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Ozone Cracking and Protection of Elastomers at High and Low Temperatures

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Ozone attack on elastomers varying in their glass transition temperatures (θ_G) by some 50°C was studied over a range of temperatures from about −20°C to +70°C. If the ambient temperature is low, the rate of crack growth due to ozone may be reduced by the high internal viscosity of the material. However, diffusion of chemical antiozonants or waxes which confer protection by forming a layer on the elastomer surface, is also slowed down. It appears more difficult to obtain good protection at low temperatures. High ambient temperatures do not greatly increase the rate of ozone crack growth and might be expected to improve antiozonant and wax protection for elastomers of high θ_G as a result of increased rates of diffusion. This was observed to some extent.

Ozone can cause cracking in vulcanised elastomers subject to constant or repeated loading. The ozone concentration in the outdoor atmosphere at ground level is typically about 1 p.p.h.m. (part per hundred million of air by volume) although levels an order of magnitude or so higher have been observed for limited periods in polluted areas. Indoors, or in enclosed spaces, ozone levels may be an order of magnitude lower unless there are electrical or other discharges, when much higher levels may result. The recommended ambient temperature for standard ozone tests is now 40°C. This is far above operating temperatures for many uses of elastomers, although it may be too low, intermittently, for articles subject to direct sunlight, particularly in tropical regions.

The effects of ozone concentration and ambient temperature on the basic ozone cracking process are well understood, as is the effect of the glass transition temperature of the elastomer[1,2,3]. However, when protective agents – chemical antiozonants or waxes – are present, these effects are much less well understood. High or low temperatures, in particular, have been relatively little studied and this paper describes work that is in progress to try to remedy this. The protective systems examined include chemical antiozonants or waxes alone and wax/antiozonant combinations, used in elastomers of various glass transition temperatures in tests over a wide range of ozone concentrations.

THEORIES FOR LAYER FORMATION

Chemical Antiozonants

When a chemical antiozonant is present in an elastomer, layer formation occurs only on exposure to ozone and is believed to be primarily the result of direct reaction between the antiozonant and ozone. In earlier work it was shown for natural rubber that under some circumstances, the rate of layer formation was consistent with control by diffusion of antiozonant across the already-formed layer to react with ozone at its outer surface[4] *(Figure 1a)*. Under these circumstances, assuming the layer to be formed entirely of reacted antiozonant and the concentration of unreacted material within it to be small, the mass per unit area

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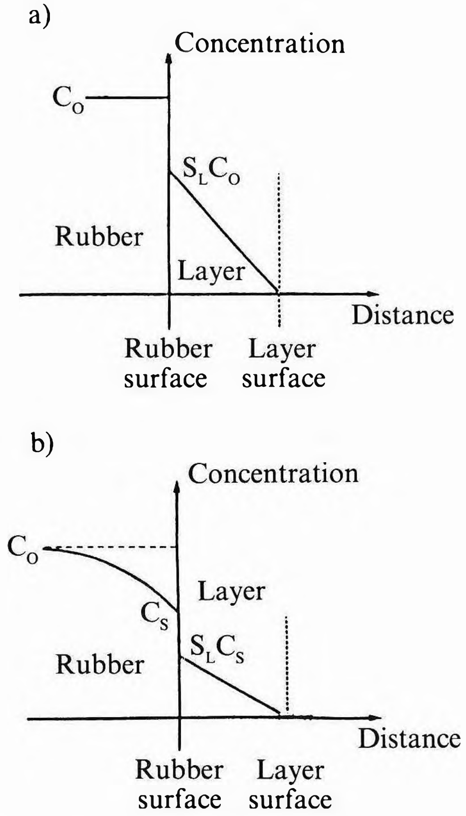

Figure 1. Diffusion models for antiozonant layer formation: a) rate governed by diffusion of antiozonant within the layer only; b) rate governed by both diffusion in the layer and in the rubber.

M_L, of layer formed after time t is given by

$$M_L = \sqrt{2\rho_L D_L S_L C_o t} \qquad \ldots 1$$

where C_o is the concentration of antiozonant in the bulk of the rubber

P_L the density of the layer material

D_L the diffusion coefficient

S_L the solubility, relative to that in the rubber, of the antiozonant in the layer.

If the product of the latter two quantities is put as

$$P_L = S_L D_L \qquad \ldots 2$$

P_L is in essence the relative permeability of the layer material to the antiozonant. Consistent with *Equation 1*, the rate of layer formation on natural rubber was observed to be independent of ozone concentration over a range from about 25 p.p.h.m. to 750 p.p.h.m. although it increased at higher concentrations.

In deriving *Equation 1*, it is assumed that the concentration of (unreacted) antiozonant in the layer is small. Under these circumstances the concentration gradient in the layer will be essentially linear (cf *Figure 1a*). If, in addition to diffusion across the layer, diffusion within the rubber is also important, a similar equation to *Equation 1* applies with C_o replaced C_s, the concentration at the rubber surface, which is lower than C_o *(Figure 1b)*. C_s is a function of C_o and the relative permeabilities of the rubber and the layer to the antiozonant, and with the assumptions made is given by

$$\frac{C_s}{C_o} = 1 + \varepsilon - \sqrt{(1+\varepsilon)^2 - 1} \qquad \ldots 3$$

where

$$\varepsilon = \frac{\pi}{4}\,\frac{\rho_L D_L S_L}{D C_o} \qquad \ldots 4$$

and D is the diffusion coefficient of the antiozonant in the rubber.

Waxes

The mechanism of layer formation by waxes is quite different from that by chemical antiozonants. Waxes are relatively inert and blooming occurs when the amount of wax incorporated in the rubber exceeds the solubility. This is possible because the melting points of the waxes used are generally within the range from about 45°C to 70°C. Thus at processing temperatures, which are normally well above the upper limit, the waxes are liquid, with relatively high solubility, whereas at operating temperatures they are crystalline solids with much lower solubility. Blooming is not simply due to super-saturation, however, but is believed to involve stress-induced diffusion due to the elastic forces acting on crystals formed inside the rubber[5]. Quantitative solution for the rate of layer formation involves, although it is not sensitive to, the distribution of crystals in the rubber. The dependence of the mass of wax layer per unit area of surface (M_w) on time (t) can be approximated by

$$M = \sqrt{\frac{F\,(C_W - S_W)\,D_W S_W G t}{\theta}} \qquad \ldots 5$$

where D_w is the diffusion coefficient

S_w the solubility of the wax in the rubber

C_w the concentration of wax incorporated,

G the shear modulus of the rubber

θ the absolute temperature

F is a function involving the wax distribution and other parameters.

Thus the rate of protective layer formation with a wax is not influenced by the ozone concentration but it is generally sensitive to temperature. This is not so much because θ appears in *Equation 5* (this small effect would in fact be balanced by the dependence of G on θ according to the statistical theory of rubber elasticity) but because of the variation of D_w and S_w with temperature. In practice, most waxes used are blends of components having different molecular weights and melting points, often over quite a wide range. (Apart from providing more consistent blooming behaviour over a range of temperatures, the bloom obtained by this means provides better protection, as pure waxes tend to give large crystals that form a very uneven bloom[6].)

EXPERIMENTAL PROCEDURE

Ozone tests were carried out using a commercially-available test cabinet[7] for concentrations within the range 50 – 200 p.p.h.m. while an apparatus similar to that described by Farlie[1] was used for concentrations of about 10^4 to 10^5 p.p.h.m. In this apparatus, ozone was generated from cylinder oxygen using an ultra-violet discharge. Intermediate concentrations from about 10^2 to 10^3 p.p.h.m. were obtained by diluting the ozonised oxygen from the 'high' concentration apparatus with a suitable flow of air. Tests in the commercial cabinet were carried out at various temperatures with a range from about 8°C to 70°C. Tests in the laboratory apparatus were carried out at about 23°C or −17°C.

Ozone cracking tests on surfaces were carried out using strip or dumbbell test-pieces held at an essentially constant strain which was normally within the range 10% to 100%. The test-pieces were examined intermittently during the course of exposure to ozone to determine whether or not cracking had occurred. In some cases, an estimate of the rate of crack growth was made by measuring the crack length (in a direction perpendicular to a major surface) at the end of a test.

Measurements of the rate of growth of a single crack by ozone were made using a test-piece in the form of a strip containing an initiating edge cut, as described by Braden and Gent[1]. Protection, by a coating of inert grease or other means, was used to prevent formation of subsidiary cracks.

The elastomers used were natural rubber (NR, glass transition temperature, θ_G *ca* $-67°C$), epoxidised natural rubber with about 50% epoxidation (ENR 50, θ_G *ca* $-21°C$) and two acrylonitrile-butadiene copolymers (34% acrylonitrile, NBR 34, θ_G *ca* $-27°C$, or 38% acrylonitrile, NBR 38, θ_G *ca* $-23°C$). Accelerated sulphur-vulcanisates of each elastomer were prepared in the form of 2 mm thick sheets from which test-pieces were die-stamped. The sheets were stored at *ca* 0°C to minimise ageing and blooming effects. When a chemical antiozonant alone was incorporated, nearly all the experiments described were carried out with di(methylethylpentyl) paraphenylenediamine (DOPPD – 'UOP88', Universal Oil Products, Illinois) which was used at levels from 2 p.h.r. to 20 p.h.r. (parts per hundred of rubber by weight). Various wax/antiozonant combinations were also employed, generally with 6 p.h.r. wax and 3 p.h.r. antiozonant.

Diffusion coefficients of the antiozonant were estimated from transfer measurements in which a test-piece containing DOPPD was pressed into contact with a test-piece of a similar vulcanisate containing, initially, no DOPPD *(Figure 2)*. The amount of antiozonant that had diffused after various times was estimated from the weight changes of the test-pieces, enabling the diffusion coefficient to be calculated according to standard diffusion theory[8].

The weight of reacted antiozonant layer formed after exposure to ozone was determined from the weight change after removal of the layer from the surface of the rubber. The layer could be removed by scraping with a blade, swabbing with tissue containing a suitable solvent or repeated application of adhesive tape. The latter method was mostly used for the chemical antiozonant layer, whereas scraping was found to be best for

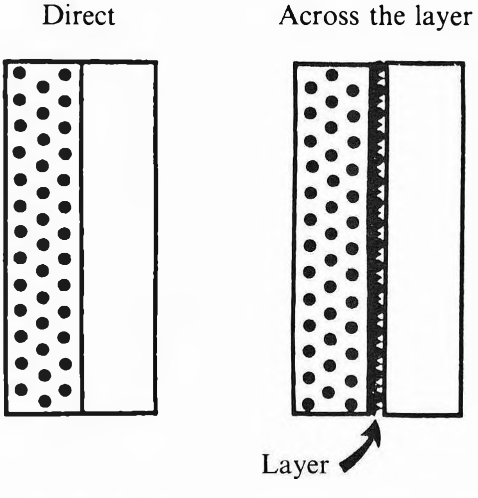

Figure 2. Arrangements for transfer measurements: direct; across the layer.

wax layers. In experiments with waxes, the elastomer sheets were vulcanised between aluminium foil or polyester sheets to minimise blooming during storage. Test-pieces for bloom formation measurements were kept at a constant temperature of either 20°C or 40°C for a fixed period. The weight of the bloom was then determined from the weight change after its removal.

Preliminary experiments to estimate the permeability of the antiozonant layer material to the (unreacted) antiozonant have been carried out by forming a layer on the surface of a test-piece by exposure to ozone and then carrying out a transfer measurement through the layer to a control test-piece pressed into contact *(Figure 2)*. In this case, the permeability was estimated by assuming the layer to act as a membrane[8]. Only a single time could be used in each experiment as the layer was disrupted when the test-pieces were separated.

RESULTS

Earlier work has indicated that two basic parameters govern the growth of a single, isolated crack by ozone: i) a threshold energy below which the crack will not grow; and, ii) the rate at which the crack grows when the threshold is equalled or exceeded[1]. For cracking in surfaces, a threshold strain corresponds to the attainment of the threshold energy at the largest flaws that are present in the surface. In the present work, the single crack growth rate and the threshold strain are being used to assess the ozone resistance of the various elastomers and protective systems studied.

Single Crack Growth Studies

Earlier work has shown that the rate of ozone crack growth is essentially proportional to the ozone concentration, provided that the test temperature is not too close to the glass transition temperature of the elastomer[1,2,3,9]. *Figure 3* shows single crack results for different elastomers without added protective agent and including various amounts of DOPPD at room temperature for high ozone concentrations of about 10^4 p.p.h.m. or 10^5 p.p.h.m. The rates of growth in the unprotected NR, ENR 50 and NBR 34 vulcanisates are very similar and are reduced to a similar extent by the incorporation of DOPPD. Consistent with earlier observations[1,9], NBR 38 shows somewhat lower rates of growth for the unprotected vulcanisate but rather smaller reductions when DOPPD is added. Bearing in mind the variability, the results for both protected and unprotected vulcanisates are broadly consistent with a rate that is essentially proportional to the ozone concentration. Observations at much lower ozone concentrations and different temperatures, made during the course of threshold strain experiments, also appear consistent with this.

Threshold Effects

Certain protective agents have the ability effectively to increase the threshold energy or threshold strain. By contrast with the reduction in rate of growth, these increases tend to be strongly influenced by ozone concentration. *Figure 4* shows results for NBR 34 vulcanisates containing 0, 5 or 10 p.h.r. DOPPD, at ozone concentrations ranging from 100 p.p.h.m. to 1500 p.p.h.m. The exposure strain is plotted against the time at which cracks were first observed (τ_1), on logarithmic scales. In order to allow results for different ozone concentrations readily to be compared, τ_1 is multiplied by the ozone concentration (this will superimpose results where the rate of crack growth is proportional to the ozone concentration and τ_1 is determined mainly by the rate). As can be seen this superimposition works quite well for the results for the nitrile vulcanisates in *Figure 4* bearing in mind particularly that considerable scatter in the results at shorter times arises because the test-pieces were inspected only intermittently). The displacement of the curves for different DOPPD levels is broadly consistent with the differences in rate of growth observed in *Figure 3*. These results show

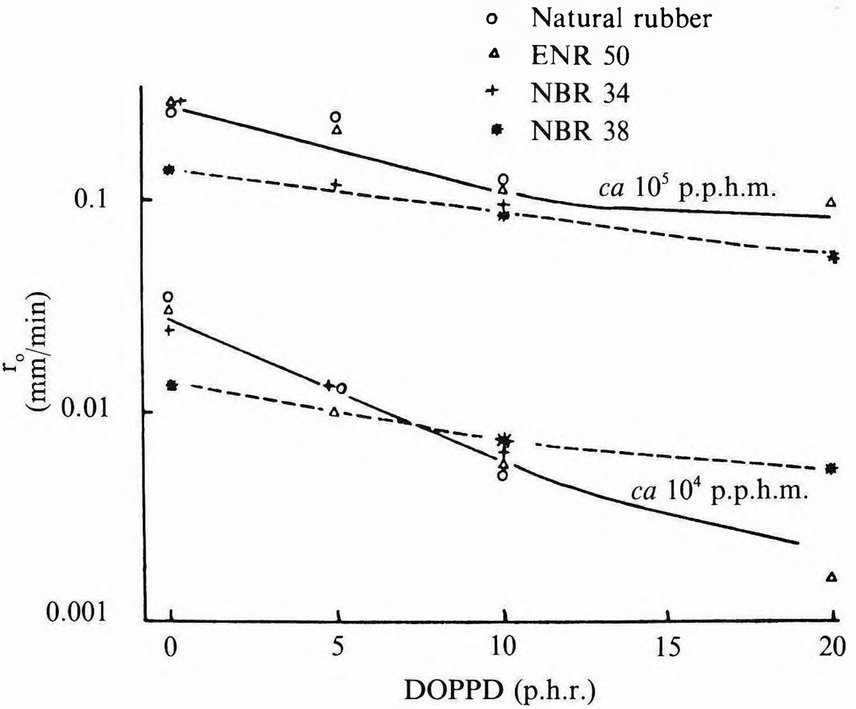

Figure 3. Rate of single crack growth (logarithmic scale) versus antiozonant level at the ozone concentrations indicated on the graph for vulcanisates of natural rubber, ENR 50, NBR 34 and NBR 38. Each point represents an average of about three results.

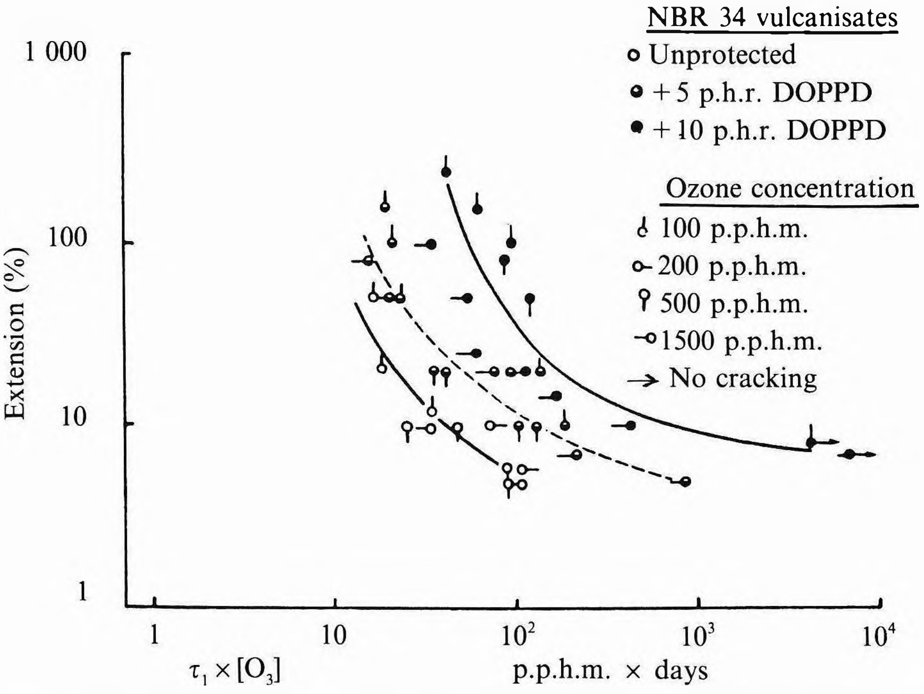

Figure 4. Time to first cracking (τ_1) for NBR 34 vulcanisates. Each point carries a flag to denote the ozone concentration used.

little or no increase in the threshold strain – even with 10 p.h.r. DOPPD the indicated threshold strain is no more than 10% which is only slightly above the level expected for the unprotected vulcanisate.

By contrast, the results for natural rubber in *Figure 5* show evidence of appreciable increase in threshold strain even with only 2.5 p.h.r. DOPPD. The effect is dependent on ozone concentration and is not significant at 1500 p.p.h.m. However, at 100 p.p.h.m. the results show no cracking at a strain of 20% after 1000 p.p.h.m. days – once a time of this order has been survived, the likelihood that cracking will occur subsequently is greatly reduced. Thus a threshold strain in excess of 20% is indicated. An interesting feature of these results is that whereas cracking occurred at 100% strain, no cracks were observed after a much longer time at 150%, indicating a second threshold strain (above which no cracking occurs) in this vicinity. This second threshold is believed to be associated with crystallisation[10]. With 5 p.h.r. DOPPD, no cracking occurred in natural rubber at all strains tested even at an ozone concentration of 1500 p.p.h.m.

With ENR 50, similar behaviour was observed to that with NBR 34. With DOPPD at the 5 p.h.r. level, no significant change in threshold strain was detected, as was the case with 10 p.h.r. at an ozone concentration of 1500 p.p.h.m., although a slight increase was indicated at 100 p.p.h.m. *(Figure 6)*. Even with 20 p.h.r. DOPPD, only a slight increase in threshold was obtained at 1500 p.p.h.m. although cracking was prevented at all strains at 100 p.p.h.m.

Antiozonant Diffusion

The above results show that while DOPPD can confer a similar reduction in rate of growth in nitrile or epoxidised natural rubber to that in natural rubber, its ability to increase the threshold condition is much less. Since the latter ability is known to be associated with the formation of a protective

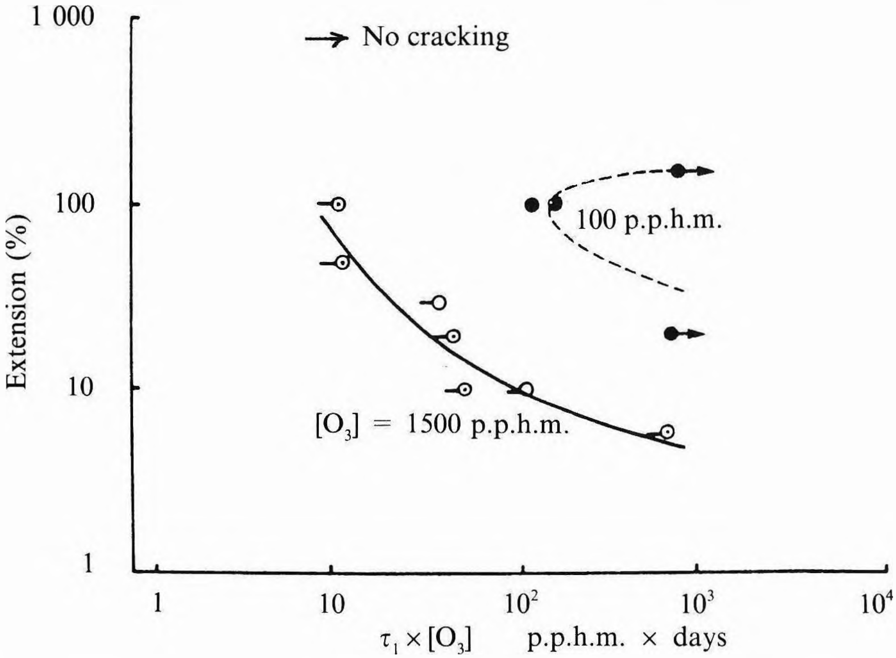

Figure 5. Time to first cracking (τ_1) for an NR vulcanisate containing 2.5 p.h.r. DOPPD at the ozone concentrations indicated on the graph.

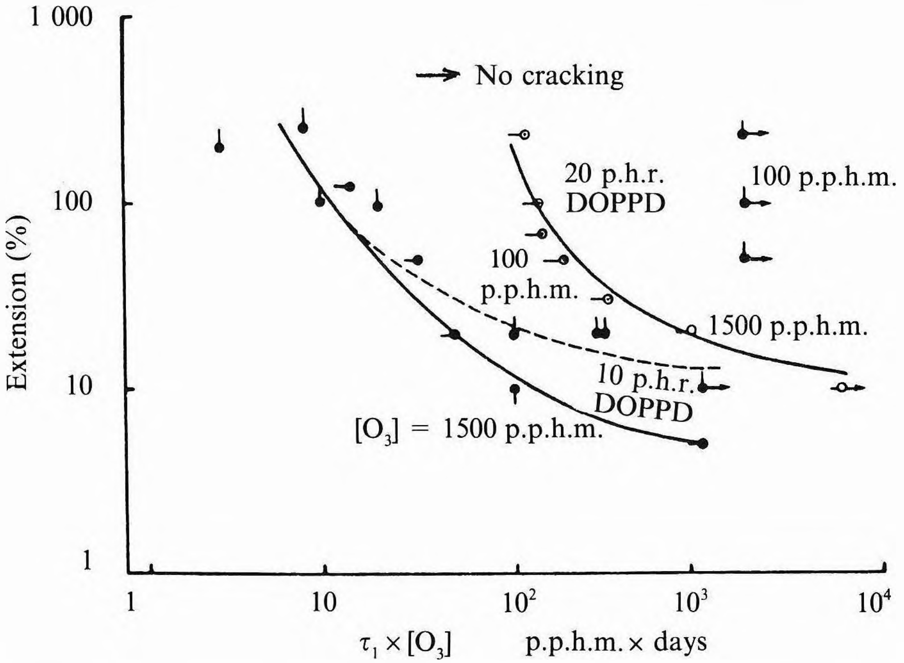

Figure 6. Time to first cracking for ENR 50 vulcanisates containing the levels of antiozonant and at the ozone concentrations indicated on the graph.

layer on the surface of the elastomer by the antiozonant following reaction with ozone[4], which must involve diffusion, it is tempting to conclude that the poorer protection of NBR and ENR is associated with slower diffusion of the antiozonant in these elastomers, which would be expected because of their higher internal viscosities. Studies of the diffusion of DOPPD in the different elastomers were made by transfer measurements, as described earlier. Results are shown in *Table 1*. At 23°C (the temperature of the earlier ozone cracking experiments), the diffusion coefficient of DOPPD in NBR 34 or ENR 50 is only about 1% of that in NR. At elevated temperatures, the diffusion coefficient of DOPPD increases for all the elastomers, although the increase is greater for the high glass transition temperature materials *(Table 1)*. Of interest is 40°C since it is the recommended temperature for standard ozone tests (although of limited relevance for practical purposes), while at 70°C the diffusion coefficient in ENR 50 or NBR 34 is similar to that in NR at 23°C.

TABLE 1. EFFECT OF TEMPERATURE ON DIFFUSION COEFFICIENTS (10 p.h.r. DOPPD)

Temperature (°C)	Diffusion coefficient (cm^2/s) in a vulcanisate of NR (θ_G *ca* -69°C)	 ENR 50 (θ_G *ca* -24°C)	 NBR 34 (θ_G *ca* -28°C)
-17	6.0×10^{-11}	*ca* 10^{-14}?	–
23	4.6×10^{-9}	5.1×10^{-11}	4.9×10^{-11}
40	2.4×10^{-8}	6.2×10^{-10}	7.2×10^{-10}
70	1.1×10^{-7}	8.8×10^{-9}	9.7×10^{-9}

Ozone Cracking Experiments at Elevated Temperatures

Results of ozone cracking tests at 40°C are shown in *Table 2*. With 5 p.h.r. or 10 p.h.r. DOPPD, the threshold strain for vulcanisates of ENR 50 or NBR 34 is higher at 40°C than at 20°C but is still well below that for NR with 5 p.h.r. DOPPD at 20°C. However, the diffusion coefficients, although increased by more than an order of magnitude at 40°C, are appreciably lower than that for NR at 20°C *(Table 1)*. Initial cracking test results at 70°C, (where the diffusion coefficients are slightly higher than for NR at 20°C) indicate the protection of ENR 50 or NBR 34 still to be inferior to that of NR at 20°C, although various difficulties have been encountered with these tests and further experiments are in progress.

Rather similar behaviour was observed with wax or wax/antiozonant protective systems for which some results are also shown in *Table 2*. For NR, all of the protective systems gave threshold strains in excess of 100% except for the paraffin wax alone at 40°C. For ENR 50 or NBR 34, by contrast, only the antiozonant/lower melting point wax combinations gave significant protection at 20°C, whereas most of the systems gave considerably better protection at 40°C.

With waxes, temperature affects the blooming rate through both the diffusion coefficient and the solubility in the rubber, which increases rapidly as the melting point of the wax is approached. These parameters also enter directly into the blooming equation (cf *Equation 5*) whereas in the case of DOPPD, the diffusion coefficient of the antiozonant in the rubber only enters somewhat indirectly into the equation for layer formation *(Equation 1) via Equations 3* and *4*. Thus a rather stronger effect of temperature on protective action might be expected for protective systems involving waxes compared with those involving a chemical antiozonant alone. The results in *Table 2* appear consistent with this.

Cracking Experiments at Low Temperatures

Experiments at 8°C and 50 p.p.h.m. ozone showed little or no protective action of a wax/antiozonant system in NBR 34 or

TABLE 2. COMPARISON OF THRESHOLD STRAINS AT 20°C AND 40°C

Protective system	Threshold strain (%) for an ozone concentration of 50 p.p.h.m. at the test temperature indicated for vulcanisates of					
	NR		ENR 50		NBR 34	
	20°C	40°C	20°C	40°C	20°C	40°C
5 p.h.r. DOPPD	> 100	> 100	< 10	*ca* 15	< 10	*ca* 15
10 p.h.r. DOPPD	–	–	*ca* 15	*ca* 50	*ca* 50	> 100
3 p.h.r. 6PPD + 6 p.h.r. Wax A	> 100	> 100	*ca* 10	*ca* 30	*ca* 10	*ca* 50
3 p.h.r. IPPD + 6 p.h.r. Wax B	> 100	> 100	*ca* 10	> 100	*ca* 30	*ca* 100
3 p.h.r. IPPD + 6 p.h.r. Wax C	–	–	*ca* 50	> 100	–	–
6 p.h.r. Wax C	> 100	*ca* 10	< 10	*ca* 10	*ca* 10	*ca* 10

6PPD is a di(methyl butyl) phenyl substituted ppd (Santoflex 13)
IPPD is an isopropyl phenyl substituted ppd (Nonox ZA)
Wax A was Antilux 600 (quoted melting point range 62°C to 65°C)
Wax B was Antilux 500 (quoted MP range 56°C to 60°C)
Wax C was a paraffin wax (MP range 48°C to 50°C)
+ Failure sometimes occurred by mechanical crack growth in NBR 34 at 40°C

TABLE 3. THRESHOLD STRAINS AT 8°C

Protective system	Threshold strain (%) for an ozone concentration of 50 p.p.h.m. at 8°C for vulcanisates of NR	ENR 50	NBR 34
10 p.h.r. DOPPD	> 100%	*ca* 50%	*ca* 50%
3 p.h.r. IPPD + 6 Wax B	> 100%	–	–
3 p.h.r. IPPD + 6 Wax C	> 100%	*ca* 10%	*ca* 10%

Waxes B and C are as in *Table 2*.

ENR 50 even with the lowest melting point wax *(Table 3)*. With 10 p.h.r. DOPPD, however, protection of either elastomer was similar to that obtained at 20°C. Thus the 8°C results are consistent with the expected temperature effects for the two systems, as discussed above. Reasonable protection of NR was obtained with either system. The rate of crack growth in the high glass transition temperature elastomers was noticeably slower at 8°C, as expected on visco-elastic grounds[2,3].

Experiments at −17°C were carried out at a high ozone concentration of about 10^5 p.p.h.m. In order to prevent cracking in natural rubber at this concentration at room temperature, about 15 p.h.r. of DOPPD is required[11]; similar protection was obtained at 10^5 p.p.h.m. at −17°C with 20 p.h.r. DOPPD.

Protective Layer Formation

Measurements of the rates of layer formation for the different elastomers correlate with threshold strain observations for either DOPPD or wax/chemical antiozonant protective systems. *Figure 7* shows results for vulcanisates containing 10 p.h.r. DOPPD after exposure to 200 p.p.h.m. of ozone at 40°C. The rate of layer formation for NR is some four times that for the other materials, paralleling the much better protection obtained in NR, while NBR 34 has a slightly higher rate than ENR 50, again in accord with the relatively small differences in the threshold strain for these materials *(Table 2)*. Similar layer formation behaviour with a wax/antiozonant system is illustrated in *Table 4*. In this case, test-pieces were stored in the absence of ozone at either 20°C or 40°C. As can be seen, the rates of layer formation are again higher for NR than for the other elastomers, with the differences being much greater at 20°C than at 40°C (as expected from the diffusion mechanism discussed earlier). NBR 34 again shows somewhat faster layer formation than ENR 50. These features parallel the differences in threshold strain illustrated in *Table 2*.

TABLE 4. EFFECT OF TEMPERATURE ON LAYER FORMATION WITH A WAX/ ANTIOZONANT[a] PROTECTIVE SYSTEM

Elastomer	Mass/unit area of layer (mg/cm^2) after 7 months ($\sqrt{t} = 70h^{1/2}$) at 20°C	40°C
NR	0.88	1.16
ENR 50	0.02	0.39
NBR 34	0.05	0.84

[a] 3 p.h.r. 6PPD and 6 p.h.r. Antilux 600

Figure 8 compares layer formation results at −17°C with those at room temperature for an NR vulcanisate containing 10 p.h.r. DOPPD. As can be seen the rates of layer formation at the two temperatures are virtually identical. The diffusion coefficient of DOPPD in NR at −17°C is very similar to that in ENR 50 or NBR 34 at 23°C

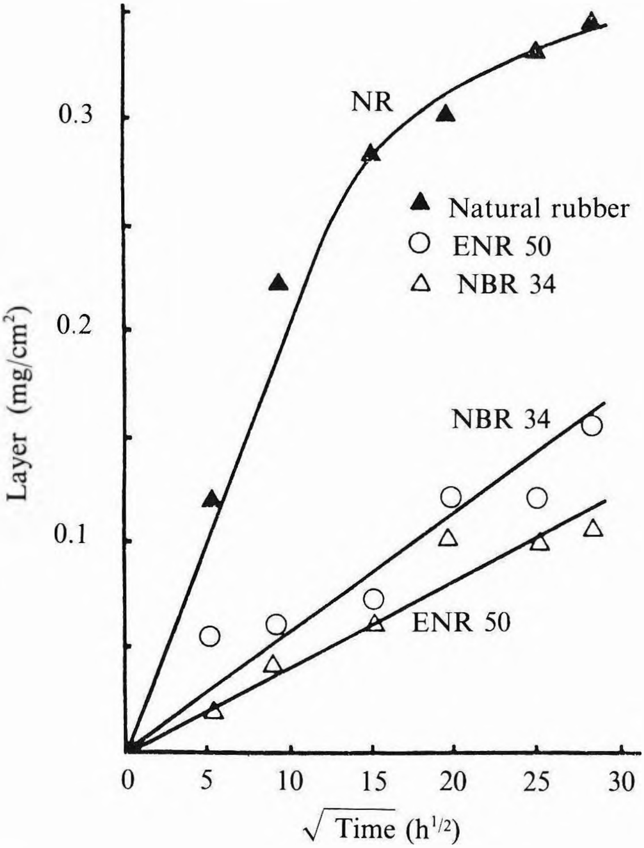

Figure 7. Dependence of the mass of antiozonant layer formed on the square root of the time of exposure to an ozone concentration of 200 p.p.h.m. at 40°C for vulcanisates of natural rubber, ENR 50 and NBR 34.

(cf *Table 1*). Thus it appears that factors other than slow diffusion of the antiozonant in the elastomer must be involved in the much lower rates of layer formation with the chemical antiozonant alone in ENR 50 and NBR 34 at 23°C.

One factor that may be relevant is the partitioning of the antiozonant between the elastomer and the layer material – S_L in *Equation 1*. Swelling and other measurements indicate the solubility of the (unreacted) antiozonant in the elastomers to vary according to NR < NBR 34 < ENR 50. Thus partitioning of the unreacted antiozonant into the layer may tend to decrease in the same order, leading to a corresponding decrease in the rate of layer formation (cf *Equation 1*). A check on this can be made by transfer measurements across the layer *(Figure 2)* to obtain the permeability of the layer material to the unreacted antiozonant (P_L in *Equation 2*). Earlier measurements[4] indicated a value for P_L of about 7×10^{-13} cm²/s for the layer on natural rubber. If it is assumed that the layer material is the same irrespective of the elastomer (as is possible if it is comprised primarily of reacted antiozonant) then differences in P_L for different elastomers would correspond to differences in S_L (cf *Equation 2*). These experiments are difficult to carry out, partly because the

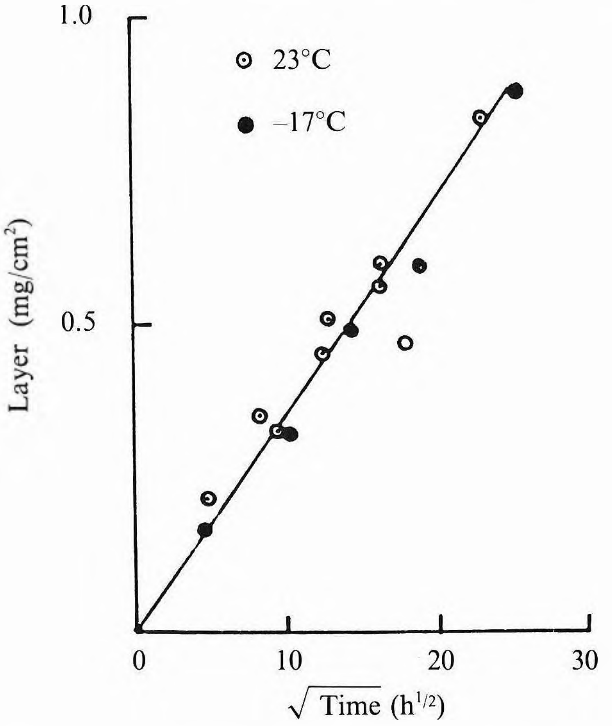

Figure 8. Mass of antiozonant layer formed versus square root of the time of exposure to 10^5 p.p.h.m. ozone for an NR vulcanisate containing 10 p.h.r. DOPPD at 23°C and 17°C.

amount of material transferred is very small. The results are therefore subject to considerable scatter but initial results with ENR 50 do not indicate any major difference in P_L compared with NR.

A further factor that may affect layer formation with DOPPD is the fact that ENR 50 and NBR 34 are more polar materials than NR. The reacted antiozonant layer is also polar in nature, dissolving very readily in polar solvents such as acetone. There may thus be a greater tendency for the layer material to diffuse back into ENR 50 or NBR 34 instead of remaining on the surface to give protection. Evidence indicating this to happen has been obtained with a light-coloured vulcanisate of ENR 50 containing DOPPD. After exposure to ozone the surface of the elastomer remained darker in colour even after removal of the layer material and repeated swabbing with acetone. A cross-section cut perpendicular to the surface showed the dark colour to extend a small distance (*ca* 0.1 mm) below the surface. Furthermore, the extent of the penetration was observed to increase with the passage of time, approximately as the square root of the time that had elapsed from the start of the ozone exposure. These kinetics indicated a diffusion coefficient of about one sixth that of unreacted DOPPD in ENR 50 (cf *Table 1*). Thus it appears that a somewhat higher molecular weight reaction product of DOPPD tends to diffuse back from the layer into ENR 50. Similar behaviour has not been observed with NR but cannot be entirely ruled out as NR vulcanisates are generally darker in colour than those of ENR 50, making any colour change more difficult to detect.

CONCLUSIONS

The work described shows that the test temperature can have marked effects on the ozone resistance of vulcanisates containing chemical antiozonant or wax/antiozonant protective systems. The effects are mainly associated with differences in threshold strain rather than rate of crack growth and vary according to the glass transition temperature of the elastomer and the protective system adopted. Elastomers with high glass transition temperature, such as epoxidised natural rubber or nitrile rubber are more readily protected at temperatures above room temperature, when the protective agents are more mobile. Protection of such materials at lower temperatures is correspondingly more difficult but a dialkyl ppd appears better than wax/antiozonant systems, for reasons that can be understood from differences in the diffusion mechanisms. Reasonable protection of natural rubber was obtained at 8°C with a wax/antiozonant system and down to −17°C with a dialkyl paraphenylenediamine.

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Properties of Rubber Compounds for Engineering Applications

A.H. MUHR*

The performance of elastomeric engineering components generally depends on their having the correct force–deformation behaviour. This can only be achieved if the stress–strain behaviour of the elastomer is appropriate and well characterised. This is taken to include the effects of frequency and hysteresis as well as the quasi-static elastic behaviour.

Extreme temperatures, oils and oxygen may alter the stress–strain behaviour of the elastomer either reversibly or irreversibly and hence degrade the performance of the component, or even result in its failure. The constraints these considerations make on the choice of compound are briefly described.

Even if the properties of the elastomer do not deteriorate, the component may fail by rupture of the elastomer. The theoretical framework for describing such failure is briefly presented and its implications for component design and choice of compound mentioned.

Most physical tests cited in specifications are appropriate to achieving quality control rather than being directly related to the performance or longevity of the component. This paper aims at restoring the balance by reviewing how the performance of the component is related to the properties of the rubber.

STRESS–STRAIN BEHAVIOUR

Quasi-static Force–deformation Behaviour of Mounts

The basic design feature of most engineering components is the stiffness, that is the ratio of force to deflection, also known as the spring rate. The stiffness of a component scales with the modulus of the rubber, that is the ratio of stress to strain. Unfortunately rubber technologists usually refer to the stress (rather than the ratio of stress to strain) at a specified strain as being the 'modulus'.

Rubber mounts are required to bear a dead load, and it is important that the static deflection should be limited to an acceptable level. However, the dynamic stiffness (determined in part by the tangent to the load-deflection curve) must be sufficiently low to achieve effective vibration isolation if the function of the component is to isolate in the same direction as the dead load is supported. These considerations mean that for a highly variable dead load (for example, for a truck suspension spring) a rising rate is appropriate *(Figure 1)* so that the natural frequency is not dependent on load (for small vibration amplitudes)[1]. For a well-defined dead load a high initial stiffness followed by a plateau region (achieved by a buckling mode of deformation) may be appropriate *(Figure 2)* so that the static deflection is small (corresponding to the high initial stiffness) as also is the dynamic stiffness for small amplitude vibrations about the static deflection[2,3,4].

Either way it is desirable that the quasi-static force–deformation behaviour of the

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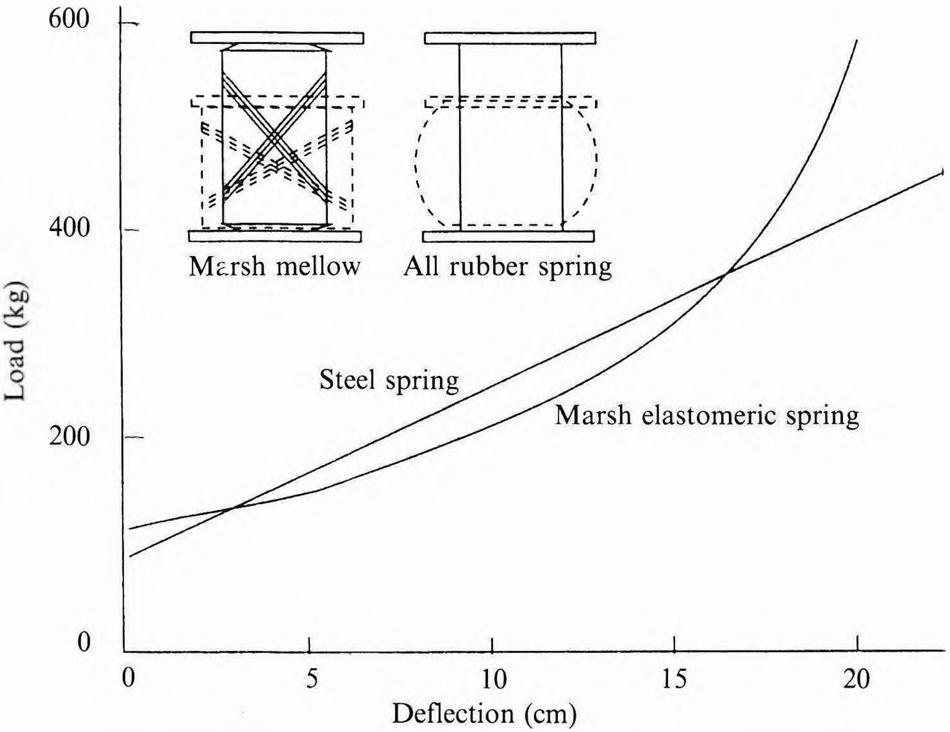

Figure 1. Load–deflection curve of Marsh Mellow spring, showing non-linearity. Insert: Marsh Mellow spring showing how pantographing cords restrict bulging of the rubber under load, causing an extra contribution to the non-linearity[1].

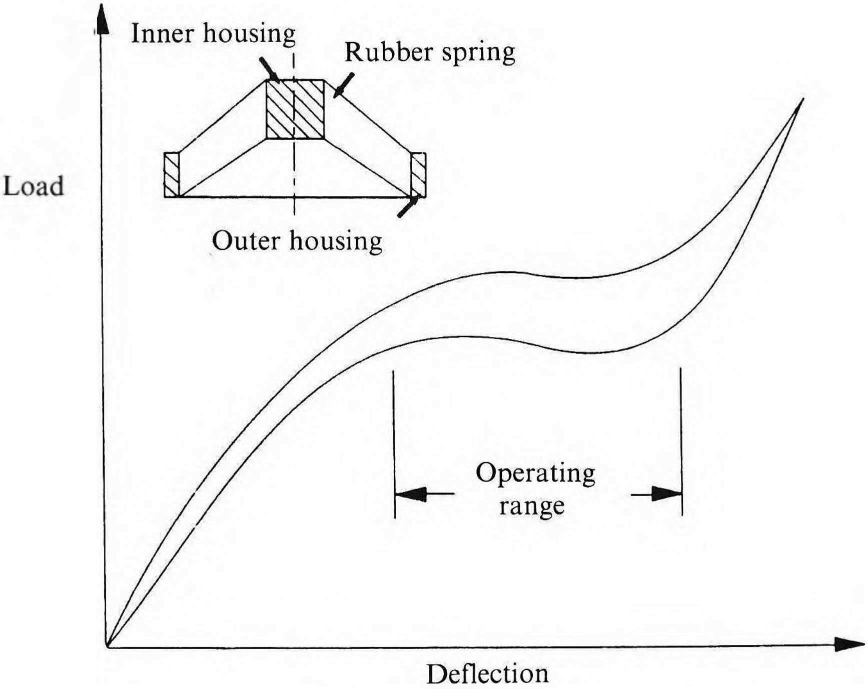

Figure 2. Load–deflection curve for axially compressed conical rubber shear bushing[3].

component is calculable. On the assumption of linear stress–strain behaviour, many formulae have been derived[5], and these are largely satisfactory for unfilled natural rubber *(Figure 3)*. However, when a reinforcing filler is used the stress–strain behaviour of the rubber is non-linear, and this results in an extra contribution to any non-linearity associated with the geometry of the component (*cf Figures 3* and *4*). At low strains, the shear modulus of filled rubber is high because of carbon black interactions which progressively break down as the strain is increased[6], resulting in a strain softening. This effect can rarely be exploited to practical advantage. One exception is the use of highly filled natural rubber for seismic isolation bearings, for which the high stiffness at low strains limits the deflection caused by wind loading to an acceptable level[7].

At high strains, the modulus of filled rubber again increases due to finite extensibility of the polymer chains, which occurs at much lower strains for a highly filled rubber because of the strain amplification effect of the filler[8]. For the rubber in *Figure 4*, it is seen that this effect occurs at shear strains above 100% whereas usually the strain levels in service are less than 100%. The detailed shape and position of the upturn in the stress–strain curve depends on the previous strain-history of the sample[8].

Non-linearity of the stress–strain behaviour means that the modulus will depend on the strain. The formulae derived assuming linear stress–strain behaviour may still provide a useful estimate for the force-deformation behaviour of non-linear rubbers if the modulus inserted into them is chosen at an appropriate strain[9]. Such a representative shear strain $\bar{\gamma}$ may be defined by

$$\bar{\gamma} = \sqrt{(k/G)/V}\, x \qquad \ldots 1a$$

where V is the volume of rubber in the component

x is the deflection of the component

and the ratio k/G is taken to be the stiffness for unit modulus derived on the basis of linear stress–strain behaviour.

Having found the representative strain $\bar{\gamma}$ using *Equation 1a*, the shear modulus to be used in predictions of stiffness of the components can be obtained from the measured shear stress/shear strain behaviour of the rubber. The modulus is calculated from the shear stress $\sigma(\bar{\gamma})$ at $\bar{\gamma}$ according to

$$G_{\text{chord}} = \sigma(\bar{\gamma})/\bar{\gamma} \qquad \ldots 1b$$

Equation 1 appears to be successful, at least at moderate deformations of the component given in *Figure 4*. Fortunately, it is the behaviour at moderate deformations which is required in practice.

Thus while reinforcing fillers cause the stress–strain behaviour to be non-linear, it is still possible to use existing formulae for the force–deformation behaviour of components, provided that the compound is characterised by the full stress–strain behaviour and not just by a single number, such as hardness.

Hardness can be used[9] to estimate the shear modulus (in Nm^{-2}) at a strain of about 5% and an equation giving an approximate fit to the theoretical relationship is

$$\log_{10}(G_{5\%}) \simeq \frac{\mathrm{IRHD} + 228}{47} \qquad \ldots 2$$

for $35 < \mathrm{IRHD} < 80$

Very rough estimates of shear modulus at other strains can only be made from the hardness value by appealing to prior experience of similar compounds.

Dynamic Stiffness and Dynamic to Static Ratio

Vibration amplitudes are typically much smaller than the quasi-static deflections of vibration isolation springs. Thus even if

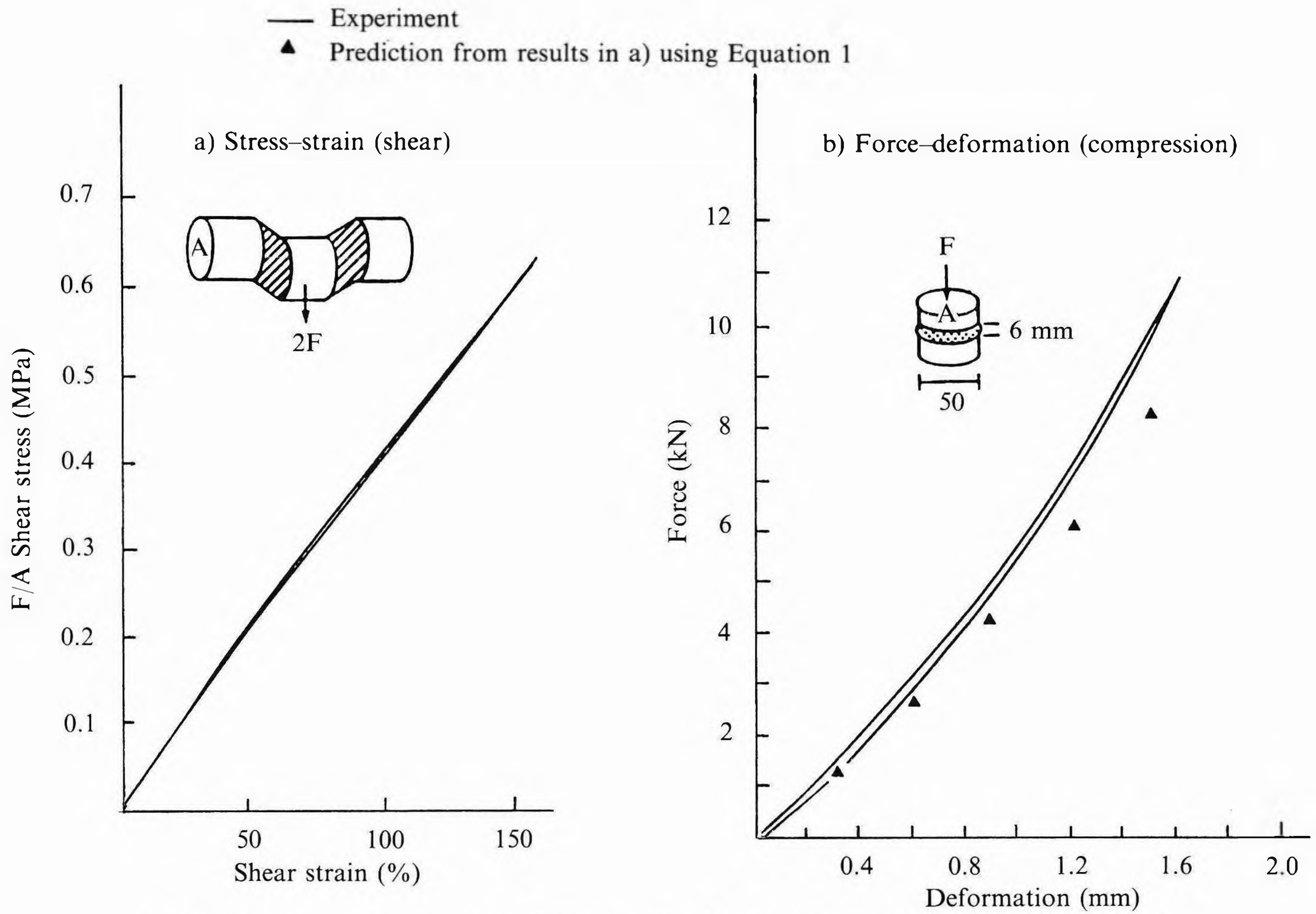

Figure 3. Tenth cycle quasi-static deformation of unfilled natural rubber a) stress–strain in simple shear and b) force–deformation in compression (shape factor $\simeq 2$).

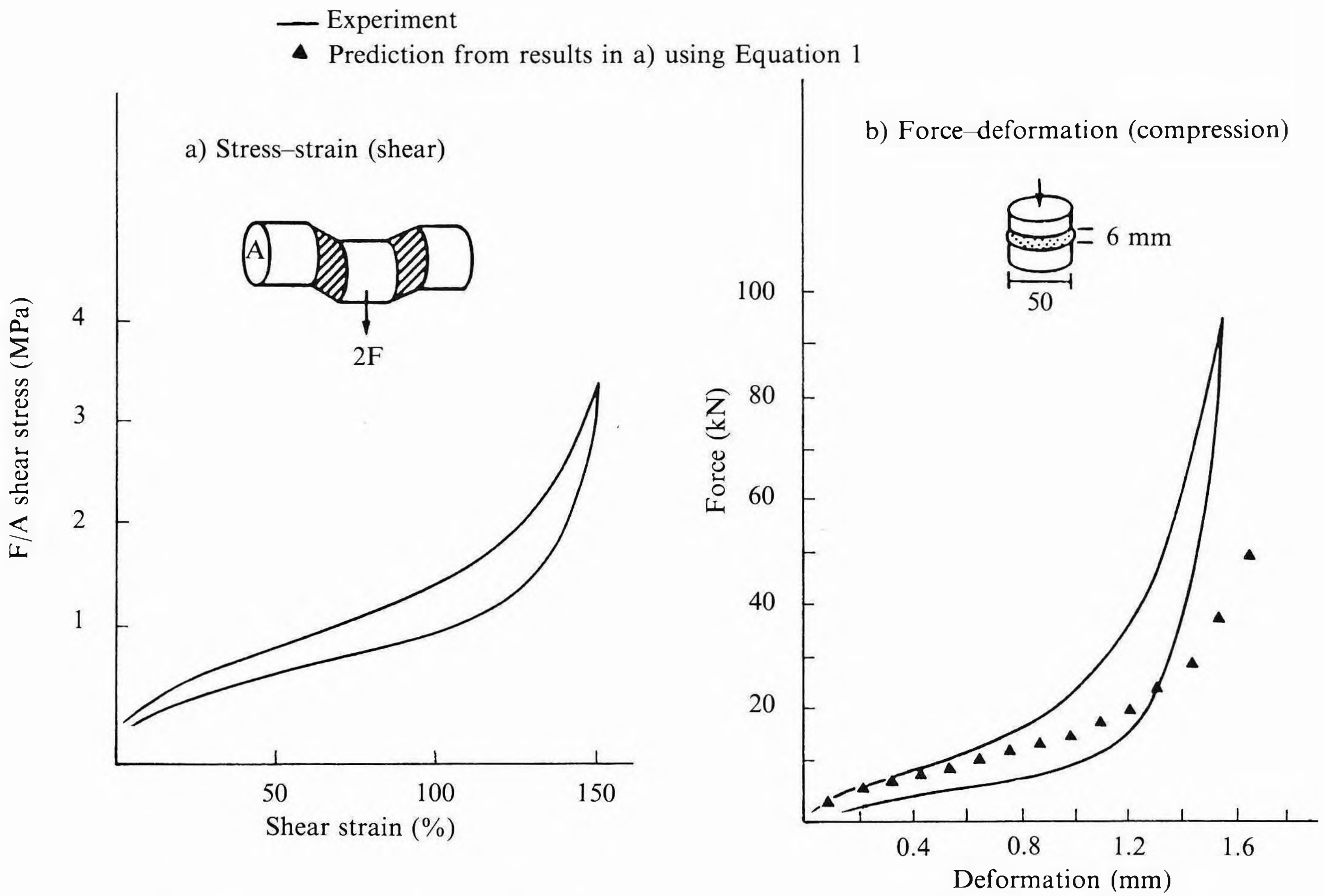

Figure 4. Tenth cycle quasi-static deformation of natural rubber + 75 p.h.r. N330 black a) stress–strain in simple shear and b) force–formation in compression (shape factor ≃ 2).

the overall quasi-static force-deformation behaviour is non-linear, vibration isolation theory based on the assumption of linearity usually suffices. The stiffness k to be used in formulae derived from this theory *(Figure 5)* must, however, be appropriate to the particular frequency and amplitude of the vibration and to the static deflection of the spring. This value of k is called the 'dynamic stiffness'.

For springs that are non-linear for geometric reasons only (*Figures 1* and *2*), the dynamic stiffness is determined by the tangent stiffness to the quasi-static force-deflection curve. Some elastomers (including all those with a high T_g, such as ENR 50) also show a strong effect of frequency, the dynamic stiffness being higher at higher frequencies, but for NR (filled or unfilled), this effect is weak.

The material contribution to non-linearity (induced by the use of reinforcing filler) affects the dynamic stiffness in a more complicated way. For clarity, this will be illustrated for simple shear *(Figure 6)*, a mode of deformation for which there is no geometric contribution to non-linearity. While for unfilled NR the dynamic modulus is equal to the quasi-static modulus *(Figure 6a)*, for filled NR, the dynamic modulus is closer to the tangent modulus at zero shear strain rather than to the tangent modulus at the specified (non-zero) static strain *(Figure 6b)*. The behaviour is reminiscent of a spring with friction, which will have an extra high stiffness at the start

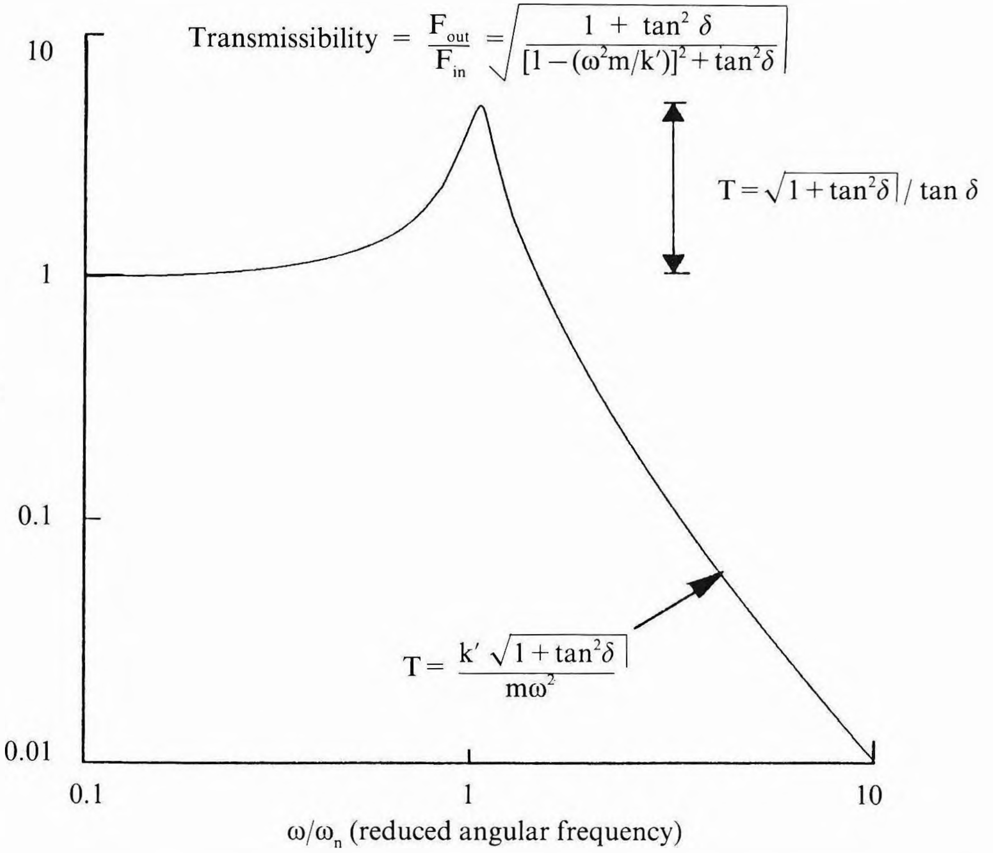

Figure 5. Schematic diagram to show effects of damping (tan δ) and dynamic storage stiffness (k') on vibration isolation.

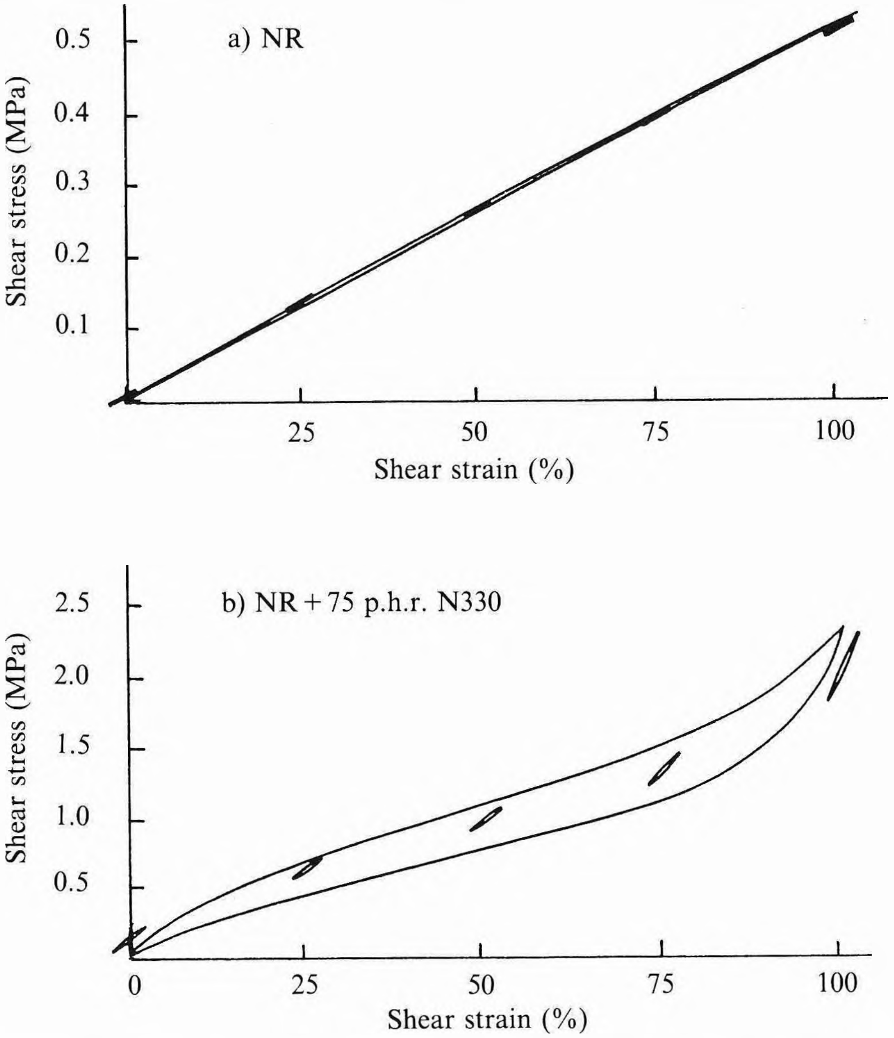

Figure 6. Tenth cycle shear stress–strain curves with cycles of ±2% shear strain superimposed about static strain of 0%, 25%, 75% and 100%; a) unfilled natural rubber and b) natural rubber + 75 p.h.r. N330 black.

of extension and at the start of retraction. The dynamic stiffness is strongly influenced by the retraction stiffness. However, carbon-black interactions cause time-dependent effects[10] as well as such 'frictional' effects.

The ratio of dynamic stiffness to the slope of the quasi-static force-deformation curve is sometimes known as the 'dynamic to static ratio'. Use of this term may imply an assumption of linearity of the quasi-static force-deformation curve, so that whether the chord or tangent slopes are specified for the static stiffness is left open.

The word 'dynamic' suggests the departure of the ratio from unity is a consequence of frequency dependence of the elastic properties. Although this may be the case for high T_g rubbers, for filled NR the amplitude effects are much more important than the frequency effects.

Figure 7 shows effects of dynamic amplitude (at zero static pre-strain) and filler loading on the 'dynamic to static ratio' in shear, choosing the tenth cycle chord modulus at 50% shear as a measure of static stiffness. The effect of static pre-strain on the dynamic modulus is relatively small and has been neglected for the purposes of *Figure 7*. Fortunately, the rise in dynamic modulus as the amplitude is decreased becomes less rapid for strains below 1%, and the dynamic modulus reaches a limiting value at about 0.1% strain according to Payne[6].

The fact that the dynamic to static ratio is greater than unity for filled rubber is not a desirable feature, since for effective isolation of noise and vibration, the dynamic stiffness must be of a specified, low magnitude. It is difficult, and perhaps impossible, to correctly design the dynamic stiffness to have the specified level, bearing in mind that it depends on dynamic amplitude (which for high frequencies may be extremely low), static amplitude, recovery time after subjection to higher amplitude and temperature. These complications may severely limit the usefulness of any constitutive equation intended to describe black filled rubber.

Thus, the use of significant amounts of reinforcing filler at best complicates the design for satisfactory performance considerably, and at worst, may prevent the optimum performance being achieved. Fortunately for natural rubber it is not essential to use filler, since the unfilled vulcanisate has adequate strength properties, unlike non-crystallising rubbers.

Damping

It is apparent from the formula in *Figure 5* that in addition to the dynamic stiffness k, the loss tangent, $\tan \delta$, is an important parameter in determining the transmissibility. The loss tangent may be defined by the following equation relating the force F required to deflect a linear rubber spring with a sinusoidal amplitude A and angular frequency ω:

$$F = kA \sin \omega t + k' \tan \delta \, A \cos \omega t \quad \ldots 3a$$

$$k = k' \sqrt{1 + \tan^2 \delta}$$

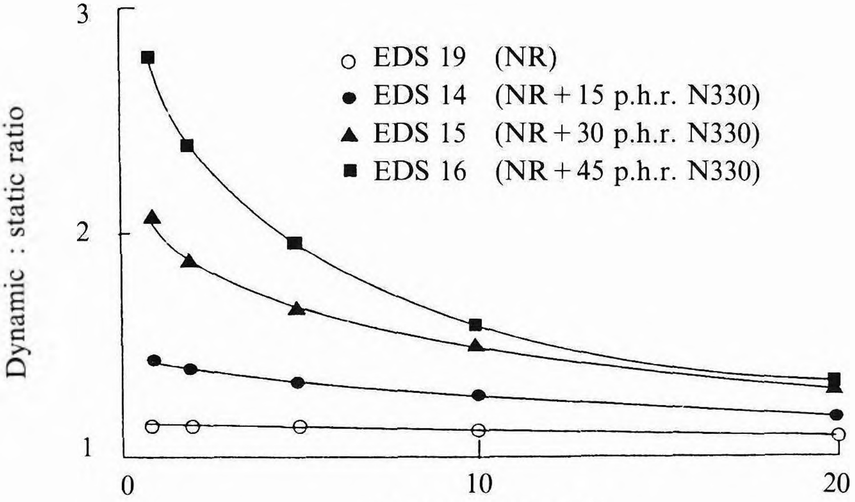

Figure 7. Effect of dynamic strain and carbon black loading on the ratio of complex dynamic shear modulus (at 1 Hz) to the tenth cycle quasi-static chord shear modulus at 50% shear[41].

An alternative definition of tan δ is in terms of the stress response to a sinusoidal strain. For example, in simple shear, the stress response $\bar{\gamma}$ to a sinusoidal strain of amplitude γ_0 is

$$\sigma = G' \gamma_0 \sin \omega t + G'' \gamma_0 \cos \omega t \qquad \text{... 3b}$$

where G' is the storage modulus and G'' is the loss modulus. In general, G' and G'' are functions of ω. The ratio G''/G' may be identified as tan δ.

Equation 3b can also be expressed as

$$\sigma = \sqrt{(G'^2 + G''^2)}\, \gamma_o \sin(\omega t + \delta) \equiv \sigma_o \sin(\omega t + \delta) \qquad \text{...3c}$$

where σ_o is the amplitude of the sinusoidal stress and δ is the loss angle.

For unfilled elastomers well above their T_g, such as natural rubber at room temperature, tan δ is fairly independent of frequency but rather low. Carbon black increases the level of tan δ, and the insensitivity to frequency is largely retained. For many applications, this is a distinct advantage over achieving high damping by choice of an unfilled high T_g polymer, since then both the in-phase modulus and tan δ increase rapidly with frequency, and the isolation of higher frequencies will be impaired *(Figure 5)*. However, if very high values of tan δ (e.g. > 0.2) are required and the temperature and frequency ranges are not too extended it may be preferable to use a high T_g elastomer (such as ENR) rather than face the problem of amplitude dependence of stiffness (discussed above) associated with high loadings of filler.

Creep and Stress Relaxation

Creep is often an undesirable feature for load bearing components such as mounts, since extra clearance is required to accommodate the creep deflections. Differential creep may also lead to redistribution of the loads on the mounts. Stress relaxation is an undesirable feature for components such as seals which are held at a constant deformation and must maintain an adequate stress for their function.

Creep and stress relaxation may be related as follows[11]. The rate of creep C at a constant stress σ is defined as the relative slope of the strain *versus* time curve:

$$C = (1/e)\,(\partial e/\partial t)_\sigma \qquad \text{... 4}$$

where e is the strain at time t after the stress σ is imposed. The rate of stress relaxation S at a constant strain e is defined similarly:

$$S = -(1/\sigma)\,(\partial \sigma/\partial t)_e \qquad \text{... 5}$$

It follows that

$$C = [(\sigma/e)/(\partial\sigma/\partial e)_t]\, .\, S \qquad \text{... 6}$$

Wherever the terms stress and strain occur in the above, they can be replaced by force and deflection respectively. It can be concluded from *Equation 6* that the creep rate of a spring depends not only on the stress-relaxation rate of the rubber but also on the degree of non-linearity of the spring's force-deformation behaviour. Springs with a falling rate will suffer more from creep than springs with a rising rate, while for linear springs the creep rate will be equal to the stress-relaxation rate.

It is generally found that the relaxation modulus $G(t)$ [that is, $\sigma(t)/e$] for rubbers well above their T_g decreases approximately linearly with $\ell n\,(t)$:

$$\partial G(t)/\partial\, \ell n t \approx -G_o \qquad \text{... 7}$$

provided the relaxation time is not very long, so that *Equation 7* is valid in the restricted time range $t_1 < t < t_2$.

Using the theory of linear visco-elasticity, it can be shown[12] that *Equation 7* corresponds to

$$G''(\omega) \approx G_o\, \pi/2 \qquad \text{... 8}$$

$$d\, G'(\omega)/d\, \ell n\, (\omega) \approx G_o$$

where $t_1 \ll 1/\omega \ll t_2$. Substitution of *Equation 7* into *Equation 5* gives

$$tS \approx G_o/G(t)$$

The right hand side can be approximately expressed in terms of tan δ [that is $G''(\omega)/G'(\omega)$] using *Equation 8*:

$$tS \simeq \frac{\pi}{2} \tan \delta/\pi \qquad \text{... 9a}$$

Because of *Equation 7*, it is customary to refer to $S_{\%} = -\dfrac{100}{\sigma}\dfrac{\partial\sigma}{\partial \log_{10} t} \equiv 100\,\ell n(10)tS$ (a dimensionless quantity having units of % relaxation per decade of time) as being the stress-relaxation rate.

Substituting *Equation 9a* into this definition of $S_{\%}$ leads to

$$S_{\%} \simeq 147 \tan \delta \qquad \text{... 9b}$$

Gent[11] has shown that for a wide range of unfilled NR vulcanisates and for an unfilled SBR vulcanisate, *Equation 9b* is reliable to 20%. However, for black filled NR vulcanisates, the measured stress-relaxation rates can be lower than those predicted by *Equation 9b* by a factor of three to five[13]. Stress-relaxation rates are increased by increase in the filler loading, but the effect is smaller than would be anticipated from the accompanying increase in tan δ. This observation shows that linear viscoelasticity is not applicable to filled rubbers, suggesting that the origin of the extra damping is 'frictional' rather than viscoelastic. The combination of relatively low stress-relaxation and creep rates with high values of tan δ may mean that the use of filler to achieve damping is preferable in some applications to using a high T_g polymer.

The above discussion concerns 'physical relaxation'. After long periods of time, it can be seen from *Equation 9a* that the physical relaxation rate becomes very small. Ultimately another process, 'chemical relaxation', which is characterised by a linear dependence of relaxation with time [rather than log (time)] becomes the dominant relaxation mechanism and the relaxation process is better described by

$$\frac{G(t_1) - G(t)}{G(t_1)} \simeq A \log_{10}(t/t_1) + B\,(t - t_1) \qquad \text{... 10}$$

A may be identified as $S_{\%}$ above. B, the chemical relaxation rate, rises more strongly with temperature than A. This makes the interpretation of accelerated tests at raised temperatures difficult. For a typical NR vulcanisate at 10°C, the rate of physical relaxation falls to about the same level as that of chemical relaxation after one or two years[14].

Applications Requiring High Damping

There is a tendency to regard creep, stress relaxation and compression set as undesirable for any rubber. However, in some applications, the primary requirement of the engineer is for high damping, and the creep rate may be of secondary importance or even irrelevant.

One such example is the 'vibration absorber'. The function of this device is to prevent the amplitude of a resonance in an engineering structure from becoming unacceptably large. This is achieved by dissipating some of the excitation energy in a sprung mass 'absorber' tuned close to the resonant frequency[15]. Often the resonant frequency is rather high, so that the static deflection of the spring is very small and creep deflections are negligible. This is fortunate, as optimising the spring properties requires very high levels of damping.

Seismic isolation bearings are another example[7]. Here the requirement is for a low horizontal stiffness together with very high damping. Creep in the horizontal direction should not occur since there is no static horizontal loading. The bearings must support a high vertical load, but the vertical creep deflections can be kept to acceptable levels by making the bearings very stiff vertically.

In other cases, it may be possible to combine a highly elastic compound in parallel with a highly hysteretic compound (the analogue of an oil-filled damper). This could be done, for example, by using multiple laminations of the flexible rubber-steel laminated spring[16] depicted in *Figure 8.*

DEGRADATION OF PERFORMANCE: EFFECT OF THE ENVIRONMENT

Swelling by Oils

If a rubber absorbs a large volume of liquid it will become very 'weak' and useless for most engineering applications. Equilibrium swelling is the conventional measure of swelling resistance. However, such a test does not take into account that throughout the life-time of bulky elastomeric engineering components only the outermost rubber will become swollen to anything like the equilibrium level. Swelling is a diffusion-controlled process and the depth of penetration of the swelling liquid is proportional to the square root of time for which the rubber has been immersed. The diffusion coefficient, and hence the time required to penetrate a certain depth, is related to the viscosity of the liquid[17] as shown in *Figures 9* and *10.* It is apparent that a natural rubber component (if 20 mm or more in thickness) will not be seriously affected by splashes of engine oil during a life-time of ten or more years. For more aggressive environments, measures may be adopted ranging from use of an oil-resistant paint to choice of an oil-resistant elastomer (ENR or NBR). Natural rubber is swollen by most hydrophobic liquids (but not by castor oil or silicone oil).

Oxidation

As with other unsaturated polymers, natural rubber and ENR are prone to oxidative degradation, at a rate which increases with temperature. However, the depth to which oxidative degradation takes place is controlled by diffusion slowed down by consumption by chemical reactions. Degradation of bulky rubber components is, therefore, generally restricted to the surface layers, this may be unacceptable

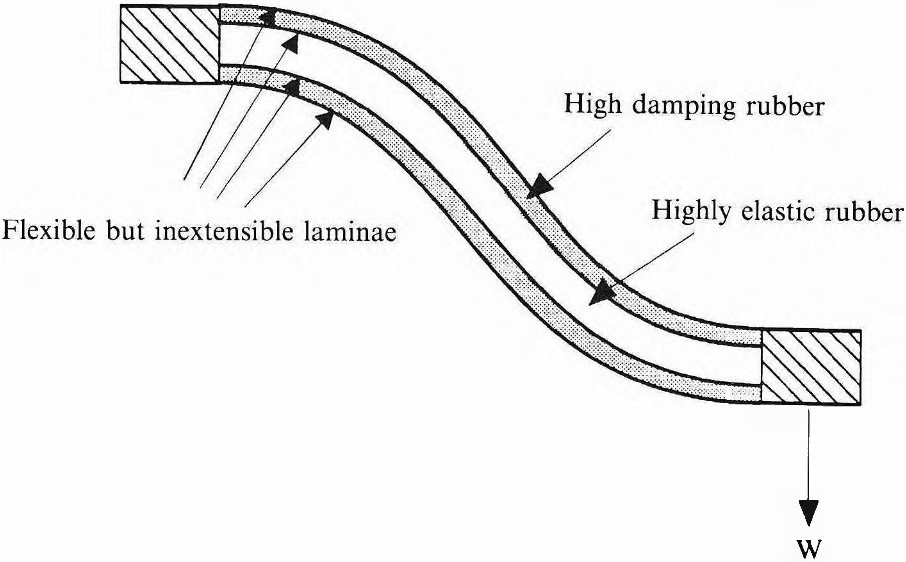

Figure 8. Flexible rubber–steel sandwich spring[16].

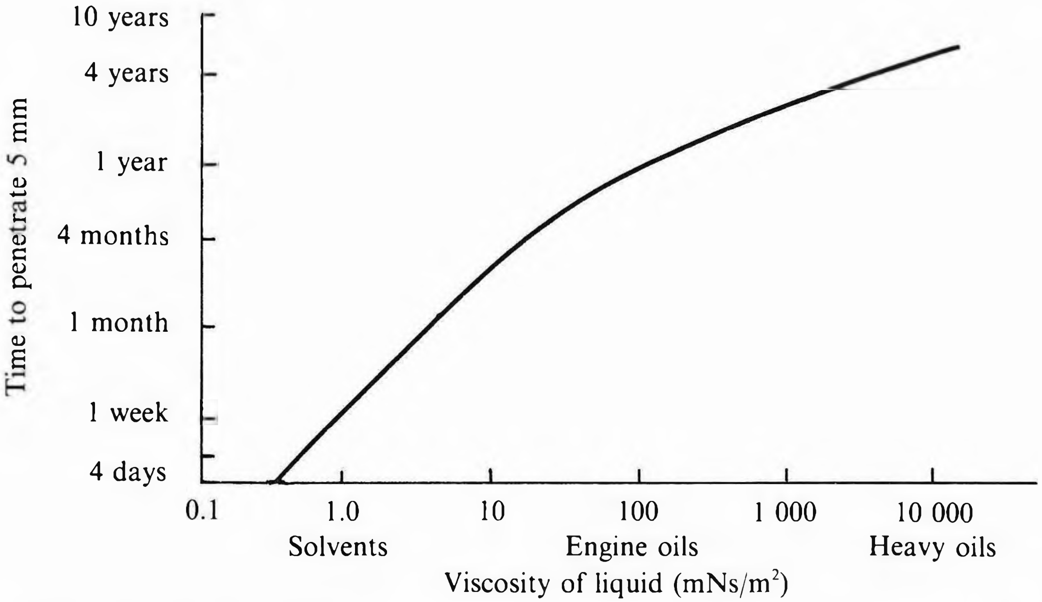

Figure 9. Effect of oil viscosity[17] *on the time taken to penetrate NR to a depth of 5 mm.*

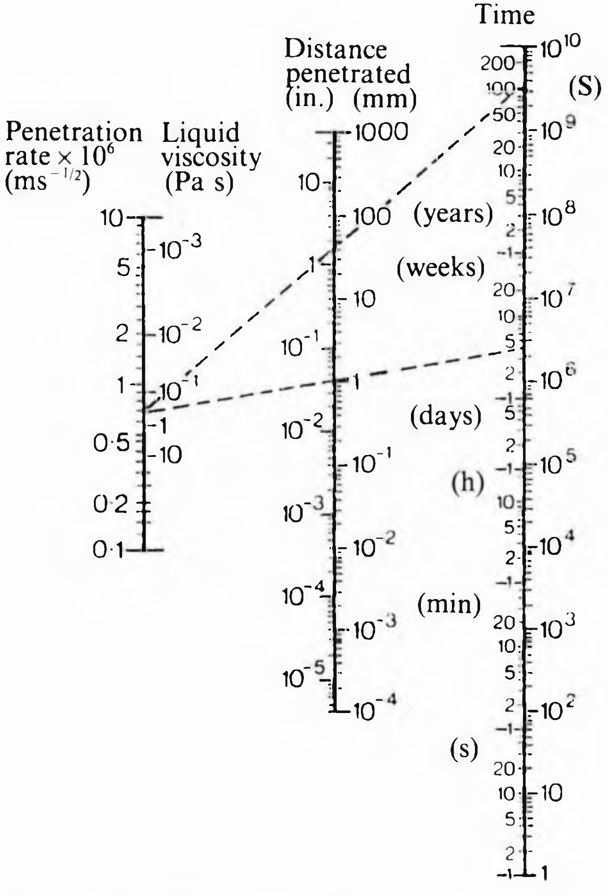

Figure 10. Nomogram relating oil viscosity, penetration distance and time for swelling of NR by oils[17].

on cosmetic grounds but may not seriously affect performance.

Effective antioxidants are available, but they generally act in a sacrificial manner. This means that their effectiveness may be under-estimated by tests on thin rubber sheets since in a bulky component, constant replenishment of the antioxidant in the surface layers can occur by diffusion from the interior. Oxidation can cause both scission and further crosslinking. The balance depends on the polymer and temperature; natural rubber softens at elevated temperatures (except when oxidation has progressed to an extreme extent[18]). but oxidation may directly cause hardening at lower temperatures. Extensive oxidation, for example at elevated temperatures, can produce a brittle surface layer, which acts as a barrier to further ingress of oxygen[19]. The time required for such a layer to form for a bulky natural rubber component formulated with antioxidant is predicted to be hundreds of years at ambient temperatures[18].

Oxidation is increased by ultra-violet radiation, but this effect can be eliminated by the presence of at least some carbon black in the compound (>1 p.p.h.r.) to make it opaque.

Ozone Attack

Ozone reacts with the carbon–carbon double bond extremely rapidly and causes direct cleavage of the bond. Attack on unstretched rubber is, therefore, restricted to a very thin surface layer, and is thus of no practical consequence. However, if the rubber is stretched above a threshold strain, cracking occurs. This results in much more efficient utilisation of the available ozone for destructive purposes. In static tests, the threshold strain at which cracking occurs may be increased substantially by dialkyl p-phenylene diamine antiozonants. However, these antiozonants may also induce a dependence of the threshold strain on the ozone concentration so that static tests in high concentrations of ozone may not be a reliable guide to performance at ambient ozone concentrations. Hydrocarbon waxes may also prevent ozone cracking under static conditions and the effect can be especially great when used together with p-phenylamine antiozonants. The effectiveness of waxes depends strongly on the temperature, and this must be borne in mind on choosing the temperature of accelerated tests. As with antioxidants, antiozonants and antiozonant waxes depend for their efficiency on diffusion to the surface. This means that bulky components will have the advantage of a large internal reservoir of antiozonant for replenishment of the surface layers[20]. If the threshold strain is exceeded, antiozonants can reduce the rate of cracking. Protection against ozone attack under dynamic conditions is more difficult than under static conditions. P-phenylenediamine antiozonants are particularly helpful in reducing the dynamic crack growth rate, but the dynamic strain below which no cracking occurs remains too low to be of practical significance.

Effect of Water

Pure polyisoprene is not swollen by water, but natural rubber does contain a few percent of hydrophilic material (such as proteins) so that some water is absorbed. This may lead to an increase in the rates of creep and stress relaxation, but other physical properties are not seriously affected for typical vulcanisates, even after immersion for forty-two years[21]. In some environments, there may be some microbial attack, paraffinic compounding ingredients such as fatty acids and wax providing the main nutrients, but the consequences for the physical properties of natural rubber are again slight. An effect of immersion that may cause problems for thin walled components (such as inflatable boats) is the leaching of antioxidants and antiozonants. Since the efficiency of antidegradants depends in part on their mobility, some of the best flex-cracking antioxidants are the most easily leached out (notably IPPD)[20].

There is a possibility that water may precipitate failure of rubber–metal bonds. For this reason, automotive bonded components are sometimes tested in an environment of salty spray. However, in a study involving immersion in sea water for two years, no degradation of natural rubber–steel bonds (using Chemlok 205/220 adhesive system) was observed even when the metal was partly exposed and had become corroded[22]. If, on the other hand, cathodic protection was used to suppress corrosion of the exposed steel then bond failure did occur. In another study, NR/steel bonds (using Chemosil 211 + 231 adhesive system) were exposed to fresh water at 70°C or 85°C; subsequent tests on the dried test-pieces did show a decline in 'stripping force' after a few days' exposure[23].

Effect of Low Temperatures

All rubbers undergo an almost instantaneous reversible increase in the storage modulus as the temperature is lowered towards the T_g, due to an increasing internal energy contribution to the work of deformation. For natural rubber (with a T_g of -72°C), this effect is small down to -40°C, while ENR 50 (with a T_g of about -20°C) is then a brittle, glassy material with a shear modulus about 1000 times greater than the value at ambient temperature. The very high dynamic stiffness of rubbers with reinforcing filler becomes even more pronounced as the temperature is lowered, as seen in *Figure 11*. It appears that the reinforcing effect becomes stronger at lower temperatures.

Another reversible but time-dependent effect of low temperature undergone by only some rubbers (notably polychloroprene and natural rubber) is stiffening due to crystallisation[24]. The rate of crystallisation of natural rubber is a maximum at around -25°C (vulcanisates then taking several days to several months to reach equilibrium). The melting temperature of the crystallites is higher the higher the temperature at which they were formed[25], but a temperature of $+30$°C is generally sufficient to cause melting. Crystallisation of natural rubber during seasonal periods of low temperature can be reduced to insignificant levels by choosing a high sulphur vulcanising system (which causes a relatively high degree of polymer chain modification)[24]. The use of efficient vulcanisation (EV) systems should be avoided in products exposed for long periods to low temperatures.

Effect of High Temperatures

As the temperature is increased, the loss modulus of a rubber decreases and the entropic contribution to the elastic modulus increases. Well above the T_g, where the entropic contribution is dominant, the elastic modulus of unfilled vulcanisates is proportional to the absolute temperature.

The strength of an elastomer generally declines as the temperature is increased *(Figure 12)*, because it is related to the amount of hysteresis (at the high strains around the crack tip). However, the effect is much more marked for non-crystallising rubber than for natural rubber, which even at temperatures as high as 140°C can apparently strain-crystallise at crack tips[26]. This aspect is not usually covered by specifications, which concentrate on the irreversible effect of exposure to high temperatures on the properties subsequently measured at room temperature.

Irreversible changes (often called 'ageing') are a consequence of both oxidation (discussed above) and anaerobic chemical reactions. The anaerobic reactions are the more serious limitations, since they proceed independently of the bulk of the rubber component. They proceed especially rapidly at temperatures of the order of the vulcanisation temperature (~150°C). For conventionally cured natural rubber, some crosslinks are lost altogether while others are reduced from polysulphides to disulphides or monosulphides. This results in a reduction in both the modulus and resilience. EV systems provide improved resistance.

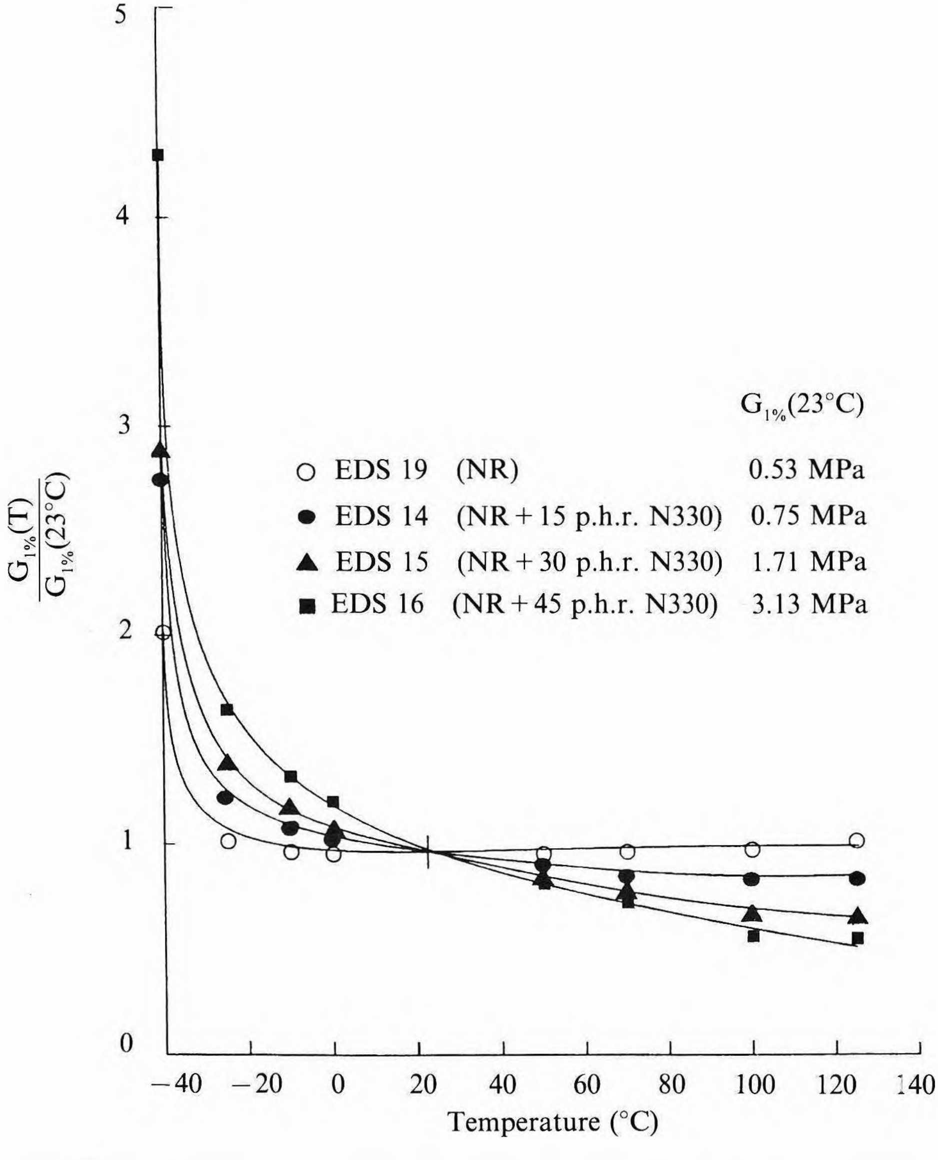

Figure 11. Effect of temperature and loading of carbon black on the complex shear modulus (1 Hz, 1% strain) of natural rubber vulcanisates[41].

Hysteretic Heating and Heat Build-up

In extreme cases (such as solid tyres) hysteretic loss can result in high temperatures, and runaway heat build-up may occur, especially if there is a tendency for the modulus and resilience to fall on exposure to high temperatures. For natural rubber compounds, the thermal runaway process begins only when the temperature reaches 120°C or more[27]. Once very high temperatures have been reached the rubber may be pyrolised to a liquid, and gaseous products may cause mechanical failure (or 'blow-out') of the surrounding rubber[28].

When a visco-elastic material is deformed in simple shear with a sinusoidal shear-strain

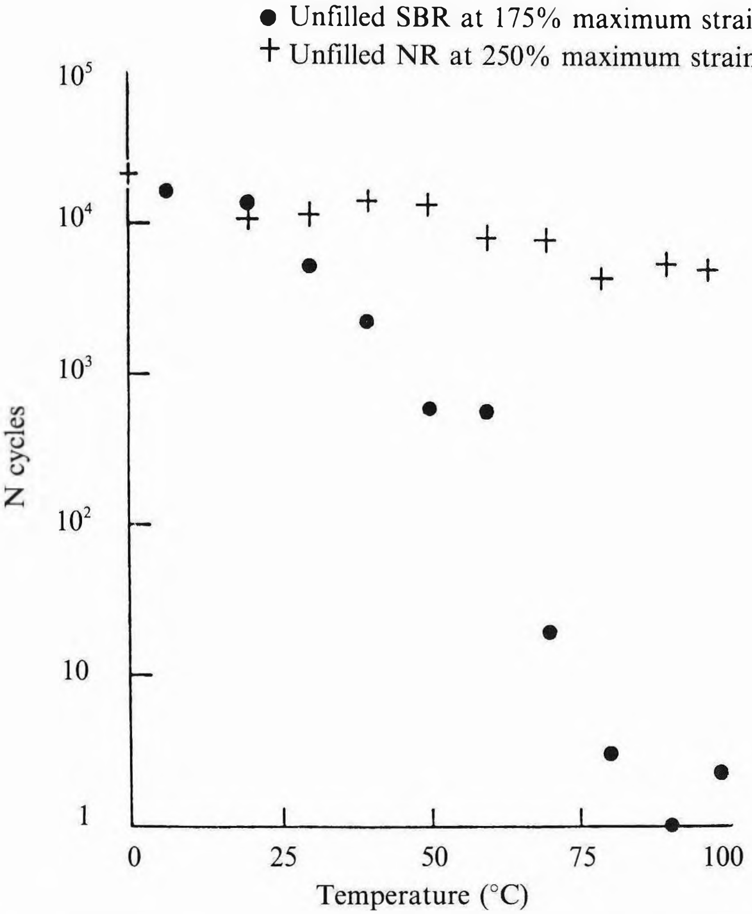

Figure 12. Effect of temperature on the cyclic fatigue life of dumbbell test-pieces at 100 cycles/min for unfilled SBR at 175% maximum strain and unfilled NR at 250% maximum strain; minimum strain zero[35].

amplitude γ_o, the energy dissipated by hysteresis per unit volume of rubber is given by

$$\pi\,\sigma_o\,\gamma_o\sin\delta = \pi\gamma_o^{\,2}\,G'' = \pi\sigma_o^{\,2}G''/(G'^2+G''^2) \qquad \ldots 11$$

where the symbols are defined as in *Equation 3*. For non-linear materials, there may be some stress response at harmonics of the displacement frequency, but *Equation 11* is still a correct expression for the energy loss provided G', G'' and δ refer to the stress response at the fundamental frequency[29].

The three expressions in *Equation 11* are useful according to whether $\sigma_o\gamma_o$, γ_o or σ_o are held constant in the application, corresponding to constant energy, constant strain or constant stress respectively. Thus, under constant energy conditions, the important parameter is the loss angle; under constant strain conditions, the modulus should also be low, while under constant stress conditions a high modulus (and low loss angle) will act to reduce heat build-up. It is important that these distinctions are considered in applications where heat build-up is a problem. Standard tests (such as Goodrich heat build-up) may not correspond to the conditions for the application of interest, and may thus give misleading information. Ideally, direct measurements of G'' and G' at the relevant elevated temperatures should be made, checking also for effects of exposure time at elevated temperatures on the dynamic properties.

MECHANICAL FAILURE OF THE COMPONENT

Specifications usually stipulate a minimum allowable value for the tensile strength and perhaps also a minimum value for the tear strength. However, in practice the only occasion when the component is likely to fail in such a catastrophic manner is when it is extracted from the mould. For maximum relevance, the tensile or tear strengths should thus be measured at the appropriate elevated temperature which is generally much higher than the service temperature. Since this is not the usual practice, the value of tensile and tear strength tests is restricted to quality control.

The property which is more appropriate to failure modes of the engineering component in service is fatigue *i.e.* the slow propagation of cracks over many cycles of deformation (or, for static applications, long periods of time). Hysteretic heating of the rubber may mean that the relevant temperature for such tests is greater than the ambient service temperature. Sometimes the hysteretic heating may be so great that failure occurs more due to heat build-up than to crack propagation, although both phenomena are sometimes referred to as fatigue.

Crack Propagation

The rate at which a crack propagates into an elastomer is governed by the strain energy release rate T:

$$T = -\frac{\partial U}{\partial A} \qquad \ldots 12$$

where U is the total elastic retraction energy, A is the area of one fracture surface, and the partial derivative is evaluated with a fixed boundary to the elastomer so that the external forces do no work.

Examples of test-pieces for which T can be simply expressed are given in *Figure 13*. In deriving the formulae in this figure, the fracture surface area A has been replaced by hc where h is the thickness of the rubber sheet and c is the crack length.

It is found that if T is less than a certain value T_o (of the order of 0.04 kNm^{-1} for unfilled NR) then the crack will not propagate except by ozone attack[30]. T_o depends on the polymer and to some extent on the vulcanisation system, and may for NR be increased somewhat by choice of a suitable antioxidant. Reinforcing blacks increase T_o for NR by about 50% but other fillers have little effect[30].

For $T > T_o$, crack propagation may proceed in a time-dependent manner for non-crystallising rubbers (e.g. SBR or EPDM) held at a constant deformation:

$$dc/dt = f(T) \qquad \ldots 13$$

where the function f must be found experimentally.

Cycling from $T = 0$ to T_{max} at moderate or high frequencies often results in a much greater increase in crack length per cycle than anticipated from *Equation 13*

$$dc/dn >> \oint f(T)\, dt \qquad \ldots 14$$

It turns out that the increment in crack length per cycle then depends on T_{max} and very little on the period of the cycle or on the detailed path from $T = 0$ to $T = T_{max}$:

$$dc/dn = g(T_{max}) \qquad \ldots 15$$

For strain-crystallising rubbers, notably natural rubber, but also some grades of polychloroprene and to a less marked extent some grades of synthetic polyisoprene, time-

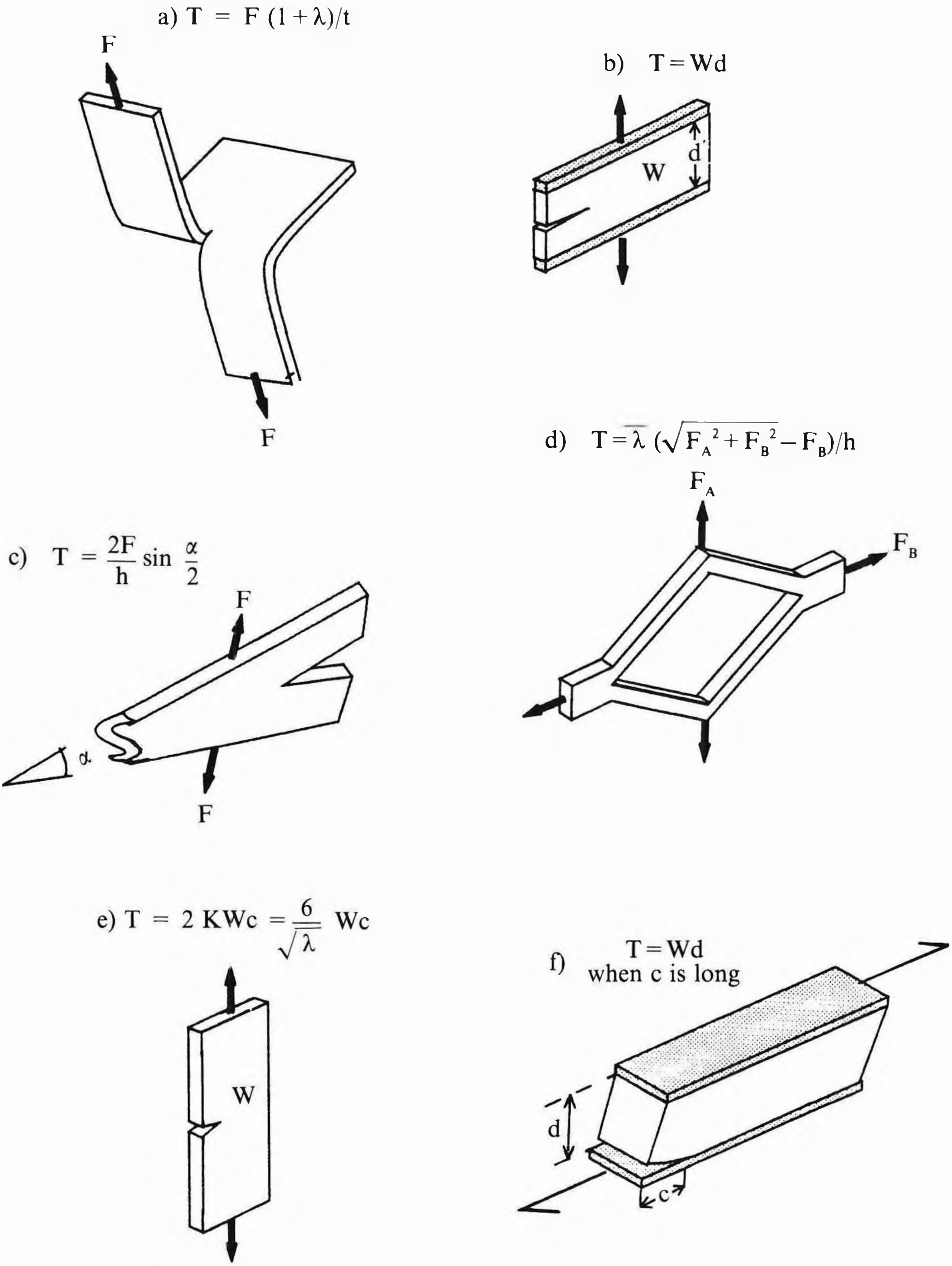

Figure 13. Various fracture test-pieces a) 'trousers', b) pure shear, c) angled, d) split, e) tensile, f) simple shear. In the formulae, h is the thickness of the rubber sheet, c is the crack length, W is the strain energy per unit volume in the region of uniform strain away from the crack and γ *is the extension ratio (in the direction of* F_B *in the case of the split test piece.*

dependent mechanical crack growth does not normally occur. The characteristic *Equation 14* is also such that *dc/dn* increases more slowly as T_{max} increases than for the non-crystallising rubbers *(Figure 14)*. Furthermore, under non-relaxing conditions (which is often the case for load-bearing components) strain-crystallising rubber shows a greatly enhanced resistance to crack growth[30]. For these two reasons, natural rubber is normally preferred for load-bearing components.

Fatigue

Provided a flaw size is put into the theory for crack propagation, the design criterion that throughout the component $T < T^*$ (where T^* is some design value — one suggestion[14] being $T^* = T_0$) may in some cases be reduced to a stress criterion $\sigma < \sigma^*$ which is amenable to finite element analysis of the component. It has been assumed that the flaw size and the strain gradients (in the absence of the flaw) are sufficiently small

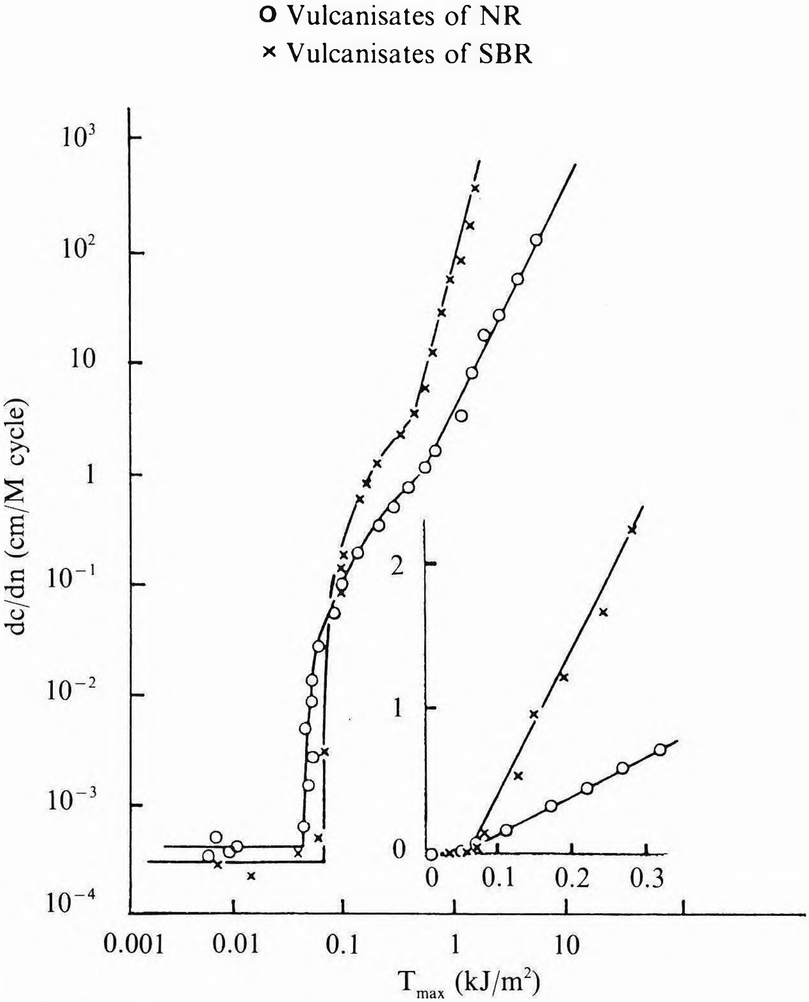

Figure 14. Cyclic crack growth rate dc/dn versus maximum tearing energy of the cycle T_{max} for vulcanisates of NR and SBR. The inset shows the region near T_0 plotted on linear scales[37].

that the flaw can be considered to reside in a field of otherwise uniform strain.

There is some hope of using a criterion of the form $\sigma < \sigma^*$ on the free surface of a component, where the rubber will be in a state of biaxial strain and its fracture behaviour may be related to experiments on sheets of rubber in biaxial extension. However, in the interior, the approach is on less certain ground since little work has been done on either calculations of available tearing energy or on fracture propagation.

A stress criterion will not be applicable to stress singularities such as at the junction of the free surface of rubber with a rigid surface to which it is bonded. Lindley and Teo[31] have analysed this situation for simple shear and reached the conclusion that T reaches a finite limit $0.4Wd$ as $c \rightarrow o$ (where d is the separation of the shear planes) and at first decreases as c increases. Eventually a minimum value of T is reached (about $0.06Wd$) and T then increases with c until a second (but higher) limiting value is reached ($T = Wd$: *Figure 13f*). The limiting value of T as $c \rightarrow o$ can be reduced by means of a fillet, but although strain analysis on fillets has been done[32] it has not been extended to cover crack initiation.

Once the locus of the maximum stress has been identified by analytical or numerical means, calculations can be made with cracks of various lengths in that position to find the T value as a function of crack length c (using *Equation 12*). This has been done in an approximate manner by Stevenson[33] for long cracks in compressed bonded blocks of rubber. T can also be found experimentally, by measuring the effect of crack length on force-deformation behaviour or by measuring the crack-opening area. The relationship of T to crack length c can then be used to estimate fatigue life once mechanical failure has been initiated.

For an edge crack in a tensile test-piece, the fatigue life can be calculated from the crack-growth characteristics as follows[34]:

$$dc/dn = BT_{\max}^{\beta} \qquad \ldots 16$$

$$T = 2KWc$$

$$=> dc/dn = B(2KW)^{\beta}c^{\beta} \qquad \ldots 17$$

Integration from $c = C_o$ (the initial flaw size) gives the number of cycles N to reach $c = C$ as

$$\mathrm{N} = \frac{1}{(\beta - 1)\, B(2KW)^{\beta}} \left[\frac{1}{C_o^{\beta-1}} - \frac{1}{C^{\beta-1}}\right] \qquad \ldots 18$$

At failure $C >> C_o$ so that the number of cycles to failure is obtained from *Equation 18* by taking only the first term in the square brackets. *Equation 18* works well at moderate and high strains[34] for which *Equation 16* is reliable. For SBR, $\beta \approx 4$ and for NR, $\beta \simeq 2$. It is apparent that for SBR, the predicted dependence on fatigue life on strain and on flaw size is much greater than for NR. At very high strains, *Equation 16* is not appropriate, since there is a value T_c of T at which the crack growth becomes catastrophic. Thus as $T_{\max}$ approaches T_c, the fatigue life will fall below that predicted by *Equation 18*. Although this regime is not relevant to most engineering components (which are normally required to have a very long fatigue life) progress has again been made in relating fatigue life to crack growth characteristics[36,37]. For strains below the mechanical fatigue limit (which corresponds to the minimum tearing energy T_o required for mechano-oxidative crack growth), the fatigue life can be quantitatively predicted by assuming the flaws will grow only by ozone cut growth until T_o is reached[35]. The cyclic rate of ozone cut growth is given by

$$dc/dn = \alpha q \tau \qquad \ldots 19$$

where τ is the time during each cycle for which the test-piece is strained

q is the ozone concentration

α is the ozone cut growth constant for the rubber under static conditions (*i.e.* the rate of growth at unit ozone concentration).

Figure 15 compares the experimental fatigue life of dumbbells of unfilled NR in tension with that predicted. The predictions are based on the experimentally determined crack-growth data for this vulcanisate[36], together with a 'single fitting' parameter of $C_o = 25\mu$; the agreement is seen to be good.

Under cyclic conditions (or static conditions for an unprotected rubber), ozone cracking only occurs if T exceeds a threshold value T_z. Unfortunately T_z is so low that it may be difficult to avoid T exceeding T_z on the surface of the component. Exposed rubber to metal bonds are also attacked by ozone[38], but only if T exceeds a threshold value T_b. Fortunately T_b is much greater than T_z and the use of fillets should enable T to be kept below T_b at the bond.

Cavitation

When subjected to a hydrostatic tension of the order of Young's modulus, naturally present cavities in the rubber open up in an unstable manner, resulting ultimately in rupture[39]. Carbon black suppresses this effect to some extent, but it is still wise to avoid subjecting rubber to hydrostatic tension of this order. Since internal damage will be initiated by a single cycle, it is important not only to avoid excessive

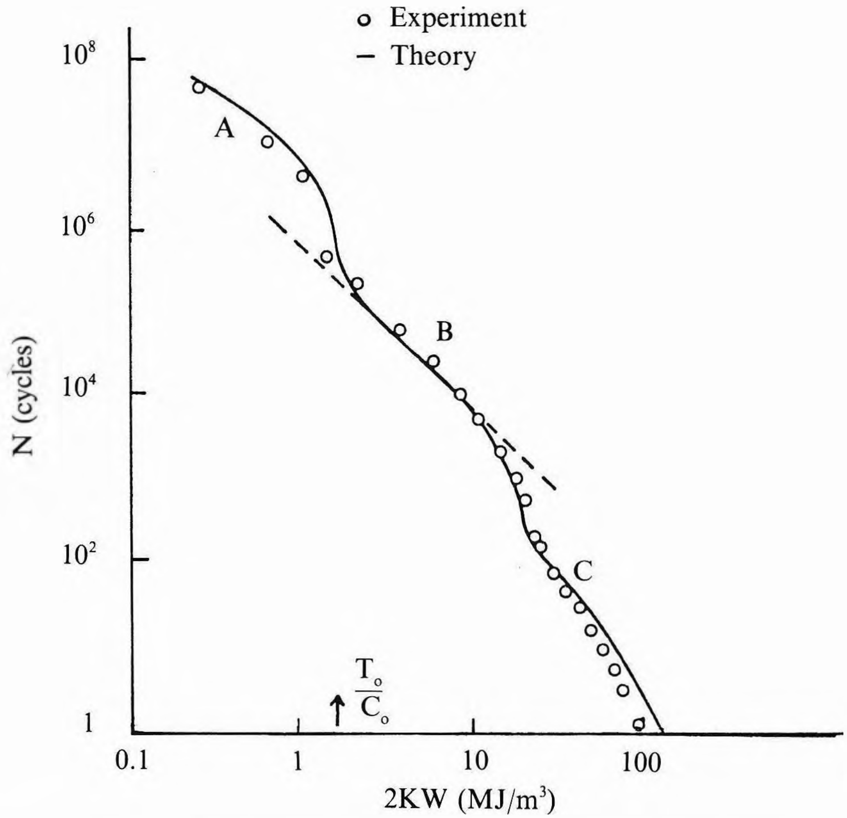

Figure 15. Tensile fatigue life N versus maximum value of strain energy density factor 2 KW (minimum value zero) for an unfilled NR vulcanisate for maximum strains from about 30% to about 700%[35]*. (Region A — fatigue dominated by ozone cracking Equation 19 Region B — crack growth fatigue mechanism, Equation 18 with $\beta = 2$, Region C — very high tearing energy regime). For the theory, a value of $C_o = 25\ \mu$ has been taken for the naturally occurring flow size.*

hydrostatic tension in service but also to restrict screening tests for poor bonding to safe levels of tension.

Component Design

Summarising the above, components should ideally be designed such that

- T, for the natural flaw size of ~25μ, is less than T_o throughout.
- Fillets and cover layers are used so that the surface values of T are lower still and preferably less than T_z, so that ozone cracking does not reduce the fatigue life or result in unacceptable appearance.
- Hydrostatic tensile stresses should be avoided, or at least be less than the modulus of extension of the rubber.

CONCLUSIONS

Performance of rubber engineering components generally depends on static and dynamic elastic properties and their dependence on amplitude, temperature and (for dynamic properties) frequency. Maintenance of satisfactory performance over long periods of time may depend on proper allowance for creep and stress relaxation. Factors which determine longevity include crack growth characteristics (especially at very low crack growth rates), diffusion rates of antioxidants/antiozonants together with effects of surface area (where the protectants may be lost by weathering, *etc*) and bulk (which provides a reservoir of protectant), diffusion rates of fluids to which the rubber is exposed (as well as equilibrium swelling levels) and leaching of protectants from the rubber by such fluids. All of these factors need to be assessed over the range of service temperatures. The measurements require skill and some may take a long time.

Standard physical tests may be favoured if they are quick, require a minimum of training to perform and yield results with little scatter. The effect of temperature tends only to be assessed in terms of measurements at laboratory temperature before and after exposure to an extreme temperature. Conditions such as temperature, ozone concentration and tearing energy tend to be so extreme as to make estimation of service performance difficult or impossible. Many standard tests are performed on 2 mm thick sheets and this might lead to rejection of materials which in the bulk form would not be seriously degraded by oxygen, ozone or a swelling fluid. The value of standard physical tests thus lies more in quality control than in providing a reliable guide to performance.

In practice, a compromise may be possible in which only those engineering properties that are crucial for the application are specified. These properties may then be supplemented by standard tests, and perhaps by specifying the polymer selected from experience in such a way as to achieve satisfactory overall performance and quality control.

There are areas where further research is required, particularly relating to longevity of engineering components. Most of the basic studies on fracture of rubber have been done on material in sheet form. Not all failure mechanisms in engineering components can be related to such studies. Another area of uncertainty concerns the durability of rubber–metal bonds. In the usual standard tests of bond strength, failure takes place away from the bond, in contrast to the appearance of automotive components after many years of service[40]. Little basic work has been done on the factors influencing longevity of the bond.

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Relationship between Resistance to Microcyclus ulei *and Clonal Foliar Phenolics of Rubber Trees*

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F. RIVANO**, A. de SAINT BLANQUAT** AND J. d'AUZAC*

The extent to which resistance of Hevea brasiliensis *to* Microcyclus ulei *may be related to the presence or absence of certain foliar phenolics analysed by High Performance Liquid Chromotography (HPLC) after selective extraction was studied. Young leaves at* M. ulei-*sensitive* (B2) *stage from fourteen clones with a broad range of resistance were used. The resistance to* M. ulei *of these clones was evaluated in the field by determining the percentage of leaf abscission during a one-year period.*

No qualitative differences were observed between the foliar phenolics of various clones of Hevea brasiliensis, *and hybrid rubber trees. Except for clones PB 235 and PB 260, the total quantity of phenols and the proportion of flavans appear to be related to resistance to* M. ulei *expressed as percentage of abscission. Multiple regression analysis showed that some compounds seemed to be closely related to the percentage of abscission whereas others appeared to be correlated with resistance (kaempferol-3-rutinoside, 3'p-coumaroylquinic acid). Classification of the fourteen clones as 'sensitive' and 'resistant' shows that some phenols are present in resistant clones and others are found in susceptible clones [(+)-catechin and rutin]. Likewise, the hydroxycinnamic derivatives (HDC)/flavonols ratio, the percentage of ratin in relation to total flavonols and the ratios of certain phenols (kaempferol-3-rutinoside: kaempferol-galactoside, rutin: kaempferol-galactoside) appeared in particular to be related to clonal resistance.*

South American leaf blight (SALB) caused by *Microcyclus ulei* (P. Henn) V. Arx, prevents the creation of large, economically profitable plantations throughout the Amazon basin, where *Hevea brasiliensis* (the only cultivated species) originated. The disease is also a threat to the other rubber-growing areas in the world as most of the high-yielding clones bred in Asia are extremely susceptible.

The earlier results of Langford[1] on the biology of the pathogen, relationships with its host and characterisation of the sensitivity and resistance of the various *Hevea brasiliensis* clones and of other *Hevea* species have been the subject of numerous references[2,3,4]

The most promising method of control appears to be based on the genetic selection for disease-resistant clones which also possess high production potential. All the resistance of the clones bred since the beginning of the century seem to have been rapidly overcome by *M. ulei*. This can be explained in part by the great physiological variability of *M. ulei*[5,6,7] and also by the types of resistance of the clones selected by breeders. The reaction of these clones[8,9] is similar to a hypersensitivity phenomenon often related to vertical resistance[10].

The current trend is to seek horizontal resistance to *M. ulei* combining as many resistant characters as possible[11]. The continuation of research on possible defence

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mechanisms may contribute to this and provide the selection criteria.

It seems that *M. ulei* penetrates the cuticle and epidermis of all clones[8,9], which seems to indicate that resistance is not related to an anatomical barrier but to biochemical features. Phenolics may contribute to resistance to phytopathogenic fungi in several ways:

- Phenols present before the infection may have an inhibitory effect on conidial germination[12].
- Many phytoalexins are phenolic[13].
- Oxidised phenolics are the cause of the brown compounds which appear during the hyper-sensitive reaction. They are also lignin precursors and thus contribute to the strengthening of cell walls.

In addition, even if phenolics have no active role in resistance, they may possibly be used as resistance or susceptibility markers.

Phenolics in *Hevea* leaves have been studied. Aqueous extracts[14] or diffusates[6] of young resistant healthy leaves have a greater inhibitory effect on conidium germination than extracts of susceptible leaves. This inhibition was attributed[14] to a compound existing before infection, identified as a flavonol, which is probably more abundant in resistant leaves. It is probably a kaempferol derivative[15] or a quercetin derivative[16]. It has also been shown[17] that diffusates of young *Hevea* leaves contain phenols which hinder the germination of *Colletotrichum gloeosporioides* conidia.

No clear relationship has been established between total phenols or each constituent phenol analysed by HPLC and the degree of resistance of *Hevea* to *C. gloeosporioides*[18] or *M. ulei*[16]. The only difference between susceptible and resistant clones concerns the flavonol contents. These compounds may, for example, be involved in the regulation of foliar auxin-oxidase[19]. Two phytoalexins have been characterised in infected *Hevea* leaves[20]; one is a coumarin and the other scopoletin[21].

All the work carried out to date[14,16,18,21] suggests that one or more particular phenols are involved — at least in part — in the resistance of *Hevea* to pathogenic fungi. A detailed HPLC study was therefore undertaken on the phenolics of fourteen clones which, under the conditions of French Guiana, displayed a broad range of susceptibility to *M. ulei.* The report concerns only results of phenolics present in young healthy leaves at the susceptible stage *(B2)* to *M. ulei.*

MATERIAL AND METHODS

Plant Material

Young leaves of the fourteen clones: FX 3864, IAN 873, RRIC 101, PB 235, IRCA 19, PB 260 and GT 1 *(Hevea brasiliensis)*; IAN 717, CD 1078, RO 38, IAN 2878, IAN 710 and IAN 713 (interspecific hybrids) and *Hevea pauciflora* PA 31 were analysed *(Table 1)*. These clones were cultivated in a plastic greenhouse and were free of any foliar contamination.

The leaves were sampled at *Stage B_2*, as defined by Dijkman[22] or at *Stage III* as defined by Blazquez and Owen[8], from plants cultivated under plastic at the IRCA Station at Kourou (French Guiana) and frequently cut back to ensure continuous growth of young leaves. The plants were not subjected to any chemical treatment and were totally free of *M. ulei*.

Samples of 1 – 2 g fresh weight (FW) were conserved in 95% ethanol and sent to Montpellier (France) for analysis. Sampling was repeated five to seven times depending on the clone to reduce the variability of sampling.

Evaluation of Susceptibility to *M. ulei*

Susceptibility was estimated using trees grown in a comparative clone trial

TABLE 1. THE DIFFERENT CLONES STUDIED

Clone	Country of origin	Species	% Abscission
PA 31	Brazil	Aspecific *H. pauciflora*	0.001
IAN 717	Brazil	Interspecific *H. benthamiana* × *H. brasiliensis*	0.001
CD 1078	Guatemala	Interspecific *H. pauciflora* × *H. brasiliensis*	0.8
RO 38	South America	Interspecific *H. benthamiana* × *H. brasiliensis*	1.5
IAN 2878	Brazil	Interspecific *H. benthamiana* × *H. brasiliensis* × *H. brasiliensis*	1.6
IAN 710	Brazil	Interspecific *H. benthamiana* × *H. brasiliensis*	4.5
FX 3864	Brazil	Intraspecific *H. brasiliensis*	4.5
IAN 873	Brazil	Intraspecific *H. brasiliensis*	4.5
RRIC 101	Sri Lanka	Intraspecific *H. brasiliensis*	11.6
PB 235	Malaysia	Intraspecific *H. brasiliensis*	20.4
IRCA 19	Ivory Coast	Intraspecific *H. brasiliensis*	30.8
PB 260	Malaysia	Intraspecific *H. brasiliensis*	40
IAN 713	Brazil	Interspecific *H. benthamiana* × *H. brasiliensis*	45
GT 1	Java	Intraspecific *H. brasiliensis*	45

The percentage of leaf abscission measured in one year was used as the criterion of sensitivity to *M. ulei*. The dividing line between resistant and sensitive clones was fixed arbitrarily at 10%. This classification is valid in the eco-climatic conditions of the French Guiana and for the *M. ulei* races present in this country.

established from 1982 to 1987 at the IRCA-CIRAD station in French Guiana.

The susceptibility score awarded to each clone is the average abscission of young leaves in 1989; this was recorded for all the trees every two months *(Table 1)*. According to Rivano (personal communication), this score gives the best description of the degree of tolerance to the disease. In *Table 1* are listed clones which appear to be resistant or susceptible to all the races of *M. ulei* collected in Guiana. It may be possible that in another eco-climatic condition or for other races of *M. ulei*, the classification will be different.

Extraction of Phenolics

The leaves conserved in 95% ethanol were drained, frozen in liquid nitrogen, crushed in a ball grinder and agitated cold for 20 min in the conservation ethanol diluted to 80% and to which 0.5% sodium disulfite had been added. After filtration through No. 4 fritted glass, the solid residue was extracted once more for 20 min in 80% ethanol containing 0.5% $Na_2S_2O_5$. The filtrates were combined and the ethanol evaporated under partial vacuum using a rotary evaporator at 35°C.

The residual aqueous phase was bleached four times with half a volume of petrol ether; $(NH_4)_2SO_4$ 40% (v/v) and metaphosphoric acid 20% (v/10) were added before four ethyl acetate (v/v) extractions. Ethanol (95%) was added during the final extraction to complete the transfer of the phenolic compounds to the ethyl acetate. The organic phase was dehydrated by filtration on 1PS Whatman paper and evaporated to dryness. The residue was re-suspended in 5 ml of HPLC methanol and filtered on a Millipore HVLP membrane (porosity 0.45 μm). The purified phenolic extract was stored at -18°C. The variability coefficient for the extraction method was 10% – 12%.

HPLC

HPLC analyses were performed with a Waters 600 E apparatus equipped with a C_{18} column (Macherey–Nagel Nucleosil 5 μm, 250 × 4 mm). The injection volume was 20 μlitre (10 μlitre of extract + 10 μlitre of phloretic acid used as an external standard; peak *E* on the chromatograms). Elution was performed with a pH 2.6 acetonitrile–water gradient. The separated components were detected by Waters 990 diode array detector.

The peak area was integrated at 280 nm for flavans (*Peaks A3* to *A22*), 314 nm for hydroxycinnamic derivatives (*Peaks B3* to *B10*) and 350 nm for flavonol (*Peaks C11* to *C21*). These wavelengths are the maximum absorption zones for the three groups of compounds. The peak areas were then converted into micromoles equivalent levels of (+)-catechin (flavans), p-coumaric acid (hydroxycinnamic derivatives, HCD) and quercitrin (flavonols). When the flavan peaks were partially superimposed on the HCD peaks, the 280 nm flavan values were corrected by subtracting the HCD absorption values at this wavelength.

In addition, an overall phenol content (in micromoles phenolic equivalent per gramme FW) was obtained by adding the quantities determined for each of the three large separation groups above.

The HPLC variability was less than 10% in all the cases.

Statistical Analyses

The analyses below take into account all the results for each of the fourteen clones using five to seven different samplings, each of which was subjected to three HPLC analyses.

Principal component analysis (PCA) was performed using the amount of each phenol in order to examine the relationship between the molecules. Supplementary variables such as combinations of the different groups of phenols and the abscission percentage were introduced in the PCA.

A second set of analyses was carried out using multiple linear regression. A relation was sought between the explained variable (the abscission percentage in this case) and the explanatory variables (abundance of phenols). Use of the method was justified as the number of observations (84) was more than double the number of explanatory variables (2 × 32).

The model is therefore written as follows:

$$\text{Percentage abscission} = a + bA1 + cA2 + dA3 + \ldots$$

in which *A*1, *A*2 ... represent the levels of the different phenols expressed in micromoles equivalent phenol per gramme FW. A stepwise procedure was used to maximise the determination coefficient R^2.

Analysis of variance was also performed on the phenols (quantitative character) according to the nature of the clone (qualitative character). When this analysis showed differences between clones for a particular phenol, the average contents of these phenols were determined using the Duncan test. The advantage of such analysis of variance is that no hypothesis concerning the sensitivity of the clones to *M. ulei* is required.

The clones were also classified as being sensitive or resistant according to their percentage of abscission (more or less than 10%). Analysis of variance was then performed to compare the phenol contents of the two classes of clones.

The results of these different analyses of variance do not necessarily correspond fully since different qualitative criteria are taken into account (clones in one case and sensitivity class in the other). Analyses of the correlation between the percentage of abscission and abundance of each of the phenols and the different ratios between phenolics were also carried out.

Computations were made with 'Statistical analysis system Inc' (USA) and SYSPAD programs from COREF (Paris).

RESULTS

Direct Analysis of HPLC Results

Analysis using thin-layer chromatography (not reported here) and HPLC co-injection with commercial standards [(+)-catechin, chlorogenic acid, rutin] or standards extracted from other plant material[23] show that *Hevea* phenolics belong to three main groups: flavans [(+)-catechin, (–)-epicatechin and derived substances] which are only detected by HPLC at 280 nm (*A* peaks), HCD whose maximum absorption is close to 314 nm (*B* peaks) and finally flavonol heterosides whose detection is maximum at 362 nm (*C* peaks). Several compounds were identified in each of these three groups when possible *(Table 2)*.

Individual study of the different peaks of phenolics on HPLC chromatograms did not reveal any qualitative differences (*Figures 1* and *2*). Only clone CD 1078 displayed an extra peak *(C12)*.

Several observations can be made concerning the quantitative results:

- The flavan group forms the largest proportion of the phenols with an average of nearly 66% resistant clones also appeared to display a high rate *(Table 3)*.
- In contrast, HCD were not abundant but the percentage in relation to the overall phenol content was greater in sensitive clones, except in clones PB 235 and PB 260 *(Table 3)*.
- The HCD/flavan ratio was also higher in sensitive clones, except PB 235 and PB 260 as above.

TABLE 2. IDENTIFICATION OF SEVERAL PEAKS OF PHENOLICS SEPARATED BY HPLC

Classes	Peak	Nature of the compounds
Flavans	A7	(+)-Catechin
	A10	(–)-Epicatechin (partly)
HCD	B5	*cis* 3' ρ-coumaroylquinic acid
	B6	*trans* 3' ρ-coumaroylquinic acid
	B9	*cis* 4' ρ-coumaroylquinic acid (+ Feruloylglucose ?)
	B10	*trans* 4' ρ-coumaroylquinic acid
Flavonols	C14	Rutin (Quercetin-3-rhamnoglucoside)
	C16	Isoquercitrin (Quercetin-3-glucoside)
	C19	Kaempferol-3-Rutinoside (= Kampferol-3-rhamnoglucoside)
	C21	Kaempferol-galactoside

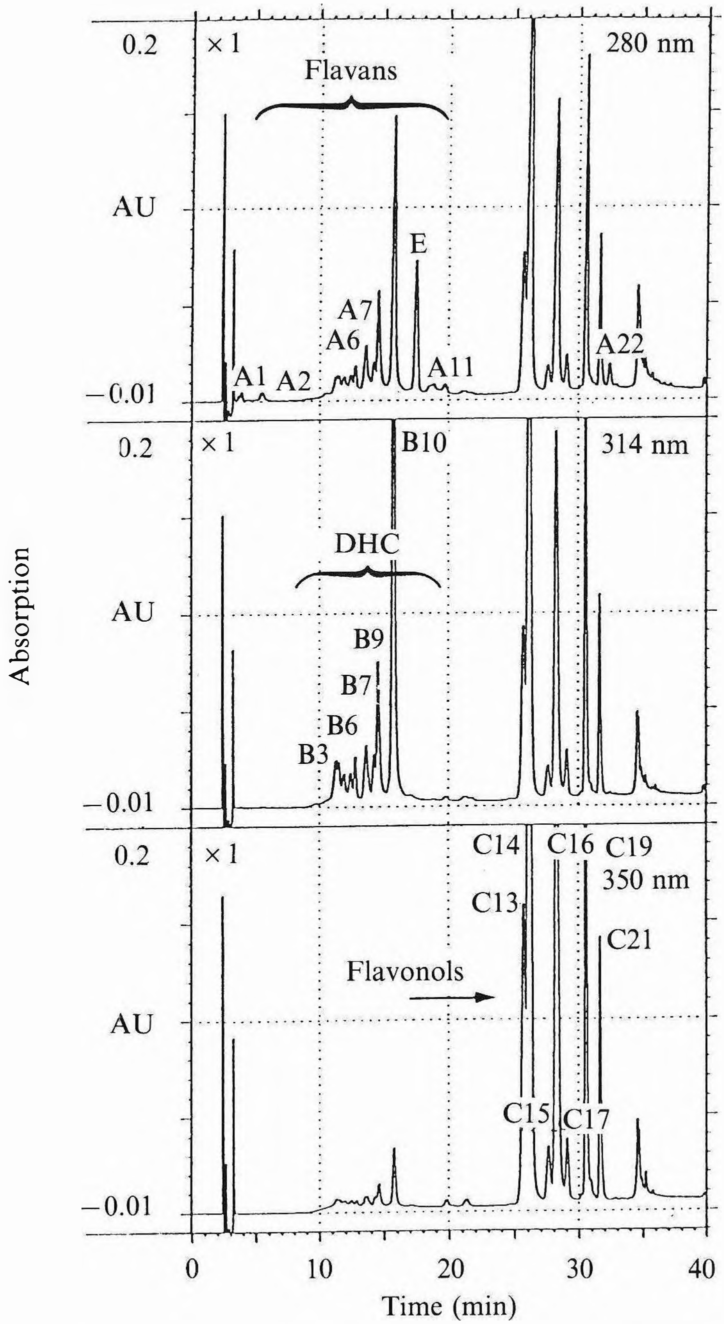

Figure 1. HPLC chromatogram of the phenols in a foliar sample of GT 1(S), (3.24 µg FW).

Individual analyses of each of the compounds separated by HPLC show that certain flavans *(A5, A7, A8, A22)* and the flavonol *(C14)* were more abundant in the resistant clones (*Tables 4* and *5*). In contrast, the HCD *B3, B4, B5, B6, B9* and *B10* and

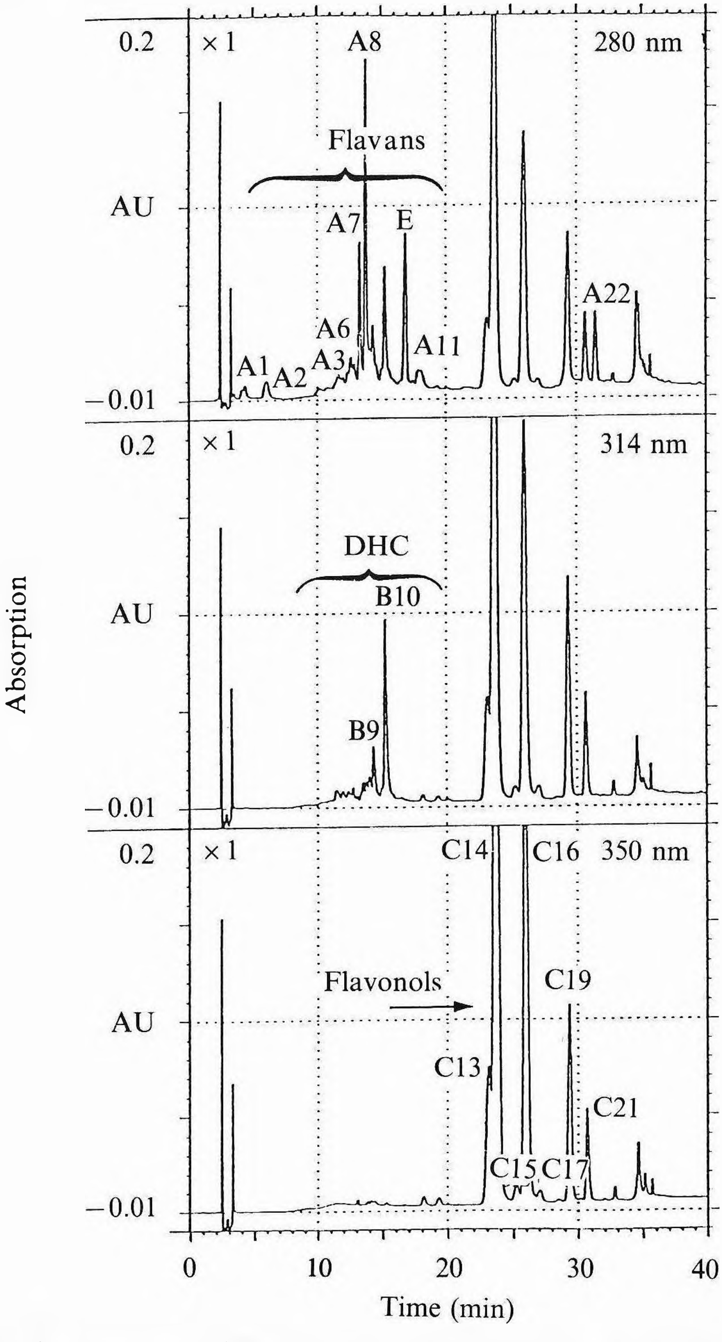

Figure 2. HPLC chromatogram of the phenols in a foliar sample of IAN 717(R), (4 μg FW).

the flavonols *C13, C15, C17* and *C18* were more abundant in the sensitive clones (*Tables 5* and *6*).

In addition, the chromatograms of the various clones appeared to show interesting variations between compounds *C19*

TABLE 3. CLONAL ANALYSIS OF THE THREE MAIN PHENOLIC GROUPS : FLAVANS, HYDROXYCINNAMIC ACIDS (HCD) AND FLAVONOLS, AND THE OVERALL PHENOL CONTENT

Clone	% abscission	Total (μmol phenol eq/ g FW)	Flavans (μmol (+) catechin eq/ g FW)	Flavans (% sum of phenols)	HCD (μmol p. coumaric acid eq/g FW)	HCD (% sum)	Flavonols (mmol quercitrin eq./g FW)	Flavonols (% sum of phenols)	HCD/ Flavans × 10^{-2}
PA 31	0.001	37.2 abcd	24.3 abcdef	66.9 ab	1.8 a	4.9 cd	11.1 ab	28.2 bcd	7.3 cd
IAN 717	0.001	36.2 abcd	26.1 abcdef	67.9 ab	0.9 e	2.9 cd	9.3 bcd	29.2 bcd	3.3 ab
CD 1078	0.8	49.7 abc	39.6 ab	77.8 a	1.5 abcd	3.2 cd	8.6 cde	19.0 cd	3.9 abc
RO 38	1.5	32.3 bcde	20.7 bcdef	62.6 ab	1.0 e	3.3 cd	10.6 abc	34.1 b	4.4 abc
IAN 2878	1.6	33.7 bcde	26.5 abcde	73.6 ab	1.6 ab	6.3 bc	5.6 de	20.1 bcd	6.3 cd
IAN 710	4.5	37.6 abcde	31.7 abcde	79.3 a	1.2 cde	5.0 cd	4.8 e	15.7 d	3.8 abc
FX 3864	4.5	51.6 ab	37.3 abc	72.2 ab	1.1 de	2.1 d	13.2 a	25.7 bcd	2.9 a
IAN 873	4.5	26.1 de	18.4 cdef	64.1 ab	1.3 bcde	6.4 bc	6.4 de	29.5 bcd	6.8 cd
Mean		37.7	27.7	70.2 A	1.3 B	4.2 B	8.8	25.6 B	4.3 A

TABLE 3. CLONAL ANALYSIS OF THE THREE MAIN PHENOLIC GROUPS : FLAVANS, HYDROXYCINNAMIC ACIDS (HCD) AND FLAVONOLS, AND THE OVERALL PHENOL CONTENT (CONTD)

Clone	% abscission	Total (μmol phenol eq/ g FW)	Flavans (μmol (+) catechin eq/ g FW)	Flavans (% sum of phenols)	HCD (μmol p. coumaric acid eq/g FW)	HCD (% sum)	Flavonols (mmol quercitrin eq./g FW)	Flavonols (% sum of phenols)	HCD/ Flavans × 10^{-2}
RRIC 101	11.6	20.9 e	9.7 f	41.2 c	1.8 a	10.3 a	9.3 bcd	48.4 a	17.7 d
PB 235	20.4	47.8 abcd	34.0 abcd	64.0 ab	1.7 ab	4.6 cd	12.1 ab	31.4 bc	5.3 bcd
IRCA 19	30.8	25.2 e	16.5 def	58.2 b	2.0 a	9.1 ab	6.8 de	32.7 bc	12.3 d
PB 260	40	57.2 a	43.9 a	73.4 ab	1.9 a	3.8 cd	11.5 ab	22.8 bcd	4.3 abc
IAN 713	45	27.8 cde	19.3 bcdef	67.6 ab	1.6 abcd	6.2 bc	7.0 cde	26.2 bcd	7.3
GT 1	45	20.5 e	13.2 ef	59.9 b	1.6 abc	9.4 ab	5.8 de	30.6 bc	12.7 d
Mean		32.4	22.0	60.3 B	1.8 A	7.4 A	8.6	32.3 A	7.9 B
Global mean		35.6	24.8	65.6	1.5	5.7	8.7	28.6	6.1
p. clone p. sens		0.0012 0.1943	0.0026 0.1286	0.0002 0.0023	0.0001 0.0001	0.0001 0.0001	0.0001 0.8254	0.0002 0.0134	0.0001 0.0001

The overall phenol content was calculated by totalling the values in micromole equivalent for the three groups. p clones, p sensitivity: probability of null hypothesis between clones and between sensitivity classes. a, b, c, *etc.*; the figure followed by the same letters refer to clones which are not statistically different (Duncan test).

TABLE 4. MEAN LEVELS OF THE MAIN FLAVANS (μMOL EQUIVALENT (+)-CATECHIN/g FW)

Clone	% abscission	A3	A4	A5	A6	A7	A8	A9	A10	A11	A22
PA 31	0.001	0.56 ab	2.41 ab	0.97 ab	1.04 cd	3.54 bc	3.98 b	2.27 bc	2.66 bcd	4.09 cdef	2.78 ab
IAN 717	0.001	0.71 ab	1.00 c	0.74 ab	2.80 abc	4.79 bc	3.83 b	2.51 bc	2.40 bcd	4.60 cdef	2.76 ab
CD 1078	0.8	0.81 ab	1.59 bc	2.22 a	3.50 a	9.57 a	9.19 a	3.24 b	3.10 bcd	1.96 ef	4.40 a
RO 38	1.5	0.31 ab	0.97 c	0.99 ab	1.93 abcd	3.68 bc	3.40 b	1.40 bc	2.15 bcd	2.88 def	3.00 ab
IAN 2878	1.6	0.14 b	0.71 c	1.87 ab	2.13 abcd	4.34 bc	3.95 b	2.71 bc	2.24 bcd	5.24 bcde	3.16 ab
IAN 710	4.5	0.13 b	1.17 bc	1.13 ab	2.01 abcd	5.60 abc	4.64 b	1.89 bc	6.25 ab	5.82 bcd	2.62 ab
FX 3864	4.5	0.56 ab	1.44 bc	1.94 ab	2.74 abc	6.91 ab	4.80 b	2.36 bc	4.75 abc	7.83 ab	3.93 ab
IAN 873	4.5	0 b	0.96 c	0.64 ab	1.40 bcd	4.31 bc	3.54 b	0.52 bc	2.45 bcd	3.43 def	1.19 ab
Mean		0.40	1.28	1.27 A	2.16	5.29 A	4.56 A	2.06	3.25	4.48	2.95 A

TABLE 4. MEAN LEVELS OF THE MAIN FLAVANS (μMOL EQUIVALENT (+)-CATECHIN/g FW) (CONTD)

Clone	% abscission	A3	A4	A5	A6	A7	A8	A9	A10	A11	A22
RRIC 101	11.6	0.22 b	0.49 c	0.24 b	0.68 d	2.63 c	3.30 b	0.35 c	0 d	1.04 f	0.79 b
PB 235	20.4	0.53 ab	1.61 bc	1.34 ab	3.04 ab	6.77 ab	4.07 b	1.28 bc	5.56 ab	7.16 abc	2.58 ab
IRCA 19	30.8	0.32 ab	0.48 c	0.40 b	1.71 abcd	3.08 bc	2.08 b	0.97 bc	0.96 cd	3.01 def	3.45 ab
PB 260	40	1.45 a	2.90 a	0.83 ab	3.26 a	3.99 bc	3.20 b	8.13 a	7.42 a	10.3 a	2.35 ab
IAN 713	45	0.38 ab	1.05 c	0.87 ab	2.18 abcd	2.64 c	1.33 b	0.97 bc	2.92 bcd	6.34 bcd	0.61 b
GT 1	45	0.07 b	0.79 c	0.47 ab	0.94 d	3.21 bc	2.37 b	0.37 c	0 d	3.25 def	1.71 ab
Mean		0.48	1.18	0.67 B	1.91	3.58 B	2.67 B	1.96	2.66	5.05	1.83 B
Global mean		0.44	1.23	0.97	2.04	4.43	3.61	2.10	2.96	4.77	2.39
p.clones p.sens		0.3087 0.6522	0.0026 0.7060	0.2914 0.0359	0.0041 0.4473	0.0063 0.0167	0.0043 0.0026	0.0001 0.8635	0.0013 0.4573	0.0001 0.4590	0.2865 0.0463

The overall phenol content was calculated by totalling the values in micromole equivalent for the three groups. p clones, p sensitivity: probability of null hypothesis between clones and between sensitivity classes. a, b, c, *etc.*; the figure followed by the same letters refer to clones which are not statistically different (Duncan test).

TABLE 5. MEAN LEVELS OF THE MAIN FLAVONOLS (μMOL EQUIVALENT QUERCITRIN/g FW)

Clone	% abscission	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C19/ C21	C14/ C21
PA 31	0.001	0.15 b	0 b	1.21 bc	3.36 cdc	0.33 bc	3.13 abc	0.31 a	0.016 bc	1.32 ab	0.05 ab	1.22 a	1.085	2.762
IAN 717	0.01	0.05 cdc	0 b	0.68 d	4.61 bc	0.10 de	2.50 abcde	0.08 defg	0.005 cd	0.83 cde	0.03 ecd	0.38 cde	2.197	12.247
CD 1078	0.8	0.44 a	0.16 a	0.72 d	1.93 f	0.41 ab	2.67 abcde	0.23 b	0 d	1.03 bc	0.03 bcd	0.97 ab	1.061	1.990
RO 38	1.5	0.02 de	0 b	0.63 d	5.32 b	0.10 de	2.89 abcd	0.10 cdef	0.013 bc	1.00 bc	0.03 bc	0.49 cde	2.032	10.813
IAN 2878	1.6	0 e	0 b	0.50 dc	2.70 def	0.08 de	1.39 ef	0.11 cde	0.017 bc	0.57 de	0.02 cd	0.20 e	2.841	13.283
IAN 710	4.5	0 e	0 b	0.30 ef	2.63 ef	0.03 e	1.08 f	0.05 efg	0.013 bc	0.54 e	0.02 cd	0.18 e	2.994	14.665
FX 3864	4.5	0.07 cd	0 b	0 f	7.71 a	0.04 e	3.38 ab	0.03 fg	0.005 cd	1.41 a	0.01 cd	0.55 bcde	2.547	13.924
IAN 873	4.5	0 e	0 f	0 f	3.18 def	0.01 e	1.39 ef	0.02 g	0 d	1.29 ab	0.01 cd	0.45 cde	3.180	7.040
Mean		0.10		0.50 B	4.03 A	0.13 B	2.33	0.11 B	0.009 B	1.01	0.03	0.55	2.242	9.603

TABLE 5. MEAN LEVELS OF THE MAIN FLAVONOLS (μMOL EQUIVALENT QUERCITRIN/g FW) (CONTD)

Clone	% abscission	C11	C12	C13	C14	C15	C16	C17	C18	C19	C20	C21	C19/ C21	C14/ C21
RRIC 101	11.6	0.01 de	0 b	1.07 c	3.41 cde	0.27 bc	2.10 bcdef	0.25 ab	0.02 b	1.41 a	0.04 bc	0.76 bcd	1.858	4.513
PB 235	20.4	0.09 c	0 b	1.54 a	4.08 bcd	0.51 a	3.51 a	0.30 ab	0.01 bed	1.28 ab	0.02 cd	0.78 bc	1.643	5.221
IRCA 19	30.8	0.03 de	0 b	0.71 d	2.49	0.22 cd	1.95 cdef	0.16 c	0.01 ecd	0.76 cde	0.02 cd	0.41	1.837	6.048
PB 260	40	0.09 c	0 b	1.47 ab	3.54 cde	0.52 a	3.82 a	0.23 b	0.04 a	0.92 cd	0.07 a	0.76 bcd	1.208	4.655
IAN 713	45	0.06 cde	0 b	0.44 de	3.31 cdef	0.12 de	1.75 def	0.07 defg	0.01 cd	0.81 cde	0.01 d	0.44 cde	1.851	7.555
GT 1	45	0 e	0 b	0.59 de	2.46 ef	0.11 de	1.37 ef	0.13 cd	0.01 bcd	0.76 cde	0.03 bcd	0.31 de	2.463	7.951
Mean		0.05		0.94 A	3.21 B	0.28 A	2.36	0.19 A	0.02 A	1.00	0.03	0.57	1.810	5.990
Global mean		0.08		0.70	3.61	0.21	2.35	0.15	0.01	1.00	0.03	0.56		
p.clones p.sens		0.0001 0.1060	0.0001 0.0465	0.0001 0.0001	0.0001 0.0282	0.0001 0.0005	0.0001 0.9027	0.0001 0.0017	0.0001 0.0152	0.0001 0.8253	0.0001 0.4052	0.0001 0.8374		

The overall phenol content was calculated by totalling the values in micromole equivalent for the three groups. p clones, p sensitivity: probability of null hypothesis between clones and between sensitivity classes. a, b, c, *etc.*; the figure followed by the same letters refer to clones which are not statistically different (Duncan test).

TABLE 6. MEAN LEVELS OF THE MAIN HYDROXYCINNAMIC DERIVATIVES (μMOL EQUIVALENT P–COUMARIC ACID/g FW)

Clone	% abscission	B3	B4	B5	B6	B7	B8	B9	B10
PA 31	0.001	0.08 dc	0.08 a	0.06 ab	0.07 abcd	0.23 a	0.22 a	0.17 abcd	0.89 abc
IAN 717	0.001	0.05 e	0.04 de	0.04 d	0.05 d	0.06 ef	0.10 def	0.11 d	0.43
CD 1078	0.8	0.05 e	0.02 e	0.03 d	0.06 bcd	0.05 ef	0.07 cd	0.17 abcd	1.05 ab
RO 38	1.5	0.08 de	0.04 de	0.04 bcd	0.05 cd	0.08 def	0.09 fe	0.11 d	0.46 e
IAN 2878	1.6	0.13 ab	0.05 bcd	0.16 a	0.07 abc	0.08 cdef	0.10 def	0.17 abcd	0.97 ab
IAN 710	4.5	0.10 cd	0.07 ab	0.07 a	0.05 cd	0.09 bcde	0.12 cde	0.12 d	0.54 de
FX 3864	4.5	0.06 de	0.04 cde	0.04 cd	0.05 cd	0.04 f	0.12 cde	0.13 cd	0.60 cde
IAN 873	4.5	0.16 a	0.08 a	0.07 a	0.07 abc	0.10 bcde	0.13 cde	0.15 bcd	0.55 de
Mean		0.09 B	0.05 B	0.05 B	0.06 B	0.09	0.12	0.14 B	0.67 B

TABLE 6. MEAN LEVELS OF THE MAIN HYDROXYCINNAMIC DERIVATIVES (μMOL EQUIVALENT P–COUMARIC ACID/g FW) (CONTD)

Clone	% abscission	B3	B4	B5	B6	B7	B8	B9	B10
RRIC 101	11.6	0.12 bc	0.06 abc	0.07 a	0.08 a	0.13 bc	0.09 fe	0.20 abcd	1.09 ab
PB 235	20.4	0.16 a	0.07 ab	0.07 a	0.08 a	0.09 cde	0.19 ab	0.25 a	0.82 abcd
IRCA 19	30.8	0.15 ab	0.05 bcd	0.06 abc	0.09 a	0.12 bcd	0.16 bc	0.22 abc	1.13 a
PB 260	40	0.14 ab	0.07 ab	0.06 ab	0.08 ab	0.13 b	0.16 bc	0.23 ab	1.05 ab
IAN 713	45	0.12 bc	0.07 ab	0.06 a	0.07 abc	0.09 cde	0.14 cd	0.19 abcd	0.81 bcd
GT 1	45	0.16 a	0.08 a	0.06 ab	0.07 abc	0.09 bcde	0.13 cde	0.21 abc	0.81 bcd
Mean		0.14 A	0.07 A	0.06 A	0.08 A	0.11	0.14	0.21 A	0.95 A
Global mean		0.11	0.06	0.06	0.07	0.10	0.13	0.18	0.81
p.clone p.sens		0.0001 0.0001	0.0001 0.0047	0.0001 0.0010	0.0002 0.0001	0.0001 0.2413	0.0001 0.0509	0.0039 0.0001	0.0001 0.0001

The overall phenol content was calculated by totalling the values in micromole equivalent for the three groups. p clones, p sensitivity: probability of null hypothesis between clones and between sensitivity classes. a, b, c, *etc.*; the figure followed by the same letters refer to clones which are not statistically different (Duncan test).

(kaempferol-3-rutinoside) and *C21* (kaempferol-galactoside). The ratio of these compounds was 25% higher on the average in the resistant clones except for PA 31 and CD 1078; these were *H. pauciflora* and an interspecific *H. pauciflora* × *H. brasiliensis* hybrid *(Table 5)*. GT 1 was the only exception among the sensitive clones. Similar results were found for the rutin/kaempferol-galactoside ratio *(C14/C12)*, which was higher in the resistant clones *(Table 5)*, and for the rutin/total flavonol ratio (45.3% instead of 38.1%), again with the exception of clones CD 1078 and PA 31.

Statistical Interpretation of the Results

The phenol contents of the fourteen clones studied, with five to seven replications per clone, were processed using different statistical methods.

Principal component analysis (PCA). PCA was applied to the phenols. Supplementary variables were introduced: total flavans *(A)*, HCD *(B)*, flavonols *(C)*, the sum of $A1+A2$ *(Y)*, the sum of total phenols $Y+A+B+C(T)$, HCD/flavonols ratio *(BC)*, flavans/flavonols ratio and the abscission percentage *(X)*.

As a whole, the first three axes accounted for 58.2% of total variability: 28.3% by *Axis 1*, 18.8% by *Axis 2* and 11% by *Axis 3*. In simple terms, *Axis 1* was characterised by total phenol content and *Axis 2* was more related to the flavans *(Figure 3)*.

Unfortunately, variable *X* (percentage abscission) was only very weakly determined by *Axis 2* ($\cos^2 = 0.1720$, *i.e.* 65.5°C). This PCA does not give an overall picture of the relation between percentage abscission and the abundance of phenols.

Comparison of clones. The abundance of each phenol was investigated by analysis of variance (GLM procedure) according to the nature of the clone and hence without pre-conceptions concerning susceptibility to *M. ulei*. *Tables 4, 5* and *6* show significant clonal differences in the various phenolics analysed. For twenty compounds, the differences were significant at $P = 0.0001 - 0.0002$.

In addition, comparison of the classification of the clones for each of the phenols given by the Duncan test with their classification of susceptibility to *M. ulei* shows that differences between susceptible and resistant clones are due, for a given phenol, to a few clones. For example, amounts of *C14* are significantly higher in CD 1078 and FX 3864 than in the other twelve clones. Likewise, significantly low levels of *B4* are noticed only in IAN 717, RO 38, CD 1078 and FX 3864. Conversely, the resistant clone like IAN 2878 displays no differences in phenolic composition with the susceptible clones *(Table 7)*.

Comparison of susceptible and resistant clones. The data above reveal interclonal differences and suggest certain links between phenolics and sensitivity or resistance to *M. ulei*. A closer approach to the preliminary information was carried out by complementary statistical analysis of the sensitive and resistant clones according to whether the abscission percentage was lower (resistant) or higher (susceptible) than 10%.

Variance analysis *(Table 8)* highlights compounds *B3, B6, B9, B10* and *C13* and to a lesser extent *B4, B5, C15* and *C17* in the most sensitive clones. In contrast, phenolics *A5, A7, A8, A22* and *C14* were more abundant in the more resistant clones.

It was also sought to correlate the abscission percentage — as a sensitivity criterion — with the amounts of flavans, HCD and flavonols, the respective percentages of the various groups in relation to total phenols and to the ratios of the different groups to each other. This analysis gave the following information *(Table 9)*:

- *A11, B3, B4, B5, B6, B8, B9* and *B10* are correlated to abcission.
- *A7, A8, C14* and *C19* are negatively correlated with it.

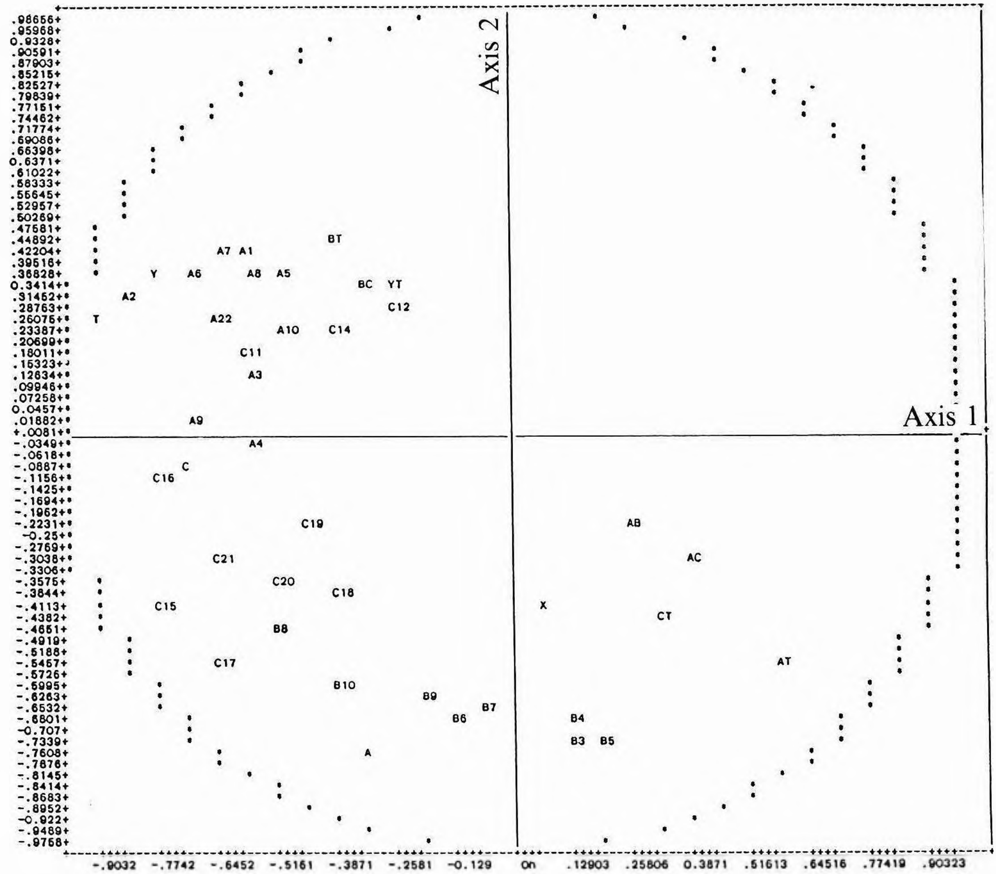

Figure 3. Correlation circle given by principal component analysis. Variable codes: A3 to A10 = flavans; B3 to B10 = hydroxycinnamic acid derivatives; C11 to C21 = flavonols; T = sum of phenols; A = sum of flavans; B = sum of HCD; C = sum of flavonols; Y = sum of A1 + A2; AB = ratio of flavans/HCD; AC = ratio of flavans/flavonols; BC = ratio of HCD/flavonols; YT = ratio of A1 + A2/total phenols; AT = flavans/total phenols; BT = HCD/total phenols; CT = flavonols/total phenols; X = % abscission. Superposed points: A2 (B), A3 (A11), C17 (C13).

- The HCD percentage was correlated positively to the abscission percentage with extremely high probability; a high level of HCD would indicate a susceptible clone. The HCD/total phenols ratio should also be noted; this varies like the HCD level. Only the HCD/total flavonols ratio was correlated negatively.

Successive multiple regression. This method showed that a linear correlation of several peaks significantly characterised the degree of susceptiblity of the clones *(Table 10)*. Starting with the HCD *B3*, which had already been noted for its abundance in susceptible clones, the following, in descending order of importance, were phenols *C19* and *A8*, which were negatively correlated

TABLE 7. ANALYSIS OF VARIANCE OF PHENOLIC COMPOUNDS

Clone	% abscission	A 5	A 7	A 8	A 22	B 3	B 4	B 5	B 6	B9	B 10	C 13	C 14	C 15	C 17	C 18
PA 31	0.001					+									+	
IAN 717	0.001					+	+	+	+	+						+
CD 1078	0.8	+	+	+	+	+	+	+					+			+
RO 38	1.5					+	+		+	+	+					
IAN 2878	1.6															
IAN 710	4.5					+				+	+	+		+	+	
FX 3864	4.5					+	+	+				+	+	+	+	+
IAN 873	4.5										+	+		+	+	+
RRIC 101	11.6	+	+		+			+							+	
PB 235	20.4					+		+	+			+		+	+	
IRCA 19	30.8	+						+		+				+		
PB 260	40							+	+			+				+
IAN 713	45		+		+											
GT 1	45					+										

Clones which have the higher contribution in the differences between sensitive and resistant clones.

TABLE 8. COMPARISON OF THE PHENOLIC LEVELS IN SENSITIVE AND RESISTANT CLONES

Phenolic level (m mol/g FW)	Sensitive clones	Resistant clones	Probability p
A5	0.67	1.27	0.036
A7	3.58	5.29	0.017
A8	2.67	4.56	0.0026
A22	1.83	2.95	0.046
B3	0.14	0.088	0.0001
B4	0.067	0.053	0.005
B5	0.062	0.049	0.001
B6	0.078	0.058	0.0001
B9	0.212	0.14	0.0001
B10	0.954	0.67	0.0001
C12	0	0.017	0.047
C13	0.94	0.50	0.0001
C14	3.20	4.03	0.028
C15	0.28	0.13	0.0005
C17	0.19	0.11	0.0017
C18	0.015	0.008	0.015

TABLE 9. CORRELATIONS BETWEEN THE PHENOLIC CONTENT AND PERCENTAGE FOLIAR ABSCISSION

Phenol	r	ρ
A5	−0.178	0.105
A7	−0.248	0.023
A8	−0.352	0.001
A11	0.225	0.040
A22	−0.195	0.076
B3	0.525	0.0001
B4	0.338	0.002
B5	0.252	0.021
B6	0.392	0.0002
B8	0.236	0.031
B9	0.409	0.0001
B10	0.300	0.006
C11	−0.170	0.122
C12	−0.208	0.057
C13	0.192	0.080
C14	−0.237	0.030
C15	0.191	0.080
C18	0.167	0.128
C19	−0.220	0.045
DHC	0.382	0.0003
DHC/Sum	0.292	0.007
DHC/Flavonol	−0.382	0.022

with abscission and hence positively with resistance, and then phenols *A11* and *B9*, which were positively correlated with abscission and hence with susceptibility. *C19* alone has only a slight influence on susceptibility *(Table 9)*, but in combination with other phenolics, the analysis shows its significance.

DISCUSSION AND CONCLUSIONS

As a preliminary approximation, this investigation did not establish simple, clearly-defined relationships between the constituent phenolic compounds in young healthy leaves of clones cultivated under plastic and the resistance of these clones to *M. ulei* estimated by the foliar abscission percentage induced by the parasite in the same adult clones in a plantation. However, a number of interesting indications emerged concerning some phenolics and some ratios of levels of the different groups of phenols.

The overall phenol content and the flavan content appear to be related to resistance, except in clones PB 235 and PB 260 which, although classified as susceptible, contain high levels of these compounds. High HCD and flavonol levels — except in the same two clones — appear to be related to susceptibility. Nevertheless, some of the flavonol ratios *(C12/C21, C14/C21)* and the rutin content appear to be related to resistance.

Overall, the various statistical analyses performed confirm these preliminary observations. Analysis by successive multiple regression characterised the phenolics positively correlated with susceptibility (compounds *B3*, *A11* and *B9*, HCD/flavan ratio, HCD level). In contrast, a number of compounds appear to be more related to resistance (kaempferol-3, rutinoside, *A8*, rutin).

The different statistical analyses result in data displaying coherence for the results as a whole *(Table 11)*. Indeed, the same phenolics are often the most abundant in susceptible clones while others are more abundant in resistant clones.

The investigation shows that there are no marked differences in the nature of the constituent phenolic compounds in the various clones or species of *Hevea*. Only quantitative differences were shown between the susceptible and resistant clones. However, certain difficulties in interpretation may result from the action of factors which increase intraclonal variability:

- sampling distributed irregularly in time

TABLE 10. ANALYSIS USING STEP-WISE MULTIPLE LINEAR REGRESSION

Step	Variable entered	Variable removed	R^2 Partial	Model	C(P)	F	Prob > F
1	B3		0.2760	0.2760	21.1096	31.2595	0.0001
2	C19		0.0494	0.3253	16.2138	5.9287	0.0171
3	C15		0.0588	0.3842	10.0049	7.6357	0.0071
4	A8		0.0533	0.4374	4.5627	7.4837	0.0077
5	A11		0.0401	0.4775	0.9686	5.9798	0.0167
6	B9		0.0233	0.5008	−0.2803	3.5881	0.0620
7	B5	C15	0.0226	0.5234	−1.4429	3.6113	0.0612
8			0.0115	0.5119	−1.8380	1.8327	0.1798
9	A3		0.0136	0.5255	−1.7390	2.1805	0.1439

Regression curve equation:

% abscission = 8.0 + 0.0027 A3 − 0.0020 A8 + 0.0011 A11 + 0.17 B3 − 0.24 B5 + 0.076 B9 − 0.010 C19

C(P) is the Mallow's statistic.

TABLE 11. COMPARISON OF THE PHENOLIC COMPOSITION OF SENSITIVE AND RESISTANT CLONES USING DIFFERENT METHODS OF STATISTICAL ANALYSIS

Table	Type of analysis	Characteristics of clones Sensitive	Resistant
3	Variance analysis (Classes of sensitivity	% Flavans HCD/Flavans	HCD HCD/Sum of phenols % Flavonol
4, 8	Variance analysis (Classes of sensitivity)		A5, A7, A8 A22
5, 8	Variance analysis (Classes of sensitivity)	B3, B4, B5, B6, B9, B10	
6, 8	Variance analysis (Classes of sensitivity)	C13, C15, C17, C18	C14
9	Linear regression	A11, B3, B4, B5, B6, B8, B9, B10 HCD HCD/Sum HCD/Flavonol	A7, A8, C14, C19
10	Step-wise regression	A3, A11, B3, B9, C15	A8, B5, C19

- physiological changes, especially in the phenolic metabolism of the plants under plastic; in this case, the material analysed may not fully express the potential of the trees in the plantation used to establish the clonal susceptible scores.

Should the importance of (+)-catechin *(A7)* and other flavans and rutin *(C14)* in relation to the flavans as a whole be confirmed, the results would agree overall with those reported in the literature. The fungi-toxicity of extracts of young leaves has been attributed to a yellow compound —

probably a flavonol — which appears to be more abundant in IAN 710 (relatively resistant here and in Guiana) than in GA 1126 (susceptible)[14]. The greatest abundance of aglycone : quercetin was reported in resistant clones[16] (with a few exceptions). This would appear to support our remark on the percentage of rutin in relation to total flavonols, assuming that the compound referred to by Figari[14] and Hashim *et al.*[16] is rutin and not kaempferol-rhamnodiglucoside, as was reported by Martins *et al.*[15], who worked on old leaves, unlike Figari[14] and ourselves.

With regard to the possible importance of (+)-catechin *(A7)* and certain other flavans *(A8, A22)*, these results might be similar to data showing that sorghum seeds resistant to grain mould complex have a higher flavan content than sensitive seeds[24]. It has been suggested that the catechin present in onion bulb coats before infection contributes to resistance to *Colletotrichum circinans* by inhibiting spore germination[25]. It has also been shown that catechin has an inhibitory effect on the germination of *M. ulei* conidia[14].

In spite of the positive aspects reported above, our results nevertheless seem to show that most constituent phenolics do not play a major role in the resistance of young *Hevea* leaves to *M. ulei*. However, they may be involved in the setting up of induced resistance mechanisms. Hashim[26] showed that it is possible to induce resistance to SALB using incompatible *M. ulei* races. He observed that the resistance remains localised in the induced zone and may therefore be caused by host cell collapse or the production of phytoalexins. The first mechanism was observed by Blazquez and Owen[8] and Hashim *et al.*[9] and the second by Gieseman *et al.*[21] for scopoletin. Scopoletin is considered a phytoalexin in *Hevea* and, logically, was not detected in our uninfected material.

As the criterion of susceptibility to *M. ulei* used here was abscission percentage, there may be links between constituent phenols and abscission which depend only indirectly on the action of pathogens in general and *M. ulei* in particular. As has been pointed out, some phenols activating auxin-oxidase[19] may thus enhance leaf abscission in leaves where pathogen attack has favourably changed the auxin-ethylene ratio.

Finally, it can be considered that even if the constituent phenolics in young *Hevea* leaves (with the possible exception of *C14, A8* and *A22*) do not participate directly in defence mechanisms against *M. ulei,* some may nevertheless be retained as sensitivity markers. Elsewhere, the total phenolics present in the leaves may contribute to passive resistance even if there is a lack of correlation in their content. Phenolics may be important especially in hypersensitive reactions.

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Total and Available Molybdenum in Some Soils under Rubber and Their Effect on Hevea

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Total and available molybdenum in nine major soil series in Peninsular Malaysia were determined. Total Mo in the soils varied from 1.13 mg/kg to 10.53 mg/kg while available Mo ranged from 155 µg/kg to 721 µg/kg soil. Among the soils, the marine clays of the Selangor series soil and the basaltic Kuantan series soil have the highest total Mo content. Within the soil profiles, the A_p *horizons appeared to have higher total and available Mo. Application of different levels of sodium molybdate to rubber seedlings grown in the glasshouse failed to induce any visual Mo toxicity symptom in the plants although dry matter yield declined at levels beyond 0.120 kg sodium molybdate/ha. Liming at rates of 2000 kg and 2800 kg ground magnesium limestone/ha enhanced the availability of Mo in the sandy Holyrood series soil. However, this effect could be marred by the rapid leaching loss of the nutrient.*

In soils, molybdenum occurs in four possible main forms: i) water-soluble; ii) in association with organic matter; iii) exchangeable anion MoO_4^{2-}, adsorbed on colloidal complex; and iv) insoluble, held in the crystal lattices of minerals[1]. The status of Mo as a plant nutrient has been well established and documented by many workers[2,3,4]. In soils with low pH, the Mo is immobilised and becomes less available to plants. Barshad[1] showed that soils with higher pH values released more adsorbed Mo into solution during plant growth than soils with lower pH values. Field experiments on Ultisols carried out by Burmester *et al.*[5] suggested that Mo deficiency in soybean was a more common factor of acid soil infertility than aluminium toxicity and liming appeared to alleviate these adverse influence on crop yield.

In Malaysia, the occurrence of Mo deficiency has not been conclusively established although growth responses by leguminous plants to added Mo were first reported by Sandison and Hanly[6]. In sand culture studies in which rubber seedlings were deprived of Mo or given a very low level of Mo supply, Bolle-Jones[7] observed that the seedlings were shorter, of smaller girth and dry weight, and developed a marginal or tip scorch in the mid-stem and upper laminae. Increased Mo supply reduced the concentration of No_3-N within the laminae as well as the concentrations of S, Mg, and K but increased that of Mn. Under Malaysian field conditions, Bolle-Jones[7] noted that Mo deficiency in rubber may occur but this does not appear to be accompanied by well defined foliar symptoms.

While there was evidence that the concentration of Mo in rubber is related to the Mo content in the soils, the Mo status of the soils was not extensively studied. Abdul Aziz[8] found that soils developed on recent alluvium in Peninsular Malaysia were adequate in total trace element content with Mo content ranging from 2 µg to 30 µg/g soil. However, the mobile or extractable components were low. Additionally, the trace elements were closely associated with the finer fractions of the soils.

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This paper attempts to determine the Mo status of some major soils where rubber is grown. The performance of rubber seedlings grown in one of the soils and receiving Mo or nil Mo in the glasshouse was assessed and discussed. In association with this study, the effect of liming on the availability and uptake of Mo by soyabean is also reported.

EXPERIMENTAL

For each soil type, samples were collected from a soil profile pit dug in the inter-row area of rubber as this is expected to be least disturbed by fertiliser inputs. The soil samples taken at 0–45 cm depth where maximum root concentration occurs[9] and at different horizons in the soil profile were air-dried at 305°K, ground to pass through a sieve (< 2 mm size) and sub-sampled for analysis[10]. In this study, nine different soils were sampled and their relevant properties are given in *Table 1*.

Determination of Total and Available Molybdenum

Total Mo was determined by the procedure reported by Grigg[11]. 5 g of ground soil (< 60 mesh) in a platinium crucible was ashed at 723°K and the ashed sample treated with hydrofluoric acid to remove silica. The residue obtained was dissolved in concentrated HCl and the solution filtered and evaporated to dryness. A further 5 ml of HCl and 1 ml of 72% $HClO_4$ was added and the mixture evaporated to dryness. This process was repeated twice before the final residue was re-dissolved in about 5 ml hot concentrated HCl and transferred to a graduated glass-stoppered test tube to which 5 ml of 5% NaF solution, 1.5 ml of 20% KCNS and 3 ml of 40% $SnCl_2$ were added. The content in the tube made up to the 32 ml mark with water followed by the addition of 10 ml isoamyl alcohol was shaken and then immersed in a hot water-bath at 353°K for 20 min. The aqueous phase was removed with a suction pipette while the amyl alcohol layer in the tube was mixed with a 25 ml portion of $SnCl_2$ solution and placed in the water-bath until all the iron colour had faded. The aqueous phase was again removed and the isoamyl alcohol layer shaken with a second portion of the $SnCl_2$ solution. After completing the washing, the aqueous layer was completely removed and the amyl alcohol solution filtered through a dry Whatman No. 1 filter paper into a dry tube. Total Mo in the soil was determined from the absorption of the alcohol solution in a 50 mm micro-cuvette with a previously calibrated spectrophotometer at a wavelength of 465 mμ.

For available Mo, 25 g of the air-dried soil was shaken with 250 ml of acid ammonium oxalate buffer containing 498 g of $(NH_4)_2C_2O_4$ and 252.1 g of $H_2C_2O_4$ per 20 litres at room temperature overnight. An aliquot of the extract which was separated by centrifugation was evaporated to dryness, ignited for 3 – 4 h at 723°K to remove oxalate and organic matter and then treated with concentrated HCl as described in the procedure for total Mo. The available Mo in the amyl alcohol solution was determined as before.

Effect of Molybdenum on Performance of Rubber Seedlings

In each 45 kg pot filled with Rengam series soil (Typic Kandiudults) that had previously been passed through a 2 mm sieve, five *Hevea* plants were grown. Molybdenum as sodium molybdate ($Na_2MoO_4.2H_2O$) was applied to the soil in a single dressing when the seedlings were two months old starting from germination and at rates of 0, 0.012, 0.12, 1.2 and 12 kg/ha. The amount of Mo was calculated on the basis of 2×10^6 kg soil/ha. To each pot, basal applications of 10 g $(NH_4)_2SO_4$, 57 g Christmas Island rock phosphate, 6 g KCl and 7 g $MgSO_4$ were made at monthly intervals. Each treatment was done in triplicate.

When the plants were four months old, micro-tapping of the seedlings commenced. To each plant, fifteen pin punctures were made on the bark at a standard height of

TABLE 1. SOME PHYSICAL AND CHEMICAL PROPERTIES OF THE SOIL (0 – 45 cm)

Soil series	Sampling area	Sub-group[a] (parent material)	Texture[b]	pH	Organic carbon (g/kg soil)	Cation-exchange capacity (cmol/kg soil)
Durian	Ayer Hitam	Plinthaquic Paleudults (siliceous shale)	sicl-cl	4.5	14.5	5.22
Holyrood	RRIM Experiment Station, Sg. Buloh	Typic Quartzisamments (sub-recent alluvium)	sl	4.6	11.8	3.42
Kuantan	Kuantan	Typic Hapludux (basalt)	sil-cl	4.6	25.3	3.28
Munchong	RRIM Experiment Station, Sg. Buloh	Typic Hapludux (schist)	cl	4.4	18.3	5.64
Prang	Seremban	Typic Hapludux (schist)	cl	4.4	13.6	4.87
Rasau	Segamat	Typic Kandiudults (sub-recent alluvium)	sl-scl	4.7	12.4	2.90
Rengam	RRIM Experiment Station, Sg. Buloh	Typic Kandiudults (granite)	coscl	4.4	15.8	4.53
Selangor	Kapar	Typic Tropaquepts (marine clay)	cl-sicl	3.9	32.7	18.32
Serdang	RRIM Experiment Station, Sg. Buloh	Typic Kandiudults (sandstone)	scl-sl	4.3	12.5	3.96

[a] According to *Keys to Soil Taxonomy* by Soil Survey Staff — USDA (1989)

[b] c = clay; si = silty; s = sandy; l = loam; co = coarse.

10 cm above soil level and the latex flowing out was absorbed by a filter paper strip (1.5 × 6.0 cm) attached to the stem. After allowing 3 h of latex flow, the wet filter paper strips were oven-dried and weighed. The increase in weight of the filter paper was taken as the yield expressed as milligramme latex per tapping per plant. A total of seven tappings spread over a period of three weeks was completed. When the seedlings were twelve months old, the girth and height of each plant were measured. The whole plant was then harvested for dry matter determination. The leaf and stem samples were separated and analysed for Mo content.

Effect of Liming on Molybdenum in the Soil

Soyabean *(Glycine max)* of the Palmetto variety and inoculated with *Bradyrhizobium japonicum* were planted on Holyrood series soil (Orthoxic Quartzipsamments) in Pilmoor Estate, Selangor. Molybdenum at rates of 0, 35 and 70 g Mo/ha and ground magnesium limestone (GML) at levels of 0, 1000, 2000 and 2800 kg/ha were applied to 2.0 × 2.0 m plots separated by a distance of 0.4 m. A randomised block design with three replications was used. Upon planting, a basal dressing of NPK fertiliser containing N, P_20_5 and K_20 at 40, 60 and 30 kg/ha, respectively, was uniformly applied to all the plots. About two months after planting, a further top dressing of N (20 kg/ha) was made. At ninety days, the plants were uprooted, seeds separated, dried and analysed for Mo. In addition, soil samples taken at different depths were analysed for available and total Mo.

Analysis of Soil and Plant Samples

Soil samples for routine determination of pH, organic carbon and cation-exchange capacity were dried at 328°K, ground and sieved (< 2 mm size). Soil pH was measured with a pH meter on a suspension of soil in distilled water, soil : water ratio being 2:5. Soil organic carbon was determined by Walkley and Black's titration method. The cation-exchange capacity of the soil was obtained from the amount of NH_4^+ ions adsorbed when the soil was leached with neutral normal ammonium acetate solution[10]. Molybdenum in plant samples were determined according to the procedure of Piper and Beckwith[12]. About 2 g of oven-dried ground sample was wet digested with HNO_3, H_2SO_4 and $HClO_4$ acids. The Mo in the digest extracted as molybdenum-dithiol complex in amyl-acetate was determined colorimetrically with a calibrated Spekker (Hilger) colorimeter using Filter No. 8 and a 4 cm cell.

RESULTS

The nine soils selected for this study originated from different parent materials and have pH values ranging from 4.7 to 3.9 *(Table 1)*. Among the soils, the Selangor series soil has the highest cation-exchange capacity attributed to the presence of significant amounts of organic matter and 2:1 expanding lattice minerals[13,14]. Elsewhere, the Holyrood, Kuantan, Munchong, Prang, Rasau, Rengam and Serdang series soils are dominated by kaolinite (> 50%) with small amounts of goethite and gibbsite. Apart from these properties, the soils also differed considerably in the proportion of sand, silt and clay. In one extreme, the Selangor, Durian and Kuantan series soils consist mainly of silt and clay while in the other, the Holyrood, Rasau and Serdang series soils were predominantly sand.

Available and Total Molybdenum in the Soils

Total Mo content of the nine soils varied from 1.13 mg to 10.53 mg/kg soil *(Table 2)*. The Mo content in the Selangor series soil was the highest and this was followed by Kuantan, Prang, Rengam, Rasau, Munchong, Serdang, Durian and Holyrood series soil. The range of total Mo content found in these soils was comparable with the values of soils from Hawaii (8.9 – 73.8 mg/kg), France (4.3 – 6.9 mg/kg), United States (0.8 – 3.3 mg/kg) and Australia (0.6 – 3.5 mg/kg) reviewed by Sauchelli[3]. The

TABLE 2. TOTAL AND AVAILABLE MOLYBDENUM IN SURFACE SOILS (0 – 45 cm)

Soil series	Total Mo (mg/kg soil)	Available Mo (μg/kg soil)	Available/ Total Mo (%)
Selangor	10.53A	721a	6.8
Kuantan	4.38B	183ed	4.2
Prang	3.84C	194ced	5.1
Rengam	3.67C	235cb	6.4
Rasau	3.07D	155e	5.0
Munchong	2.95D	228cbd	7.7
Serdang	2.69D	247b	9.2
Durian	1.72E	207cbd	12.0
Holyrood	1.13F	190ced	15.5
Mean	3.78	262	8.1
CV(%)	7.7	10.4	

Values in the same column followed by the same letter(s) are not significant at LSD (0.5).

high Mo content of the Selangor series soil is attributed to the marine deposits which are composed of kaolinite, smectite, mica, quartz and gibbsite. Examination of the soil under a scanning electron microscope showed that the clays of the Selangor series, besides the clay minerals, also consist of fragmented micro-structures which had been found to associate with biorelicts from shells of molluscs and echinoderms and plant remains[15]. The Kuantan series soil has moderately high Mo content as it is derived from basalt which was reported by Mitchell[16] to contain high levels of Mo. As for the sandy Holyrood series soil, prolonged leaching probably results in the depletion of its native Mo.

Unlike total Mo, available Mo in the soils varied within narrow limits, *viz.* 155 – 721 µg/kg soil. It is of interest to note that the level of available Mo is not closely related to the total Mo content in the soil. For all the soils, available Mo constitutes about 8.1% of the total Mo content. These results suggested that a major portion of the Mo in soil is either associated with the parent materials or occurs in forms which are not readily available. In the acid soils[17] with mean pH 4.5 and dominated by Fe and Al sesquioxides, fixation of Mo as MoO_4^{2-} through adsorption on the sesquioxides or clay minerals probably contributes to the low available Mo. This observation applies to soils like the Selangor and Kuantan series which have relatively high total Mo content but a low degree of availability.

Molybdenum Status of Some Profiles

The distribution of available and total Mo in four soil profiles are shown in *Table 3*. Values for total and available Mo in surface horizons (A_p) ranged from 3.90 mg/kg to 1.45 mg/kg and 267 μg/kg to 219 μg/kg soil, respectively, while the mean pH value was 4.5. Both total and available Mo did not appear to show any regular pattern with depth of the profiles although the surface horizons consistently had higher values. These data suggest that organic matter accumulated in the surface soils has a strong influence on the Mo status of the soils. The Mo could be associated with the soil organic matter. Additionally, the

TABLE 3. TOTAL AND AVAILABLE MOLYBDENUM IN FOUR PROFILE SOIL SAMPLES

Soil series	Horizon	Depth (cm)	pH	Mo	
				Total (mg/kg)	Available (μg/kg)
Durian	A_p	0–7	4.5	2.03	219
	B_{1t}	7–22	4.4	1.89	207
	B_{21t}	22–48	4.2	1.75	182
	B_{22cn}	48–51	4.3	1.67	175
	B_3	51–104	4.1	1.82	190
	C	104–137	4.4	1.58	184
Holyrood	A_p	0–18	4.6	1.45	245
	A_c	18–46	4.9	1.32	198
	C_1	46–89	4.9	1.27	180
	C_2	89–130	5.0	1.19	211
Munchong	A_p	0–5	4.5	3.20	267
	B_{21ox}	5–50	4.3	3.31	225
	B_{22ox}	50–137	4.4	2.98	203
Rengam	A_p	0–10	4.4	3.90	251
	B_{21t}	10–74	4.5	3.52	238
	B_{22t}	74–135	4.3	3.78	210

organic matter could interact with the Fe and Al hydrous oxides resulting in less adsorption of Mo[17]. Despite higher total Mo in the Munchong and Rengam series soils, available Mo in these two soils was moderately above those in the Durian and Holyrood series soils. With the exception of the Holyrood series soil, available Mo forms less than 10% of total Mo.

Effect of Molybdenum on Performance of Rubber Seedlings

The data for uptake, growth, yield and dry matter content of rubber seedlings treated with different levels of sodium molybdate in the glasshouse are presented in *Table 4*. At rates of 0.012 kg/ha and 0.120 kg/ha, there was a significant increase in girth over that of the control plants. However, beyond 0.120 kg/ha, there was a significant drop in girth. The decrease in girth at 1.20 kg/ha and 12.0 kg/ha implies that the plants suffered toxicity at these high rates of sodium molybdate application. Besides girth, mean height and total dry matter content also declined. In terms of percentage increase in girth and height determined from values at the beginning before molybdate was applied and at the end of the experiment, the control pots appeared to show better performance than the treated pots. This discrepancy shows that the effect of Mo based on growth of rubber seedlings alone could not be convincingly established. Generally, there is an increase in yield of latex in the molybdate treated pots although the yield values as shown in *Table 4* were not significantly related to the levels of molybdate applied *(Table 4)*.

The effects of molybdate application on Mo content in the rubber seedlings were examined *(Table 5)*. Evidently increasing

TABLE 4. EFFECT OF APPLICATION OF MOLYBDENUM ON GIRTH, HEIGHT, DRY MATTER CONTENT AND LATEX YIELD OF RUBBER SEEDLINGS GROWN IN THE GLASSHOUSE

Treatment[a] with sodium molybdate (kg/ha)	Mean girth[b] (cm)	Girth increment[c] (%)	Mean[d] height (cm)	Height increment[c] (%)	Total dry matter content[d] (g/plant)	Yield[b] (mg)
0	1.31	299.2	131.7	127.7	119.2	15.0
0.012	1.44	286.8	141.7	107.5	144.2	19.6
0.120	1.48	263.5	137.1	112.6	153.3	18.2
1.200	1.34	288.8	123.6	113.1	128.4	26.3
12.000	1.23	282.3	128.6	122.9	110.4	19.7
S.E. of difference between mean	±0.053	–	–	–	±7.87	±3.27
L.S.D. ($P < 0.05$)	0.113	–	–	–	21.8	6.4

[a] Source of Mo is sodium molybdate.

[b] Measurement taken when the seedlings were four months old. Yield is expressed as milligramme per plant per tapping.

[c] Girth and height increments were recorded when the plants were twelve months old, increments expressed as percentages of the initial values before Mo was applied.

[d] Mean height and dry weight of whole plants at twelve months inclusive of senescent leaves.

TABLE 5. MOLYBDENUM CONTENT IN LEAVES AND STEMS OF RUBBER SEEDLINGS

Treatment with sodium molybdate (kg/ha)	Molybdenum content (mg/kg material)				Total Mo per plant[b] (μg)
	Leaves				
	Top whorl	Bottom whorl	Total	Stems[a]	
0	0.19	0.17	0.36	0.06	8.21 –
0.012	0.17	0.16	0.32	0.12	17.93 (8.70)
0.120	0.42	0.53	0.95	0.12	24.31 (1.45)
1.200	0.89	1.39	2.28	0.77	103.36 (0.85)
12.000	6.30	20.40	26.70	15.50	1 706.89 (1.51)

[a] Include petioles and senescent leaves

[b] Figures within brackets are Mo uptake expressed as percentages of applied Mo.

the amount of sodium molybdate applied gave rise to a higher Mo content in the leaves (top and bottom whorls) and stems which include the senescent leaves and petioles. The combined amount of Mo in the leaves which varied from 0.32 mg/kg to 26.70 mg/kg was generally greater than that in the stem which ranged from 0.06 mg/kg to 25.50 mg/kg. At Mo concentration of 26.70 mg/kg in the leaves, no visual foliar toxicity symptoms were recorded although total dry matter content indicated the toxicity effect of Mo. Additionally, the uptake of Mo does not increase proportionally with the level of molybdate applied. The increase was gradual at lower molybdate levels, and steep at rates of 1.20 kg/ha and 12.0 kg/ha, respectively. At higher levels, there is a possibility that the Mo applied is in excess of what the soil can retain either through adsorption or reaction with the iron and aluminium hydrous oxides and is also more than sufficient for the plants requirement. After all, the amount of applied Mo taken up by the seedlings came to about 8.70%–1.45% and 0.85%–1.51% of applied Mo for levels 0.012–0.120 kg/ha and 1.20–12.0 kg/ha, respectively.

Effect of Liming on Molybdenum Status of the Soil and Soyabean

The effects of liming on Mo status of the soil and soyabean are summarised in *Table 6*. An increase in the level of Mo applied to the soils results in the corresponding higher content of total and available Mo in both top and sub-soils ($P < 0.001$ and $P < 0.01$, respectively). The high total and available Mo content in the treated soils at 15–60 cm and 60–90 cm depths indicate that there is considerable downward movement of the applied Mo. Liming at rates of 2000 kg/ha and 2800 kg/ha raised the pH of the top soils by 1.0 to 1.7 units and also appeared to enhance the availability of Mo in the soil ($P < 0.05$). At 2800 kg GML/ha, there was a slight increase in available Mo in the top soil (about 8%) over that of the control plot. The influence of lime on the movement of Mo to lower depths was not observed as available Mo at lower soil depths was not markedly increased. In fact, for soils at 60–90 cm depth, available Mo was marginally lower than in the control plots.

Besides raising the available and total Mo content in the soil, application of Mo also increases the amount of Mo in soyabean ($P < 0.001$). At 70 g Mo/ha, there was a four-fold increase in plant Mo. As for rubber seedlings, the amount of Mo in soyabean less that of the control forms about 7.0% of the applied Mo. Application of lime generally improve growth and yield of soyabean and hence leads to more Mo being absorbed by the plants ($P < 0.05$).

TABLE 6. EFFECT OF MOLYBDENUM AND APPLICATION OF LIME ON MOLYDENUM STATUS IN THE SOIL AND SOYABEAN

Treatment	Soil depth (cm)	pH (H_2O)	Soil Mo		Total Mo in plants/treatment[a] (mg)	Percentage uptake[b]
			Available (μg/kg)	Total (mg/kg)		
Molybdenum (g/ha)						
Nil	0–15	4.6	190	1.10	0.58	
	15–60	4.5	172	0.75		
	60–90	4.4	114	0.45		
35	0–15	5.2	225	3.03	1.63	7.5
	15–60	4.9	198	2.87		
	60–90	5.1	208	2.33		
70	0–15	5.3	291	4.86	2.31	6.2
	15–60	5.1	234	4.36		
	60–90	4.8	256	4.22		
GML (kg/ha)						
0	0–15	4.4	235	2.93	0.72	
	15–60	4.4	196	2.57		
	60–90	4.3	190	2.33		
2000	0–15	5.4	210	2.69	1.92	
	15–60	5.0	208	2.82		
	60–90	4.9	182	2.59		
2800	0–15	6.1	253	2.87	1.84	
	15–60	5.5	201	2.66		
	60–90	5.2	177	2.71		
F-Test						
Mo		NS	**	***	***	
Lime		**	*	NS	**	
Mo × Lime		NS	NS	NS	NS	
C.V. (%)		7	25	18	28	

[a] Total Mo in plants per plot per treatment (each value is a mean of three plots with about forty plants each).

[b] Total Mo in plants expressed as percentage of Mo applied.

Significant at $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ levels. NS = Not significant.

GML = Ground magnesium limestone

Linear regression analysis of the data showed that plant dry mass (gramme per plot per treatment) of soyabean was related to available Mo in the soil at $P < 0.05$ level but not total Mo. No linear relationship between seed yield of soyabean and Mo status (both available and total) of the soil was obtained.

DISCUSSION

The methods developed by Grigg[11] were found to be suitable for determining the total and available Mo content in Malaysian soils. Despite the complexities of the methods which require the isolation of Mo from the soil extracts by amyl alcohol for colorimetric determination and the presence of Mo in minute quantities, good agreement between replicate determinations was obtained. Mean coefficients of variation of total and available Mo computed from replicate determinations over the nine soils were 10.4% and 7.7%, respectively. The chemical determination of Mo in plant samples is precisely that described by Piper and Beckwith[12] and adopted by Bolle-Jones[7] for the determination of Mo in the laminae of *Hevea* seedlings. For values of 0.87 µg/kg to 3.38 µg/kg in the laminae, the coefficient of variation was in the range 3.3% to 12.8%.

Although the nine soils have different levels of total Mo and mean pH values less than 4.5, available Mo in all the soils are above the critical value of 150 µg/kg soil determined by Grigg[18]. It is probably for this reason that Mo deficiency symptoms in *Hevea* plants could not be established. Bolle-Jones[7] found that severe visual symptoms of Mo deficiency would not be consistently obtained until the concentration of Mo in the dried laminae was well below 0.07 mg/kg, probably at a level of about 0.010 mg/kg. In this study, the Mo concentration in the leaves was in the range 0.16 mg/kg to 20.40 mg/kg and therefore Mo deficiency symptoms would least be expected. Furthermore, the *Hevea* plants could tolerate high levels of Mo application without exhibiting any toxicity effect. The fact that Mo concentration in leaves at 12.0 kg/ha is about eighty-four times that at 0.012 kg/ha lends support to this observation. Although the adverse effect of high Mo application was not manifested visually by the leaves, the decline in dry matter content beyond the level of Mo application at 0.120 kg/ha did indicate its toxicity effect. The employment of pin puncture tapping in this study was the closest to the conventional method of tapping by bark removal currently in practice. The lack of relationship between latex yield by pin-puncturing and Mo input or Mo concentration in the leaves was contrary to that reported by Bolle-Jones[7] who took latex concentration in the petioles as yield. In his sand culture experiments, it was reported that the concentration of rubber in the petioles diminished significantly as the Mo status in the plant improved. The reduced rubber concentration in the petioles was caused by the decrease in concentration of N, S, P, Mg and K in the laminae and an increase in Mn content.

Unlike the other micro-nutrients, the availability of Mo has been known to increase as the soil pH approaches neutrality or increases above it. Kariman and Cox[17] found that adsorbed Mo was negatively correlated with pH and P levels. An increase in pH through liming would reduce the activity of the hydrous iron oxides and hence increase the mobility of Mo which is present mainly as the MoO_4^{2-} or $HMoO_4^{-1}$ ions. In the liming experiment, results indicate that liming has no appreciable effect on the availability of Mo in the soil although it enhances crop yield and consequencely Mo content in the plants. This is partly attributed to the sandy nature of the soil that leads to the leaching loss of Mo which is made available by liming.

CONCLUSION

The available and total Mo content of Malaysian soils depend on the parent materials from which they orginate. Soils derived from marine alluvial clays and basalts have higher Mo status. The lack of visual Mo deficiency or toxicity symptoms in rubber seedlings and soyabean is attributed to the high tolerance of Mo in the plants and the adequate amount of the nutrient which is inherent in Malaysian soils. The toxicity or deficiency symptoms, if at all present, can only be detected through proper determination of the biomass production and nutrients that are immobilised in the plant. Although liming has been known to improve the Mo status of the soils, its effect could not be observed in the sandy Holyrood series soil as leaching loss could possibly be the over-riding factor. Finally, there is a need to improve on the existing methods of determining Mo content in soil and plant samples. Methods for direct determination of Mo in soil and plant extracts by graphite furnace atomic-absorption spectrometry and inductively coupled plasma spectrometry would form the core of future studies. Without this development, it is difficult to monitor the effect of Mo in *Hevea* cultivation on a regular basis.

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Dissolution of Phosphate Rocks in Malaysian Soils under Rubber and Their Availability to Plants

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The dissolution of four phosphate rocks (Christmas Island, North Carolina, Togo and Jordanian) in five soils under rubber was studied. Determination of exchangeable calcium in the soils by $BaCl_2$*-triethanolamine at pH 8.1 showed that rapid dissolution of phosphate rocks took place in the initial fourteen days. For the more reactive North Carolina and Jordanian phosphate rocks, higher dissolution was observed. In the least reactive Togo rocks, the dissolution was low even at pH 5.2. In soils with high silt and clay content, the dissolution of phosphate rock is enhanced even though it is made up of coarser materials. Repeated extraction of exchangeable calcium in the soils after the incubation period indicated that about 84% of calcium from the phosphate rock added to the soils was recovered compared with 64% for soluble P by the Bray II procedure. When triple superphosphate was applied, 87%–94% of added calcium was accounted for, in contrast with 25%–31% for Bray II P. Although these results suggest that there is a tendency for the Bray II P to under-estimate the dissolution of phosphate rocks in Malaysian soils, glasshouse experiments with* Pueraria javanica *as an indicator crop showed that the Bray II P provides a better index for assessing the availability of soil phosphorus.*

On the basis of agronomic effectiveness and cost per unit nutrient, the insoluble phosphate rock is generally preferred over the soluble superphosphate as a source of phosphorus (P) for rubber[1-4]. In recent years, extensive agronomic evaluations of phosphate rocks (PR) originating from different geographical locations have been conducted on a legume cover crop, *Pueraria javanica* and rubber with little reference to the reaction of PR in the soils[5-8].

The low pH of the highly weathered Malaysian soils which is favourable to the slow dissolution of PR and the characteristics of *Hevea* which do not require an immediate high concentration of P in the soil solution during the immature and mature phases, favour the use of PR as fertilisers. It is common observation that the dissolution of PR results in higher calcium and phosphorus contents in the soil. The rate of dissolution of PR varies depending on their origins and the soils to which they are applied and consequently their availablilty to plants[9-12]. For soils with high phosphate-sorption capacity, Chien *et al.*[10] found that the dissolution of PR was strongly favoured.

This paper reports on the dissolution of four PR in five common soils under rubber. Changes in soil calcium and phosphorus contents were monitored using known chemical methods, and the data were then related to glasshouse croppings.

EXPERIMENTAL

Top-soils of 0–15 cm depth were collected (in bulk) from the interrows of rubber trees as these areas are expected to be least disturbed by fertiliser inputs. The five locations from which the soils were sampled are shown in *Table 1*. The soil samples were air-dried at ambient temperature (299°K) and crushed to pass through a sieve (< 2 mm size). The chemical characteristics of the four phosphate rocks are given in *Table 2*.

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TABLE 1. SOME CHARACTERISTICS OF THE SOILS

Soil series	Location	Sub-group (parent material)	Texture[a]	pH (H_2O)	Organic C (g/kg)	Total N (g/kg)	P(mg/kg)		Exch Al[b] (cmol/kg)	Exch Ca[c] (cmol/kg)	Acid extractable Ca (cmol/kg)
							Total	soluble			
Rengam	Kluang	Typic Kandiudults (Granite)	coscl	4.4	23.7	2.6	175	9	1.90	0.44	0.49
Bungor	Kuantan	Typic Kandiudults (Arenaceous shale)	sc -c	4.1	10.1	1.1	153	7	5.01	0.30	0.32
Durian	Kluang	Typic Plinthudults (Argillaceous shale)	sc	4.3	21.4	1.2	64	6	4.12	0.30	0.30
Serdang	Kahang	Typic Kandiudults (Sandstone)	scl	4.3	9.1	0.9	187	13	1.36	0.24	0.35
Rasau	Gambang	Typic Quartzisamments (Sub-recent alluvium)	scl	5.2	15.1	1.5	101	7	0.92	0.26	0.26

[a] co = coarse; s = sandy; si = silty; c = clay; l = loam

[b] 1*M* KCl extractant at pH 4.0

[c] 1*M* neutral ammonium acetate

TABLE 2. CHARACTERISTICS OF ROCK PHOSPHATES STUDIED

Phosphate sources	Total P_2O_5 (%)	CS[a]	NACS[b]	CaO (%)	Fe_2O_3(%)	Al_2O_3(%)	F(%)	Particle size[c] (< 250 μm) (%)
CIRP	36.0	27.2	11.5	45.0	3.5	4.0	2.0	86.5
NCRP	30.5	34.8	21.9	48.7	0.9	0.4	1.5	77.3
TRP	36.2	18.2	NA	51.2	1.5	1.0	3.9	89.0
JRP	31.0	27.4	15.2	50.0	0.3	0.5	3.5	73.2

NA: Not available
[a] Citric acid soluble P as percentage of total P_2O_5
[b] Neutral ammonium citrate soluble P as percentage of total P_2O_5
[c] Percentage of total weight less than 250 μm by dry sieving

Incubation Study

Air-dried soil of 600 g lots were taken in plastic containers and thoroughly mixed with each of the four phosphate rocks, *viz.* Christmas Island phosphate rock (CIPR), North Carolina phosphate rock (NCPR), Togo phosphate rock (TPR) and Jordanian phosphate rock (JPR). The moisture content of the soil-phosphate rock mixture was brought to field capacity (for Rengam, Bungor and Durian series soils 35% by weight and for Rasau and Serdang series soils 25% by weight). For comparison, triple superphosphate (TSP) was also included in the study. Two levels of the phosphate rocks at 350 mg P/kg soil (*L*1) and 700 mg P/kg soil (*L*2) were added and each treatment was triplicated. The rates correspond approximately to 340 g and 680 g PR recommended for each stand of rubber[13]. The soil-phosphate rock mixtures were maintained at field moisture capacity and incubated at room temperature (299°K). Samples were taken at suitable intervals over a period of about 244 days for the determination of pH, exchangeable calcium and soluble phosphorus. Soil pH was measured with a pH meter on a suspension of soil in distilled water, the soil: water ratio being 2:5. For exchangeable calcium, 1 g soil was extracted with 10 ml of 2*M* barium chloride-triethanolamine ($BaCl_2$-TEA) solution buffered at pH 8.1 and the calcium in the extract determined[14]. Soluble P in the soil was determined by the procedure of Bray and Kurtz[15] using 2 g soil to 20 ml of the extracting solution. The whole procedure was repeated on the same five soils without the addition of PR.

The extent of dissolution of PR in the soil was determined from the difference in exchangeable calcium and soluble P values ($\triangle$Ca and $\triangle$P) between the PR-treated and untreated soils.

Repeated Extraction of Ca and P in the Soils

At the end of the incubation period, 1 g soil was repeatedly extracted with the $BaCl_2$-TEA solution and a further 2 g soil continuously shaken with 20 ml portions of the Bray and Kurtz's ammonium fluoride-hydrochloric acid extracting solution to determine the residual Ca and P in the soils. These procedures were repeated until the concentrations of Ca and P in the extractants remained low and constant. Precautions were taken to minimise the loss of soil particles during the separation of the soil and extractant by centrifugation.

Glasshouse Experiments

Pre-germinated *Pueraria javanica* seeds were planted in pots containing 200 g of soil pre-mixed with different PR. The rates of P application were 0, 50 and 200 mg

P_2O_5/pot which corresponded to 0, 100 and 400 kg P_2O_5/ha, respectively. Each treatment was triplicated. The rate of P_2O_5 represented the recommended range of P_2O_5 applied during the establishment of leguminous covers in *Hevea* cultivation. Besides PR, minus P nutrient solutions containing nitrogen, potassium and magnesium were uniformly applied at the beginning of the experiment. After about eight weeks, the plant tops were harvested and the dry matter yield determined. Following this and after removing the roots from the soils, two additional croppings were carried out on the same pots (without the addition of fresh P_2O_5) and the dry matter yield determined. On completion of the cropping experiment, the soil from each pot was sampled for the determination of exchangeable Ca and soluble P. The relationships between crop yield and soil data were statistically analysed.

Analysis of Soil and Solutions

Total nitrogen, organic carbon and phosphorus contents in the soil were determined by the micro-Kjeldahl digestion method, Walkley and Black's titration method and digestion in a mixture of perchloric-sulphuric acid, respectively[16]. Soluble P was determined by the Bray and Kurtz II method[15], exchangeable aluminium was determined by extracting with 1 *M* KCl solution and acid-extractable calcium by 6 *M* HCl digestion[16]. The amounts of calcium in all the soil extracts were determined by atomic absorption spectrophotometry and phosphorus by the molybdenum blue method with ascorbic acid as the reducing agent.

RESULTS

The five soils selected for this study varied considerably in their textural properties *(Table 1)*. The Durian series soil consists mainly of silt and clay while the Serdang and Rasau series soils have a loamy sand texture with the sand fraction constituting more than 50% of the soil. Intermediate between these two groups of soils are the sandy clay Rengam and Bungor series soils. Besides textural differences, the soils also contained different amounts of organic carbon, total nitrogen, total and soluble phosphorus and exchangeable cations.

Of the soils, the Bungor and Durian series soils have the highest level of exchangeable aluminium with values of 5.01 cmol/kg and 4.12 cmol/kg soil, respectively. With the exception of the Rasau series soil, the soil pH falls within a narrow range of 4.4 – 4.1. A pH value of 5.1 in the Rasau series soil is attributed to the low clay content in the soil ($<20\%$) and low exchangeable aluminium which have little influence on the acidity of the soil.

The chemical characteristics of the four PR as specified by the suppliers are shown in *Table 2*. The total P_2O_5, 2% citric acid and neutral ammonium citrate soluble P and the percentage of fineness of the fertilisers were further verified by laboratory analysis. Both CIPR and TPR contain higher total P_2O_5 and proportion of fineness (<250 µm) compared with NCPR and JPR. Nevertheless, the reactivities of PR as indicated by citric-P were NCPR > CIPR ~ JPR > TPR.

Dissolution of Phosphate Rocks

The dissolution of PR as measured by the increase in exchangeable calcium content ($\triangle$ Ca) in the soils is typified by the graphs of the Durian, Rengam and Rasau series soils shown in *Figure 1*. For all the soils, the dissolution was rapid from the day of preparing the soil-phosphate rock mixtures. The results were similar to those of Hughes and Gilkes[12] for PR applied to thirty soils of Brazil, Columbia, Australia and Nigeria. During the initial fourteen days, the rate of dissolution of PR decreased in the order: Durian > Bungor > Rengam > Serdang > Rasau. This sequence closely relates to the silt and clay contents of the soils. Particularly in the Durian and Bungor series soils, the fineness of grinding of CIPR and TPR did not have any advantage over NCPR and JPR. The dissolution of the

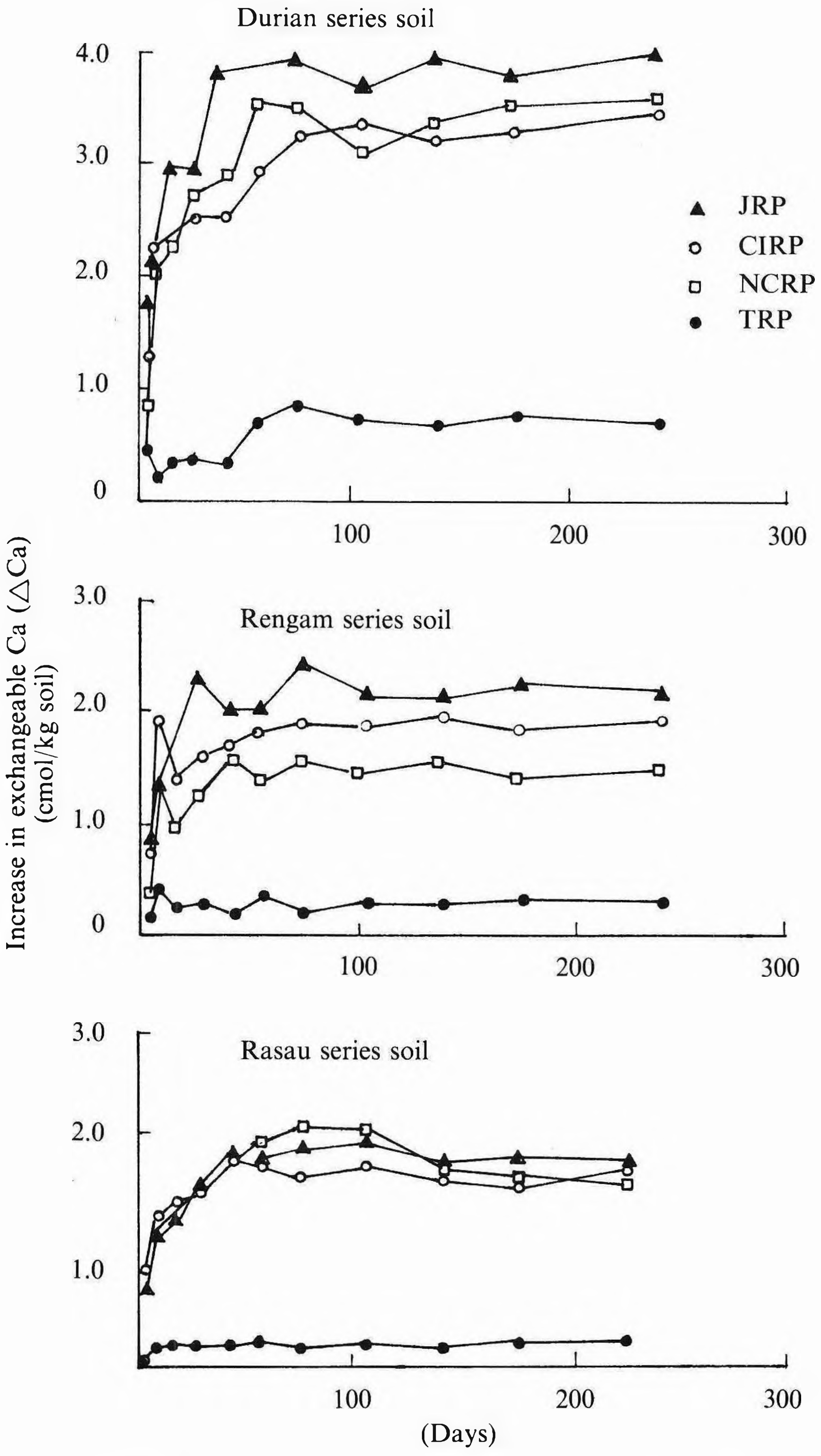

Figure 1. Increase in exchangeable Ca (△Ca) with time.

coarser NCPR and JPR in these soils were comparable if not better than the finer CIPR and TPR. Of the four phosphates, TPR showed the least reactivity and solubility.

After an incubation of about 30 days, the amount of △Ca remains fairly constant and this trend continues till the duration of the experiment is over at 224 days (32 weeks). The lack of increase in △Ca after 30 days is ascribed to the reactant products, especially Ca and P blocking any further dissolution of PR[17]. The amount of △Ca determined at 224 days and expressed as percentage of total Ca in the PR added to the soils are given in *Table 3*. Dissolution of PR as measured by △Ca (%) was higher in the Durian and Bungor series soils than the Rengam, Serdang and Rasau series soils. The average △Ca (%) over all levels of PR in these two soils came to about 57.7%–53.2% compared with 30.0% for the Rengam, Serdang and Rasau series soils. Although there was an increase in exchangeable Ca from *L*1 to *L*2, △Ca (%) values in *L*2 were only slightly higher than those in *L*1. These results further suggest that in a closed system the presence of dissolved Ca hinders the dissolution of PR. Determination of soil pH showed that there was a significant increase in pH values at *L*2 *viz*., from a mean of 4.5 to 7.2. Under this condition, the dissolution of the PR is reduced. Unlike the phosphate rocks, △Ca from TSP added to the soils was readily recovered by $BaCl_2$-TEA. The dissolution of TSP was immediate and complete once the soil-TSP mixture was moistened. In *Table 3*, results indicate that about 90.0% of the added Ca from TSP could be accounted for, irrespective of the levels of TSP and also the characteristics of the soils.

The increase in △P determined by the Bray II extractant followed a pattern similar to those shown in *Figure 1* for △Ca. The difference between △Ca and △P as indicators of PR dissolution is that △P is lower than △Ca in all the soils. This observation is particularly significant in the Bungor and Durian series soils with △P values of 23.8% and 25.5% and △ Ca values of 57.7% and 53.2%, respectively. The lower values of △ P (%) is due to either the incomplete extraction of dissolved P by the Bray II solution or the rapid P fixation in the soil. As a result, △ P could underestimate the extent of PR dissolution. Among the phosphate sources, P from the soluble TSP was readily fixed in the soil. In this instance, fixation of P had resulted in very low △ P with values of 25.0%–30.5% compared with △ Ca with values of 87.0%–93.7%. Because of the high P fixation in the soil, the advantage of TSP as a source of P over CIPR, NCPR and JPR is not significant.

Influence of Number of Extractions on △Ca and △P

After the incubation period of 224 days, cumulative amounts of △Ca and △P in the soils which were repeatedly extracted with $BaCl_2$-TEA and Bray II extractant, respectively, were determined. Of the soils, only the results of the NCPR treated Durian, Rengam and Rasau series soils are shown in *Figures 2* and *3*. Values of △Ca showed marked differences in the three classes of soils. Where there is greater dissolution of PR as in the Durian series soil, the calcium extraction curve reached a plateau after the tenth extraction. In contrast, △Ca from the Rengam and Rasau series soils continued to increase at a moderate rate even after twenty extractions. For the least reactive TPR, △Ca increased very slowly with the number of extractions. At the higher level of PR (*L*2), more extractions are needed to obtain the maximum amount of △Ca. However, in this study no further attempts were made to fully recover the amount of calcium added to the soils through prolonged extractions.

The △P against number of extraction curves for NCPR *(Figure 3)* show that after twenty-two extractions, only about 64% of the original quantity of P in the phosphate added to the soils was recovered as compared with about 84% for △ Ca. Furthermore, the

TABLE 3. EFFECT OF LEVELS OF ROCK PHOSPHATES ON △P, △Ca AND TOTAL DRY MATTER YIELD OF *PUERARIA JAVANICA*

Parameter	Soil type		Rock phosphates										
			CIRP		NCRP		TRP		JRP		TSP		Overall[c] Mean
			L1	L2	L1	L2	L1	L2	L1	L2	L1	L2	
△P(%)[a]	Rengam		33.0	35.3	29.0	25.4	10.2	14.9	31.7	32.8	24.6	32.3	26.9
	Bungor		20.6	27.9	28.3	26.7	14.3	18.8	23.5	29.3	20.3	28.3	23.8
	Durian		28.4	31.2	30.8	33.4	8.6	13.8	25.9	31.8	23.4	27.7	25.5
	Serdang		19.0	26.3	31.4	31.3	11.3	15.7	28.4	33.5	26.8	33.1	25.1
	Rasau		22.5	30.8	33.8	35.6	12.6	13.2	30.6	28.4	29.7	31.0	26.8
		Mean	24.7	30.3	30.7	30.5	11.4	15.3	28.0	31.2	25.0	30.5	
		L.S.D.	(P 0.05) = 3.82										
△Ca(%)[a]	Rengam		47.1	41.6	32.8	21.4	6.0	2.8	48.4	40.6	94.5	96.8	30.1
	Bungor		81.4	63.7	85.0	61.7	14.6	9.4	84.0	61.7	95.8	74.4	57.7
	Durian		83.9	63.2	79.0	60.7	12.9	10.5	48.2	67.0	90.9	90.2	53.2
	Serdang		33.2	36.4	47.6	26.0	6.3	4.4	48.2	37.5	91.5	89.4	29.9
	Rasau		43.6	35.2	37.2	31.5	4.8	2.8	41.0	44.5	95.5	84.4	30.1
		Mean	57.8	48.0	56.3	40.3	8.9	6.0	54.0	50.3	93.7	87.0	
		L.S.D.	(P 0.05) = 17.43										
DMY[b]	Rengam		2.20	5.52	2.67	8.75	2.70	4.26	3.12	8.02	3.16	8.11	4.85
	Bungor		3.01	5.92	3.35	7.72	3.55	4.95	4.32	7.04	4.17	6.64	5.06
	Serdang		3.03	6.48	3.49	8.16	4.39	5.47	3.83	7.34	2.85	6.45	5.15
	Rasau		1.60	4.49	2.48	7.32	1.15	2.82	2.06	4.44	3.22	9.09	3.87
		Mean	2.40	5.60	3.00	7.99	2.95	4.38	3.33	6.71	3.35	7.57	
		L.S.D.	(P 0.05) = 0.84										

[a] △P(%), △Ca(%) – Soluble P and exchangeable Ca expressed as percentages of P and Ca in RP added to the soil after 32 weeks

[b] DMY – Total dry matter yield of three croppings (g)

[c] Mean △Ca(%) and △P(%) over all levels of RP

L1, L2 refer to 100 kg and 400 kg $P_2 0_5$/ha, respectively

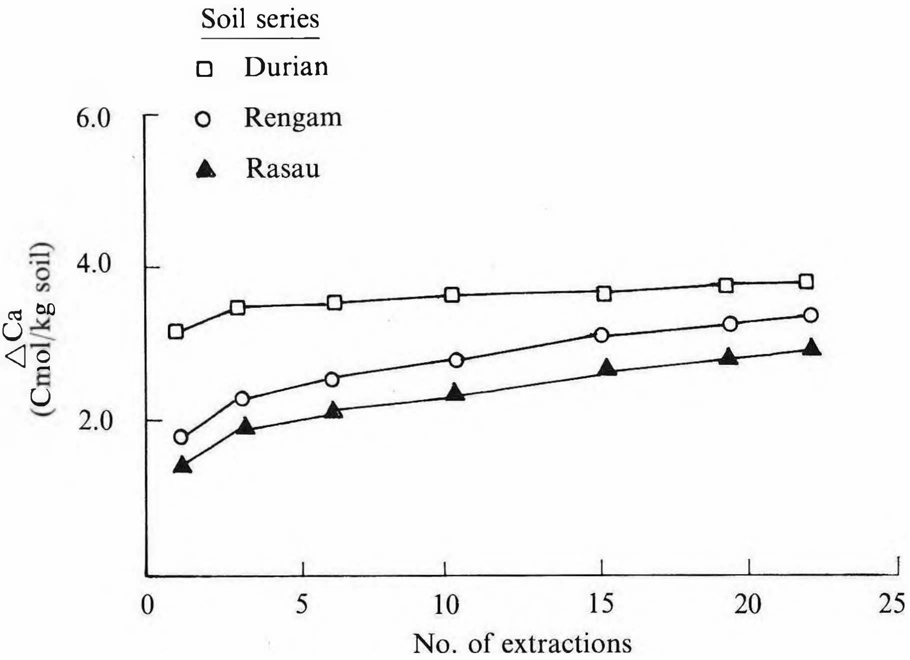

Figure 2. Cumulative amount of exchangeable Ca (△Ca) against number of extractions for NCRP.

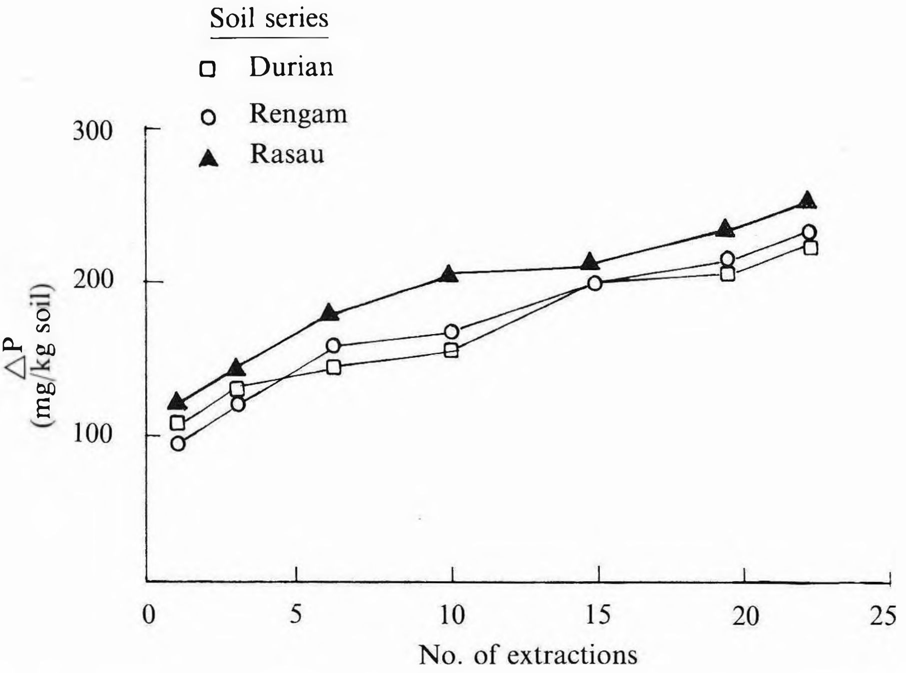

Figure 3. Cumulative amount of soluble P (△P) against number of extractions for NCRP.

results suggest that the soils are high P retention soils and the P content could not be readily extracted by the Bray II extractant. With the higher PR level (*L*2), all extraction curves still showed a general similarity as those in *Figure 3* except that △P in the initial extraction was higher. After the long incubation period, △P in the TSP-treated soils (especially the Durian and Bungor series soils) appeared to be lower than those receiving PR. The higher △P values for the PR-treated soils could be explained by the fact that Bray II extractant determines not only the soluble P in the soil but also part of the PR. In the case of TSP, complete dissolution and P fixation had led to lower P being extracted.

Glasshouse Study

In the glasshouse experiment, exhaustive croppings with *Pueraria javanica* could be established in four of the five soils *viz*., the Rengam, Bungor, Serdang and Rasau series soils. The inability of the leguminous creeper to grow in the Durian series soil is mainly due to the poor physical conditions of the soil. The compact Durian soil does not favour proper root development and hence plant growth. In his study on a wide range of soils of varying physical properties, Soong[18] had rated the Durian series soil as a Class IV soil. Rubber grown on this category of soils generally have poor growth and yield. During the dry spell, the soil is compacted while in the wet season, the soil is soggy and saturated with water.

The dry matter yield (DMY) of *Pueraria javanica* in the four soils increased in the order: Serdang > Bungor > Rengam > Rasau *(Table 3)*. Comparing the sources of phosphate, particularly at *L*2, the performance of NCPR appeared to surpass that of TSP and this was followed by JPR, CIPR and TPR. Even at the lower level of phosphate (*L*1), the superiority of TSP over the less soluble PR like JPR, NCPR and TPR was not conclusive. On examining the DMY in each cropping (not shown), it was found that TSP gave the highest yield in the first crop when compared with PR. In subsequent croppings without further addition of fresh phosphate, DMY from treated soils dropped drastically. This trend was also reported by Pushparajah *et al.*[19] and Zaharah and Sharifuddin[20] when *Pueraria phaseoloides* and maize (*Zea mays* L.) were grown in the glasshouse.

DISCUSSION

The highly acidic nature of Malaysian soils enhances the dissolution of phosphate rocks. However, the extent of dissolution, as measured by △Ca would vary considerably depending on the soils to which they are applied and their characteristics. Results obtained in this study show that soils with high percentages of clay and silt like the Durian series soil enhance the rapid dissolution of PR. The fine clay and silt fractions provide a greater area of contact between the applied PR and the soil particles thus increasing the reaction of PR and the soil.

Apart from textural differences in the soils, the degree of aluminium saturation in the soils have a profound effect on PR dissolution. Of the five soils, the Durian and Bungor series soils have the highest exchangeable aluminium (Al) values *(Table 1)*. The presence of Al and also Fe (iron) as exchangeable bases or oxides would provide a bigger sink for P thus favouring the dissolution of PR. Zaharah[21] had shown that the maximum amount of P that could be absorbed by the Bungor and Durian series soils were 1.08 mg and 0.75 mg P/g soil, respectively, and the absorbed P was found to contain more Al-P and Fe-P. Since P-sorption capacity was shown by Chien *et al.*[10] to have a major influence on PR dissolution, the dissolution of PR in the Durian and Bungor series soils would be strongly favoured as these soils have very high phosphate-fixing capacity.

With the increasing level of PR addition (*L*2), the percentage dissolution △Ca (%) decreased significantly although the absolute amounts of Ca increased. These results were consistent with those reported by Khasawneh and Doll[9] and Hughes and Gilkes[11,12]. Assuming a mean maximum P-sorption capacity of 1.02 mg P/g soil determined from the data reported by Zaharah[21] for the soils in this study, complete dissolution of PR at *L2* would be possible as the absorption sites in the soils could more than accommodate all the phosphate ions from PR dissolution. As the percentage dissolution of PR based on △Ca (%) amounted to about 30%–58% of the added PR, it is estimated that a considerable amount of PR still remains as discrete phosphate rock particles which can undergo slow dissolution. The curves in *Figure 1* showed that the reaction of the PR had slowed down and equilibrium was attained fourteen days after the start of the incubation experiment. One other possible reason for the lower dissolution at the higher level of PR application is the increase in pH of the soils. As shown by Chien[22], the increase in pH of the soils from an average value of 4.5 to 7.2 clearly does not favour the dissolution of PR. In a field experiment where CIPR equivalent to 4165 kg P_2O_5/ha was continuously applied to rubber over a period of seventeen years, Pushparajah *et al.*[19] observed that a significant amount of the soil phosphorus was calcium-bound and concluded that the long-term residual P effect in the soil is influenced by the accumulation of the applied CIPR.

In contrast to exchangeable calcium (△Ca), soluble P (△P) determined by the Bray II procedure did not show significant differences between the five soils. In all the soils, △P varied between 23.8% and 26.9%. These results indicate that there is no preference of the Bray II extractant for any of the phosphate sources in the soils. The Bray II extractant not only measures the amount of adsorbed P in the soil but also the amount of P associated with the unreacted PR. Where there is complete dissolution of the applied phosphate as in the case of TSP and also the more reactive NCPR and JPR, the phosphate is readily retained in the soil as aluminium or iron-bound phosphates which were shown by Pushparajah *et al.*[19] and Owen[23] to be less soluble and available for plant uptake. Depending on the types of soil and crop species used, Hammond[24] and Chien *et al.*[25] had shown that soil P determined by the Bray I procedure might under-estimate or over-estimate the available P from phosphate rock with respect to that from TSP. In this study, the values for △P (%) were found to be significantly lower than those of △Ca (%). It thus appears that △P tends to under-estimate the dissolution of PR.

In the glasshouse croppings, a significant correlation between DMY of *Pueraria javanica* and Bray II extractable P in the soil was obtained ($P < 0.001$) despite △P under-estimating the dissolution of PR *(Table 4)*. The suitability of the Bray II procedure for soils under *Hevea* over other soil test methods had been extensively evaluated by Owen[26] and Lau *et al.*[27] It must be emphasised that there are marked differences in exchangeable Ca and Bray II P in determining the dissolution of PR and the availability of P in the soil. Unlike P which is readily held in the soil, the calcium originated from PR exists predominantly as the exchangeable cation, Ca^{2+} which can be easily displaced or leached under Malaysian conditions. Therefore, its usefulness for assessing soil P availability would be limited. Furthermore, with the presence of carbonate apatite in varying quantities in the

TABLE 4. CORRELATION COEFFICIENTS BETWEEN BRAY II EXTRACTABLE PHOSPHORUS, EXCHANGEABLE CALCIUM AND DRY MATTER YIELD OF *PUERARIA JAVANICA*

Extractant	
Bray II Soluble P	Exchangeable Ca
0.39***	0.23*

Number of pots: 132
Treatment: 0, 100, 400 kg P_2O_5/ha
*** $P < 0.001$; *$P < 0.05$

TABLE 5. ANALYSIS OF VARIANCE FOR THE EFFECT OF ROCK PHOSPHATE ON THE SOILS

Source		Mean square		
	d.f.	$\triangle$Ca	$\triangle$P	DMY
Soil	4 (3)	3 481.014***	48.141***	10.495***
Level of RP	9 (9)	11 789.587***	735.590***	51.587***
Soil × level of RP	36 (27)	375.531***	32.724***	2.289***
Error	100 (80)	21.103	5.575	0.270
Total	149 (119)			
Coefficient of variation		9.16	9.17	10.97

Figures within brackets refer to determination of DMY in the glasshouse.
*** Significant at $P < 0.001$

PR, it is difficult to obtain consistent results based on $\triangle$Ca alone. The continuous leaching of the soil with $BaCl_2$-TEA and the rapid recovery of calcium re-affirmed the observation that calcium in the soils is loosely held and fluctuations under tropical climate are bound to occur. Therefore, it would appear that exchangeable Ca may be useful as an index for determining the dissolution of PR but not for assessing the availability of P in the soil.

The effect of PR on the soils and DMY of *Pueraria javanica* are summed up in *Table 5*. Different levels of PR had significant influence ($P < 0.001$) on $\triangle$Ca, $\triangle$P and DMY. Of the five phosphate sources, the agronomic potential of NCPR and to a lesser extent, JPR are comparable with that of TSP. These findings suggest that the agronomic effectiveness of PR is dependent on the chemical and mineralogical composition of PR and the properties of the soils to which they are applied. Knowing the conditions of Malaysian soils and the properties of most PR that are in the market, its choice is determined by the cost.

CONCLUSION

The extent of dissolution of phosphate rock is dependent on the source from which it originates and the soil to which it is applied. Incubation studies showed that rapid dissolution of the phosphate rock occurred in the initial fourteen days. Beyond this period, the rate of dissolution is slow suggesting that the soil-phosphate rock reaction has reached an equilibrium. The significant difference in values for exchangeable Ca and soluble P with respect to the levels of phosphate rock applied indicated that the soils have very high P-fixing capacity and that soluble P determined by Bray II procedure tends to under-estimate the dissolution of PR. Although the dissolution of phosphate rocks could not be reflected by the Bray II soluble P, the significant correlation ($P < 0.001$) between dry matter yield of *Pueraria javanica* and Bray II soluble P compared with that of exchangeable calcium shows that Bray II soluble P provides a better index for determining the availability of soil P. Further work is needed to investigate the various forms of soil phosphorus which are affected by the continuous application of phosphate rocks.

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