Title: **Evaluation and Control of Ground Sucker Formation in Burley Tobacco Varieties**

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Lay Summary: The use of hydroponically produced tobacco transplants, commonly referred to as "float plants", has been widely adopted by tobacco producers over the last several years. One negative aspect of float plants is their increased propensity to form basal axillary buds or shoots, commonly referred to as "ground suckers". The primary objective of the current research project was to determine if the difference in root to shoot ratio between traditional versus hydroponically produced transplants results in a significantly different auxin to cytokinin ratio, thereby affecting the development of ground suckers. A secondary objective was to investigate whether the normal auxin to cytokinin ratio can be restored by adding auxins to the float water, resulting in a reduction of ground suckers when the treated plants are grown in the field. Initial greenhouse studies indicated that the addition of 1-naphthaleneacetic acid (NAA) to hydroponic solutions was successful in reducing ground sucker formation. However, hydroponic applications of NAA resulted in no significant decrease in ground sucker formation in subsequent field studies.

Introduction

Rationale and Background

There is no clear understanding as to why ground suckers are so much more prevalent in hydroponically produced transplants. One possibility is that there is a difference in levels of specific levels of plant hormones between traditional versus hydroponically produced transplants. Of particular interest is the ratio of auxins, which are produced primarily in apical meristems, to cytokinins, which are primarily produced in the roots of plants. Cytokinins are involved in cell division and shoot and root morphogenesis. They are known to regulate axillary bud growth and apical dominance, and the cytokinin to auxin ratio is known to affect the formation of axillary buds. In tobacco, sucker formation in tobacco plants is repressed prior to topping, but suckers grow prolifically following topping. Before tobacco plants are topped, auxin from apical buds travels down the stalk to inhibit axillary bud (sucker) growth. This promotes primary shoot growth, and restricts lateral branching. Cytokinin moves from the roots into the shoots, eventually signaling lateral bud growth. When the apical bud is removed from tobacco plants at topping, the auxin to cytokinin ratio is altered and the growth of suckers increases dramatically if they are not physically or chemically removed.

The root to shoot ratio in hydroponically grown plants is much greater compared to plants grown in traditional plant beds. At transplanting, only a very small root system is maintained in plants pulled from traditional plant beds; in comparison, hydroponically produced plants have an intact root system that is much larger, with exact size determined by the size of the tray cells used to produce the pants. The hypothesis that the proposed research investigated was that this difference in root to shoot ratio between traditional versus hydroponically produced transplants results in a significantly different auxin

to cytokinin ratio, thereby affecting the development of ground suckers. In other words, the ratio between these two phytohormones in float plants may approximate the differences observed in topped versus non-topped mature tobacco plants. The research investigated tobacco a variety known to have a propensity to form ground suckers (TN 86) versus one where ground suckers are rarely observed (Hybrid 403). The initial research was done in the laboratory utilizing tissue culture to determine the response of TN 86 and H 403 plantlets grown on media containing varying levels of auxins and cytokinins. 1-naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BA) were the chosen synthetic auxin and cytokinin hormones, respectively. Based on results of the tissue culture studies, a follow-up study was conducted in the greenhouse to determine optimal levels of hormone concentrations in hydroponic solutions that could possibly minimize the formation of adventitious axillary buds without significantly altering stem elongation or plant growth. The laboratory and greenhouse studies were followed by field research trials that were conducted in 2015 and 2016 to determine the effectiveness of treatment with plant auxins and/or cytokinins in minimizing or eliminating ground sucker formation without adversely affecting plant growth or yield potential.

Summary of Progress

Brief review of 2015 Research Studies

Initial laboratory studies were conducted to determine initial levels of hormones to use for greenhouse and field studies. The experimental methods utilized and results obtained for these laboratory studies were presented in the 2015 Interim Report. Based on the results from the laboratory studies, greenhouse studies conducted in January 2015 evaluated hormone treatments of 2nM, 10nm, and 50nM concentrations of NAA; 2nM, 10nm, and 50nM concentrations of BA; and a Control with no added hormone. The experimental methods utilized and results obtained from these initial greenhouse studies were also presented in the 2015 Interim Report. To summarize, for the control plants that had no added hormones, TN 86 produced significantly more axillary shoots than did H 403. For H 403, which essentially did not produce any axillary shoots in the greenhouse control treatment and also normally does not produce a significant number of ground suckers when grown in the field, all of the hormone treatments resulted in no change or an increase in the number of axillary shoots produced in comparison to the untreated control. For TN 86, all of the NAA concentrations resulted in a decrease in axillary shoot formation. However, the reduction in sucker formation was statistically significant only for the 2nM NAA treatment; this was the case regardless of when the hormone treatments were added. None of the BA concentrations appeared to be effective in reducing axillary shoot formation.

TN 86 and H 403 that had been treated with hormones during transplant production were compared in field studies conducted in Lexington and Versailles, KY and Greeneville, TN during 2015. The experimental methods utilized and results obtained from the 2015 field studies were also presented in the 2015 Interim Report Tobacco. Plants of the two varieties were produced on hydroponic solutions containing three auxin and three cytokinin concentrations. To summarize the results of the 2015 field studies, ground sucker pressure was extremely light at both Lexington and Woodford County in 2015, likely as a result of plentiful rainfall that occurred at both locations. H 403 had virtually no ground suckers at either location. TN 86 produced an average of one to two ground suckers per plant at Lexington and Versailles, respectively, but on average less than half of the plants produced suckers that grew to be more than six inches in length, so ground suckers that did form were of no consequence. At Greeneville, it was relatively drier immediately after transplanting and ground sucker formation was

somewhat more prevalent. H 403 still produced on average of less than one ground sucker per plant, and virtually none of those grew to a length of six inches. TN 86 produced between one and two suckers per plant, but only one or two suckers per every ten plants exceeded six inches in length. Although the incidence of ground suckers was extremely low in all three 2015 field studies, incidence was high enough to clearly demonstrate that none of the hormone treatments were effective in reducing ground sucker incidence. Although the results were unexpected and certainly disappointing, there were three possibilities for the lack of results. The first was that the initial concentrations of auxins and/or cytokinins were simply not high enough to affect sucker formation. The second possibility was that the initial concentrations were high enough, but that as water was added to the beds as necessary during the transplant season, hormone levels were diluted far below their initial levels. This was a possibility because much more water evaporated from float beds during April and May than occurred in December and January when the greenhouse studies were conducted; in April and May the increased evaporative demand required the addition of water to the one tray treatment beds on a near daily schedule, which could have resulted in ineffective hormone concentrations. The third possibility was that auxins, which are normally produced primarily in the apical meristem and then translocated down the stalk through phloem vessels to suppress the formation of axillary buds, cannot be effectively absorbed through the roots and translocated upward through xylem cells. In that scenario, the addition of auxins to hydroponic solutions may not be a possibility for the control of axillary buds.

To test possibilities one and two, another set of greenhouse experiments was conducted in the fall of 2015. Only one variety, TN 86, was utilized for this second study. Much higher concentrations of NAA and BA were utilized; in the first greenhouse study and in the 2015 field studies, 2, 10, and 50nM concentrations were evaluated, while in the second greenhouse study the auxin concentrations evaluated were 200nM, 1000nM (1uM), and 5uM. The BA concentrations in the second study were 50nM, 200nM, and 1250nM. One application of hormones, applied approximately four weeks after seeding, was compared to repeated applications of hormone every time water had to be added to the float beds; this was achieved by adding stock solutions having the desired hormone concentrations to the beds rather than adding only water.

Data from this second greenhouse demonstrated that higher levels of NAA did substantially reduce the formation of axillary buds in comparison to untreated control plants, but the addition of BA cytokinin had little or no apparent effect (**Table 1**). More importantly, only one application of NAA was necessary to control the formation of axillary shoots even in plants that were approximately two feet tall. The most effective rate of NAA, 1uM, reduced the formation of all axillary shoots by 85% in comparison to the untreated control without altering the overall growth characteristics of the plants. The reduction in axillary shoots that were 1” - 6” in length were reduced by 95% in comparison to the control; this is a particularly significant result as suckers exceeding 4” – 6” in length are much more likely to continue to grow and become problematic in comparison to shoots that do not exceed 1” in length at transplanting. The highest rate of 5uM NAA also resulted in a significant reduction in axillary shoots, but the plants were approximately 50% taller than control plants. However, leaf number of the plants was not increased by the high NAA level; only the internode length was increased. This is not surprising since one of the primary functions of auxins is stem elongation.

2016 Field Studies

As a result of the latest greenhouse study, a second field study was conducted in 2016. The new study looked primarily at the effects of higher levels of NAA on ground sucker incidence in TN 86, but a control treatment of variety H 403 was also included in the study. In addition to determining the effects of auxin concentrations on axillary bud formation and overall plant growth, the effects of tray cell size which dramatically alters the root to shoot ratio was also evaluated in the 2016 study. If the original hypothesis that the increased incidence of ground suckers in float plants is a result of altered auxin to cytokinin balance resulting from an increase in the root to shoot ratio is in fact true, there should be a significantly higher incidence of suckers in non-hormone treated plants grown in 128 cell trays as compared to plants grown in 338 cell trays.

The 2016 field studies were conducted at Lexington and Versailles, KY and Greeneville, TN. The individual treatments are given in **Table 2**. The five NAA levels were added to recommended hydroponic solutions at 21 days after seeding (first clipping). A split split-plot experimental design arranged in a randomized complete block with three replications was utilized for the study. Main plots were varieties, sub plots were NAA concentrations, and sub sub-plots were tray/cell size. Data were collected for number and length of ground suckers at 50 days after transplanting.

Results from the 2016 field studies are presented in **Tables 3** and **4**. There was a variable but relatively low incidence of ground suckers at all test locations; the average total number of suckers per plant was approximately 1 - 1.5 in Lexington, compared to 1 - 2.5 in Greeneville, and 2 - 2.5 in Versailles (**Table 3**). As seen in previous studies conducted as part of this project, there was a pronounced difference between burley varieties H 403 and TN 86 in their propensity to produce ground suckers, with H 403 producing an average of less than one ground sucker per plant at all locations. The effects of NAA, applied hydroponically during the production of transplants, on decreasing or eliminating subsequent development of ground suckers in the field was much less than what was expected based on the second 2015 greenhouse study. In that study, a 1uM concentration of NAA reduced the formation of all axillary shoots by 85% in comparison to the untreated control. Unfortunately, that level of effectiveness was not expressed in the field. A positive response to increasing levels of NAA on decreased ground sucker formation was observed only at Greenville, where a decrease in sucker formation was observed for the two highest NAA concentrations; this was particularly true for the number of suckers exceeding 12” in length. In contrast, at Lexington higher levels of ground suckers were actually observed for all NAA concentrations in comparison to the control. However, virtually all suckers at Lexington were less than 12 inches in length, with the majority being less than 6 inches in length. At the Versailles location, there was essentially no effect of any of the NAA treatments on sucker development or elongation. This was also the case when all three locations were averaged.

The effects of tray/cell size on ground sucker formation are presented in **Table 4**. The original hypothesis for this project was that the increased formation of ground suckers in hydroponically produced tobacco transplants was the result of altered shoot to root ratios, with a subsequent altered auxin to cytokinin ratio. If this hypothesis was correct, one would expect an increase in ground suckers among plants that had larger root systems. Although that trend was observed somewhat at Lexington,

and for H 403 at Greeneville, that pattern was not observed in Versailles, or when data from all locations were combined.

In the two years that this study was conducted, ground sucker incidence in the field was very low even in TN 86, and almost all suckers that did develop failed to elongate beyond 12" in length. This was true for the control plots as well as the hormone treated plots. Suckers of this size are of little agronomic or financial consequence. This demonstrates that ground suckers may not be a significant problem for the majority of growers during most seasons. Unfortunately, the results from this project clearly show that the addition of auxins to hydroponic transplant systems is not effective in eliminating ground sucker formation in the field. There is no clear explanation as to why the 1Um NAA treatment was so effective in the greenhouse, but not in the field. It is known that environmental growing conditions greatly affect ground sucker formation, and pot-grown greenhouse plants are inherently different from field grown plants.

Although it is somewhat difficult to draw conclusions from research that produces negative results, there are two possibilities as to why the addition of auxin to the float water was ineffective in eliminating ground sucker formation. One possibility is that auxin is not actively transported from the roots to the shoots. Auxins are normally produced in the tops of the plants and transported down to the roots through phloem sieve elements; it is not normally transported through the xylem, which moves water and nutrients from the roots to shoots. This possibility was considered before the initiation of this project, but because of the chemical similarity of NAA to known herbicides, it was decided that there was a considerable risk of leaf damage occurring from foliar applications of NAA or similar compounds.

The second possibility as to why application of NAA was not effective in reducing ground suckers is simply that the initial hypothesis was incorrect. If every hypothesis that a scientist formulated was in fact correct, there would be no need to actually conduct research; scientists could simply sit in the office and think. In the current research project, the relative lack of differences in sucker incidence among plants produced in differing cell sizes suggests that differences in root to shoot ratios are not the driving force in ground sucker formation. An alternative hypothesis is the possibility that ground sucker formation is essentially a response to excessive water in the hydroponic soil mix, and any changes in hormone levels are actually a secondary response to near drowning. Over the last 35 years of conducting numerous variety trials, differential responses among tobacco varieties to excessive soil moisture have been observed in flooded fields on several occasions. Just as some varieties tend to stand drought conditions relatively better than others, there is also a striking difference among varieties as to how much water they can tolerate before "flopping". It is well known that plant stress from any source will lead to increased sucker formation. It is possible that the differential tendency to form ground suckers observed among different tobacco varieties is associated with their ability to withstand excessive soil moisture conditions, rather than their inherent ratios of auxins to cytokinins.

Plans for Future Work:

Appreciation is expressed for funding provided for this project. No additional work is anticipated.

Table 1. Growth Hormone Effects on Axillary Shoot Formation In Tobacco

Greenhouse Study 2, Fall 2015

Treatment	Rate	Height (cm)	Suckers < 1"	Suckers 1-6"	Suckers > 6"	Total No.
Auxin Treatments						
1	NAA 5 µM 1 Time	72.0	1.1	0.4	0	1.5
2	NAA 1 µM 1 Time	51.6	0.7	0.1	0	0.8
3	NAA 200 nM 1 Time	48.4	2.3	0.6	0	2.9
7	NAA 5 µM each watering	76.7	2.8	0.1	0	2.9
8	NAA 1 µM each watering	42.0	2.3	0.6	0	2.9
9	NAA 200 nM each watering	48.4	2.3	0.5	0.1	2.9
13	Control None	46.5	0 3.2	1.9	0	5.1
Cytokinin Treatments						
4	BA 1250 nM 1 Time	53.2	2.8	1.4	0.2	4.4
5	BA 250 nM 1 Time	46.5	3.7	0.6	0	4.3
6	BA 50 nM 1 Time	53.9	3.1	1.8	0	4.9
10	BA 1250 nM each watering	49.3	3.8	1	0	4.8
11	BA 250 nM each watering	48.6	3.9	1.3	0	5.2
12	BA 50 nM each watering	46.5	4	0.6	0	4.6
13	Control None	46.5	0 3.2	1.9	0	5.1

Table 2. 2016 Growth Regulator Field Study
Treatment Codes

Variety TN 86 (H 403 Check)
Whole plots: Hormone Concentration
Sub-plot : Tray Cell Size
*** Treatment with NAA 21 days after seeding**

Treatment No.	Variety	NAA Conc.	Tray Size	Cell Size
1	H 403LC	H 403 Check	128	2.6
2	H 403LC	H 403 Check	200	1.9
3	H 403LC	H 403 Check	242	1.6
4	H 403LC	H 403 Check	288	1.2
5	H 403LC	H 403 Check	338	0.7
6	TN 86LC	TN 86 Check	128	2.6
7	TN 86LC	TN 86 Check	200	1.9
8	TN 86LC	TN 86 Check	242	1.6
9	TN 86LC	TN 86 Check	288	1.2
10	TN 86LC	TN 86 Check	338	0.7
11	TN 86LC	500nM	128	2.6
12	TN 86LC	500nM	200	1.9
13	TN 86LC	500nM	242	1.6
14	TN 86LC	500nM	288	1.2
15	TN 86LC	500nM	338	0.7
16	TN 86LC	1uM	128	2.6
17	TN 86LC	1uM	200	1.9
18	TN 86LC	1uM	242	1.6
19	TN 86LC	1uM	288	1.2
20	TN 86LC	1uM	338	0.7
21	TN 86LC	3uM	128	2.6
22	TN 86LC	3uM	200	1.9
23	TN 86LC	3uM	242	1.6
24	TN 86LC	3uM	288	1.2
25	TN 86LC	3uM	338	0.7
26	TN 86LC	5uM	128	2.6
27	TN 86LC	5uM	200	1.9
28	TN 86LC	5uM	242	1.6
29	TN 86LC	5uM	288	1.2
30	TN 86LC	5uM	338	0.7

Table 3. NAA Concentration Means Across Tray Sizes - 2016 Field Study

Greeneville

Variety	NAA Concentration	Ground Suckers (Avg No. / Plant)			
		< 6"	6-12"	> 12"	Total
H403	0	0.51	0.14	0.14	0.79
TN86	0	1.24	0.62	0.59	2.45
TN86	500 nM	0.93	0.57	0.41	1.91
TN86	1uM	1.33	0.61	0.51	2.45
TN86	3uM	0.77	0.39	0.27	1.43
TN86	5uM	0.73	0.29	0.12	1.13

Lexington

Variety	NAA Concentration	Ground Suckers (Avg No. / Plant)			
		< 6"	6-12"	> 12"	Total
H403	0	0.02	0.01	0.02	0.05
TN86	0	0.59	0.13	0.03	0.74
TN86	500 nM	0.80	0.23	0.06	1.09
TN86	1uM	0.89	0.37	0.07	1.33
TN86	3uM	0.97	0.38	0.06	1.42
TN86	5uM	1.02	0.33	0.06	1.41

Versailles

Variety	NAA Concentration	Ground Suckers (Avg No. / Plant)			
		< 6"	6-12"	> 12"	Total
H403	0	0.21	0.30	0.10	0.62
TN86	0	0.87	1.09	0.36	2.32
TN86	500 nM	1.00	1.12	0.49	2.62
TN86	1uM	0.77	0.79	0.33	1.89
TN86	3uM	0.82	0.97	0.39	2.18
TN86	5uM	0.80	1.03	0.61	2.44

Mean Across Locations

Variety	NAA Concentration	Ground Suckers (Avg No. / Plant)			
		< 12"	6-12"	> 12"	Total
H403	0	0.24	0.15	0.09	0.48
TN86	0	0.90	0.61	0.33	1.84
TN86	500 nM	0.91	0.64	0.32	1.87
TN86	1uM	1.00	0.59	0.30	1.89
TN86	3uM	0.86	0.58	0.24	1.68
TN86	5uM	0.85	0.55	0.26	1.66

Table 4. Tray Size Means Across NAA Concentrations

Greeneville

Variety	Tray Size	Ground Suckers (Avg No. / Plant)			
		< 6"	6-12"	> 12"	Total
H403	128	0.38	0.19	0.07	0.64
H403	200	0.89	0.33	0.13	1.35
H403	242	0.54	0.07	0.29	0.91
H403	288	0.53	0.02	0.12	0.67
H403	338	0.19	0.10	0.08	0.37
TN86	128	0.93	0.51	0.36	1.80
TN86	200	0.78	0.48	0.30	1.56
TN86	242	1.01	0.59	0.44	2.05
TN86	288	1.07	0.50	0.44	2.00
TN86	338	1.21	0.40	0.36	1.98

Lexington

Variety	Tray Size	Ground Suckers (Avg No. / Plant)			
		< 6"	6-12"	> 12"	Total
H403	128	0.02	0.05	0.03	0.10
H403	200	0.00	0.02	0.02	0.03
H403	242	0.02	0.00	0.02	0.03
H403	288	0.02	0.00	0.02	0.03
H403	338	0.03	0.00	0.00	0.03
TN86	128	0.90	0.47	0.09	1.46
TN86	200	0.90	0.24	0.04	1.18
TN86	242	0.83	0.32	0.05	1.20
TN86	288	0.80	0.25	0.05	1.11
TN86	338	0.83	0.15	0.05	1.03

Versailles

Variety	Tray Size	Ground Suckers (Avg No. / Plant)			
		< 6"	6-12"	> 12"	Total
H403	128	0.15	0.33	0.12	0.60
H403	200	0.13	0.35	0.17	0.65
H403	242	0.33	0.28	0.13	0.75
H403	288	0.23	0.13	0.00	0.37
H403	338	0.20	0.42	0.10	0.72
TN86	128	0.86	1.19	0.64	2.70
TN86	200	0.86	1.15	0.42	2.43
TN86	242	0.99	1.03	0.52	2.54
TN86	288	0.78	0.82	0.33	1.93
TN86	338	0.76	0.81	0.27	1.84

Mean across Locations

Variety	Tray Size	Ground Suckers (Avg No. / Plant)			
		< 6"	6-12"	> 12"	Total
H403	128	0.18	0.19	0.07	0.45
H403	200	0.34	0.23	0.11	0.68
H403	242	0.30	0.12	0.15	0.56
H403	288	0.26	0.05	0.04	0.36
H403	338	0.14	0.17	0.06	0.37
TN86	128	0.90	0.72	0.36	1.98
TN86	200	0.85	0.62	0.25	1.72
TN86	242	0.94	0.65	0.34	1.93
TN86	288	0.88	0.52	0.27	1.68
TN86	338	0.93	0.45	0.23	1.62