ALKALOID CONTENT AND GERMINATION PERFORMANCE OF SEED PRODUCED BY CYTOPLASMIC MALE-STERILE TOBACCO[1]

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Seed alkaloid content, germination rate and percent germination were investigated to determine their possible contribution to seedling performance of cytoplasmic male-sterile tobacco, **Nicotiana tabacum** L. Seed produced on cytoplasmic male-sterile plants contained double the total alkaloid content of those produced on normal, male-fertile counterparts. Under laboratory conditions seed of cytoplasmic male-sterile genotypes germinated as quickly as normals, and total germination percentages, although reduced, were not significantly different from the control. We have concluded, therefore, that the reduced seedling vigor observed for some male-sterile genotypes in field-grown seed beds cannot be explained by alkaloid content or germination performance as measured under laboratory conditions.

INTRODUCTION

Cytoplasmic male sterility in tobacco, *Nicotiana tabacum* L., has been developed from a series of interspecific crosses in which the tobacco nucleus was incorporated in the cytoplasm of a number of species of the *Suaveolentes* section of the *Nicotiana* genus (3, 4, 5, 7). In addition to their effect on sex expression and flower morphology, male-sterile inducing cytoplasms have exhibited deleterious effects on other phenotypic characters in dark-fired (12), flue-cured (2, 6, 14), and Maryland (1) tobacco. These deleterious effects were not observed for burley tobacco male-sterile lines and hybrids with *N. megalosiphon* cytoplasm (13). Male-sterile flue-cured tobacco with cytoplasms from *N. suaveolens* Lehm., *N. megalosiphon* Heut. and Muell. and *N. plumbaginifolia* Viv. exhibited a retardation on growth in plant beds (10) suggesting that the cytoplasms involved may produce their initial effect on seed germination and seedling vigor.

Tobacco is highly self-pollinated and the only seed which develops on a male-sterile plant is that which results from hand cross-pollination. Normal male-fertile inflorescences are capable of developing hundreds of capsules with numerous seeds per capsule. Continuous hand cross-pollination of male-sterile plants would probably provide as many seeds on male steriles as on normals; normally only a few flowers are pollinated to provide sufficient seed for experimental work. In the present study we have investigated germination performance and seed alkaloid content as possible factors contributing to the reduced vigor of cytoplasmic male-sterile types.

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MATERIALS AND METHODS

The cytoplasmic male-sterile material used in this investigation consisted of five sources produced by transferring *N. tabacum* chromosomes of 'Hicks Broadleaf' (Hicks) into the cytoplasm of the following *Nicotiana* species: *N. bigelovii* (Torr.) Wats., *N. undulata* R. & P., *N. megalosiphon, N. suaveolens* and *N. plumbaginifolia*. All male-sterile entries were in the BC_{11} generation. Normal male-fertile Hicks, *N. tabacum* nucleus in its own cytoplasm, served as the control.

Seeds of all entries were assayed for their alkaloids by gas-liquid chromatography (GLC). Five hundred mg of seeds were ground to a gummy black paste with pestle and mortar and extracted with 25 ml of 2.5% (v/v) HCl in methanol. The mixture was boiled for one minute, cooled, and filtered. Methanol was evaporated from the filtrate and the acidic aqueous phase made basic with 10 ml of 2N NaOH. This mixture was shaken and then extracted with 5 ml of diethyl ether. The organic phase was removed, the aqueous phase extracted three additional times with diethyl ether, and each subsequent organic phase removed and combined with the original organic partition. The combined organic phase was evaporated in air, and the remaining residue dissolved in 1 ml of diethyl ether and subjected to GLC analysis following the procedure of Weeks, Davis, and Bush (17). Two injections were made for each cytoplasmic source, and the experiment was replicated four times.

Seeds of each of the six cytoplasmic entries were examined for germination percentage and mean days-to-germination. Two germination media, distilled water and 200 ppm gibberellic acid (GA_3), were imposed over the six cytoplasmic entries and are referred to as a set. Two hundred seeds of each entry were washed in 1% sodium hypochlorite solution, rinsed, dried and placed on two thicknesses of filter paper in petri dishes containing 5 ml of germination media. Each entry within a given germination medium and each medium within a set was randomized and replicated four times. Seeds were germinated under 107.6 hlx (1000 foot-candles) continuous light or in the dark at 26C. Each day seed that germinated were counted and removed to alleviate competition among remaining seed. Germination was considered to have occurred when both cotyledons had protruded from the testa and mean days-to-germination were calculated by the procedure described by Haroon, Long, and Weybrew (9).

RESULTS AND DISCUSSION

Means for total alkaloids, nicotine, and nornicotine

Table 1. Mean values for alkaloid content in mature air-dried tobacco seed for male-fertile Hicks and five cytoplasmic male-sterile entries.

Cytoplasm	Total Alkaloids	Nicotine	Nornicotine
		µg/g	
Hicks (N. tabacum) Control	208.1	124.2	57.2
CMS Hicks (N. bigelovii)	461.5*	210.7	207.6*
CMS Hicks (N. undulata)	425.0	201.4	164.4
CMS Hicks (N. megalosiphon)	457.8*	254.8**	178.4
CMS Hicks (N. suaveolens)	430.4	219.5*	176.3
CMS Hicks (N. plumbaginifolia)	493.9*	254.4**	143.3
LSD .05	248.3	88.1	125.0
LSD .01	333.2	118.3	167.7

*, ** Significantly different from the control at the 0.05 and 0.01 probability levels, respectively.

concentrations of mature, air-dried seed are given in **Table 1**. Although not always statistically significant, seed of male-sterile entries showed higher levels of total alkaloids, nicotine, and nornicotine than the control. This might be expected, since seeds serve as sinks for metabolites. The only capsules that develop on male-sterile plants are those that result from hand-pollinations, while many more capsules form on normal, male-fertile types. Therefore, a given amount of alkaloids being translocated from the roots to the flowers of male-sterile plants would have fewer reservoirs to fill and thus the seeds could possibly contain more alkaloid than seed of the control. The higher alkaloid content of the seed is probably not a cytoplasmic effect *per se*, but a maternal effect induced by the reduction of seed capsules formed on male-sterile plants. Although the physiological role of alkaloids in tobacco seed is unknown, high levels of alkaloids, especially nicotine, could have a detrimental effect on germination. Nicotine contains a nitrogen with a slightly positive charge in the pyridine moiety of the molecule and may form bonds with negative charges on protein and nucleic acids. Indeed, if tobacco seed nicotine might complex and inactivate protein, then nicotine content of seed could have an influence on germination and subsequent seedling vigor. In view of the extraction procedure used and the fact that heat and HCl were required to release alkaloids, it appears that the alkaloids are bound in some complex involving protein and are liberated upon germination, a conclusion advanced by Il'in and Lovkova (11).

Experimental means for average days-to-germination and percentage germination for each germination medium under both light and dark regimes are presented in **Table 2**. Seed of male-sterile Hicks (*N. plumbaginifolia*) treated with light and placed in water showed a highly significant reduction in germination percentages when compared to the control. Small but non-significant differences in germination performance were associated with seed of male-sterile entries with cytoplasm of *N. undulata* and *N. megalosiphon* also. Except for the entry containing the *N. suaveolens* cytoplasm, male-sterile entries treated with GA_3 varied little in germination percentage from the control. Consequently, the application of GA_3 can overcome the suppressed germination observed under normal conditions, although days-to-germination may be increased.

Entries subjected to darkness germinated only under the GA_3 treatment. This supports the conclusion of Spaulding and Steffens (15), that some tobacco cultivars have a light requirement for germination and that GA_3 eliminated the requirement. These results also show that

Figure 1. Plant beds of normal Hicks, *N. tabacum* cytoplasm (A and D), adjacent to male-sterile Hicks, *N. megalosiphon* cytoplasm (B), and male-sterile Hicks, *N. plumbaginifolia* cytoplasm (C).

Table 2. Mean values for days-to-germination and percentage germination of mature air-dried tobacco seeds germinated under continuous light and dark regimes in water and gibberellic acid (A_3).

Cytoplasm	In Light				In Dark			
	Water (100.0%)		Gibberellic Acid (A_3)-200 ppm		Water (100.0%)		Gibberellic Acid (A_3)-200 ppm	
	Days-to-germination	% germination	Days-to-germination	% germination	Days-to-germination	% germination	Days-to-germination	% germination
Hicks (N. tabacum) Control	6.1	96.3	9.0	95.4	—	—	14.2	98.3
CMS Hicks (N. bigelovii)	6.1	96.0	9.3	92.9	—	—	13.9	98.3
CMS Hicks (N. undulata)	6.6	80.9	8.3	94.4	—	—	14.4	97.5
CMS Hicks (N. megalosiphon)	6.2	78.6	8.5	97.7	—	—	13.0	98.5
CMS Hicks (N. suaveolens)	6.0	96.1	9.5	84.1	—	—	14.3	99.3
CMS Hicks (N. plumbaginifolia)	8.5**	32.4**	9.1	95.3	—	—	13.9	97.3
LSD .05	1.1	27.1	1.8	11.5			1.6	2.2
LSD .01	1.5	37.6	2.4	15.9			2.2	3.0

** Significant at 0.01 level of probability.

the light requirement is probably under nuclear control rather than cytoplasmic.

In field-grown plant beds, male-sterile Hicks with cytoplasm from *N. megalosiphon*, *N. plumbaginifolia*, or *N. suaveolens* frequently exhibits seedling performance characteristic of that shown in **Figure 1**. In the present laboratory study only the entry with *N. plumbaginifolia* cytoplasm showed a statistically significant reduction in days-to-germination and percent germination, although all entries with alien cytoplasm possessed higher seed alkaloid levels than the controls. Consequently, definitive evidence to support the hypothesis that nicotine content of seed adversely affects germination performance was not obtained. Nevertheless, the laboratory germination experiments were conducted under optimum temperature conditions. Under field conditions seeds are frequently subjected to adverse temperature and moisture regimes and the small differences in germination performance observed in this experiment could have a large direct bearing on seed bed performance of field planted male steriles.

Gibberellic acid enhanced percent germination of seeds of most cytoplasmic entries in light, substantiating other reports (8, 16) on the effect of exogenous gibberellins on germination.

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