

EFFECT OF ETHEPHON, PURE TEA SEED SAPONIN, AND PARTIALLY PURIFIED TEA ROOT SAPONIN ON THE GROWTH OF TEA IN THE NURSERY AND THE FIELD

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Application of ethephon to tea plants resulted in a marked greening of foliage. When applied as a spray solution to pruned plants the yield of flush, on recovery, was improved. The effect on nursery plants of appropriate concentrations of ethephon was to increase shoot growth and root weight, but not root length, but application of excessive amounts of ethephon was injurious to growth. Treatment of pruned tea bushes with pure tea seed saponin also led to increased yield of flush on recovery, and the effect on nursery plants was increased root weight and increased shoot growth. Spraying nursery plants with partially purified tea root saponin solution caused a remarkable increase in root weight and root length. Mixtures of ethephon with pure tea seed saponin, or with partially purified tea root saponin, influenced the growth of tea in a manner which could not be predicted from their individual effects.

Saponins are widely distributed in the plant kingdom (Amarasingham, Bisset, Millard & Woods 1964; Basu & Rastogi 1967), and have been reported to exert varying effects on the growth of different plants. The application of saponin solutions to leaves stimulated the development of shoots and roots in Begonia (Balansard & Pellesier 1943a), but inhibited root growth of tomato (Balansard & Pellesier 1945), garden cress and barley (von Euler 1946). The germination of seeds was found to be promoted by low concentrations of saponins but high concentrations were inhibitory (Balansard & Pellessier 1943b). More recently, Mayevsky and Marchaim (1972) found that immersion of cotton seeds in aqueous 0.5% lucerne saponin led to inhibition of seed respiration, and saponins have also been found to inhibit growth of fungi (Zentmeyer & Thompson 1967; Olsen 1971; Gestetner, Assa, Henis, Birk & Bondi 1971) and rice weevils (Su, Speiers & Mahany 1972). Part of this investigation describes the effect on nursery and field tea plants of pure tea seed saponin (de Silva & Roberts 1972), and the effect on nursery plants only of partially purified tea root saponin.

The effect of ethephon (2-chloroethylphosphonic acid)* alone, and in combination with pure tea seed saponin, and with partially purified tea root saponin, on the growth of tea plants is also described. Ethephon releases ethylene directly to the plant tissues, and ethylene controls many physiological processes such as activation of enzymes (Rhodes & Woollorton 1971), rooting (Kawase 1971; Smith & Robertson 1971), apical dominance, general growth, greening of foliage (Sachs & Hackett 1972) acceleration of fruit ripening (McGlasson 1970; Oyebade 1971) *etc.*

Each of the experiments carried out is described separately, as the conditions, materials and methods were varied.

* Trade name 'Ethrel' marketed by Amchem Products, Ambler, Pa, USA

EXPERIMENT 1

The effect of 'Ethrel Paste' on recovery after pruning.

Materials and Methods

The Ethrel paste (containing 10% ethephon) supplied for use on rubber trees was used in this trial. Sixty mature bushes (TRI 2024), 11 years old, two years after the last prune, were divided into two lots of 30 bushes each. One lot of bushes was clean pruned, and the other lung pruned, leaving four lungs. Alternate bushes in each lot were then treated with Ethrel paste, by lightly applying the paste on cut ends of the bushes with a small brush; the remaining bushes served as untreated controls. Lungs were removed after 2 months, and all the bushes tipped three months later. Plucking was commenced in about four weeks time, continued at weekly intervals for 13 weeks, and the weight of flush from each of the four groups of bushes recorded.

Results

It was observed that the application of Ethrel paste reddened the cut ends of the bushes, whereas the untreated control bushes did not show this effect. It was also observed that after about six weeks, the foliage of the treated bushes was much greener than that of the controls, indicating a higher content of chlorophyll, which would be expected to yield a blacker made tea (Wickremasinghe & Perera 1966). This higher content of chlorophyll, as well as the above-mentioned reddening effect, are both due to activation of enzymes by ethylene.

The results shown in Table 1, indicate that a single treatment of pruned bushes with 10% Ethrel paste does not significantly affect the yield of flush.

TABLE 1 — Yield of flush after recovery of pruned bushes (TRI 2024) treated with Ethrel paste, containing 10% ethephon

Yields (mean of 13 plucks) expressed as oz flush per 30 bushes

	Clean pruned	Lung pruned
Treated	63.7	47.8
Control	55.5	51.3

EXPERIMENT 2

The effect of various concentrations of ethephon sprays on the yield of flush of different clones of tea.

Materials and Methods

Flush was plucked at weekly intervals for eight weeks from four clones of tea TRI 2023, TRI 2151, TRI 2025 and DT 1, ten years old, growing in blocks of 18 bushes each. After each pluck, the bushes were sprayed with 250 ml aliquots of solutions of 'Ethrel Concentrate' (39.56% ethephon), diluted with water to contain 50, 100, 200, 250, 500 and 1000 ppm ethephon. The flush from each group of sprayed bushes was weighed separately, and the mean weights of flush from the eight plucks were compared with those of two groups of control bushes which had been sprayed with 250 ml water.

Results

The results (Table 2) show the weights of flush from the different blocks divided by the number of plucks, and indicated that weekly sprays of ethephon at 100 ppm and above reduced the yield of flush and that this effect of yield reduction is marked in clones TRI 2025, TRI 2151 and DT 1, and slight in clone TRI 2023. It was observed, that ethephon treatment led, in all clones, to a marked increase in the greenness of the foliage, as observed in Experiment 1 with the use of Ethrel paste. The results also indicate that frequent, weekly sprays (250 ml per 18 bushes) of ethephon at levels of 100 ppm and above are detrimental to the yield of flush, and that this effect varied with the clone.

TABLE 2 — *Effect of ethephon sprays at various dilutions on yields of flush from four different clones—Sprayed at the rate of 250 ml per 18 bushes at weekly intervals for 8 weeks*

Yield (mean of 8 plucks) expressed as oz per plot of 18 bushes

Dilution	Clone			
	TRI 2023	TRI 2025	TRI 2151	DT 1
Control	21.6	18.5	21.3	13.0
50	21.7	20.5	22.0	14.6
100	19.8	18.0	16.6	9.0
200	17.4	14.3	15.2	8.2
250	19.9	NT	12.4	12.8
500	19.1	NT	13.0	8.0
1000	19.4	NT	9.0	9.0
LSD ($P=0.05$)	3.06	2.42	2.89	3.53

NT=Not tested

EXPERIMENT 3

The effect of spraying different levels of ethephon solutions and pure tea seed saponin solutions, respectively, on recovery after pruning.

Materials and Methods

Nine years old lung-pruned plants of clone TRI 2026 were used in nine groups of 25 bushes each and lung-pruned. Two groups of bushes served as untreated controls, and the remaining seven groups were respectively sprayed with 500 ml aliquots of solutions of Ethrel Concentrate, diluted with water to contain 50, 100, 200 and 400 ppm ethephon, and solutions of pure tea seed saponin containing 100, 200 and 400 ppm saponin. The spray treatments were repeated twice more at intervals of three weeks, and the plants tipped one month after the last spray treatment. At this time a severe drought set in and no further observations were made for a period of four months. At the end of the drought, and with the onset of rains, the bushes were tipped again and thereafter plucked at weekly intervals for 13 weeks. The flush from each group of bushes was weighed separately.

Results

The mean weights of flush from the 13 plucks, expressed as g/bush, from the sprayed and control bushes are shown in Table 3.

TABLE 3 — *Effect of pure tea seed saponin, and ethephon sprays, at various dilutions, on yield of flush after pruning (TRI 2026)*

Treatment	Mean weights of 13 plucks expressed as g/bush
Control 1	19.8
Control 2	21.7
S 400	32.9
S 200	28.8
S 100	16.4
E 400	26.5
E 200	28.7
E 100	15.9
LSD ($P=0.05$)	5.43

S 400, S 200, S 100=pure tea seed saponin sprays at 400, 200 and 100 ppm respectively.
 E 400, E 200, E 100=ethephon sprayed at 400, 200 and 100 ppm respectively.
 Spray—500 ml per 25 bushes, sprayed three times at intervals of 3 weeks.

It is seen that the conditions of treatments resulted in statistically significant increases in yield of the bushes sprayed with solutions containing 200 and 400 ppm tea seed saponin and 200 ppm ethephon, respectively. The latter result taken in conjunction with the results of Experiments 2, indicates that the rate and the frequency of application of ethephon determine its effect on the yield of tea. Similar conclusions have been reported by workers on the effect of ethephon on other crops.

EXPERIMENT 4

The effect on nursery plants of treatment with spray solutions containing respectively, ethephon, pure tea seed saponin, and a mixture of ethrel and pure tea seed saponin.

Materials and Methods

Four hundred plants clone TRI 2023, four months old, were divided into four lots of 100 plants each. Each lot was subdivided into two groups of 50 plants each, and the plants in one of the groups were 'thumbnailed'. The four groups of 100 plants each consisting of 50 thumbnailed and 50 free-growing plants, were sprayed, respectively with 100 ml aliquots of water, pure tea seed saponin (4000 ppm), ethephon (300 ppm), and a mixture containing tea seed saponin and ethephon at these levels. A total of seven spray treatments, given at fortnightly intervals, was imposed. Fertilizer (T 65) was applied at the usual rate of 28g/4546 ml/100 plants, also at fortnightly intervals, on the first five occasions, and at double this rate for the final two applications. Two weeks after the final treatment, *ie* when the plants were eight months old, 20 plants from each treatment were assessed for length and weight of shoots and roots.

Results

It was observed that two weeks after the first spray treatment, all treated plants were healthier in appearance than the control plants. The latter were dull in appearance, whereas the former appeared succulent. One month later, the ethephon, saponin, and ethephon + saponin treated plants appeared to be growing better than the control plants. The leaves of the ethephon and ethephon + saponin treated plants were much greener, but somewhat smaller, than those of the other two groups, and saponin appeared to have enhanced the effect of ethephon in respect of greenness of foliage. It was also observed that the height of ethephon + saponin treated

plants was less than that of the controls during the first few weeks of the experiment but later surpassed that of the controls—evidently because the presence of ethephon, resulted in a reduction of bud dormancy. The ethephon+saponin treated plants appeared to be the best at the time of assessment of the eight month old plants for length and weight of shoots and roots. The results of this assessment are shown in Table 4.

TABLE 4 — *Effect of ethephon, tea seed saponin, and a mixture of ethephon and tea seed saponin sprays on the growth of nursery (TRI(2023))*

Control	Shoot weight (g)	Shoot length (cm)	Root weight (g)	Root length (cm)
T	10.5	14.7	4.2	7.8
F	9.7	17.3	3.7	7.9
Ethephon				
T	12.2	14.8	4.7	7.3
F	13.3	21.3	4.3	7.4
Tea seed saponin				
T	12.6	14.5	5.3	7.6
F	12.5	20.3	4.1	7.9
Ethephon + tea seed saponin				
T	13.1	15.1	4.9	8.7
F	13.1	18.1	4.1	8

T= thumbnailed; F=free growing

Spray—100 ml per 50 plants (4 months old), sprayed seven times at fortnightly intervals

Spray solutions contained ethephon at 300 ppm and Saponin at 400 ppm

Values are means of 20 plants.

Free-growing plants showed statistically significant increases in shoot length, as well as shoot weight, as a result of all treatments. In addition, as expected the shoot length of free-growing plants was significantly greater than that of thumbnailed plants, but there was no corresponding increase in shoot weight. Root weights of all treated plants were greater than those of the controls, but the differences were not statistically significant. Root weights of thumbnailed plants were greater than those of free-growing plants, but root length was unaffected by thumbnailing. These findings suggest, firstly, that the judicious use of ethephon and tea seed saponin sprays may be used to accelerate the growth of nursery plants and, secondly, that thumbnailing favours the formation of a greater root/shoot ratio.

EXPERIMENTS 5A & 5B

The effect on nursery plants of adding drops of various dilutions of ethrel and pure tea seed saponin to the cut ends of thumbnailed plants.

Materials and Methods

Experiment 5A

One hundred plants, clone TRI 2026, six months old, were divided to 10 rows of 10 plants each. All plants were thumbnailed and one drop (0.04 ml) of the following solutions were added to the cut ends of the respective rows of plants (Treatment 1T). T 65 fertilizer was applied at fortnightly intervals at the rate of 28g/4546 ml/100 plants.

TABLE 5A — *Effect of adding drops (0.04 ml) of various dilutions of ethephon to the cut ends of thumbnailed nursery plants (TRI 2026)*

	No. of shoots	1 T				2 T				
		Shoot wt (g)	Root wt (g)	Shoot length (cm)	Root length (cm)	No. of shoots	Shoot wt (g)	Root wt (g)	Shoot length (cm)	Root length (cm)
Control	8.6	32.4	16.4	17.6	6.9	10.7	27.6	10.8	17.5	7.7
Ethephon (ppm)										
137 12	10.0	36.3	20.1*	21.8*	6.2	13.2	33.6	13.0*	20.5	6.8
25	8.2	35.5	15.3	22.2*	7.0	10.6	31.1	10.6	20.3	7.3
98	6.8	30.2	8.6	20.4*	6.6	10.2	27.7	10.2	18.1	7.1
390	7.8	31.1	11.3	17.7	7.7	13.7	26.6	13.7*	16.5	7.3
1569	8.2	33.4	11.4	18.3	7.2	13.1	28.5	13.1	17.6	6.8
12500	8.2	29.4	10.8	18.1	6.4	10.3	30.9	10.3	17.9	7.9
25000	7.4	28.7	10.1	16.5	7.8	8.1	25.8	8.1	15.0	7.0
100000	9.6	27.8	8.9	17.5	7.5	6.3	21.8	6.3	14.4	7.6
400000	6.2	23.2	6.2	14.5	7.8	5.3	20.3	5.3	15.6	7.4
(LSD $P=0.05$)	NS	NS	3.7	1.9	NS	NS	7.3	2.9	3.24	NS

1T—plants thumbnailed at commencement of experiment, and treated once.

2T—plants thumbnailed at commencement of experiment and five weeks later, and treated after each thumbnailing.

Initial age of nursery plants=6 months

Plants assessed 8 months after first treatment.

TABLE 5B — *Effect of adding drops (0.04 ml) of various dilutions of pure tea seed saponin to the cut ends of thumbnailed nursery plants (TRI 2026)*

	No. of shoots	1 T				2 T				
		Shoot wt (g)	Root wt (g)	Shoot length (cm)	Root length (cm)	No. of Shoots	Shoot wt (g)	Root wt (g)	Shoot length (cm)	Root length (cm)
Control	7.6	29.2	19.5	18.8	6.9	7.8	26.4	19.0	16.6	6.2
Saponin (ppm)										
500	8.0	33.6	23.0	18.5	6.3	8.2	29.4	24.0	18.5	6.3
1000	8.8	31.6	35.5*	18.3	6.8	6.6	34.0	21.5	19.8	6.4
2000	8.0	30.8	27.5*	21.0	7.8	7.0	38.4	32.0*	22.7	6.1
4000	7.4	22.6	31.5*	17.2	7.8	7.2	37.8	28.5*	18.8	7.2
(LSD ($P=0.05$))	NS	NS	8.0	NS	NS	NS	NS	9.1	NS	NS

1 T—Plants thumbnailed at commencement of experiment, and treated once

2 T—plants thumbnailed at commencement of experiment, and five weeks later, and treated after each thumbnailing.

Initial age of nursery plants—6 months

Plants assessed 8 months after first treatment.

Control—water

Ethephon (ppm)—12, 25, 98, 390, 1560, 6750, 25000, 100000, 400000

Experiment 5B—This experiment was similar to Experiment 5A, except that five rows of ten plants each were treated respectively with drops of water, and 500, 1000, 2000 and 4000 ppm tea seed saponin.

Five weeks later, five plants from each row or plants were again thumbnailed, and treated for a second time as before (Treatment 2T). When the plants were 14 months old, the five plants from each of the two treatments were assessed for growth by measuring the length and weight of shoots and roots, and number of shoots.

Results

The general appearance of the leaves of the control plants was always duller than that of the plants treated with ethephon or saponin. Plants treated with 390 ppm ethephon and above showed a decreased stature, due to shortening of the internodes. The foliage was, however, very much greener and thicker than that of the control. The saponin-treated plants were healthier in appearance, and taller, but not greener, than the control plants.

The effect of the various treatment on the length and weight of shoots and roots are shown in Tables 5A and 5B.

The results are summarized as follows:

1. The number of shoots formed is not affected by any of the treatments.
2. Thumbnailing twice is detrimental to shoot weight and root weight, but does not affect elongation of shoots and roots.
3. Shoot weight is unaffected by low concentrations of ethephon or tea seed saponin, but is reduced by relatively high levels of ethephon.
4. Root weight is increased by one treatment with drops containing 12 ppm ethephon, or 1000, 2000 or 4000 ppm tea seed saponin. However levels of ethephon greater than 12 ppm resulted in a progressive and marked reduction of root weight. In the plants treated twice (2T) there was a marked reduction of root weight of control and some of the treated plants as compared to 1T, but even here the plants treated twice with 12 ppm (and 390 ppm) ethephon, or the higher levels of saponin showed significantly greater root weight than the corresponding control plants.
5. Shoot length was increased by low levels of ethephon, decreased by higher levels of ethephon, and unaffected by tea seed saponin.
6. Root length was unaffected by all treatments.

EXPERIMENT 6

The effect on nursery plants (TRI 2023) of spraying with partially purified tea root saponin, and a mixture of partially purified tea root saponin and ethephon.

TABLE 6—*Effect of sprays of partially purified tea root saponin, and a mixture of partially purified tea root saponin and ethephon on the growth of thumbnailed nursery plants*

	Root weight (g)			Root length (cm)			Shoot weight (g)			Shoot length (cm)		
	1	2	3	1	2	3	1	2	3	1	2	3
Control	2.48	3.6	3.8	4.7	6.8	6.3	6.9	11.7	21.5	11.2	14.4	19.4
Ethephon (300 ppm)	2.31	4.2	4.0	5.2	5.5	5.6	5.9	14.9	19.9	10.0	15.5	19.6
Tea root saponin extract (0.4%)	2.04	4.1	7.39**	5.2	8.1	8.2*	6.7	12.7	20.7	11.3	13.7	19.1
Ethephon (300 ppm) + Tea root extract (0.4%)	2.2	4.4	4.83	4.4	5.0	5.2*	6.9	12.1	16.9	9.2	13.7	16.9*
LSD ($P=0.05$)			1.48			0.89						1.9
LSD ($P=0.01$)			2.83			1.6						

Spray—100 ml per 50 plants (initially 5 months old) applied at intervals of four weeks. 1, 2, 3=6, 12 and 20 weeks respectively after initiation of the experiment. Values are Means of (1 and 2) 10 plants, (3) 15 plants.

Materials and Methods

Partially purified tea root saponin was prepared by boiling 0.5 Kg of chopped tea roots with 4.5 l of boiling, 50% (v/v) aqueous methanol for 3 hr. The root extract was freed of debris by centrifugation, and the saponins in the clear supernatant extracted into n-butanol (1½ l). The n-butanol was then removed on a rotary vacuum evaporator, and yielded 11.4 g of dry powder. This dry powder, without any further purification, was the material used in this experiment.

Two hundred nursery plants of clone TRI 2023, five months old, were divided into four lots of 50 plants each. All plants were thumbnailed and sprayed at intervals of four weeks with 100 ml aliquots of (a) water (b) ethephon (300 ppm). (c) 0.4 % (w/v) dry tea root solids, (d) a mixture of 0.4% (w/v) dry tea root solids and ethephon (300 ppm). Fertilizer (T 65) was applied to the plants at fortnightly intervals at the usual rate of 28g/4546 ml/100 plants.

Ten plants from each treatment group were assessed for length and weight of shoots and roots, as well as for number of branches, (1) two weeks after the first spray treatment (2) four weeks after the third spray treatment. Finally, (3) fifteen plants were similarly assessed four weeks after the fourth spray treatment. The remaining 15 plants were resprayed with the respective solutions and planted in the field, and are under observation for any differences in growth. It is proposed that a comparison be made of their growth characteristics, especially yields, when they come into plucking.

Results

At the time of the first assessment for weight of shoots and roots (*ie* six weeks after the initiation of the experiment) there were no marked differences between the four groups of plants. However, at the time of the second assessment, (*ie* 12 weeks after the experiment was initiated), it was observed that growth of the plants sprayed with tea root extract, was very much better than that of those sprayed with ethephon or ethephon + tea root extract, although the growth of these two latter groups of plants was better than that of the controls. At the time of the third assessment (*ie* 20 weeks after the initiation of the experiment), the plants sprayed with tea root extract appeared to be the best of the four groups of plants.

The results of the three assessments, shown in Table 6, are in agreement with the visual appearance of the plants, and indicate that spraying with tea root saponin caused highly significant increases in root weight and root length. However, root length, as well as shoot length, were greatly reduced by the mixture of ethephon and tea root saponin, although the reduction in root length was not reflected in a corresponding reduction of root weight. It appeared, that the effect of ethephon was to increase root branching at the expense of root elongation. None of the treatments had any significant effect on the extent of branching of the aerial portion of the plant.

* Quoted by Heftmann, E. (1965). In *Plant Biochemistry*. Chapter 27—Steroids (Eds J. Bonner and J. E. Varney), Academic Press, London and New York p. 703.

DISCUSSION

Ethephon, tea seed saponin and tea root saponin were all found to affect the growth of tea plants in the field and in the nursery. Their effect depended on the frequency and concentration at which they were applied, and this was particularly so with ethephon. At relatively low levels, ethephon was beneficial in promoting the greening of foliage, and this would be useful for the production of blacker made teas. Root weight was increased by ethephon, but root length was decreased, suggesting that root branching rather than elongation was encouraged. Tea seed saponin, and to a much greater extent, partially purified tea root saponin, increased root weight as well as root length of nursery plants. A remarkable improvement in root growth was caused by the latter (which was found to be chemically different to tea seed saponin), suggesting that this effect may be useful in increasing the rate of growth and the resistance to drought of young plants. However the length of time for which this beneficial effect will persist is unknown, and a field experiment is in progress to determine the possible practical usefulness of spraying nursery plants with partially purified tea root saponin.

SUMMARY

1. A single application of 10% Ethrel paste to pruning cuts resulted in the production of greener foliage on recovery of the bushes (TRI 2024), and did not cause any yield reduction.
2. Tea bushes which were sprayed frequently with relatively large volumes of relatively high concentrations of ethephon showed a reduction in yield of flush. This effect of yield reduction showed variations with clone, and was not observed with frequent sprays of ethephon at relatively low concentrations. Spraying with ethephon invariably resulted in production of markedly greener foliage.
3. Spraying lung-pruned TRI 2026 bushes with the appropriate concentrations of ethephon and pure tea seed saponin, respectively, caused significant increases in yield of flush, on recovery.
4. Judicious spraying of thumbnailed nursery plants (TRI 2023) with ethephon, pure tea seed saponin, or a mixture of ethephon and pure tea seed saponin improved the growth of the plants. When free-growing nursery plants were sprayed with these solutions, the improvement in shoot growth was significant.
5. Addition of drops of relatively low concentrations of ethephon to the cut ends of thumbnailed nursery plants (TRI 2026) increased root weight and shoot length. Relatively higher levels of ethephon were, however, injurious to the growth of the plants. The addition of drops of pure tea seed saponin also caused a significant increase in root weight.
6. The effect of ethephon appeared to be increased root branching and root weight at the expense of root elongation.
7. Shoot branching was not affected by ethephon, tea seed or tea root saponins.
8. Judicious spraying of nursery plants (clone TRI 2023) with partially purified tea root saponin caused increases in root weight and root length which were highly significant.

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