controlled conditions, good agriculture practices and proper post-harvesting handling techniques provides growers with an opportunity to produce a plant product of real need to the industry. Moreover "branded" South Carolina medicinal botanicals, which exceed current standards for marker compounds, purity, and clinical efficacy will identify these plants as a "first choice" for buyers and distributors. It s anticipated that recognition of branded products will result in the creation of contract farming arrangements an possibility to the creation of a nutraceutical growers cooperative. Evidence supporting the success of this approach can be found in states such as Georgia in which the trade name "Vidalia" has resulted in a dramatic increase in onion production over the last 20 years. A second example comes from Oregon and Trout Lake's announcement of a new Echinacea selection with the trade name "Magical-Ruth". This cultivar was in part responsible for the dramatic increase in sales of Echinacea in the 1990's and in the rapid rise in Trout Lake's and Oregon's rise to premier status as a producer of quality medicinal botanicals.

8. Impact on marketing strategies for distributors

The following comments have been contributed by Mr. Edward Fletcher, VP for Marketing, Strategic Sourcing Inc, Boone, NC. Strategic Sourcing is one of the largest distributors of medicinal plant materials and Mr. Fletcher chairs the American Herbal Plant Association's Advisory Committee on raw material standards. Strategic Sourcing's goal is to provide customers and partners with an integrated strategy for utilizing cost-effective, top quality medicinal botanicals. This is done through more stringent crop production programs that produce raw materials with higher MC's on a consistent basis. The company has achieved this goal with several medicinal botanicals. One example is Hydrastis canadensis, goldenseal, which is a CITES, Appendix II listed plant. Strategic Sourcing has collaborated with grower organizations to establish a 'crop production program' that has been awarded a CAPP (Certificate for Artificially Propagated Plants) permit from the United States Department of the Interior, Fish and Wildlife Service. Customers appreciate the benefit of knowing they are supporting sustainable practices of an endangered species and enjoy the consistent high quality material the company provides. Strategic Sourcing looks forward to developing similar programs with grower organizations in South Carolina acting as both the liaison and the marketing arm of future projects to produce high quality medicinal botanicals.


Overton
Overton
PHOTO 5


Regal
Regal
PHOTO 5


Sweet
White
Photo 6

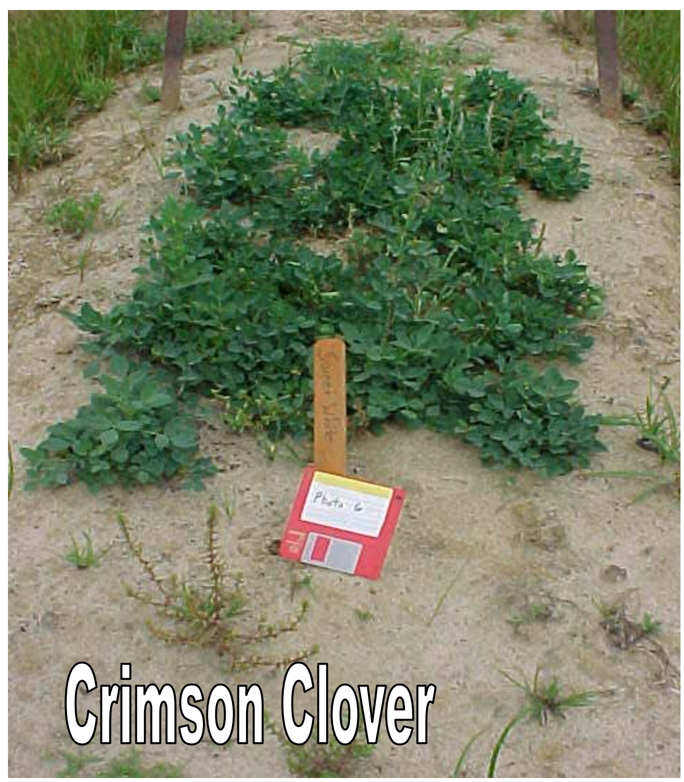
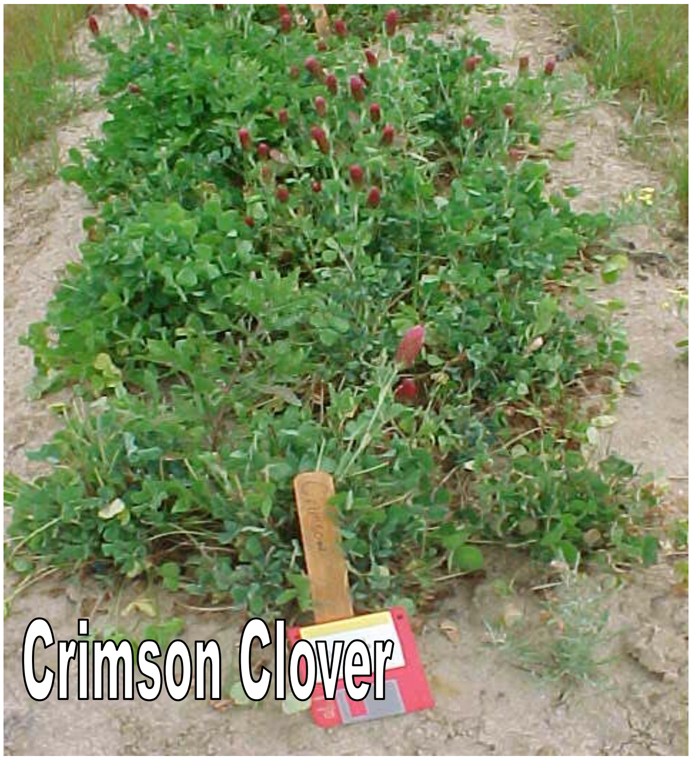


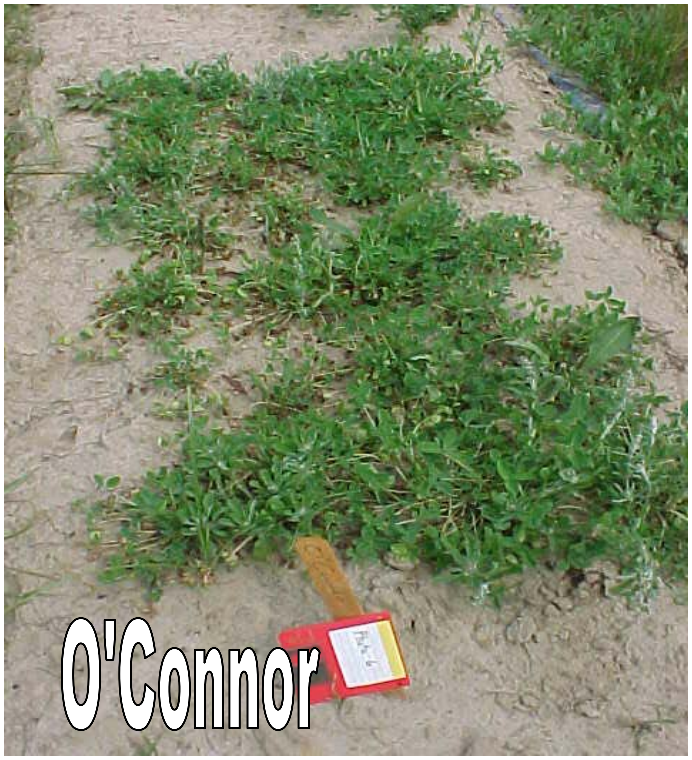
Photo 6
Crimson Clover


Crimson Clover


Dutch White

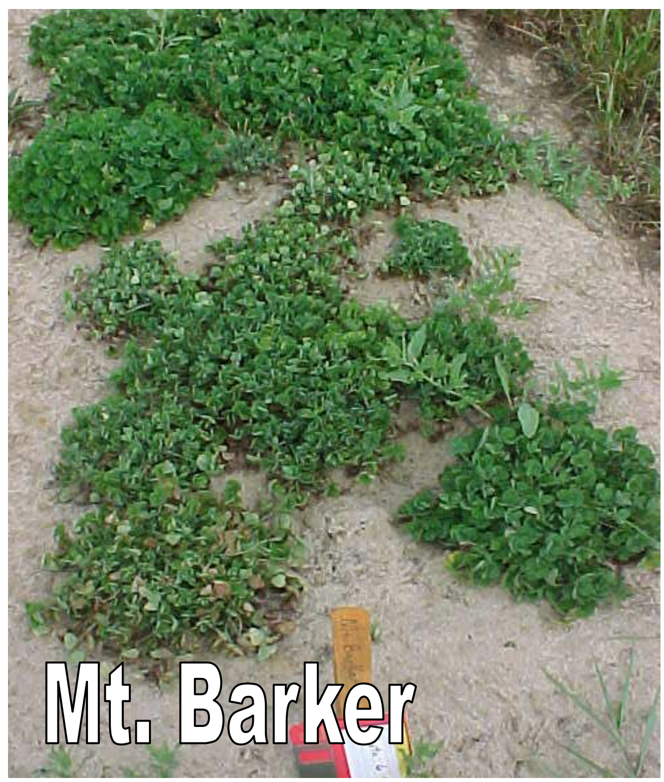

Kenland


Rose


O'Connor


Nitro


Sweet Yellow


Mt. Barker


Aliske


New Zealand


Berseem

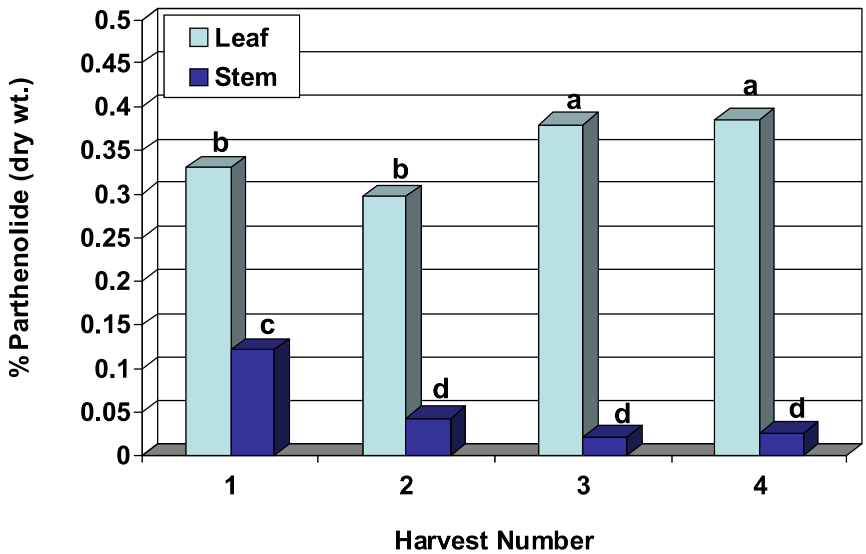


Fig. 1. Data from all drying temperatures were pooled and plotted to show the influence of harvest no., e.g. stage of plant development, on the parthenolide (% dry wt.) content of leaf and stem tissue from feverfew. Mean separation by LSD at P=0.05.

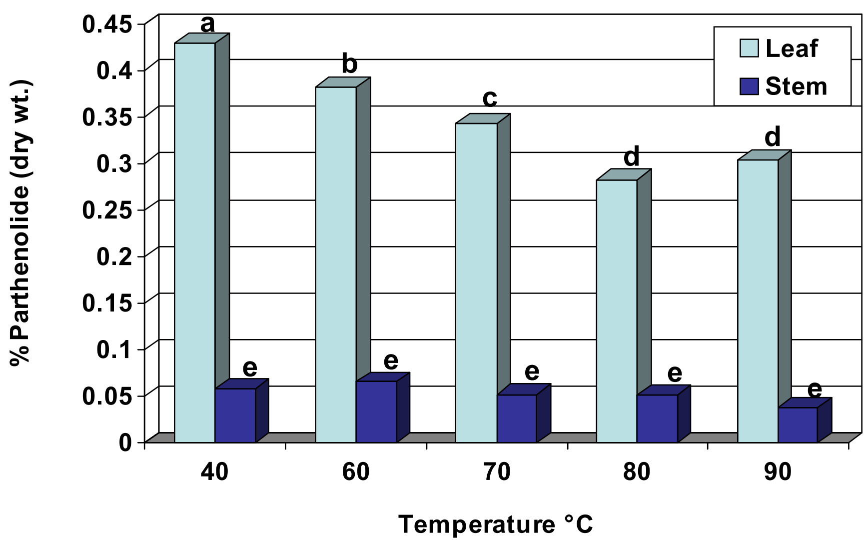


Fig. 2. Data from all four harvests were pooled and plotted to show the influence of drying temperature on the parthenolide (% dry wt.) content of leaf and stem tissue of feverfew. Mean separation by LSD at P=0.05.

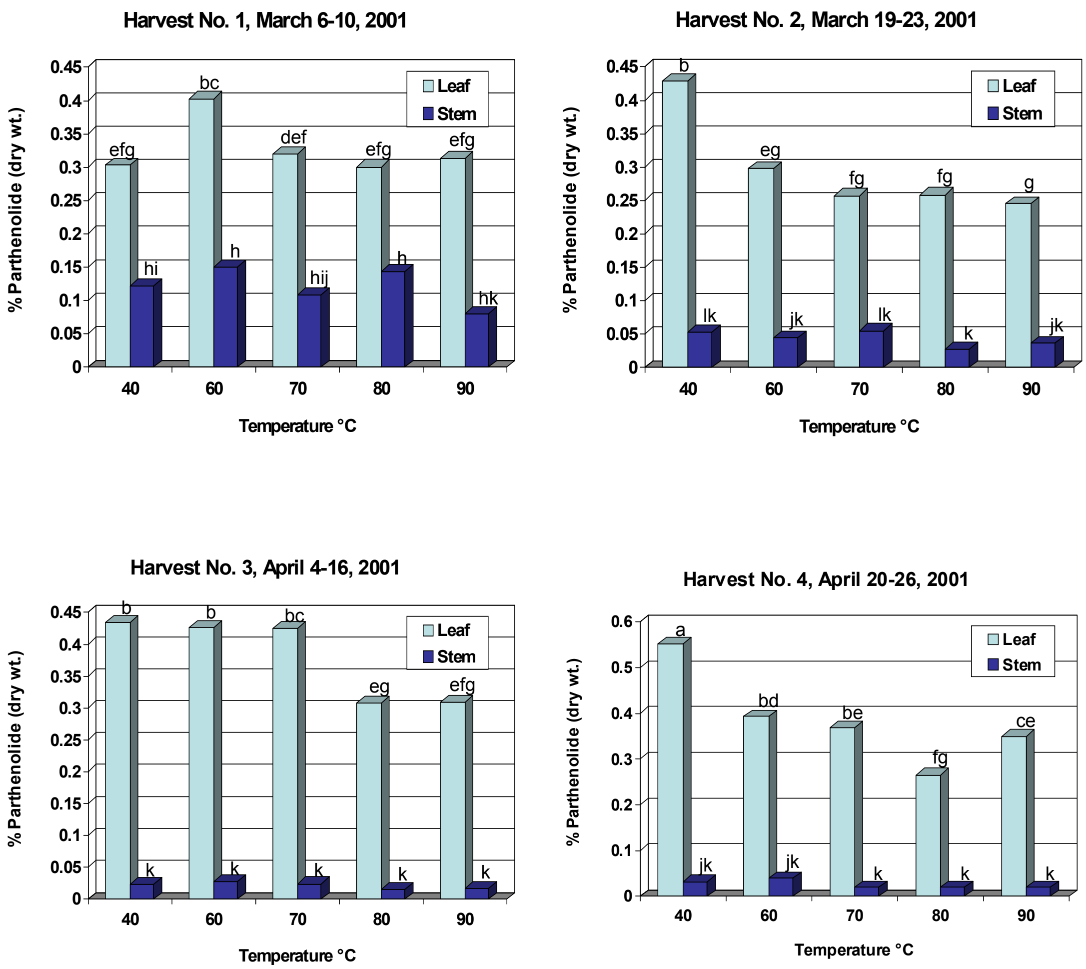


Fig. 3. The effect of drying temperature on parthenolide (% dry wt.) content of leaf and stem tissue for each harvest date. Mean separation by LSD at P=0.05.