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Effects of Ethephon Stimulation on Latex Invertase in Hevea

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Ethephon stimulation on previously unstimulated trees caused an increased invertase activity in whole latex as well as in C serum. The speed and extent of this response varied with clones. Repeated ethephon stimulation resulted in an eventual decline in invertase activity, which at times was lower than that of the unstimulated controls, even though the yield response was positive. The response of long-term repeated ethephon stimulation and the introduction of stimulation rest to latex invertase are discussed. Some properties of Hevea *invertase are also described. Invertase activity did not appear to be correlated with latex output.*

The importance of invertase in latex physiology and production has been a subject of much study[1–4]. It has been suggested that invertase, by virtue of its extremely low activity[5] is a possible pace-maker enzyme in the glycolytic pathway of *Hevea* latex. Certainly, since latex invertase controlled the utilisation of sucrose–the primary carbohydrate substrate in latex – it might have an important role in controlling the overall metabolic activity of latex[2]. This is a report on the effects of ethephon stimulation on latex invertase activity as well as the invertase-latex output relationship in stimulated trees.

MATERIALS AND METHODS

Experimental Materials

Details of the trees used in the experiment on the immediate effects of ethephon stimulation are given in *Table 1*.

The long-term effects of repeated ethephon stimulation were studied in two separate experiments. Details of these experiments are summarised in *Table 2*.

Experimental Methods

Invertase (β-fructofuranosidase, E.C. 32.1.26) activities in C serum and whole latex were measured according to the methods described by Yeang *et al.*[4] One unit of invertase activity was defined as 1 μmole of sucrose hydrolysed by 10 ml undiluted latex or serum per 30 min at 30°C.

After electrophoresis of C serum in 7.5% polyacrylamide gels (prepared by the method of Ornstein and Davis[8,9]), invertase activity in these gels was located by staining in 0.1% 2,3,5-triphenyltetrazolium chloride (TTC) in *M* NaOH[10]

Sucrose content in latex was measured by the procedure described by Low[11].

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TABLE 1. DETAILS OF TREES USED IN THE STUDY OF THE IMMEDIATE EFFECTS OF ETHEPHON STIMULATION

Item	PR 107	Tjir 1	RRIM 501	RRIM 701
Number of trees/treatment	6	10	10	5
Panel in tapping	*BO-2*	*BI-1*	*BI-1*	*BO-1*
Field in RRIM Experiment Station, Sg. Buloh	*22*	*22*	*22*	*37*
Stimulation history	Nil	Nil	Nil	Nil
Ethephon applied (%)	10	10	10	10
Stimulation method	Scraped bark[a]	Tree lace[b]	Tree lace[b]	Scraped bark[a]
Tapping system	½S d/2	½S d/2	½S d/2	½S d/2

[a] After Abraham *et al.*[6]

[b] After P'ng *et al.*[7]

TABLE 2. DETAILS OF TREES USED IN THE EXPERIMENTS ON LONG-TERM EFFECTS OF REPEATED ETHEPHON STIMULATION

Item	Expt. A RRIM 701	Expt. B Tjir 1, GT 1, PB 86, RRIM 600, RRIM 501
Number of trees/treatment	20	16
Randomised on	Girth	Yield
Field in RRIM Experiment Station, Sg. Buloh	*37*	*14D*
Panel in tapping	*BO-1*	*BO-2*
Tapping system	½S d/2	½S d/2
Stimulation history	Nil	Bimonthly for 4 years
Ethephon applied (%)	10	10
Stimulation method	Tree lace[a]	Scraped bark[b]
Duration of expt. (months)	12	14

[a] After P'ng *et al.*[7]

[b] After Abraham *et al.*[6]

RESULTS

Immediate Effects of Etheplon Stimulation on Invertase Activity

The immediate effects of ethephon stimulation on previously unstimulated trees were studied in two high-plugging clones (Tjir 1 and PR 107) and two low-plugging clones (RRIM 501 and RRIM 701). Ethephon stimulation was shown to increase the invertase activities in C serum and whole latex in two of these four clones. These were Tjir 1 and RRIM 501 for increased C serum invertase activity *(Figure 1)* and Tjir 1, RRIM 501 and RRIM 701 for increased whole latex invertase activity *(Figure 2)*. Thus, Tjir 1 and RRIM 501 were the only two clones out of the four examined which manifested an increased invertase activity in C serum as well as in whole latex. Comparing the extent of increased invertase activity between C serum and whole latex, the

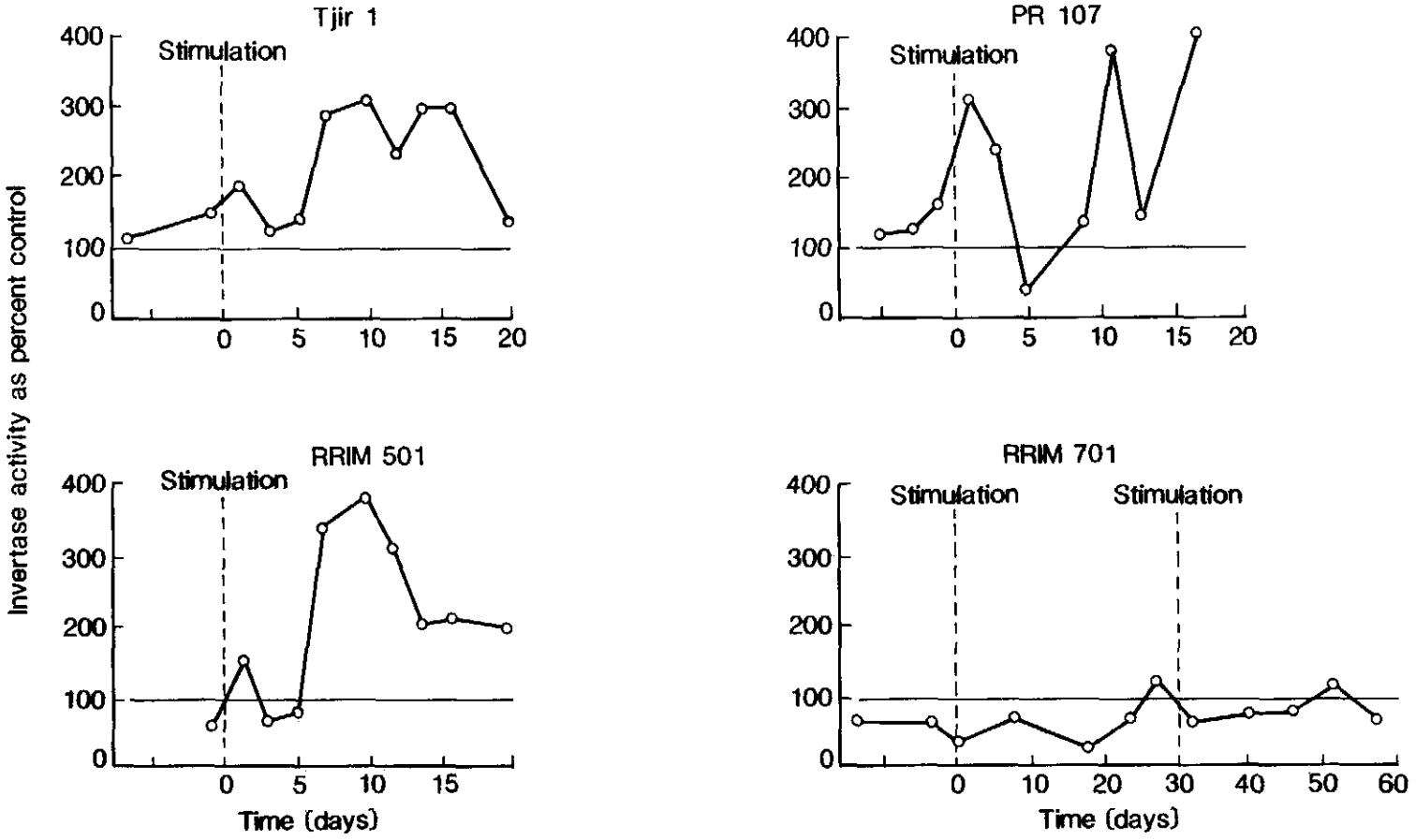

Figure 1. Effect of ethephon stimulation on invertase activity in C serum.

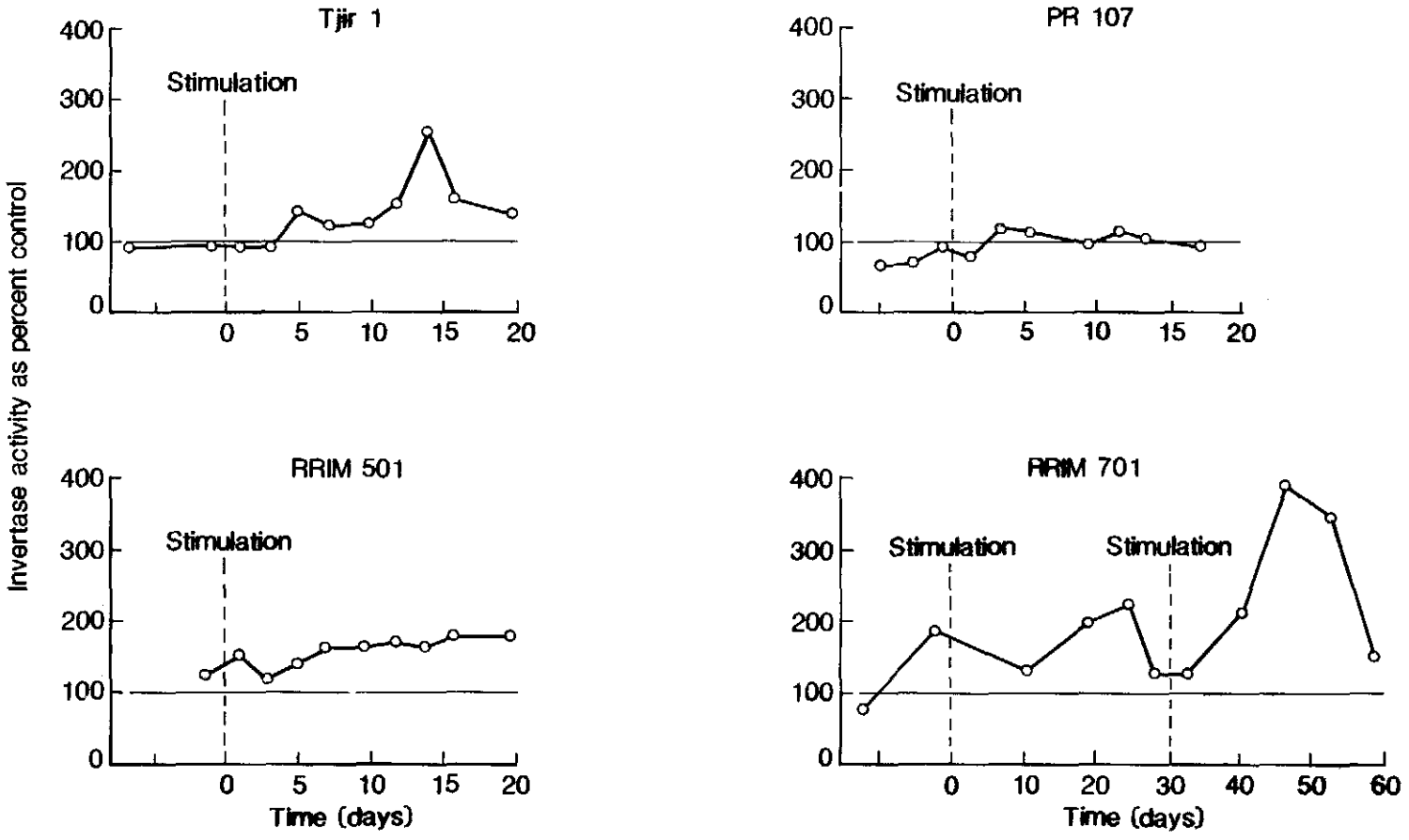

Figure 2. Effect of ethephon stimulation on invertase activity in latex.

increase in C serum appeared to be much higher (compared to the controls) than that in whole latex.

Ethephon stimulation in PR 107 resulted in a variable invertase activity in the C serum. The activity in the whole latex however, appeared to be relatively unaffected by ethephon stimulation. Invertase activity in RRIM 701, on the other hand, was increased in whole latex, but not in C serum after ethephon stimulation *(Figures 1* and *2).*

Long-term Effects of Ethephon Stimulation on Invertase Activity

The response to repeated monthly ethephon stimulation in previously unexploited RRIM 701 trees was observed over a period of one year. After an initial lag period of about a month, invertase activity appeared to be enhanced with repeated ethephon stimulation at the initial few months *(Figure 3).* However, as the experiment progressed, a gradual decline in invertase activity was evident, so that six months after the commencement of the experiment, the invertase activity in the stimulated latex serum was sometimes less than that in the control latex serum *(Figure 3).*

This negative response of invertase to repeated ethephon stimulation was not accompanied by a similar negative response to yield *(Figure 3).* In fact, in the last two months of the experiment, while the invertase activity was at its lowest level, the simultaneous yield response was at its highest for the entire period of the experiment.

The long-term effects of repeated ethephon stimulation on invertase activity were investigated in another experiment. After four years of repeated bimonthly ethephon stimulation, three out of the five clones under study displayed a depressed invertase activity compared to controls *(Figure 4).* These were Tjir 1, RRIM 600 and GT 1. On the other hand, the invertase activity in stimulated PB 86 and RRIM 501 remained elevated in comparison to controls. The yield response to repeated ethephon stimulation in these five clones did not however, coincide with that of invertase activity. Only one clone (PB 86) out of five displayed a positive yield response to ethephon stimulation. When expressed as percent control, Tjir 1, RRIM 600 and GT 1 had a yield response of 99%, 47% and 92% respectively, while PB 86 and RRIM 501 were responding at 106% and 78% respectively[12].

Cessation of ethephon application, *i.e. stimulation rest, for a period of eight* months, appeared to result in an increase in invertase activity in the previously stimulated trees. The percentages of invertase activity in the previously stimulated trees to that of controls were shown to increase in four clones (Tjir 1, PB 86, RRIM 600 and GT 1). The invertase activity in RRIM 501 latex was unchanged with stimulation rest *(Figure 4).*

Some Properties of *Hevea* Invertase

A comparison of some properties of invertase in *Hevea* latex after ethephon stimulation with that of unstimulated latex was performed to determine whether ethephon stimulation affected latex invertase properties.

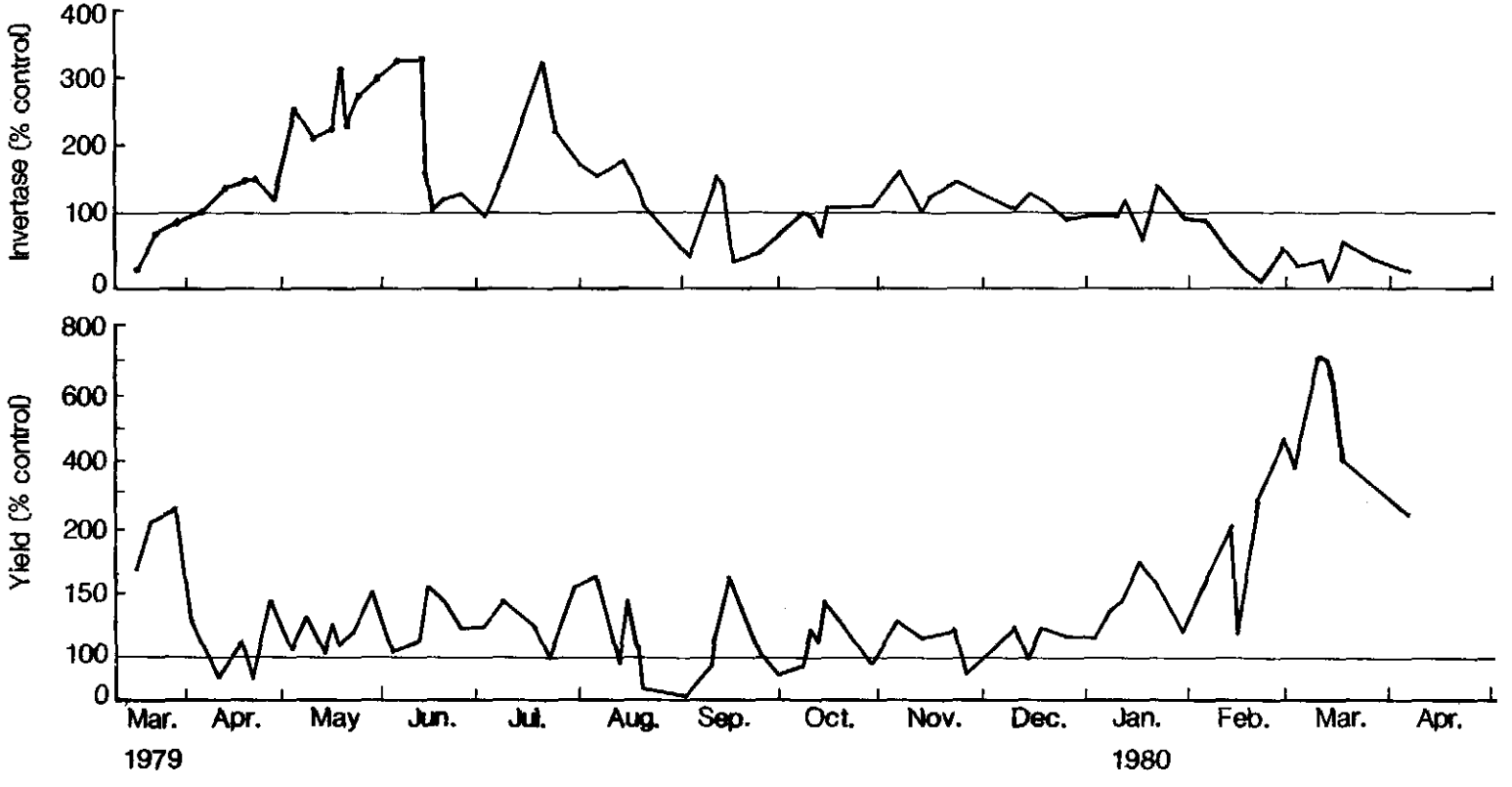

Figure 3. Effects of repeated ethephon stimulation on C serum invertase activity and yield.

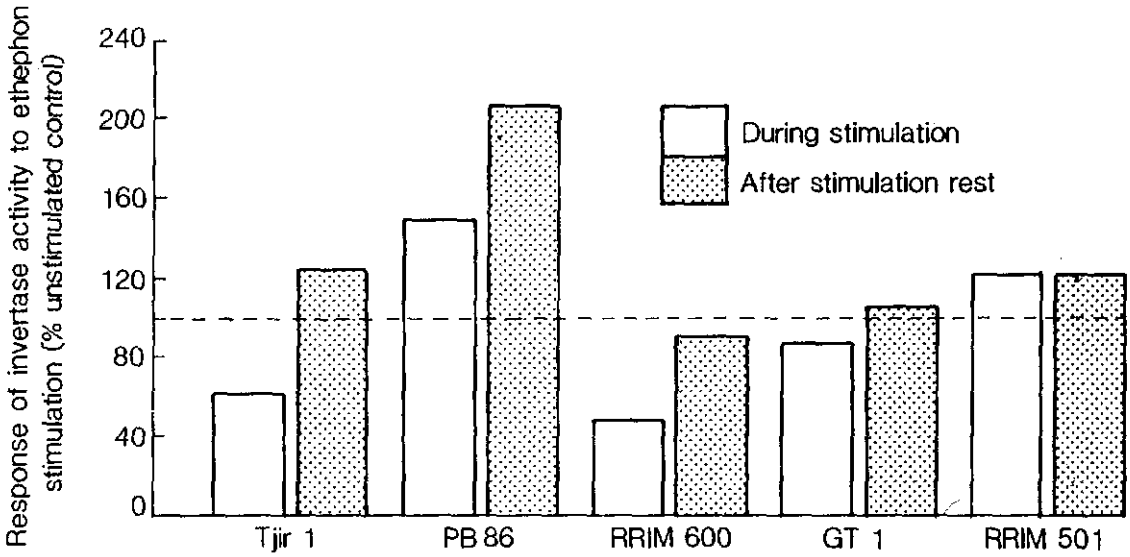

Figure 4. Effect of stimulation rest on invertase activity.

The pH optimum of invertase in stimulated and unstimulated latex of RRIM 701 trees was found to differ when measured ten months after stimulation. When assayed in glycine-sodium hydroxide buffer, the pH optimum of invertase in the stimulated latex was 9.1, compared to 8.9 in the unstimulated latex *(Figure 5)*. At optimum pH, the invertase activity in stimulated latex was estimated to be 1000 invertase units, in comparison to 600 invertase units in the unstimulated latex.

Ammonium sulphate fractionation failed to detect any differences in invertase activity between the C sera from stimulated and unstimulated trees. Most of the invertase activity was precipitated at 0%–50% saturation of ammonium sulphate in both the stimulated and unstimulated treatments. Negligible invertase activity was precipitated at 75% saturation of ammonium sulphate in both treatments.

Electrophoresis in polyacrylamide gels revealed similar protein patterns in both the stimulated and the unstimulated latex sera. A total of seventeen protein bands were separated from both these sera, of which only *one* band was associated with invertase activity. This band of invertase activity was also the slowest-migrating protein band. In comparison to the unstimulated control, the invertase activity in the stimulated sera was stained more intensely.

Correlation between Invertase Activity and Latex Yield, pH and Sucrose Content

Simple correlation studies between invertase activity and latex yield, pH and sucrose content were carried out over ten months, in the experiment with RRIM 701 trees. The results *(Table 3)* show that invertase activity was significantly correlated to yield (r = 0.524***)

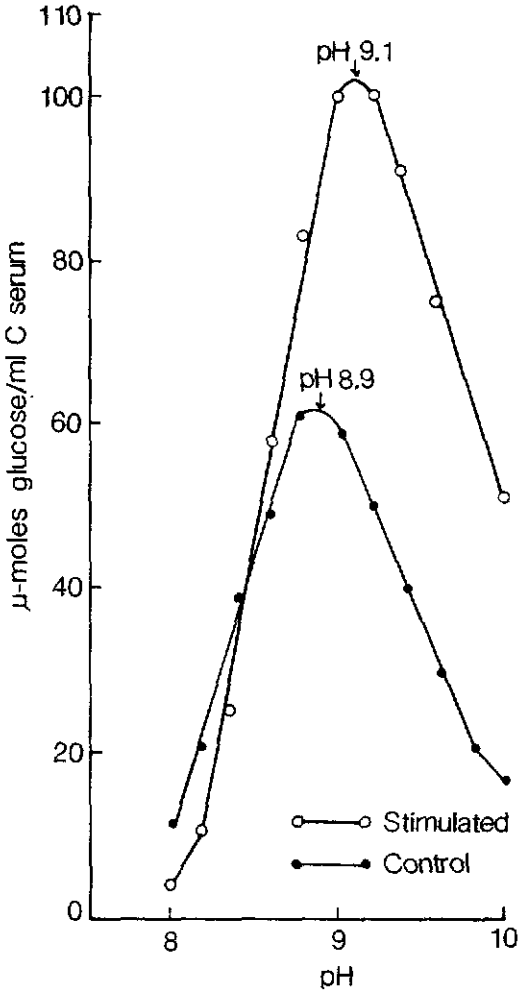

Figure 5. Effect of stimulation on the pH optimum of invertase.

and weakly correlated to latex pH and sucrose content ($r = 0.222^{P < 0.1}$ and $-0.209^{P < 0.1}$ respectively) in the unstimulated control. On the contrary, all these parameters were not correlated at all in the stimulated sera.

A similar simple correlation study between latex yield and invertase activity, and latex yield and the sucrose content of latex was performed on five clones. The results *(Table 4)* demonstrated that only Tjir 1 displayed a significant correlation ($r = 0.674^{**}$) between yield and invertase activity for the unstimulated control, while a less significant ($r = 0.567^{*}$) correlation was established between these two parameters in the stimulated treatment.

As far as correlation between latex production and latex sucrose content was concerned, only the stimulated treatments of Tjir 1 and RRIM 501 displayed some significance ($r = -0.424^{*}$ and 0.502^{*} respectively). Correlations with all other clones, both the stimulated and control treatments, were shown to be not significant *(Table 4)*.

TABLE 3. SIMPLE CORRELATIONS BETWEEN INVERTASE ACTIVITY AND YIELD, LATEX pH, AND LATEX SUCROSE CONTENT IN RRIM 701

Sources of variables	Yield		Latex pH		Latex sucrose content	
	n	r	n	r	n	r
½S d/2 control	67	0.524***	61	$0.222^{P<0.1}$	66	$-0.209^{P<0.1}$
½S d/2 + ethephon	67	−0.170 NS	61	0.195 NS	66	0.090 NS

TABLE 4. CORRELATION COEFFICIENTS BETWEEN YIELD AND SUCROSE CONTENT AND BETWEEN YIELD AND INVERTASE ACTIVITY

Source of variables	*Correlation coefficient*[a] yield : sucrose content	*Correlation coefficient*[b] yield : invertase activity
Tjir 1 control	– 0.0517 NS	0.674**
Tjir 1 stimulated	– 0.424*	0.567*
PB 86 control	– 0.124 NS	– 0.078 NS
PB 86 stimulated	– 0.119 NS	0.451 $P < 0.1$
RRIM 600 control	– 0.080 NS	0.211 NS
RRIM 600 stimulated	– 0.027 NS	0.073 NS
GT 1 control	– 0.234 NS	0.333 NS
GT 1 stimulated	0.190 NS	0.285 NS
RRIM 501 control	0.276 NS	0.150 NS
RRIM 501 stimulated	0.502*	0.359 NS

[a] n = 23 pairs

[b] n = 18 pairs

DISCUSSION

While the response of invertase activity to ethephon stimulation fluctuated on occasions, certain clear trends were evident. Invertase activity in the C serum was shown to increase with ethephon stimulation in Tjir 1, RRIM 501 and RRIM 701, although in RRIM 701 *(Figure 3)*, the response was somewhat delayed. On the other hand, the response was immediate in Tjir 1 and RRIM 501 *(Figure 1)*. There was also an increase in C serum invertase activity in PR 107, but this was not so distinct. Comparing the C serum invertase activity on the first day with that on the second day of tapping after ethephon stimulation, a decrease in invertase activity in the second day of tapping after ethephon stimulation was recorded in Tjir 1, PR 107 and RRIM 501. This dip was followed by a large increase in invertase activity. Reasons for this temporary decline in C serum invertase activity are not known.

With the exception of PR 107, an increase in latex invertase activity following ethephon stimulation was recorded in Tjir 1, RRIM 501 and RRIM 701. However, unlike the C serum invertase activity, the response of latex invertase activity was less immediate. Other than RRIM 701, the increase in latex invertase activity in the other three clones was less than their increase in C serum invertase activity when expressed as percent of control. It is not clear why RRIM 701 responded much more positively to ethephon stimulation at the whole latex rather than at the C serum level.

Working with PR 107 trees, Tupy[1-3] did not observe any differences between the stimulated and the unstimulated latices, when invertase was assayed in buffered conditions. In our present studies with PR 107, the invertase activity in whole latex was measured under buffered conditions *(Figure 2)*. As seen in *Figure 2*, negligible differences in whole latex invertase activity were observed in PR 107 between the stimulated and the unstimulated latices. This observation is in agreement with that of Tupy's[1-3]. However, PR 107

is the exception rather than the rule. Three other clones namely, Tjir 1, RRIM 501 and RRIM 701, in the same experiment, exhibited increased whole latex invertase activity after stimulation. Thus an actual increase in the enzyme present was suggested, rather than the pH effect as proposed by Tupy[1-3]

Studies on the long-term effects of repeated ethephon stimulation on C serum invertase activity have shown that the increased C serum invertase activity following ethephon stimulation was not sustained throughout the period of investigation *(Figures 3* and *4)*. Observations of repeated monthly applications of ethephon on RRIM 701 had shown that after several months, the enhancement of C serum invertase activity declined, until the activity in stimulated latex was less than that in controls. The decline in invertase activity over an extended period of repeated stimulation is a new observation. This decline in invertase activity with long-term repeated ethephon stimulation did not appear to be associated with yield response. A positive yield response was recorded during this period of negative invertase response. Hence, the general statement that invertase activity was correlated with latex production[2] deserves some reservations.

The declining invertase response to repeated ethephon stimulation was not confined to only one clone (RRIM 701) but was also observed in other clones (Tjir 1, RRIM 600 and GT 1) in another experiment *(Figure 4)*. After four years of repeated bimonthly ethepton stimulation, three clones out of the five examined exhibited a depressed invertase activity in the stimulated latex, when compared to controls. This depressed invertase activity appeared to be associated with repeated stimulations because an increase in invertase activity was noted after the introduction of eight months of stimulation rest (*i.e.* no application of stimulant). Even PB 86 which was responding positively before the stimulation rest appeared to be further enhanced in its invertase activity after stimulation rest.

The association of depressed invertase activity with repeated ethephon stimulation appears evident though the reasons are less apparent. Correlation studies between yield and invertase, and yield and sucrose levels did not suggest that these parameters were significantly related, except in Tjir 1 *(Table 4)*. Hence the role of invertase in latex production might have been over-emphasised in the past[2] and needs further examination in the future.

Ethephon stimulation resulted in a shift of the pH optimum of invertase in RRIM 701 latex. When assayed in glycine-sodium hydroxide buffer, the pH optimum of invertase in the stimulated latex was 9.1, compared to 8.9 in the unstimulated latex. Though an increased pH optimum with ethephon stimulation was observed, simple correlation studies suggested that latex pH and latex productivity were not significantly correlated *(Table 3)*.

The *Hevea* latex invertase did not exist in multiple iso-enzymic forms. The appearance of only a single band of invertase activity, which was associated with the least mobile protein band in both the stimulated and the unstimulated control suggested that invertase did not exist as isoenzymes and that stimulation

did not cause the formation of these. The more intense invertase band in the stimulated latex suggested an enhanced invertase activity on stimulation – an observation verified from other quantitative measurements.

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