Evidence for the Presence of Growth Inhibitory Substances in Mikania cordata (Burm. f.) B.L. Robinson*

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Laboratory and pot culture tests have shown that Mikania cordata *contains substances which can depress growth of* Hevea brasiliensis, *tomato and* Pueraria phaseoloides, *and also strongly inhibit* in vitro *growth of the fungus* Fomes lignosus *and depress nitrification in the soil. In the field the harmful effect of* M. cordata *on other plants appears likely to be the combined result of a direct toxicity and of indirect effects via the soil microflora. Essential oils separated by steam distillation showed inhibitory activity, and chromatographic examination of petroleum ether extracts suggests that the growth inhibitory substances include phenolic and flavonoid constituents, either or both of which may be responsible for the observed effects.*

Observations that toxic substances released into the soil by the roots of certain plants could affect the subsequent growth of the same or other species were made over a hundred years ago by DE CANDOLLE (1832). However this work and later studies by SCHREINER and his associates (1908), PICKERING (1917) and others, was largely ignored, and mutual influences between plants were considered more from the point of view of competition for nutrients until the 1930's when renewed interest in 'allelopathy', or the effect of one plant on another through its metabolic products, was stimulated as a result of the investigations of MOLISCH (1937). Some of these metabolic products have been chemically identified (BODE, 1940; GRAY AND BONNER, 1948), and recent work on the subject has been extensively reviewed by BORNER (1960), GARB (1961) and WINTER (1961).

In rubber cultivation, plants of different species are grown as ground covers between the tree rows in the early years after planting and their effect on growth of the rubber is of practical importance. In field experiments comparing different cover plants, *Mikania cordata*, a herbaceous creeper belonging to the family Compositae, has been observed to depress growth of rubber in comparison with leguminous creeping covers consisting mainly of *Pueraria phaseoloides* Benth. and certain grasses such as *Paspalum conjugatum* Berg. Trees grown together with *M. cordata* have shown depressed contents of nitrogen and phosphorus in their leaves, depressed rooting in the litter layer and A horizon, and have developed relatively small canopies, but since covers of *M. cordata* show lower dry matter production and nutrient immobilisation than the other covers, it has been difficult to ascribe the effects to simple competition for soil moisture and nutrients (WATSON *et al.*, 1964a; 1964b). In one experiment lower levels of soil nitrate-nitrogen were recorded under a cover of *M. cordata* than under covers of creeping legumes and grasses (WATSON *et al.*, 1964a) while in pot incubation studies depressed rates of nitrification, not easily explained by reference to C/N ratios, were found in mixtures of soil with *M. cordata* leaf and stem material (GUHA AND WATSON, 1958).

Observations on the effect of *M. cordata* on rubber in Malaya have been confirmed by a report from Indonesia (TAN *et al.*, 1961), while a similar effect on oil palms has been reported

* Formerly known as *Mikania scandens*, Willd.

in Malaya where it has depressed palm vigour, frond nitrogen and phosphorus contents, and the yield of fruit bunches (GRAY, 1963).

P. phaseoloides is widely planted as a ground cover and it is common experience that unchecked invasion by *M. cordata* quickly results in its displacement. This could be an effect of competition for light, water and nutrients but in view of the effect of *M. cordata* on rubber and oil palm, other factors might also be involved.

The present investigations were carried out to determine whether some of the observed effects might be due to growth inhibitory or toxic substances produced by the *M. cordata*.

EXPERIMENTAL METHODS AND RESULTS

The work described below has necessarily been of an exploratory nature, commencing with rather crude tests with rubber in pot culture and progressing to the use of a rapid and convenient fungal bioassay test.

Pot Culture Experiments with Rubber Seedlings

In preliminary experiments, efforts were made to duplicate conditions in the field. In one trial the three main covers tested in the field, *Mikania cordata*, *Paspalum conjugatum* and *Pueraria phaseoloides*, were grown together with Tjir 1 seedlings in 18 inch pots, but vigorous root competition by the covers made interpretation difficult. However, four months after commencement of the experiment, rubber seedlings grown with *P. phaseoloides* were significantly larger than those grown with *P. conjugatum* or *M. cordata;* as in previous pot work (WATSON, 1957), the growth and rooting of *M. cordata* was very poor compared with that of the other covers, and this plant might have been expected to exert less competition with the rubber than did the other two covers.

In a second experiment, rubber seedlings grown in soil showed no differential growth effects when watered with leachates from the three cover plants grown in sand culture, possibly as a result of excessive dilution of the leachates. In a third experiment, rubber seedlings growing in soil were mulched with oven dried leaf and stem material of the three cover plants (hereafter referred to as 'green material'), but few differential growth effects were recorded although plants mulched with *P. phaseoloides* material tended to show the best growth.

In a further experiment water extracts of the three covers were prepared by soaking oven dried and ground green material for up to 18 hours in water followed by filtration. These extracts, at concentrations of 1.65% and 3.30% (based on the number of grams of dried material used for preparation of 100 ml), with and without nutrients added to bring all extracts to the same nutrient content, were watered at weekly intervals at 150 ml per pot on to rubber seedlings grown in 10-inch-diameter pots of soil. The application of increasing levels of the extracts of *P. phaseoloides* and *P. conjugatum* increased dry weights of rubber seedlings, but the application of *M. cordata* extracts produced little growth response (*Figure 1*). The same pattern of response was evident in dry weight production of rubber leaves, stems and petioles, and roots, and this was true whether the extracts were balanced for nutrient content or not. Analysis of the rubber seedlings at the end of the experiment did not indicate any interference with nutrient uptake by the rubber seedlings in any of the treatments, although nitrogen deficiency symptoms were observed in the *M. cordata* treatments for the first six weeks of the experiment.

Pot Culture Experiments with Tomato Seedlings

The slow growth, variability and size of rubber seedlings made pot culture tests laborious and imprecise, and tests were carried out with tomato seedlings in an attempt to obtain more rapid and reproducible results.

Effect of water extracts. Tomato seedlings were grown in pots holding 600 g of sand and treated twice weekly with a basal complete nutrient solution and 1% or 2% water extracts of oven dried green material and roots of three cover plants, *M. cordata*, *P. conjugatum* and *P. phaseoloides*, at the rate of 50 ml per pot. The plants were sampled at the end of

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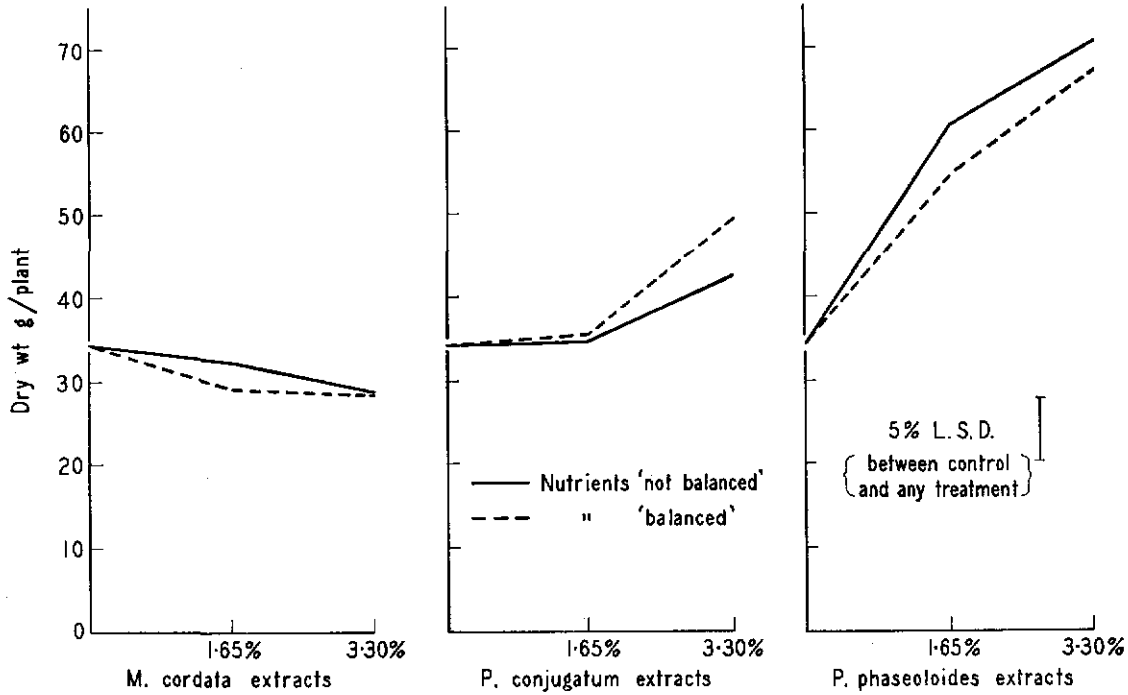

Figure 1. Effect of water extracts of oven dried green material of M. cordata, P. conjugatum *and* P. phaseoloides *on dry weight production of* H. brasiliensis *seedlings, after 24 weeks of treatment.*

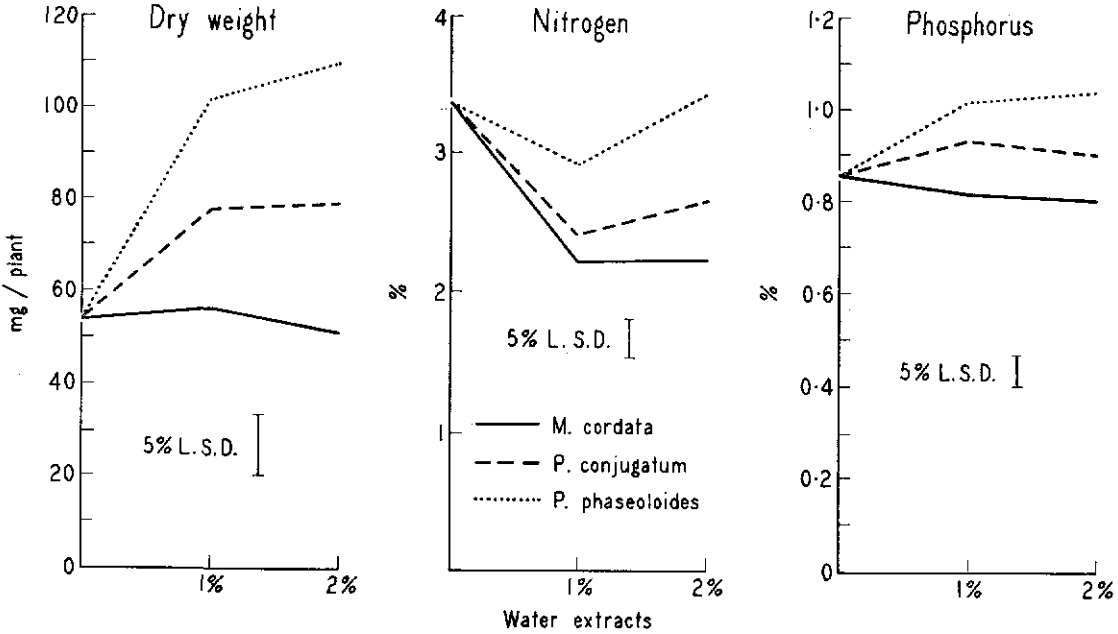

Figure 2. Effect of water extracts of oven dried green material of M. cordata, P. conjugatum *and* P. phaseoloides *on dry weight production and percentage nitrogen and phosphorus of tomato seedling tops, after 3 weeks of treatment.*

three weeks, weighed and analysed. A similar pattern of effects to that found in the rubber seedlings tests was recorded: application of *P. phaseoloides* and *P. conjugatum* extracts increased the dry weight of the tomato seedlings tops, whereas the application of *M. cordata* extracts produced little growth response (*Figure 2*). Analysis of the test plants showed comparatively low nitrogen and phosphorus contents in the *P. conjugatum* and *M. cordata* treatments, possibly the result of a dilution effect in the case of the former. Similar patterns of response were recorded for both green material and root extracts.

In another experiment to be discussed elsewhere, similar results were obtained with tomato seedlings grown in soil culture.

Effects of petroleum ether extract of M. cordata. Oven dried *M. cordata* green material was extracted in a Soxhlet apparatus for eight hours with petroleum ether (boiling point 56–70°C). One gram of material was found to yield approximately 25 mg of crude extracts. These crude extracts were mixed with 600 g of sand at six levels in small plastic pots and tomato seedlings were planted in the treated sand. A basal complete nutrient solution was applied to all pots. After three weeks, the plants were sampled and it was found that dry weight production of the tomato tops decreased as the concentration of petroleum ether extracts increased, while analysis showed a slight increase in the potassium content but little effect on the other nutrients (*Figure 3*).

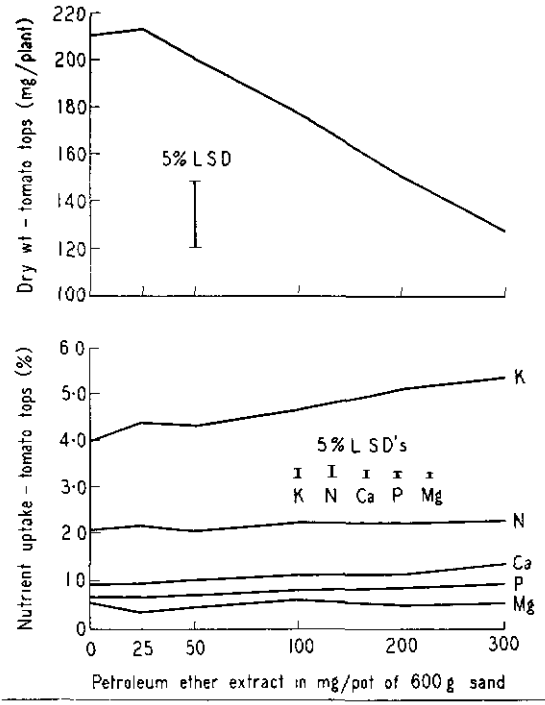

Figure 3. Effect of petroleum ether extracts of oven dried M. cordata *green material on growth and nutrient composition of tomato seedlings, 3 weeks after commencement of test.*

POT CULTURE EXPERIMENT WITH PUERARIA PHASEOLOIDES

Seedlings of *P. phaseoloides* were grown in small plastic pots each containing 600 g of soil, and treated twice weekly with 50 ml per pot of 2.5% and 5.0% water extracts of oven dried green material and of roots of *M. cordata*, *P. conjugatum* and *P. phaseoloides*. Application of both green material and root extracts of *M. cordata* produced a marked depression in dry weight production by the *P. phaseoloides* (*Figure 4*); the nitrogen content of the plants was increased, but the contents of phosphorus and calcium were markedly decreased especially at the higher concentration.

Similar results were obtained in a further experiment in which sampling was carried out one week earlier than in the first test, except that in this case the nitrogen content of the plants was slightly decreased—possibly a dilution effect in those plants receiving *P. phaseoloides* extracts. In this experiment *P. conjugatum* extracts depressed growth by comparison with the effect of *P. phaseoloides* extracts, but a much more severe depression was recorded in the treatments receiving extracts of *M. cordata* (*Table 1*).

Bio-assay Tests With Fomes Lignosus

In an attempt to improve methods of detecting possible growth-inhibitory activity, the effect of *M. cordata* extracts was tested on a

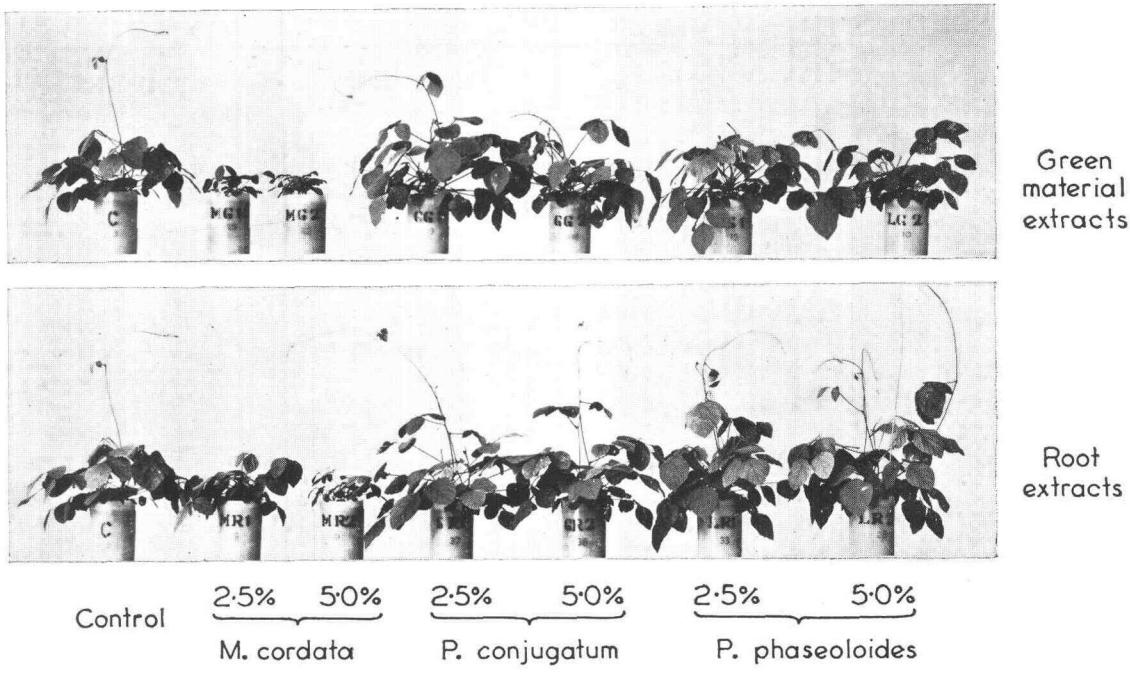

Figure 4. Effect of water extracts of oven dried M. cordata, P. conjugatum *and* P. phaseoloides *on growth of* P. phaseoloides, *after 4 weeks of treatment. 50 ml of extract per pot per week was applied at concentrations of 2.5 and 5%.*

TABLE 1. EFFECT OF WATER EXTRACTS OF OVEN DRIED GREEN MATERIAL OF MIKANIA CORDATA, PASPALUM CONJUGATUM AND PUERARIA PHASEOLOIDES ON DRY WEIGHT PRODUCTION AND NUTRIENT CONTENT OF P. PHASEOLOIDES SEEDLINGS

(Plants sampled after receiving extract treatments for 3 weeks)

Treatments	Dry weight grams per pot			Nutrient content of tops (% dry matter)		
	Tops	Roots	Nodules	Nitrogen	Phosphorus	Calcium
2.5% *P. phaseoloides* extracts	1.04	0.62	0.067	3.61	0.34	0.96
5.0% *P. phaseoloides* ,,	1.13	0.71	0.044	3.60	0.33	0.78
2.5% *P. conjugatum* ,,	0.80	0.50	0.056	3.60	0.33	1.13
5.0% *P. conjugatum* ,,	0.64	0.45	0.038	3.33	0.34	1.22
2.5% *M. cordata* ,,	0.43	0.26	0.009	3.29	0.23	0.76
5.0% *M. cordata* ,,	0.41	0.25	0.003	3.40	0.18	0.60
Control	0.95	0.61	0.066	3.95	0.30	0.91
s.e.	±0.044	±0.015	±0.0050	±0.057	±0.009	±0.020
5% l.s.d.	0.13	0.04	0.015	0.17	0.03	0.06

lower plant, *Fomes lignosus*, the fungus responsible for white root disease of rubber and used as a test organism at the Institute. Preliminary work showed that water extracts of *M. cordata* could inhibit growth of the fungus and two rapid and sensitive test techniques were developed.

Technique 1. Water soluble extracts of oven dried plant materials were incorporated into 20 ml of 2% malt agar media in standard 9 cm Petri dishes. An inoculum of *F. lignosus*, 0.2 inch in diameter, was embedded in the media at the centre of each dish and the dish was then incubated; growth of the fungus was measured across two diameters at right angles, four days after inoculation.

Technique 2. Water insoluble extracts were tested by an adaptation of the 'paper disc' method. Sterilised filter paper discs were treated with known amounts of test materials and placed on the medium at the centre of each dish. Two inocula of *F. lignosus* were embedded in the media opposite each other and at approximately equal distances from the discs. The distances between the leading edges of the growth and the edges of the discs were measured four days after inoculation.

In two preliminary tests using Technique 1, growth of the fungus was retarded when oven dried and ground roots and water extracts of oven dried green material and roots of *M. cordata*, were incorporated into the malt agar media; extracts of *P. conjugatum* and *P. phaseoloides* showed no effect. The developing edges of the mycelial mat in tests with *M. cordata*, but not those with the other plants, showed a characteristic curling up.

Water extracts of oven dried fresh leaves, stems and roots, and dead leaves and dead stems of *M. cordata* were next prepared and incorporated into the culture media at concentrations equivalent to the extracts from 1, 0.5 and 0.25 g dried material per dish. Dead roots were not available for testing as they are thin and fragile and decay too rapidly on death to be easily collected. All extracts inhibited growth of the fungus, with root extracts showing greatest activity, and leaf and stem extracts following in decreasing order; inhibition increased with increase in concentration, and total or partial lysis of the mycelium occurred at the higher levels (*Table 2* and *Figure 5*). Similar results were obtained in tests with macerated living tissue.

In further work petroleum ether extracts of oven dried roots were prepared and separated into hot-water-soluble and hot-water-insoluble fractions. Growth inhibitory activity was found to be present in both fractions, and the former was more active than water extracts of the same roots (*Table 3*); limitations of technique prevented a comparison of the hot-water-insoluble fraction with water extracts. Root residues after extraction with petroleum ether or water both showed growth inhibitory activity and to a similar degree.

Pot Incubation Tests on Nitrogen Mineralisation.

Using the technique described by GUHA AND WATSON (1958), 350 g samples of a sandy loam soil were mixed with sufficient water, or alternatively with 2.5, 5.0 and 10% water extracts of oven dried green material of *M. cordata*, *P. conjugatum* and *P. phaseoloides*, to bring the soil moisture content to one-third saturation. The soils were incubated at 32°C and sub-samples were analysed at convenient intervals for nitrate and ammonium nitrogen. Small moisture losses occurring during incubation were replaced with either water or plant extracts. Rapid ammonification took place in all treatments, followed by nitrification in the test with *P. phaseoloides* and *P. conjugatum* extracts; in the case of the former the rate of nitrification increased with increase in concentration of plant extracts but with *P. conjugatum* an increase in concentration had little effect (*Figure 6*). Extracts of *M. cordata* significantly decreased the rate of nitrification which was almost totally suppressed in the pots receiving 10% plant extracts.

Preliminary Tests to Identify Growth Inhibitory Substances

M. cordata roots have a characteristic smell indicating the presence of essential oils, and steam distillation was carried out to separate

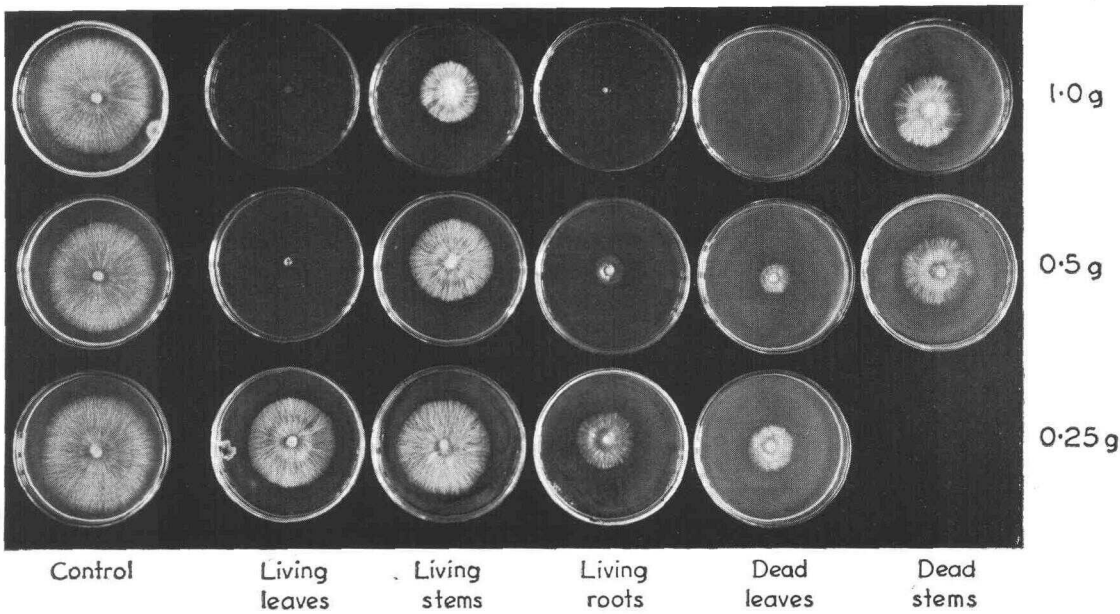

Figure 5. Effect on F. lignosus *of water extracts (from 0.25, 0.5 and 1.0 g dried material per dish) of oven dried* M. cordata *leaves, stems and roots, 4 days after inoculation.*

TABLE 2. EFFECT ON GROWTH OF FOMES LIGNOSUS OF WATER EXTRACTS OF OVEN-DRIED MIKANIA CORDATA LEAVES, STEMS AND ROOTS

(Technique 1. Measurements taken at 4 days after inoculation)

Treatments, equivalent weight of material in grams per Petri dish		Increase in diameter of *F. lignosus* inocula (cm)
Fresh leaves	1.0	0.00
	0.5	0.00
	0.25	2.03
Fresh stems	1.0	1.28
	0.5	2.88
	0.25	2.93
Fresh roots	1.0	0.00
	0.5	0.00
	0.25	0.46
Dead leaves	1.0	0.00
	0.5	0.71
	0.25	1.57
Dead stems	1.0	2.39
	0.5	2.33
	0.25	not tested
Control		4.37
s.e.		±0.1567
5% l.s.d.		(0.45)

TABLE 3. EFFECT OF WATER AND PETROLEUM ETHER EXTRACTS OF OVEN DRIED MIKANIA CORDATA ROOTS, AND THE RESIDUES AFTER SUCH EXTRACTION, ON GROWTH OF FOMES LIGNOSUS

(Measurement taken at 4 days after inoculation)

COMPARISON A

Treatment (Test using Technique 1)	Increase in diameter of *F. lignosus* inocula (cm)
Water extracts of 1.0 g roots per Petri dish†	2.53
Water-soluble fraction of petroleum ether extracts of 1.0 g roots per Petri dish	0.35
1.0 g water-extracted root residues per Petri dish	3.46
1.0 g petroleum-ether-extracted root residues per Petri dish	3.46
Control	5.61
s.e.	±0.3504
5% l.s.d.	1.04

COMPARISON B

Treatment (Test using Technique 2)	Distance between growing edge of *F. lignosus* inocula and treated paper discs (cm)
Water insoluble fraction of petroleum ether extracts of 0.9 g roots per paper disc†.	0.85
,, ,, ,, ,, ,, ,, ,, ,, 0.6 g ,, ,, ,, ,,	0.75
,, ,, ,, ,, ,, ,, ,, ,, 0.3 g ,, ,, ,, ,,	0.77
Control	0.00
s.e.	±0.017
5% l.s.d.	0.06

†10 mm diameter filter paper disc.

the essential oils. Distillates of green material smelt strongly and contained droplets of pale yellow oil which had little effect on soil nitrification and growth of tomato seedlings, but produced significant depressive effects on growth of *H. brasiliensis* seedlings. Root distillates smelt strongly and contained droplets of oil; these distillates were found to inhibit growth of *F. lignosus*, and the remnant water in equilibrium with steam distilled material was also found to show activity. These experiments are to be discussed more fully elsewhere.

Since the bio-assay test with *F. lignosus* had shown that roots were the most active portion of the plant, and that petroleum ether was the most convenient extractant, further work on the identification of these substances was carried out with petroleum ether extracts of roots. Petroleum ether extracts of oven dried fresh roots were obtained and separated into the hot-water-soluble and insoluble fractions; these were then re-extracted with petroleum ether, and dried under reduced pressure, to yield strong-smelling, brownish-yellow, viscous liquids. These liquids were studied by descending paper chromatography separation, carried out over about 18 hours: the materials were taken up in ethanol, spotted on to Whatman No. 1 filter paper, and developed with the upper phase of a 4:1:5 mixture of *n*-butanol, glacial acetic acid and water. Both

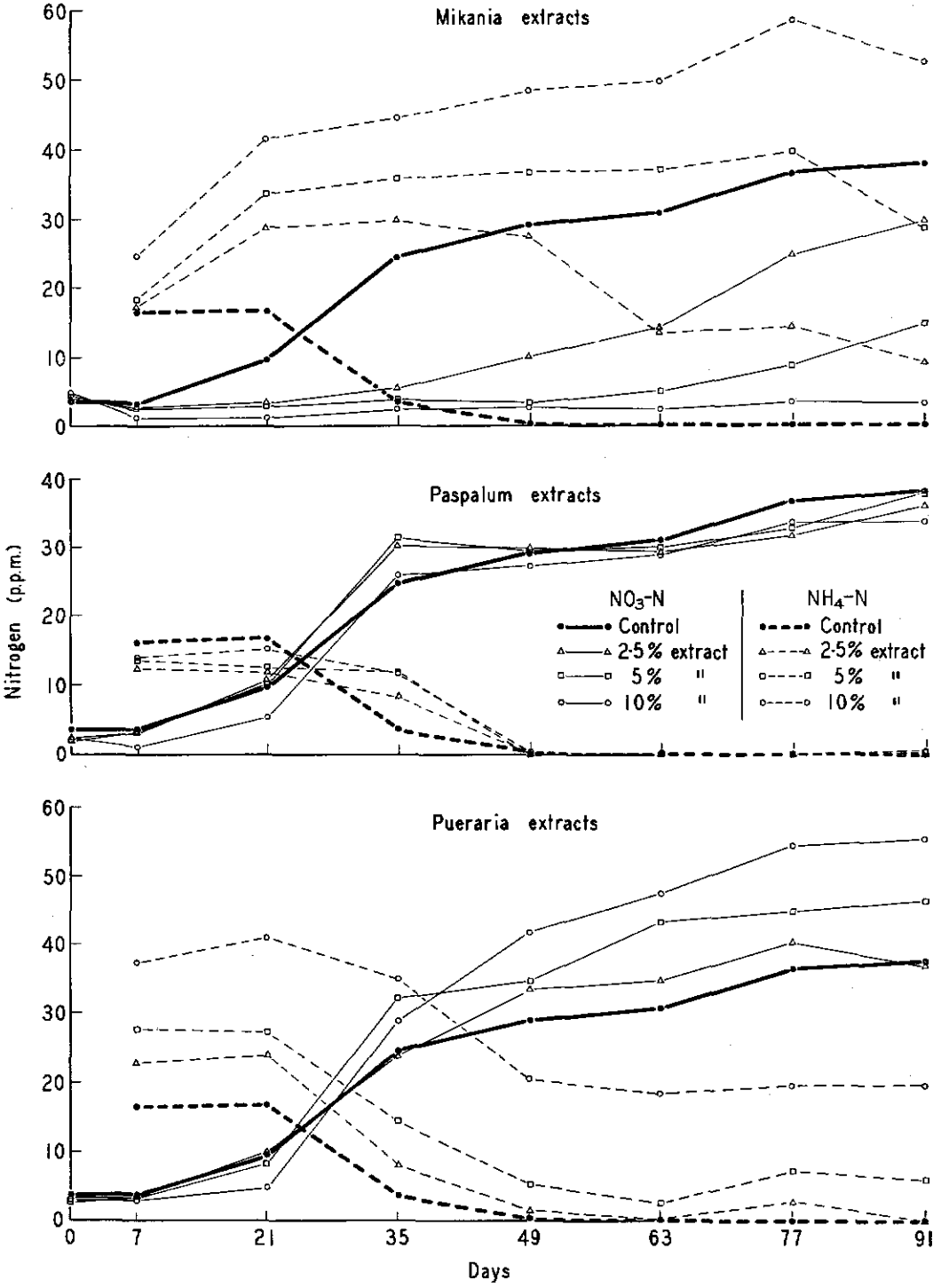

Figure 6. Effect of water extracts of oven dried green material of M. cordata, P. conjugatum *and* P. phaseoloides *on nitrification of the soil.*

chromatograms gave a single yellowish spot, Rf 0.94±0.01, that turned reddish brown, except at the central portions, after spraying with diazotised sulphanilic acid and exposure to ammonia. The spots fluoresced a pale purple on ultraviolet examination after exposure to ammonia.

It was concluded that the petroleum ether extracts contained a mixture of phenolic and flavonoid substances both of which were only slightly soluble in hot water. The unsprayed spot from the chromatogram of the hot water-insoluble fraction was eluted with ethanol and tested for activity against *F. lignosus*. Inhibitory activity was shown, and the curling of the mycelia, noted in tests with Technique 1, was again observed (*Figure 7*).

DISCUSSION

The above work seems to show quite conclusively that *Mikania cordata* contains substances which, on leaching or excretion, can depress the growth of *Hevea brasiliensis*, tomato and *Pueraria phaseoloides*, and also strongly inhibit the *in vitro* growth of *Fomes lignosus* and depress nitrification in the soil. As far as the soil microflora is concerned there is some indication of specificity in effect, for in the incubation tests *M. cordata* extracts did not suppress ammonification, and in the fungal bioassay tests contaminants sometimes thrived even where *F. lignosus* was inhibited. The fact that inhibitory activity was shown by dead material collected from the field indicates that artefacts produced in sampling and extraction were unlikely to be responsible for the observed effects, and that a true growth inhibition was involved.

The effect on nitrification observed in pot incubation tests confirms field studies in which low nitrate-nitrogen levels were found in soil under *M. cordata*, when compared with those under covers of grasses or leguminous creepers, and in which trees grown with a *M. cordata* ground cover showed depressed leaf nitrogen contents (WATSON *et al.*, 1964a). At the same time this particular effect may not occur to the same extent on all sites, nor at all times, for a sampling in two other experiments showed similar levels of nitrate-nitrogen under *M. cordata* as under leguminous creepers and grasses, and at five years after planting the leaf nitrogen contents of the trees concerned were not depressed by *M. cordata* (WATSON *et al.*, 1963). The pattern of results obtained in the field experiments seems to suggest that in the early years after planting *M. cordata* can depress soil nitrification and leaf nitrogen content, but as its vigour wanes under developing rubber, and perhaps also because the soil microbial population acquires some tolerance, the level of nitrification and leaf nitrogen content may recover to some extent. Such a suggestion would be supported by unpublished data showing that in incuba-

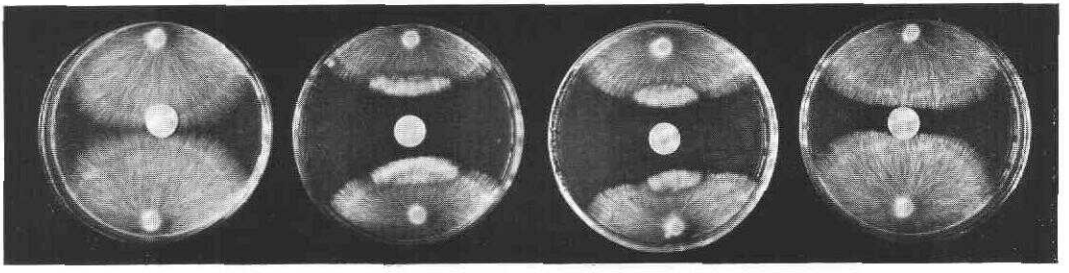

Figure 7. Effect on F. lignosus *of substances eluted from chromatogram of hot-water-insoluble fraction of the petroleum ether extract of* M. cordata *roots, at 4 days after inoculation. Eluates applied at 0.01 g, 0.001 g, 0.0001 g per disc (0.01 g eluate ≃1–2 g oven dried roots).*

tion tests soil from plots of *M. cordata* with an initiality lower nitrate-nitrogen than soil from plots of grasses and leguminous covers, subsequently showed a higher rate of nitrification over a period of 30 days—possibly because removal of the soil from the field, and probable subsequent degradation of inhibitory substances by soil microflora, permitted recovery of the previously inhibited population of nitrifiers.

The effect of *M. cordata* on phosphorus uptake by plants, as recorded in the above experiments and in field experiments, might also be the result of some effect similar to that found in the case of the nitrifiers, but more detailed studies will be required to reach any firm conclusion. However, it does seem likely that an indirect effect on the soil microflora contributes to the overall depressive effect of *M. cordata* on rubber.

Only tentative work has been carried out on the identification of the growth inhibitory substances, but it seems likely that they include phenolic and flavonoid compounds either or both of which may be responsible for the observed effects of *Mikania cordata*; growth inhibitory activity has been shown by such compounds before (NAGHSKI *et al.*, 1947; BORNER, 1960).

ACKNOWLEDGEMENTS

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