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Effects of 1-Aminocyclopropane-1-Carboxylic Acid and Aminoethoxyvinylglycine on Ethylene Formation and Latex Flow in Hevea brasiliensis

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A marked increase in ethylene synthesis was obtained when Hevea *bark tissues were treated with 1-aminocyclopropane-1-carboxylic acid (ACC), applied either to excised* Hevea *bark discs or to the tapping cut on a tree. ACC was effective in promoting ethylene synthesis both on virgin and renewed bark of a number of clones. Aminoethoxyvinylglycine (AVG) inhibited ethylene formation of excised* Hevea *bark discs. However, AVG was ineffective in inhibiting ACC-stimulated increases in ethylene synthesis in* Hevea *bark tissues.*

The auxins, NAA and 2,4,5-T induced formation of significant amounts of ethylene in excised bark discs. The auxin-stimulated increase in ethylene levels was effectively inhibited by AVG, when AVG was included together with the auxins in the incubation media. The significance of the effects of ACC and the selective inhibitory action of AVG on ethylene synthesis are discussed and the suggestion is advanced that the pathway of ethylene biosynthesis in Hevea *bark tissues may be similar to that established for other plants.*

The application of ACC either in a buffer solution or palm oil to the tapping cut stimulated yield increases in a number of clones. The responses to ACC were generally lower than those of ethephon at concentrations applied. AVG had no effect on ACC-stimulated increases in yield when applied together with ACC to the tapping cut.

It has recently been reported[1, 2, 3] that 1-aminocyclopropane-l-carboxylic acid (ACC) is the immediate precursor of ethylene in tissues of a number of plants. The pathway of ethylene biosynthesis has been shown as methionine ⟶ S-adenosylmethionine ⟶ ACC and ethylene[4]. A similar pathway has been suggested for auxin-induced[5] and stress-induced[6] ethylene production. These steps in the biosynthetic pathway have been shown to be enzyme-regulated processes[7,8]. However, while the enzymes involved in the conversion of methionine to S-adenosylmethionine and the formation of ACC from S-adenosylmethionine have been crystal-

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lised and identified[7,8], those involved in ethylene formation from ACC have not been identified. It is also reported[9,10] that aminoethoxyvinyl-glycine (AVG) effectively prevents the conversion of S-adenosylmethionine to ACC thus acting as an inhibitor of ethylene formation in plant tissues.

In view of the importance of ethylene in latex flow physiology of *Hevea* trees[11], it was considered of interest to study the effects of ACC treatment on ethylene formation in excised bark discs and on latex flow when applied to the tapping cut. The effects of AVG treatment on wound ethylene formation of excised bark discs and ACC-treated bark discs were investigated. This investigation was also extended to cover auxin-treated bark discs since it has been shown that auxins promote ethylene biosynthesis in excised *Hevea* bark discs[12]. This paper discusses data obtained from the above investigations.

MATERIALS AND METHODS

The trees used in this investigation were those at the RRIM Experiment Station in Sungai Buloh. Trees of either clone RRIM 600 or PB 5/51 or GT 1 were used for all investigations with excised bark discs while RRIM 600, tapped on *Panels BO-2* and *BI-1* and RRIM 701 and GT 1 tapped on *Panel BI-1* were used for studies on application of ACC to the tapping cut. All trees were tapped on ½S d/2 system. A tree plot design was used with generally ten trees per treatment.

Bark discs were excised by driving a 1.2 cm bark-borer up to the cambium with a hammer on high virgin panel above the renewed panel (183 cm above the union). Bark shavings were collected from the tapping cut immediately following tapping.

ACC was purchased from Calbiochem and Sigma while AVG was given by Maag Chemicals, Switzerland. NAA and 2,4,5-T were purchased from Sigma.

The relevant concentrations of ACC were made up in a medium consisting of 10 mM phosphate buffer (pH 7) and 600 mM sorbitol solution (PBS). Four to five excised bark discs were incubated in 10 ml of this medium contained in 130 ml quick-fit conical flasks equipped with rubber septa in the glass stoppers. The auxins, NAA and 2,4,5-T were made upto the desired concentration in 0.02 *M* tris-HCl buffer (pH 6). Three to five excised bark discs were incubated in 10 ml of the auxin solution for 6 h in 50 ml conical flasks. At the end of 6 h incubation the bark discs were removed, blotted dry and incubated in 130 ml quick-fit conical flasks equipped with rubber septa in the glass stopper. The incubation conditions were varied in one experiment with NAA, where the auxin dissolved in a few drops of ethanol was made upto the relevant concentration in distilled water. The bark discs were then incubated in 10 ml of this solution, contained in 130 ml quick-fit conical flasks. AVG was made upto the required concentrations in water and excised bark discs were incubated in 10 ml of the respective solutions contained in quick-fit conical flasks. For studies on AVG effects on ACC and auxin-treated bark discs, the relevant concentrations of AVG were added directly to the respective media used for ACC or auxin treatments.

For applications to the tree, a concentration of 50 mM ACC in 10 mM phosphate buffer (pH 7) plus 600 mM sorbitol solution was applied. Five millilitres of the above solution was applied per tree with a pasteur pipette along the groove of the tapping cut after removal of tree lace. The run-off from the cut was collected and reapplied two to three times. The excess solution was finally applied by absorption in a pad of tissue paper lining the groove and which was sealed by cellophane tape. AVG was incorporated in the ACC formulation where indicated. In subsequent trials, the relevant concentrations of ACC dissolved in a few millilitres of water were formulated in palm oil and applied to the groove[13].

Bark shavings from the tapping cut were collected separately for each treated tree 20 h after application during the first tapping, transferred immediately to 130 ml quick-fit conical flasks and generally incubated for a period of 24 h before sampling for ethylene.

The air in the incubation flasks containing either bark discs or bark shavings was sampled, analysed for ethylene and ethylene concentrations determined as previously reported[12]. Ethylene concentration is expressed as per gramme fresh weight of tissue.

The method of yield recording inclusive of both No. 1 crop (normal flow) and late drip, measurements of initial flow rates and d.r.c. determination were similar to those described previously[14].

RESULTS

Effect of Different Concentrations of ACC on Ethylene Production

The results of an experiment to test the effects of a range of ACC concentrations on ethylene production of excised *Hevea* bark discs are given in *Figure 1*. Increased ethylene production for all concentrations of ACC tested was evident after 4 h of incubation but marked increases were only obtained at 24 h. There was further increase in ethylene levels on prolonged incubation to 48 h. The optimum concentration for maximum production of ethylene was 1 mM ACC; there were no further increases for higher concentrations of 5 mM and 10 mM ACC. The bark discs treated with 1 mM ACC induced a sixteen-fold increase in ethylene production, above that of bark discs treated with either water or phosphate sorbitol buffer at the end of 48 h of incubation.

ACC Application to the Tapping Cut in Two Formulations

The effects of ACC application to the tapping cut on ethylene formation and yields were studied in three trials on clones RRIM 600, RRIM 701 and GT 1, tapped on *Panel BI-1 (Figure 2, 3 and 4)*.

In the first trial, ACC was applied in phosphate buffer (pH 7) plus sorbitol for the first two applications carried out during the first and second months, while for the third application made at the end of three-and-a-half months ACC was applied formulated in palm oil as a carrier.

The bark shavings obtained from the fisrt tapping following ACC treatment produced ethylene which was manifold higher than that produced by shavings treated with just buffer or shavings from untreated trees *(Figure 2)*. The eight-to-ten-fold increase was obtained for all

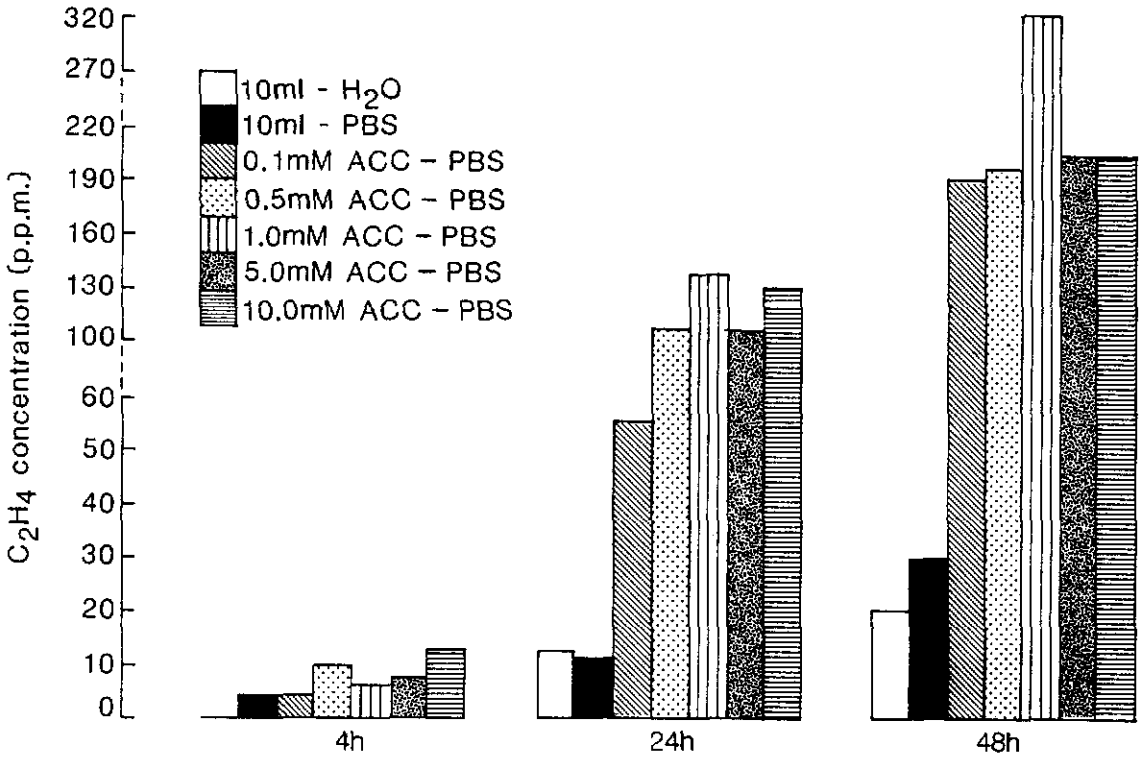

Figure 1. Effect of different ACC concentrations on C_2H_4 production of excised Hevea *bark discs. (Results given are representative of two separate determinations.)*

three applications for both ACC applied in buffer and in palm oil.

There were positive yield responses to ACC treatment for all three applications. The yields of trees treated with ACC were higher than those of untreated control trees throughout five months of recording *(Figure 2)*. During the first week following each application of ACC in either buffer or palm oil, there was marked increase in yields with high peak responses of 50% to 100% above that of control trees. These peak responses were recorded when a eight-to-ten-fold increase in ethylene production was detected in the excised bark shavings. The responses generally declined after the first few tappings, till the next application when a resurgence in response was recorded. There was no yield response to application of phosphate buffer plus sorbitol, with yields being marginally lower or comparable to those of unstimulated control trees.

In the second trial on clone RRIM 701 *(Panel BI-1)*, there was only one application of ACC in phosphate buffer (pH 7) plus sorbitol *(Figure 3)*. The ethylene production of bark shavings obtained from the first tapping of ACC-treated trees was two-to-three-fold higher than that of bark shavings obtained from trees treated with buffer or that obtained from unstimulated trees. ACC treatment resulted in positive yield responses ranging from 15% to 44%, with yields being higher than those of unstimulated control trees

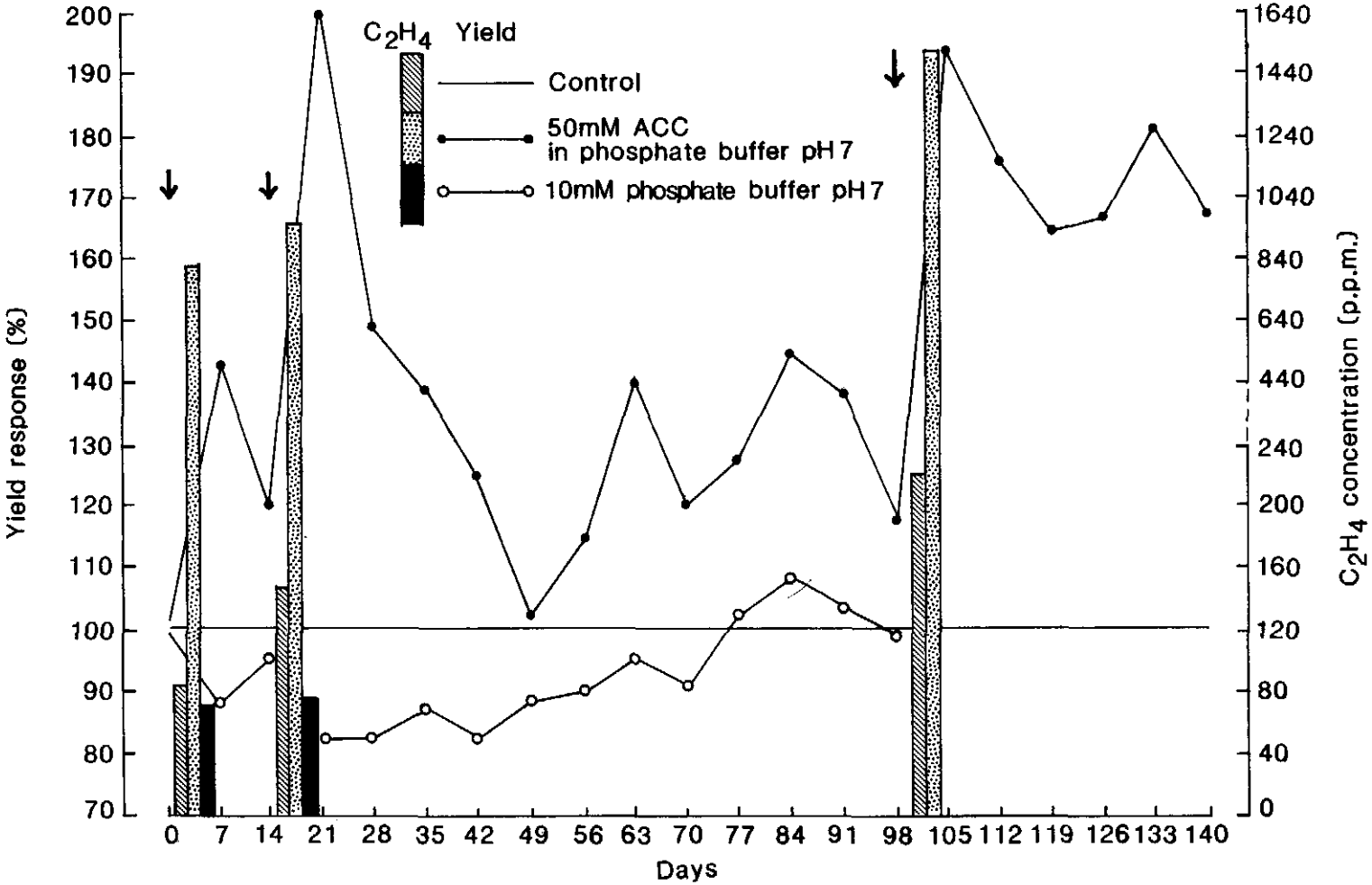

Figure 2. Effect of ACC on latex yields of clone RRIM 600 tapped on renewed bark. First two applications of ACC in phosphate buffer, third application in palm oil; arrows indicate dates of application; ethylene concentrations given refer to mean of four replicates (four trees).

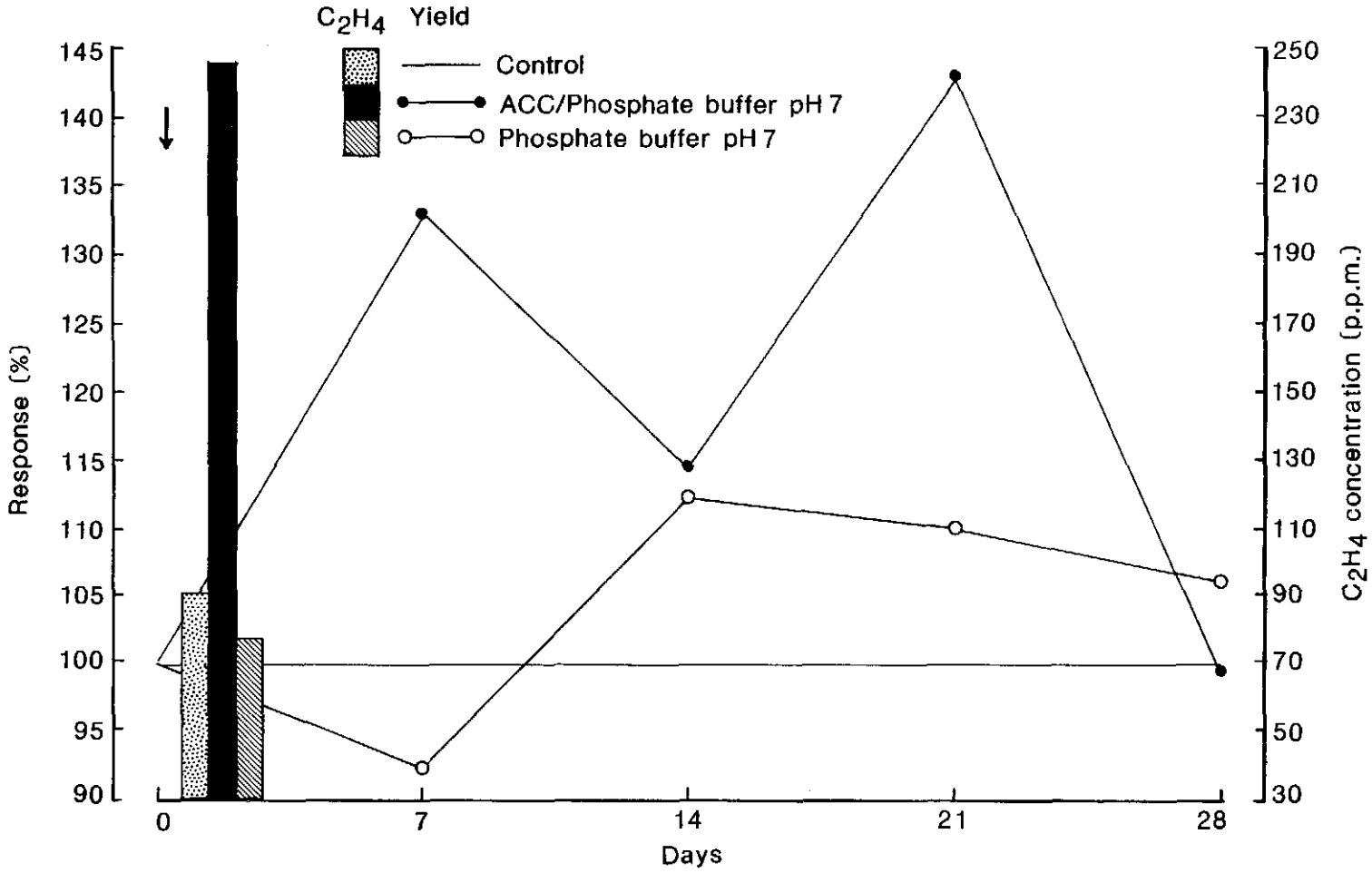

Figure 3. Effect of ACC on latex yields of clone RRIM 701 tapped on renewed bark. Arrow indicates date of application; ethylene concentrations given refer to mean of four replicates (four trees).

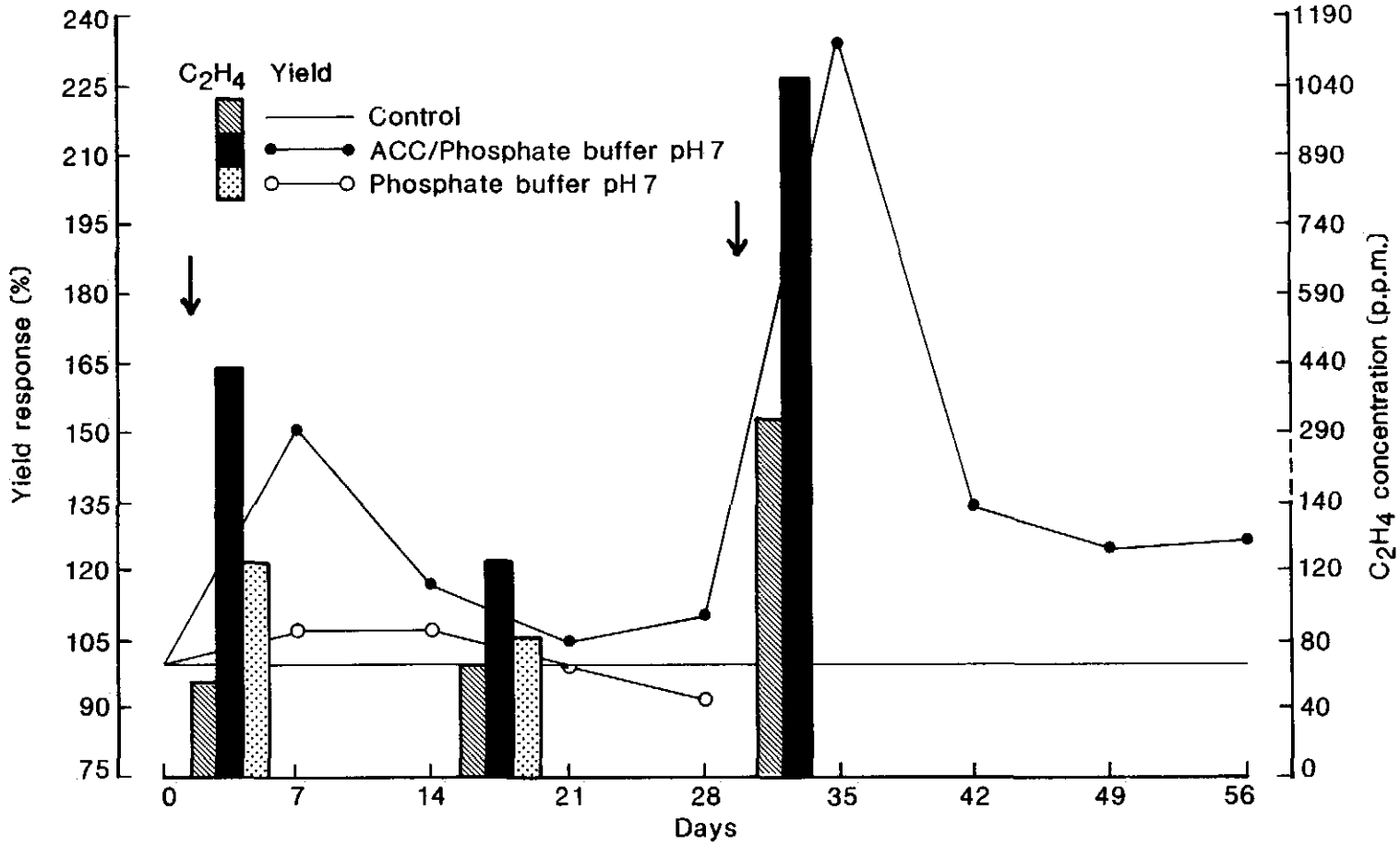

Figure 4. Effect of ACC on latex yields of clone GT 1 tapped on renewed bark. First application of ACC in phosphate buffer, second application in palm oil; arrows indicate dates of application; ethylene concentrations given refer to mean of five replicates (five trees).

(Figure 3). A marginal response of 5% to 10% was obtained from trees treated with buffer but this was much lower than that of ACC-treated trees.

In the third trial on clone GT 1, there were two ACC applications with one applied in phosphate buffer and the other in palm oil. As observed for the other two clones, both ACC applications induced a three-to-four-fold increase in ethylene levels in bark shavings relative to that produced by bark shavings treated with buffer or that produced by bark shavings of unstimulated trees. There was a marked drop in ethylene levels produced by bark shavings obtained two weeks after ACC treatment *(Figure 4)*. However, the levels were still higher than those of control trees or trees treated with buffer.

The yields increased in response to ACC treatment, with yields being higher than those of unstimulated control trees. A marked increase in response of 50% to 130% was recorded for the initial tappings during the first week following each application. This marked increase in response appears to be related to the three- to four-fold increase in ethylene levels detected in bark shavings. However, a decline in response was evident after the first week, reflecting the fall with time of ethylene levels induced by ACC treatment. The yield responses to ACC were well above that of unstimulated control trees and trees treated with buffer throughout the two months of recording.

Comparison of ACC with Ethephon

ACC as a yield stimulant was compared with ethephon in two trials on clones RRIM 600 *(Panel BO-2)* and RRIM 701 *(Panel BI-1)*. The results of both trials are summarised in *Tables 1* and *2* respectively. ACC treatment induced a marked increase in ethylene production in bark shavings obtained from the first tapping following each application in both clones *(Tables 1* and *2)*. The increase in ethylene levels was more than ten-fold when compared with the ethylene production of bark shavings obtained from untreated trees for clone RRIM 701. For RRIM 600, the first ACC application gave a five-fold increase while the second application gave more than a ten-fold increase.

Both clones gave positive responses above those of respective controls to each application of ACC applied in palm oil. In clone RRIM 600, these responses (20% to 25%) were lower than those obtained from application of 2.5% ethephon (45% to 50%). However, for clone RRIM 701, the responses obtained were comparable for both ACC and ethephon. The responses of both clones to ACC and ethephon declined to below control yields when no reapplication was made after the second and third months respectively.

Effect of AVG on ACC-enhanced Ethylene Production

The results of an experiment to study effects of AVG on ACC-induced ethylene production in excised bark discs are given in *Figure 5*. ACC increased ethylene production of bark discs six-fold when compared with that of bark discs incubated in phosphate buffer plus sorbitol without ACC. The incorporation of AVG in the incubation medium consisting of buffer

TABLE 1. COMPARISON OF RESPONSES OF CLONE RRIM 600 TO ACC AND ETHEPHON

Treatment	Ethylene levels (p.p.m.)		Yield during 6 months (ml/tree/tapping)						
	1st month (1st appl.)	2nd month (2nd appl.)	1st	2nd	3rd	4th	5th	6th	Mean (6 months)
Control	165.9	214.8	99.3 (100)	113.4 (100)	118.8 (100)	149.8 (100)	154.4 (100)	157.7 (100)	132.2 (100)
2.5% ET in Amchem carrier			148.5 (150)	164.8 (145)	175.4 (148)	168.9 (113)	164.4 (106)	159.3 (101)	163.6 (124)
500 mM ACC in palm oil	791.8	2 520.3	123.3 (124)	135.6 (120)	148.7 (125)	147.0 (98)	152.4 (99)	150.2 (95)	142.9 (108)

Stimulation was only carried out during first, second and third months.
Ethylene levels given refer to that of bark shavings obtained from first tapping after stimulation and are the mean values of ten replicates (ten trees).
Ten trees of clone RRIM 600 tapped on ½S d/2 on *Panel BO-1* were used per treatment.
Figures within brackets are percentage values.

TABLE 2. COMPARISON OF RESPONSES OF CLONE RRIM 701 TO ACC AND ETHEPHON

Treatment	Ethylene levels (p.p.m.)		Yield during 6 months (ml/tree/tapping)						
	27/5	29/5	*1st*	*2nd*	*3rd*	*4th*	*5th*	*6th*	Mean (6 months)
Control	85.5	79.1	137.2 (100)	224.8 (100)	189.5 (100)	222.0 (100)	211.8 (100)	238.6 (100)	204.0 (100)
2.5% ET[a] in Amchem carrier			172.1 (125)	255.2 (114)	191.2 (101)	187.1 (84)	169.9 (80)	181.5 (76)	192.8 (95)
500 mM ACC in palm oil	1 125.5	1 103.6	173.8 (127)	253.9 (113)	200.9 (106)	210.1 (95)	209.1 (99)	209.5 (88)	209.6 (103)

Stimulation was only carried out during first and second months.
Ethylene levels given refer to that of bark shavings obtained from first tapping after stimulation and are the mean values of ten replicates (ten trees).
Ten trees of clone RRIM 701 tapped on ½S d/2 on Panel BI-1 were used per treatment.
Figures within brackets are percentage values.
[a]Second stimulation for ethephon changed to 5%.

plus ACC, did not affect the magnitude of increase in ethylene levels induced by ACC. However, when bark discs were incubated in buffer solutions consisting of only AVG, there was a marked reduction in ethylene levels when compared with that produced by bark discs incubated in buffer solutions.

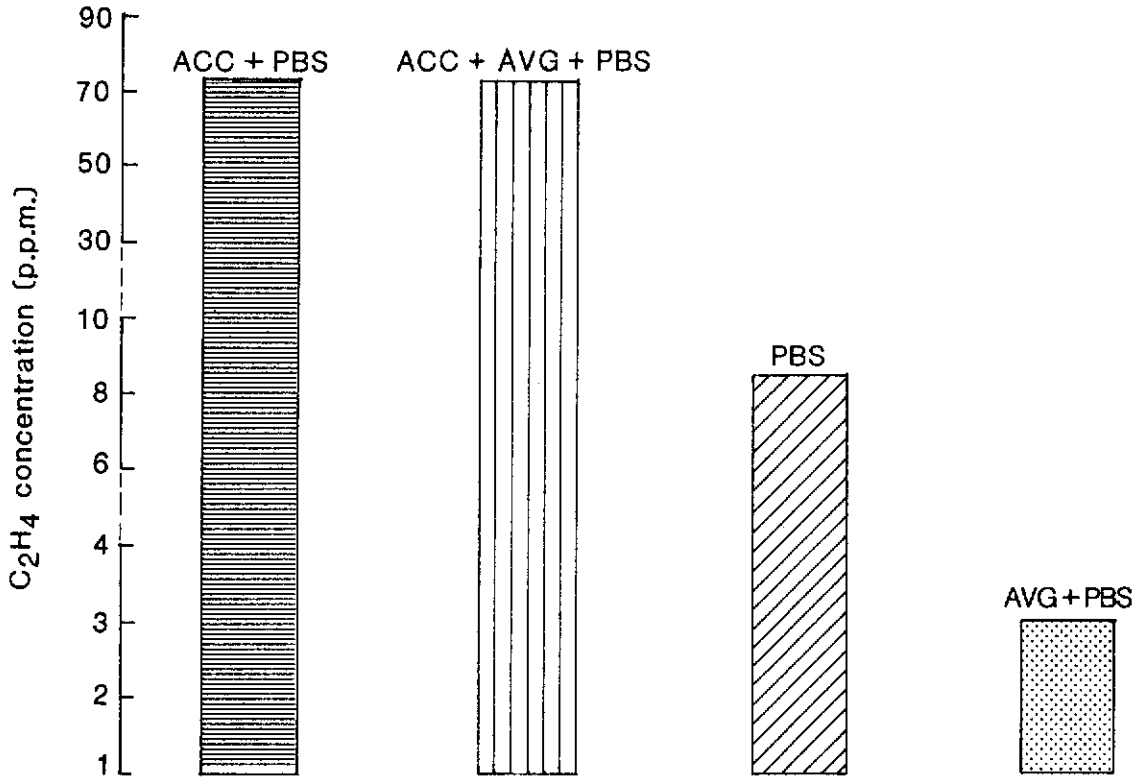

Figure 5. Effect of AVG on ACC-enhanced C_2H_4 production of excised Hevea *bark discs. (The results refer to mean of two replicates per treatment.)*

ACC plus AVG Application to the Tapping Cut

A trial was carried out to study the effects of applying AVG in combination with ACC on yield responses. The results are given in *Figure 6*. ACC as noted in earlier experiments induced a marked increase in ethylene production of bark shavings obtained from the first tapping following application when compared with that produced by bark shavings obtained from unstimulated control trees. Incorporation of AVG in the ACC formulation did not affect the level of ethylene produced. Thus, bark shavings obtained from tapping cuts treated with formulation consisting of ACC plus AVG produced ethylene levels comparable to those obtained from treatment with ACC only.

AVG applied together with ACC did not affect the magnitude of increase in yield responses. This is evident from the similar order and pattern of positive responses obtained for both treatments consisting of ACC plus AVG and ACC only. Thus the marked increase in yields of 32% obtained during the first few tappings following application for trees treated with ACC plus AVG was comparable to 40% increase in response obtained from application of just ACC. The yields of both treatments showed a progressive decline after the fourth week. As observed in earlier experiments, trees treated with

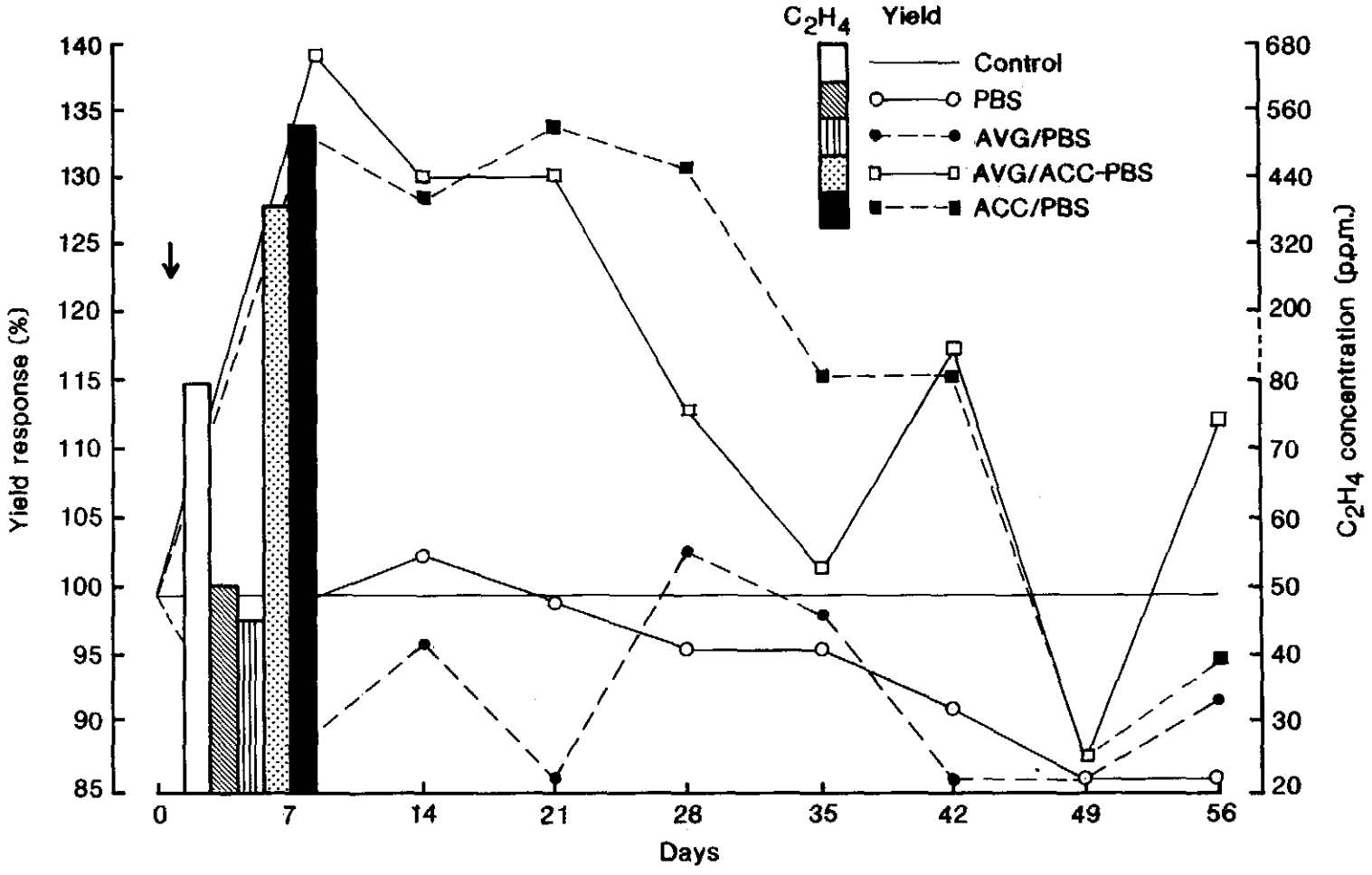

Figure 6. Effect of AVG on ACC stimulation of enhanced latex yields in clone RRIM 600 tapped on renewed bark. Arrow indicates date of application. The results for C_2H_4 concentrations refer to the mean of ten replicates (ten trees).

buffer showed no response with yields being comparable or marginally lower than that of unstimulated control trees. The trees treated with AVG despite fluctuations had yield levels which were lower than that of untreated control trees for most of the eight weeks of recording.

Effect of AVG on Auxin-induced Ethylene Production

The auxins NAA and 2,4,5-T have been shown to induce increased ethylene production in excised *Hevea* bark discs. The effects of AVG treatment on auxin-induced increase in ethylene levels in excised bark discs were investigated in three experiments. The results of these investigations are given in *Figures 7, 8* and *9*.

There was a two-fold increase in ethylene levels produced by bark discs treated with 10 p.p.m. NAA, above that of bark discs treated with water *(Figure 7)*. The NAA-induced increase in ethylene levels was inhibited when bark discs were incubated in a medium consisting of 10 p.p.m. NAA and 100 p.p.m. AVG. This inhibition was further enhanced when AVG concentration was increased to 1000 p.p.m. Thus, in a medium consisting of 10 p.p.m. NAA and 1000 p.p.m. AVG, ethylene levels were reduced to less than half of those produced by bark discs incubated in water. AVG on its own at both concentrations was effective in reducing the ethylene produced by bark discs incubated in water (control). Thus the ethylene production of bark discs incubated in AVG solutions was reduced to one-third of that produced in a water medium.

Treatment of bark discs with 10 p.p.m. 2,4,5-T in tris-HCl buffer increased ethylene production more than three-fold relative to that produced by bark discs treated with just the buffer *(Figure 8)*. AVG at a concentration of 100 p.p.m. was effective in inhibiting 2,4,5-T-induced increases in ethylene production. Thus bark discs incubated in medium consisting of 10 p.p.m 2,4,5-T and 100 p.p.m. AVG in tris-HCl buffer produced ethylene which was only one-sixth of that produced by bark discs treated with 2,4,5-T. AVG was just as effective in inhibiting ethylene formation of bark discs not treated with 2,4,5-T. Thus bark discs incubated in a medium consisting of 100 p.p.m. AVG in tris-HCl buffer reduced ethylene levels to half that produced in tris-HCl buffer.

The results of another experiment to study AVG effects on NAA- and 2,4,5-T-induced ethylene production in excised bark discs are given in *Figure 9*. 2,4,5-T and NAA increased ethylene production, two-and three-fold respectively relative to that produced by bark discs incubated either in water or tris-HCl buffer solution. The ethylene levels produced by bark discs were similar in both water and tris-HCl buffer. AVG effectively inhibited 2, 4, 5-T- and NAA- induced increase in ethylene production. Thus ethylene produced from bark discs incubated in media consisting of either 2,4,5-T plus AVG or NAA plus AVG, was only a fraction of that produced in media without AVG. AVG also inhibited normal ethylene production of bark discs in non-auxin solution. Thus ethylene production of bark discs in tris-HCl buffer solution plus AVG was only one-fifth of that produced in water or tris-HCl buffer solutions without AVG.

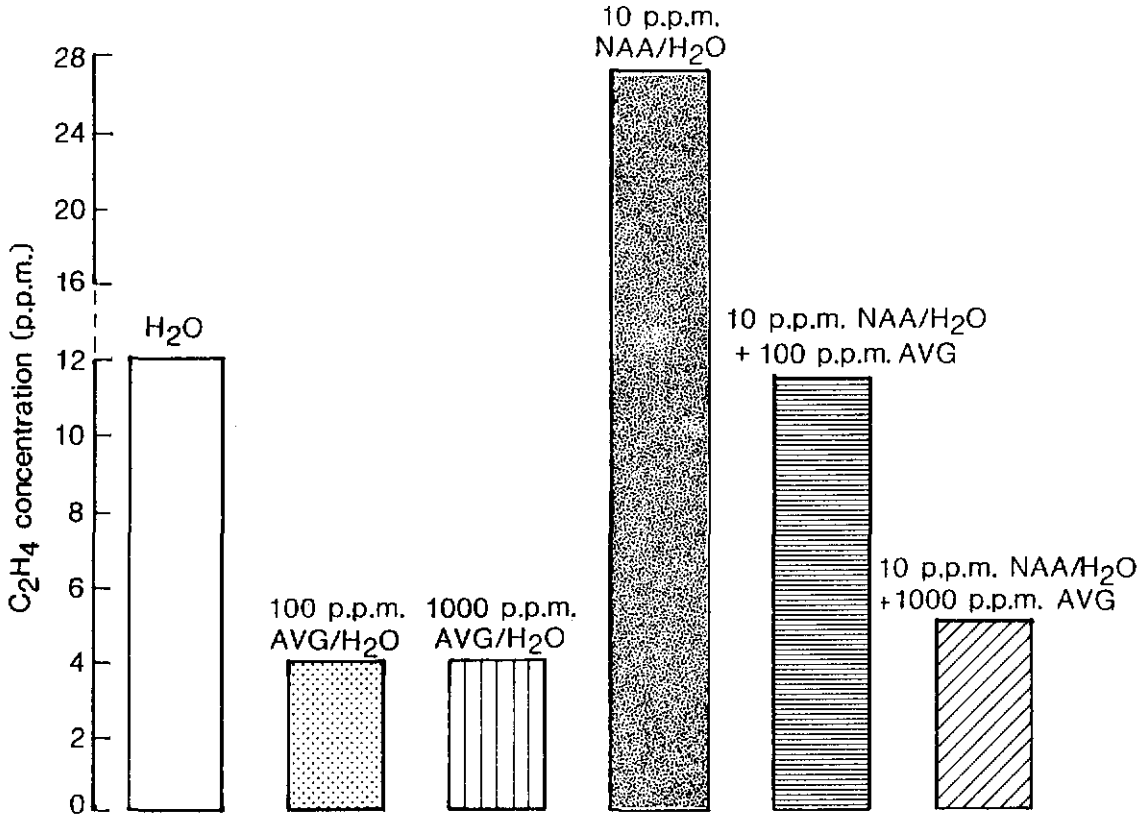

Figure 7. Effect of AVG on NAA-induced C_2H_4 production in excised Hevea *bark discs. (The results refer to mean of three replicates per treatment.)*

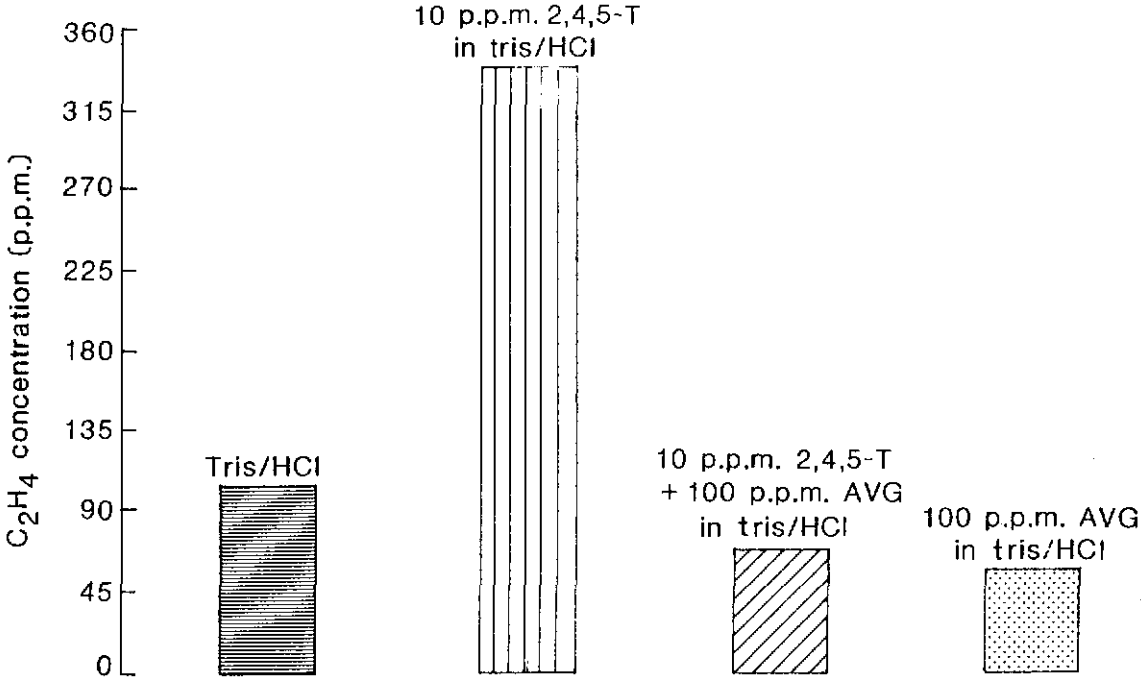

Figure 8. Effect of AVG on 2,4,5-T- induced C_2H_4 production. (The results refer to mean of four replicates per treatment.)

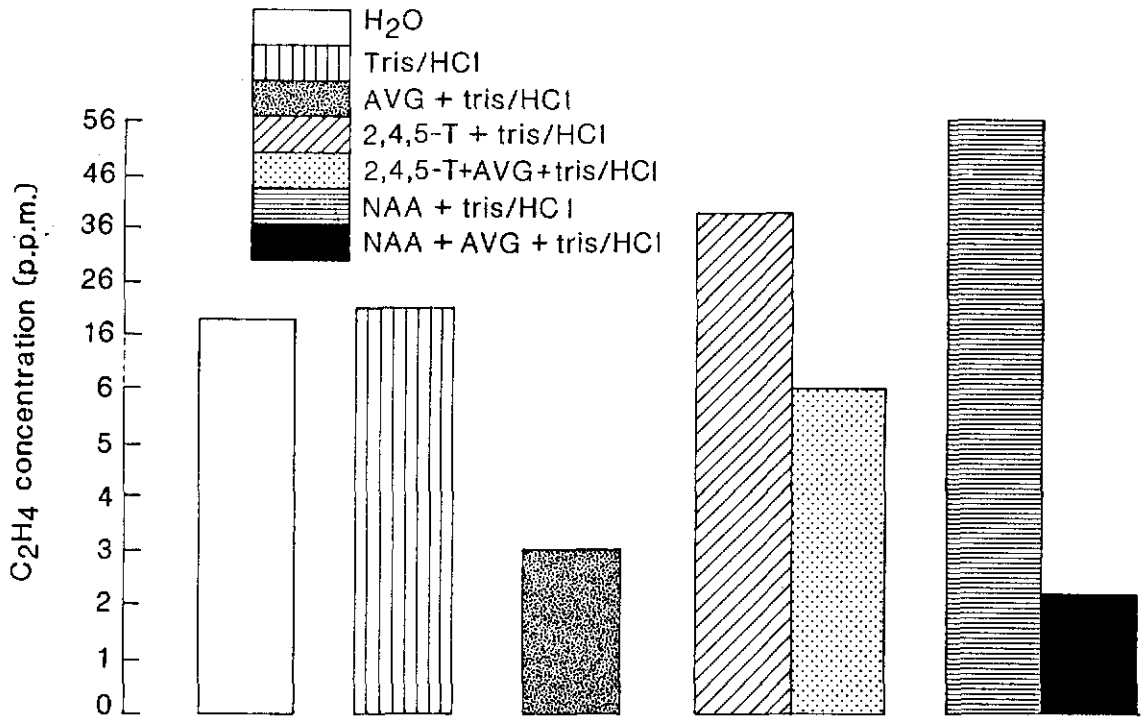

Figure 9. Effect of AVG on NAA and 2, 4, 5-T induced C_2H_4 production of excised Hevea *bark discs. (The results refer to mean of three replicates per treatment.)*

DISCUSSION

ACC treatment stimulated ethylene production considerably in *Hevea* bark tissues. ACC was effective on both virgin and renewed bark tissues of a number of different clones. However, the responses of *Hevea* bark tissues to ACC as observed for other plant tissues were mediated by the concentration of ACC applied[3]. Thus increasing the concentration of ACC beyond a maximum for periods of incubation of 24 h or 48 h did not result in further increase in production of ethylene. It yond a maximum for periods of incubation of apple discs in high concentrations of ACC had an inhibitory effect. It is likely that this was also true for *Hevea* bark discs. The exception is when ACC was applied to the bark of the tree. The concentrations applied were five-fold higher to overcome possible limitations of penetration and uptake of ACC.

It has been shown that ACC stimulates increased ethylene production in a variety of plants and different tissues[2]. It is now established for a number of plants that ACC is the immediate precursor in the ethylene biosynthetic pathway from methionine[15,16,17]. It seems reasonable to suppose that ACC is also the immediate precursor of ethylene in *Hevea* bark tissues. This is supported by a number of observations adduced from data obtained in these investigations. Thus, the marked increase in ethylene production following ACC treatment, the rapid response to

ACC, the concentration effect and the effectiveness of ACC on a number of cultivars and bark of different ages, when taken together and coupled with the knowledge that ACC is the precursor in other plants, suggest that ACC could be the precursor of ethylene in *Hevea* bark tissues.

AVG is a potent inhibitor of ethylene synthesis in plant tissues[18,19] . However, it has been shown that AVG is ineffective in inhibiting conversion of ACC to ethylene thus implying that it acts at a step preceding ACC formation in the ethylene biosynthetic pathway[20] . This step is now identified as the conversion of S-adenosylmethionine to ACC[21] . Data obtained in this investigation show that AVG treatment causes a marked reduction in ethylene formation of *Hevea* bark tissues. However, AVG was ineffective in inhibiting ACC-induced increase in ethylene production either in excised *Hevea* bark discs or when applied to the tree on the tapping cut. This lends further support to the suggestion that ACC may be the immediate precursor of ethylene in *Hevea* bark tissues.

It is known that several auxin compounds stimulate ethylene synthesis in most plants including *Hevea* bark tissues[12,22,23] as confirmed in this investigation where a marked increase in ethylene production was obtained when excised *Hevea* bark discs were treated with NAA and 2,4,5-T. However, AVG treatment inhibited the auxin-stimulated increases in ethylene levels in *Hevea* bark discs, this being similar to that shown for other plants[21] . The AVG inhibition was very marked since ethylene levels produced by bark discs treated with NAA or 2,4,5-T in the presence of AVG was reduced to levels below that produced by control bark discs treated with just the incubation medium. The step inhibited by AVG in the pathway of ethylene synthesis stimulated by auxins has been identified. Thus, a manifold increase in ACC levels has been detected in auxin-treated tissue implying that its inductive effect occurs at a step preceding conversion of ACC to ethylene[21] . It has now been demonstrated that auxins increase conversion of S-adenosylmethionine to ACC by stimulating the synthesis of the enzyme involved in this reaction[20,24] . Since AVG does not affect the conversion of methionine to S-adenosylmethionine, it is apparent that AVG inhibits the conversion of S-adenosylmethionine to ACC, the same step upon which auxin exerts its inductive effect[10,21] . The similarity in AVG effects on auxin-treated *Hevea* bark tissues suggests that its inhibitory action occurs at a step preceding ACC formation in *Hevea* bark tissues.

It is apparent from evidence presented in relation to ethylene synthesis in *Hevea* bark tissues that there are a number of similarities to the ethylene biosynthetic pathway established for other plants. However, confirmation of this pathway for *Hevea* bark tissues is outside the scope of this paper.

The yield stimulant activity of ACC can be attributed to its promotion of ethylene synthesis in treated bark tissues and the consequent action of ethylene as a yield stimulant[11] . ACC application induced yield increase in a number of clones and was equally effective when applied either in buffer solutions or formulated in oil-based carriers. The characteristic pattern

of yield response typical of most ethylene-based yield stimulants was also seen with ACC. Thus initially following application, there was a marked increase in response during the first few tappings followed by a gradual decline over subsequent tappings till the next application when the same pattern was repeated. The initial peak yield responses appear to correspond to the massive synthesis of ethylene immediately following ACC application. The lack of continued ethylene synthesis after the initial period may account for the subsequent decline in yield response. The responses to ACC were generally lower than that of ethephon at concentrations tested. The similar pattern of yield responses obtained from trees treated with ACC and ACC plus AVG suggests that AVG has no effect on the ACC-induced yield increases. This supports the earlier conclusion that AVG has no inhibitory effect on ACC-promoted ethylene synthesis.

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