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ALLELOPATHIC POTENTIAL OF *Macaranga tanarius* (L.) MUELL.–ARG.

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Abstract—*Macaranga tanarius* is widely distributed in the abandoned lowlands of Taiwan where substantial amounts of leaves accumulate on the ground. A unique pattern of weed exclusion underneath trees is often found and thought to result from allelopathic interactions. Density-dependent phytotoxicity analysis of *Lactuca sativa* L. (lettuce) growing in soil mixed with the powder of *M. tanarius* leaves showed a significant deviation from the expected yield–density relationship. Lettuce growth was most suppressed in the low seed density experiment suggesting that the phytotoxins produced during leaf decomposition inhibit the growth of lettuce seedlings. *Bidens pilosa* and *Leucaena leucocephala*, growing in soil mixed with the leaf powder of *M. tanarius* were also suppressed. Aqueous leaf extracts were bioassayed against lettuce and *B. pilosa*, and exhibited a significant suppression in radicle growth. Compounds identified from leaves included nymphaeol-A (**1**), nymphaeol-B (**2**), nymphaeol-C (**3**), quercetin (**4**), abscisic acid (ABA) (**5**), blumenol A (**6**), blumenol B (**7**),

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roseoside II (**8**), tanariflavanone A (**9**), and tanariflavanone B (**10**). ABA was the major growth inhibitor. At concentrations of 20 ppm, ABA suppressed lettuce germination, while at 120 ppm it inhibited the growth of *Miscanthus floridulus*, *Chloris barbata*, and *Bidens pilosa*. At 600 ppm, quercetin, blumenol A, and blumenol B, caused 20–25% inhibition of radicle and shoot growth of *M. floridulus*. The amount of ABA in *M. tanarius* leaves was approximately 3–5 μg g^{-1} dry weight, significantly higher than previously reported. We conclude that the pattern of weed exclusion underneath stands of *M. tanarius* and its invasion into its adjacent grassland vegetation results from allelopathic interactions.

Key Words—Phytotoxicity, allelopathy, allelochemicals, *Macaranga tanarius*, abscisic acid, fallen leaves, prenylflavanones, quercetin, blumenols, nymphaeols.

INTRODUCTION

Allelopathic compounds are released from plants that can be beneficial or detrimental to the growth of receptor plants. The source of allelopathic compounds includes decomposition and leaching of plant debris, volatilization, leaf leachate, or root exudates (Putnam and Tang, 1986; Chou and Leu, 1992; Chou et al., 1998; Mallik and Pellissier, 2000; Chaves et al., 2001). In habitats with limiting environmental conditions, dominant species can suppress the growth of neighboring plants and compete for more resources by releasing allelochemicals into the environment. Allelopathy has increasingly been recognized as playing an appreciable role in plant dominance, succession, formation of plant communities and climax vegetation, and crop productivity (Muller, 1969; Rice, 1984; Chou, 1999a).

Macaranga tanarius, an endemic species, is commonly distributed in abandoned areas throughout Taiwan. It is an evergreen species, but leaf-fall may take place at any time of year, resulting in large quantities of fallen leaves underneath tree stands. *M. tanarius* is an early succession tree and often spreads into adjacent grasslands, resulting in a secondary forest with few other species growing in the understory. *Alocasia macrorrhiza* is common beneath *M. tanarius* stands. On the basis of field observation, it was hypothesized that fallen *M. tanarius* leaves might release allelopathic compounds that inhibit the growth of weeds adjacent to *M. tanarius* stands. Experiments based on bioassay and the yield–density relationship for lettuce and *A. macrorrhiza* were conducted to evaluate the allelopathic potential of *M. tanarius*. Bioassay of the responsible phytotoxic compounds released from the plant was conducted in order to confirm the allelopathic effect.

METHODS AND MATERIALS

Study Sites. Four study sites were selected for field experiments. Data were obtained from two sites, namely Yuansan and Chauchou, which were previously

abandoned by the Taipei Water Department and by local farmers, and representing different climate types. The Yuansan site is located in Taipei City and belongs to the northeast humid zone, while the Chauchou site is located in Pingtung County in southern Taiwan and has a long drought season in winter. Precipitation in southern Taiwan is usually concentrated in the summer. Typhoons frequently hit Taiwan from August to October. Northeastern Taiwan receives a substantial amount of rainfall in winter in addition to summer rain; however, in the south it is often dry in autumn and winter.

Field Observation. M. tanarius. is widely distributed over the island and commonly found in thickets forming pure secondary forests in abandoned fields at low elevations. *M. tanarius* is a fast-growing, light-demanding species with the characteristics of early seral trees. A unique pattern, with relatively few species growing in the understory, is found in the Yuansan site (Figure 1A), and the pattern is particularly pronounced in the Chauchou site in southern Taiwan during the winter season (Figure 1B). Light intensity, relative humidity, and temperature underneath *M. tanarius* stands and its adjacent grassland were measured by a light intensity logger (StowAway), and a relative humidity and temperature logger (HOBO H8 Pro Series). Light intensity ranged from 20 to 110 μmol $m^{-2}s^{-1}$ at noon in summer under the canopy of *M. tanarius* stand, while it was 220–240 μmol $m^{-2}s^{-1}$ in its adjacent grassland. The temperature underneath the *M. tanarius* stand was 29–32°C, and 36–38°C outside of the stand. Relative humidity ranged from 47 to 52% under *M. tanarius*, higher than in that grassland (40–42%), indicating that soil moisture was not a limiting factor in regulating the understory species (Tseng, 2001).

Sampling and Field Measurement. In order to understand the influences of the invasion of *M. tanarius* into grassland, the vegetative compositions beneath the canopy and the grasslands were compared. In each site, 20 plots (1×1 m^2 each) were randomly selected in *M. tanarius* stands and 20 plots in the vicinity adjacent to the *M. tanarius* stand. Plant species in each plot were identified, and the coverage of each species per plot was measured.

Greenhouse Pot Experiments. Seedlings of *Bidens pilosa* and *Leucaena leucocephala* were planted in pots containing a mixture of *M. tanarius* fallen leaf powder and treated soil (1 g leaves/30 g soil), or untreated soil (vermiculite-perlite-peat moss, 3:1:1, v/v/v) as control. Each set of experiments consisted of three replicates. Pots were placed into a greenhouse of Academia Sinica, Taipei, Taiwan.

Density Dependent Phytotoxic Effects. Treated soil mixed with or without the powder of fallen leaves was transferred into $30 \times 36 \times 10$ cm^3 plastic seed trays. Lettuce and *Alocasia macrorrhiza* seeds were sown in the trays and the emerging seedlings were thinned to allow three levels of density, namely low (4500 seedlings m^{-2}), medium (8500 seedlings m^{-2}), and high (17,500 seedlings m^{-2}) for lettuce, respectively. Each treatment was set with three replicate trays and placed in the greenhouse. Approximately 5 weeks after treatment, plants were

FIG. 1. The understory of *M. tanarius* at the Yaunsan site (A) and the Chauchou site (B), showing the relatively few species present, except *Alocasia macrorrhiza*.

harvested and soil was carefully washed out of roots. Roots and shoots were separated, oven-dried, and weighed.

Preparation of Aqueous Extracts. Fallen leaves were allowed to air dry, then ground to powder for water extraction. A series of aqueous extracts, 0.5 (0.5 g plant sample + 99.5 ml distilled water), 1.0, 1.5, and 2.0% were prepared as described by Chou and Muller (1972) and Chou (1999b). The ionic concentration of the aqueous extract was determined by using a Fiske Osmometer (model G-66). The osmolarity of the 0.5, 1.0, 1.5, and 2.0% aqueous extracts of *M. tanarius* fallen leaves was 20, 24, 26, and 32 milliosmols, respectively.

Bioassays. To determine the allelopathic potential of *M. tanarius*, aqueous extracts of fallen leaves were bioassayed using lettuce and *Bidens pilosa* as test plants. Extracts were bioassayed separately by a modification of Muller's standard sponge bioassay as described by Chou (1999b). In addition, a chromatographic bioassay described by Chou (1999b) was employed to determine the presence of phytotoxic compounds in the ether fraction of the aqueous extract. Washed paper strips spotted with a methanol solution of the ether fraction or an unspotted control strip were developed simultaneously with 2.0% acetic acid. Phytotoxicity was evaluated by bioassaying relevant R_f segments of the paper chromatogram. The pure or crude compounds were dissolved in spectroscopic grade methanol to prepare variable concentrations, and spread onto silica gel TLC sheets (1.5×5 cm^2). The methanol was allowed to evaporate completely in a laminar flow hood. Prior to bioassay, the TLC sheets were placed into Petri dishes and moistened with distilled water, then surrounded by wet sponges without contact. The test seeds were soaked in distilled water for 2 hr, and then placed onto the TLC sheets containing the pure compound or onto untreated control sheets. Petri dishes were sealed with parafilm "M" (American National Can^{TM}), and placed in an incubator at 25°C for 48 hr in the dark.

Extraction and Isolation. Four kilogram of crushed *M. tanarius* fallen leaves were twice extracted with 100 l MeOH at room temperature for 7 days. The extract was evaporated *in vacuo*, the residue was suspended in 1 l H_2O, and partitioned with hexane, ethyl ether, and finally with butanol. Extracts were chromatographed on silica gel (Merk 70-230, 230-400 mesh, ASTM). Crude compounds from the ether fraction were eluted with 25–100% ethyl acetate in dichloromethane. Further purification was accomplished with Sephadex LH 20, TLC, HPTLC, and HPLC [Merk Lichro CART 250-10 Cat. 1.50179 Lichrosorb Si 60 (7 μm)]. For each separation, the fractions obtained were concentrated to dryness *in vacuo* at 40 ± 2°C, then bioassayed against lettuce. The isolation procedures of the phytotoxic fraction are schematically presented in Figure 2.

Identification. All purified compounds were subjected to spectroscopic identification using IR (Perkin-Elmer 983G Spectrophotometer), ^{1}H and ^{13}C NMR (Varian Unity Plus 400 spectrometer), MS (JEOL JMS-HX 300 mass spectrometer), and specific rotations (JASCO DIP-1000 digital polarimeter), respectively.

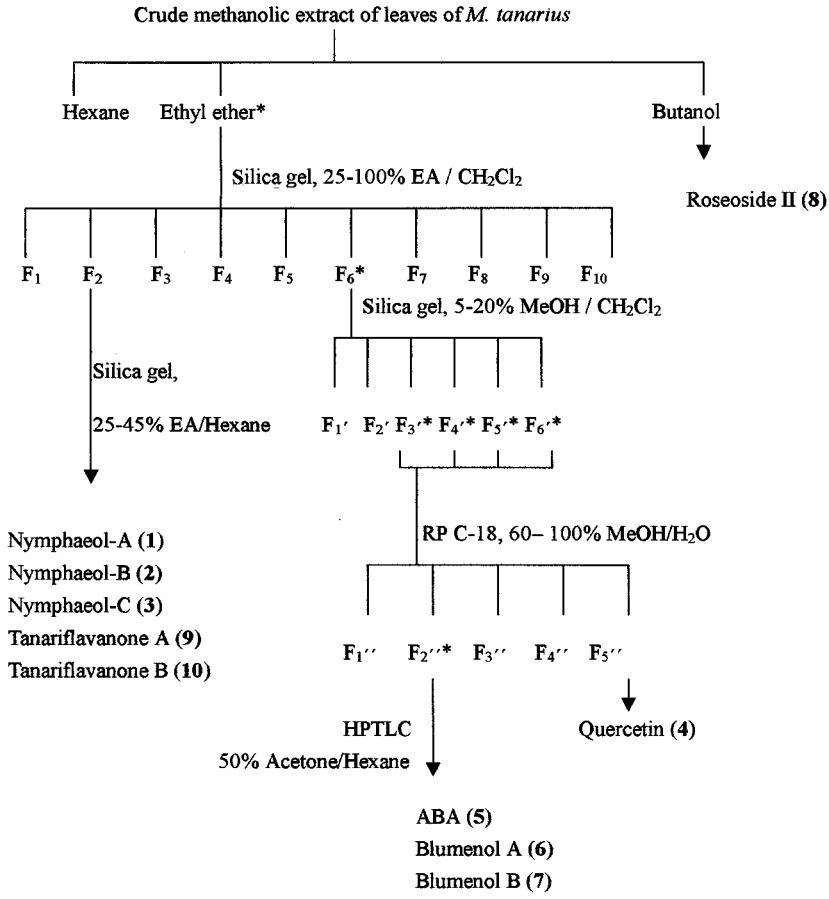

FIG. 2. Scheme for isolating phytotoxic active components from crude methanolic extract of *M. tanarius* leaves. *High phytotoxic active fraction.

Statistics. Data were analyzed by the Duncan's multiple range test (Gomez and Gomez, 1976), *t* test, and SAS for Windows v. 6.12.

RESULTS

Comparison of Vegetation Composition. The floristic composition and absolute coverage of each species per plot in *M. tanarius* stands and the adjacent grassland areas are given in Table 1. The vegetation composition on the *M. tanarius* floor at the two sites was similar, but the grasslands at the two sites were different.

TABLE 1. FLORISTIC COMPOSITION AND COVERAGE OF SPECIES PRESENT IN CHAUCHOU AND YAUNSAN STUDY SITES

Species name	Actual coverage (%)/m^2			
	Chauchou		Yaunsan	
	Grassland	*M. tanarius*	Grassland	*M. tatarius*
Aeschynomene indica	20.0	-	-	-
Alocasia macrorrhiza	-	43.0	6.0	61.1
Alpinia formosana	-	-	-	6.3
Areca catechu	-	-	-	0.3
Bidens pilosa	70.0	-	-	-
Broussonetia papyifera	-	1.0	-	-
Callicarpa remotiserrulata	-	-	-	5.0
Cassia mimosoides	2.0	-	-	-
Cenchrus echinatus	0.3	-	-	-
Chloris barbata	5.0	-	-	-
Christella acuminata	-	0.7	-	7.0
Cinnamomum camphora	-	0.07	-	-
Crassocephalum rabens	-	-	10.0	-
Cyperus rotundus	-	0.4	-	-
Elaeocarpus serratus	-	-	-	11.5
Eragrostis amabilis	0.3	-	-	-
Euphoria longana	-	-	-	1.5
Ficus pumila	-	-	-	1.5
Ipomoea batatas	-	-	1.0	-
Ipomoea digitata	-	-	4.5	0.4
Ipomoea obscura	7.0	0.2	5.0	-
Lygodium japonicum	-	-	-	1.1
Macaranga tanarius	-	-	4.5	-
Machilus kusanoi	-	-	4.0	5.7
Mallotus paniculatus	-	-	-	2.3
Malvastrum coromandelianum	5.0	-	-	-
Melanolepis multiglandulosa	3.3	6.0	-	-
Mimosa invisa	35.0	-	-	-
Mimosa pudica	3.3	-	-	-
Momordica charantia	1.0	-	-	-
Miscanthus floridulus	7.0	-	50.0	
Murraya paniculata	-	-	-	2.1
Musa sapientum	-	-	30.0	-
Oplismenus compositus	-	2.0	50.0	-
Panicum repens	4.0	-	-	-
Paspalum conjugatum	-	0.7	81.0	-
Passiflora foetida	3.0	-	-	-
Pennisetum alopecuroides	5.0	-	-	-
Peristrophe japonica	-	-	20.0	-
Polygonum chinense	-	-	10.0	-
Psychotria rubra	-	-	-	5.3
Pueraria lobata	5.0	0.48	-	-

TABLE 1. CONTINUED

Species name	Actual coverage (%)/m^2			
	Chauchou		Yaunsan	
	Grassland	*M. tanarius*	Grassland	*M. tatarius*
Rhaphidophora aurea	-	6.6	-	40.0
Rhynchelytrum repens	5.0	-	-	-
Schefflera octophylla	-	-	-	8.6
Solanum capsicastrum	-	0.4	-	-
Typhonium divaricatum	-	0.004	-	-
Wedelia chinensis	-	0.07	-	-
Total coverage (%)	196.2	62.0	276.0	159.7
% of grassland control	100.0	32.0	100.0	58.0
Number of species	18.0	14.0	12.0	16.0

Note: "-" indicates that plant was not found.

Most plants under *M. tanarius* canopy were shade-tolerant plants; for example, the mean coverage of dominant species *Alocasia macrorrhiza* was above 40%, and *Christella acuminata* and *Rhaphidophora aurea* were also dispersed on the floor of *M. tanarius* stands at the two sites. In the grassland plots at the Chauchou site, the coverage of *B. pilosa* and *Mimosa invisa* reached 70 and 35%, respectively. *Aeschynomen indica*, *Chloris barbata*, *Malvastrum coromandelianum*, *Pennisetum alopecuroides*, *Rhaphidophora aurea*, and *Rhynchelytrum repens* were found. Coverage of *Miscanthus floridulus* and *Oplismenus compositus* was significantly higher than 50%, and *Peristrophe japonica*, *Polygonum chinense*, *Musa sapientum*, and *Crassocephalum rabens* were also found at the Yaunsan site. Some seedlings of *Machilus kusanoi* were dispersed under the *M. tanarius* stand at the Yaunsan site, but were absent in the Chauchou site. Differences could be due to different weather conditions at the two sites. It is concluded that total coverage of understory species on the *M. tanarius* floor was significantly lower than that of the grassland area at the two sites (Table 1).

Density-Dependent Phytotoxicity. Lettuce growth was reduced in soil containing *M. tanarius* leaf powder (Table 2). The highest reduction of lettuce growth was observed in the low-density treatment. Total dry weight per plant was reduced by 68% in soil containing leaf powder compared to the control (Table 2). Growth was reduced by 55% in the medium-density treatment, and only by 23% in the high-density treatment. The results of this experiment may be better understood by studying the slope of the log yield–log density relationship. Harvesting at the 5th week, the slope was reduced in the soil mixed with *M. tanarius* leaves as compared to the soil alone control (0.004 vs. -0.53, Figure 3A), showing significance below the 5% level ($F = 44.86$). In contrast, there was no reduction of slope in the growth of *A. macrorrhiza* in soil mixed with *M. tanarius* leaf powder (-0.46

TABLE 2. GROWTH PERFORMANCE OF LETTUCE SEEDLINGS AT THREE DENSITIES IN SOIL CONTAINING *Macaranga tanarius* LEAF POWDER OR SOIL ALONE AS CONTROL

Seedling density (no./m^2)	Dry weight per plant (mg)				% reduction of control
	Soil	Shoot	Root	Total ± SE	
Low (4500)	Soil (control)	8.00 a	2.70 a	10.65 ± 0.07	
	Soil + leaf powder	2.20 d	1.20 b	3.45 ± 0.07	68 a
Medium (8500)	Soil (control)	4.75 b	2.40 a	7.10 ± 0.14	
	Soil + leaf Powder	2.05 d	1.15 b	3.20 ± 0.28	55 a
High (17,500)	Soil (control)	3.05 c	1.45 b	4.55 ± 0.21	
	Soil + leaf Powder	2.35 d	1.20 b	3.50 ± 0.71	23 b

Note: Means in the column followed by the same letter are not significantly different at the 0.05 level by Duncan's multiple range test. The measurements were taken 5 weeks after planting.

vs. −0.44, Figure 3B), indicating that the growth of this plant was not affected by phytotoxins released by *M. tanarius*.

Phytotoxicity of Aqueous Extracts. Aqueous extracts (0.5, 1.0, 1.5, and 2.0%) of *M. tanarius* leaves were bioassayed by using lettuce and *B. pilosa* seeds as test material. In general, radicle growth of lettuce was suppressed by the extract at as low as 0.5%, and the inhibition increased with greater concentrations (Figure 4). The same inhibition was observed when *B. pilosa* grew in the aqueous extract of *M. tanarius* leaves (Figure 4). Radicle growth of both lettuce and *B. pilosa* was suppressed by increased concentrations of the aqueous leaf extract. The 2.0% aqueous extract of *M. tanarius* leaves caused 95 and 70% inhibition of the radicle growth of lettuce and *B. pilosa*, respectively. The aqueous extract was not active at the 0.5% concentration for *B. pilosa*, and more nutrients were in the extract than in the pure water control. Therefore, response of the low concentration of extract promoted the growth of *B. pilosa*. One might question whether the inhibition might be due to the effect of osmolarity and pH of the solutions. The osmolarity of the 0.1% extracts of *M. tanarius* leaves was 18 milliosmols, while that of 2.0% extracts was 32 milliosmols. The pH of the 0.1–2.0% aqueous extracts ranged mostly above 4.0 (Tseng, 2001). Chou and Young (1974) indicated that osmolarity of extracts below 30 milliosmols would not cause a significant osmotic inhibition, but a pH below 4.0 or above 8.0 did cause significant inhibition of lettuce radicle growth. They also indicated that when the osmolarity of extracts exceeds 50 milliosmols, it might cause 20–30% inhibition. In the present study, even at 2.0%, the osmolarity of extracts was below 35 milliosmols, and the pH ranged from 4.0 to 4.5. The 2.0% (or above) extract caused both osmotic inhibition and phytotoxic effects; however, the osmotic inhibition was about 20% while the total inhibition was up to 70%. Thus, phytotoxic inhibition was about 50% (70–20%). In addition to bioassays of the aqueous extract, soil containing *M. tanarius* leaf powder also suppressed the growth of *B. pilosa* and *L. leucocephala* (Figure 5).

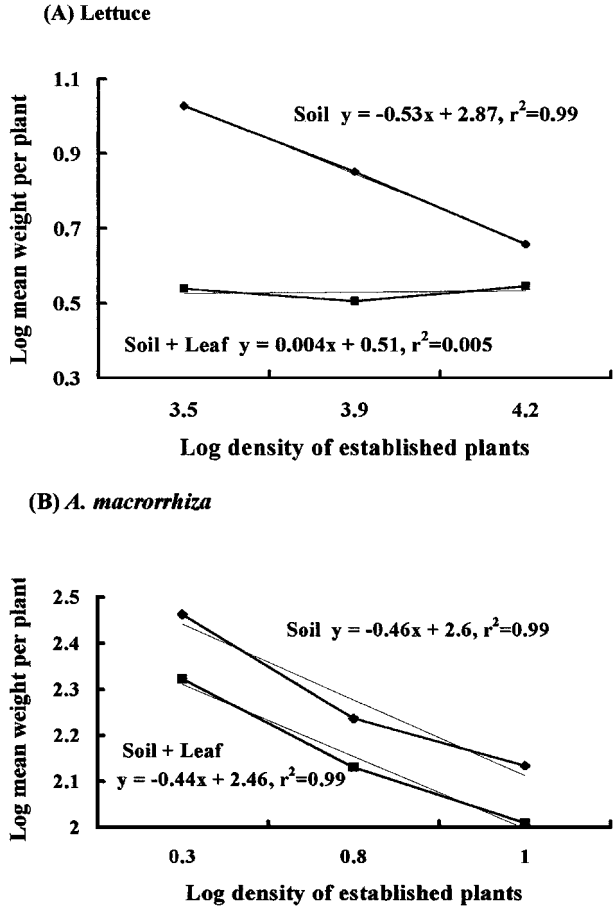

FIG. 3. Relationship between log mean weight per plant and log plant density for lettuce and *A. macrorrhiza* grown in soil containing leaf powder and soil alone: (A) lettuce, (B) *A. macrorrhiza.* Solid dark lines indicate the observational data while the light lines indicate the statistical linear regression on the basis of experimental data.

Phytotoxicity of Ether Extracts. The result of chromatographic bioassay of the ether fraction of an aqueous extract of *M. tanarius* leaves showed significant suppression of radicle growth at R_f 0.42–0.49 and R_f 0.76–0.84 (Figure 6). These findings led us to isolate phytotoxins from the ethyl ether fraction (Figure 2). Silica gel filtration of the ethyl ether fraction (80 g) gave 10 fractions (F_1–F_{10}). Fraction F_6 showed the most inhibition of lettuce germination. Further purification of F_6 using silica gel resulted in six fractions ($F_{1'}$–$F_{6'}$). At 500 ppm, fractions $F_{3'}$–$F_{6'}$ inhibited lettuce seed germination (Tseng, 2001). The active fractions ($F_{3'}$–$F_{6'}$)

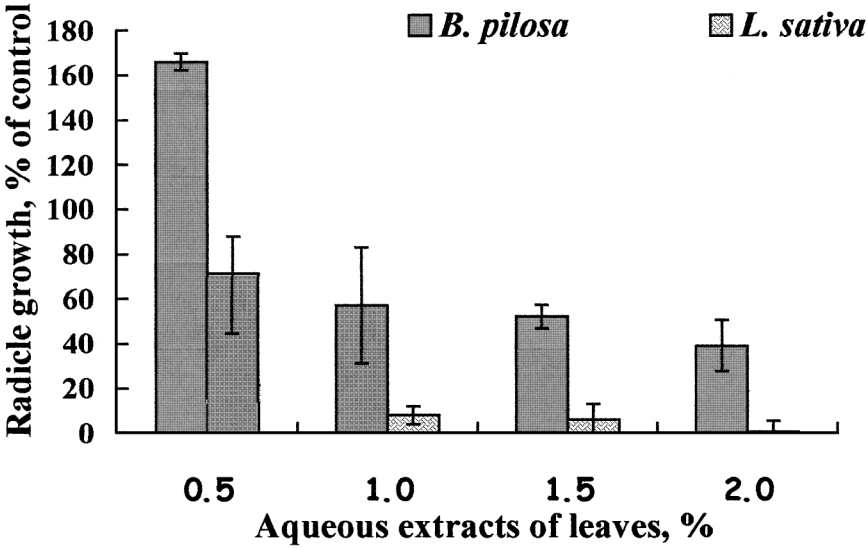

FIG. 4. Effect of aqueous extracts (0.5–2.0%) of *M. tanarius* leaves on radicle growth of lettuce and *B. pilosa*.

were combined and further fractionated by reverse-phase chromatography into 5 fractions ($F_{1''}$–$F_{5''}$). Fraction $F_{2''}$, at 500 ppm, exhibited the most inhibitory effect on germination. Fraction $F_{2''}$ was further purified by HPTLC, and three bands were visualized. The pure compound from the first band (R_f value 0.3) was eluted and bioassayed against lettuce. Lettuce germination was remarkably suppressed by the isolated compound at 20 ppm (Tseng, 2001).

Identification. The spectroscopic data were checked with those of Yakushijin et al. (1980) and confirmed the structure of compound **1** as nymphaeol-A, compound **2** as nymphaeol-B, and compound **3** as nymphaeol-C (see Figure 7). The spectroscopic data for compound **4** were in agreement with Lin et al. (2000) and confirmed the structure as quercetin (Figure 7). The spectroscopic data of compound **5** agree with Ohkuma et al. (1965) and Constantino et al. (1989) and confirm the structure of the first band (R_f value 0.3) as abscisic acid (ABA) (Figure 7). The spectroscopic data obtained for compound **6**, and **7** agree with Galbraith and Horn (1972) and confirm the structures as blumenol A and blumenol B, respectively (Figure 7). The spectroscopic data of compound **8** agree with Otsuka et al. (1995) and confirms the structure as roseoside II (Figure 7).

Phytotoxicity of Pure Compounds. Pure compounds isolated from *M. tanarius* leaves, namely quercetin (**4**), abscisic acid (**5**), blumenol A (**6**), blumenol B (**7**), and roseoside II (**8**) were prepared separately at various concentrations and bioassayed against *Miscanthus floridulus*, *Bidens pilosa*, and *Chloris barbata.* Results

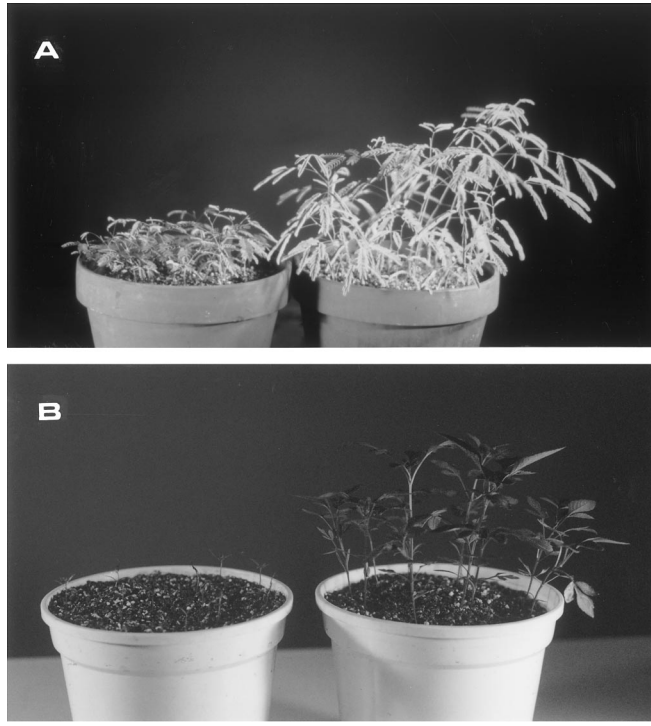

FIG. 5. Comparison of inhibitory effect of soil mixed with leaf powder (left) and soil alone as control (right) on the seedling growth of *L. leucocephala* (A) and *B. pilosa* (B). The measurement was taken 1 month after planting.

varied with different compounds (Table 3). ABA (**5**) exhibited the most inhibitory effect. At a concentration of 120 ppm, ABA caused about 75% inhibition of shoot growth of *M. floridulus* and *C. barbata*, and about 55% inhibition of radicle growth of both weeds. At 40 ppm, the radicle growth of *B. pilosa* was inhibited up to 45% compared to the distilled water control. The other compounds, including quercetin, blumenol A, and blumenol B, at 600 ppm showed 20–25% inhibition of radicle and shoot growth of *M. floridulus* (Table 3). Nymphaeol-A (**1**), nymphaeol-B (**2**), nymphaeol-C (**3**), and roseoside II (**8**) did not inhibit lettuce or weed germination (data not shown).

DISCUSSION

Analysis of yield–density relationships can be used as a tool to understand the resource competition and allelopathic interference between plants prior to a

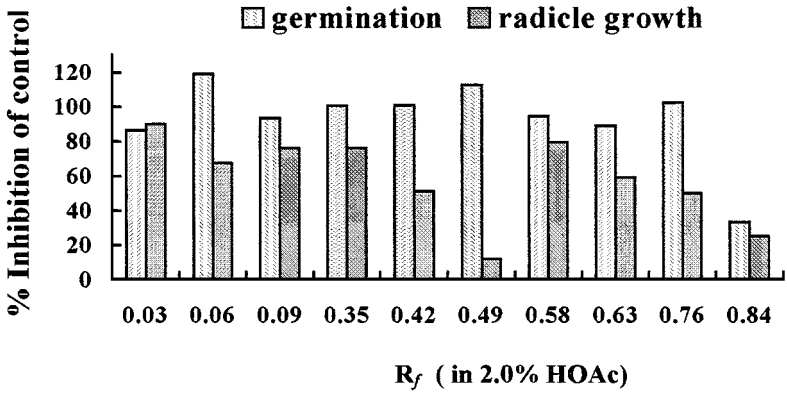

FIG. 6. Chromatographic bioassay of compounds isolated from the ether fraction of aqueous extract of *M. tanarius* leaves. The corresponding R_f segments of chromatogram with or without spotting extract were used as test and control, respectively. The paper strip was developed with 2.0% acetic acids.

prolonged and expensive phytochemical investigation of a suspected toxic species (Weidenhamer et al., 1989). As plant density increases, total yield increases linearly. Beyond a certain density, reductions of individual plant growth caused by density-dependent mortality and resource competition result in a constant total yield per unit area (Kira et al., 1953). Studies of both allelochemicals and herbicides have shown that phytotoxic effects are density-dependent (Skipper, 1966; Hoffman and Lavy, 1978; Winkle et al., 1981; Weidenhamer et al., 1987). These studies suggest that the presence of high phytotoxin concentrations may cause a reversal in the slope of predicted log yield–log density relationships at low plant densities, in contrast to the expected consequences of increased density and resource competition (Weidenhamer et al., 1989). The relationship of log mean weight per plant and log plant density for tomatoes exhibited a reversal in slope of the predicted log yield–log density relationship, indicating the presence of a toxic substance in walnut soil (Weidenhamer et al., 1989). The retrogressive slope of the log weight–log density relationship in lettuce indicated a positive response of plant growth to phytotoxins from the leaves of *M. tanarius* (Figure 3A). In a given soil volume containing a finite amount of leaf powder, each lettuce plant growing at low density might uptake a higher amount of available phytotoxins than at high density. In other words, lettuce growing at low density would be exposed to higher levels of phytotoxins, leading to a greater inhibition of growth. Total dry weight per plant was most reduced at low density in soil containing leaf powder (Table 2). The presence of phytotoxins in leaves was further confirmed by the standard sponge bioassay against seed germination of lettuce and *B. pilosa*. *Alocasia macrorrhiza* found in the understory of Australian tropical forests and

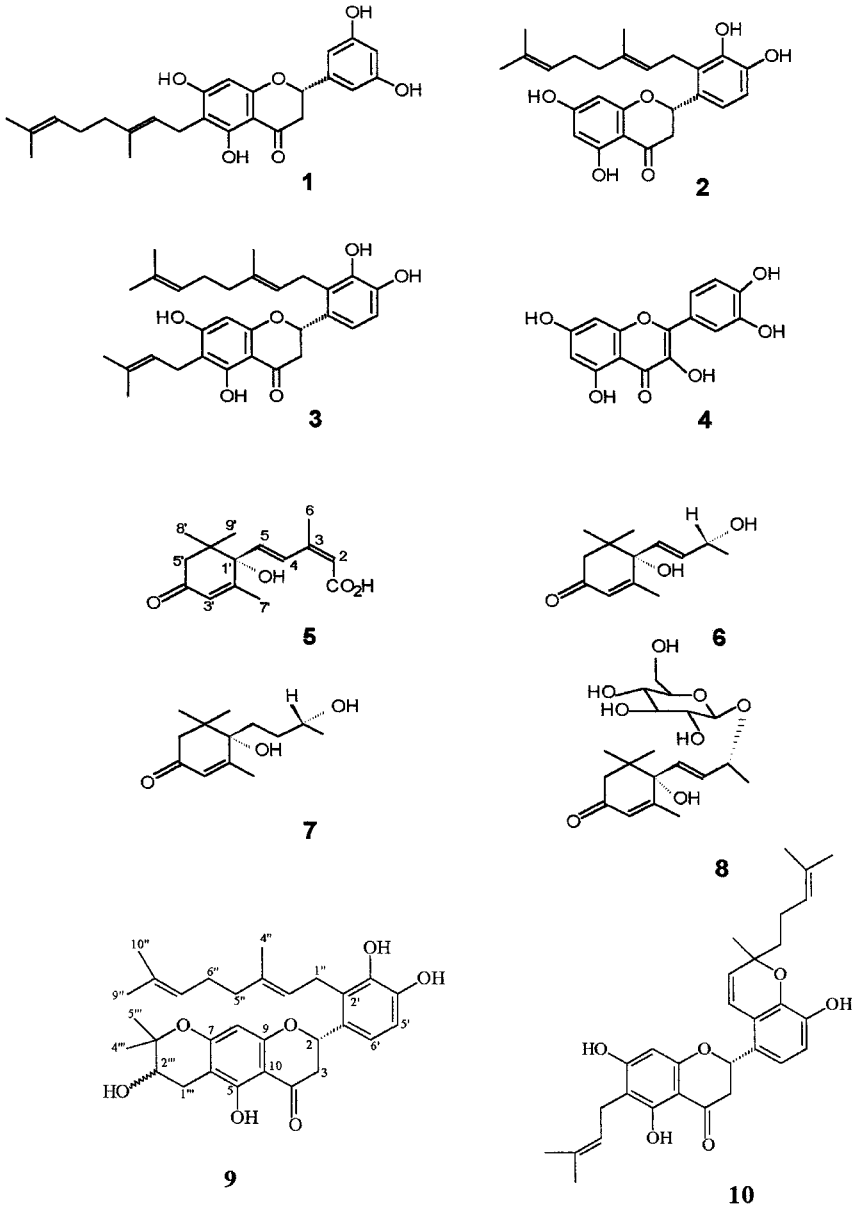

FIG. 7. Chemical structures of the 10 identified compounds from *M. tanarius* leaves. (**1**) nymphaeol-A; (**2**) nymphaeol-B; (**3**) nymphaeol-C; (**4**) quercetin; (**5**) abscisic aicd; (**6**) blumenol A; (**7**) blumenol B; (**8**) roseoside II; (**9**) tanariflavanone A; (**10**) tanariflavanone B. The compounds are structurally confirmed by ^{1}H NMR, ^{13}C NMR, and MS spectra.

TABLE 3. EFFECTS OF DIFFERENT CONCENTRATIONS OF COMPOUNDS ISOLATED FROM *M. tanarius* LEAVES ON RADICLE AND SHOOT GROWTH OF TESTED PLANTS

Compound	Conc. (ppm)	Radicle growth (% of control)	Shoot growth (% of control)	Tested species
Abscisic acid	10	80*	-	*Biden pilosa*
	20	82*	-	
	30	67**	-	
	40	55**	-	
Abscisic acid	40	71**	57**	*Chloris barbata*
	80	65**	62**	
	120	41**	25**	
Abscisic acid	40	142**	68*	*Miscanthus floridulus*
	80	86	49**	
	120	44**	23**	
Quercetin	200	106	82	*M. floridulus*
	400	98	77	
	600	72*	80	
Blumenol A	200	90	96	*M. floridulus*
	400	75*	81	
	600	71*	70	
Blumenol B	200	98	86	*M. floridulus*
	400	82	89	
	600	81*	87	

Note: "-" indicates that the shoot length of test plants was not visualized.
* Values are significant from control ($P < 0.05$) according to t test.
** significant at $P < 0.01$.

subtropical forests has been extensively studied in terms of adaptation to extreme shade conditions (Boardman, 1977). On the basis of our field observation, the canopy of *M. tanarius* seems to provide a moderate habitat for *A. macrorrhiza*. In the greenhouse, *A. macrorrhiza* was apparently not sensitive to the phytotoxins released from the leaves of *M. tanarius*.

Compounds identified from *M. tanarius* leaves, namely quercetin (**4**), ABA (**5**), blumenol A (**6**), blumenol B (**7**), tanariflavanone A (**9**), and tanariflavaone B (**10**) (Tseng et al., 2001), exhibited inhibitory effects on radicle growth of lettuce and weeds. The major growth inhibitor, however, was ABA, which is well recognized as a plant growth regulator (Zeevaart and Creelman, 1988; Leung and Giraudat, 1998). Wheat straw collected from the soil surface after harvest also contains ABA (Hall et al., 1986). The existence of ABA in straw residues from preceding crops was the cause for poor growth of successive crops (Hall et al., 1986). Leachates of tall fescue grass (*Festuca arundinacea*) contained three principal allelochemicals including ABA, caffeic acid, and *p*-coumaric acid. After quantitative analysis, ABA was found to be a predominant inhibitor (Buta and Spaulding, 1989). The concentrations of ABA in grass leachates of desiccated tall fescue were

about 390 ng g^{-1} fw and approximately the same as from straw leachates (Buta and Spaulding, 1989). The concentration of ABA in leaves of *M. tanarius* was much higher. Since ABA inhibits seed germination and influences stomatal control (Zeevaart and Creelman, 1988; Leung and Giraudat, 1998), nanogram quantities of ABA found in dried grass leachates were sufficient to cause inhibition (Buta and Spaulding, 1989). Likely, a certain quantity of ABA would be leached from senescent or dehydrated leaves after the extended desiccation of 30 days (Buta and Spaulding, 1989). ABA is a potent phytotoxin and its concentration was higher in *M. tanarius* leaves than that of previous reports, suggesting that ABA is involved in the allelopathy of *M. tanarius*. Although it is probable that blumenol A (**6**), blumenol B (**7**), and roseoside II (**8**) are biosynthetically derived from abscisic acid (Bhakuni et al., 1974), they did not inhibit radicle growth of lettuce like ABA (**5**). The different biological activities are probably due to structural differences, suggesting that C-1, C-2, C-3, and C-6 of ABA are important for biological activity. Tanariflavanone B (**10**) inhibited lettuce germination more effectively than nymphaeol-A (**1**), nymphaeol-B (**2**), nymphaeol-C (**3**), and tanariflavanone A (**9**), suggesting the importance of the pyran ring for biological activity, but this remains to be confirmed.

When *M. tanarius* invades grasslands, its fallen leaves accumulate on the ground over time and can inhibit the growth of the nearby weeds allowing the plant to compete for more resources. One or two years after invasion of the grassland, development of a *M. tanarius* canopy leads to a gradual reduction in light and increases the concentration of allelopathic compounds, resulting in a change in the floral composition. *M. tanarius* has larger leaves with a dense canopy that would lead to shading, resulting in lower temperature and higher humidity in the understory. The dense shade might provide moderate habitat for shade-adapted plants, such as *A. macrorrhiza*, that could resist the *M. tanarius* phytotoxins. *M. tanarius*, therefore, becomes a dominant in the community after invasion into grasslands.

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REFERENCES

BHAKUNI, D. S., JOSHI, P. P., UPRETY, H., and KAPIL, R. S. 1974. Roseoside—A C_{13} glycoside from *Vinca rosea*. *Phytochemistry* 13:2541–2543.

BOARDMAN, N. K. 1977. Comparative photosynthesis of sun and shade plants. *Annu. Rev. Plant Physiol.* 28:355–377.

BUTA, J. G. and SPAULDING, D. W. 1989. Allelochemicals in tall fescue— abscisic and phenolic acids. *J. Chem. Ecol.* 15:1629–1636.

CHAVES, N., SOSA, T., ALÍAS, J. C., and ESCUDERO, J. C. 2001. Identification and effects of interaction phytotoxic compounds from exudate of *Cistus ladanifer* leaves. *J. Chem. Ecol.* 27:611–621.

CHOU, C. H. 1999a. Roles of allelopathy in plant biodiversity and sustainable agriculture. *Crit. Rev. Plant Sci.* 18:609–636.

CHOU, C. H. 1999b. Methodologies for allelopathic research: From fields to laboratory, pp. 3–24, *in* A. M. Macías, J. C. G. Galindo, J. M. G. Molinillo, and H. G. Cutler (eds.). Recent Advances in Allelopathy, Vol. I: A Science for the Future. International Allelopathy Society. Servicio De Publicaciones Universidad de Càdiz, Spain.

CHOU, C. H., FU, C. Y., LI, S. Y., and WANG, Y. F. 1998. Allelopathic potential of *Acacia confusa* and related species in Taiwan. *J. Chem. Ecol.* 24:2131–2150.

CHOU, C. H. and LEU, L. L. 1992. Allelopathic substances and interactions of *Delonix regix* (Boj. Raf.). *J. Chem. Ecol.* 18:2285–2303.

CHOU, C. H. and MULLER, C. H. 1972. Allelopathic mechanism of *Arctostaphylos glandulosa* var. *zacaensis*. *Am. Midl. Nat.* 88:324–347.

CHOU, C. H. and YOUNG, C. C. 1974. Effect of osmotic concentration and pH on plant growth. *Taiwania* 19:157–165.

CONSTANTINO, M. G., LOSCO, P., and CASTELLANO, E. E. 1989. A novel synthesis of (±)- abscisic acid. *J. Org. Chem.* 54:681–683.

GALBRAITH, M. N. and HORN, D. H. S. 1972. Structures of the natural products blumenols A, B, and C. *J. Chem. Soc. Chem. Comm.* 3:113–114.

GOMEZ, K. A. and GOMEZ, A. A. 1976. Statistical Procedures for Agricultural Research with Emphasis on Rice. The International Rice Research Institute, Los Banos, Philippines.

HALL, K. C., CHAPMAN, S. J., CHRISTEAN, D. G., and JACKSON, M. B. 1986. Abscisic acid in straw residues from autumn-sown wheat. *J. Sci. Food Agric.* 37:219–222.

HOFFMAN, D. W. and LAVY, T. L. 1978. Plant competition for atrazine. *Weed Sci.* 26:94–99.

KIRA, T., OGAWA, H., and SAKAZAKI, N. 1953. Intraspecific competition among higher plants. I: Competition–density yield interrelationship in regularly dispersed populations. *J. Inst. Polytechn. Osaka City Univ. D* 4:1–6.

LEUNG, J. and GIRAUDAT, J. 1998. Abscisic acid signal transduction. *Annu. Rev. Plant Pysiol. Plant Mol. Biol.* 49:199–222.

LIN, Y. L., WANG, W. Y., KUO, Y. H., and CHEN, C. F. 2000. Nonsteroidal constituents from *Solanum incanum* L. *J. Chin. Chem. Soc.* 47:247–251.

MALLIK, A. U. and PELLISSIER, F. 2000. Effects of *Vaccinium myrtillus* on spruce regeneration: Testing the notion of coevolutionary significance of allelopahy. *J. Chem. Ecol.* 26:2197–2209.

MULLER, C. H. 1969. Allelopathy as a factor in ecological process. *Vegetatio* 18:348–357.

OHKUMA, K., ADDICOTT, F. T., SMITH, O. E., and THIESSEN, W. E. 1965. The structure of abscisin II. *Tetrahedron Lett.* 29:2529–2535.

OTSUKA, H., YAO, M., KAMADA, K., and TAKEDA, Y. 1995. Alangionosides G-M: Glycosides of megastigmane derivatives from the leaves of *Alangium premnifolium*. *Chem. Pharm. Bull.* 43:754–759.

PUTNAM, A. R. and TANG, C. S. 1986. The Science of Allelopathy. Wiley, New York.

RICE, E. L. 1984. Allelopathy. Academic Press, Orlando, Florida.

SKIPPER, H. D. 1966. Microbial degradation of atrazine in soils. MS Thesis, Oregon State University.

TSENG, M. H. 2001. Allelopathic potential of *Macaranga tanarius* (L.) Muell.-Arg. PhD dissertation, National Taiwan University, Taiwan.

TSENG, M. H., CHOU, C. H., CHEN, Y. M., and KUO, Y. H. 2001. Allelopathic prenylflavanones from the fallen leaves of *Macaranga tanarius*. *J. Nat. Prod.* 64:827–828.

WEIDENHAMER, J. D., HARTNETT, D. C., and ROMEO, J. T. 1989. Density-dependent phytotoxicity: Distinguishing resource competition and allelopathic interference in plants. *J. Appl. Ecol.* 26:613–624.

WEIDENHAMER, J. D., MORTON, T. C., and ROMEO, J. T. 1987. Solution volume and seed number: Often overlooked factors in allelopathic bioassays. *J. Chem. Ecol.* 13:1481–1491.

WINKLE, M. E., LEAVITT, J. R. C., and BURNSIDE, O. C. 1981. Effects of weed density on herbicide absorption and bioactivity. *Weed Sci*. 29:405–409.

YAKUSHIJIN, K., SHIBAYAMA, K., MURATA, H., and FURUKAWA, H. 1980. New prenylflavanones from *Hernandia nymphaefolia* (Presl) Kubitzki. *Heterocycles* 14:397–402.

ZEEVAART, J. A. D. and CREELMAN, R. A. 1988. Metabolism and physiology of abscisic acid. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 39:439–473.