Table III. Apparent NAA Residues on Untreated Olive Samples

(25-gram aliquots)

Sample No.[a]	Absorbance (360 mμ)	Apparent P.P.M.
1084A	0.090	0.08
842A	0.068	0.06
1269A	0.073	0.06
843A	0.078	0.07
1142A	0.070	0.06

[a] See Tables I and II for description.

published methods for the analysis of NAA (*1, 8*) to olives proved unsuccessful. Bache *et al.* (*1*) analyzed apples for NAA by extraction and a single-column cleanup using silica gel. Although Bache's colorimetric method was finally adopted, the cleanup would not remove olive oil. The gas chromatography–ultraviolet spectrophotometry technique (*7*) also proved inadequate for this purpose, again due to high oil content and interfering absorbances at 224 and 281 mμ.

The method finally adopted for NAA analysis in olives is a modified combination of the published methods.

After the initial extraction of olives with acidified chloroform, column chromatography on basic alumina permitted the complete removal of olive oil. This procedure was first suggested by Daoud and Luh (*4*) and was based on the quantitative adsorption of organic acids from nonpolar solvents. The alumina was washed with copious amounts of chloroform which removed all of the oil and some fat-soluble green pigments. Naphthaleneacetic acid was quantitatively eluted from alumina with 1% sodium bicarbonate solution.

However, the base also removed a water-soluble, purple pigment from the column which interfered with the colorimetric or spectrophotometric methods if applied after this step. This pigment could be removed by silica gel chromatography, and the acid was quantitatively eluted with 5% *n*-butanol in chloroform (*2, 3*). This step was checked by chromatographing 1000 μg. of NAA and reading the absorbance of 5-ml. fractions at 281 mμ (*7*). Based on the theoretical absorbance of 1000 μg. of NAA, 94.3% of the acid was recovered between 40 to 100 ml. elutriate (Figure 1).

Even after silica gel chromatography, direct colorimetry or spectrophotometry yielded relatively high blanks which did not yield an over-all sensitivity of 0.1 p.p.m. Consequently, the final solution was further purified by gas-liquid chromatography, analogous to one of the published methods (*7*). Since the colorimetric procedure based on nitration yielded considerably lower blanks than a direct reading at 281 mμ, the colorimetric technique was chosen for final analysis. The extinction coefficient of the color resulting from the nitrated naphthalene ring was low and optical cells with a 10-cm. light path were chosen to give ultimate sensitivity. If an absorbance of 0.11 per 2.5 μg. NAA was arbitrarily chosen as minimum readability, a sensitivity of 0.1 p.p.m. with 25-gram aliquot samples was achieved.

Residue Analysis. Olive trees were sprayed 2 weeks after bloom with an emulsifiable concentrate of NAA containing 150 p.p.m. active ingredient. These tests were conducted in May 1960 and 1962, but all analyses were performed in 1962. Samples from the 1960 series were stored at −10° C. Recoveries of NAA added to fruit prior to extraction were studied at three levels—0.1, 0.5, and 1.0 p.p.m. Recoveries of added NAA at the 1.0 and 0.5 p.p.m. levels ranged from 76 to 104% and at 0.1 p.p.m. from 50 to 76% (Tables I and II). Apparent NAA residues in untreated samples ranged from 0.06 to 0.08 p.p.m., which was below the stated sensitivity of the method (Table III).

Results for residues found in treated olives and harvested at various periods after treatment up to actual harvest time (mid-October) are shown in Tables I and II and plotted in Figure 2. Residues of NAA from the 1960 test were generally lower than those of 1962. However, in both cases no detectable residues (below 0.1 p.p.m.) were found at harvest time. The reason for lower 1960 residues may be the slow volatilization of NAA during almost 2 years' storage. If, however, the straight-line portion of the 1962 series (Figure 2) is extrapolated to 0.1 p.p.m., a calculated waiting period of 100 days after treatment is obtained.

Acknowledgment

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SUCKER CONTROL CHEMICALS

Detection of Compounds that Inhibit Vegetative Bud Growth of Tobacco

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Removing the flower parts of tobacco as the plants approach maturity is a recommended procedure to improve quality of the leaves (*2*). In commercial practice, a few days after the top has been removed the dormant buds at the base of the leaf petioles begin to grow vigorously and develop new shoots that must be removed, sometimes repeatedly at several weekly intervals to maintain high quality of the salable leaves. Mineral oil emulsions and maleic hydrazide preparations were developed to control sucker growth chemically and reduce labor costs (*9*). These methods have not proved entirely satisfactory, however. A new method of evaluating additional chemicals for sucker control is described here, and results obtained with the method are presented.

Methods

Preparation of Chemicals. Many of the chemicals were available for these tests in minute quantities. For this reason, lanolin paste was used as a carrier to conserve the amount of chemical required and thus make it possible to test a wide range of chemicals. Each chemical was prepared at approximately 1.0%

A rapid method for evaluating the bud-inhibiting properties of chemicals in the greenhouse is described. A small-leaf type of tobacco, Xanthi-nc, which rapidly develops an elongated stem, was used. With this method, the bud-inhibiting properties of a chemical were evaluated 10 days after application treatment of the axillary buds of decapitated plants. Thirty-seven growth-regulating chemicals were selected for evaluation from several families of compounds which had not been previously tested on tobacco. Two of these, namely, 3-chloro-isopropyl *N*-phenylcarbamate and ammonium, 3-chloro-α-methoxyphenylacetic acid, reduced lateral sprout growth by an amount equal to that induced with maleic hydrazide which was used for comparison. These results have not been fully evaluated in experiments using commercial varieties of tobacco growing in the field.

Figure 1. Typical Xanthi-nc tobacco plants used in screening for sucker-inhibiting compounds

Left, plant showing sucker growth 10 days after decapitation
Right, comparable plant *treated with 3-chloro-isopropyl N-phenyl carbamate at the time of decapitation*

concentration in lanolin by placing 6 mg. of the chemical in a shell vial and dissolving it in approximately 0.25 mg. of acetone; then 0.5 gram of melted lanolin was added and the mixture stirred until cool, forming a creamy paste. This procedure was facilitated by use of a buret for the acetone. Another buret containing melted lanolin was wrapped with a tape heater connected to a rheostat to maintain constant temperature and droplet delivery.

Culture of Xanthi-nc Plants. This plant is a hybrid of *Nicotiana glutinosa* and *N. tabacum* var. Xanthi produced by R. E. Clausen and D. R. Cameron, Department of Genetics, University of California, Berkeley, Calif. The plant is day-length neutral, producing an abundance of flowers and seeds at all times of the year either in the greenhouse or in the field. These plants develop suckers relatively soon after decapitation, and the internodes are elongated in comparison with tobacco plants of most other varieties (Figure 1). In preliminary studies, growth of five types of commercial varieties was compared with that of Xanthi-nc. Varieties having larger leaves required almost twice as much bench space and remained in a compact rosette condition for 1 to 2 months longer than did Xanthi-nc. All types appeared to respond approximately the same to maleic hydrazide treatment for sucker inhibition under greenhouse conditions.

The following procedures were developed to ensure a steady year-round supply of plants. A fertile loam soil was mixed with coarse building sand (1 part to 2, respectively) to provide good drainage. Seed pans filled with the mixture were sterilized with steam, seeded lightly, and the seed was covered with a thin layer of sifted, sterilized soil. Rapid seed germination and emergence were brought about by subjecting the seeded pans to water mist at intervals. The mist system was regulated by a time clock set to operate for a period of about 10 seconds in every 10 minutes during the day. Another timer turned the system off at night. A relatively high greenhouse temperature (75° to 85° F.) was maintained. A 300-watt Mazda bulb suspended 3 feet above the flats provided continuous illumination. Under these conditions, Xanthi-nc tobacco seed germinated, emerged, and developed into small plants within 5 to 6 days.

The young seedlings were then removed from the mist, treated with a fungicide to protect them against damping-off organisms, and allowed to grow in a warm (75° to 80° F.) greenhouse until they were 1 inch tall. This required a period of 25 days. Uniform seedlings with leaves about $^1/_2$ inch across were then transplanted to a similar but unsterilized soil mixture contained in 4-inch clay pots. Each seed pan generally produced 1200 or more uniform seedlings. The growth of plants was retarded when necessary, by placing the pans in a cool (55° to 65° F.) greenhouse. A fungicidal treatment was included in the first watering after transplanting. The plants were fertilized with a complete fertilizer and allowed to grow in a warm greenhouse until they were 6 to 8 inches tall. About 25 days after transplanting, uniform plants were selected and made ready for chemical treatment. The period from sowing of the seed until plants were suitable for use in tests was about 56 days.

Method of Conducting Test. When test plants were 6 to 8 inches tall with four, partially expanded lower leaves, the upper fourth of the stem was removed. A lanolin-paste mixture containing approximately 1 mg. of the test chemical was placed directly on the axillary buds of the first leaf immediately below the cut stem surface. An equal amount was similarly placed on the second and third leaves below the cut stem surface so that each plant received a total of 0.3 mg. of chemical.

Seven to 10 days after application of the chemical, the three treated axillary buds on each plant were removed and weighed as a composite sample. Each chemical treatment was replicated four times. Untreated decapitated tobacco plants, which had developed suckers 3 to 4 inches long together weighing 15 to 20 grams per plant, were included in each experiment along with others treated with a 1% mixture of maleic hydrazide. Chemical regulators that retarded bud growth in an amount equal to that of maleic hydrazide without visibly injuring the plants were evaluated further.

Results and Discussion

The 38 compounds shown in Table I, representing several different families of chemicals, were selected on the basis of previous experiments in which they had shown bud-inhibiting effects on test plants such as bean, sunflower, or cucumber. With the exception of maleic hydrazide, none of these substances has been reported previously as an inhibitor of sucker growth of tobacco. The compound, 3-chloro-isopropyl *N*-phenylcar-

Table I. Results of Screening Tests with the Xanthi-nc Tobacco Plant for Detection of New Chemical Sucker Inhibitors

Compound, Family and No.	Chemical Name	% Reduction in Weight of Suckers	Other Responses[a]
Maleic hydrazide			
1	6-Hydroxy-3-(2*H*)-pyridazione (treated control)	98	0
Carbamate			
2	α-Carboxyethyl *N*-3-chlorophenylcarbamate	90	0
3	3-Chloro-isopropyl *N*-phenylcarbamate	98	0
Indole			
4	Methyl-α-methoxy-propionyl-3-indoleacetate	82	++
5	Morpholine-3-indoleacetate	60	++
Methoxy-phenylacetic			
6	Ammonium-3-chloro-α-methoxyphenylacetate	97	+
7	Ammonium-3-fluoro-α-methoxyphenylacetate	92	+
8	Ammonium-α-2,3-trimethoxyphenylacetate	94	+
9	α-Methoxy-3,4-dichloro-phenylacetic acid	94	+
10	α-Methoxyphenylacetic acid	86	++
11	3-Nitro-α-methoxyphenyl-acetic acid	90	+
Nicotinium			
12	4-Chlorobenzylnicotinium chloride	87	0
13	2,4-Dichlorobenzylnicotinium chloride	80	0
14	α-Naphthylmethylnicotinium chloride	84	0
Phenoxy			
15	2,5-Dichlorophenoxyacetamide	88	+++
16	2,5-Dichlorophenoxyacetic acid	74	+++
17	2,6-Dichlorophenoxyacetic acid	5	+
18	2,4,6-Trichlorophenoxyacetic acid	4	+
Phenylacetic			
19	2,4-Dichlorophenylacetic acid	86	+
20	2,3,6-Trichlorophenylacetic acid	84	+
Phosphonium			
21	Tributyl 2,4-dichlorobenzyl-phosphonium chloride	90	++++
22	Triphenylmethylphosphonium chloride	84	++++
Picolinium			
23	2,4-Dichlorobenzyl-2-picolinium chloride	86	++++
24	2,4-Dichlorobenzyl-3-picolinium chloride	98	++++
25	2,4-Dichlorobenzyl-4-picolinium chloride	96	++++
Quaternary ammonium			
26	Ammonium chloride, (5-hydroxycarvacryl) trimethyl, 1-piperidinecarboxylate	60	
27	Ammonium iodide, (5-hydroxycarvacryl) trimethyl, 1-piperidine-carboxylate	65	++++
28	2-Chloroethyltrimethyl ammonium chloride	60	0
29	2-Chloroethyltrimethyl ammonium *p*-toluene sulfonate	72	0
30	2,4-Dichlorobenzyl, triethyl ammonium chloride	1	0
31	2,4-Dichlorobenzyl, trimethyl ammonium chloride	6	0
32	2-Fluoroethyltrimethyl ammonium tosylate	70	0
Miscellaneous			
33	*N*-2-Chlorophenyl-phthalmic acid	20	0
34	2,2-Dichloropropionic acid	74	0
35	*N*-Dimethylamino maleamic acid	67	0
36	Methyl 2,3,5-triiodobenzoate	39	+++
37	Pentachloropentadienoic acid	40	0
38	Trichloroacrylic acid	55	0

[a] 0 = None observable; + = slight epinasty of petioles or gall formation; ++ = moderate epinasty of petioles or gall formation; +++ = severe epinasty of petioles or gall formation; ++++ = treated buds discolored, indicating contact burning.

bamate (No. 3), commonly known as chloro-IPC, retarded sucker growth by an amount equal to that of maleic hydrazide without distorting the plant when applied in lanolin paste. However, preliminary field experiments with chloro-IPC, conducted cooperatively with R. N. Jeffrey, indicated that this compound may cause moderate to severe crinkling of the leaves of the Catterton variety of tobacco when applied as a spray at relatively high dosage levels.

Limited field experiments were also conducted with aqueous spray applications of the chemicals shown in Table I. Two-hundred milligrams of each chemical was applied per plant in 30 ml. of aqueous spray suspension in order to wet the upper third of each of five plants per chemical treatment. Ethanol (0.2%) and Tween-20 (0.5%) were used to dispense chemicals in water. Relatively mature plants of the Catterton variety growing at Beltsville, Md., were used. Greenhouse results obtained with paste application of Xanthi-nc, as compared with field-sprayed Catterton, consistently showed the following trends. A reduction of 95% or more in sucker growth in the greenhouse gave satisfactory sucker control for 3 to 4 weeks in the field. Chemicals that induced 90 to 95% reduction in the greenhouse controlled suckers for a period of 1 to 2 weeks in the field. Perhaps this period could be extended by a repeated application or by increasing the applied dosage. Compounds that caused slight epinasty in the greenhouse did not always induce similar effects on the more mature field plants. Both the moderate and severe formative effects caused by a chemical applied under greenhouse conditions resulted in malformed plants when these chemicals were used in field experiments. Chemicals that caused localized discoloration of buds in the greenhouse usually injured foliage of field plants severely.

Applied in appropriate amounts, chloro-IPC gave a sucker-retarding effect equivalent to that produced by the conventional MH treatment, and there was no apparent injury. Further evaluation of chloro-IPC under field conditions using commercial varieties is, of course, necessary.

Compounds No. 8 to 11, inclusive, belong to a family of methoxyphenylacetic acids, some of which are known to translocate readily in a downward direction in plants, which is an important characteristic of a sucker-inhibiting compound (*1*, *3*, *5*). Ammonium-3-chloro-α-methoxyphenylacetate reduced sucker growth 97% with only slight epinasty of the petioles. Also included in Table I are compounds that are now being evaluated rather widely as retardants for plants other than tobacco (*4*, *6–8*). These chemicals were relatively ineffective as sucker inhibitors of tobacco. No recommendations are made for the use of any of these or other new chemicals mentioned for sucker control of field-grown tobacco.

Although the method described has been applied to only the tobacco plant at present, with modifications, it may possibly be useful in developing a chemical method for retarding lateral bud growth of other crops, such as chrysanthemums, that are now disbudded by hand labor.

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FERTILIZER CONSISTENCY

Bulk Blending of Fertilizer Material: Effect of Size, Shape, and Density on Segregation

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The tendency of dry-blended fertilizers to segregate during handling and spreading was shown to result chiefly from differences in particle size of the various components of the blend. Variations in particle density had little effect, and shape had practically no effect. Segregation can be held to a minimum by using materials of matching size distribution and by avoiding the coning of blends during handling.

THE SIMPLE dry blending of granular fertilizer materials is gaining popularity as a method of producing mixed fertilizers. Such blends are distributed widely in bulk (*3–5*) and are being bagged to an increasing extent.

The major technical problem connected with dry blending appears to be that of segregation. Unless certain precautions are observed, the components of blends may segregate severely during handling and distribution. Such segregation not only causes difficulties in sampling and in meeting guaranteed analysis but also results frequently in spotty crop response in the field.

The components of a blend tend to segregate when they differ in physical properties to such an extent that they respond differently to mechanical disturbance. The physical properties of possible significance in this respect have been recognized to be particle size, shape, and density, but little information has been reported as to quantitative effects of these properties on segregation. The need for such information as a guide to the preparation of blends with good handling properties led to the study reported here—an evaluation of the relative contributions of size, shape, and density differences to segregation of granules in dry blends.

Handling procedures that may induce segregation include coning (as occurs when mixtures are allowed to drop into sloping piles in storage areas, hoppers, or truck beds), vibration (as occurs in bulk spreader trucks being driven to and across fields), and ballistic action (as imparted by fan-type spreaders). Since it was recognized that the effects of particle size, shape, and density on segregation might differ with the different handling procedures, each procedure was studied separately. Most of the work pertained, however, to coning and to ballistic action. Exploratory tests indicated that vibration was only a minor cause of segregation; also, work by Smith (*6*) indicated that most mixtures could be transported 30 miles in a spreader truck with little segregation due to vibration.

Size, Shape, and Density of Fertilizer Materials

Size distributions, granule shapes, and granule densities were determined for various fertilizer materials commonly available for bulk blending. Typical measurements are shown in Table I.

All the materials fell almost entirely within the 6- to 16-mesh size range, but the distribution within this range varied widely. For example, a prilled urea and a high-density prilled ammonium nitrate contained 80 to 90% of −10+16-mesh particles, whereas an 18–20–0 (18–46–0) ammonium phosphate and a triple superphosphate had about the same pro-