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THE ALLELOPATHIC POTENTIAL OF *MISCANTHUS FLORIDULUS* (1) (2)

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Abstract

An unique pattern of herb exclusion by a dominant species of *Miscanthus floridulus* is found ubiquitously throughout the Island. It is found that the aqueous leaf leachate of *Miscanthus floridulus* exhibits the significant inhibition on the growth of lettuce. The aqueous extracts of leaves and soils collected from the *Miscanthus* area also show toxic effect. Furthermore, toxic spots are found on the chromatogram of the ether fraction of aqueous extract of *Miscanthus* leaves. Seven phytotoxins are identified by means of paper chromatography. They are cis and trans *p*-coumaric acid, ferulic, vanillic, syringic, *p*-hydroxybenzoic, and (*o*-hydroxyphenyl) acetic acids. In addition, one toxic spot on chromatogram has not yet been identified. Thus, it appears that the bare areas associated with *Miscanthus* stands are primarily due to allelopathy.

Introduction

Miscanthus floridulus (Labill.) Warb, a dominant grass, distributes widely in the abandon area of mountains hill throughout Taiwan. There is an unique pattern of herb exclusion by the *Miscanthus* stands, which occupies a large area in the mountains, particularly in Nankang, suburb of Taipei. The mechanism of dominance of this vegetation has not been elucidated. Field observation suggests that the physical factor seems unlikely to be a limiting factor in determining the process of dominance; however, the biotic interaction seems likely to play an important role in the process. Thus, a hypothesis of allelopathic effect of *Miscanthus floridulus* was proposed, and the experimentation was set to test this hypothesis.

Allelopathy is defined as the detrimental effect of one plant upon another mediated by a toxic chemical (Molisch, 1937). Recently the concept of allelo-

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pathy has received as a fundamental ecological function (Muller, 1966; Rice, 1967; Whittaker, 1970). In addition many research articles concerning this study were published in a variety of journal (Davidonis and Ruddat, 1973; McPherson and Muller, 1969; Lodhi and Rice, 1971; del Moral and Muller, 1970; Chou and Muller, 1972). Most of their investigations were based on the effect of shrub on the associated grasses; however, only a few examples of allelopathic effect among grasses were reported (Naqvi, 1969). Particularly the allelopathic interaction between *Miscanthus* and other herbaceous plants has not been studied. Thus it is the purpose of the study to evaluate allelopathic nature of the vegetation. As a consequence, phytotoxic properties of leachate, various kind of extracts, and the identity of responsible toxins in *Miscanthus* are reported.

Materials and Methods

Study site

Although *Miscanthus floridulus* is widely distributed throughout the island, it is easier for us to center the study site near the research institute of Academia Sinica, Nankang, Taipei. At the elevation of 200–300 meter of mountain hill of Nankang, the area previously occupied by *Acasia confusa* was completely disturbed by coal miner around 1967. Since that time the area has been dominanted by an extensive pure stands of *Miscanthus floridulus.* In the basin area adjacent to the *Miscanthus* stands, many herbaceous plants were found.

Climate

The study area receives the northwestern monsoon in winter and the southern monsoon in summer, thus rainfall is almost throughout a year and the annual precipitation is over 4000 mm. The average temperature is from 15 to 23°C (Li, 1963). This mild climate is the consequence of the development of luxuriant vegetation along the mountain slopes.

Materials

Plant materials including fresh leaves leaf litter, and root residues were collected from the area. Parts of samples were air-dried in the greenhouse, and the rest of them were stored in a cold room for the specific use.

The soils were sampled in November, 1972, from various locations of the study area. They were taken by the upper 5–10 cm layer of ground, brought back to laboratory and allowed to air-dry. Then they were screened with a 2.5 mm sieve before use.

Preparation of aqueous leachate from ***Miscanthus***

An experiment was designed to determine whether toxins were present in raindrip from the *Miscanthus* foliar crowns. Fresh leaves of *Miscanthus floridulus* were spreaded over a large, shallow polyethlene funnel, and sprayed with distilled water, and the resulting leachate collected. An effort was made to keep the volume of leaves constant between batches, and the same amount of water was used each time. To 10 kg of *Miscanthus* leaves, 2500 ml (equivalent to 2.5 mm rainfall) of distilled water was used to simulate rainfall for 3 hours. About 500 ml of leachate was obtained, and filtered with Selecta No. 597 paper. Part of the original leachate (1×) was concentrated to one tenth (10×) the original volume by a rotatoy flash evaporator at a temperature below 50°C.

Aqueous extracts of ***Miscanthus*** *leaves and soils*

After leaves of *Miscanthu* were air-dried, they were chopped into small pieces about 2.5 cm long. To 50 g of chopped leaves, 350 ml of distilled water was added to soak for the time intervals of 6, 12, 18, 24 and 48 hr. The extract was obtained by filter with 2 layers of cheesecloth and with Selecta filter paper No. 597. The filtrate was then concentrated to 5× or 10× the original volume by using a rotatory evaporator.

To 100 g of the soil, 200 ml of distilled water was added. Then the mixture was shaken for 2 hr before the filtering process. The filtrate obtained from the suction filter was further centrifuged by 5000 rpm. The final clean soil extract was concentrated to 5× and 10× the original volume, using the same techniques.

Measurement of osmotic concentration and pH of extracts

Seed germination and growth can be suppressed when the osmotic concentration of test solution is too high, usually the concentration of solution can not above 50 milliosmols (Chou, 1971). The concentration of leachate and extract was determined by using a Fiske Osmometer (model G-66) cryoscopically. In addition, the pH of extract and leachate was determined by using a pH meter (Chemtrix, type 40).

Bioassay techniques

Using the following bioassay techniques, the phytotoxic effects of the aqueous extract and leachate were examined. The bioassay method called "sponge bioassay" was a modification of "standard sponge bioassay" described by Muller (1966), and a method called "chromatographic bioassay" was described by Chou and Muller (1972). Because the germination of lettuce seed

(*Lactuca sativa* var. Great Lake 366) was high, usually over 95% and very stable, the seeds were used as the test material. In the sponge bioassay, the leachate and plant extracts or soil extracts were used as the test solution, and the distilled water was served as the control. While in the chromatographic bioassay, the chromatograms obtained from the paper strips spotted with the ether fraction of extracts were used as the test sample and the chromatogram without spotting was served as the control. The bioassays were allowed to incubated at 25–27°C for 72 hr. The results were taken by counting the number of seed germination and measuring the length of radicle growth in millimeter.

In the chromatographic bioassay results, the data were computed by the formular $I = \frac{I_t - I_c}{I_c} \times 100$; where I_t represents the data of treatments and I_c represents the control, thus, the negative value of I indicates the % inhibition, and the positive value indicates the % stimulation. The final data were then analyzed statistically by means of analysis of variance, and the value of least significant difference (L. S. D.) at 5% and 1% level of confidence was obtained.

Identification of phytotoxins in ***Miscanthus***

To identify the responsible toxin in extracts, Whatman 3 MM chromatographic paper was used. The paper strip (Wang, *et al.*, 1967) was conducted, and was developed descendingly with two solvents, such as 2% acetic acid, and *n*-butanol: acetic acid: water (4:1:5, v/v/v, called BAW). After papers were developed, they were examined under the short wavelength of ultraviolet light and detected under two spray reagents: (1) DPNA, diazotized *p*–nitroaniline (Hais and Macek, 1963) followed by 10% sodium carbonate, (2) DQC, 0.1% ethanolic 2, 6–dichloroquinone chlorimide followed by saturated sodium borate solution (Vásquez, *et al.*, 1968). Phenolic compounds appear as absorbing or variously fluorescing spots under the short wavelength u. v. light and also appear in the distinguishable colors after the spray reagents.

Results

Field observation and measurement

Relatively pure stands of ***Miscanthus floridulus*** occur in a large area of mountain hill of Mt. Shih-Hsing, suburb of Taipei (Fig. 1). Underneath of the vegetation, there is almost absent of other herbaceous plants (Fig. 2).

To understand the dominance of *Miscanthus floidulus* in field, number of seedlings per square meter was counted from ten quadrates (one meter square of each quadrate). It was found that there was about 30% canopy free of

Fig. 1. Thick and relatively pure stands of *Miscanthus floridulus*, on mountain hill at 200-300 meter in Mt. Shih-Hsing, Nankang, Taipei, Taiwan.

Fig. 2. Underneath the vegetation of *Miscanthus* and between the bunches of that, there is almost free of other plants.

any plant between the bunches of *Miscanthus*. The samplying data were furthermore analyzed by means of the analysis of variance, thus the value of LSD (least significant difference) was obtained. The percent of each species per square meter is given in Table 1, such as 65% of *Miscanthus floridulus*, 17% of *Lactuca indica*, 4% of *Eupatonium formosanum*, 2.8% of *Brachiaria distachya* and of *Sporobolus fertilis*, 2.2% of *Pouderia seandens*, and less than one percent of *Cyperus pilosus*, *Digitaria violascens* and three unknown grasses. It is obvious from the Table 1 that the *Miscanthus floridulus* and *Lactuca indica* are the most dominant species in the area. However, the mortality of *L. indica* is high when it grows with *Miscanthus*, and can survive only within a year. Therefore, the *Miscanthus* becomes a dominant species.

Table 1. *Presence of* ***Miscanthus floridulus*** *and its associated species occurring in ten quadrates*

Species	No. of seedlings/m²	% presence
Miscanthus floridulus	25.2**	65
Lactuca indica	6.1*	17
Eupatonium formosanum	1.4	4
Brachiaria distachya	1.0	2.8
Sporobolus fertilis	1.0	2.8
Pouderia seandens	0.8	2.2
Digitaria violascens	0.3	0.8
Cyperus pilosus	0.2	0.6
Unknown sp. 1	0.9	2.5
Unknown sp. 2	0.4	1.1
Unknown sp. 3	0.3	0.8

L. S. D. *(5%)=5.71 **(1%)=7.55

Phytotoxicity of aqueous leachate and extracts in leaves and soils

To demonstrate the phytotoxicity of *Miscanthus*, the leachate obtained from the artificial raindrip was tested. The original leachate and its concentrated 10× leachate were bioassayed against lettuce seeds by using the sponge bioassay techniques. The distilled water was used as the control. The corresponding osmotic concentration of mannitol solution at pH 6 was also bioassayed to make inhibition comparison. Results expressed as the percentage of control are given in Table 2. The results indicated that the original leachate gave no inhibitory effect on the growth of tettuce. However, the concentrated part exhibited significant toxicity on both the germination and radicle growth, being significant difference at 1% level of confidence, using the analysis of variance. The osmotic concentration of leachates was determined. It was 4 milliosmols in the original leachate of *Miscanthus* and 50 in the 10× concentrated part. Adjusting the 50 milliosmols of the mannitol solution to pH 6, it exhibited stimulation effect on lettuce growth. It is clearly

Table 2. *The effect of aqueous leachate of **M. floridulus** on the germination and growth of lettuce*

Solution	pH	Osmotic concentration (milliosmols)	Germination		Radicle growth	
			%	% of control	length (mm)	% of control
Distilled water (Control)	6.5	0	95		6.0	
M. floridulus						
1×	6.6	4	85	89	6.5	107
10×	7.0	50	31	36	3.8**	63
Mannitol solution[1]						
1	6.0	4	95	100	8.5**	140
2	6.0	50	100	105	7.3*	121

L. S. D. *(5%)=1.13 mm **(1%)=1.71 mm

[1] See Chou and Young (in press).

indicated that the inhibition of leachate is due to toxic substances but not due to the osmotic concentration. It was also found that the subsequent leachate obtained after 48 hr leaching still exhibited toxicity (Chou, 1973 unpublished data). This indicates that toxic substances in *Miscanthus* can continuously be leached out by rainfall, resulting in the suppression of other herbs in field.

In addition, the aqueous extracts of *Miscanthus* (50 g/350 ml) and its 5× concentrated solution were bioassayed by using the same techniques as described above. It was found that the extracts revealed significantly toxic effect on the growth of lettuce (Fig. 3). The 5× extracts gave 100% inhibition,

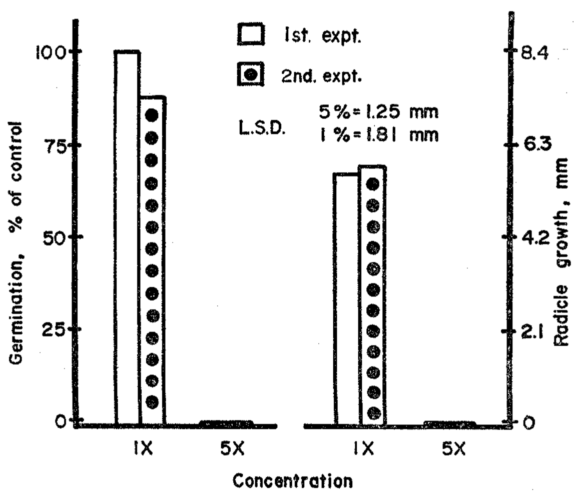

Fig. 3. The effect of aqueous extracts of *Miscanthus floridulus* on the germination and growth of *Lactuca sativa* var. Great Lake.

resulting in the death of seeds. These dead seeds were unable to germinate when they were washed by distilled water and re-germinated under the normal conditions.

Leachability of phytotoxicity in ***Miscanthus***

Although the toxicity of *Miscanthus* has been well demonstrated, the information of toxin leachability is still lacking. Thus an experiment was designed that the chopped leaves of *Miscanthus* were soaked with distilled water for a period of time, such as 6, 12, 18, 24, and 24 hr. By using the same techniques described as above, the extracts were bioassayed. Results are given in Figure 4. It was found that the inhibition of radicle growth was increased with the increase of soaking time period, except the result of the 24 hr treatment which could be a result of experimental error. The results suggested that the quantity or quality of toxins leached out was varied with different soaking times.

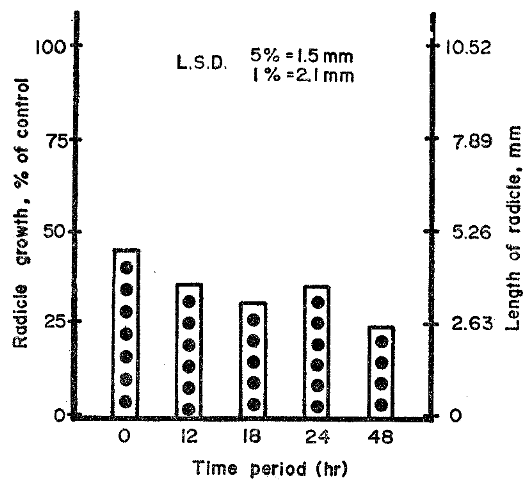

Fig. 4. The toxic effect of aqueous leaf extract of *M. floridulus* on the growth of lettuce. The extracts were made at different time periods of soaking.

Bioassay of soil extracts

The soil samples were collected from various locations in the study area, such as sample 1 was from the area underneath *Miscanthus* bunches, sample 2 from the area between two bunches, sample 4 from the area adjacent to the rice field, sample 5 from the *Miscanthus* root area, and sample 6 from the disturbed open area. To 100 grams of soil, 200 ml of distilled water was added to each soil to make soil extract. The extraction procedures were described in the earlier section. The extracts then bioassayed by using sponge bioassay techniques. Results are computed and given in Table 3. It was shown that the original extracts did not give inhibition, but the concentrated parts of 5× and 10× provided significant toxicity. Among them, soils collected from the adjacent rice field and from the disturbed open area gave inhibition only at 10× concentration; however, soils collected from the *Miscanthus* area including root soil exhibited inhibition highly at 5× concentration. The inhibition was shown not due to the osmotic concentration because the osmotic concentration of soil extracts at 10× was below 21 milliosmols. At which concentration of mannitol solution, it failed to give significant inhibition (Chou and Young, in press). The pH of soil extracts, except sample 5, was between 7.2–8.5. Within such a pH range, vegetation change is not different. The low pH value of soil sample 5 is probably due to organic acids released from *Miscanthus* roots.

Table 3. *The effect of aqueous soil extracts on the radicle growth of lettuce* Symbols of 1×, 5×, and 10× represent the various concentration by reducing the original volume to one fifth, and one tenth respectively[1)]

	Distilled water (control)	Sample 1			Sample 2		
		1×	5×	10×	1×	5×	10×
pH	6.5	7.2	7.2	7.8	7.2	7.6	7.6
Osmotic concn. (milliosmols)	0	0	5	14	0	1.5	9
Radicle growth (millimeter)	20	23	16	10	21	15	9.1
% of control		118	80*	52**	106	75*	46**

	Sample 4			Sample 5			Sample 6		
	1×	5×	10×	1×	5×	10×	1×	5×	10×
pH	7.5	7.8	8.4	4.6	4.3	3.9	7.6	8.1	8.5
Osmotic concn. (milliosmols)	0	1	10	0	8	21	0	5	15
Radicle growth (millimeter)	25	20	12	20	7	5	21	20	10
% of control	129	101	59**	102	35**	22**	104	99	49**

L. S. D. *(5%)=1.12 mm **(1%)=1.57

1) The description of soil samples was cited in the text.

*Chromatographic bioassay of **Miscanthus** extract*

It has been clearly demonstrated that phytotoxicity is present in the *Miscanthus* leachate, extracts of leaves, and soils. An attempt was made to find out the location of toxic spot on chromatogram. Thus 50 μl (0.05 g/μl) of the ether fraction of aqueous extract was chromatographed, and the chromatogram was divided into 10 segments according to the Rf value. The blank unspotted chromatogram was also treated with the same way to serve as controls.

Lettuce seeds were used as the test material. Thus results expressed as the percent inhibition and percent stimulation of radicle growth were shown in Figure 5. There were two stimulation and two inhibition spots on the chromatogram. The stimulation spots were found at Rf 0.00–0.20, while the inhibition spots were found at Rf 0.80–1.00. The last segment exhibited the highest toxicity, thus the seeds turned to black in color and swelled in shape, and the tip of root was demaged. The identity of toxic spots was furthermore studied by paper chromatography.

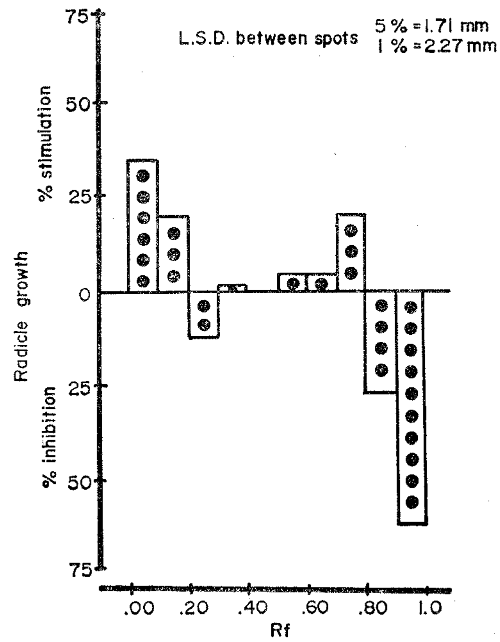

Fig. 5. The radicle growth of lettuce seeds on separated paper chromatogram segments. The ether fraction of aqueous leaf extract of *Miscanthus* was chromatographed on paper strips with 2% acetic acid.

Identification of growth inhibitors in ***Miscanthus***

To find out the responsible phytotoxins in *Miscanthus,* the aqueous extract was subjected to ether extraction. Thus the ether fraction of aqueous leaf extract of *Miscanthus floridulus* was chromatographed by using two solvents. The chromatograms were then detected under the short wavelength of u. v. light and sprayed with two different color reagents, such as DPNA and DQC, described in detail in the earlier section. The results of Rf values in two solvent systems and of color reaction were given in Table 4. Seven phytotoxic substances were found. They are ferulic, trans *p*–coumaric, cis *p*–coumaric, vanillic, syringic, *p*–hydroxybenzoic, and (*o*–hydroxypheny)acetic acids, and one unknown. Among them, (*o*-hydroxypheny)acetic acid and the unknown exhibited the most toxic effect on lettuce growth. The (*o*-hydroxypheny)acetic acid gave over 70% inhibition on the growth of lettuce at concentration below 100 ppm (Chou, 1973). The rest of identifitable toxins provided significant inhibition at 200 ppm or above (Chou and Muller, 1972). The unknown spot was thought to be non-phenolics. Its identification by using a sophisticated instrument, such as gas-liquid chromatograph, is in progress.

Table 4. *Identification of phytoxins in aqueous extract of* ***Miscanthus floridulus***
Data were obtained from 3 to 4 runs.

Compound	Rf value		Color reaction		
	2% AA	BAW (4:1:5)	s. u. v.	DPNA	DQC
Phytotoxin 1	0.37	0.92	bl-fl	bl	bl
Ferulic acid	0.36	0.89	bl-fl	bl	bl
Phytotoxin 2	0.44	0.91	ab	dk-bl	bl to yel
trans-*p*-Coumaric a.	0.45	0.91	ab	dk-bl	bl to yel
Phytotoxin 3	0.55	0.90	ab	vi	sk-bl
Vanillic a.	0.56	0.91	ab	vi	sk-bl
Phytotoxin 4	0.51	0.85	ab	bl	bl
Syringic a.	0.48	0.87	ab	bl	bl
Phytotoxin 5	0.62	0.93	ab	red	bl-gr
p-Hydroxybenzoic a.	0.62	0.92	ab	red	bl-gr
Phytotoxin 6	0.74	0.90	ab	dk-bl	bl to yel
cis-*p*-Coumaric a.	0.75	0.91	ab	dk-bl	bl to yel
Phytotoxin 7	0.84	0.93	—	vi	bl
(*o*-Hydroxyphenyl) acetic acid	0.83	0.93	—	vi	bl
Unknown A	0.91	0.95	ab	—	—

ab=absorption bl=blue dk=dark fl=fluorescence
gr=gray sk=sky vi=violet yel=yellow
—: not sensitive to detection

Discussion

There is almost lack of an understory of herbaceous plants in *Miscanthus* stands. The stands accumulate toxic substances on or near the surface of their leaves and stems. The toxic substances are leached out by rainfall, and are probably held by soil colloid in the upper layer of soil for a period of time. There is a continuous supply of toxic leachate provided during a long rainy season particularly in the Nankang area. Seeds of the other herbs are usually distributed in the upper 5 cm of soil layer, and should germinate during the rainy season. Meanwhile, the toxins have built up to a sufficient quantity to inhibit the germination of herb seeds and suppress the root growth of these which do germinate. As a consequence, the *Miscanthus* becomes a dominant species and its associated herbs are suppressed. This phenomenon of biochemical interaction among plants has been recognized as an important ecological process.

The process of toxins leached out is by means of four ways. First, leaves and other plant parts absciss and fall to the ground releasing large amount of various kinds of metabolites (Tukey, 1966). Second, a variety of substances can be exuded from roots (Rovira, 1971; Woods, 1960). Third, volatile terpene are released from plant foliage (Muller, 1966). Fouth, water soluble compound can be leached out of the intact plant (Tukey, 1966; Muller and Chou, 1972). The allelopathic substances of *Miscanthus* are mainly released into the environment by means of the first and fourth processes. Although we do not have the direct evidence to show the toxins are exuded from the *Miscanthus* roots, the root soil evidently reveals toxicity (Table 3). It was also demonstrated that the toxic effect was not due to the osmotic concentration. It is therefore concluded that the toxins are mainly leached out from the leaves and stems of *Miscanthus* by rainfall.

Seven phytotoxic substances were identified in plant tissue and soils (Table 4). Among them, (*o*-hydroxypheny)acetic acid and one unknown show the most toxic effect on the growth of lettuce. However, the spots on chromatogram, Rf 0.30–0.70 where were identified as ferulic, *p*-coumaric, vanillic, syringic, and *p*-hydroxybenzoic acids, failed to give the significant inhibition (Figure 3). This is due to the low concentration of these chemicals on chromatogram. Chou and Muller (1972) indicated that these compounds exhibited phytotoxicity only at the concentration of 200 ppm or above. We had a technical difficulty to chromatograph with a large amount of extract, of above 50 μl because of the capacity of paper and impurity of extract. However, authors believe that the concentration of these compounds in soil would be higher than 200 ppm. We also failed to isolate the toxin from the soil. This problem is probably based on the complication of soil colloid. The toxin could be binded with inorganic substrates, or fixed with the soil humic acid (Wang, *et al.*, 1971). It is possible that the toxins found in the *Miscanthus* leachate can be converted into other kind of chemicals, resulting in a difficulty to isolate them. Investigation based on the conversion of phytotoxins in soil needs to be performed.

Acknowledgement

We are indebated to Dr. C. C. Hsu, Prof. of Botany, National Taiwan

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五節芒中 Allelopathy 的潛能

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五節芒（*Miscanthus floridulus*）是遍佈臺灣山坡地極廣的一種草原植物。當它侵入後，整片地爲之佔有。研究結果顯示此植物的水溶濾出液，水溶萃取液及其土壤萃取液都含有毒性。經紙色層分析，鑑得七種植物毒物質，另一毒物質尙未被鑑定出。從五節芒分泌出來的毒物質是使其他草原植物生長不好的主要原因之一。