Table V. Penetration of *N*-Isopropyl-α-chloroacetamide through Chloroform- or Alkali-Soaked Apricot Leaf Cuticle

Cuticle Treatment	Intact Cuticles	Penetration, %
None	10	15.5 ± 1.05
$CHCl_3$[a]	5	63.5 ± 5.24
1% NaOH[b]	5	16.6 ± 1.12

[a] Presoaked 4 days in $CHCl_3$.
[b] Presoaked 4 days in 1% NaOH.

surfactant concentrations. The dye test, however, also eliminates this variable.

Pretreatment with Surfactant. Since little change in permeability was observed in the conventional test, leaf cuticle disks were soaked for 4 days in 1% solutions (or suspensions) of the surfactants at 25° C. After removal and rinsing with water, the cuticle disks were tested in the normal manner. The low incidence of defective disks by the dye test showed that gross damage to the cuticle had not occurred. Table IV also shows that only the treatment with sodium *n*-dodecane sulfonate significantly altered the permeability of the cuticle disks to the test compound *N*-isopropyl-α-chloroacetamide, and the increase was not large.

Pretreatment with $CHCl_3$ and NaOH. To show that removal of cuticular waxes would increase permeability, cuticle disks were soaked in chloroform, a good solvent for cuticular waxes (*10*), and in 1% NaOH. Although the latter treatment was without effect, soaking in chloroform increased the penetration of *N* - isopropyl - α - chloroacetamide from 16 to 64%—almost the equilibrium distribution—in the 4-hour test period (Table V). The permeability to the dye, however, was increased only slightly.

These results show that the surfactants tested, including commercial types, do not increase the permeability of hydrated leaf cuticle, and are not good solubilizers of leaf waxes. The result with chloroform shows that inclusion of a solubilizer in pesticide formulations where penetration is desired could increase at least tenfold the penetration rate and activity of nonpolar compounds. The lack of excessive penetration of the fluorescein dye after chloroform treatment of the cuticle suggests that cutin is a major barrier to the penetration of cuticle by polar compounds. However, it is not established that the chloroform treatment will completely remove nonpolar constituents embedded or enmeshed intimately in the cutin framework.

A possible disadvantage of the cuticle disk test as used here is that the cuticle disks are maintained and tested in aqueous media and therefore represent fully hydrated cuticle. Since hydrated cuticle is considered more permeable than that of plants under water stress (*1*), surfactants could affect cuticle permeability in the field by influencing the degree of hydration of cuticular tissue. Similarly, the 40× concentration range of sodium dodecyl sulfate tested in Table II is only an approximation of the field situation where evaporation exposes the cuticle to increasing concentrations of surfactant.

Acknowledgment

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TOBACCO SUCKER CONTROL

Inhibition of Tobacco Axillary Bud Growth with Fatty Acid Methyl Esters

T. C. TSO, G. L. STEFFENS, and M. E. ENGELHAUPT

Crops Research Division, Agricultural Research Service, Beltsville, Md.

Experiments were conducted employing methyl esters of fatty acids with chain lengths from C_6 to C_{18} for the inhibition of axillary bud growth of tobacco. Results from four tobacco types showed that the methyl esters of fatty acids with eight to 14 carbon atoms gave a high degree of inhibition. Methyl pelargonate and undecenoic acid were also effective. Generally, the methyl ester of the C_{10} acid was the most effective, effectiveness gradually being reduced with either increased or decreased carbon chain lengths. The exact mode of action of these fatty acid esters is not yet known. Only the meristematic and differentiating cells of axillary buds were destroyed when they came in contact with these fatty acid esters.

In tobacco production, decapitation is a necessary process in obtaining leaves with desired physical properties and chemical composition. The consequent growth of axillary buds, or "suckers," and the problem of inhibiting such growth are of academic interest and economic importance. A number of compounds, synthetic or naturally occurring, are known to be effective inhibitors of tobacco sucker growth (*3, 8, 9*). Some compounds, however, induce further metabolic changes which are considered undesirable to leaf quality (*2*). Since many fatty acids and esters have been found in tobacco plants (*4, 6*), compounds of this group, if effective as sucker control agents, would be more desirable than other compounds which are entirely foreign to the tobacco plants. Methyl esters of fatty acids with various carbon chain lengths were used in this experiment to study their effectiveness in sucker growth inhibition.

Materials and Methods

Four major types of tobacco (*Nicotiana tabacum* L.) including Hicks, Catterton, Burley 21, and Connecticut Broadleaf were used. These plants were grown

in tobacco fields at the USDA Research Farm, Beltsville, and the University of Maryland Tobacco Experimental Farm, Upper Marlboro, Md.

Methyl esters of fatty acids, including caproate (C_6), caprylate (C_8), caprate (C_{10}), laurate (C_{12}), myristate (C_{14}), palmitate (C_{16}), stearate (C_{18}), oleate (C_{18}, —2H), and linoleate (C_{18}, —4H), were obtained from Emery Industries, Inc.

Test plants were decapitated at the medium to full flower stage. Esters were applied with a shoulder-type sprayer with a fan-type nozzle. The spray was directed downward over the stalk of the plant covering an area of about 8 inches. The solution drained down along the stem of the plant and thus came in contact with axillary buds which had the potential to develop into suckers. The spray solution was a mixture of the methyl ester of the fatty acid, a wetting agent (Tween 20), and water (4:1:55, v./v.). Each plant received approximately 30 ml. of this solution.

Effectiveness of sucker inhibition of each compound is expressed as the percentage of reduction in green sucker weight in comparison with the untreated control. Results reported here represent an average of 100 plants.

Field appearance evaluations were based on the general plant appearance, leaf development, visible damage, and/or other abnormalities. A score of 1 to 5 was given to each treatment, 5 being the best rating. Desirability index is the product of per cent sucker inhibition and field appearance.

Results

The first experiment employed methyl esters of fatty acids with an even number of carbon atoms from C_6 to C_{18}. Figure 1 shows the different treatments and the compositions of the various fatty acids methyl esters. Effectiveness of sucker inhibition is shown in Table I. Figure 2 shows the effectiveness of treatment 2.

Methyl esters of fatty acids with eight to 14 carbon atoms gave the most effective sucker control. Separate tests with the methyl esters of pelargonic acid (C_9) and undecenoic acid (C_{11}) also showed their effectiveness in sucker inhibition. In general, the methyl ester of the C_{10} fatty acid was the most effective in sucker inhibition. Such effectiveness is gradually reduced with increase or decrease of carbon chain length.

Under certain conditions, it may be of interest to prolong the growth inhibition effects of the fatty acid derivatives. The initial application of the esters inactivates the primary axillary bud, but the secondary and tertiary buds (*5*) will soon develop. To inhibit the growth of secondary axillary buds, a second application of the fatty acid esters may be made as shown in treatment 3 of Table I or the inhibiting activity of the

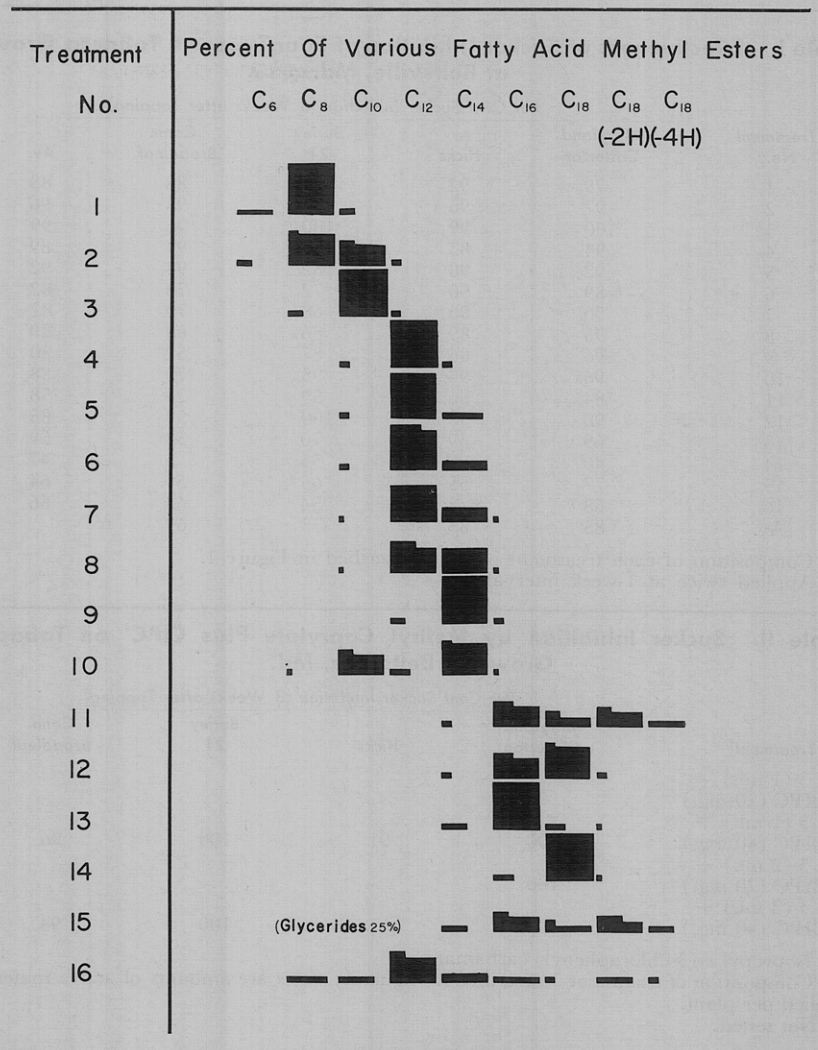

Figure 1. Composition of various fatty acid methyl esters in different treatments

Figure 2. Sucker growth of *N. tabacum* plants 4 weeks after treatment with methyl caprylate and methyl caprate

Treatment 2, Figure 1. All leaves, except those developed from suckers, removed.

1. Catterton control
2. Catterton
3. Hicks
4. Burley 21
5. Conn. Broadleaf
6. Hicks control

Table I. Effectiveness of Sucker Inhibition of Four Types of Tobacco Grown at Beltsville, Md.

Treatment No.[a]	Per Cent Sucker Inhibition (3 Weeks after Topping)				
	Maryland Catterton	Hicks	Burley 21	Conn. Broadleaf	Av.
1	79	92	73	88	83
2	97	96	92	98	96
3[b]	100	99	100	96	99
4	94	83	85	94	89
5	92	98	82	94	92
6	89	90	72	78	82
7	90	90	68	79	82
8	95	89	86	88	89
9	96	86	87	52	80
10	96	94	95	70	88
11	84	66	59	23	58
12	90	90	100	59	85
13	69	39	70	58	59
14	40	90	55	3	47
15	77	55	65	59	64
16	68	78	54	63	66
Av.	85	83	78	69	

[a] Composition of each treatment number described in Figure 1.
[b] Applied twice at 1-week interval.

Table II. Sucker Inhibition by Methyl Caprylate Plus CIPC[a] on Tobacco Grown at Beltsville, Md.

Treatment[b]	Per Cent Sucker Inhibition (3 Weeks after Topping)			
	Maryland Catterton	Hicks	Burley 21	Conn. Broadleaf
No. 3 (1 ml.) + CIPC (20 mg.)	98	...[c]	...	...
No. 3 (1 ml.) + CIPC (40 mg.)	100	91	100	96
No. 3 (2 ml.) + CIPC (20 mg.)	100	...	...	...
No. 3 (2 ml.) + CIPC (40 mg.)	100	...	100	94

[a] Isopropyl *n*-(3-chlorophenyl) carbamate.
[b] Composition of treatment 3 described in Figure 1; rates are amounts of active material applied per plant.
[c] Not tested.

Table III. Desirability Evaluation of Some Fatty Acid Treatments in Comparison with Maleic Hydrazide

Treatment[a]	% of Sucker Inhibition, A	Field Appearance Rating, B	Desirability Index,[b] A × B
Control (topped, not suckered)	0	5	0
Maleic hydrazide (170 mg. per plant)	99	5	495
No. 2	100	5	498
No. 4	99	4.9	481
No. 5	100	4.9	488
No. 6	99	4.9	480
No. 7	100	5	500
No. 8	100	5	498
No. 16	100	5	499

[a] Composition of each treatment number described in Figure 1.
[b] Average of four replications.

methyl ester may be enhanced by mixing it with another growth regulator. Table II represents results from several fatty acid methyl esters mixed with isopropyl *n*-(3-chlorophenyl) carbamate (CIPC). CIPC in high concentration inhibited tobacco axillary bud growth but induced leaf deformities. As a mixture with the methyl ester of fatty acids, the amounts of CIPC needed are only about one tenth to one fifth of that normally required; thus leaf abnormalities are eliminated and effective sucker control is retained (Figure 3).

The desirability and effectiveness for sucker control of some of these fatty acid esters were compared with maleic hydrazide, a widely used growth-regulating chemical. Results in Table III indicate that effective sucker control (17 days after treatment) was obtained under field conditions with the methyl esters of fatty acids which exhibited the most promise in the first experiment (Table I). The field appearance ratings show that applications of these materials caused little or no adverse effects. The "desirability index" values are not greatly different and all are near the maximum rating.

Discussion

In animal tissue (*1*) it was observed that fatty acids *per se* may decrease respiratory activity and may inhibit the oxidation of pyruvate. Whether such effects are upon the enzyme system or upon the cell membrane is not clear.

In the present experiment, there was

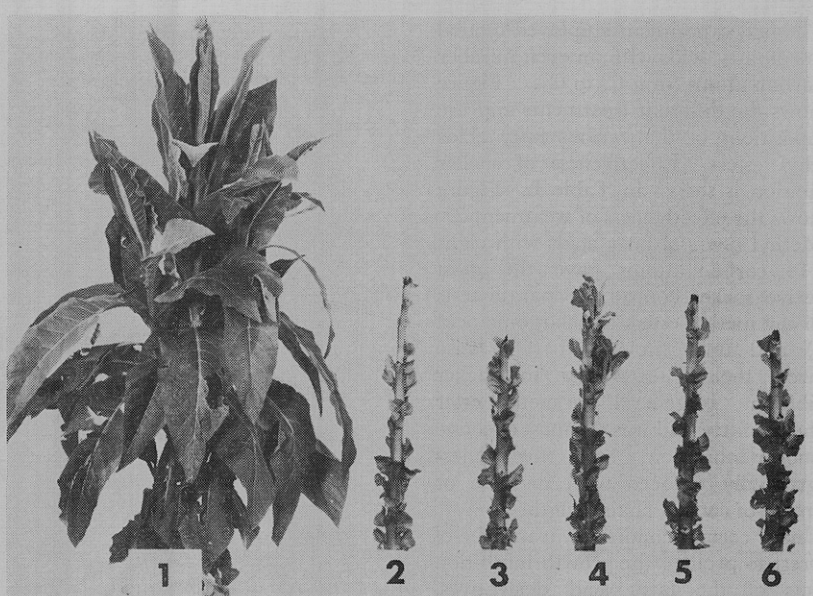

Figure 3. Sucker growth of N. tabacum cv. Catterton 5 weeks after treatment with methyl caprate (A) and CIPC (B)

Treatments described in Table II, except plant 2 which received treatment 3 of Table I. All leaves, except those developed from suckers, removed

1. Control
2. 2.2 ml. A applied twice
3. 1 ml. A + 40 mg. B
4. 1 ml. A + 20 mg. B
5. 2 ml. A + 40 mg. B
6. 2 ml. A + 20 mg. B

no indication that the methyl esters of fatty acids were translocated or metabolized. The meristematic and differentiating cells of axillary buds are destroyed when in contact with these fatty acid materials (*7*), whereas physiologically more mature cells are not damaged under most conditions.

The occurrence of a wide variety of fatty acids in the tobacco plant is well established (*6*). In addition, many fatty acids are found in tobacco seeds (*4*). The methyl esters of fatty acids, therefore, are not completely foreign compounds to tobacco plants.

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NITRATE IN FORAGE

Influence of Stage of Growth and Soil Nitrogen on Nitrate Content of Herbage of Alfalfa, Red Clover, Ladino Clover, Trefoil, and Bromegrass

DALE SMITH and J. M. SUND

Department of Agronomy, University of Wisconsin, Madison 6, Wis.

Alfalfa accumulated nitrate in high amounts at young stages of growth. Red and ladino clover contained less nitrate than alfalfa. Per cent nitrate increased in these legumes as soil nitrogen was increased. The nitrate content of birdsfoot trefoil usually was never more than a trace. Bromegrass contained large amounts of nitrate when soil nitrogen was high. Per cent nitrate decreased with advance in maturity in species where it was high in the young stages of growth.

The nitrate (NO_3) content of plants is of particular importance since consumption of forage containing excessive amounts can cause injury to livestock. Species, stage of maturity, and soil nitrogen level are important factors influencing nitrate accumulation.

Numerous investigators have studied nitrate accumulation in corn, sorghums, cereal grains, tobacco, vegetables, and weed species. Few studies have been conducted on perennial forage species, especially legumes, which have been regarded as the least likely to store nitrate. Crawford and Kennedy (*4*) listed alfalfa among the nonaccumulators. However, Olson and Whitehead (*9*) reported that alfalfa and other forage legumes contained "appreciable amounts of nitrate" but gave no supporting data.

Case (*3*) reported that alfalfa contained 0.11 to 0.66% KNO_3 during a year when corn contained 8.04% KNO_3. Tucker *et al.* (*13*) found 1.22% KNO_3 in alfalfa and 4.48% KNO_3 in pigweed growing together in the same field. Kretschmer (*6*) found much less nitrate in legumes than in oats and ryegrass. Wilson (*15*) measured the expressed sap of 56 plant species collected from a variety of locations and conditions and found that legumes sometimes contained more nitrate than grasses.

Nitrate poisoning of livestock and nitrogen dioxide silage gas formation have been observed from grass-legume silage, as well as from corn silage, in certain years in drought areas of Wisconsin. Peterson *et al.* (*10*) reported that each of two 10- × 30-foot silos filled with alfalfa and equipped with drain basins gave off visible fumes of NO_2 gas. High concentrations of gas were detected from each silo.

No data were found regarding the nitrate content of perennial forage legumes at various stages of growth or when fertilized with nitrogen. Some data are available for perennial forage grasses. Carey, Mitchell, and Anderson (*2*) found that the nitrate content of bromegrass increased markedly with increasing rates of application of NH_4NO_3 fertilizer. Ramage (*11*) reported that orchardgrass harvested in the very early spring contained 0.08, 0.22, and 2.23% KNO_3 when fertilized with 100, 200, and 400 pounds of N per acre, respectively.

This paper provides information on the nitrate content of alfalfa, medium red clover, ladino clover, birdsfoot trefoil, and smooth bromegrass as influenced by stage of growth and soil nitrogen level.

Material and Methods

Forage samples were from three separate experiments. One set of samples was obtained during 1955 and 1956, and was used also for other analyses (*1, 7, 14*). Details of the experiment and data on plant heights and hay yields have been reported (*14*). The herbage samples came from plots seeded to Vernal alfalfa (*Medicago sativa* L.), Canadian Common smooth bromegrass (*Bromus inermis* Leyss.), a mixture of these two species, Wisconsin Common medium red clover (*Trifolium pratense* L.), and Wisconsin Common ladino clover (*Trifolium repens* L.). Samples were obtained during 1955 from the first crop beginning in early spring and from the second crop following cutting on June 15, and at each of six stages of growth. This was repeated during 1956.

The second set of samples was obtained during 1959, 1960, and 1961, and was used also for other analyses (*8, 12*). Details of the experiment and data on plant heights and hay yields have been given (*12*). Samples came from plots seeded to Vernal alfalfa, Dollard medium red clover, Oregon Common ladino