Evaluation of Potential Sources of Allelochemicals in Lalang (Imperata cylindrica)

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ABSTRACT

Aqueous leachates from fragmented lalang (I. cylindrica (L) Raeuschel var. major) dead leaves in situ, caused delayed germination in biossays of all six species of pasture legumes tested, while radicle and shoot growth were inhibited in two of the test species. Phytotoxicity was dependent on the quantity of plant residues available in the proximity of germinating phytometer species. Leachates from rhizome and root fragments exhibited strong inhibitory activity even at the 5% (w/v) level while leaf leachates were inhibitory at the 10% level and higher. In pot experiments with soil containing living lalang tillers or incorporated with rhizome or root fragments, phytotoxicity development in the soil extracts was evident at four weeks of growth or decay. In soils with decaying roots or rhizomes there was a sub-

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sequent loss in activity over the eight week incubation period. Extracts of soils with living tillers showed loss of activity at six weeks of growth followed by a significant stimulatory response at eight weeks. The use of two controls (control-soil extract and distilled water control) enabled detection of inherent stimulatory activity in the control soil. Comparison with both controls to eliminate such influences showed only living tillers, and decaying rhizomes and roots as major potential sources of inhibitory substances. The common soil organism, Bacillus cereus (Frankland & Frankland), Alcaligenes faecalis Castellani & Chalmers} and a Trichoderma sp. were found to be associated with the decay of rhizomes resulting in an initial stimulatory growth response in the phytometer species followed by inhibition and subsequent loss in activity. The nature of these organisms suggest that the release of phytotoxins through decay of rhizomes as a common, natural phenomenon in most soils. The dead leaves accumulating as litter in mature lalang vegetation may serve as an additional source of water leachable allelochemicals if sufficient quantities were available in contact with the soil.

INTRODUCTION
Lalang (Imperata cylindrica) (L.) Rübschel var. major) is a noxious weed causing serious problems in many countries (Holm et al., 1977). It presents major problems in plantation crops (Tempany, 1951) and in pastures (Cabanilla, 1949; Yoshida, 1953). Aided by fire, cultivation and other disturbance, the weed rapidly colonizes large areas to the virtual exclusion of other species, thus becoming a dominant feature in many situations in most countries where it is found.

The literature suggested that allelopathy may play an important role in the overall interference of lalang with other species (Eussen and Wirjahardja, 1973; Sajise and Lales, 1975; Eussen et al., 1976, Eussen, 1978). In the above studies involving mixed and mono-cultures, allelopathy was implicated based on the observation that the species in mixtures were not mutually exclusive. However, the methods used present difficulties in separating allelopathy from other neighbour effects (Trenbath, 1974; Dekker et al., 1983): Further, in the soil leachate experiments, continuous treatment of phytometer species with leachates for extended periods does not allow for a control where nutrient or other effects can be completely eliminated.

The phytotoxic activity in extracts of lalang plant parts have also been investigated (Dhanmanonda, 1973; Eussen and Soerjani, 1976; Eussen, 1977). However, the use of macerated tissue extracts in those studies raises the question as to whether the biologically active substances present within tissues become available in leachates of intact or fragmented residues in sufficient quantities to exert phytotoxic effects.

Eussen (1978) also demonstrated phytotoxic activity in soil leachates collected at two and four weeks following foliage removal in mature plants grown in pots, a phenomenon which inhibitory potential of the different plant parts foliage destruction by fire, herbicides, slashing or moving. However, the activity of the decaying residues beyond the fourth week and the relative inhibitory potential of the different plant parts during decay following soil incorporation are not known. Studies with decomposing plant residues indicate disappearance of phytotoxicity after one to several weeks (Cochran et al., 1977; Elliot et al., 1978). Besides environmental factors, development and extent of toxic effects are also dependent on the amount and type of residues

Toai and Linscott, 1979).

Eussen and Soerjani (1976) also implicated phytotoxins from lalang leaves in the observed growth reductions in plants grown in soils surface-mulched or incorporated with the leaves; but the possible influence of other factors (e.g. nutrients) was not discounted.

The limitations mentioned above warranted further study using alternative methods, to investigate the relative importance of potential sources of phytotoxins in lalang, and evaluate their significance.

Observations on an old lalang vegetation showed considerable accumulation of leaf on the soil surface, and an abundance of rhizomes in various stages of decay to a depth of 15 cm in the soil. Hence, under such vegetation, two most likely sources of inhibitory substances are the dead leaves accumulating and decaying on the soil surface, and decaying rhizomes just below the surface. Further, two possible mechanisms may be operative with the dead leaves (a) direct leaching out of water soluble compounds and (b) release of substances during subsequent
microbial decay. Where renovation or improvement of lalang grasslands is attempted with cultivation, seeds of sown species may be exposed to three possible sources of inhibitory substances: (a) leachates from fragmented residues, (b) chemicals released following decay of the residues, or (c) exudates from plants regenerating from rhizomes. A similar situation would exist when attempting to establish leguminous cover-crops in plantation agriculture. Seeds of species direct-drilled into chemically killed swards may be exposed to toxins leaching out of decaying rhizomes and roots, in addition to substances leaching out of surface litter. Result of investigations in phytotoxic activity in the lalang rhizosphere in relation to its growth, the influence of soil factors under field conditions, and characterization of associated phytotoxins will be reported later. This paper presents results of studies on the potential sources by which phytotoxic substances may be introduced into the soil environment.

MATERIALS AND METHODS

Information available on the nature of potential inhibitors associated with the Poaceae (and the levels of such inhibitors reported to be present in the natural environment) suggest delay or inhibition in germination and early seedling growth as the most obvious effect of pyhtotoxins associated with most species, including lalang. Hence, in all bioassays phytometer species were treated with plant or soil leachates only for the first few days of growth. This also enabled the inclusion of control treatments to differentiate nutrient and other chemical effects in bioassays where phytometer species were treated with soil leachates. In all bioassays indicator plants used were generally legume species commonly used as ground covers in plantations or for improving pastures.

The lalang materials used in all experiments were obtained from a lalang-dominant pasture on a property at Enmore (30 45′50″S, 151 45′20″E), 40 km south-east of Armidale, New South Wales, located 1000 m above sea-level. All data were statistically analysed and most parameters measured did not require transformations to stabilise the variance. The AOV comparisons presented were generally based on original data unless otherwise specified.

Experiment 1: Inhibitory Activity in Dead Leaf Leachates

The following experiment was conducted to study the effect of leachates from dead lalang leaves accumulating as surface litter, as a potential source of inhibitors during germination and early growth of the test species.

The dead lalang leaves were cut into 4 cm pieces and 1 g (3.3% moisture) samples were placed at the bottom of 90 mm diameter petri dishes. Ten ml distilled water was added and allowed to stand for 2 h. The leaves were then covered with Whatman 3 MM filter paper and 50 seeds of each species to be tested were placed on the paper. Another filter paper was placed on top of the seeds. All test species were thus placed in contact with the leachates in the presence of the leaf material to simulate the field situation. The controls had glass-wool (0.5 g) at the bottom of the dishes. All dishes were incubated at 24°C in the dark for 5 d.

The study was conducted in two sets of bioassays.


(b) In the second set T. subterraneum, T. repens, P. atropurpureus, D. uncinatum, M. sativa and L. sativa were used.

There were four replicates for each of the test species in both bioassays. In the first set, the dishes were not disturbed for the five days, at the end of which radicle and shoot lengths were measured. In the second set changes in germination rate were recorded daily for the five days.

Experiment 2: Inhibitory Activity in Green Leaf, Dead Leaf, Rhizomes and Root Leachates

The following experiment was conducted to assess the relative phytotoxic potential of leachates of the different plant parts. The primary objective
was to study their relative importance and assess activity at levels of materials that were realistic with respect to quantities available in the field.

The experiment was conducted in petri-dishes using the procedure described in Experiment 1. Lalang materials collected fresh from the field were separated into green leaves, dead leaves, rhizomes and roots; and cut into 4 cm pieces. Samples of green leaves, dead leaves and rhizomes of 0.5, 1.0, 1.5 or 2.0 g fresh weight and root samples of 0.5, 1.0 or 1.5 g were placed at the bottom of 90 mm petri dishes, 10 ml distilled water added and allowed to stand for 2 h. The control had glass-wool (0.5 g) at the bottom of the dish. Fifty seeds of *Trifolium subterraneum* cv. Woogenellup were set in each dish using the sandwiched filter paper technique described earlier. The dishes were incubated at 24°C for 72 h, at the end of which radicle lengths were measured. There were eight replicates in a completely randomised design. Moisture content of plant parts were 45.2, 3.3, 62.5 and 10.6% for the green leaves, dead leaves, rhizomes and roots, respectively.

The amount of plant materials available in the field was determined from six random 60 x 60 cm quadrat samples in an old uniform lalang vegetation at Enmore during mid-September.

**Experiment 3: Inhibitory Activity in Soils with Living Tillers and Decaying Plant Parts.**

The following experiment was conducted to study the changes in biological activity during decay of the various plant parts in a soil medium and to determine the more likely source of inhibitory substances. A living lalang tiller was included as one of the treatments for observation of inhibitory activity in the rhizosphere soil. The effects of all treatments on seedling radicle growth were evaluated on a tropical and a temperate pasture legume.

There were six soil treatments: four with the different plant parts, one with a living tiller and one control (soil only) with four replicates of each treatment. The phytotoxicity due to treatments was evaluated after 2, 4, 6 and 8 weeks of decay or growth in bioassays treated with the respective aqueous soil extracts. The levels of plant parts were 10 g per pot for fresh leaves, dead leaves or rhizomes and 2 g per pot for roots. The tillers were established from three-node rhizome cuttings, grown to a height of 5 cm in the glasshouse, and transplanted (1 tiller per pot). The fresh plant parts were prepared as described in Experiment 2, mixed into 200 g of soil and placed into 8 cm pots lined with polyethylene film. The lining had six equally spaced 2 mm perforations at the bottom to permit drainage. The soil was a 1:1 mixture of sandy loam and sand, sieved (2 mm) prior to mixing.

The pots were set in 4 blocks, with treatment pots randomized within blocks, in a growth chamber at 29°C (12 h, day) and 23°C (night). Relative humidity was maintained between 68-76% and the light density flux was 300 uE m⁻² s⁻¹. Pots were watered daily with 40 ml distilled water and no nutrients were provided.

At the completion of each incubation/growth period, 1 pot of each treatment was sampled from every block. The contents were transferred into 500 ml beakers, 200 ml distilled water added and allowed to stand for 30 min, stirred for 2 min and allowed to stand for a further 30 min. With the living tillers, plants were gently shaken to loosen soil adhering to roots and removed, prior to stirring the remaining contents. The aqueous soil extracts were filtered through glass-wool (to remove large debris), chilled to 4°C and centrifuged at 3000 rpm for 30 min.

Bioassays for phytotoxic activity were performed using the sandwiched filter paper technique described in Experiment 1, on both *Trifolium subterraneum* and *Calopogonium mucunoides*, with 40 seeds per dish. Two dishes per extract (pot), for each of the six treatments in replicates, were set at each sampling time. A distilled water control (2 x 4 dishes) was included in the bioassays to differentiate the inherent biological activity in the control soil. Five ml extract or distilled water was used per dish with *T. subterraneum* incubated for 72 h, and 7 ml was used with *C. mucunoides* incubated for 96 h, both incubations being performed at 24°C in the dark. (Preliminary studies suggested a 96 hour bioassay to be suitable for *Calopogonium* bioassay due to its slower radicle emergence, and also a larger volume of water was found to be required for that period). At the end of the incubation period dishes and contents were frozen and held for radicle length measurement. Electrical con-
ductivity and pH values of fresh extracts were also determined.

On a dry weight basis, the levels of plant parts used in this study were 2.7, 4.9, 2.0 and 0.9% (residue wt/soil wt) for the green leaves, dead leaves, rhizomes and roots, respectively. However, taking into account the 1:1 extraction, the soil extracts for the decaying green leaves, dead leaves and rhizomes were equivalent to the 5% leachates (fresh weight/volume) used in experiment 2, while the decaying root extracts were equivalent to a 1% leachate.

Experiment 4: Inhibitory Activity in Leachates of Decaying (Surface-Sterilised) Rhizomes

It was noted earlier that a large proportion of rhizomes under field conditions were at various stages of decay. The decaying rhizomes could contribute a continuing source of phytotoxins. However, the nature of the organisms responsible for the decay is not known. The following experiment was conducted primarily to (a) obtain supporting evidence for the responses observed during decomposition of rhizomes in soils (Experiment 3), and (b) to isolate and identify the microorganisms involved. Leachates of surface sterilised rhizomes allowed to decay for 1, 2, 3, 5, 9 or 13 weeks, were evaluated for phytotoxic activity in seedling biossays. There were a total of seven treatments in the biossays including a distilled water control, with four replicates per treatment.

Freshly collected rhizomes (65% moisture) were washed thoroughly with water, rinsed in distilled water, cut into 4 cm pieces, surface sterilised in 10% calcium hypochlorite solution for 3 min, and washed thoroughly with sterile distilled water. Eight g of surface sterilised rhizomes were placed in 90 mm petri dishes, 10 ml sterile distilled water was added and incubated at 28°C. The water was replenished as required at weekly intervals. A sufficient number of dishes was prepared to enable sampling at 1, 2, 3, 5, 9 and 13 weeks from the start of the experiment. At each sampling time 10 dishes from each of the four replicates were removed, the contents filtered through glass-wool and the leachates pooled. The dishes and rhizomes pieces were washed by applying sterile water dropwise to extract as much of the original leachate as possible, and the volume made up to 100 ml (total of ten dishes). The pooled leachates were filtered through 0.2 um Millipore filters.

The phytotoxicity of the leachates was evaluated, using the sandwiched filter paper technique on *T. subterraneum* and *C. mucunoides* with 40 seeds per dish. Five ml leachate or distilled water (control) was used per dish with *T. subterraneum* incubated for 72 h and 7 ml with *C. mucunoides* incubated for 96 h, both incubations being performed at 24°C in the dark. At the end of the incubation period, radicle lengths were measured.

At the end of the second week, small pieces of rhizome tissues and droplet samples of leachates were taken and cultured on Oxoid tryptone soy agar (4%) or malt-yeast extract-agar (17:1:15 g/L) containing aureomycin (5 mg/L) to obtain pure cultures of bacteria and fungi, respectively. The isolated organisms were identified by the Commonwealth Mycological Institute, England.

RESULTS AND DISCUSSION

Experiment 1: Inhibitory Activity in Dead Leaf Leachates

Inhibitory effects of dead leaf leachates on germination of the six species tested are presented in Figure 1. Germination was found to be significantly delayed in all species tested but the degree of inhibition varied between species. Percent inhibition in germination on the second day varied from 9.4% in *M. sativa* to 86.5% in *T. subterraneum*. Four of the six legume species tested showed complete recovery in percent germination by the fifth day. *M. sativa* and *D. uncinatum* recovered completely after three days and *P. atropurpureus* and *L. sativa* recovered after five days. However, *T. repens* and *T. subterraneum*, while showing trends towards recovery were still inhibited on the fifth day.

The effects of the dead leaf leachates on seedling growth are presented in Table 1. The leachate treatment had no effect on seedling growth in six of the ten species tested. In tomato seedlings both radicle and shoot growth were stimulated. Shoot and radicle growth were both significantly inhibited in the temperate legume *T. subterraneum* and in the tropical legume *C. mucunoides*. In *D. uncinatum*, shoot was inhibited while radicle growth was not affected.
Figure 1:  Effect of lalang dead leaf leachates on germination of six test species  (—— Control; ——— Dead Leaf Leachates)

vertical bars denote S.E.
### Table 1: Effect of leachates from dead lalang leaves on seedling growth of test species after 5 days

<table>
<thead>
<tr>
<th>Species</th>
<th>Radicle Length (mm)</th>
<th>Shoot Length (mm)</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Leachate</td>
<td>n.s.</td>
</tr>
<tr>
<td><em>M. sativa</em> (Hunter River)</td>
<td>26.1&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n.s.</td>
</tr>
<tr>
<td><em>T. semipilosum</em> (Safari)</td>
<td>15.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n.s.</td>
</tr>
<tr>
<td><em>T. repens</em> (Huia)</td>
<td>14.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>15.0&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n.s.</td>
</tr>
<tr>
<td><em>T. subterraneum</em> (Woogenellup)</td>
<td>21.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>18.21&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><em>D. uncinatum</em></td>
<td>16.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n.s.</td>
</tr>
<tr>
<td><em>P. atropurpureus</em> (Siratro)</td>
<td>20.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>21.3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n.s.</td>
</tr>
<tr>
<td><em>S. guyanensis</em> (Schofield)</td>
<td>21.7&lt;sup&gt;a&lt;/sup&gt;</td>
<td>19.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n.s.</td>
</tr>
<tr>
<td><em>C. mucunoide</em></td>
<td>21.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>16.6&lt;sup&gt;b&lt;/sup&gt;</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td><em>L. sativa</em> (Great Lakes)</td>
<td>31.4&lt;sup&gt;a&lt;/sup&gt;</td>
<td>31.9&lt;sup&gt;a&lt;/sup&gt;</td>
<td>n.s.</td>
</tr>
<tr>
<td><em>L. esculentum</em> (Grosse Lisse)</td>
<td>9.1&lt;sup&gt;b&lt;/sup&gt;</td>
<td>15.2&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Between treatment means with the same subscript are not significantly different at p < 0.05 (Duncan's Multiple Range Test).

n.s. = non-significant at p < 0.05.

The results suggest delay and inhibition of germination and early seedling growth as important mechanisms by which establishment of some species will be affected when seeds are located around lalang surface litter. On a per unit surface area basis, the quantity of dead leaves found under established lalang stands at Enmore was 20 to 60% higher than that used in this study. In addition, the concentration of leachates used in the study was only 10% (i.e. 1 g/10 ml). Under lower soil moisture conditions in the field, significantly higher leachate concentrations would occur and increased phytotoxic activity can be expected. Thus, under mature undisturbed lalang vegetation, the litter leachates would constitute a significant source of inhibitors though actual activity may fluctuate with environmental factors and microbial activity.

Levels of accumulation of inhibitory compounds within plant tissues are also known to vary depending on environmental factors (Koeppe et al., 1970; Hall et al., 1983). Winter killed foliage would have a different chemical composition compared to foliage dying through normal senescence. Hence, some variation in responses observed above may be expected depending on source of samples.

**Experiment 2: Inhibitory Activity in Green Leaf, Dead Leaf, Rhizome and Root Leachates**

Rhizomes and root leachates caused significant root reductions even with the 5% leachate concentration (0.5 g/10ml), whereas the green and dead leaf leachates were inhibitory with the 10% concentration and higher (Table 2).
TABLE 2
Effect of increasing levels of *Imperata* leaf, rhizome and root leachates on *Trifolium subterraneum* radicle growth (mm)

<table>
<thead>
<tr>
<th>Plant Part</th>
<th>Levels of <em>Imperata</em> leachates (g/10 ml water)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 (control)</td>
</tr>
<tr>
<td>Green leaves</td>
<td>14.86&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dead leaves</td>
<td>13.27&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Rhizomes</td>
<td>14.59&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Roots</td>
<td>15.05&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

Means within rows with the same subscripts are not significantly different at \( p < 0.05 \) (Duncan's Multiple Range Test).

With the 5% leachates the percent inhibition (% of control) obtained with the green leaves, dead leaves, rhizomes and roots were —1.6, 13.6, 20.4 and 35.5%, and with the 10% leachates the corresponding values were 24.0, 26.0, 35.7 and 44.5%, respectively. Thus, relative importance in order of decreasing phytotoxicity over these two levels was roots, rhizomes, dead leaves and green leaves.

The mean fresh weights of rhizomes, roots, dead leaves (including surface litter) and green leaves available in the field per 60 x 60 cm ground surface were 655, 55, 459 and 314 g, respectively. Thus, taking into account both the relative amounts present in the field, and the relative phytotoxic effect of each component, and assuming uniform distribution of the various plant parts within a 20 to 25 cm soil depth in the field following cultivation, the rhizomes would be the plant part most likely to exert inhibitory effects on germinating seeds. However, residues are never uniformly distributed in soils (Patrick, 1971; Cochran et al., 1977; Elliot et al., 1978). Hence, in concentrated pockets, dead and green leaves could also be potential sources of inhibitory leachates. Root leachates were relatively more phytotoxic than the other leachates, but due to the small amounts available in the field the root material by itself will not be of any significance.

**Experiment 3: Inhibitory Activity in Soils with Living Tillers and Decaying Plant Parts**

Radicle growth in *T. subterraneum* in general, showed strongest phytotoxic effects in the fourth week (Figure 2). Compared to the control soil (CS), the decaying dead leaves (DL), rhizomes (RZ) and roots (RT) all showed suppressed root growth in *Trifolium* in the fourth week, while the green leaves (GL) showed growth inhibition at the second and sixth week. With the exception of GL, the other plant parts were not inhibitory at the second, sixth or eighth week. Extracts of soils with the living tiller (LT) showed strong phytotoxic effects in the fourth week, and to a lesser degree in the second week.

However, the CS extract showed significant stimulation of *Trifolium* root growth in the second, sixth and eighth week when compared to the distilled water control (CW). In these instances the absolute inhibitory potential of a treatment will certainly be over-estimated if quantified with respect to CS particularly if the activity inherent in the soil was due to stimulatory levels of potentially inhibitory substances; and the reverse will also be true where the control soil exhibits inhibitory activity relative to the distilled water control. The use of the two controls was thus useful in differentiating inherent factors that may influence the growth response in the soil medium. Comparison with both CS and CW clearly suggest only the LT, RT and RZ treatments to have the potential for inhibitory activity.

The LT extract in the second week was inhibitory with respect to CS, but stimulatory with respect to CW. The inhibitory effect was more pronounced and significant compared to both CW and CS extracts at the fourth week when the plants were visually observed (during soil extraction) to have attained near maximum root development. There was a marked difference...
EVALUATION OF POTENTIAL SOURCES OF ALLELOCHEMICALS IN LALANG

Figure 2: Effect of aqueous extracts of soils with decaying lalang plant parts or living tillers on Tifton 85 radicle growth.

1. Control - Distilled water (DW)
2. Control - Soil (CS)
3. Dead Leaves (DL)
4. Green Leaves (GL)
5. Rhizomes (RZ)
6. Roots (RT)
7. Living Tillers (LT)

Between treatment means (within TIME) with the same superscripts are not significantly different at P < 0.05 (Duncan's Multiple Range Test).

Radicle length (mm transformed to log x + 1)
in root growth between the second and fourth week tillers. Over the six and eight weeks the foliage of tillers showed nutrient deficiency symptoms, and there was no marked increase in root development after four weeks. At eight weeks LT extract showed significant stimulation relative to both CW and CS extracts. These observations strongly suggest that inhibitory activity with the living lalang plants was associated with the plants growth activity. Release of organic substances via root exudation has been reported to be directly related to growth of the root system (Prikryl and Vancura, 1980). Over the eight weeks, it appears that the level of inhibitory substances produced was low initially, increased to a maximum at four weeks and subsequently declined to levels sufficient to exert stimulatory effects. The response was characteristic of the general concentration effect often observed with many growth-regulatory chemicals, i.e., stimulatory at low and inhibitory at higher concentrations (Kaindl, 1956; Lovett, 1982).

In relation to the amount of materials available in the field, the results of the experiments in this study suggest that the dead leaves (including surface litter) and rhizomes are the major sources of inhibitory substances in mature lalang vegetation. While the effects are short-lived with residue incorporation, the amounts of decaying materials under old undisturbed lalang stands is not a fixed quantity. The accumulation and decay of surface litter, and the death and decay of old rhizomes and roots is a dynamic process. Further, effects in the field would be the net or combined effect of all possible sources of inhibitors.

In *Calopogonium mununoides*, inhibition was observed only with the decaying root treatment at four weeks (Figure 3). The decaying rhizomes at four weeks appeared to have some inhibitory effect on growth, but the effect was not significant. The LT extracts also had no effect. Compared to *T. subterraneum*, the tropical legume appeared to be more tolerant of the inhibitory extracts.

The change in pH of the aqueous soil extracts showed a general increase at the fourth week, both with treatments involving decaying materials and the living tillers (Figure 4). The maximum pH range observed was from 7.2 (CS extract) to 8.0 (RZ extract) in the fourth week of decay or growth, but the pH increase was not directly related to inhibitory activity (Figure 2, 3 and 4). Patrick and Koch (1958) reported more acid conditions during decomposition of plant residues incorporated into soils, but the crocks containing the soil and residues were covered after watering to saturation to prevent moisture loss during the decay process. The production of inhibitors in their experiment could have been due to more anaerobic conditions under which volatile organic acids can be expected to accumulate (Gotoh and Onikura, 1971). Further, they observed loss in activity of extracts when stored without a layer of toluene on the surface, suggesting that inhibitors were volatile substances.

The pH increases observed in this study are in agreement with those observed by Kanchan and Jayachandra (1979). The change in pH of extracts observed by Patrick and Koch (1958) were on the average from 6.5 to 5.0, and that observed by Kachan and Jayachandra (1979) was from 7.5 to 8.3. In both these studies it was demonstrated that pH changes had no direct effect on growth of the test species.

The electrical conductivity (EC) values were measured to monitor salinity and ionic concentrations in the extracts (Giffin and Jurinak, 1973) in order to ensure that it was well within levels that would not exert any direct effects on the bioassays. Significant changes in EC values were observed with all plant residues and there was a general rise in EC values from two to eight weeks of decay or growth (Table 3). Highest values were obtained in extracts of soils containing the green leaves. In all cases, the values recorded were well below the levels reported to have any direct effect on seedling growth (Patrick et al., 1964). The LT extracts which were strongly inhibitory to *T. subterraneum* radicle growth during the fourth and stimulatory during the eighth week) showed no difference in EC from corresponding CS extracts at two, four, six or eight weeks. The increase in EC values was also not directly related to relative inhibitory activity of decaying plant parts.

**Experiment 4: Inhibitory Activity in Leachates of Decaying (Surface-Sterilised) Rhizomes**

Leachates of surface sterilised decaying rhizomes showed initial stimulation, followed by inhibition
TABLE 3
Influence of decaying lalang plant parts and living tillers on electrical conductivity values of soil extracts

<table>
<thead>
<tr>
<th>Time (weeks)</th>
<th>Control Soil (CS)</th>
<th>Dead Leaves (DL)</th>
<th>Green Leaves (GL)</th>
<th>Rhizomes (RZ)</th>
<th>Roots (RT)</th>
<th>Living tillers (LT)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>0.107&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.160&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.320&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.121&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.292&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.123&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>4</td>
<td>0.134&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.233&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.323&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.277&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.280&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.140&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>6</td>
<td>0.177&lt;sup&gt;d&lt;/sup&gt;</td>
<td>0.199&lt;sup&gt;cd&lt;/sup&gt;</td>
<td>0.361&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.236&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.305&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.197&lt;sup&gt;ed&lt;/sup&gt;</td>
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<tr>
<td>8</td>
<td>0.278&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.298&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.445&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.390&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.381&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.266&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

<sup>1</sup>Electrical Conductivity in mS cm<sup>-1</sup>
Between treatment means within rows with the same subscripts are not significantly different at p < 0.05 (Duncan’s Multiple Range Test).

As decay progressed to the ninth week, and loss in activity at 13 weeks in both test species (Table 4). The results suggest a gradual increase in growth-regulatory substances to inhibitory levels at around nine weeks, and subsequent inactivation of these substances within the next four weeks. The growth effects on <i>T. subterraneum</i> over the first nine weeks was typical of the concentration effect on biological responses by growth-regulatory substances including allelochemicals (Kaindl, 1956; Lovett, 1982).

The longer time taken for the production of substances at inhibitory levels, compared to the experiment 3 is probably due to the need for the microbial population to build-up to sufficient levels from the inoculum within the tissues.

In the <i>Calopogonium</i> bioassay, significant stimulation in radicle growth was only obtained with the fifth week leachates, while <i>Trifolium</i> responded earlier. This slight delay in the stimulatory response suggests that slightly higher concentrations were required to exert the growth effect on <i>Calopogonium</i>. The greater tolerance of <i>Calogonium</i> to the inhibitory substances was also suggested by the results with decaying plant parts in soils.

The organisms isolated from the decaying rhizomes were <i>Bacillus cereus</i> (Frankland and Frankland) and <i>Alcaligenes faecalis</i> (Castellani and Chalmers), both non-pathogenic bacteria, very common in soils; and a fungus, <i>Trichoderma</i> sp., a very common saprophyte in soils and on

TABLE 4
Effect of leachates from decaying rhizomes on root growth response in <i>Trifolium subterraneum</i> and <i>Calopogonium mucunoides</i>

<table>
<thead>
<tr>
<th>Species</th>
<th>Period of decay (weeks)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>&lt;i&gt;T. subterraneum&lt;/i&gt;</td>
<td>9.13</td>
</tr>
<tr>
<td>&lt;i&gt;C. mucunoides&lt;/i&gt;</td>
<td>3.06</td>
</tr>
</tbody>
</table>

<sup>1</sup>Reduction (+) or Stimulation (-) in radicle length expressed as percent of control.

* Significant at p < 0.05 (compared to distilled water control).
** Significant at p < 0.01 (compared to distilled water control).
1. Control - Distilled water (CW)
2. Control - Soil (CS)
3. Dead Leaves (DL)
4. Green leaves (GL)
5. Rhizomes (Rz)
6. Roots (RT)
7. Living tillers (LT)

Between treatment means (within TIME) with the same superscripts are not significantly different at p < 0.05 (Duncan's Multiple Range Test).

Figure 3: Effects of aqueous extracts of soils with decaying lalang plant parts or living tillers on Calopogonium mucunoides radicle growth.
1. Control - soil (CS)
2. Dead leaves (DL)
3. Green leaves (GL)
4. Rhizomes (Rz)
5. Roots (RT)
6. Living tillers (LT)

Between treatment means (within TIME) with the same superscripts are not significantly different at p 0.05 (Duncan's Multiple Range Test).

Figure 4: Influence of decaying lalang plant parts and living tillers on pH of soil extracts.
woody tissues. Hence, the production of phytotoxic substances through decay of rhizomes is likely to be a common and natural phenomenon in most soils.

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