Fatty Alcohol Inhibition of Tobacco Axillary and Terminal Bud Growth

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Fatty alcohols with chain lengths of C_9, C_{10}, and C_{11} were highly active in selectively killing or inhibiting axillary and terminal bud growth of tobacco. Fatty alcohols with chain lengths shorter than C_9, or longer than C_{11}, were less effective. On an equal molar basis, the most effective fatty alcohols are more active than the corresponding fatty acid methyl ester. Without the proper type and amount of surfactant, the C_9, C_{10}, and C_{11} alcohols and esters exhibit nonselective tissue kill. With the aid of surfactants, the emulsions become phytotoxic only to young meristematic tissue but cause little or no visible injury to more mature tissue.

The effectiveness of fatty acid methyl esters for the inhibition of tobacco axillary bud (sucker) growth has previously been reported (Tso, 1964; Tso *et al.*, 1965). The alkyl esters of the C_8 to C_{12} fatty acids effectively and selectively killed the axillary meristems without damaging mature leaf tissue. Among them, the C_{10} and C_{11} methyl esters are most effective; however, the C_{11} compound showed considerable phytotoxicity. Growth inhibition or tissue death occurs only when the sprayed emulsion comes in direct contact with the meristematic tissue (Tso *et al.*, 1966). When an emulsified fatty acid ester is applied to plants, the ester, per se, is not translocated, but is restricted to the general area of application. Therefore, growth of meristematic tissues appears to be inhibited because of the selective penetration of the active agents into the areas of rapidly dividing cells. The selective inhibition of meristematic growth on a wide range of plants has important implications which are brought out by Cathey *et al.* (1966).

This paper reports on another group of compounds, C_4 to C_{18} fatty alcohols, several of which also effectively and selectively inhibit axillary and terminal bud growth of tobacco.

MATERIALS AND METHODS

Test Plants. The fatty alcohol sprays were applied to *Nicotiana tabacum* cv. Xanthi-nc, Connecticut Broadleaf, Hicks flue-cured, and Burley-21 plants. The Xanthi and Connecticut Broadleaf plants were grown in the greenhouse, and the Hicks and Burley plants were field-grown at Beltsville, Md. Xanthi plants were grown essentially as described by Marth and Mitchell (1964). Connecticut Broadleaf seedlings were produced as the Xanthi but they were transplanted to a soil mixture in 7-inch clay pots. Hicks flue-cured and Burley seedlings were produced in seed beds and then transplanted to the field during the 1966 growing season. Each type was fertilized and produced according to normal field practices.

All greenhouse experiments were conducted in duplicate and, in each case, there were four plants per treatment. For the field experiments, each treatment consisted of 10 plants for both the Hicks and Burley types. The results reported are averages of the two types.

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Spray Solutions. The fatty alcohols used in these experiments are insoluble in water. Therefore, emulsions were prepared with the aid of a surfactant. The effectiveness of the fatty acid derivatives in selectively killing meristematic tissue depends on the use of suitable amounts and types of surfactants; polyoxyethylene-20-sorbitan monooleate (Tween-80) is a suitable surfactant for obtaining a relatively stable emulsion with the fatty alcohols.

The following 10 fatty alcohols were sprayed onto plants as emulsions: 1-butanol (C_4); 1-hexanol (C_6); 1-octanol (C_8); 1-nonanol (C_9); 1-decanol (C_{10}); 1-undecanol (C_{11}); 1-dodecanol (C_{12}); 1-tetradecanol (C_{14}); 1-hexadecanol (C_{16}); and 1-octadecanol (C_{18}). For tests with Xanthi plants, the concentration of alcohols was 0.16*M* with 1.5% Tween-80; for Connecticut Broadleaf plants, 0.19*M* with 1.5% Tween-80; and for field-grown Hicks and Burley plants, the alcohols were 0.32*M* with 3% Tween-80. Emulsions were prepared by weighing the required amount of alcohol and surfactant into a container, warming the mixture on a steam bath, and thoroughly mixing. Warm water was then added until the mixture thickened or formed a gel. Warm water was slowly added while stirring to make the emulsion up to the required volume. The emulsions thus formed were relatively stable, but all solutions were remixed before application.

Test Methods. Tests of fatty alcohols on Xanthi plants were conducted in two ways. In one case, the terminal buds were not removed before treatment to determine the effectiveness of the emulsions for inhibiting terminal bud growth. In the other case, Xanthi plants were treated after removal of the terminal bud to determine the effectiveness of the emulsion in inhibiting the growth of axillary buds. The emulsions were applied to both topped and untopped Xanthi with an air pressure sprayer regulated to deliver a relatively fine spray at a low pressure (7 to 10 p.s.i.). The leaves were wetted by the spray, and a portion was allowed to cover the terminal bud (when present) and to run down the stalk so that it came in contact with the axillary buds. Approximately 5 ml. of spray solution were applied to each plant.

The emulsions were applied to Connecticut Broadleaf, Hicks, and Burley plants which had been topped. A coarse spray at approximately 25 p.s.i. was directed downward over the stalk of the plant with the spray covering an area of 8 to 12 inches in diameter. The emulsion

covered portions of the upper leaves and drained down along the stem of the plant and thus also came in contact with the small axillary buds. Approximately 20 ml. of spray were applied to each plant.

The results are expressed as per cent reduction in weight of suckers from topped plants and per cent reduction in top growth of untopped plants. This was done by comparing the weight of suckers or top growth of treated plants with suckers or tops from untreated controls. Preliminary tests showed that sprays containing only Tween-80 at the rates employed in this study caused little or no growth reduction and no visible phytotoxicity. Tops and the upper four suckers from Xanthi and all suckers from Connecticut Broadleaf plants were harvested 14 days after treatment. All suckers from Burley plants were removed and weighed 15 days after treatment and from Hicks plants 27 days after treatment.

RESULTS AND DISCUSSION

The C_9, C_{10}, C_{11}, C_{12}, and C_{14} alcohols were highly active on both topped and untopped Xanthi plants (Figure 1*A*). The C_4, C_6, C_8, C_{16}, and C_{18} alcohols are relatively inactive at 0.16*M* concentrations. The extent of leaf injury to Xanthi plants was slight with the alcohols having carbon chain lengths less than 11. The C_{11} alcohol caused a large number of small necrotic areas, and some larger necrotic areas were observed from treatment with the C_{12} alcohol. Several days after treatment with the C_{14} alcohol, leaves on Xanthi plants became very rough and puckered, and their margins turned upward and inward. The C_{16} and C_{18} alcohols left white residues on the leaf surfaces but caused little actual leaf injury.

The activity curve obtained with the fatty alcohols applied to Connecticut Broadleaf plants (Figure 1*B*) closely resembles the curve obtained when these materials were applied to Xanthi. The C_4 and C_6 alcohols showed no activity, and the C_8 alcohol reduced axillary bud growth by about 50%. The C_9, C_{10}, and C_{11} compounds gave 100% inhibition of sucker growth but the C_{12} and C_{14} alcohols were less effective. The C_{16} and C_{18} alcohols gave poor growth control. The alcohols with carbon chain lengths less than 10 caused no marked leaf injury, the C_{10} and C_{11} compounds caused some small necrotic spots, and the C_{12} and C_{14} alcohols caused leaf and stalk necrosis. White residues remained on the leaf surfaces but no injury was evident from the C_{16} and C_{18} emulsion sprays.

The activity of the C_8, C_{12}, and C_{14} fatty alcohols equaled that of the C_9, C_{10}, and C_{11} alcohols when applied to field-grown plants at a concentration of 0.32*M* (Figure 1*C*). The C_4, C_6, C_{16}, and C_{18} alcohols again resulted in poor inhibition of sucker growth. Under field conditions, the C_4 and C_6 alcohols caused no injury, C_8, C_9, and C_{10} alcohols caused very slight injury, and the C_{11}, C_{12}, and C_{14} treatments caused moderate to severe leaf necrosis. A white residue remained on the leaf surfaces after treatment with the C_{16} and C_{18} emulsions. The C_{14}, C_{16}, and C_{18} emulsions were sprayed onto the plants while still warm to avoid formation of gels. The warm C_{14} alcohol emulsion caused severe leaf necrosis and chlorosis, but no injury was observed after treatment with the C_{16} and C_{18} emulsions.

The greenhouse results on terminal bud growth inhibition of Xanthi plants (Figure 2) led to field tests with the fatty alcohols and esters for chemically topping commercial tobacco types. In tobacco production, decapitation or topping is necessary to obtain leaves of high commercial value. Preliminary field tests show that these materials destroy the terminal bud if applied at an early stage in their development. In addition, the axillary buds are killed if the applied spray is allowed to drain down along the entire length of the stalk. The fatty alcohols were more effective than the methyl esters, and 1-decanol was the most effective alcohol tested (Figure 3). To obtain complete inhibition of terminal growth, it is very important that the buds be treated before the flowers open.

As with the esters, the most active fatty alcohols for the inhibition of tobacco meristematic tissue were those with

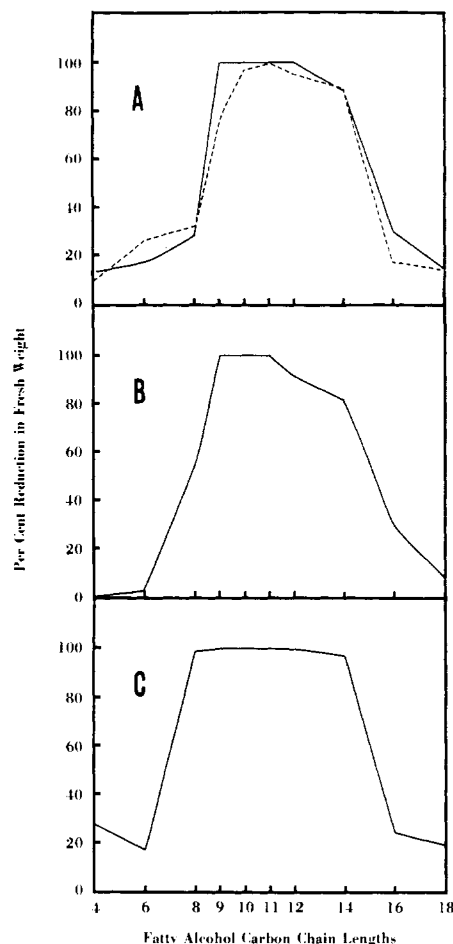

Figure 1. Effect of fatty alcohols on the inhibition of meristematic growth of *Nicotiana tabacum* L.

A. Axillary bud growth inhibition (topped plants——) and terminal bud growth inhibition (untopped plants - - - -) of cv. Xanthi-nc 14 days after treatment with 0.16*M* alcohol emulsions containing 1.5% Tween-80
B. Axillary bud growth inhibition (topped plants) of cv. Connecticut Broadleaf 14 days after treatment with 0.19*M* alcohol emulsions containing 1.5% Tween-80
C. Axillary bud growth inhibition (topped plants) of cv. Hicks and Burley-21 field grown plants 27 and 15 days after treatment, respectively (average), with 0.32*M* alcohol emulsions containing 3% Tween-80

Figure 2. Effect of fatty alcohols on terminal bud growth

N. tabacum cv. Xanthi-nc plants 14 days after treatment with 0.16*M* alcohol emulsions containing 1.5% Tween-80

Figure 3. Effect of 1-decanol on terminal and axillary bud growth of *N. tabacum* cv. Hicks field grown plants 14 days after treatment

A. Topped check showing secondary suckers (primary suckers were removed 7 days after treatment)
B. Treated with 0.32*M* 1-decanol and 3% Tween-80 showing inhibited terminal bud and the absence of axillary buds

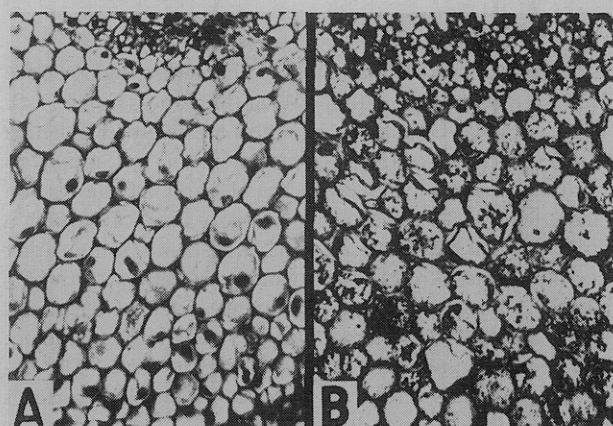

Figure 4. Cross section of axillary buds from *N. tabacum* cv. Connecticut Broadleaf plants (246×)

A. Normal cells of an untreated bud
B. Cells with disrupted nuclei from a bud treated with a 0.16*M* methyl decanoate emulsion containing 1% Tween-20 (fixed 4 hours after treatment)

carbon chain lengths of 9, 10, and 11. On an equal molar basis, the most effective fatty alcohols are more active than the corresponding fatty acid methyl ester. The fatty alcohol activity curves show that they tend to be either highly active or relatively inactive. The fatty alcohol and ester emulsions showing the most activity appear to penetrate young tissue rapidly. Once penetration has occurred, the tissue darkens and desiccation occurs, which is followed by cell death. Figure 4 shows cells of untreated and methyl decanoate-treated young axillary bud tissue. In treated tissue, the nuclei of most cells are no longer in well-defined units but nuclear particles are scattered throughout the cell. This indicates rupture of the nuclear membrane by the applied emulsion.

The apparent relationship between fatty acid and fatty acid derivative carbon chain length and inhibitory activity on different biological materials has been demonstrated by a number of investigators, although satisfactory interpretations have not yet been found. Burström (1950) found the C_{12} fatty acid slightly more active than the C_{11} acid for inhibiting meristematic growth of wheat roots. His studies show that these two acids only affect immature cells actually in the progress of elongation. The C_9 fatty acid (on a gram per liter basis) was the most active in the series from C_2 to C_{19} for inhibiting the germination of mustard seed (Le Poidevin, 1965).

Booij and Bungenberg De Jong (1949), Booij and Veldstra (1949), and Veldstra and Booij (1949) describe work they conducted in an effort to relate structure of plant growth regulators to activity. They found that certain saturated fatty acids strongly influence the turgescent (opening) activity of oleate coacervates (aggregates of colloidal droplets held together by electrostatic attractive forces). These workers used coacervates as models of protoplasmic membranes. The C_{11} fatty acid gave the greatest response in opening the oleate coacervate, but activities of the C_8 and C_{15} acids were very low. The acids below C_7 showed no opening action on the coacervates. In addition, the C_{11} acid was the most active in releasing the pigment from beet tissue after 22 hours

($4 \times 10^{-4}M$). The acids with chain lengths greater or less than 11 were less effective under these conditions. In the present study, the shape of the fatty alcohol activity curves for the selective kill of meristematic tissue on tobacco compares rather well with those obtained by these workers for the fatty acids in opening oleate coacervates and releasing the pigment from beet tissue. These studies indicate that the fatty acid esters and alcohols most effective in destroying meristematic tissue may directly affect the structure and thus the permeability of cellular membranes. If this is the case, the physicochemical properties of the compounds containing 8 to 12 carbon atoms would be of importance in explaining their activity for inhibiting growth of meristematic tissue.

In developing suitable emulsions of fatty acid derivatives for selectively inhibiting meristematic growth of tobacco tissue, it was found that the fatty alcohols and esters are much less toxic than the corresponding acids. The C_9, C_{10}, and C_{11} alcohols and esters exhibit nonselective tissue kill without the proper type and amount of surfactant. With the aid of surfactants, the phytotoxic effects of the C_9, C_{10}, and C_{11} alcohols and esters can be controlled so that the emulsions become toxic only to young meristematic tissue but cause little or no visible injury to more mature tissue.

The exact role surfactants play and why their rates need be so high in these emulsion systems is not yet known. Lower surfactant rates decrease the stability of the emulsions formed, and reduce the effectiveness of the more active fatty acid derivatives. Possibly, the surfactants are involved in the penetration of the fatty alcohols and esters through leaf surfaces as well as through cellular membranes. The hydrophilic-lipophilic balance of the surfactant is an important property which relates to the activity of the final emulsion. The hydrophilic-lipophilic balance of leaf surfaces also may be directly involved in this phenomenon.

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