

INVESTIGATIONS ON NEW FUNGICIDAL SYSTEMS FOR CONTROL OF BARK ROT ON *HEVEA BRASILIENSIS*

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SUMMARY

The efficacy of Bark Rot fungicides is significantly impaired by frequent rains. The present investigation was therefore undertaken to examine the possibility of increasing the efficacy of panel fungicides by incorporating them in rubber latex.

Several dithiocarbamates containing metal ions, chemicals such as zinc oxide and formaldehyde and fungicides recommended for Bark Rot control were used singly or in combination, in in vitro trials. Zinc oxide and Zinc dithiocarbamates were of limited use, while others gave satisfactory results. The influence of rubber serum, on the activity of the chemicals and fungicides was then examined. It was shown that all the materials tested except Ziram inhibited the growth of the fungus. Pre-vulcanised latex was not found to be suitable as an adjuvant under laboratory conditions. However, satisfactory results were obtained when fungicides were incorporated into high ammonia field latex and high and low-ammonia centrifuged latex.

The use of preserved latex as an adjuvant with Antimucin and Thiram (TMTD), singly or in combination, was investigated under field conditions. Infection occurred on tapping panels which were inoculated after removal of scrap, although fungicides were applied prior to inoculation. Disease also occurred on panels, when the inoculum was removed a week after inoculation, but its spread was arrested when fungicides were applied, indicating that continued application of fungicides could limit the spread of the disease. Panels which were tapped and protected with Antimucin at the time of collection of latex, when inoculated 4 hours later, without removal of scrap, showed less infection. The significance of these findings, in relation to the control of Bark Rot and the influence of these fungicides on the yield are discussed.

INTRODUCTION

Bark Rot or Black Stripe caused by the fungus *Phytophthora spp.* is the most important panel disease of *Hevea brasiliensis* in Sri Lanka. The fungus gains entry through a fresh tapping wound and could cause considerable damage to the tapping panel (Figs. 1 & 2). If the disease is not detected in the initial stages, infection spreads above and below the tapping cut, affecting wide areas of the renewing and virgin bark. This could lead to uneven callusing of the renewed panel, rendering it unsuitable or difficult for subsequent tapping (Fig. 3). Under local conditions, the disease assumes epidemic proportions, in the presence of infected pods, only in certain localities during the South-West monsoon season, when heavy rainfall and high atmospheric humidity are prevalent.

Bark Rot is controlled by the regular application of water miscible fungicides such as Antimucin, Brunolinum plantarium or Fylomac followed by sealing off the panel with a water proof dressing. The efficacy of these fungicides is significantly impaired by frequent rains (Peries, 1975). Therefore, the development of a cheap systemic fungicide or a formulation which is highly persistent (tenacity) and having good redistribution (leaching) properties would be most desirable.

Natural rubber (NR) latex has given promising results when used as an adjuvant for controlling pink disease (Yeoh & Jan, 1974). The use of NR latex assumes greater importance because of the possibility of water insoluble organic fungicides

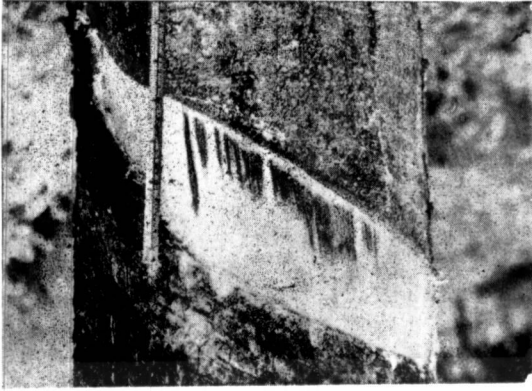


Fig. 1. Early symptoms of bark rot infection showing Black vertically parallel lines on the wood.



Fig. 2. Advanced stage of bark rot infection, condition referred to as 'Canker'.



Fig. 3. Tapping panel affected with bark rot showing uneven callusing.

becoming soluble in the hydrocarbons of NR. The present investigation was therefore undertaken to examine the possibility of increasing the efficiency of panel fungicides by incorporating them in NR latex formulations.

MATERIALS AND METHODS

Fungicides

The following fungicides were tested, at the concentrations given :

<i>Trade Name</i>	<i>Chemical Name</i>	<i>Active ingredient (%)</i>
Vondozeb	Manganese, Zinc ethylene bisdithiocarbamate	62.4
Zineb	Zinc ethylene, 1, 2 bisdithiocarbamate	75.0
Antimucin	Phenyl mercuric acetate (PMA)	16.5
Actidione	Cycloheximide (3- (2 - (3, 5 - dimethyl-2-oxycyclohexyl) -2 hydroxyethyl) - glutarimide)	4.2
Thiram	Tetramethyl thiuram disulphite (TMTD)	75.0
Ziram	Zinc dimethyl dithiocarbamate (ZDDC)	80.0
Zinc oxide	Zinc oxide	100.0
Formaldehyde	Formaldehyde	30.0

Base latex formulations

The type of latex used as a carrier for the fungicide is listed below :

<i>Code</i>	<i>Type of base latex</i>
A	High-ammonia field latex (1.4% ammonia)
B	High-ammonia centrifuged latex (0.7% ammonia)
C	Low-ammonia centrifuged latex (0.2% ammonia + 0.1% Thiram) (Pre-treatment of field latex with 0.35% ammonia + 0.025% Thiram)
D	Low-ammonia centrifuged latex (0.2% ammonia + 0.1% Antimucin) (Pre-treatment of field latex with 0.35% ammonia)

Field latex and centrifuged latex were diluted five and ten times respectively to obtain 12% dry rubber content.

Laboratory Experiments

The fungicides, singly or in combination, were tested *in vitro* for their toxicity to mycelial growth, ability to diffuse through the agar medium and contact fungicidal properties using the method adopted by Peries *et al.* (1962) with slight modifications.

The fungicides were separately incorporated into Lima Bean Agar (LBA) or Rubber Serum Agar (RSA) in the presence or absence of latex, to give dilutions of 0.0025%, 0.02% and 0.1% of the fungicide. All calculations were based on the active ingredient present in each fungicide. Discs, 0.4 cm diameter, were obtained from the leading edge of a five day old culture of *Phytophthora* spp. grown in 10 cm. diameter petri dishes each containing 5 ml of LBA (standardised inoculum discs). The inoculum discs were placed in the centre of petri dishes, each containing 17.5 ml of the medium in which the fungicides were incorporated separately to give the desired strength. The plates were incubated at room temperature ($28^{\circ} \pm 2^{\circ}\text{C}$), for seven days. The linear growth of the fungus was measured daily across two diameters at right angles to each other.

The ability of the fungicide to diffuse through the agar medium was tested, by placing two drops of a known concentration of a fungicide in eight wells, 0.7 cm diameter, placed equidistantly from the centre of the plate and arranged on the circumference of a circle of radius 3 cm. A standardised inoculum disc was placed in the centre of each plate and incubated at room temperature. The colony diameter was measured daily for seven days as described previously. The activity of the fungus was not tested by re-isolation.

Contact fungicidal properties of the chemicals were assessed by inverting standardised inoculum discs, on sterile filter paper discs, 0.6 cm diameter, impregnated with a known concentration of the fungicide. These were kept in a petri dish containing 5 ml of LBA. Five such discs, with the same concentration were placed in each petri dish and incubated at room temperature. Sterile filter paper discs dipped in sterile distilled water served as controls. The number of discs showing growth of the fungus was recorded daily up to seven days.

Field Experiments

Method of inoculation: The technique developed by Satchuthananthavale *et al.* (1974) was modified to suit the inoculation of the tapping panels. A zoospore suspension was prepared by scraping the sporangia from a few plates of 7–9 day old cultures of *Phytophthora* spp. grown in Difco LBA at room temperature $28 \pm 2^{\circ}\text{C}$. The zoospore suspension was standardized to give 150,000 zoospores/ml for inoculation trials to determine the efficacy of fungicides and for other field investigations a standard suspension of 100,000 zoospores/ml was used.

Sterile absorbant cotton wool strips (15 cm \times 1 cm) were saturated, each with 6 ml of the zoospore suspension before placing the inoculum strips on the tapping panel. These were protected with budding tape, the upper edge of which was pasted to the tree leaving the bottom end free to allow for air circulation. The wounds were exposed three weeks after inoculation and the resulting lesions above the tapping cut were measured.

Effect of fungicides on the establishment and spread of Phytophthora spp: Three hundred PB 86 trees were selected from the 1959 clearing at Galewatta Estate to determine the effect of fungicides on the establishment and spread of the disease. Two fungicides, Thiram and Antimucin, were used singly and in combination using four base latex formulations. These were compared with Antimucin mixed in water and a water control. Thus, there were 14 treatments. Ten trees were used for the water control and all the other treatments had 5 trees each.

The three hundred trees were divided into 4 equal groups, each having 75 trees and were subjected to the following treatments, prior to inoculation :—

- (1) Fungicides were applied every alternate day, for 8 days. The trees were tapped on the day before the inoculation. The inoculum was placed after removal of the panel scrap.
- (2) Fungicides were applied every alternate day, for 13 days. The trees were tapped on the day before the inoculation. The inoculum was placed after removal of the panel scrap. The inoculum strip was removed 9 days after inoculation. Trees were tapped and application of fungicides on the panel was continued until 3 weeks after inoculation.
- (3) Fungicides were applied every alternate day, for 14 days. The trees were tapped on the day before the inoculation. The inoculum was placed after removal of the panel scrap.
- (4) Fungicides were applied every alternate day, for 16 days. The trees were tapped on the day of the inoculation, followed by the application of fungicides at the time of collection of latex. The inoculum was placed about 4 h after application of fungicides without the removal of scrap.

The number of trees infected and the maximum lesion length above the tapping cut were recorded three weeks after inoculation.

Effect of fungicides on the yield : Seventy PB 86 trees tapped on the S/2, d/2 system were selected from the 1959 clearing at Galewatta Estate. The number of treatments were the same as for the previous experiment *i.e.* 14. Each treatment was replicated 5 times. Pre-treatment yield assessments were taken for 19 days using the cup coagulation method. This was spread over a period of 43 days. The fungicides were applied thereafter on every tapping day at the time of collection of latex. Post-treatment yield assessments were also taken for 16 days spread over a period of 83 days.

Effect of panel scrap on the incidence of Bark Rot : Ninety PB 86 trees were selected from the 1959 clearing at Galewatta Estate. All the trees selected for the experiment were tapped, followed by inoculation of 5 trees per treatment, using a zoospore suspension standardized to give 100,000 zoospores/ml, with and without the removal of panel scrap, 3, 9, 24, 48, 72 and 96 h after tapping. The observations were made 3 weeks after inoculation.

RESULTS

Table 1 shows that most of the fungicides tested *in vitro* had little or no toxic effect on the growth of *Phytophthora* spp. at a concentration of 0.0025%. A mixture of Thiram and Formaldehyde completely inhibited mycelial growth at this concentration but its effect did not last for long periods. Formaldehyde alone was also effective.

TABLE 1

EFFECT OF VARIOUS FUNGICIDES AT DIFFERENT CONCENTRATIONS ON GROWTH OF *PHYTOPHTHORA* SPP. ON LIMA BEAN AGAR

Fungicide	0.1% a.i.						0.02% a.i.						0.0025% a.i.					
	Linear growth (mm)		Diffusibility (mm)		Contact		Linear growth (mm)		Diffusibility (mm)		Contact		Linear growth (mm)		Diffusibility (mm)		Contact	
	72h	168h	72h	168h	72h	168h	-72h	168h	72h	168h	72h	168h	72h	168h	72h	168h	72h	168h
Vondozeb	—	—	—	—	—	—	0	0	24	44	NG	NG	22	53	25	63	NG	NG
Zineb	29	62	21	50	NG	G	—	—	—	—	—	—	—	—	—	—	—	—
Thiram	0	0	17	22	NG	G	0	0	23	35	NG	NG	20	45	23	55	G	G
Ziram	0	0	18	23	NG	NG	0	0	26	36	NG	NG	19	52	28	64	G	G
Thiram + Zinc oxide (1 : 1)	0	0	17	21	NG	G	0	0	24	34	NG	NG	28	70	24	57	G	G
Thiram + Formaldehyde (1 : 1)	0	0	0	0	NG	NG	0	0	19	33	NG	NG	0	13	18	50	NG	NG
Thiram + Bark extract	0	0	19	40	NG	NG	—	—	—	—	—	—	—	—	—	—	—	—
Zinc oxide	31	72	23	58	G	G	—	—	—	—	—	—	—	—	—	—	—	—
Formaldehyde	—	—	—	—	—	—	0	0	22	47	NG	NG	0	0	26	69	NG	NG
Control	35	82	23	64	G	G	37	82	32	63	G	G	32	80	30	63	G	G

G = Fungal growth

NG = no fungal growth

It is possible that accumulation of formaldehyde vapour in the petri dishes would have been lethal to the fungal mycelium. At higher concentrations however all the fungicides tested except Zinc oxide and Zineb were found to be effective. This shows that Zinc based chemicals and fungicides are not suitable for Bark Rot control. At the highest concentration, a mixture of Thiram and formaldehyde diffused rapidly through the agar medium, restricting the growth of the fungus, whereas others diffused relatively slowly through the medium enabling the fungus to grow for some time. The bark extract appeared to have an adverse effect on the diffusibility of Thiram. A result similar to that shown for linear growth was obtained when contact fungicidal properties were assessed, except with Vondozeb which did not show any growth even at the lowest concentration.

The laboratory tests are merely rapid and convenient methods of evaluating the fungitoxicity of a number of products. When fungicides are applied to the tapping panel, they get mixed with rubber serum. This could alter the performance of fungicides applied to the tapping panel. Therefore, the promising fungicides were tested *in vitro* by incorporating rubber serum into the agar medium and these results are presented in Table 2. These studies showed that, in the presence of rubber serum, all fungicides except Antimucin and Actidione were rendered ineffective at low concentrations. At higher concentrations however all the fungicides except Ziram, which is also a zinc based compound, were effective. Although mycelial growth was arrested at higher concentrations, by a mixture of Thiram and Zinc oxide, Thiram and formaldehyde and formaldehyde alone, these gave unsatisfactory results in contact fungicidal tests.

TABLE 2 : ACTIVITY OF FUNGICIDES AT DIFFERENT CONCENTRATIONS ON *PHYTOPHTHORA* SPP. IN THE PRESENCE OF RUBBER SERUM IN AGAR

Fungicide	0.02% a.i.		Contact		0.0025% a.i.	
	linear growth (mm)				Linear growth (mm)	
	72h	168h	72h	120h	72h	168h
Vondozeb	0	0	NG	NG	27	46
Antimucin	0	0	NG	NG	0	0
Actidione	0	0	NG	NG	0	0
Thiram	0	0	NG	NG	37	55
Ziram	28	52	G	G	42	60
Thiram + Zinc oxide (1 : 1)	0	0	G	G	35	—
Thiram + Formaldehyde (1 : 1)	0	0	G	G	40	59
Formaldehyde	0	0	G	G	41	53
Control	33	60	G	G	39	53

G = fungal growth
NG = no fungal growth

The use of latex as an adjuvant was then examined. The results of this experiment are presented in Table 3. Field latex and ammoniated (1.2%) latex were used

initially. Field latex was not suitable as it leads to bacterial contamination. Although ammoniated latex inhibited mycelial growth at higher concentration, it did not prevent growth of the fungus when inoculum plugs were placed on sterile discs dipped in ammoniated latex. Antimucin and Actidione when incorporated into latex, prevented mycelial growth. Although Antimucin showed persistent contact fungicidal properties, Actidione was found to be ineffective after 168 h from the time of inoculation.

TABLE 3 : ACTIVITY OF FUNGICIDES AT DIFFERENT CONCENTRATIONS ON *PHYTOPHTHORA* SPP. IN THE PRESENCE OF LATEX IN AGAR

Fungicide	0.02% a.i.		Contact		0.0025% a.i.	
	linear growth (mm)				linear growth (mm)	
	72h	168h	72h	168h	72h	144h
Field latex	BC	BC	—	—	BC	BC
Ammoniated (1.2%) latex	0	0	G	G	37	55
Antimucin + Ammoniated (1.2%) latex	0	0	NG	NG	0	0
Actidione + Ammoniated (1.2%) latex	0	0	NG	G	0	0
Thiram + Formaldehyde + Ammoniated (1.2%) latex	0	0	G	G	36	64
Lima Bean Agar	—	—	—	—	40	76
Control	31	59	G	G	32	56

G = fungal growth

NG = no fungal growth

BC = bacterial contamination

The results of field experiments, summarised in Table 4, indicate that fungicides play an important role in minimising and preventing the establishment of infection. The number of trees infected by the fungus was reduced when Antimucin, incorporated in a base latex formulation, was applied just prior to inoculation. The application of Antimucin as a water miscible formulation, just before inoculation, completely prevented the establishment of the fungus. Thiram and a mixture of Thiram and Antimucin appeared to be inferior to Antimucin.

These results also showed that once the disease is established, the spread of the disease could be arrested by continuous fungicide application. It was also shown that lesion lengths were very restricted, when Antimucin incorporated into base latex formulations were continuously applied on the panels suggesting that Antimucin leaches out with the rains. The use of Antimucin as a water miscible formulation was not quite as effective as the former. It is possible that much of the fungicide applied in this manner is washed off with the rains. Thiram and a mixture of Thiram and Antimucin also helped in curtailing the spread of the disease, though not quite as effectively as Antimucin.

TABLE 4 : EFFECT OF FUNGICIDES ON THE PREVENTION AND CONTROL OF BARK ROT

Fungicide	Trees infected				Mean maximum lesion length above the tapping cut (mm)				Range of lesion length above the tapping cut (mm)			
	1	2	3	4	1	2	3	4	1	2	3	4
Thiram 0.1% + A	100	80	100	100	41	40	108	80	30—65	10—77	70—160	15—120
Thiram 0.1% + B	100	80	100	100	48	37	138	95	30—80	20—48	45—305	45—165
Thiram 0.1% + C	100	80	80	100	40	43	49	97	30—65	20—108	30—73	45—115
Thiram 0.1% + D	100	60	100	60	52	32	93	94	33—80	17—45	55—160	50—160
Antimucin 0.1% + A	100	40	100	60	43	24	71	48	20—95	08—40	63—85	30—70
Antimucin 0.1% + B	100	60	80	40	58	32	47	26	30—90	20—39	30—75	25—27
Antimucin 0.1% + C	100	60	80	80	58	21	60	47	25—165	16—33	50—75	40—55
Antimucin 0.1% + D	100	60	80	20	39	25	70	33	20—80	13—38	50—89	0—33
Thiram + Antimucin 0.1% + A	100	60	60	80	63	42	127	88	35—100	30—50	57—145	70—100
Thiram + Antimucin 0.1% + B	100	80	100	100	59	43	86	93	45—90	30—55	50—165	60—100
Thiram + Antimucin 0.1% + C	100	80	80	80	79	64	77	72	50—115	30—70	50—101	40—125
Thiram + Antimucin 0.1% + D	100	80	100	100	97	30	61	70	60—160	10—50	33—85	25—118
Antimucin 0.1% + water	100	60	100	0	73	64	40	0	40—164	40—80	30—60	0—0
* Water control	100	80	80	90	56	70	93	99	45—110	45—91	50—130	20—175

Each figure is a mean of five replicates except in the control (water) where it is a mean of ten replicates.

*Average of 10 measurements, All the other figures represent a mean of 5 measurements

A—D % — see the text for details

1—4 W%

The results shown in Table 5 indicate that a tapping cut remains susceptible for 3 days, when inoculated after the removal of panel scrap. However, when the inoculation was carried out without the removal of panel scrap, the critical period for infection was found to be the first 48 h after bark injury confirming the findings of Peries (1975). Thereafter, the percentage infection was reduced rapidly and in 4 days no infection was recorded. Peries (1975) has shown that it is due to the formation of callus tissue over the injury.

TABLE 5 : EFFECT OF REMOVAL AND RETENTION OF PANEL SCRAP ON THE INCIDENCE OF BARK ROT

h After tapping	% infection	
	Without scrap	With scrap
3	100	100
9	100	100
24	100	100
48	100	80
72	100	40
96	0	0

Table 6 summarises the effect of the fungicide on the yield of rubber. These results generally show that there is a slight reduction in the yield of rubber when the fungicides were continuously applied on the tapping panel. There was a marked reduction in yield when Antimucin was applied mixed with a base latex formulation which already contained Antimucin. However, this effect was not shown when Antimucin was mixed with Thiram. Application of Antimucin mixed in water also reduced the yield. It has been shown that organo-mercurials could diffuse into the latex vessels to react with the enzymes which play an important role in rubber biosynthesis and this could lead to a reduction in the yield.

TABLE 6 : THE EFFECT OF FUNGICIDES ON THE YIELD OF RUBBER

Fungicide	Mean yield g/tree/tapping	
	Pre-treatment	Post-treatment
Thiram 0.1% + A	42.6	39.3
Thiram 0.1% + B	39.3	33.3
Thiram 0.1% + C	30.7	29.4
Thiram 0.1% + D	22.1	24.1
Antimucin 0.1% + A	28.4	26.0
Antimucin 0.1% + B	28.6	27.2
Antimucin 0.1% + C	30.6	27.1
Antimucin 0.1% + D	42.9	36.4
Thiram + Antimucin 0.1% + A	23.8	23.8
Thiram + Antimucin 0.1% + B	53.7	51.7
Thiram + Antimucin 0.1% + C	33.5	31.4
Thiram + Antimucin 0.1% + D	41.5	41.7
Antimucin 0.1% + water	35.6	27.8
Water control	44.7	42.5

DISCUSSION

The tapping knife used locally has two cutting edges, the horizontal one removes a shaving of the bark exposing the latex vessels and the vertical edge causes damage to the renewing area of the bark. It is clearly evident from these studies that the fungus gains entry into the bark at the regions just above the tapping cut and this is probably why the initial symptoms of vertically parallel depressions are seen on this area. The risk of infection could be greatly minimised either by carrying out carefully controlled tapping or by modification of the tapping knife so that its vertical edge does not cause any damage to the renewing bark. The critical period for protection of the tapping cut is the first 3 days after tapping.

These investigations have also clearly shown that the application of Antimucin as a water miscible fungicide soon after collection of latex helped to prevent the establishment of the pathogen, provided there is no interference with rain.

Peries (1975) had shown that 2.5 mm of rain reduced the efficacy of most water miscible fungicides as they are washed away from the panel. This necessitates repeated application of fungicides to protect the panels, even if small amounts of rainfall are recorded following the first application, during the period when infected pods are present on the trees. Therefore, the use of water miscible fungicides becomes uneconomical, unless the chemical used is extremely cheap.

The application of fungicides mixed with latex, on the bark after each tapping is expected to build up the concentration of fungicides and retain them for some period, to be leached out with rain. However, the expected results were not obtained and several reasons could be attributed to this. It is possible that the optimum amounts of chemicals required to stabilise the latex to be used as an adjuvant may have been insufficient. These chemicals could either enhance the penetrating properties by delaying the drying time or their persistency and the ability to leach out may be affected by excessive use of the chemicals. A part of the active ingredient of Thiram, (Tetramethyl thiuram disulphide) gets crosslinked with the rubber thus reducing the available fungicide, and this could also be a reason for their ineffectiveness. Furthermore, as a result of the increased level of crosslinking the leaching effect may have been markedly reduced. It could also be possible that these chemicals, though absorbed into the bark, are converted by reaction with other substances such as proteins into chemicals which are inactive against the fungus. Thus, these fungicides would have no effect on their build up over a period of time and their fungicidal effect could only be expected, at the time of application. These studies provided results to prove this as the spread of the fungus was arrested when fungicides were applied continuously.

Antimucin is a mercury-based formulation and it could promote coagulation of latex as it denatures proteins. Therefore, only a part of the Antimucin is expected to be retained by the rubber to be leached out with rains. This was probably another drawback in the efficient functioning of this system.

Different fungicides which contained dithiocarbamates and bisdithiocarbamates as their active ingredients are known to control diseases caused by *Phytophthora* spp. Horsfall (1956). In these studies Zineb and Zinc oxide did not inhibit the growth of the fungus even at 0.1% concentration. Similarly, Vondozeb and Ziram were found to be ineffective at lower concentrations. It can therefore be concluded that generally none of the Zinc based formulations are suitable to control Bark Rot caused by *Phytophthora* spp.

The most significant factor that emerged from these studies is that the application of fungicides, using this novel approach could limit the spread of the lesion provided the fungicides are applied on each tapping day. Unfortunately, Antimucin which was used in this study was not the ideal fungicide for the proper evaluation of this system. The use of other fungicides should be investigated.

The general recommendation that wet trees should not be tapped, could be reversed by the discovery of a systemic fungicide or a formulation which has the ability to persist and release the active ingredient slowly into the tapping cut. Although these preliminary investigations indicate that natural rubber latex could be used as a carrier, further work has to be done before conclusive results could be obtained.

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