SARE FNE12-766
Annual Report 2013

Project description

Artemisia annua is a medicinal herb used extensively in the treatment of malaria[i], and there are indications that it may have wider benefits for other health concerns. The active ingredient, artemisinin, is derived from the leaves of *A. annua* and is reported to be allelopathic as well as providing a medicinal benefit. Artemisinin is already in short supply, and if additional medicinal uses are developed there will be increased demand. This project will provide information on the possibility of *A. annua* as a new high value crop that can double as a cover crop with some weed control efficacy. The project will evaluate the productivity of Artemisia annua in order to provide yield estimates. It will also evaluate the effectiveness of the crop in weed suppression.

Although we completed most of the work on this project in 2012, there were some unexpected results so we decided to repeat some of the work at a reduced level in 2013. There were reports in the literature that a dual harvest would increase productivity of artemisinin, the anti-malarial compound found in *A. annua*. However, our results showed no such effect, and yields in this study were not statistically different between the single harvest plots and the double harvest plots. Also, in the second year we expanded the scope of the study by using different planting densities to determine the effect on biomass productivity.

Results of first year work

Analysis of the 2012 harvest was done in 2013 by Professor Weathers and students at WPI. Leaves from individually harvested plants were used to extract artemisinin using the method of Weathers and Towler[ii]. Flavonoids were also extracted[iii], since they seem to play a role in enhancing the therapeutic effect of the whole plant[iv]. Artemisinin content was 13.96 mg/g dry weight leaves (1.4%) for both the half-season and final harvested plants. Flavonoids were about 35% higher in the half-season harvested leaves compared to leaves from the final harvest. This is attributed to the higher proportion of shoot tips in the smaller plants at half-season. The shoot tips contain proportionally about four times more flavonoids than mature leaves (Weathers and Towler, unpublished data). Although the flavonoid content may vary with harvest time, the artemisinin content is determined to be consistent between harvests.

Second year activities

We had planned to use the other side of the field in which the A. annua was planted in 2012, but we were unable to produce a usable seedbed due to heavy rains just prior to planting time. We decided to use an adjacent field, with plots about the same distance from the 2012 planting as the area which we had planned to use. The replacement field was slightly higher and drier so we were able to produce a good seedbed for planting. We did not add any fertilizer or bone meal to this plot, nor did we install a drip line since the 2012 planting did not really require water beyond normal rainfall.

The SAM cultivar was again produced by Professor Weathers at WPI. The planting density in 2012 was 12 plants per square meter. In 2013 we tried $10/m^2$, $8/m^2$, and $4/m^2$. The seedbed was prepared June 6th, and flame weeded immediately prior to setting out the plants on June 12th. The 1 square meter plots were separated by wooden stakes as in the 2012 planting, but we did not leave a buffer between the plots. We did leave a small buffer between different density zones, just for easier identification of the zones.

Half-season harvest was done July 17th, but instead of harvesting 1 m^2 blocks, the harvest was done by pruning every other plant to half height. As before, the plants were labeled at harvest so that the different density planting zones could be compared. These plants were taken to WPI and dried in the laboratory, where the leaves were subsequently removed, sieved, and stored for later analysis.

Final harvest was done September 21st, when the plants were just starting to develop flower buds. These plants were bagged, dried in the greenhouse, then transported to the laboratory for processing as drying progressed.

Analysis of data

Combining data from 2012 and 2013 work we found that planting densities of 8 plants/m^2 yielded the highest biomass per plant. Yield dropped off after 8/m^2, with 12 plants/m^2 giving the lowest yield per plant. The 4 plants/m^2 zones gave yields per plant similar to 8/m^2, but the total biomass per acre was significantly reduced because of the lower number of plants (Table 1). Funding was insufficient to complete analysis of artemisinin content for the 2013 harvest, but that was not within the expected scope of this project.

Part of the study was to determine the value of A. annua as a cover crop. As far as weed suppression is concerned, there was no evidence for any allelopathy in this study. However, weed suppression was considerable by shading, for all densities tried in this study.

The value of A. annua as a crop (other than pure weed suppression) is based on the quantity of artemisinin that can be derived from the crop. The global value of the dried leaves of *A. annua* for the extraction market varies considerably. At this point we should point out that the SAM cultivar used in this study was selected for consistently high artemisinin content. Moreover, this cultivar is currently vegetatively propagated. Work is ongoing to develop a cultivar that can be produced from seed and still maintain consistently high artemisinin content.

Table1. Comparison between 2012 and 2013 field trials for *Artemisia annua* dry leaf productivity at different planting densities.

Parameter	2012	2013		
	12 plants m^{-2}	10 plants m^{-2}	8 plants m^{-2}	4 plants m^{-2}
Avg. leaf DW per plant (g/plant)	72.71 ± 26.01a	85.11 ± 23.65b	112.44 ± 34.48 b,c	112.29 ± 32.32 b,c
Leaf DW (g/m^2)	872.52	851.13	899.51	449.16
Leaf DW/cultivated area:				
(kg/ha)	8,725	8,511	8,995	4,491
(T/ac)	3.93	3.79	4.00	1.99
Global market value @ $1,000/T	3,930	3,790	4,000	1,990
Artemisinin (mg gDW^{-1})		Not yet Assayed.	Not yet Assayed.	Not yet Assayed.
July	14.47a			
Sept.	13.96a			
Total flavonoids (mg gDW^{-1})		Not yet Assayed.	Not yet Assayed.	Not yet Assayed.
July	6.37a			
Sept.	4.71b			

Different letters after data points indicate statistically significant results at $p \geq 0.05$.

[i] Elfawal MA, Towler MJ, Reich NG, Golenbock D, Weathers PJ, et al. (2012) Dried Whole Plant Artemisia annua as an Antimalarial Therapy. PLoS ONE 7(12): e52746. doi:10.1371/journal.pone.0052746 http://dx.plos.org/10.1371/journal.pone.0052746

[ii] Weathers, P.J., Towler, M.J. (2012) The flavonoids casticin and artemetin are poorly extracted and are unstable in an *Artemisia annua* tea infusion. Planta Medica. 78:1024-1026.

[iii] Arvouet-Grand A., Vennat B.,Pourrat A.,Legret P. (1994) Standardization of a propolis extract and identification of the main constituents. Journal de pharmacie de Belgique 49:462-468

[iv] Elford, B.C., Roberts, M.F., Phillipson, D., Wilson, R.J.M. (1987) Potentiation of the antimalarial activity of qinghaosu by methoxylated flavones. Transactions of the Royal society of tropical Medical Hygiene. 81, 434-436.