Stimulation of Latex Flow in Hevea Brasiliensis by 4-Amino–3,5,6-Trichloropicolinic Acid and 2-Chloroethanephosphonic Acid

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Application of 4-amino–3,5,6-trichloropicolinic acid, alone or in mixtures with 2,4,5-T, gave larger and more persistent yield responses than application of 2,4,5-T alone in one experiment, but this was not confirmed in another. One morphactin was moderately effective but another was ineffective. N*-dimethylamino-succinamic acid did not stimulate longer flow or give larger yields.*

Acetylene, ethylene, halogenoparaffins and 2-chloroethanephosphonic acid (which releases ethylene in plant tissues) were all effective in prolonging flow and increasing yields. All effective non-gaseous stimulants of latex flow seemed to have one common feature, namely, production of ethylene.

The stimulation of latex flow by application of suitable formulations of 2,4-D and 2,4,5-T to obtain greater yields of rubber has been successful experimentally and commercially on *Hevea* trees at least 15 years old, whose low panels are tapped on renewed bark (BLACKMAN 1961; DE JONGE, 1961; RUBBER RESEARCH INSTITUTE OF MALAYA, 1963; ABRAHAM AND TAYLER, 1967). Copper sulphate also stimulates greater yields of latex and rubber when injected into the tree (TIXIER, 1951; MAINSTONE AND TAN, 1964; LOWE, 1965; BANCHI AND POLINIERE, 1968a). However, caution in the use of copper sulphate is advisable owing to risks of contaminating the rubber or damaging the trees, unless the application is competently supervised.

ABRAHAM *et al.* (1968) tested 89 compounds by application to the bark, and 23 of them showed significant activity; but, only 2,4-dichloro 5-fluoro-phenoxyacetic acid—which is not yet available commercially—was shown to be definitely superior to 2,4,5-T at its optimum concentration. Most of the active compounds were synthetic growth substances of similar physiological action to auxin or 3-indolylacetic acid, which was itself effective. Four other types of compounds were significantly active: three herbicides (2-methoxy-4-diethylamino-6-isopropylamino-1,3,5-triazine, *N*,*N*-diallyl-α-chloroacetamide and paraquat), two bactericides (neomycin and dichloro-*m*-xylenol), two organomercurials (phenyl mercury acetate and chloride) and copper sulphate. Paraquat and copper sulphate were effective only when applied by injection. Increased yields of latex and rubber have been obtained by the application of gases to the trunk of the tree: ethylene oxide (TAYSUM, 1961), chloroform (BANCHI, 1967) and acetylene (BANCHI AND POLINIERE, 1968b).

Recent investigations have had a variety of objectives: firstly, to discover new active compounds; secondly, to develop improved methods of formulation and application; thirdly, to extend the use of these procedures for the effective exploitation of trees of different ages; and, fourthly, to investigate the underlying mechanisms responsible for the increased yield of latex and, in particular, to elucidate whether there is a common principle involved for compounds of high activity. To obtain such information both on the nature of the compounds increasing the flow of latex and on the mechanisms by which this is achieved, many kinds of experimental treatments must perforce be

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tried, which would clearly be uneconomic in commercial practice.

METHODS AND MATERIALS

Trees planted in Fields 48 and 49 of the R.R.I.M. Experiment Station at Sungei Buloh and budded in 1950/51 with mixed clones derived from legitimate seedlings were used, except that the final experiments with halogenoparaffins employed budded trees of similar age of clone Tjir 1 only in Field 47. All have been tapped on the S/2.d/2.100% system, throughout their previous history and during the experiments. The first panel of bark of first renewal was tapped during the experiments, except that some of the Tjir 1 trees were tapped at the foot of the second panel of virgin bark. The total yield of dry rubber per tree per tapping of every tree was recorded prior to the application of the treatments, except for the Tjir 1 trees for which volume of latex was recorded, so that trees of similar yield could be allocated to each treatment and appropriate adjustments made for the small differences in pre-treatment yields.

The solid and liquid chemicals, or concentrated solutions of them in carriers as supplied by the manufacturers, were diluted for application. The diluent in the first experiment was a mixture of three parts liquid paraffin and one part petroleum jelly by weight. In the other experiments, palm oil was used. The concentrations are given as the percentage of the acid equivalent of the active ingredient in the mixture as applied. The mixtures so obtained were applied thinly to the lightly scraped bark below the tapping cut.

The gases were applied under jackets of polythene sheet about the tapping panel or in chambers cemented onto the bark of the tree. Acetylene was obtained by evolution from calcium carbide suitably lodged under the polythene jacket in such a way as to allow contact with the moisture condensed under the sheet. The source of ethylene was evaporation from a previously chilled alcoholic solution of ethylene as it warmed up to ambient temperature. The ethylene was prepared by dehydration of ethanol with concentrated sulphuric acid. The halogenoparaffins were also allowed to evaporate under the hood or in the chamber.

Yields were recorded by conventional methods such as coagulation, drying and weighing the whole crop including the 'late drip' portion, or by measuring the volume of latex and determining the dry rubber content of samples and weighing the dried 'late drip' separately. Turgor pressures were determined by the method of BUTTERY AND BOATMAN (1964) and osmotic concentrations were assessed by a vapour pressure osmometer (PAKIANATHAN, 1967).

RESULTS

Experiment 1

The first experiment compared ten treatments including the control, where the bark was scraped. There were 50 trees per treatment. The n-butyl ester of 2,4,5-T at 1% *a.e.*, and a proprietary formulation containing 1.5% 2,4-D were included to provide comparisons with commercial practice. The experimental treatments were: three concentrations (0.11, 0.33 and 0.99% *a.e.*) of the iso-octyl ester of 4-amino–3,5,6-trichloropicolinic acid (picloram), two mixtures of picloram and 2,4,5-T (respectively 0.11 and 0.89%, and 0.33 and 0.67%) and two morphactins A and B at 1%, which were respectively n-butyl-9-hydroxyfluorene-(9)-carboxylate and methyl-2-chloro-9-hydroxyfluorene-(9)-carboxylate. Treatments were applied three times at intervals of six months. Yields for the eighteen months following the first application are given in *Table 1* and for the treatments of most interest in *Figure 1*.

During each of the six-month periods and over the whole eighteen months the two treatments giving the biggest responses were 0.99% picloram and the mixture of 0.33% picloram and 0.67% 2,4,5-T. These were followed by the standard formulations in commercial practice, which gave a typical response for trees of this age, then by the picloram/2,4,5-T mixture of lower picloram content, morphactin B at 1% and the lower concentrations (0.33 and 0.11%) of picloram. Morphactin A produced hardly any effect.

After the second application, all treatments showed a distinct delayed response in reaching maximum yield expressed as percentage of control until the second month (Month 8 in

TABLE 1. MEAN YIELDS IN GRAMS OF RUBBER PER TREE PER TAPPING ADJUSTED FOR DIFFERENCES BEFORE TREATMENT AND EXPRESSED IN PARENTHESIS AS PERCENTAGE OF CONTROL

Treatment	Months after first application																		Mean for first		
	1*	2	3	4	5	6	7**	8	9	10	11	12	13***	14	15	16	17	18	6 months	12 months	18 months
Control	37.7	35.8	49.0	47.0	46.1	49.1	52.4	33.4	22.1	27.5	32.2	36.1	39.3	46.4	43.9	46.2	52.7	36.6	44.1	39.0	40.8
1.5% 2,4-D	76.1 (202)	60.1 (168)	61.0 (124)	53.1 (113)	50.1 (109)	50.2 (102)	79.1 (151)	62.3 (187)	24.8 (112)	25.8 (94)	29.9 (93)	35.2 (98)	69.2 (176)	62.3 (134)	56.2 (128)	43.1 (93)	53.9 (102)	37.4 (102)	58.4 (132)	50.6 (130)	51.7 (127)
1% 2,4,5-T	69.5 (184)	52.4 (146)	57.8 (118)	51.5 (110)	48.5 (105)	48.5 (99)	68.9 (131)	54.3 (163)	31.1 (141)	35.1 (128)	35.2 (109)	35.7 (99)	62.1 (158)	50.9 (110)	52.1 (119)	40.8 (88)	49.3 (94)	36.4 (99)	54.7 (124)	49.0 (126)	48.9 (120)
0.11% picloram	48.7 (129)	50.1 (140)	56.5 (115)	51.8 (110)	50.3 (109)	52.8 (108)	54.6 (104)	43.2 (129)	34.2 (155)	38.5 (140)	42.2 (131)	42.1 (117)	55.1 (140)	58.3 (126)	48.3 (110)	51.1 (111)	57.2 (109)	43.0 (117)	51.7 (117)	47.1 (121)	48.8 (120)
0.33% picloram	56.4 (150)	54.3 (152)	54.6 (111)	49.4 (105)	48.2 (105)	53.9 (110)	62.6 (119)	55.2 (165)	31.9 (144)	32.2 (117)	32.4 (101)	33.2 (92)	49.6 (126)	53.7 (116)	47.5 (108)	48.0 (104)	51.8 (98)	38.9 (106)	52.8 (120)	47.0 (120)	47.4 (116)
0.99% picloram	67.3 (179)	65.0 (182)	67.3 (137)	58.2 (124)	57.6 (125)	59.9 (122)	74.6 (142)	53.3 (160)	25.8 (117)	29.4 (107)	34.1 (106)	38.0 (105)	71.1 (181)	71.5 (154)	50.2 (114)	49.2 (106)	54.5 (103)	45.2 (123)	62.6 (143)	52.5 (135)	54.0 (132)
0.11% picloram +0.89% 2,4,5-T	66.4 (176)	52.6 (147)	53.8 (110)	50.6 (108)	47.7 (103)	49.6 (101)	65.0 (124)	48.5 (145)	31.6 (143)	31.3 (114)	37.4 (116)	37.5 (104)	68.2 (174)	59.2 (128)	48.7 (111)	46.3 (100)	48.2 (91)	35.5 (97)	53.5 (121)	47.7 (122)	48.8 (120)
0.33% picloram +0.67% 2,4,5-T	74.5 (198)	61.1 (171)	62.5 (128)	61.3 (130)	53.4 (116)	56.0 (114)	70.9 (135)	52.1 (156)	27.9 (126)	35.1 (128)	35.9 (111)	40.6 (112)	80.1 (204)	64.0 (138)	46.5 (106)	46.0 (100)	56.9 (108)	46.9 (128)	61.5 (139)	52.6 (135)	54.0 (132)
1% Morphactin A	48.9 (130)	46.1 (129)	55.8 (114)	49.4 (105)	44.7 (97)	45.8 (93)	49.9 (95)	42.4 (127)	25.3 (114)	25.1 (91)	29.5 (92)	31.1 (86)	43.4 (110)	46.9 (101)	43.7 (100)	46.2 (100)	51.1 (97)	40.7 (111)	48.5 (110)	41.2 (105)	42.6 (104)
1% Morphactin B	51.7 (137)	53.9 (151)	58.2 (119)	54.4 (116)	53.3 (116)	55.6 (113)	57.1 (109)	49.6 (149)	36.0 (163)	35.1 (128)	36.3 (113)	38.9 (108)	47.8 (122)	54.6 (118)	50.1 (114)	56.0 (121)	57.5 (109)	47.6 (130)	54.5 (124)	48.3 (124)	49.7 (122)

*First application at the beginning of the 1st month **Second application at the beginning of the 7th month ***Third application at the beginning of the 13th month

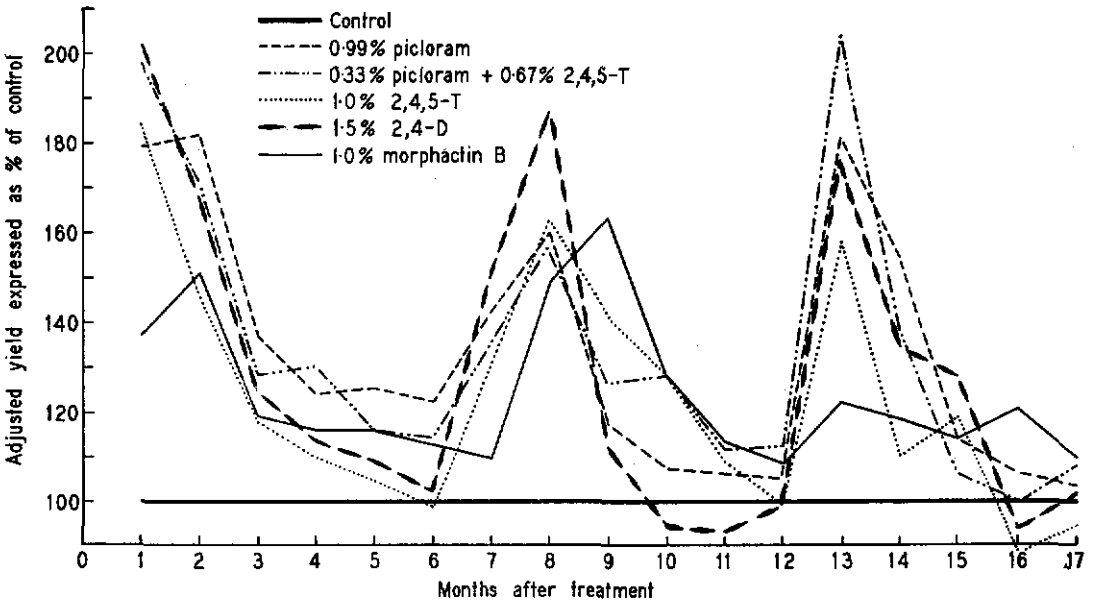

Figure 1. Five treatments of interest compared with control in Experiment 1.

Table 1). However, this was in part due to the sharp drop in the yield of the control at this time, which coincided with the annual leaf shedding or 'wintering' and subsequent refoliation. Actual yields exhibited a maximum during the first month after that application. Inspection of *Figure 1* reveals that picloram alone and the morphactins showed a tendency towards a more pronounced delay in positive response than 2,4,5-T alone, even when allowance is made for seasonal effects. The mixtures of picloram and 2,4,5-T followed a similar but less pronounced trend.

Experiment 2

The second experiment compared treatments with 1.5% 2,4-D, 1% 2,4,5-T (n-butyl ester), 1% picloram (iso-octyl ester), 3:1 mixtures with combined acid equivalent of 1% of 2,4-D and picloram and 2,4,5-T and picloram, 1% *N*-dimethylamino-succinamic acid, CEPA (2-chloroethanephosphonic acid) at 0.3, 0.9 and 2.7%, with control trees which were scraped and to which only the palm oil carrier was applied. There were 45 trees per treatment. The yields, adjusted for pre-treatment differences for the first eight months after the first application, are given in *Table 2*. The applications were repeated six months after the first. The highest percentage responses over the whole period were from 2,4,5-T mixed with picloram, the highest concentration of CEPA and 2,4,5-T alone. These were followed by the mixture of 2,4-D and picloram, and then by either 2,4-D or picloram alone. CEPA at 0.9% (*cf.*, 1.0% for the others) gave a moderate response during the first month after the first application and a fair but rapidly declining response after the second. Both applications of *N*-dimethylamino-succinamic acid at 1% were ineffective. The lowest concentration of CEPA at 0.3% gave only a brief response to the second application.

The results of this experiment differed in some respects from those of the previous experiment. Firstly, the ranking of 2,4-D at 1.5% and of 2,4,5-T at 1% was reversed. Secondly, picloram was not superior to 2,4,5-T. Thirdly, no markedly delayed response was evident with picloram.

The delayed response was most evident in the

TABLE 2. MEAN YIELDS IN GRAMS OF RUBBER PER TREE PER TAPPING ADJUSTED FOR DIFFERENCES BEFORE TREATMENT AND EXPRESSED IN PARENTHESIS AS PERCENTAGE OF CONTROL

Treatment	Months after first application								Mean for first	
	1*	2	3	4	5	6	7**	8	6 months	8 months
Control	55.7	64.2	56.2	52.5	58.0	55.2	60.8	47.0	57.0	56.2
1.5% 2,4-D	84.6 (152)	74.2 (116)	59.8 (106)	52.4 (100)	50.6 (87)	50.3 (91)	78.0 (128)	45.2 (96)	62.0 (109)	61.9 (110)
1.0% 2,4,5-T	93.0 (167)	83.6 (130)	65.2 (116)	57.5 (110)	62.8 (108)	60.1 (109)	83.3 (137)	42.8 (91)	70.4 (124)	68.5 (122)
1.0% picloram	74.4 (134)	76.1 (119)	64.5 (115)	57.0 (109)	62.6 (108)	57.7 (105)	77.2 (127)	50.8 (108)	65.4 (115)	65.0 (116)
0.25% picloram +0.75% 2,4-D	88.2 (158)	79.7 (124)	59.1 (105)	53.1 (101)	57.3 (99)	56.0 (101)	91.2 (150)	49.3 (105)	65.6 (115)	66.7 (119)
0.25% picloram +0.75% 2,4,5-T	96.2 (173)	88.1 (137)	63.8 (114)	54.5 (104)	59.7 (103)	57.5 (104)	98.0 (161)	56.0 (119)	70.0 (123)	71.7 (128)
1.0% *N*-dimethylamino-succinamic acid	57.8 (104)	62.5 (97)	58.4 (104)	52.1 (99)	57.2 (99)	56.3 (102)	64.1 (105)	41.4 (88)	57.4 (101)	56.2 (100)
0.3% CEPA	53.3 (96)	60.7 (95)	56.6 (101)	52.7 (100)	56.1 (97)	49.1 (89)	78.3 (129)	44.7 (95)	54.8 (96)	56.4 (100)
0.9% CEPA	64.4 (116)	63.8 (99)	58.9 (105)	56.0 (107)	59.8 (103)	54.7 (99)	87.5 (144)	43.5 (93)	59.6 (105)	61.1 (109)
2.7% CEPA	88.8 (159)	77.4 (121)	60.4 (107)	55.9 (106)	62.7 (108)	60.1 (109)	104.7 (172)	56.7 (121)	67.6 (119)	70.8 (126)

* First application at beginning of the 1st month

** Second application at beginning of the 7th month

first experiment when the control yield was declining or steady, but it was less marked after the third application when the control yield was rising as in the second experiment. The applications which seem to have given a delayed response from picloram were made in July 1967 and January 1968 when relatively dry weather prevailed, whereas the first and second applications in the second experiment and the third application in the first experiment were made at the beginning of May, the beginning of November and mid-July 1968 when rainfall was comparatively heavy.

Experiment 3

The third experiment compared 1% 2,4,5-T, CEPA at 2.7, 8.1 and 24.3%, acetylene and ethylene applied as gases in unknown quantities and concentrations, with control trees scraped and treated with palm oil alone. There were only 10 trees per treatment—this small number was dictated by the limited amounts available of ethylene and CEPA. The quantity of ethylene applied was probably considerably less than that of acetylene, so the treatments with gases must be judged qualitatively. The applications were made in September; during the latter part of the experiment, rain interfered frequently. The results for the first two months after treatment are given in *Table 3*. During the second month after treatment, the total yield in all experimental treatments fell below the control level in relative yield and almost to the pre-treatment level in actual yield. This trend has

TABLE 3. TOTAL YIELD IN GRAMS OF RUBBER PER TREE PER TAPPING ADJUSTED FOR DIFFERENCES BEFORE TREATMENT AND EXPRESSED IN PARENTHESIS AS PERCENTAGE OF CONTROL, MEAN PERCENTAGE DRY RUBBER CONTENT OF THE LATE DRIP AND PERCENTAGE OF THE TOTAL CROP HARVESTED AS LATE DRIP DURING THE FIRST (A) AND SECOND (B) MONTH AFTER TREATMENT

Treatment	Total yield, g		Dry rubber content %		Late drip %	
	A	B	A	B	A	B
Control	47.6	52.9	40.1	40.9	9.5	18.7
1.0% 2,4,5-T	73.4 (154)	54.0 (85)	36.9	39.5	30.6	27.9
2.7% CEPA	74.5 (157)	37.8 (71)	33.7	37.4	31.8	20.3
8.1% CEPA	79.8 (168)	42.5 (80)	32.9	36.8	33.3	17.4
24.3% CEPA	73.7 (155)	49.2 (93)	31.5	34.7	35.3	23.9
Acetylene	88.1 (185)	42.2 (80)	36.1	40.2	30.8	23.4
Ethylene	53.3 (112)	35.7 (67)	37.6	42.4	11.1	9.2

sometimes been experienced before when treatments were applied at an unfavourable period for maximum response and has complicated the interpretation of the results. The small number of trees also detracted from precision.

All experimental treatments increased yield during the first month. The small response to ethylene may have been due to the probably low initial concentration and subsequent losses by diffusion. The greatest response was to acetylene, which was renewed for at least some days by further evolution from the calcium carbide lodged under the enveloping polythene jacket. CEPA, at 8.1%, gave the second largest increase. This was followed closely by 2.7% CEPA, 24.3% CEPA and 1% 2,4,5-T. The yield from 24.3% CEPA was not much less than that from 8.1% CEPA and probably did not indicate any adverse effect from this highest concentration of CEPA, because of all the experimental treatments this suffered the least depression in yield during the second month.

The dry rubber content (d.r.c.) of the latex harvested at the normal hour for collection (about 4 hours after tapping) was depressed by all experimental treatments (except for ethylene during the second month when yields were depressed). The CEPA treatments depressed the d.r.c. most during both months. Acetylene caused the greatest increase in yield during the first month and 2,4,5-T gave an increase similar to CEPA, but neither acetylene nor 2,4,5-T depressed the d.r.c. as much as CEPA. All experimental treatments increased during the first month the proportion of the crop harvested after the normal time of collection, *i.e.*, the percentage 'late drip', indicating longer periods of flow. The percentage of late drip and yield increase were correlated.

The total yields, adjusted for pre-treatment differences for each tapping during the first month after treatment, are plotted as percentage of control in *Figure 2* for the treatments with acetylene, ethylene, 2.7% CEPA and 1% 2,4,5-T. The two gases, acetylene and ethylene, and CEPA, whose hydrolysis releases ethylene in plant tissues, gave the maximum yield response on the first tapping, which was the third

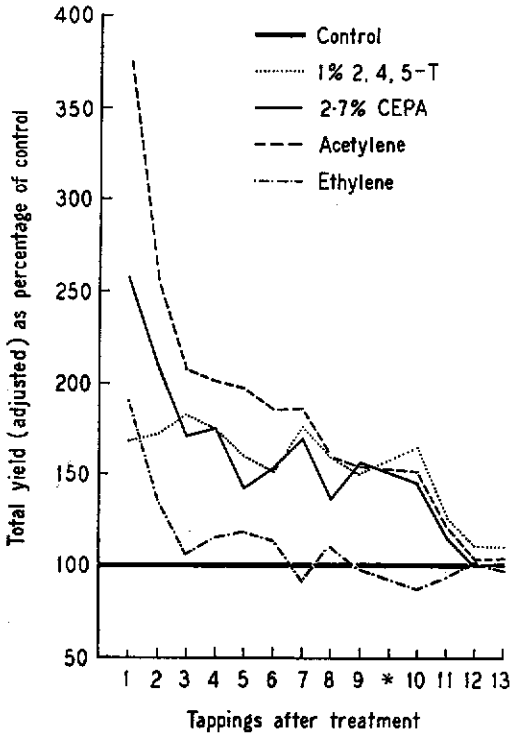

Figure 2. Four treatments of interest compared with control in Experiment 2 (No tapping on usual day due to rain).*

day after application owing to the unexpected intervention of a special holiday. The yield of the acetylene-treated trees fell almost to control level by the twelfth tapping, the yield for 2.7% CEPA likewise fell somewhat erratically over the same period, the 8.7 and 24.3% CEPA treatments (not shown in *Figure 2*) did not decline quite so rapidly. By the third to seventh tapping, the yield had fallen rapidly in the ethylene treatment. The maximum yield was not attained until the third tapping in the 2,4,5-T treatments, thereafter a moderate level was maintained somewhat erratically until the decline at the end of the month, *i.e.*, the fourteenth tapping. The data were too variable for precise conclusions, but the delay of several tappings before attaining the maximum yield with 2,4,5-T was consistent with previous reports on 2,4-D and 2,4,5-T (BAPTIST AND DE JONGE, 1955; DE JONGE, 1955).

The dry rubber content of the latex fell rather erratically from the beginning to the middle of the month, then showed a slight recovery and ended at a lower value than at the beginning. This was so in all treatments including control. The d.r.c. in the ethylene, acetylene and CEPA treatments had already fallen at the first tapping after treatment and the fluctuations in d.r.c. for these treatments were roughly parallel to those of the control. The 2,4,5-T treatment was exceptional in that the d.r.c. at the first tapping was the same as control and did not fall markedly below control until the third tapping; it then followed similar fluctuations as in the other treatments and at the end of the month had a value between those for the gases and those for the CEPA treatments, all less than the control level. Thus the response in d.r.c. as well as in yield seemed to show a slight delay in the 2,4,5-T treatment compared with the other treatments. CEPA was similar to the gases in exhibiting an immediate response in d.r.c. and a similar pattern of yield response, but differed in the much lower d.r.c. attained.

Experiment 4

The fourth experiment was also on a small scale and of short duration to test for synergism between 2,4,5-T and CEPA. There were 10 trees in each treatment. In addition to the control, the treatments were 0.5 and 1.0% 2,4,5-T, 1.5 and 3.0% CEPA, and mixtures of 2,4,5-T and CEPA at 0.5 and 1.5%, and 1.0 and 3.0% respectively. The results are given in *Table 4*. The greater depression in d.r.c. caused by 1.5% CEPA than by 3.0% CEPA is anomalous. This may be due in part to the small number of trees. CEPA alone or in mixtures seemed to produce more rapid responses in yield and d.r..c than 2,4,5-T. The effects of 2,4,5-T and CEPA may be additive but are not powerfully synergistic.

Experiment 5

In the fifth experiment, the osmotic concentrations and turgor pressures were determined before tapping and the turgor pressures were again recorded 5 minutes after tapping. For

TABLE 4. TOTAL YIELD IN GRAMS OF RUBBER PER TREE PER TAPPING ADJUSTED *FOR DIFFERENCES BEFORE TREATMENT AND EXPRESSED IN PARENTHESIS AS* PERCENTAGE OF CONTROL AND MEAN PERCENTAGE DRY RUBBER CONTENT OF THE LATEX, DURING THE FIRST (A) AND SECOND (B) MONTH AFTER TREATMENT

Treatment	Total yield		Dry rubber content %	
	A	B	A	B
Control	50.5	45.7	42.8	41.7
0.5% 2,4,5-T	57.6 (114)	49.0 (107)	42.2	41.9
1.5% CEPA	67.2 (133)	52.4 (115)	38.3	38.0
0.5% 2,4,5-T +1.5% CEPA	74.9 (148)	57.1 (125)	37.4	36.7
1.0% 2,4,5-T	74.0 (147)	55.6 (122)	41.1	40.2
3.0% CEPA	92.8 (184)	67.8 (148)	40.7	40.0
1.0% 2,4,5-T +3.0% CEPA	90.6 (179)	59.0 (129)	38.6	39.2

these purposes, trees representative of the four treatments were selected for measurement on the day before and the third tapping (*i.e.*, on the fifth day) after application of the several treatments. Flow rates and total solids contents were also determined for the third tapping. The four treatments were: control, bark scraped below the cut and palm oil only applied, compared with 1% 2,4,5-T, 2.7% CEPA and acetylene evolved from 30 g calcium carbide.

The results are given in *Table 5*. Before tapping the osmotic concentrations and turgor pressures agreed closely, although the latter were consistently slightly higher. Save for two minor exceptions there was a marked fall in turgor pressure after tapping. Again, with one trivial exception, the fall in turgor pressure was greater the closer to the tapping cut. In general, the fall was greater below the cut than above. The experimental treatments did not effect any major change in this pattern, except that the pressure fall after treatment, was greater above the cut, especially close to the cut, than in the control or before treatment. 2,4,5-T and acetylene also exhibited greater decreases in turgor pressure 80 cm distant below the cut. The experimental treatments caused only very slight reductions in the osmotic concentration and the turgor pressure of the latex in the bark before tapping.

The flow characteristics given in *Table 6* may be somewhat misleading because they are based on single trees. However, the decline in total solids, longer periods of flow and greater yields in latex volume and total solids in the experimental treatments are consistent with previous results. CEPA does not appear to have altered the initial flow rate or perhaps even slightly reduced it, whereas 2,4,5-T and acetylene seem to have increased the initial flow rate. These two treatments caused greater falls in turgor pressure distant from the cut.

Experiment 6

This was a pilot experiment to ascertain whether other gases reported to cause formative and epinastic growth responses in etiolated pea seedlings could stimulate latex flow. Bromoethane (ethyl bromide) was tested at five concentrations on 1 tree each. The data for volume of latex harvested only, are given

TABLE 5. OSMOTIC CONCENTRATION (OP), TURGOR PRESSURE (TP) AND CHANGE IN TURGOR PRESSURE (DT) IN ATMOSPHERES AT VARIOUS DISTANCES ABOVE AND BELOW THE TAPPING CUT BEFORE AND 5 MINUTES AFTER TAPPING 1 DAY BEFORE AND 5 DAYS AFTER TREATMENT

Treatment period	Distance* from tapping cut, cm	Control				1% 2,4,5-T				2.7% CEPA				Acetylene			
		Before tapping		After tapping		Before tapping		After tapping		Before tapping		After tapping		Before tapping		After tapping	
		OP	TP	TP	DT	OP	TP	TP	DT	OP	TP	TP	DT	OP	TP	TP	DT
Before treatment	+80	10.2	10.4	10.5	−0.1	9.8	10.8	9.3	1.5	9.2	9.5	9.6	−0.1	9.5	9.8	9.2	0.6
	+20	10.1	10.2	8.7	1.5	9.3	10.2	8.7	1.5	9.4	9.6	8.2	1.4	8.9	9.7	8.1	1.6
	+ 5	9.7	9.5	7.5	2.0	9.2	9.4	5.8	3.6	9.2	9.4	7.7	1.7	9.8	10.1	7.7	2.4
	− 5	9.6	9.2	6.0	3.2	9.6	10.0	6.6	3.4	8.6	9.3	3.5	5.8	9.1	10.0	4.4	5.6
	−20	9.7	9.8	7.1	2.7	10.0	10.5	7.3	3.2	8.7	9.7	4.6	5.1	9.2	9.6	6.5	3.1
	−80	9.8	9.3	8.1	1.2	9.8	10.2	9.8	0.4	8.8	9.4	6.9	2.5	9.4	10.1	8.1	2.0
		Yield 75 ml				Yield 90 ml				Yield 90 ml				Yield 90 ml			
After treatment	+80	10.2	10.7	9.9	0.8	9.8	10.3	7.6	2.7	9.3	10.0	8.0	2.0	9.2	10.0	8.1	1.9
	+20	10.1	9.7	8.3	1.4	9.8	9.8	5.2	4.6	9.2	8.7	6.3	2.4	9.0	9.4	6.8	2.6
	+ 5	9.5	9.1	6.8	2.3	9.4	8.2	4.4	3.8	9.8	9.9	4.6	5.3	8.9	8.5	4.2	4.3
	− 5	10.1	10.2	4.4	5.8	9.1	10.0	4.1	5.9	9.2	9.6	4.1	5.5	8.9	9.1	2.7	6.4
	−20	10.4	10.4	6.3	4.1	9.3	10.2	5.3	4.9	9.3	10.0	4.7	5.3	9.0	9.0	3.9	5.1
	−80	9.8	10.7	8.9	1.8	9.4	10.0	6.7	3.3	9.0	9.5	7.3	2.2	9.0	9.4	6.1	3.3
		Yield 71 ml				Yield 160 ml				Yield 165 ml				Yield 840 ml			

* (+) indicates distances above and (−), distances below, the tapping cut

TABLE 6. FLOW RATE, TOTAL SOLIDS CONTENT OF LATEX, FLOW PERIOD, VOLUME AND YIELD MEASURED 5 DAYS AFTER TREATMENT

Treatment	Control	1% 2,4,5-T	2.7% CEPA	Acetylene
Flow rate ml/min				
First 1 ml	2.2	3.8	2.8	4.1
First 8 ml	3.0	4.0	2.0	3.6
Second 8 ml	1.6	2.0	1.2	3.1
Average	1.2	0.7	0.4	1.3*
Total solids content of latex, %				
First drop	65.5	53.6	36.9	35.8
First 1 ml	61.7	53.6	40.3	37.2
Total flow	55.7	47.7	39.3	36.3
Total flow period, min	58	234	370	660*
Total flow volume, ml	71	160	165	840
Total solids harvested, g	39.5	76.3	64.8	304.9

* Final cessation of flow not known accurately for more than 11 hours

in *Table 7*. Although the experiment was not replicated the results are convincing. There was seepage of latex from bark wounds above the panel encased in the polythene jacket which was reminiscent of that observed by TAYSUM (1961) after application of ethylene oxide, although in his experiment the bleeding was from more distant wounds. The trees treated with bromoethane have not yet shown signs of defoliation or death, although the yield fell severely on the 18th and 19th tappings after treatment with 40 g bromoethane, but the tree treated with 50 g still maintained its yield.

The results of three further exploratory trials of halogenoparaffins are given in *Table 8*. Although none of the halogenoparaffins caused increases in flow greater than that due to acetylene, nevertheless they all produced considerable and significant increases except for chloroform. BANCHI (1967) reported chloroform to be effective in promoting latex flow; however, the average of all ten post-treatment tappings in this experiment showed virtually no difference from control. The yield was unaccountably depressed for the third, fourth and fifth tappings after application of chloroform in most of the treated trees, but, even when these are omitted—as given in parenthesis in *Table 8*—the response to chloroform is poor compared with the others.

TABLE 7. LATEX YIELDS BEFORE AND AFTER TREATMENT WITH BROMOETHANE

Amount of bromoethane placed under polythene hood, g	Mean yield of latex		Ratio of mean yields after and before treatment, %
	8 tappings before treatment ml/t/t	19 tappings after treatment ml/t/t	
0	77	71	92
10	75	239	319
20	48	133	277
30	77	216	281
40	89	166	187
50	70	204	291

TABLE 8. YIELD OF LATEX IN MILLILITRES PER TREE PER TAPPING ADJUSTED FOR DIFFERENCES BEFORE TREATMENT AND EXPRESSED AS PERCENTAGE OF CONTROL AFTER APPLICATION OF HALOGENOPARAFFINS AS GASES OR VAPOURS TO THE BARK OF THE TREES

Name of Halogenoparaffin	Amount per tree	Number of trees	Number of tappings before treatment	Number of tappings after treatment	Mean yield after treatment ml/t/t	Mean yield after treatment %
Control	0	5	4	18	68	100
Acetylene	From 15g Ca carbide	5	4	18	214	315
Bromomethane	20g	5	4	18	160	235
Dichloromethane	20g	5	4	18	180	265
Iodoethane	20g	5	4	18	164	241
1,2-dichloroethane	20g	5	4	18	176	259
Control	0	3	5	10	52	100
Iodomethane	20g	3	5	10	136	261
Iodopropane	20g	3	5	10	124	237
Control	0	5	5	10 (7)*	133 (136)*	100 (100)*
Chloroform	30ml	5	5	10 (7)*	134 (158)*	100 (116)*

* After discarding three tappings when the yields were unaccountably depressed

DISCUSSION

Practical Applications

The greater yield responses induced by picloram alone or by mixtures with 2,4,5-T compared to 2,4,5-T alone which are reported in Experiment 1 suggest that picloram might be more effective than existing chemicals currently employed commercially. The delayed maximum response and the spreading of response over a longer period could be advantageous economically. Wage agreements, processing facilities and marketing all indicate a preference for steady production rather than flush yields alternating with depressions. However, Experiment 2 did not confirm the findings of Experiment 1. Further experiments are planned to determine if picloram alone or with 2,4,5-T can provide better formulations, especially if applied during favoura' '

Most of the literature on picloram concerns its use as a herbicide. There is considerable evidence that picloram is much more persistent than for example 2,4-D. Thus a possibly slower rate of decomposition of picloram compared to 2,4-D in *Hevea* might account for its more persistent effect in Experiment 1. Differences in translocation may also play a part. MERKLE AND DAVIS (1967) reported more rapid translocation of picloram than 2,4,5-T in bean plants and that with increasing water stress the rate of translocation of 2,4,5-T was relatively more depressed. DAVIS *et al.* (1968) found that mesquite leaves absorbed picloram more rapidly than 2,4,5-T, less picloram was absorbed as water stress increased although the amount still exceeded that of 2,4,5-T, the absorption and movement of 2,4,5-T was less affected by water stress. In winged elm, the amount of picloram did not exceed that of 2,4,5-T greatly

and water stress did not affect uptake very markedly. Although picloram seemed more effective than 2,4,5-T when applied to *Hevea* during conditions of moisture stress, suggesting more rapid uptake, these were also the periods when the delayed response to picloram was most evident, suggesting less rapid movement.

The first use of a gas to increase latex flow from *Hevea brasiliensis* was by TAYSUM (1961). Ethylene oxide applied within a polythene jacket to the trunk above the tapping panel produced prolonged flow and a greater yield of latex when tapped the next day. Within one to four tappings the trees were apparently exhausted; latex flow ceased and necrosis of the bark and fungal attack followed. Wounds distant from the site of application also bled latex for a period. Apart from its phytotoxicity to *Hevea*, ethylene oxide is a relatively expensive and dangerous chemical. The need for enveloping jackets about the tree reduces the practicability of applying any gas. Acetylene produced from calcium carbide is doubtless the cheapest and produces dramatic yield increases; even so, those (BANCHI AND POLINIERE, 1968b) with the longest experience of application of acetylene (from cylinders) are doubtful of its possibilities. They warn also of increasing dryness after applications of long duration.

The innovation of 2-chloroethanephosphonic acid, which releases ethylene in plant tissues on hydrolysis (COOKE AND RANDALL, 1968), has opened up new prospects and methods of getting ethylene to the site of action in plant tissues. Nevertheless, with conventionally tapped trees and conventional methods of application, it would appear that higher concentration of CEPA are required than of other chemicals to obtain similar responses, moreover the effect seems less persistent. This lack of persistence is not surprising since there is as yet no evidence that CEPA induces ethylene production by plant tissues and thus once hydrolysis is complete the ethylene content within the tissues will fall abruptly by diffusion within the tissues and to the atmosphere. These considerations and the likelihood of CEPA costing more per unit than other growth regulators argue against its use as a conventional stimulant. Possibly, cheaper substances will be discovered which release ethylene or other gases in a similar manner in the plant tissues, and have the other necessary properties for incorporation in formulations which can be applied to the tree so that the substances penetrate without undue phytotoxic effect.

Meanwhile, CEPA promises to be of greatest value in local application to the small excisions suggested by SOUTHORN (1968) as a novel tapping technique, dependent for its success on the prevention of plugging, which is a process arresting flow. Heavy exploitation, especially large flows of latex with lowered contents of dry rubber and total solids as induced by chemical treatment, risks brown bast or dry trees (CHUA, 1967). This condition has been described as accelerated senescence of the phloem. Ethylene is associated with senescence of fruit and leaves (COOPER *et al.*, 1968). Existing compounds have been used on older trees without increasing the incidence of dry trees, but this has not always been so with younger trees (HO AND PAARDEKOOPER, 1965). Empirical solutions to these problems can be sought, but this research might be better directed if the modes of action of the established and novel active compounds were better understood.

Fundamental Aspects

The chemicals which enhance a longer flow of latex and greater yields of rubber in *Hevea* are diverse and the first point is whether they achieve their effect by any common agency or mechanism. The growth regulators 2,4-D and 2,4,5-T have been shown to produce ethylene in plant tissues, by MORGAN AND HALL (1962) and by MAXIE AND CRANE (1967) respectively. Cupric ethylene diamine tetracetate (CuEDTA) at 1.2 to 2.3 $\times$ 10^{-3} M increased the evolution of ethylene from calamondin fruit by several fold probably in response to minor injury (COOPER *et al.*, 1968). Copper sulphate causes ethylene production in plant tissues (RASMUSSEN AND COOPER, 1968). Injection of copper sulphate increases rubber yield, CuEDTA is also effective but EDTA itself is not (LOWE, 1965 and 1968). Therefore effective copper compounds may also cause the production of ethylene in plant tissues. CEPA hydrolyses to

produce ethylene in plant tissues (COOKE AND RANDALL, 1968).

In this paper, ethylene itself has been shown to enhance flow. The stimulation of flow by acetylene reported by BANCHI AND POLINIERE (1968b) has been confirmed, but it is not possible to determine whether this is an effect of acetylene itself or of small proportions converted to ethylene in the plant tissues or of small amounts of ethylene contaminating the acetylene evolved from carbide. CROCKER *et al.* (1932) found that acetylene caused epinasty, but the concentration required was five hundred times that of ethylene. Ethylene is also likely to be present as an impurity in ethylene oxide because of the method of manufacturing the latter. Although it is possible to imagine that some of the halogenoparaffins contain small amounts of ethylene as an impurity or that they may be converted to ethylene within plant tissues, it is unlikely that this is true of the whole of this group of stable, saturated compounds. It is more likely either that they have a direct effect independent of ethylene or that by slight damage to the tissues they induce ethylene production (*cf.*, the effect of CuEDTA discussed above).

COOPER *et al.* (1968) have reported that maleic hydrazide and 2,3,5 triiodobenzoic acid did not induce ethylene formation in calamondin fruit. These were found ineffective in *Hevea* by ABRAHAM *et al.* (1968). *N*-dimethylaminosuccinamic acid was found to inhibit ethylene production in plants, according to ABELES AND RUBINSTEIN (1964); HICKS AND BROWN (1968) reported an initial inhibition of ethylene production by this compound but a stimulation 101 days later; the authors found this substance to be inactive in prolonging latex flow. All these observations are consistent: substances which enhance latex flow in *Hevea* have been reported to produce ethylene in plant tissues, whereas several substances inactive for increasing ethylene production in plants have failed to enhance latex flow in tests on *Hevea*.

Despite this apparent common factor of ethylene production it does not follow that all the active compounds achieve their effect by similar means. WARNER AND LEOPOLD (1967) found that at 10 and 100 p.p.m., CEPA was about seven times more effective than IAA in causing ethylene production by pea stem sections. Both CEPA and IAA caused swelling of pea stem sections which in degree was proportional to the amount of ethylene produced. However, in the *Avena* straight growth test, BURG AND BURG (1966) have reported that ethylene was inactive, IAA promoted elongation but CEPA had no promotive, inhibitory or synergistic action with IAA or alone.

SHARMA AND LEOPOLD (1968) tapped plants of *Ficus elastica* by severing the petiole of a mature leaf, reopening the cut twice by further cuts and collecting the latex which flowed out during a total period of two hours from the cut end of the petiole attached to the plant. Turgor pressures were measured by manometers attached to severed petioles. Plants were treated by spraying with solutions of CEPA or 2,4-D while controls were sprayed with water and carrier only. The volume of latex obtained increased with concentrations of 10 to 1000 p.p.m. of CEPA, the yield fell with successive tappings to control level by the eighth day. At 1000 p.p.m., 2,4-D gave a somewhat more sustained increase in yield. The yield of control plants diminished slightly. No synergistic effect of CEPA and 2,4-D was detected. Plants treated with 1000 p.p.m. CEPA had an average turgor pressure double that of control plants. Ethylene production from leaves excised four days after treatment was determined by gas chromatography. Ethylene production was greatly enhanced by CEPA application but only slightly increased by 2,4-D treatment. Different mechanisms of 2,4-D and CEPA stimulation were suggested, the latter being by increased turgor pressure through the mediation of ethylene. Some of these findings differ from ours, which may in part be due to the use of different species and different methods of measuring the turgor pressures. The differences may be associated also with time, CEPA evolves ethylene rapidly in quantity, whereas 2,4-D or 2,4,5-T promotes the cellular production of ethylene over a period.

The cessation of latex flow due to plugging of the latex vessels and the action of 2,4,5-T

in delaying plugging, described by BOATMAN (1966), has been ascribed by PAKIANATHAN *et al.* (1966) to the effects of 2,4,5-T on the stability of the particles found in the bottom fraction of latex after high speed centrifugation. Possible mechanisms are discussed in detail by SOUTHORN (1968). The alternative suggestion that a greater flow results from an increase in turgor pressure was dismissed by BUTTERY AND BOATMAN (1967), because 2,4,5-T caused only small increases in turgor pressure in untapped trees and had no effect on the turgor pressure in regularly tapped trees. They obtained a larger fall in turgor pressure after tapping in trees treated with 2,4,5-T compared with controls. These effects and the greater and more extensive dilution of the latex are regarded as consequences rather than causes of prolonged flow.

The results reported here indicate that the general pattern is the same whatever compound is used to enhance latex flow, whether it is an auxin which may have other effects in addition to ethylene production, or ethylene itself, or a substance such as CEPA which produces ethylene or a gas like acetylene which also causes epinasty like ethylene. These is no appreciable effect of any of these treatments on osmotic concentrations or turgor pressure in the bark prior to tapping. Greater falls in turgor pressure at greater distances from the cut are obtained after tapping for all effective treatments. The total solids and dry rubber contents of the latex are decreased by all active compounds. CEPA is exceptional in reducing the d.r.c. rapidly and to lower levels than might be predicted from other data. All effective compounds increase the period of flow and the volume of latex harvested. Such differences as the time taken to attain maximum yield response and the persistence of the responses may be related to differences in the rate of translocation, production of ethylene and breakdown of the active ingredient. No synergism has been detected between 2,4,5-T and CEPA as representatives of the two main groups of effective compounds.

Numerous substances have been tested and found either effective or inactive as stimulators of latex flow. Although it may be premature to generalise, the results are consistent with the view that all active compounds have the capacity to produce ethylene. Moreover, the enhancement of latex flow may be expected by application of substances causing evolution in plant tissues of ethylene or other gases with similar physiological characteristics.

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