Final Report to the Council for Burley Tobacco (December 2014)

Title: Production and Purification of Nicotine from Green Tobacco for Emerging Tobacco Products

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Report type: Final report

Introduction

The market for electronic cigarettes continues to grow with most major US cigarette manufacturers now marketing an e-cigarette. In addition there are other innovative, non-combustible nicotine –containing products that are either under development or are already on the market. Such products create a potential new opportunity for Kentucky tobacco growers. However, these products are not likely to contain the typical burley tobacco that is currently produced in Kentucky. Other tobacco cultivars or other *Nicotiana* species may be better suited for these products and novel production methods from green of dried tobacco biomass will require economical purification. This project will evaluate tobacco types for alkaloid production. Purification from green tobacco biomass must be competitive with current suppliers and there is commercial interest in a domestic supply of nicotine. Currently the nicotine used in e-cigarettes and other non-combustible products comes from foreign sources where there may be concerns about product quality and consistency. With FDA regulation of all tobacco products, there is an expectation that domestic production and purification may be desirable.

Summary of Progress

Objective

The work this year was an extension of the study conducted in 2013 (see status report from Dec. 2013). The goal was to define the two best tobacco lines for the production of pure nicotine. Desirable characteristics for this line will include high nicotine content; high biomass and growth characteristics that would permit machine topping and harvesting.

Methods

Design

The experimental design was four randomized compete blocks of a split plot design with seven main plots (plant line), and two subplots (harvest date) i.e. 14 treatments and 56 plots.

Varieties

1. T1 401 - germplasm selected for high nicotine during preliminary field screening in 2013.
2. T1 1275 - germplasm selected for high nicotine during preliminary field screening in 2013.
3. T1 464 - germplasm selected for high nicotine during preliminary field screening in 2013.
4. TI 959 - germplasm selected for high nicotine during preliminary field screening in 2013.
5. NL Madole – commercial dark variety, reported to have highest nicotine of dark varieties
6. PH 1-2 – flue-cured variety reported to have high nicotine
7. TN 90LC – check

Harvest date

1. 4 weeks after topping (WAT)
2. 7 weeks after topping (WAT)

Agronomic details

Transplants were produced in 242 cell float trays following University recommended production practices. Transplants were set in the field on June 16. The field was fertilized with 200 lb/acre N pre-plant. University recommendations for conventional tobacco production were utilized for plant spacing and weed and pest control. Drip irrigation was installed.

The plants were topped when 80% of the plants were in bud at the selected leaf number (Table 1). Immediately after topping the plants were treated with a ½ rate of fatty alcohol. Follow up suckeride treatments were made one week and two weeks post-topping with a tank mix of full rate fatty alcohol/ butralin and butralin/maleic hydrazide, respectively. Plots were monitored weekly to remove ground or axillary suckers that were not controlled by the chemical treatment.

Fourteen plants from each plot were hand harvested four weeks and seven weeks after topping (WAT). The harvested leaves were split into 4 sub-groups; bottom, middle, top and 4^{th} leaf. The leaves in each group were counted, weighed, bagged and dried in a forced air drier set at 60^{o}C. After drying, the leaves were ground to a particle size that would pass through a 1 mm sieve and then analyzed for nicotine content.

Results

Biomass Yield

The earliest lines flowered 40 days after transplanting (TI 401 and TI 1275) and the latest line flowered 58 days after transplanting (TN 90LC). Early flowering may be desirable as it might allow 2 crops per season.

Dry weight biomass yields varied greatly between the lines with a range of 963 lb/acre (TI 1275) to 3783lb/acre (PH1-2) for the 4 WAT treatment and 1,155 lbs/acre (TI 1275) to 3806 lb/acre (PH1-2) for the 7 WAT treatments (Figure 8).

With the exception of line PH1-2, dry leaf biomass yield increased on average approximately 17% across the lines from the 4 WAT to the 7 WAT harvest. PH1-2 had less than a 1% increase between the harvests.

The following growth characteristics were observed from each line;

1. TI 1275. Early flowering, low biomass, weak stalk that contributed to severe lodging, short in stature, many ground and axillary shoots.

2. TI 401. Early flowering, low biomass, strong upright stalk, very little lodging, short in stature, limited ground and axillary suckers.

3. TI 464. Normal flowering time, medium biomass, weak stem with some lodging, medium in stature, some ground suckers.

4. TI 959. Normal flowering time, medium biomass, weak stem with some lodging, medium in stature, some ground suckers.

5. NL Madole. Normal time to flower, high biomass, strong stem, no lodging, tall in stature, no ground or axillary suckers

6. PH1-2. Later flowering, very high biomass, strong stem, no lodging, tall in stature, no ground or axillary suckers, brittle leaves.

7. TN 90LC. Later flowering, high biomass, strong stem, no lodging, tall in stature, no ground or axillary suckers. (See photos)

Nicotine and yield results

Nicotine concentration over the whole plant also varied between the lines (Figure 5) with a whole plant range of 3.1% DM (PH1-2) to 6.6% DM (TI 1275) for the 4 WAT treatment and 3.1% DM (PH1-2) to 6.6% DM (TI 464) for the 7 WAT treatment.

For both the 4 WAT and 7 WAT harvest, the top leaves contained the highest level of nicotine (Figure 3) followed by the middle (Figure 2) and bottom (Figure 1) leaves. The top leaves produced approximately twice as much nicotine as the bottom leaves. This was expected as the many of the bottom leaves were perished. In a harvest system that just removed the leaves the bottom leaves would most likely not be harvested because it would not be economically feasible to process them.

Generally, the nicotine content did not increase between the 4 WAT to the 7 WAT harvest (Figures 5A and B). This was unexpected as it is generally accepted that, after topping, nicotine content will continue to accumulate in the leaves the longer the plant is allowed to grow in the field. One explanation may have been excessive soil moisture. The field received a great deal of rain between the 4 WAT and the 7 WAT harvest and this may have affected the nicotine levels as dry conditions tend to promote higher nicotine levels. This year was the second consecutive year where nicotine content did not increase between the 4 WAT and 7 WAT harvests. This trend may indicate that 4 to 5 WAT may be sufficient time to maximize nicotine accumulation in the leaf. One benefit to the shorter time to harvest would be improved leaf quality. Secondly, it is very difficult to maintain good sucker control for 7 WAT. After 4 to 5 WAT the chemical treatment loses effectiveness and ground and axillary shoots begin to grow. For our study we removed the suckers manually. On a commercial scale it would not be economically feasible to do this and the presence of suckers on the plant negatively affects the accumulation of nicotine. Further research will be necessary to determine optimal harvest time.

We purposely obtained the line identified as PH1-2 as it had been reported to produce tobacco with very high levels of nicotine (50% above the check). We were very surprised and disappointed to find that it was the line that had the lowest nicotine levels in the leaves (Figure 5). The reason for the poor results is unknown.

Plans for Future Work

With the exception of TI 401 all of the TI lines (See photos) were very poor from an agronomic standpoint and even though they were generally higher in nicotine concentration, they would not be suited for field production. Conversely, TI 401 (See photos) possesses agronomic characteristics and nicotine content that would make it a suitable for field production. The dark tobacco cultivar NL Madole was the top line in the test. The nicotine content per unit of dry matter for the 4 WAT and 7 WAT was good at 4.7% DM and 5.3% DM, respectively, and the dry leaf biomass yield was very good for the 4 WAT and 7 WAT at 3,042 lb/acre and 3,672 lb/acre, respectively. Using these numbers to calculate yield of nicotine per acre, NL Madole is the highest in the study producing 144 lb /acre 4WAT and 188 lb/acre 7 WAT (Figure 6).

We plan to seek funding to continue the research on nicotine production research utilizing the lines TI 401 and NL Madole. We feel the next step in the development of a nicotine production system would

be to incorporate these two lines into a fully replicated study or studies designed to define the optimal fertilization, topping height and harvest date. It is generally understood that high nitrogen fertilization can increase the nicotine in the plant and that topping height can also influence nicotine content. In addition a small separate replicated study will be designed to evaluate mechanical topping vs. hand topping. Economically, it would be advantageous if topping for nicotine production could be done mechanically.

Figures and Tables

Table 1: Topping and harvest dates

Variety	Topping	Harvest 1	Harvest 2
T1 401	July 25	August 22	Sept. 12
T1 1275	July 25	August 22	Sept. 12
T1 464	August 04	Sept. 02	Sept. 22
TI 959	August 06	Sept. 03	Sept. 24
NL Madole	August 07	Sept. 04	Sept. 25
PH 1-2	August 11	Sept. 08	Sept. 29
TN 90LC	August 12	Sept. 09	Sept. 30

Table 2: Wet weight for each stalk position (lb/acre)

Variety	Harvest 1			Harvest 2		
	Bottom	Middle	Top	Bottom	Middle	Top
TN 90LC	1,607	10,663	13,562	1,558	10,614	13,759
NL Madole	1,944	11,302	12,965	962	10,164	14,222
TI 959	716	5,117	6,093	611	4,738	6,641
T1 401	877	4,366	5,026	779	4,514	5,770
T1 1275	674	2,822	3,007	674	3,082	2,738
T1 464	695	5,068	6,809	449	5,096	6,823
PH 1-2	2,218	12,186	14,917	1,839	10,452	14,608

Table 3: Dry weight for each stalk position (lb/acre)

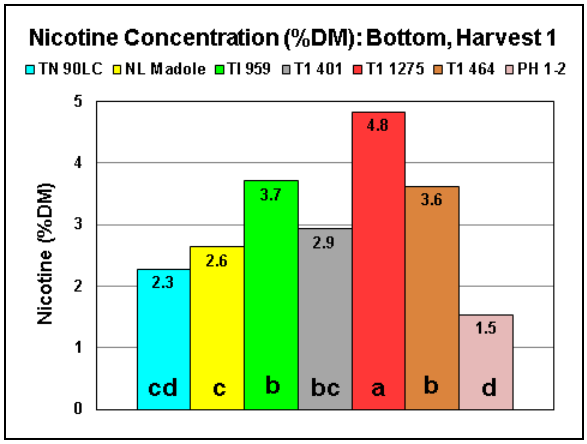
Variety	Harvest 1			Harvest 2		
	Bottom	Middle	Top	Bottom	Middle	Top
TN 90LC	248	1,074	1,699	394	1,296	1,928
NL Madole	272	1,174	1,596	249	1,298	2,125
TI 959	67	611	797	99	627	972
T1 401	99	558	712	165	714	909
T1 1275	100	409	454	158	537	461
T1 464	85	582	872	92	699	1,027
PH 1-2	440	1,323	2,019	457	1,245	2,104

Table 4: ANOVA table: significance levels for variety, harvest and interaction

Variable	Variety	Harvest	Var x Harv
Nicotine (Bottom)	<0.0001	0.0012	0.031
Nicotine (Middle)	<0.0001	NS	NS
Nicotine (Top)	<0.0001	0.0009	0.0093
Nicotine (4^{th} Leaf)	<0.0001	NS	0.05
Nicotine (Whole Plant)	<0.0001	0.013	0.033
Nicotine Yield / Ac *	<0.0001	<0.0001	0.0168
Wet Weight *	<0.0001	NS	NS
Dry Weight *	<0.0001	0.044	NS
Leaf Number *	<0.0001	<0.0001	0.004

* Log transformed

NS Treatments are not significantly different at $p < 0.05$



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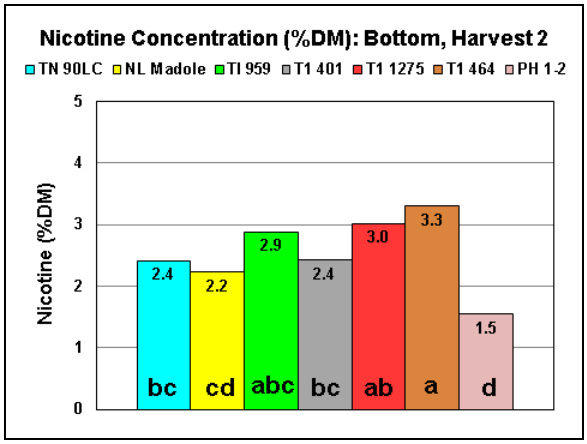
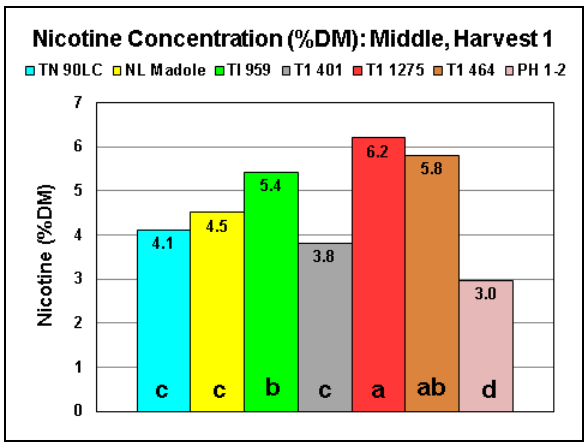


B

Figure 1: Nicotine concentration (% DM) for the bottom stalk position **A.** Harvest 1 **B.** Harvest 2



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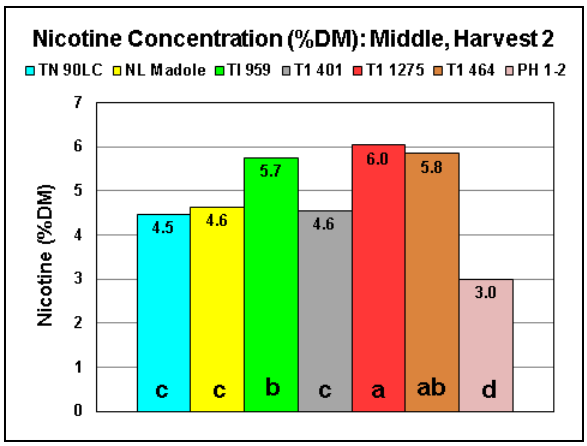

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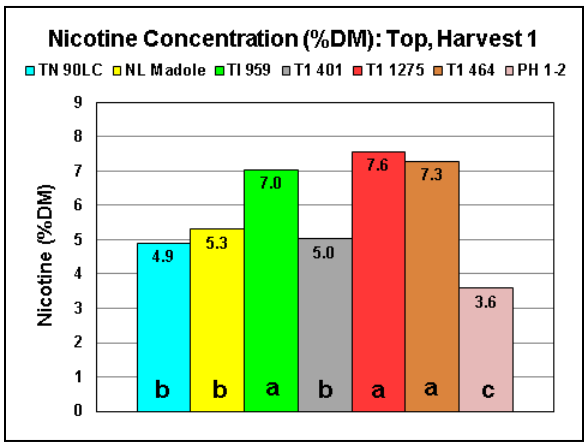
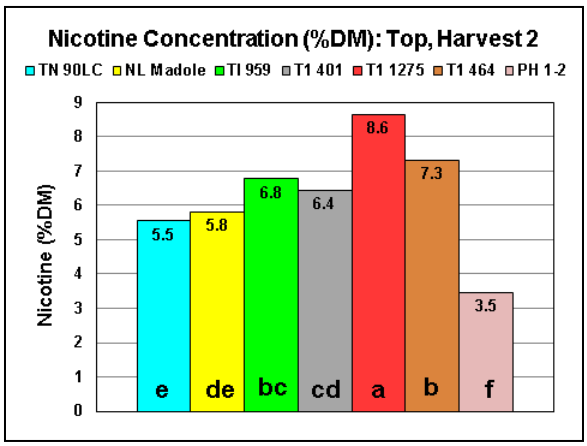
Figure 2: Nicotine concentration (% DM) for the middle stalk position **A.** Harvest 1 **B.** Harvest 2



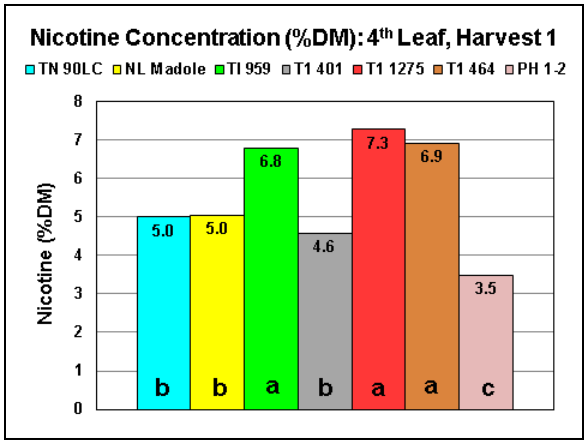
A



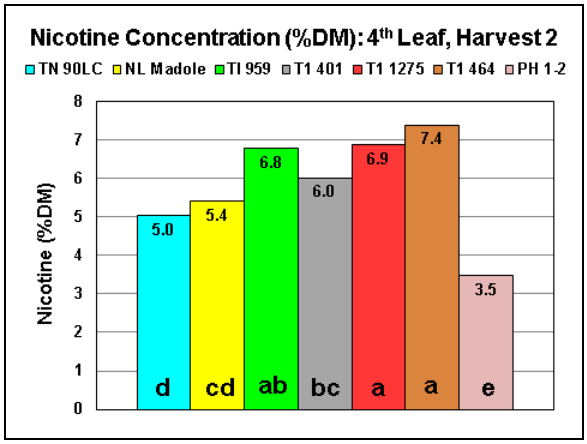
B

Figure 3: Nicotine concentration (% DM) for the top stalk position **A.** Harvest 1 **B.** Harvest 2

Bars with the same letter are not significantly different at $p < 0.05$

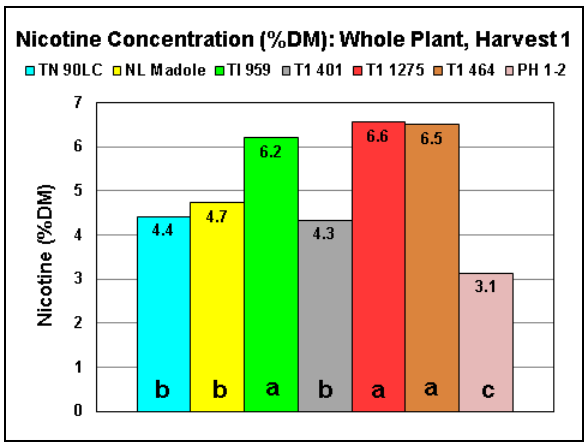


A

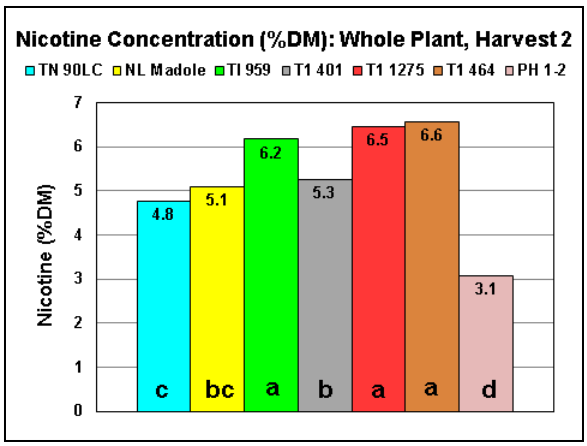


B

Figure 4: Nicotine concentration (% DM) for the 4th leaf **A.** Harvest 1 **B.** Harvest 2

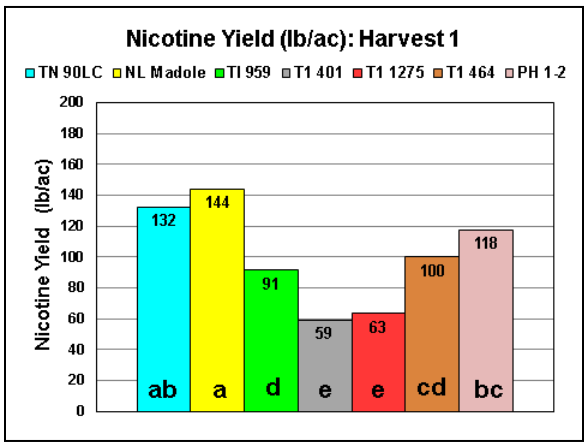


A

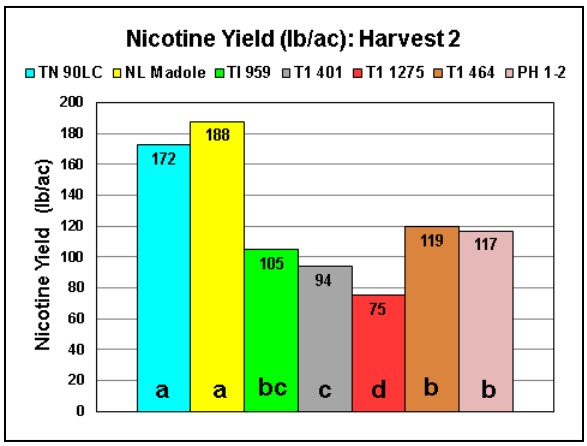


B

Figure 5: Weighted nicotine concentration (% DM) for whole plant **A.** Harvest 1 **B.** Harvest 2



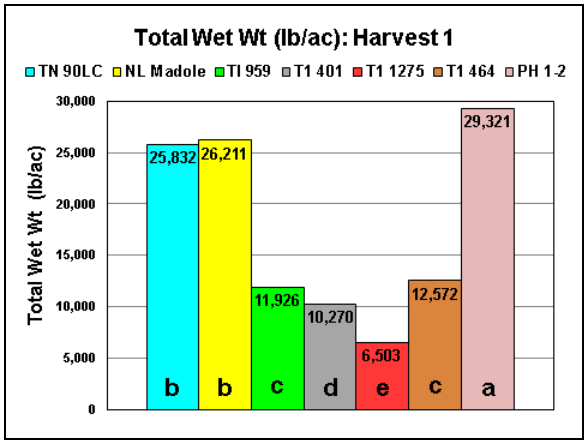
A



B

Figure 6: Nicotine yield (lb/ac) **A.** Harvest 1 **B.** Harvest 2

Bars with the same letter are not significantly different at $p < 0.05$



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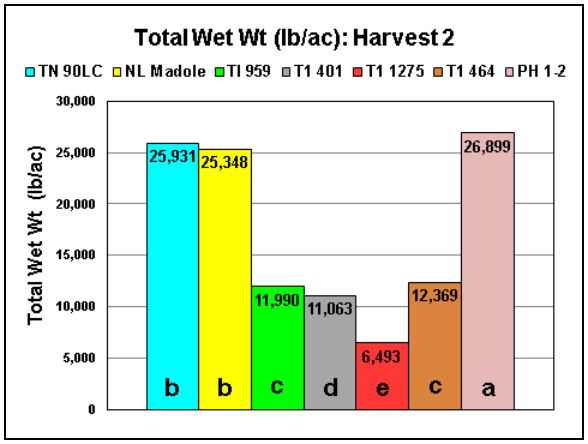
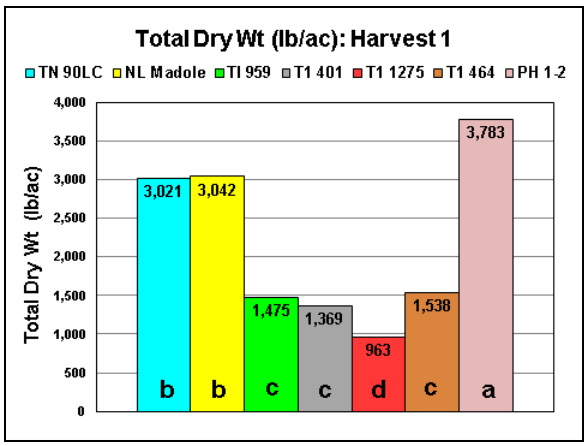


B

Figure 7: Total wet weight (lb/ac) **A.** Harvest 1 **B.** Harvest 2



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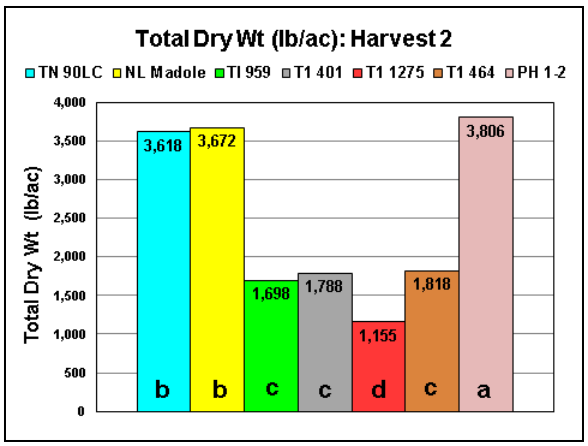
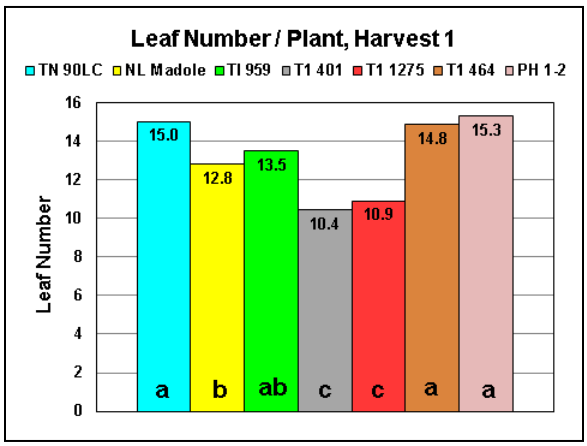


B

Figure 8: Total dry weight (lb/ac) **A.** Harvest 1 **B.** Harvest 2



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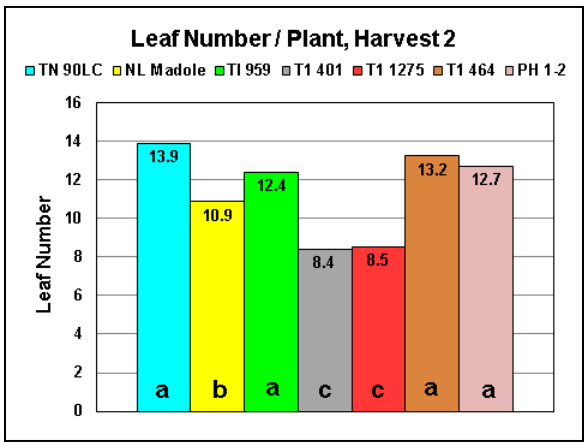

B

Figure 9: Leaf number / plant **A.** Harvest 1 **B.** Harvest 2

Bars with the same letter are not significantly different at $p < 0.05$

Photo 1 TI 464

Photo 4 TI 959

Photo 2 TI 401

Photo 5 TI 1275

Photo 3 PH1-2

Photo 6 NL Madole

Photo 7 TN90 LC

Photo 10 Post Harvest

Photo 8 Hand harvesting the leaves

Photo 9 Hand harvesting the leaves