NUTRACEUTICALS AS ALTERNATIVE CROPS PART II – IMPLICATIONS FOR CERTIFICATION AND BRANDING

Work completed under this project included development of guidelines for identifying and producing certain medicinal plants (feverfew and Echinacea) with high marker compounds (components valued in the nutraceutical market); criteria to evaluate the suitability of the plants for commercial production; weed control protocols; and quality control protocols for harvesting and handling the crops to maximize and/or preserve the medicinal properties valued by the nutraceutical industry.

Final Report

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Production and Development of Nutraceuticals as Alternative Crops: Refinement of Quality Standards for Certification and Branding Part II

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E. A synopsis of the current state of commercial medicinal plant production research

A. Identify selection of *Echinacea* species and feverfew with high marker compounds, climatic tolerance and potential in breeding programs

Situation:

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 constitute quality: the first is high biomass per acre and the second is high marker compound (chemicals desired by dietary supplement industry) per unit harvested product. With feverfew, the marketable product is the foliage since that is the plant part used for extraction of the marker compound. With *Echinacea*, depending on species, the roots and/or leaves are the products harvested. It is necessary that the correct production practices enhance the production of very high tonnage of biomass per acre, but not at the expense of high marker compound. The biomass must be highly fortified with marker compounds and without this, the tonnage is worthless to the processor. Grower guidelines that outline how to enhance biomass and marker compounds are nonexistent for most medicinal crops. Therefore, before any of these crops become a commercial endeavor, we have to understand how to grow these crops to insure quality. 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. 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 has been to evaluate the available germplasm in seed catalogs 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, would be to cross and breed these excellent ecotypes and then, in time, produce new cultivars that can be patented and protected. 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. 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 that 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 affects the expression and production of marker compounds in the plants. Any new line of medicinals that may be 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, such as drought, heat, etc. It is therefore imperative in our research to monitor the genesis of marker compounds over the times that the plants are harvestable to learn to understand why they react as they do. Our

goal in these studies was to identify 'super' ecotypes of feverfew and *Echinacea purpurea* worthy of being graduated into a breeding program for new cultivar development.

Topic 1. Change in parthenolide content over time in feverfew super selections - Individual cuttings of six feverfew super selections were field grown at the Coastal Research Center in Charleston and sampled three times over a year to determine how stable parthenolide content is over different times during the growing season.

Results: Parthenolide content is not a constant. Table 1 indicated that in some selections like Richter's Green (1-31) selection, parthenolide increased almost 69% from July 2003 to October 2003; however, USDA Mexican 502 selection (38-12 and 38-20) increased 28% and 52% respectively, but the other selections remained the same or decreased by October sampling. By the following July, 2004 sampling, all parthenolide concentrations had dramatically decreased from the October 2003 levels, except USDA Chelsea Physic Garden selection (notoriously low in parthenolide). It may be possible that: 1) old feverfew plants do not retain high parthenolide content and the reduction is part of increased longevity; or 2) the climate during July 2004 may have negatively impacted parthenolide content.

Table 1. Change in parthenolide content in feverfew super selections over June 2003 to July 2004.

Selection	Number	Date Sampled	Parthenolide[1]	Date Sampled	Parthenolide[1]	Date Sampled	Parthenolide[1]
Richter's Green	1-31	7/8/03	0.54	10/2/03	0.91	7/8/04	0.36
Horizon Green	2-45	6/27/03	0.53	10/2/03	0.53	7/8/04	0.44
USDA Germany 161	33-63	7/30/03	0.77	10/2/03	0.72	7/8/04	0.60
USDA Chelsea Physic	34-28	7/29/03	0.24	10/2/03	0.15	7/8/04	0.22
USDA 502 Mexican	38-12	7/31/03	0.85	10/2/03	1.09	7/8/04	0.67
USDA 502 Mexican	38-20	7/31/03	0.64	10/2/03	0.97	7/8/04	0.73

(mg/g dry leaf material)

It is apparent that parthenolide concentration is not a constant chemical principle and its concentration is dynamic. More work is needed to understand how climate and age affect the vacillation of parthenolide. Cloned feverfew plants need to be grown in the field and sampled every three weeks for parthenolide and all weather data gathered (such as temperature, day length, rainfall, humidity, light quality) and then these data need to be correlated and modeled to determine a prediction model. Once a suitable strong model is derived, production sequences can be developed to pinpoint when parthenolide peaks and what environmental factors enhance content.

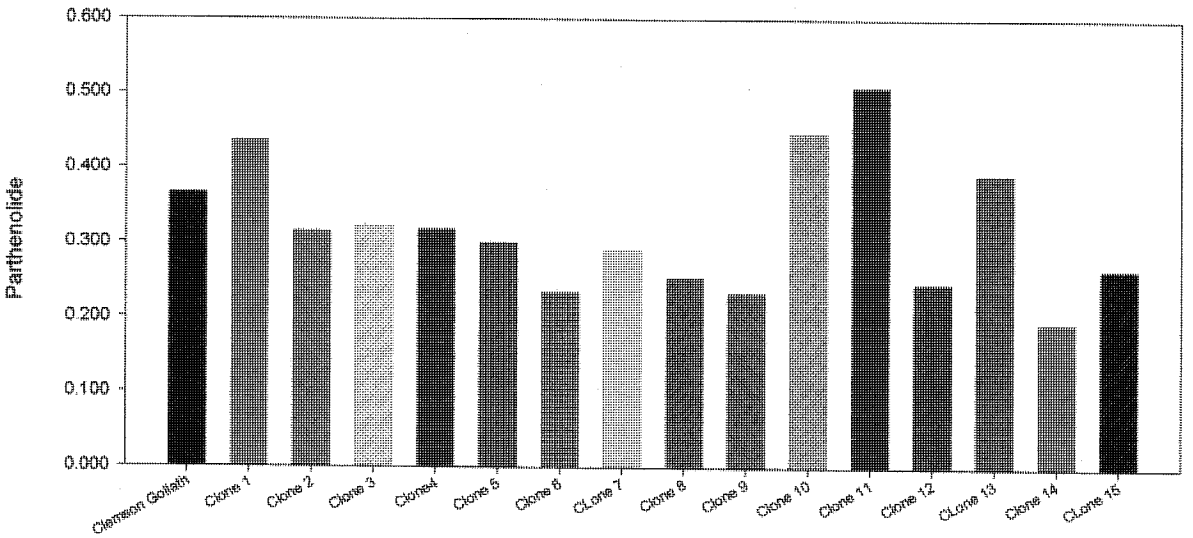
Topic 2. Variation in marker compound content within 'Goliath' feverfew- One selection from USDA Chelsea Physic Garden feverfew was found to have huge biomass, however, low parthenolide level and refuses to flower in Charleston, but will flower in the upstate of SC (see following). We have called this selection 'Goliath' and consider it an excellent biomass parent. In 2004, we took many cuttings of the 'Goliath' mother plant producing many clonal plants. All the cuttings of 'Goliath' were planted in the field in a straight contiguous row and allowed to grow. In mid-May 2004, we sampled 15 clonal 'Goliath' plants as well as the 'Goliath' mother. Figure 1 shows the diversity of parthenolide within these genetically-identical plants.

Mike Watkins, Foundation Seed, Clemson University with five 'Goliath' plants in upstate SC.

Results: Theoretically, all of the clones in Fig. 1 are genetically-identical yet the parthenolide content among all these plants ranged from as low as 0.19 to 0.51 with a

mean of 0.32. The clones in Fig. 1 are numbered from one to fifteen and that number corresponds to its number and placement in the planted row, i.e., the row ran west to east and 'Goliath' mother was planted in the first position in the row on the west end, followed by clone plant one, then two, then three, etc. until clone plant 15 was the last plant in the row on the east. It is an enigma why parthenolide varied so much in clonal material and the diversity in content may be possibly affected by position in the row, yet no apparent physical soil characteristics changed in the row to affect parthenolide content. This variation is not understood and cannot be explained at this point in time. The problem these results illuminate now is that even if a high parthenolide cultivar can be developed in a breeding program, we can expect wide

Fig. 1. Parthenolide of Goliath mother plant and her clonal cuttings sampled in Mid-May, 2004



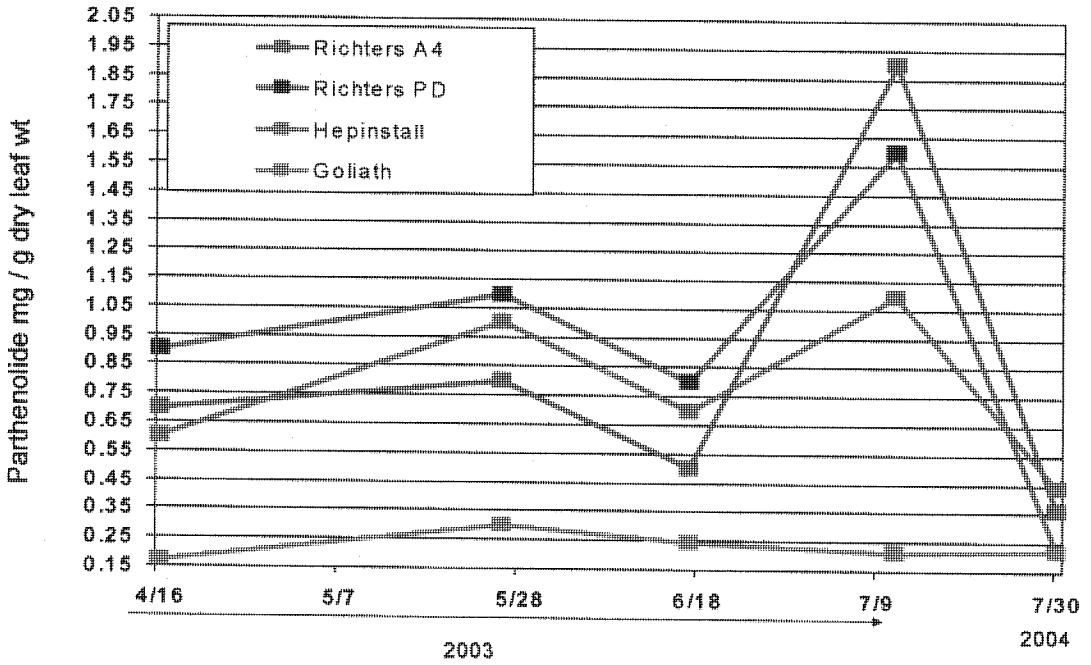
Variation in parthenolide content even within those selections, since 'Goliath' clonal material is so widely variable. It may be possible that once a new cultivar is developed, cultural practices of known parthenolide enhancement can be superimposed over the new cultivar in production and possibly increase parthenolide content without depending totally on genetic superiority.

Topic 3. Change in parthenolide in clonal selections over time - Cuttings of feverfew super selections (2 of Richter's Green Feverfew, Hepinstall Green Feverfew, and Goliath super selection from USDA Chelsea Physic Garden) were propagated to produce genetically identical plant populations. These rooted cuttings were field planted and monitored from April 2003 to July 2004 for parthenolide content.

2 **Results**: In this second study, we again found that parthenolide content is not a constant. The figure that follows indicated that from April 16, 2003 through June 18, 2003, parthenolide tended to increase with all selections into the summer up until May 28, 2003 and then decreased significantly by June 18, 2003. However, parthenolide content dramatically spiked by June 9, 2003 in all selections except Goliath super

selection. This outcome suggested that parthenolide content may be enhanced: 1) by hotter summer field temperature and/or other climatic features; or 2) parthenolide naturally increased and spikes at a certain plant age from transplanting. These same plants were allowed to continue growing through Fall 2003 and into Spring and Summer, 2004 at which time they were again sampled during the suspected parthenolide spiking period seen in July 2003. To our dismay, parthenolide content in all selections of feverfew (except Goliath), had plummeted to very low undesirable levels. Similar to our previous observation above, it may be possible that old feverfew plants do not retain high parthenolide content and the reduction is part of increased longevity.

Fig. 1. Change in parthenolide in feverfew clonal selections over time.



The same conclusions reached in Topic 2 apply to the present study: 1) parthenolide is not a constant chemical principle but dynamic; and 2) aged feverfew plants have inferior parthenolide content than younger plants

Topic 4. Notes on feverfew breeding program - As a commercial crop, feverfew production is limited by the industry's whims. In the last 18 months, the demand for feverfew has been minimal although we have proceeded with variety development. Surprisingly, there has been a world shortage of feverfew and the industry now is in great need of immediate supplies of feverfew and has looked to growers to supply this need. This reinforces the idea of high risk with these crops, fickleness of the industry and the absolute need for grower contracts if anything will be grown for the medicinal industry. Our germplasm development with feverfew has been slow but steady. About 2 years ago, we identified a single plant of extremely high biomass with a constant parthenolide concentration of 0.28%. We named this selection 'Goliath'. This parthenolide level is similar to Hepinstall parthenolide content that established

parthenolide as the marker compound to identify feverfew plant material. Parthenolide, however, may not confer clinical value----this still is unproven, yet the medicinal industry still wants higher parthenolide in their products, ideally ranging from 0.5 to 1.0%. To satisfy their desires for "quality" feverfew (although we think clinical efficacy will not be improved), we have selected five individual plants that have produced very high parthenolide from a diversity of germplasm available from commercial varieties and the National Germplasm Repository. All these individual plant selections, have been propagated multiple times and are in the process of being crossed to develop totally new ecotypes. Mike Watkins from the Clemson University's Foundation Seed Association, has participated in this work by growing isolation plots for feverfew crosses in the Clemson, SC area. This year, Mike has grown isolation plots that combine the parents of high biomass feverfew with high parthenolide parents. He will collect the seed in fall, 2004 and we will plant the progeny in 2005 in Charleston.

Our main parent is 'Goliath' a selection of USDA Chelsea Physic Garden feverfew noted for its huge biomass however, low parthenolide level. 'Goliath' refuses to flower in Charleston, but will flower in the upstate. The following crosses of high parthenolide selections with 'Goliath' are ongoing at present:

Cross number	Selection versus Selection
1	Goliath vs Richter's Green
2	Goliath vs Horizon Green
3	Goliath vs USDA Germany 161
4	Goliath vs USDA Mexican 502 plant 12
5	Goliath vs USDA Mexican 502 plant 20

From these plants, we will have to search for that one new plant that combines extremely high biomass as well as high parthenolide. Once identified, we can proceed to releasing this new variety and commercial production of a protected variety.

Topic 5. Notes on *Echinacea angustifolia* ecotolerance - Of the three medicinal *Echinacea* species, *purpurea, pallida* and *angustifolia*, *E. angustifolia* is the most highly desired *Echinacea* for the dietary supplement and has the highest income potential for the grower. We decided to look more carefully at *E. angustifolia* because its dual value to farmers and the industry. Our first exposure to *E. angustifolia* was disheartening since most of the plants died in the field from only two seed sources of the plant. However, in 2004, we decided to screen all the *E. angustifolia* germplasm selections in the USDA Germplasm Repository for *Echinacea* located in Ames, Iowa. The following lists the vendor (seed source), descriptive numbering of the selection, and the state where the wild *Echinacea* was found growing (USDA sources only).

Echinacea angustifolia germplasm evaluation			
No.	Vendor	Description	Locale
1	USDA	633657	ND
2	USDA	Ames 26428	MN
3	USDA	633653	ND
4	USDA	633654	ND
5	USDA	633655	ND
6	USDA	633656	ND
7	USDA	312814	NB
8	USDA	421331	OK
9	USDA	421372	NB
10	USDA	597601	KS
11	USDA	631266	OK
12	USDA	631267	OK
13	USDA	631268	OK
14	USDA	631269	OK
15	USDA	631270	OK
16	USDA	631271	OK
17	USDA	631272	OK
18	USDA	631273	OK
19	USDA	631283	IA
20	USDA	631284	IA
21	USDA	631285	IA
22	USDA	631286	IA
23	USDA	631287	IA
24	USDA	631288	IA
25	USDA	631289	IA
26	USDA	631317	KS
27	USDA	631318	KS
28	USDA	631319	KS
29	USDA	631320	OK
30	USDA	633650	IA
31	USDA	633651	IA
32	USDA	633652	ND

E. angustifolia seedlings were grown in the greenhouse and field planted in spring 2004. Seedlings were transplanted on plastic mulched beds and allowed to grow through the summer without any additional weed control or pest management. Our goal was to determine as quickly as possible, any selection that could tolerate our benign neglect. In late August, numbers of survivors within each replicated plot were flagged, counted,

dug and moved to a new nursery containing other superior survivors from other experiments.

Results: The majority of selections died or showed very poor vigor. The following selections showed potential of having some environmental tolerance and worthy of further study.

Selection	Number of vigorous survivors in all plots
421331	1
597601	8
631266	1
631267	1
631271	4
631272	9
631273	8
631319	1
631320	19

USDA 631320, originating from OK, is the best selection of *E. angustifolia* for future exploration. Most of the plants within this selection were very large and vigorous in comparison to even the other selections that showed some promise. Although the plot population per selection was more than 80 individual plants, survival of these plants in the open-pollinated illustrated that there is a genetic difference in stress tolerance and future selection work will concentrate on this specific USDA line.

Topic 6. Notes on *Echinacea purpurea* marker compound variation in spring and summer, 2004 - The major marker compound of interest with *Echinacea purpurea* is chicoric acid (CA). Ideally, in any new variety of *E. purpurea* we desire that the marker compound be stable over a wide variety of environmental conditions. If not, we wish to better understand under which climatic conditions, the marker compounds surge or recede. Twenty-three super selections of *E. purpurea* were field transplanted in spring 2003 and leaf samples gathered in Sept. 2003, Nov. 2003, July 2004 and assayed for CA content.

Results: The desirable range of CA by the dietary supplement industry is from 2% to 4%. All super plants were chosen in previous field evaluations because they initially had a CA content of 3% or greater. There was not a strong trend for CA content to fluctuate similarly in all 23 super selections. Generally, CA decreased from Sept. 2003 to Nov. 2003 sample dates in 9 selections, remained the same in 5 selections or increased in concentration in 3 selections. The significance of these November readings is that commercially, the roots and/or leaves of the plants would be harvested at this time period before cold weather sets in. Based solely on the Nov., 2003

samples, the three selections that show the best potential (CA greater than or equal to 5%) for high CA content and worthy of closer attention in breeding program are super plants 1, 5 and 6. To a lesser degree (CA greater than or equal to 3.3%), plant 2, 7, 8, 10 (Richter's common *E. purpurea*) and 23 (Jelitto Bright Star) are considered promising selections for further evaluation. The rest of the selections were lower in CA but still within the acceptable range for the dietary supplement industry. *E. purpurea* plants were allowed to overwinter and grown for another growing season and sampled during July, 2004 which was the hottest time of the growing season and most stressful to the plants. All selections decreased in CA content except selection 13 which remained about the same from the November sampling; however, selection 1 showed the best potential with the highest CA content of 3.9%. This solitary plant selected from Richter's common green *E. purpurea* has the best potential for enduringly high CA of all super selections.

Super no.	"Variety"	Plant no.	% Chicoric acid		
			Sept 2003	Nov. 2003	July 2004
1	Richter's common lot 1	1	5.02	**5.05⇒**[1]	**3.86↓**
2	Richter's common lot 1	2	3.58	**3.50⇒**	**1.95↓**
3	Richter's common lot 1	3	3.08	----	**2.37↓**
4	Richter's common lot 1	4	3.34	**2.44↓**	**3.24↑**
5	Richter's common lot 2	1	4.48	**4.95↑**	**1.31↓**
6	Richter's common lot 2	2	3.18	**5.12↑**	**1.86↓**
7	Richter's common lot 2	3	3.06	**3.37⇒**	**1.12↓**
8	Richter's common lot 2	4	4.23	**4.02⇒**	**1.98↓**
9	Richter's common lot 2	5	3.50	**2.28↓**	**1.14↓**
10	Richter's common lot 2	6	4.03	**3.48↓**	**2.79↓**
11	Richter's common lot 2	7	2.99	**3.21↑**	**1.57↓**
12	Richter's common lot 2	8	3.65	**2.97↓**	**1.94↓**
13	Richter's common lot 2	9	3.30	**2.30↓**	**2.05⇒**
14	Jelitto Magnus	1	3.12	**3.15⇒**	**1.75↓**
15	Jelitto Magnus	2	----	----	**1.99**
16	Jelitto Magnus	3	4.19	----	**1.37**
17	Jelitto Magnus	4	5.49	**2.48↓**	**1.29↓**
18	Jelitto Magnus	5	2.98	----	**1.75**
19	Jelitto Rubinstern	1	3.58	**2.51↓**	**1.87↓**

20	Jelitto Bright Star	1	3.08	----	**2.97**
21	Jelitto Bright Star	2	3.20	----	**2.06**
22	Jelitto Bright Star	3	3.69	**2.87**↓	**2.02**↓
23	Jelitto Bright Star	4	4.65	**3.97**↓	**1.94**↓

[1] =, ↓, ↑ CA concentration stayed the same, decreased or increased, respectively.

B. Develop specific guidelines for production of medicinal plants

Topic 7. Results of fertility experiment contrasting organic and inorganic fertility on *Echinacea purpurea* - 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. Organically-grown products are in full synchrony with the philosophy of using herbs which is to have a total natural approach to improve health and this should start from the seed in the ground. 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 each 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 nor conventional production methodology allows chemical pesticides since nothing is labeled for these very minor, specialty crops.

Approach: Richter's common *E. purpurea* seedlings were transplanted in March and fertilized with equivalent amounts of nitrogen, phosphorus and potassium from: 1) organic fertilizers (bat guano [nitrogen], soft rock phosphate, and greensand [potassium]) and 2) synthetic 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 white plastic. *Echinacea* roots were harvested in January after dormancy. Root tissue was analyzed for CA. We were interested if biomass and marker compound content differed by fertility regime.

Results: The yield of *E. purpurea* root biomass and marker compounds were differentially affected by the fertilizer source and rate of fertilizer used. There was a significant interaction between fertilizer source and rate on root fresh weight but not CA content. With synthetic fertilizers, root fresh weight was greater at the lower fertility rate, but with organic fertilizer, root fresh weights were the same at either rate. For heaviest roots, low rates of synthetic fertility should be used in synthetic production. In contrast, CA content generally was 12% higher with the organic fertility than with synthetic chemical fertilizer.

Influence of organic and synthetic fertility on *Echinacea purpurea* root fresh weight

Fertility source	Rate	Root weight (g)
Synthetic	Low	174 a
Synthetic	High	140 b
Organic	Low	147 b
Organic	High	154 ab

Influence of organic and synthetic fertility on *Echinacea purpurea* chicoric

Fertility source	Chicoric Acid
Synthetic	.58 b
Organic	.77 a

Topic 8. Notes on the correlation of feverfew leaf and flower mineral content with parthenolide content - It was of interest to determine if feverfew leaf mineral content could be used as an indicator of parthenolide content. Ideally, if such an indicator could be found that highly correlated with parthenolide, possibly a rapid tissue analysis assay using portable meters (i.e. nitrate, potassium, sodium handheld meters, etc.) might give a grower some idea how parthenolide content is progressing in the field immediately without expensive chemical analyses. This information may be used to signal the best time to harvest. Richter's common green feverfew seedlings were transplanted in March and fertilized with equivalent amounts of nitrogen, phosphorus and potassium from: 1) organic fertilizers (bat guano [nitrogen], soft rock phosphate, and greensand [potassium]) and 2) synthetic 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 white plastic. Feverfew leaf tissue was harvested in June and leaf samples taken for parthenolide and elemental analysis. Parthenolide content was correlated with leaf mineral content and R^2 and probability values determined. The only mineral that correlated with parthenolide was calcium and that was considered a weak correlation after all. Apparently as calcium level decreased, parthenolide content increased

Correlations of leaf and flower mineral content and parthenolide content (from organic study...all feverfew fertility data pooled)												
Correlations of	%N	%P	%K	%Ca	%Mg	Na ppm	%S	Fe ppm	Mn ppm	Cu ppm	Al ppm	Zn ppm
Leaf parth R^2	.01	-.01	.09	-30	.06	-.11	-.01	-.21	-.20	-.15	-.09	-.18
prob	.98	.95	.55	.04	.68	.47	.93	.15	.17	.30	.57	.22
Flower parth R^2	-.18	-.02	-.01	-.01	-.01	-.02	-.06	.21	-.02	.16	.23	.15
prob	.22	.91	.97	.93	.93	.92	.68	.15	.88	.29	.13	.30

Topic 9. Risk assessment of commercial scale plantings of feverfew, *Echinacea purpurea* and *pallida* - There are two appropriate times that *Echinacea* and feverfew can be planted in the Charleston area. The ideal time to plant a fall feverfew crop in the field is about mid-September for a winter harvest near the New Year's holidays or in late winter (provided the feverfew plants do not send up long stems). With shorter days and longer nights, some cultivars of feverfew will stretch out, grow waist high, with nothing but woody stems (a crop failure). Ideally, feverfew should be planted in Sept. and be prime for harvesting before Christmas. Feverfew should be considered a cool season crop in our location even though we grow it through the summer, but it gets very stressed in the heat of our summers. ***Orders by a dietary supplement company to a grower for a fall feverfew crop need to be placed by June 1st to allow enough time to grow 8 week old transplants.***

A spring feverfew crop needs to be harvested before July 4th to avoid the really hot rainy summer months that stress these plants. In mid summer, the leaf tips of feverfew plants will turn brown and the growth will be stunted, halted and not resume till cooler fall weather in late September. The ideal time to spring plant feverfew is by early February. ***Therefore, orders for feverfew production by a dietary supplement company to a grower need to occur about Dec. 1st to secure seed and grow 8 week old transplants.*** Planting feverfew later than February is possible but the risk of stressful field conditions at crop maturity increases the risk of failure which was observed in the 2004 growing season.

For *Echinacea*, the same fall planting and order dates suggested for feverfew are appropriate; however, harvest of *Echinacea purpurea* leaf tissue may occur about May and roots in November, approximately 14 months after transplanting. We feel that the first leaf harvest in early summer and the second just before root digging in November is about all we can expect from very juvenile *Echinacea*. If a grower has patience, *Echinacea* leaf production would be much greater if the plants were allowed to mature into a two-year-old plants. Older plants can tolerate harvest pressure much better than immature, developing plants. A spring *Echinacea* crop follows the same production schedule as feverfew.

The major problem facing large scale commercial herb production is uncontrollable weed infestations and major disease outbreaks that cannot be controlled with pesticides (none labeled). In fall 2003, we made an acre large scale planting to experience the potential problems a grower may experience. With small plot research, minor problems

within those plots can be crippling on a large scale. A major unforseen and unanticipated problem was weeds germinating within the planting holes during later winter and early spring. The black mulch enhanced the soil temperature in fall inducing summer as well as winter weeds to emerge which severely competed with *Echinacea*. Since the soil sterilant, methyl bromide, is not registered for herbs, herb growers face a struggle with expensive hand weeding, unless some alternative is developed to kill weed seeds and pathogens as well.

Although the funding for this research has terminated, we continue to correct the problems outlined above in experiments from 2004-2006.

Experiment 1. We will attempt to passively kill weed seed and pathogens in next year's fall 2005 *Echinacea* planting using solarization. A large block of land has been selected and preparations are in progress now to implement this work. The scheme we will use is to cover the planted rows with clear plastic with buried drip irrigation lines in mid- to late-March 2005. The bare soil between plastic rows will be planted to a nitrogen-fixing clover (Regal Ladino) to naturally fertilize the soil. During the summer, the soil under the plastic will be watered and the heat of the sun will pasteurize the soil and naturally cleanse it of weed seed and pathogens. I have done this scheme for fall planted strawberries with rousing success. In fall, 2005, the plastic will be painted with a gloss exterior black plant and *Echinacea* and feverfew planted at close populations. Then, winter feverfew will be harvested in Dec. and *Echinacea* pallida roots in Dec., 2006. To do these procedures correctly, requires a lot of time and months of preparation.

Experiment 2. We are preparing a field for a spring planting of *Echinacea* and feverfew with the idea of avoiding weed infestations in the past. This fall we are preparing a large block of land with black plastic mulch and seeding the bareground between the rows with Regal Ladino clover we have determined will suppress weeds, including nutgrass, maintain a short height and can tolerate Charleston's hot, humid summer climate. The field will "grow" clover over the winter and in Feb., *Echinacea* and feverfew will be planted in the field and with warm temperatures, the plants should grow rapidly and fill up the planting holes before the weeds can compete. The feverfew will be ready for harvest by July 4, 2005 and *Echinacea* roots may be harvested in Nov-Dec, 2005.

C. Formulate weed control protocols without need for herbicides

Topic 11. Results of the evaluation of clover cover crops as "living mulches" in the production of organic medicinal plants- Herbicides are not labeled for medicinal crops and whether organically-fertilized or conventionally-fertilized, 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 heat be tolerant and long-lived. The ideal candidates are clover crops since they also fix nitrogen and can provide natural sources of fertility.

Six varieties of clover, selected from preliminary screening, were direct-seeded in Sept. 2003 in the field and cultured throughout the winter, spring and summer. The varieties used were: Aliske, Red Kenland, Regal Ladino, Alfalfa, Redland III, and Sweet

Yellow. Occasionally, the clovers were mowed (mower set high) to determine their tolerance to mowing. We visually rated the potential of these as living mulches (see table below for specific rated qualities) on April 30 and Aug. 8, 2004. Each subjective quality criterion was rated according to the following subjective measure of intensity:

100	**= Outstanding quality**	**No Effect**
90	**= Slightly less than the best desired quality**	
80	**= Some obvious loss of desired quality**	**Slight effect**
70	**= More pronounced reduction in desired quality**	
60	**= Deficient level of desired quality**	
50	**= Deficient to Moderate loss of desired quality**	**Moderate effect**
40	**= Moderate loss of desired quality**	
30	**= Heavy loss of desired quality**	
20	**= Nearly all plants undesirable**	**Severe effect**
10	**= All plants undesirable**	

Results - The most desirable living mulch should cover most of the ground area to suppress weeds, be a vigorous grower, be short to avoid competing with medicinals, suppress especially nutgrass, resist mowing pressure, and be heat tolerant and not die during summer months. The value of the late April 2004 ratings is that they indicate those living mulches that either excelled at establishing themselves through the winter and spring or did not.

April 30, 2004 ratings

Variety	% Flowered by 4/30/ 2004	Height (Inches)	Ground coverage (%)	Weed competition (%)	Vigor (%)	Stand (%)	Mulch potential
1. Aliske	100	7.3	68	65	64	65	65
2. Red Kenland	50	10.3	97	92	98	97	97
3. Regal Ladino	25	7.3	93	87	89	90	91
4. Alfalfa	0	10.8	73	66	69	75	70
5. Redland III	25	10.0	91	90	92	92	94
6. Sweet yellow	0	4.3	14	10	14	21	0

August 5, 2004 ratings

Variety	% Flowered by 8/5/2004	Height (Inches)	Ground coverage (%)	Weed competition (%)	Sedge control (%)	Vigor (%)	Stand (%)	Mulch potential
1. Aliske	yes	5.8	50	40	65	43	49	45
2. Red Kenland	yes	12.0	45	78	75	43	45	73
3. Regal Ladino	yes	7.3	91	93	90	90	90	90
4. Alfalfa	yes	15.3	74	25	85	68	74	45
5. Redland III	yes	9.3	50	68	69	45	50	50
6. Sweet yellow	Dead							

<u>April 30 ratings</u> - Of the six clovers evaluated for living mulch potential, only Red Kenland, Redland III and Regal Ladino were considered outstanding at that time of the year. All of the plant qualities evaluated were about the same magnitude, but Regal Ladino

was the shortest of the three clovers which was a very desirable quality. Sweet yellow clover had no potential because all of the ratings were extremely unacceptable ratings across all the criteria.

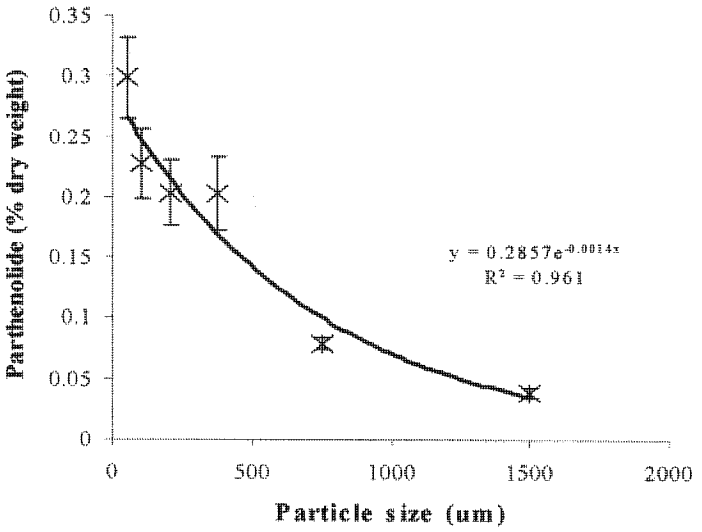
August 5, 2004 ratings - **(SEE PICTURES AT END OF REPORT)** By this date, the weather had been very hot and humid for months and the clovers were exposed to the most stressful climate by the time of the ratings. Of the original six selections, only Regal Ladino still persisted as an outstanding living mulch with excellent ratings in all quality categories. Those that did well in the April ratings failed in one or more categories by August. For example, the stands in Red Kenland and Redland III declined to the point that vigor and ground coverage was very poor probably an indicator of poor heat tolerance.

Bottom line - Regal Ladino is the best choice for a living mulch for weed control in medicinal plant beds. Another obvious advantage of this variety is its ability to suppress yellow nutsedge growth to a very high degree. Apparently this variety's massive ground cover suppressed nutgrass emergence or it possesses a level of allelopathy that suppressed nutlet growth and development.

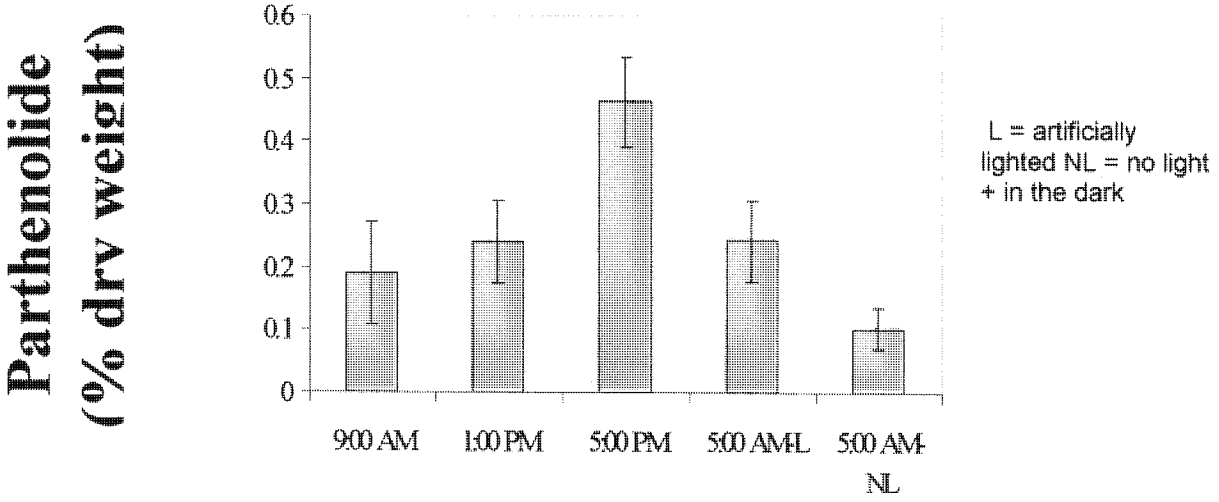
D. Establish quality control protocols for harvest and storage of medicinal plants

Topic 12. Report from James Rushing on postharvest handling of feverfew - Feverfew historically is valued for its use to control pain and fever. In recent years, studies have been focused on its utility for the treatment of migraine headaches. 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 suspected 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, i.e. production and postharvest management protocols, that maximize the parthenolide content of feverfew. Our objectives were to identify the most appropriate processing techniques as well as climatic stress agents that may maximize and/or preserve parthenolide in feverfew tissues.

Particle size versus parthenolide content in feverfew - Greenhouse and laboratory studies were conducted to determine how processing, environmental factors, and plant status affect marker compounds in feverfew. Research was completed on the influence of particle size on parthenolide extraction yields. Feverfew leaves must be ground to powder in the production of dietary supplement pills. It was found that feverfew ground to a powder with particle size of 500 µm or smaller yielded up to 5 times more parthenolide than particle size of 1000 µm or larger and for the best quality product, uniform small particle sizes are mandatory. A survey of herbal compounds available in the market place indicated a wide variability of particle size in all the herbal products and this commonly found aspect may reduce herbal potency and quality.



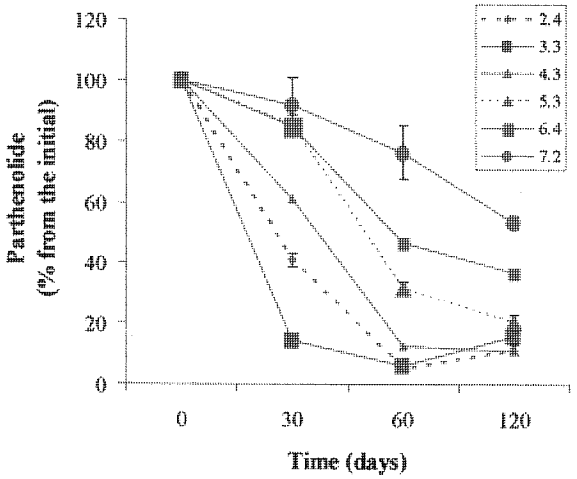
Effect of light availability on parthenolide content in feverfew - We wanted to determine if parthenolide differs by the time of day harvests may occur. If parthenolide is very stable, harvest time would not affect parthenolide. However, if parthenolide is transient during the day and affected by sunlight, harvest should be planned during periods of maximum parthenolide content. We found that parthenolide was lowest at 5 am before dawn and that by adding artificial light, parthenolide content could be increased, indicating a strong role of light in parthenolide synthesis. We also found that with continued light exposure up to 5pm, that parthenolide increased almost 5 fold from the 5am concentration.



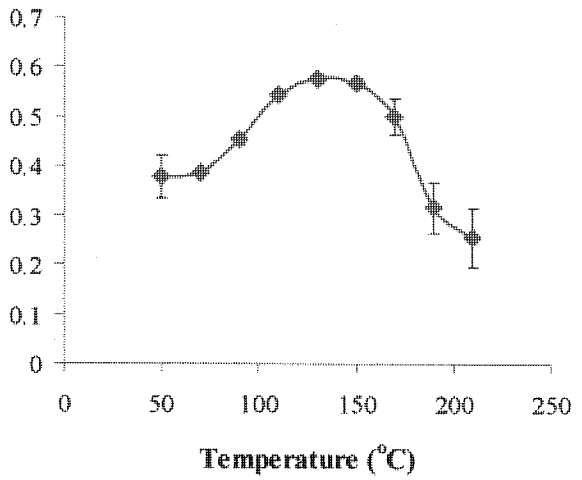
Water stress and parthenolide content - Feverfew plants were water-stressed repeatedly and then samples were collected and assayed for parthenolide. The parthenolide content was higher in water-stressed plants, but only after the second water stress cycle. Similar to above, we found that parthenolide content was at its peak at harvest conducted immediately prior to dusk and after the plants were under water stress. The diurnal fluctuations exposed in this research strongly indicated that parthenolide is very illusive and field production practices can have a profound effect on product quality. If parthenolide cycles during a normal day, from the commercial aspect, harvest should be delayed under late in the afternoon to maximize parthenolide content and any irrigation should be avoided prior to harvest.

Effect of pH and temperature during feverfew processing on parthenolide content - The pH of the solution may have activity on the parthenolide content and choice of solution pH may be a very important decision to preserve potency and quality. Feverfew powder and parthenolide standards mixed in citrate buffers at selected pHs (2.4 to 7.2) were stored for four months. Extracts with pH < 5 had more than 40% parthenolide loss within 30 days of storage, whereas solutions with pH > 5 had less than 15% loss in the same period. Extracts with pH < 5 had less than 15% loss in the same period. Light did not appear to affect parthenolide in stored feverfew products. Parthenolide declined in all treatments with the greatest loss in solutions with pH below 5. Optimal pH for parthenolide stability was 7.2. Parthenolide in dry samples declined 30% after 320 days storage. Degradation of parthenolide in feverfew solutions exposed to 40°, 60° and 80°C for 24hours increased with increasing temperature. Dry samples exposed to various temperatures revealed stability of parthenolide at temperatures up to 130°C for short time periods.

pH versus parthenolide



Drying temperature versus parthenolide



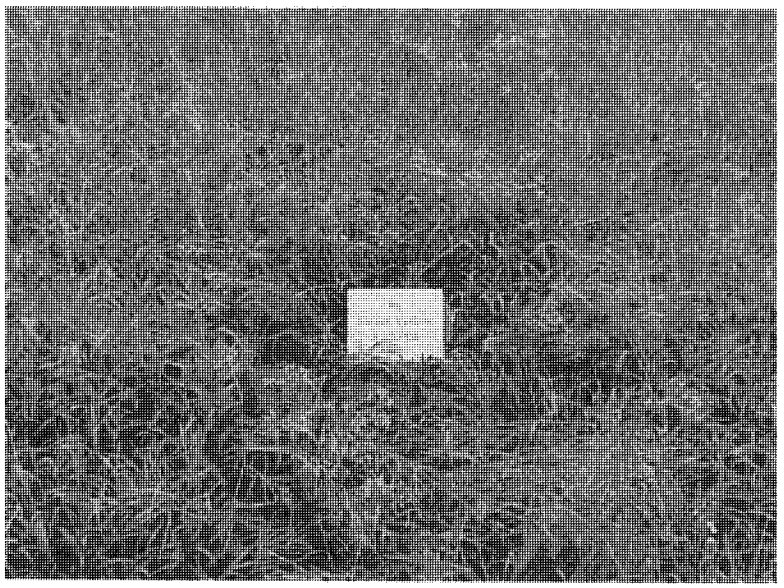
Conclusion. It is apparent at this time that many factors contribute to the quality of the medicinal plants grown for processing by the dietary supplement industry. The

production of medicinal plants requires care during the entire growing process to stimulate higher marker compound content during field production; however, research has shown that improper processing and handling of the harvested product can negate all the efforts employed during field production.

E. Synopsis of the current state of commercial medicinal plant research

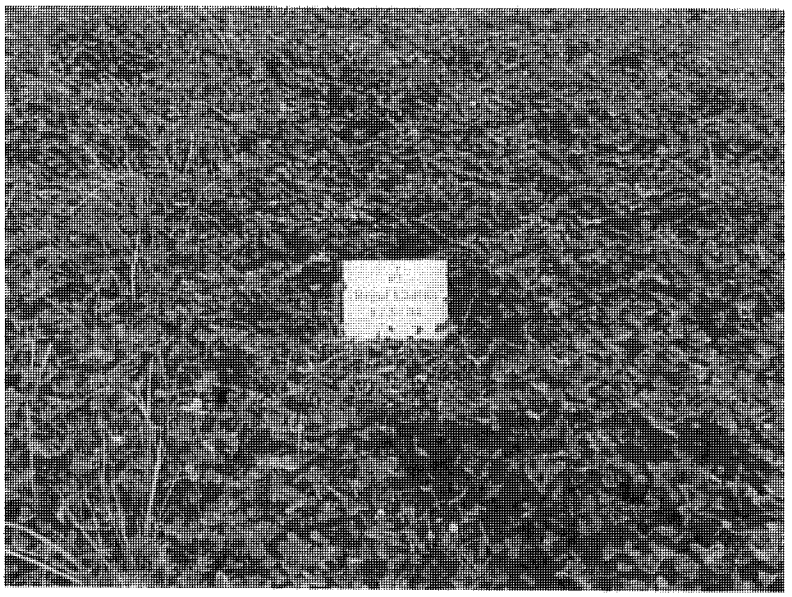
Production and postharvest research have conclusively indicated that the marker compounds in the medicinals studied are ephemeral, dynamic, and unpredictable. In order for the production of medicinals to be successful, it is mandatory that the marker compound content be as high as possible, yet we have found that marker compounds vacillate over time even from morning to late afternoon. It is critical that we understand more thoroughly the effect of the climate on marker compounds. It is necessary to monitor the marker compound content every two to three weeks throughout the field growth period, and this data must be correlated with climate (temperature, rainfall/irrigation, day length, etc.) to produce prediction models. A more thorough understanding of the genesis of marker compounds and how climate affects concentration will guide sequencing of production seasons and prediction of ideal harvest periods that maximize marker compounds and biomass production. Although high quality, fortified germplasm is required for a successful medicinal industry, the genotype x environment interaction of any new varieties must be fully understood concurrently or in other words, we must understand how a known variety of medicinal will react to a wide diversity of field conditions possible in SC. After developing new varieties, field trials are needed to monitor marker compound geneses before release of these varieties for commercial production.

Another major complication of commercial medicinal production is the lack of labeled herbicides and pesticides. Without these "tools," the commercial production of medicinal herbs is extremely complex. It is a "natural" observation that medicinal herbs require organic cultural practices for commercial production. Weed pressure in mid summer, however, renders medicinal production cost prohibitive because of manual weeding costs. Since disease epidemics caused predominantly by *Schlerotium rolfsii* can destroy plant quality, growth and reduce stands significantly, the most logical way to successfully grow any medicinal crop is to avoid those seasons when these pest pressures are extreme. Additional work is ongoing utilizing solarization from March to September to kill soil organisms (weed seed and pathogen propagules) prior to a fall planting of Echinacea spp. Feverfew may be planted in early Sept. for December harvest; however, short days and long nights increase stem production which reduces product quality (stems need to be discarded since they do not contain marker compound). Spring planting of feverfew must be field planted late Jan. to early Feb., for harvests before July 4th to avoid summer heat stress and crop losses. Later plantings of feverfew will seriously decline in quality in the heat of midsummer since feverfew is a cool season crop and resents heat stress. We have also concluded that production of *Echinacea purpurea* is a low value crop to our growers, considering all the labor needed to control weeds and harvest leaf material. The value of *Echinacea pallida* is more than 5 times higher and this crop is grown strictly for its roots. We suggest that SC farmers should



concentrate production of E. pallida because of its value and ease of harvesting with root digging machinery similar to potato diggers.
August 5, 2004 ratings

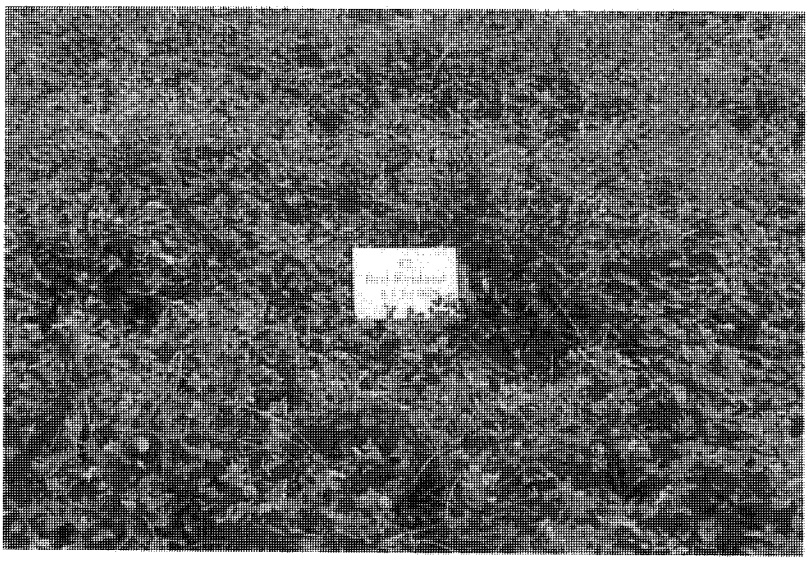
Sweet Yellow Clover was overrun by weeds especially grasses. Although the picture indicates "greenery," the majority of vegetation is weeds with very poor stands and very few sweet yellow clover survivors by this rating date.



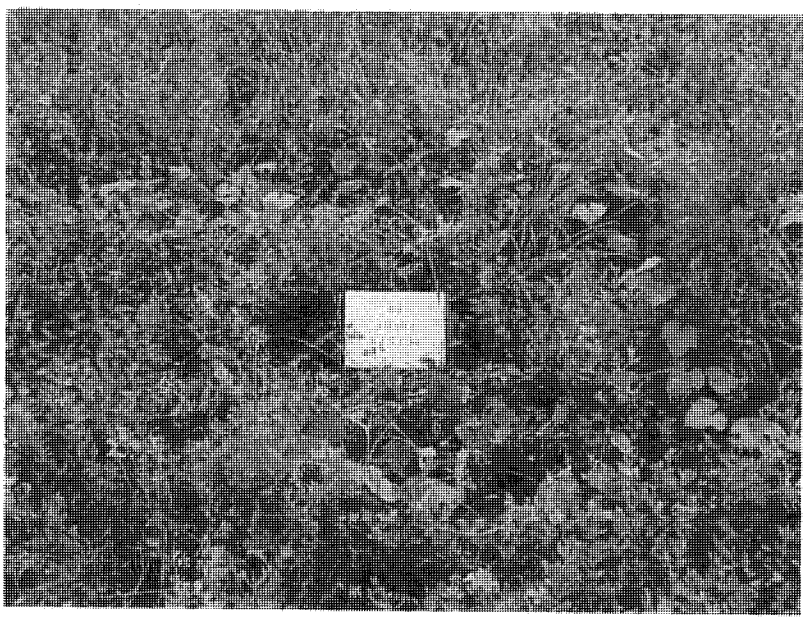
Regal Ladino Clover was short, and had a thickly-matted growth habit that suppressed most grasses and inhibited nutgrass growth. This clover was the best choice for a living mulch of all clovers.

Redland III Clover, although strong early in the season, was weaker by late summer and in many areas of the plots, the plants had declined, weeds invaded the plots and was not a long-term successful living mulch.

Aliske Clover was poor in April ratings and declined even more in potential as summer progressed. Poor plant survival allowed weeds to enter the plots and flourish.



Red Kenland Clover was an excellent choice as a living mulch in first ratings in April, but by August, the clover had thinned out, lost vigor and weeds invaded with unacceptable mulch potential compared to Regal Ladino



Alfalfa was barely fair in April ratings and declined even more in potential as summer progressed. Poor plant survival allowed weeds to enter the plots and flourish.

State of South Carolina
Department of Agriculture

Hugh E. Weathers
Interim Commissioner

Wade Hampton Office Building
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December 29, 2004

Ms. Janise Zygmont, Staff Officer
Federal-State Marketing Improvement Program
USDA-AMS
1400 Independence Ave, SW
Room 4009-S
Washington, DC 20250

Received by FSMIP
1-4-05
Date

Dear Ms. Zygmont:

Please find enclosed the final report for the project "Production and Development of Nutraceuticals as Alternative Crops: Refinement of Quality Standards for Certification and Branding, Part II."

Thank you for your assistance with this project. If we can provide additional information, please contact us.

Sincerely,

Larry J. Boyleston
Assistant to the Commissioner

State of South Carolina
Department of Agriculture

Charles R. Sharpe - Commissioner

Wade Hampton Office Building
P.O. Box 11280
Columbia, S.C. 29211

Telephone: (803) 734-2210
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AGENDA

GROWERS MEETING
August 13, 2003
10:00 a.m. – 2:30 p.m.
Clemson Sandhills Station
Main Conference Room

10:00 – 10:15 a.m.	Overview of activities since last meeting- Dave Gangemi
10:15 – 10:45	SC Dept. Ag./ USDA marketing grant- Larry Boyleston
10:45 – 11:15	Coastal REC medicinal plant program/Triarco contacts- Bob Dufault/Rich Hassell
11:15 – 11:45	Branding SC medicinal plant products- Mike Watkins and Kelly Springs
11:45 - 12:00	Update: The legislative front- Allison Dalton
12:00 - 12:30	Lunch
12:30– 1:00	"FDA Sabbatical happenings" and USP possibilities- Jim Rushing
1:00 – 1:45	Opportunities for contract growing- Ed Fletcher (Strategic Sourcing)
1:45 – 2:00	"The muscadine project" and Horry County happenings- Greg Hyman
2:00 – 2:20	The nutraceutical lectureship series and update on black cohosh cultivation in the Upstate- Dwight Camper
2:20 – 2:30	Open discussion- Where are we now and where should we be at the start of the New Year? New funding opportunities.

Noon

Nutraceutical Lectureship Series
Fall Semester 2003

Date	Speaker and Topic
September 5	Mark Farnham, USDA, Charleston. Nutraceutical Benefits of Broccoli
October 10	Jim Affolter, State Botanical Garden of Georgia. Medicinal Plant Production and Conservation (Bloodroot, Lemon Verbena, Echinacea and Ginseng)
October 24	Jeanine Davis, North Carolina State University Mountain Research Station. Production of Quality Medicinal Plants
November 7	John Riddle, North Carolina State University. Kidney and Urinary Therapeutics in Early Medival Monastic Medicine
Novermber 21	Laura Fox, USC. Pharmacokinetics and Drug Metabolism: Cytochrome P450 Studies With Medicinal Plant Extracts

Note: All seminars will be held in the Auditorium of Lethotsky Hall on the Campus of Clemson University