Response of Tobacco Root-Tip Cells to Various Sucker Control Chemicals*

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Decapitation (topping) at onset of flowering and subsequent debranching (suckering) are common practices in production of tobacco. The effects include improved quality and increased size and weight of leaf having a higher alkaloid content. Attempts to bypass manual suckering began with the use of synthetic compounds (1) to inhibit cell division or to balance the loss of apical dominance in the plant after topping. Maleic hydrazide (diethanolamine salt of 6-hydroxy-3-[2H]-pyridazinone) is a synthetic compound which prevents the development of axillary buds through systemic effects (2). Recently, several other compounds, primarily fatty acid derivatives, were found to inhibit sucker growth by contacting and damaging the axillary bud (3, 4, 5). Surfactants are admixed in various amounts in these treatments. It is of academic interest and practical importance to examine the effects of these compounds on plant cells, especially meristematic cells. A study was made of cells within root-tip meristems which had been treated with various concentrations of systemic and contact sucker control chemicals. This paper reports the response of dividing and differentiating tobacco root-tip cells upon treatment with these chemicals.

MATERIALS AND METHODS

The sucker control materials used in this study are shown in Table 1. Two different tests were employed, one for seed germination and the other for root treatment by solution culture of intact plants.

Seeds and plants from several tobacco varieties were used including *Nicotiana tabacum* L. cv. Coker 298, Maryland Catterton, and a low nicotine selection designated as "Lonibaw".

The seed germination studies were conducted under controlled environment conditions. Seeds were sown in

Table 1 Sucker control materials tested

MH – Maleic hydrazide, diethanolamine salt of 6-hydroxy-3-(2H) pyridazinone
Penar – Dimethyldodecylamine acetate
T-43 – Methyl caprate (95% C ₁₀ , 3% C ₈ and 2% C ₁₂). Applied with 3 parts of ester and 1 part of Tween-20.
T-148 – Mixture of 1-octanol and 1-decanol (approx. 40–50% C ₈ and 50–60% C ₁₀). Applied with 5 parts of alcohol to 3 parts of Tween-80.
Tween-80 – Polyoxyethylene (20) sorbitan monooleate
Tween-20 – Polyoxyethylene (20) sorbitan monolaurate

petri dishes on a double layer of #2 Whatman** filter paper soaked in solutions of chemicals at concentrations from $5 \times 10^{-2} \text{M}$ to $1.25 \times 10^{-8} \text{M}$ as described in Table 2. Frequent observations were made during the germination and emergence period. The root-tip study, based on intact plants, was conducted in the greenhouse; chemicals were added to the nutrient solution culture, in which a maturing plant was growing, to make a final concentration of $5 \times 10^{-2} \text{M}$ or $5 \times 10^{-8} \text{M}$. In one test, root tips were harvested from the solution culture at approximately 2-hour intervals between 8 A. M. and 5 P. M., for 2 days. In another test they were harvested at zero, 1, 2, 3, 7, 8, 9, 10, and 13 days post treatment. Root tips were killed and fixed in a 1:3 mixture of propionic acid and 95% ethyl alcohol for 24 hours and then were stored in 70% ethyl alcohol. Root tips were hydrolyzed in 1N HCL at 60° C for 5 minutes, macerated and stained in propionic-carmin. Slides were permanized by a process involving freezing, treating with 95% ethanol, and coating with an alcohol miscible resin (Diaphane).

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RESULTS AND DISCUSSION

Observations were made on visible appearance of root tips and on microscopic studies of cells. Table 2 shows the visible effects of treatments with sucker control agents. At concentrations below $5 \times 10^{-3}M$ level, all test chemicals failed to produce any noticeable effects either on seed germination or on root-tip development. Even at a higher concentration, $5 \times 10^{-2}M$, maleic hydrazide and two surfactants, Tween-20* and Tween-80* did not cause any visible damage, while three other compounds including Penar*, T-43 (methyl caprate), and T-148 (mixture of 1-octanol and 1-decanol) induced discoloration of root tips on germinating seeds, and resulted in complete collapse after prolonged treatment. Root tips from intact plants submerged in nutrient solution containing sucker control agents showed similar effects. At $5 \times 10^{-2}M$ concentration Penar, fatty ester T-43 and fatty alcohol T-148 treated materials were so discolored and disorganized that they were no longer suitable for cytological examination. At that high a concentration, meristematic cells had a burned appearance which reflects the efficiency of these contact chemicals in the selective destruction of meristematic tissue. At a concentration of $5 \times 10^{-3}M$, the root tip showed less discoloration and some changes in meristematic cells were observed. It was also noted that root tips which emerged after a treatment had been initiated, and which were separated from or no longer immersed in test solutions, would show little evidence of distortion, discoloration or cytological disturbances. Therefore, care was taken to base cytological observations on root tips which had been continuously submerged in the treatment solution. However, the effectiveness of the contact chemicals to cause distortion was considerably diminished after the eighth day which prob-

ably resulted from a gradual decomposition of the chemicals in the solution by microorganisms.

The cytohistophysiological effects of growth regulators on roots are widely studied. An earlier examination by *Burstrom* (6) of *n*-diamylacetic acid on wheat root growth showed that at $10^{-4}M$ concentration a delay in cell elongation and progressive decrease in the cell multiplication of the epidermis occurred. Maleic hydrazide treated root tips of *Lens culinaris* showed a decrease in mitotic activity as well as an inhibition in growth (7). A more detailed observation showed that tobacco root growth ceased four days after treatment with $10^{-3}M$ maleic hydrazide, and that the root hair zone was found to advance to the region of the meristem (8).

Typical examples of effects by sucker control chemicals are shown in Figure 1 from A through I, as described. Figures 1-A and 1-B show normal cells and cells not affected by the treatment, respectively. Figures 1-E, F, G, and I show distortion and much enlarged polynucleate cells. The probable onset of the binucleate condition is seen in Figures D and H, in which nuclear division is not accompanied by cytokinesis.

Effects on cell division and differentiation by systemic and contact sucker control chemicals reported here verified the efficiency of these compounds in inhibiting axillary bud (sucker) development. The primary mode of action of the systemic, MH-30, is through interference with the mechanism of cell division. Except for the absence of cell divisions, meristematic cells treated with MH-30 have a near normal appearance (Figure 1-C). However, MH-30 produces secondary effects on differentiating cells by causing fragmentation or duplication of nuclei (Figure 1-F). The cells which we have examined tend to support the idea that the nucleus is often fragmented rather than duplicated.

The contact compounds, in contrast to systemic compound MH-30, selectively destroy meristematic cells at the point of application (5). Concentrations at $5 \times 10^{-3}M$ of the contact compounds tend to be chronic

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Table 2 Visible effects of treatment with sucker control agents on seed germination and root-tip development

Tests (varieties used)	Concentrations of chemicals	Visible appearance
Seed germination (Coker 298, Lonibaw, and Catterton)	Control	Normal appearance
	$1.25 \times 10^{-3}M$	Normal appearance
	$2.5 \times 10^{-3}M$	Normal appearance
	$5 \times 10^{-3}M$	Normal appearance
	$5 \times 10^{-2}M$	MH, Tween-80, Tween-20 appeared to be normal. All others inhibited some germination and emergence. Other effects were that Penar caused browning of root tips, T-43 caused blackening of root tips, and T-148 produced discoloration in the zone of division. All resulted in complete collapse after prolonged treatment.
Root treatment of intact plant (Catterton and Lonibaw)	Control	Normal appearance in root tip
	$5 \times 10^{-3}M$	Generally normal. Only slight suggestion of root tip discoloration.
	$5 \times 10^{-2}M$	Entire root system collapsed in less than two days of all treatments except MH, Tween-20 and Tween-80.

Figure 1-A thru I

A, control; B, Tween-80 after 26 hrs; C, MH-30 after 4 days; D, T-148 after 6 days; E, T-148 after 8 days; F, MH-30 after 8 days; G, Penar after 4 days; H, Penar after 8 days; and I, Penar after 9 days. Initial concentration of chemicals was $5 \times 10^{-3}M$.

rather than critical and thus induced secondary effects on living cells (Figures 1-D, E, F, H, I). The immediate effects of fatty materials (T-43 and T-148) include swelling of the nucleus after approximately two days of exposure and a general cessation of cell divisions after 5 days of exposure. Penar, like MH-30, causes enlargement of differentiating cells and nuclear fragmentation or duplication (Figure 1-G and I). Penar was also found to induce a higher incidence of binucleate cells than T-148 or even MH-30. Figures 1-E and 1-F respectively show binucleate cells induced by T-148 and MH-30. Certain chemicals, such as the herbicide "nitralin" are known to induce a high frequency of binucleate cells (9). Apparently, only certain kinds of differentiating cells were sensitive to the cell enlarging and endomitotic action of these chemicals.

The concentrations of chemicals used to observe changes reported here were comparatively lower than those used in the field for effective sucker control. For field application, the concentrations were 2.2×10^{-2} M Penar, 7.6×10^{-2} M for maleic hydrazide, 2.2×10^{-1} M for fatty ester T-43, and 1.4×10^{-1} M for fatty alcohol T-148. Under field conditions, the concentration of the chemicals has to be high enough for immediate action whether by contact or by systemic action.

The compounds tested here, including MH, Penar and T-148, induced a rather low incidence of polynucleate cells or cells with fragmented nuclei, and such an endomitotic action apparently was also limited to certain kinds of differentiating cells. Such information is of academic interest, and may not attribute any significance to other biological systems under different circumstances.

SUMMARY

Effects of sucker control chemicals on cell division and differentiation were studied by observing changes in root-tip cells continuously submerged in 5×10^{-3} M solutions of MH-30 (maleic hydrazide), fatty ester T-43 (methyl caprate), fatty alcohol T-148 (mixture of 1-octanol and 1-decanol), Penar (dimethyldodecylamine acetate), and surfactants Tween-20 (polyoxyethylene sorbitan monolaurate) and Tween-80 (polyoxyethylene sorbitan monooleate). No adverse cytological effects were induced by surfactants. Meristematic cells treated with MH-30 appeared to be normal but there was no cell division. MH-30 also caused fragmentation or duplication of nuclei on differentiating cells. The effects of fatty materials (T-43 and T-148) include immediate swelling of the nucleus and then a general cessation of cell division. Penar caused enlargement of differentiating cells and nuclear fragmentation or duplication, a higher frequency of binucleate cells was found to be induced by Penar than T-148 or even MH-30. In general, sucker control compounds tested in this study induced a rather low incidence of endomitotic action which was limited to certain differentiating cells.

ZUSAMMENFASSUNG

Der Einfluß von Chemikalien, die das Geizenwachstum kontrollieren, auf Zellteilung und Zelldifferenzierung wurde untersucht. Dazu wurden Wurzelspitzen in 5×10^{-3} molare Lösungen von MH-30 (Maleinsäurehydrazid), des Fettsäureesters T-43 (Caprinsäuremethylester), des Fettalkohols T-148 (Mischung aus Octanol-1 und Decanol-1), von Penar (Dimethyldodecylaminacetat) sowie der oberflächenwirksamen Substanzen Tween-20 (Polyoxyäthylensorbitanmonolaurat) und Tween-80 (Polyoxyäthylensorbitanmonooleat) eingebracht, und es wurde geprüft, ob dadurch Zellveränderungen eintreten. Die oberflächenaktiven Stoffe bewirkten keine ungünstigen Zellveränderungen. Nach Behandlung mit MH-30 erschienen die Meristemzellen normal, es wurde aber keine Zellteilung beobachtet. Im Verlaufe der Zelldifferenzierung rief MH-30 außerdem Brüche und Verdoppelungen der Zellkerne hervor. Die Fetten nahestehenden Substanzen (T-43 und T-148) bewirkten eine sofortige Schwellung des Zellkernes und das Aufhören der Zellteilung. Durch Penar kam es zu Zellvergrößerungen während der Differenzierung sowie zur Fragmentbildung und Verdoppelung der Zellkerne. Der Anteil zweikerniger Zellen war nach Behandlung mit Penar höher als unter dem Einfluß von T-148 oder selbst von MH-30. Allgemein wurde eine endomitotische Wirkung bei den hier untersuchten Mitteln nicht sehr häufig beobachtet, und sie blieb auf die Differenzierungsphase bestimmter Zellen beschränkt.

RÉSUMÉ

L'effet produit sur la division et la différenciation de cellules par des composés chimiques de lutte contre la croissance des bourgeons du tabac a été étudié par observation des cellules des bouts de racine du tabac submergées de façon continue dans des solutions 5×10^{-3} M de MH-30 (1,2-dihydro-3,6-pyridazindione), d'ester gras T-43 (méthylcaprate), d'alcool gras T-148 (mélange d'octanol-1 et de décanol-1), de Pénar (diméthylodécylamine-acétate) et de mouillants Tween-20 (polyoxyéthylènesorbitanmonolaurate) et Tween-80 (polyoxyéthylènesorbitanmonooleate). Aucune influence adverse n'a été exercée sur les cellules par des mouillants. Les cellules méristématiques traitées au MH-30 paraissaient normales, mais il n'y avait pas de division. Le MH-30 causait aussi le fractionnement ou la dichotomie des noyaux de cellules différenciantes. Les matières grasses (T-43 et T-148) causaient le gonflement immédiat du noyau et ensuite une cessation générale de la division des cellules. Le Pénar provoquait la croissance de cellules différenciantes et le fractionnement ou la dichotomie des noyaux. On a trouvé qu'une fréquence plus haute de cellules bi-nucléaires avait été causée plutôt par le Pénar que par le T-148 ou même le MH-30. En général les composés chimiques appliqués pour le contrôle des bourgeons du tabac étudiés ici, provoquaient une assez faible action endomitotique qui était limitée à certaines cellules différenciantes.

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