121

Ann. appl. Biol. (1968), **62**, 159–173
Printed in Great Britain

Effects of plant growth regulators and other compounds on flow of latex in *Hevea brasiliensis*

BY P. D. ABRAHAM AND S. G. BOATMAN*
Rubber Research Institute of Malaya, Kuala Lumpur, Malaysia

AND G. E. BLACKMAN AND R. G. POWELL
Agricultural Research Council Unit of Experimental Agronomy, Department of Agriculture, University of Oxford

(*Received* 9 *April* 1968)

SUMMARY

Eighty-nine compounds have been tested for their ability to promote the flow of latex from *Hevea brasiliensis* after tapping. The twenty-four substances which showed significant activity were: twelve chloro- and fluoro-phenoxy alkylcarboxylic acids substituted in the 4-, 2,4- and 2,4,5- positions, two chlorinated benzoic acids, α- and β-naphthaleneacetic acid, 3-indolylacetic acid, 2-methoxy-4-diethylamino-6-isopropylamino-1,3,5- triazine (methoxy-ipazine), *N*,*N*-diallyl-α-chloroacetamide (CDAA), 1,1′-dimethyl-4,4′-bipyridilium-2A (paraquat), neomycin, dichloro-*m*-xylenol, and phenyl mercury acetate and chloride. Despite the many replications the high variability of the yield data does not allow of a precise classification of the relative activities. The most active compounds were 2,4-dichloro-5-fluorophenoxyacetic acid and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T), although 2,4-dichlorophenoxyacetic acid (2,4-D) gave increases in yield comparable to those of 2,4,5-T in some of the trials.

INTRODUCTION

Abraham & Tayler (1967) have described the use of 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) on trees of *Hevea brasiliensis* to increase the flow of latex after tapping. On suitable trees large increases in yield of rubber can be brought about at little extra expense and the practice has been widely adopted in Malaysian plantations.

Compagnon & Tixier (1950) published the first account of the promotion of the flow of latex in *H. brasiliensis* by the application of synthetic compounds. A range of inorganic and organic chemicals was introduced for application through holes drilled into the trunk; yield increases were reported following the application of copper sulphate or 2,4,-D. Chapman (1951) lightly scraped a strip of bark immediately below the tapping cut and painted various compounds dissolved in palm oil. 2,4,-D proved to be most effective in promoting latex flow at a concentration of 1 %. Other compounds showing activity included 2-methyl-4-chlorophenoxyacetic acid and α-naphthaleneacetic acid. Baptiste & de Jonge (1955) confirmed the effectiveness of copper sulphate injection

---

* Present address: Plant Protection Ltd., Jealott's Hill Research Station, near Bracknell, Berks.

THE RUBBER RESEARCH INSTITUTE OF INDIA
Acc. No. ....
Date 3. Apr. 1970
LIBRARY

and reported significant increases in yield with 2,4,5-T, 2,4-D, 4-chlorophenoxyacetic acid, α-naphthaleneacetic acid and β-naphthoxyacetic acid, employing techniques similar to those of Chapman. The highest yields were obtained with 2,4,5-T, 2,4-D and by copper sulphate injection. The copper sulphate treatment was thought to entail risks of bark damage and of an increased copper content of the latex which might have deleterious effects on the technological properties of rubber.

The commercial use of 2,4-D and 2,4,5-T was based on the comparative trial of a relatively few substances and a much wider range from diverse groups of biologically active compounds was therefore tested. A preliminary report on the first part of the investigation has already been given (Blackman, 1961).

MATERIALS AND METHODS

Baptiste & de Jonge (1955) established that the *n*-butyl ester of 2,4,5-T was highly active and was easily incorporated into suitable carriers. In the present trials, the substituted phenoxyalkylcarboxylic and benzoic acids have therefore been tested as the *n*-butyl esters whenever possible. In other groups of compounds, it was either inconvenient or impracticable to employ esters. All experiments contained comparable 2,4,5-T treatments, formulated as either *n*-butyl ester or the free acid.

The activity of 2,4,5-T is limited to a comparatively narrow range of concentrations around 1% (Baptiste & de Jonge, 1955). The optimum concentrations of other active compounds do not appear to vary greatly from this. Hence, a range of concentrations was tested whenever possible, against 2,4,5-T as a standard.

Carriers

Commercial mixtures of 2,4-D and 2,4,5-T are usually compounded with palm oil, which has itself been reported to induce small increases in yield (Baptiste, 1955) and was therefore avoided in most of the present work. In Expts L1–L5, white petroleum jelly was employed as carrier, while for most of the remaining trials a thinner and more convenient mixture was prepared from liquid paraffin and petroleum jelly (3:1 by weight). Certain compounds could not easily be incorporated into this carrier and these were mixed with lanoline. The comparative 2,4,5-T treatments were formulated in the same manner.

Methods of application

The compounds tested were applied by one of the following methods:

(1) *Method A.* Application, at monthly intervals, was made to a narrow strip of recently tapped bark immediately above the tapping cut. The compound, in its carrier, was painted thinly and evenly along a strip, about 1·9 cm in width, left after the previous month's tapping. Care was taken not to overlap one application with another.

(2) *Method B.* A single application, in the same type of carrier, was made to a strip of lightly scraped bark, 6 cm in width, immediately below the tapping cut.

(3) *Method C.* Injection of the compound itself into the wood via two holes, drilled in the trunk below and on either side of the tapping cut. Either 2·5 or 5·0 g of the substance was introduced into each hole, avoiding, as far as possible, direct contact with the phloem tissue.

Method C was rarely used and then only for a substance, such as copper sulphate, which was believed to be active but which failed to give a significant response when applied by the other two methods.

When active growth regulators, such as 2,4,5-T, are applied above the tapping cut, as in Method A, meristematic activity is induced, which may be detected by an increased thickness of the regenerating bark. This abnormal growth may result in sufficient swelling and cracking to render the bark unfit for future tapping. Such difficulties are avoided in Method B, since the treated strip of bark is removed within 3–4 months by the normal process of reopening the tapping cut. Method A was, however, used in a number of the present trials, especially with the substituted phenoxyalkylcarboxylic and benzoic acids, as it was desired to compare the ability to induce meristematic activity with the effectiveness in promoting latex flow. A slight disadvantage of Method B is that the light scraping which is necessary to assist penetration can also induce small increases in yield (Baptiste, 1955), but this can be overcome by comparing yields with a control series scraped and painted with the carrier.

Design of field trials

All the trials employed a randomized single-tree-plot design similar to that described by Baptiste & de Jonge (1955). Three series of experiments will be mentioned. The main series of 'large-scale' trials, designated L1–L4, employed 16–52 replications per treatment and yields were recorded for 6 months after treatment. The first experiment (L1) was on a population of 7-year-old buddings, predominantly of one variety, PR107, using sixteen replications. A good increment in yield was obtained with satisfactory precision. More recent work indicates that this was somewhat fortuitous, because such young trees frequently show small and erratic responses (Abraham & Ng, 1968; Ho & Paardekooper, 1965; Ng, Abraham, Gill & P'ng, 1965). Later experiments were conducted on older populations (18–30 years), planted with genetically heterogeneous material, i.e. either trees originating from seed or mixed buddings of a wide range of clones. This was done partly because such areas were more readily available and also to obtain results of general application independent of the characteristics of any particular variety. This material had large tree-to-tree variability, both in the yield of the controls and in responses to treatment. Thus, in Expts L2 and L3, even with thirty replicates, the yield data were very variable. Increasing the number of replicates to 46–52 in the remaining experiments improved the position. Thickness of the bark was much less variable and differences could be detected even in Expts L2 and L3.

The large-scale trials were costly in time, labour and materials and attempts were made to obtain approximate indications of the activity of compounds in smaller 'preliminary trials' (P1–20), using eight replicates (on somewhat less heterogeneous material than in the large-scale trials). These preliminary trials were superseded later by the 'small-scale screening trials' (S1–60) which employed 10–12 replications and slightly modified techniques of recording. Both series were recorded for about 2 months after the application. The main purpose of these smaller experiments was to select promising materials for large-scale trials, but the results of a few are cited here, so that data can be presented on compounds which were not included in larger trials.

Recording of results

In the large-scale trials the yield of each tree was obtained by adding sufficient dilute formic acid to the collecting cup to coagulate the rubber hydrocarbon. The resulting coagulum was squeezed and threaded onto a labelled wire for subsequent drying and weighing. In the smaller experiments the volume of latex from each tree was measured and the rubber content of the bulked latex from each treatment was determined at intervals by a standard technique (A.S.T.M. D 1076/59).

In some large-scale trials, when growth regulators were applied by Method A, the thickness of the bark was measured 6 months after treatment with a Schlieper bark gauge (Abraham & Boatman, 1964).

Analysis of data

The mean yields for each treatment, expressed on a per tree and per tapping basis, were corrected for yield differences before treatment by covariance analysis. The corrected yield increases were then obtained by subtraction of the control yields. Differences in response between experiments were eliminated by dividing the yield increases of the experimental compounds by that obtained with 2,4,5-T in the same experiment. The resulting comparative 'activities' have as their base A (2,4,5-T) = 1·0, while A = 0·0 indicates no gain over the level of control. Occasional yields below that of the control are indicated by negative signs. Where a range of concentrations of each compound was simultaneously tested, two methods of calculating 'activities' were compared. In the first method the mean yield increase for all concentrations (both for the test compound and 2,4,5-T) was used in the calculation, whereas in the second method, the yield increase at the most effective concentration was selected. The second approach generally appeared more satisfactory and results are presented here on this basis. When only a single concentration (usually 1%) was tested, it was compared with the same concentration of 2,4,5-T. 'Activities' in increasing the thickness of the treated bark were calculated in the same manner.

Sources of compounds tested

The following compounds were prepared at Oxford. The *n*-butyl esters of: 2-chlorophenoxyacetic acid, 4-chlorophenoxyacetic acid, 2,4-dichlorophenoxyacetic acid, 2,4,5-trichlorophenoxyacetic acid, 4-fluorophenoxyacetic acid, 2-chloro-4-fluorophenoxyacetic acid, 2,4-dichloro-5-fluorophenoxyacetic acid, 2,4-dimethylphenoxyacetic acid, (±) α-(4-chloro-2-methylphenoxy) propionic acid, (±) α-(2,4-dichloro-5-fluorophenoxy)propionic acid, 2,3,6-trichlorophenylacetic acid, 2-chlorobenzoic acid, 2,5-dichlorobenzoic acid, 2,3,6-trichlorobenzoic acid, 2,3,5-triiodobenzoic acid and 3,6-dichloro-2-methoxybenzoic acid, together with the octyl ester, cetyl ester and 2,4-dichlorophenoxyethyl ester of 2,4,5-T. Additional compounds which were prepared include (2,4,5-trichlorophenyl)octyl ether, (2,4,5-trichlorophenyl)tetradecyl ether, (2,4,5-trichlorophenyl)octadecyl ether, phenyl mercury chloroacetate and 2,5-dichlorophenyl mercury acetate.

The methods employed for preparation may be summarized as follows:

(i) Direct esterification of the acids was possible in all except two instances. The acid was treated with the appropriate alcohol in benzene solution, the reaction being catalysed by *p*-toluenesulphonic acid and forced to completion by entraining the water formed in a Dean and Stark apparatus (Migrdichian, 1957). Both 2,3,5-triiodobenzoic acid and 3,6-dichloro-2-methoxybenzoic acid resisted esterification by this method and therefore the silver salts of these acids were reacted with iodobutane in butanol solution (Migrdichian, 1957). The esters were purified by appropriate methods and finally by distillation.

(ii) Most of the acids were available commercially and some of the substituted phenoxyacetic acids could readily be prepared from the appropriate phenol. A method devised by Klaasens & Schoot (1956) was used for the preparation of 2,4-dichloro-5-fluorophenoxyacetic acid and a modification of this method yielded the corresponding propionic acid.

(iii) The 2,4,5-trichlorophenyl ethers were prepared using the Williamson ether synthesis by reaction of the sodium salt of 2,4,5-trichlorophenol with the relevant bromo paraffin (Howard, McLane & Weintraub, 1958).

(iv) Phenyl mercury chloroacetate was prepared by interaction in boiling xylene of equivalent amounts of phenyl mercury chloride and the silver salt of chloroacetic acid.

(v) 2,5-Dichlorophenylmercury acetate was prepared by a method originally devised by Nesmejanow & Kahn (1929). The diazonium salt of 2,5-dichloroaniline was treated in acetone solution at 0 °C with mercuric chloride and copper powder in the presence of strong hydrochloric acid. The product was crystallized from acetone, m.p. 205 °C.

RESULTS

The compounds tested have been arranged under convenient headings in a series of tables: 'activities' cited under the same experimental serial number were included in a single experiment. The method of application, already outlined in the Materials and Methods section, and the concentration range employed are listed. (A single figure under the latter heading indicates that only one concentration was tested.)

Table 1. *Relative activities of monosubstituted phenoxyacetic acids and the parent acid*

Expt. no.	L2	L4	P1	P5
Application method ...	A	A	B	B
Concentration range (%) ...	0·3–2·7	0·3–2·7	1·0	1·0
(*a*) Relative increase in latex yield (2,4,5-T = 1·0)				
Phenoxyacetic acid	—	—	0·1	—
2-Chloro-	0·4	—	—	0·1
3-Chloro-	—	—	—	0·3
4-Chloro-	—	0·4	—	0·6
4-Fluoro-	0·8	0·3	—	—
Significant difference ($P = 0·05$)	0·85	0·26	0·49	0·57
(*b*) Relative increase in bark thickness (2,4,5-T = 1·0)				
2-Chloro-	0·0	—	—	—
4-Chloro-	—	0·7	—	—
4-Fluoro-	0·3	0·1	—	—
Significant difference ($P = 0·05$)	0·22	0·15	—	—

Monosubstituted phenoxyacetic acids

Of the four experiments cited in Table 1, the large errors in yields in Expt L makes the results unreliable. The remaining experiments show that the 4-fluoro- an 4-chloro- compounds have similar activities, perhaps half that of 2,4,5-T. Th 3-chloro- compound may also be slightly active, but the 2-chloro- and parent phenoxy acetic acid are seemingly not.

The errors in the measurements of bark thickness are less, especially in Expt L The 4-chloro-derivative is highly significantly more active than the correspondin fluorine compound; this contrasts strikingly with the results for flow of latex. Th 2-chloro compound appears to be inactive.

Table 2. *Relative activities of disubstituted phenoxyacetic acids*

Expt. no. ...	L1	L2	L4	L5	L11	L12	L13	P1	P5
Application method ...	A	A	A	A	B	A	B	B	B
Concentration range (%) ...	0·9	0·3–2·7	0·3–2·7	0·1–0·9	0·5–1·5	0·5–1·5	0·5–1·5	1·0	1·0
(*a*) Relative increase in latex yield (2,4,5-T = 1·0)									
2,3-Dichloro-	—	—	—	—	—	—	—	—	0·1
2,4-Dichloro-	0·4	1·4	0·5	0·5	1·1	0·8	0·8	0·6	1·4
2-Chloro-4-fluoro-	—	—	0·6	—	—	—	—	—	—
4-Chloro-2-methyl-	—	—	—	—	—	—	—	0·5	—
2,4-Dimethyl-	—	0·6	—	—	—	—	—	—	—
2,5-Dichloro-	—	—	—	—	—	—	—	0·4	0·4
2,6-Dichloro-	—	—	—	—	—	—	—	0·0	—
Significant difference ($P = 0·05$)	0·36	0·85	0·26	0·31	0·61	0·38	0·44	0·49	0·57
(*b*) Relative increase in bark thickness (2,4,5-T = 1·0)									
2,4-Dichloro-	0·7	0·6	1·0	0·8	—	—	—	—	—
2-Chloro-4-fluoro-	—	—	0·6	—	—	—	—	—	—
2,4-Dimethyl-	—	0·1	—	—	—	—	—	—	—
Significant difference ($P = 0·05$)	0·23	0·22	0·15	0·21	—	—	—	—	—

Disubstituted phenoxyacetic acids

2,4-D was included in nine experiments (Table 2); in three it gave smaller yiel increases than 2,4,5-T (activity 0·4–0·5), but in the remaining six (activity 0·6–1·4) t difference was not significant. Of the four experiments in which effects on bark thic ness were measured, 2,4- D was less effective than 2,4,5-T in two, but there was litt difference between them in the others. Although on average 2,4-D may be less acti both in its effects on yield and regeneration of bark, it is sometimes equal to 2,4,5-

Considering the group as a whole, 2,4-D, the 2-chloro-4-fluoro- and the 4-chlor 2-methyl-compounds appear to be the most active in promoting latex flow, with litt to choose between them. Although the results of Expt L2 are unreliable they sugge that the 2,4-dimethyl-derivative is less active than 2,4-D. The 2,5-dichloro-compoun also appears to be less active while the 2,3-dichloro- and 2,6-dichloro- compounds a inactive. In the bark measurements, substitution of fluorine for chlorine in t 4-position again decreased meristematic activity, in contrast with the yield result The 2,4-dimethyl- derivative had little or no effect.

Tri- and penta- substituted phenoxyacetic acids

Table 3 shows that pentachlorophenoxyacetic acid is probably inactive. Of the trisubstituted compounds, the 2,4,5-configuration is clearly the most active. The 2,4-dichloro-5-fluoro-derivative outyielded 2,4,5-T in three of out of four trials, but the differences were barely significant. The 2,4-dichloro-5-fluoro compound was much more active than 2,4,5-T in one trial in increasing bark thickness but less active in another. The 2-chloro-4,5-difluoro- compound, tested in only one small-scale trial, appeared similar to 2,4,5-T in promoting latex flow. The 2,3,4-trichloro- and 2,4,6-trichloro-derivatives had no effect on yield and slightly increased bark thickness. The increase in thickness induced by the 2,4,6-trichloro- compound can probably be ascribed to the fact that in this first experiment, application of the carrier alone was not included; even petroleum jelly can promote bark renewal possibly by protection from desiccation (de Jonge, 1957).

Table 3. *Relative activities of tri- and penta- substituted phenoxyacetic acids*

Expt. no. ...	L1	L2	L4	L5	L14A	P1	S43A
Application method ...	A	A	A	A	B	B	B
Concentration range (%) ...	0·9	0·3–2·7	0·3–2·7	0·1–0·9	1·0–2·0	1·0	1·0
(*a*) Relative increase in latex yield (2,4,5-T = 1·0)							
2,3,4-Trichloro-	—	—	—	0·1	—	—	—
2,4,5-Trichloro-	1·0	1·0	1·0	1·0	1·0	1·0	1·0
2,4-Dichloro-5-fluoro-	1·3	1·7	0·8	—	1·3	—	—
2-Chloro-4,5-difluoro-	—	—	—	—	—	—	1·0
2,4,6-Trichloro-	0·1	—	—	—	—	—	—
2,3,4,5,6-Pentachloro-	—	—	—	—	—	0·1	—
Significant difference ($P = 0·05$)	0·30	0·85	0·26	0·31	0·34	0·49	0·64
(*b*) Relative increase in bark thickness (2,4,5-T = 1·0)							
2,3,4-Trichloro-	—	—	—	0·2	—	—	—
2,4,5-Trichloro-	1·0	1·0	1·0	1·0	—	—	—
2,4-Dichloro-5-fluoro-	1·1	1·6	0·8	—	—	—	—
2,4,6-Trichloro-	0·3	—	—	—	—	—	—
Significant difference ($P = 0·05$)	0·27	0·22	0·15	0·21	—	—	—

In view of the reported anti-auxin properties of 2,4,6-trichlorophenoxyacetic acid (McRae & Bonner, 1952), the effect of additions of it to 2,4,5-T was tested. An equimolar concentration approximately halved the activity of 2,4,5-T.

Substituted phenoxypropionic acids

The substituted phenoxypropionic acids are slightly less effective in increasing latex flow than the corresponding acetic compounds (Table 4), although the difference was significant only for the 2,4,5-trichloro-substitution in Expt L4. The trends for bark thickness in this experiment are similar.

Esters of 2,4-D and 2,4,5-T

In comparisons of the *n*-butyl, *n*-octyl, *iso*-octyl, cetyl and butoxyethanol esters, the *n*-butyl ester has on average given the highest yield, but was significantly superior only over the *n*-octyl and *iso*-octyl esters. The 2,4-dichlorophenoxy ethyl ester and *n*-butyl ester of 2,4,5-T did not differ in activity.

Substituted phenoxyacetoxy propionamides

The *n*-butyl esters of 2,4,-D, 2,4,5-T and 2,4-dichloro, 5-fluorophenoxyacetic acid were compared with the corresponding 2-(phenoxyacetoxy)propionamides. There were no significant differences, either between the three compounds within each group or between the two groups.

Table 4. *Comparison of activities of substituted phenoxyacetic and α-(phenoxy) propionic acids*

Expt no. ...	L2	L4	L11	L12	L13
Application method ...	A	A	B	A	B
Concentration range (%) ...	0·3–2·7		0·5–1·5		
(*a*) Relative increase in latex yield (2,4,5-T = 1·0)					
2,4-Dichlorophenoxyacetic acid	1·4	0·5	1·1	0·8	0·8
(±)α-(2,4-Dichlorophenoxy) propionic acid	—	—	0·8	0·7	0·6
(±)α-(2-Methyl-4-chlorophenoxy) propionic acid	0·7	—	—	—	—
2,4,5-Trichlorophenoxyacetic acid	1·0	1·0	1·0	1·0	1·0
(±)α-(2,4,5-Trichlorophenoxy) propionic acid	—	0·7	1·0	0·7	0·9
2,4-Dichloro-5-fluorophenoxyacetic acid	1·7	0·8	—	—	—
(±)α-(2,4-Dichloro-5-fluorophenoxy) propionic acid	—	0·6	—	—	—
Significant difference ($P = 0·05$)	0·85	0·26	0·61	0·38	0·44
(*b*) Relative increase in bark thickness (2,4,5-T = 1·0)					
2,4-Dichlorophenoxyacetic acid	0·6	1·0	—	—	—
(±)α-(2-Methyl-4-chlorophenoxy) propionic acid	0·7	—	—	—	—
2,4,5-Trichlorophenoxyacetic acid	1·0	1·0	—	—	—
(±)α-(2,4,5-Trichlorophenoxy) propionic acid	—	0·6	—	—	—
2,4-Dichloro-5-fluorophenoxyacetic acid	1·6	0·8	—	—	—
(±)α-(2,4-Dichloro-5-fluorophenoxy) propionic acid	—	0·3	—	—	—
Significant difference ($P = 0·05$)	0·22	0·15	—	—	—

2,4,5-trichlorophenylalkyl ethers

A large-scale trial of the 2,4,5-trichlorophenyl octadecyl, tetradecyl and octyl ether indicated that the first two compounds appeared to have a slight activity but the increases were not significant. Somewhat surprisingly, the octyl ether was completel inactive.

Substituted benzoic acids

The results of the four experiments covering the seven compounds are given i Table 5. Unfortunately 2,4,5-T failed to produce a significant increase in yield i Expt L3 and few conclusions can be drawn from the data, except that 2,5-dichloro benzoic acid was active. The 2,5-dichloro- and 2,3,6-trichloro- compounds were als included in L4 but failed to increase the yield significantly. The 2-chloro- and 2,4 dichloro- compounds did not promote any increase in bark thickness, the 2,5-dichlor and 2,3,6-trichloro- derivatives showed some activity, but 2,3,5-triiodobenzoic aci was inactive. In the other experiments, 3-amino-2,5-dichlorobenzoic acid was inactiv and 3,6-dichloro- 2-methoxybenzoic acid was moderately active.

α- and β-naphthaleneacetic acids

That α-naphthaleneacetic acid will enhance latex flow was observed by both Chapman (1951) and Baptiste & de Jonge (1955). In the present investigation both α- and β-naphthaleneacetic acids were about half as active as 2,4,5-T. Various combinations with 2,4,5-T were tested for additive or synergistic effects. Such mixtures occasionally gave larger increases than 2,4,5-T alone, but the differences were not significant.

Table 5. *Relative activities of substituted benzoic acids*

Expt no. ...	L3	L5	L24A	S20
Application method ...	A	A	B	A
Concentration range (%) ...	0·3–2·7	0·1–0·9	0·2–5·0	0·1–1·0
(*a*) Relative increase in latex yield (2,4,5-T = 1·0)				
2-Chloro-	0·6	—	—	—
2,4-Dichloro-	0·7	—	—	—
2,5-Dichloro-	1·4	0·1	—	—
2,3,6-Trichloro-	0·6	0·2	—	—
2,3,5-Triiodo-	—	0·0	—	—
3-Amino-2,5-dichloro-	—	—	—	−0·5
3,6-Dichloro-2-methoxy-	—	—	0·6	—
Significant difference ($P = 0·05$)	1·06	0·31	0·34	0·69
(*b*) Relative increase in bark thickness (2,4,5-T = 1·0)				
2-Chloro-	0·0	—	—	—
2,4-Dichloro-	0·0	—	—	—
2,5-Dichloro-	0·2	0·2	—	—
2,3,6-Trichloro-	0·4	0·2	—	—
2,3,5-Triiodo-	—	0·1	—	—
Significant difference ($P = 0·05$)	0·13	0·21	—	—

Table 6. *Relative activities of indole compounds*

	Relative increase in latex yield (2,4,5,-T = 1·0)			
Expt no.	P3	P4	S34	S45
Application method ...	A	B	A	B
Concentration range (%) ...	1·0	1·0	1·0–4·0	1·0
3-Indolylacetic acid	0·3	0·7	0·3	—
3-Indolylacetonitrile	—	0·5	—	—
3-Indolylpropionic acid	—	0·4	—	—
3-Indolylbutyric acid	—	0·3	—	—
L-Tryptophane	—	—	—	−0·2
Significant difference ($P = 0·05$)	0·65	0·58	0·49	0·57

Indole compounds

L-Tryptophane, which is converted to indole auxins by several plant tissues, appears to be inactive (Table 6). 3-Indolylacetic acid (IAA) increased yield in Expt P4, but its activity was less than that of 2,4,5-T in two other trials. The remaining indole compounds are probably less active than IAA, but the errors are too high for precise conclusions. None of the indole compounds appeared to induce meristematic activity during the removal of bark.

Miscellaneous biologically active compounds

A heterogeneous group of substances including gibberellic acid, kinetin, β-(2-furyl)acrylic acid, traumatic acid, (2-chloroethyl)trimethylammonium chloride (CCC), 2,4-dichlorobenzyltributyl phosphonium chloride (Phosfon-D), maleic hydrazide, ascorbic acid, *N*,*N*′-diphenylurea, coconut milk, ethylene diamine tetraacetic acid, 2-hydroxyquinoline, 8-hydroxyquinoline, 2,2′-dipyridyl, 4,4′-dipyridyl, 2,4-dichlorophenol, 2,4,5-trichlorophenol, and the *n*-butyl ester of 2,3-6-trichlorophenylacetic acid, were tested without significant effects. The butyl ester of 2,3,6-trichlorophenylacetic acid showed little activity although the acid is highly active in inducing extension growth (Pybus, Wain & Wightman, 1959).

Table 7. *Relative activities of selected herbicides*

Relative increase in latex yield (2,4,5-T = 1·0)

Compounds*	Expt. no.	Application method	Concentration range (%)	Activity	Significant difference (P = 0·05)
Dalapon, diethylene glycol ester	P7	B	0·1–1·0	0·3	0·61
Chlorpropham	S48	B	0·2–2·0	0·3	0·47
Dicryl				0·1	
Ipazine				0·4	
Methoxyipazine				0·5	
CDAA				0·5	
Eptam				0·4	
Paraquat				0·2	
Paraquat	S50	B	0·5–2·0	0·2	0·55
Paraquat		C	—	0·6	

* Dalapon (2,2-dichloropropionic acid); chlorpropham (isopropyl-*m*-chlorocarbanilate); dicryl (*N*-(3,4-dichlorophenyl) methacrylamide); ipazine (2-chloro-4-diethylamino-6-isopropylamino-1,3,5-triazine); methoxyipazine (2-methoxy-4-diethylamino-6-isopropylamino-1,3,5-triazine); CDAA (*N*,*N*-diallyl-α-chloroacetamide); eptam (ethyl-*N*,*N*-dipropylthiolcarbamate); paraquat (1,1′-dimethyl-4,4′-bipyridylium-2A).

Table 8. *Experiment L10. Relative activities of antibiotics and bactericides*

Relative increase in latex yield (2,4,5-T = 1·0)

Application method ...	B	Application method ...	B
Concentration range (%) ...	1·0	Chloramphenicol	0·2
Streptomycin eluate	0·1	2,4,5-T + chloramphenicol	1·4
2,4,5-T + streptomycin eluate	0·7	Dichloro-*m*-xylenol	0·6
Copper streptomycin	0·3	'Vaseline Green' mixture*	0·6
2,4,5-T + copper streptomycin	0·6	'Streptomycin Vaseline Green'*	0·1
Neomycin sulphate	0·5	Significant difference (P = 0·01)	0·46
2,4,5-T + neomycin sulphate	1·3		

* For composition see text.

Substituted dithiocarbamates

Ferric dimethyldithiocarbamate promotes the rooting of clonal cuttings of *Hevea* species (Tinley, 1961) and it was thought of interest to test it and various other dithiocarbamates in a small-scale trial, but there was no evidence that they increased yield.

Selected herbicides

Three experiments concerned with testing a range of herbicides are summarized in Table 7. In the first trial, the diethylene glycol ester of dalapon showed only slight activity. In the second, however, methoxyipazine and CDAA induced yield increases and were about half as active as 2,4,5-T. Ipazine, eptam and chlorpropham may also have some activity. Paraquat was almost inactive, together with dicryl. Reports that attempts to poison *Hevea* trees by injection of paraquat resulted in yield increases led to the setting up of a further experiment to compare application by Methods B and C. Injection caused significant increase in yield. None of the treatments caused any symptoms suggesting acute toxicity.

Fatty acid esters, glycerides and fatty alcohols

Various fatty acid esters, glycerides and fatty alcohols have been reported to have growth-promoting properties, either alone or acting synergistically with auxins (Stowe, 1958, 1960; Crosby & Vlitos, 1959; Vlitos & Crosby, 1959). Methyl oleate, glycerol trioleate, phytol, docosanol and 2-heptadecanone were tested either alone or in combination with 1% 2,4,5-T, but none showed a significant gain in activity.

Antibiotics and bactericides

Taysum (1961) reported that substantial increases in yield may be obtained by applying a bactericidal mixture designated 'Vaseline green' by a technique similar to Method B. This mixture contains dichloro-*m*-xylenol, neomycin, chloramphenicol, sulphanilamide, sulphathiazole and Malachite Green in a base of petroleum jelly. For comparative purposes, the results of an experiment conducted in co-operation with Taysum are quoted in Table 8. In this trial, various antibiotics and bactericides were tested either alone or in combination with 2,4,5-T, together with the 'Vaseline green' mixture and a similar mixture in which the neomycin had been replaced by the much-less-expensive streptomycin eluate. Neomycin, dichloro-*m*-xylenol and the 'Vaseline green' mixture containing neomycin, gave significant yield increases, but both streptomycin preparations, the 'Vaseline green' mixture containing streptomycin, and chloramphenicol failed to do so. In combination with 1% 2,4,5-T, neomycin and chloramphenicol showed some evidence of additive effects, which, however, were not significant. Extensive trials over a range of concentrations, required to confirm the existence of an additive effect of this type, have not yet been carried out.

Mercury compounds

A number of organic and inorganic mercury compounds have been screened (Table 9). The activity of phenyl mercury acetate and chloride are confirmed and probably ethyl mercury chloride also is active. Methyl mercury acetate, mercuric acetate and mercuric chloride have little or no effect. Phenyl mercury acetate applied by Method B gave increase in yield comparable to those of 2,4,5-T in the first two trials but less in later experiments. Phenyl mercury chloride gave comparable results. In three other experiments, these two compounds were applied above the tapping cut (Method A) to test whether they induced meristematic activity of the treated bark. No significant effects on either latex flow or bark thickness were detected, suggesting first that the compounds do not induce meristematic activity and secondly that they are not translocated readily to their probable site of action in the area of bark drained by tapping. Shimshi (1963) reported that phenyl mercury acetate is relatively immobile in plants, so the effectiveness of application below the tapping cut (Method B) may be ascribed to the shorter distances over which the compounds must be transported.

Table 9. *Relative activities of mercury compounds*

Relative increase in latex yield (2,4,5-T = 1·0)

Expt. no. ...	S41	S44	S52	S53	L7	L14A
Application method ...	B	B	B	B	B	B
Concentration range (%)	1·0	0·2–5·0	1·0	0·3–0·9	1·0	1·0
Mercuric acetate	0·0	—	—	—	—	—
Mercuric chloride	0·3	—	—	—	—	—
Methyl mercury acetate	0·1	—	—	—	—	—
Phenyl mercury acetate	1·1	1·4	0·4	0·6	0·5	0·5
Ethyl mercury chloride	0·5	0·3	—	—	—	—
Phenyl mercury chloride	0·9	1·1	0·5	—	0·7	—
Phenyl mercury chloroacetate	—	—	—	0·4	0·5	—
2,5-Dichlorophenyl mercury chloride	—	—	—	0·3	0·4	—
Significant difference ($P = 0·05$)	0·66	0·63	0·59	0·61	0·63	0·34

DISCUSSION

Out of the eighty-nine compounds tested by application to the bark, twenty-three showed significant activity and these may be subdivided into twelve halogen-substituted phenoxyacetic or phenoxypropionic compounds, two chlorinated benzoic acids, α- and β-naphthaleneacetic acids, 3-indolylacetic acid (IAA), two herbicides (methoxyipazine and CDAA), two bactericides (neomycin and dichloro-*m*-xylenol) and two organo-mercurials (phenyl mercury acetate and chloride). Tests by injection into the trunk add copper sulphate and paraquat to this list.

Some of the remaining compounds were apparently, but not certainly active, because of the high variability of the data. Other compounds might have shown activity if applied by a different technique; only paraquat and copper sulphate were applied by injection (Method C). The site of application relative to the tapping cut may also be important, as the interaction between the activity of the organo-mercurial compounds and the method of application shows. Fortunately most of the compounds reported to be inactive have been tested by Method B, which is apparently the more sensitive

In tests of ability to induce meristematic activity in the bark, nine out of the thirteen 'phenoxy' compounds and two of the five substituted benzoic acids were active. However, two compounds, α-(2-methyl-4-chlorophenoxy) propionic acid and 2,3,6-trichlorobenzoic acid, produced thicker bark but failed to enhance yield. This apparen contradiction may arise from the higher errors associated with the yield data. Within the two series of substituted phenoxyacetic and benzoic acids active compounds usually both increase yield and induce a thicker bark, but such a correlation is no always observed; thus the organo-mercurials do not increase bark regeneration despite their relatively large effects on yield.

The measurements of yield increases were precise enough only to permit broad and approximate classifications of relative activity. Nevertheless, the varying capacity of the phenoxyalkylcarboxylic acids to enhance yield matches their activities in most bioassays of growth regulation (Zimmerman & Hitchcock, 1942; Hansch & Muir, 1950; Fawcett, Wain & Wightman, 1955; Pybus, Smith, Wain & Wightman, 1959). Thus all the compounds that increased yield are substituted in the 4-, 2,4- or 2,4,5-positions, whereas the 2,6-dichloro- and 2,4,6-trichlorophenoxyacetic acids appear to be inactive. The mean activities determined for 4-chlorophenoxyacetic acid, 2,4-D and 2,4,5-T are 0·5, 0·8 and 1·0 respectively, although 2,4-D was at least as active as 2,4,5-T in some experiments.

Substitution of fluorine in place of chlorine had variable effects; 2,4-dichloro-5-fluorophenoxyacetic acid is probably the most active compound tested, as it out-yielded 2,4,5-T in three out of four trials, although not significantly. In contrast, replacement of chlorine by fluorine in the 4-position, as in 4-fluorophenoxyacetic acid and 2-chloro-4-fluorophenoxyacetic acid, has little effect on the ability to promote latex flow but decreases ability to increase bark thickness. This divergence supports the previous conclusion that the induction of a greater flow of latex is not invariably correlated with the enhancement of bark thickness.

The results support the current preference for substituted phenoxyacetic acids rather than substituted phenoxypropionic acids, and for formulating them as the *n*-butyl esters. Although the main experiment on the chlorobenzoic acids was inconclusive the group as a whole appears less active than the chlorophenoxyacetic acids; 3,6-dichloro-2-methoxybenzoic acid had the highest activity, but was less active than 2,4,5-T.

Effects on latex yield can be detected over only a narrow range of concentrations. Also, the difference between the yield increase induced by very active and slightly active compounds is small—less than tenfold—compared with a range of about a thousandfold in most bioassays of extension growth (Fawcett *et al.* 1955; Fawcett *et al.* 1959; Pybus *et al.* 1959).

The activities of bactericidal compounds, such as neomycin and dichloro-*m*-xylenol, have been explained by their effects on the bacterial population that inhabits the surface the tapping cut and penetrates the cut ends of latex vessels (Taysum, 1961). However, a direct effect on the metabolism of the tree is also possible, especially in view of the heterogeneous range of active compounds. It may be significant that all the active compounds are to some degree phytotoxic; 2,4,5-T makes an excellent arboricide for *Hevea* at a concentration of 5% in diesel oil. The common factor may be a selective and limited toxicity. Recent studies on the mode of action of 2,4,5-T in promoting latex flow (Boatman, 1966; Pakianathan, Boatman & Taysum, 1966; Buttery & Boatman, 1967) indicate that the major effect is to delay sealing of the cut ends of the latex vessels.

Various mixtures of compounds were tested in these experiments but none was conclusively shown to be superior to 2,4,5-T alone at its optimum concentration. The results support the continued use of 2,4,5-T and 2,4-D in commercial practice, with the qualification that 2,4-dichloro-5-fluorophenoxyacetic acid might be a profitable alternative, if available at a competitive price.

We are grateful to Amchem Products Inc., Dow Chemical Co., Mirvale Chemic
Co., I.C.I. (Malaya) Ltd., Murphy Chemical Co. and Badische Anilin und Sod
Fabrik AG (BASF) for gifts of chemicals. We thank the manager and staff of R.R.
Experiment Station, where the experimental work was carried out, for their c
operation, Mr D. R. Westgarth, Mr A. Fong Chu Chai, Mr G. C. Iyer and Mr
Narayanan for assisting with the statistical analyses, and Messrs T. S. V. Aiya
R. Perumal, B. Manikam, K. Vasudevan, S. Tharmalingam, M. Zain and others f
technical help and Mr H. P. Bauer for synthesizing a number of the compounds teste

REFERENCES

ABRAHAM, P. D. & BOATMAN, S. G. (1964). The influence of formulation on yield response a
bark damage following the application of yield stimulants above the tapping cut. *J. Rubb. Re*
Inst. Malaya **18**, 211.

ABRAHAM, P. D. & NG, E. K. (1968). Stimulation of young rubber. Private communication.

ABRAHAM, P. D. & TAYLER, R. S. (1967). Stimulation of latex flow in *Hevea brasiliensis*. *Ex*
Agric. **3**, 1.

BAPTISTE, E. D. C. (1955). Stimulation of yield in *Hevea brasiliensis*. I. Prewar experimen
with vegetable oils. *J. Rubb. Res. Inst. Malaya* **14**, 355.

BAPTISTE, E. D. C. & DE JONGE, P. (1955). Stimulation of yield in *Hevea brasiliensis*. II. Effe
of synthetic growth substances on yield and bark renewal. *J. Rubb. Res. Inst. Malaya* **1**
362.

BLACKMAN, G. E. (1961). The stimulation of latex flow by plant growth regulators. *Proc. Na*
Rubb. Res. Conf. Kuala Lumpur 1960, p. 19.

BOATMAN, S. G. (1966). Preliminary physiological studies on the promotion of latex flow
plant growth regulators. *J. Rubb. Res. Inst. Malaya* **19**, 243.

BUTTERY, B. R. & BOATMAN, S. G. (1967). Effects of tapping, wounding and growth regulato
on turgor pressure in *Hevea brasiliensis*. *J. exp Bot.* **18**, 644.

CHAPMAN, G. W. (1951). Plant hormones and yield in *Hevea brasiliensis*. *J. Rubb. Res. In*
Malaya **13**, 167.

COMPAGNON, P. & TIXIER, P. (1950). Sur une possibilité d'améliorer la production d'*Hev*
brasiliensis par l'apport d'oligo-elements. *Rev. Gen. Caoutch.* **27**, 525.

CROSBY, D. G. & VLITOS, A. J. (1959). Growth substances from Maryland Mammoth tobacc
long chain alcohols. *Contrib. Boyce Thompson Inst.* **20**, 283.

DE JONGE, P. (1957). Stimulation of bark renewal of *Hevea* and its effect on the yield of late
J. Rubb. Res. Inst. Malaya **15**, 53.

FAWCETT, C. H., PASCAL, R. M., PYBUS, M. B., TAYLOR, H. F., WAIN, R. L. & WIGHTMAN,
(1959). Plant growth regulatory activity in homologous series of ω-phenoxy alkane carboxyl
acids and the influence of ring substitution on their breakdown by β-oxidation with pla
tissue. *Proc. roy. Soc.* B **150**, 95.

FAWCETT, C. H., WAIN, R. L. & WIGHTMAN, F. (1955). Studies on plant growth-regulati
substances. VIII. The growth-promoting activity of certain aryloxy- and arylthio-alkan
carboxylic acids. *Ann. appl. Biol.* **43**, 342.

HANSCH, C. & MUIR, R. M. (1950). The ortho effect in plant growth regulators. *Pl. Physiol.* **2**
389.

HO, C. Y. & PAARDEKOOPER, E. C. (1965). Application of stimulants to the virgin bark in clo
trials. *Rubb. Res. Inst. Malaya Planters' Bull.* no. 80, p. 150.

HOWARD, W. L., MCLANE, S. R. & WEINTRAUB, R. L. (1958). Halogenated phenyl alkyl ethe
found effective as herbicides and also for the production of sterility. U.S. Patent 2,839,58

KLAASENS, K. H. & SCHOOT, C. J. (1965). Some fluoro-substituted phenoxyacetic acids. *R*
Trav. Chim. **75**, 186.

MCRAE, D. H. & BONNER, J. (1952). Diortho substituted phenoxyacetic acids as anti-auxi
Pl Physiol. **27**, 834.

MIGRDICHIAN, V. (1957). *Organic syntheses*, p. 311. New York: Reinhold.

NESMEJANOW, A. N. & KAHN, E. J. (1929). Aus dem Gebeit der quecksilberorganischen Verbindungen, I. Mitteilung: Eine neune Methode zur Synthese von aromatischen quecksilberorganischen Salzen. *Ber. dt. Chem. Ges.* **62**, 1010, 1018.

NG, E. K., ABRAHAM, P. D., GILL, D. S. & P'NG, T. C. (1965). Exploitation of young rubber: preliminary results. *Rubb. Res. Inst. Malaya Planters' Bull.* no. 80, p. 177.

PAKIANATHAN, S. W., BOATMAN, S. G. & TAYSUM, D. H. (1966). Particle aggregation following dilution of *Hevea* latex. A possible mechanism for the closure of latex vessels after tapping. *J. Rubb. Res. Inst. Malaya* **19**, 259.

PYBUS, M. B., SMITH, M. S., WAIN, R. L. & WIGHTMAN, F. (1959). Studies on plant growth-regulating substances. XIII. Chloro- and methyl-substituted phenoxyacetic and benzoic acids. *Ann. appl. Biol.* **47**, 173.

PYBUS, M. B., WAIN, R. L. & WIGHTMAN, F. (1959). Studies on plant growth-regulating substances. XIV. Chloro-substituted phenylacetic acids. *Ann. appl. Biol.* **47**, 593.

SHIMSHI, D. (1963). Effect of chemical closure of stomata on transpiration in varied soil and atmospheric environments. *Pl. Physiol.* **38**, 709.

STOWE, B. B. (1958). Growth promotion in pea epicotyl sections by fatty acid esters. *Science* **128**, 421.

STOWE, B. B. (1960). Growth promotion in pea stem sections. I. Stimulation of auxin and gibberellin action by alkyl lipids. *Pl. Physiol.* **35**, 262.

TAYSUM, D. H. (1961). Yield increases by the treatment of *Hevea brasiliensis* with antibiotics. *Proc. Nat. Rubb. Res. Conf. Kuala Lumpur* 1960, p. 224.

TINLEY, G. H. (1961). Effect of ferric dimethyldithiocarbamate on the rooting of cuttings of *Hevea brasiliensis. Nature Lond.*, **191**, 1217.

VLITOS, A. J. & CROSBY, D. G. (1959). Isolation of fatty alcohols with plant-growth promoting activity from Maryland Mammoth tobacco. *Nature, Lond.* **184**, 462.

ZIMMERMAN, P. W. & HITCHCOCK, A. E. (1942). Substituted phenoxy and benzoic acid growth substances and the relation of structure to physiological activity. *Contrib. Boyce Thompson Inst.* **12**, 321.