Proyecto de Desarrollo de Agroquimicos con Especies Alelopaticas Amazonicas

15:00-15:30, Martes 28 de Junio del 2011

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National Institute for
Agro-Environmental Sciences

1) Who am I and about our Institute, NIAES

National Institute for Agro-Environmental Sciences

given name family name

Dr. Yoshiharu FUJII

- ☺ **Born: Jan 18, 1955 in Japan**
- ☺ **Graduate: Kyoto University**
- ☺ **Ph-D (Agr): Kyoto University**

Research at

National Institute for Agro-Environmental Sciences

(1981-2011now, 30 years service)

Visiting Professor: Tokyo University for Agriculture (2008-now)

Call me as FUJI-san

International Allelopathy Society

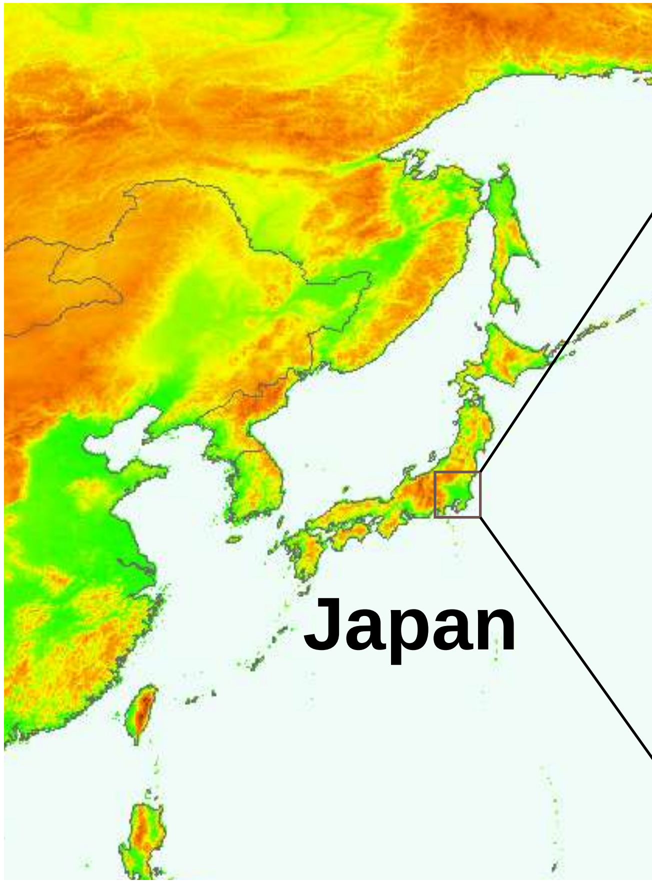
Past President : Dr. Fujii (Japan) 2005-2008

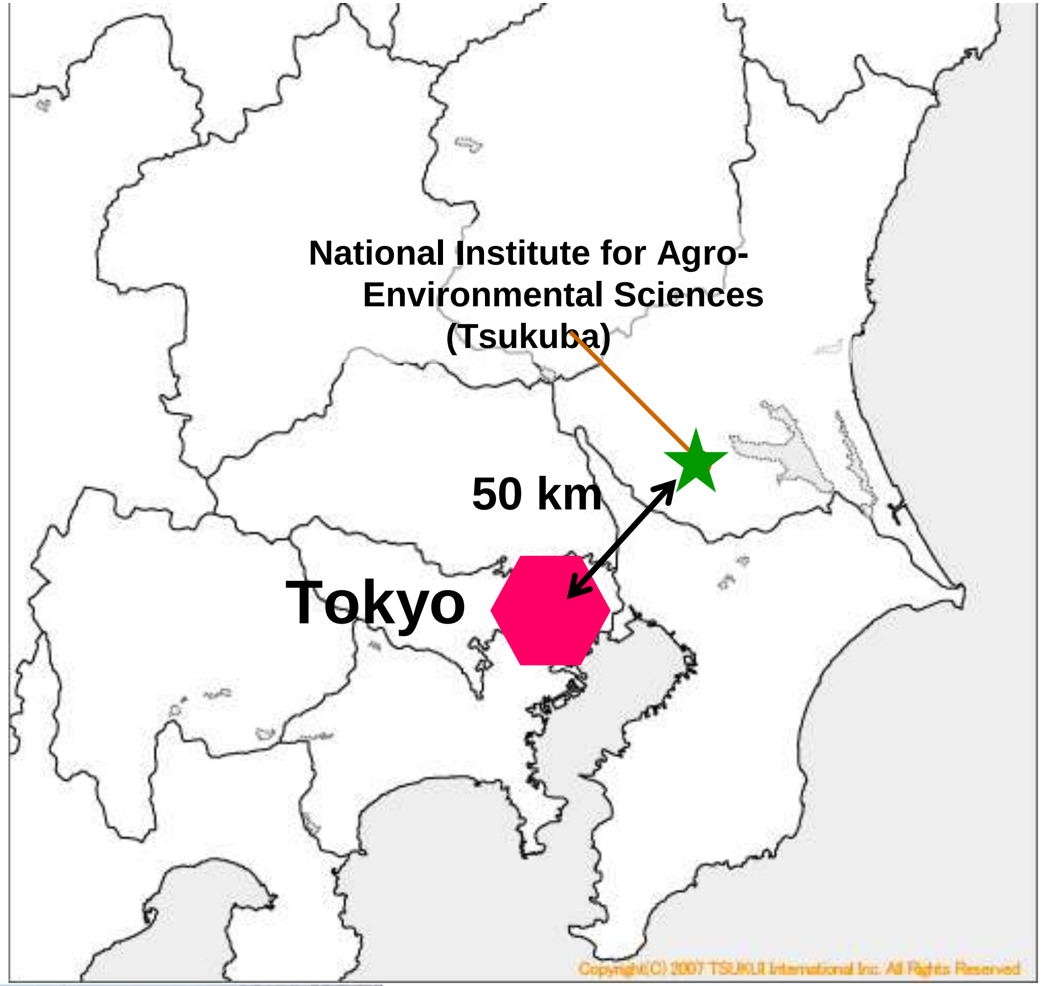
President : Dr. S. Duke (USA) 2008-2011

President Elect: Dr. Weston (Australia) 2011-2013

In Tsukuba, I organized 4th IAS Meeting

NIAES





Japanese Oldest National Institute for Agriculture (117 years old)

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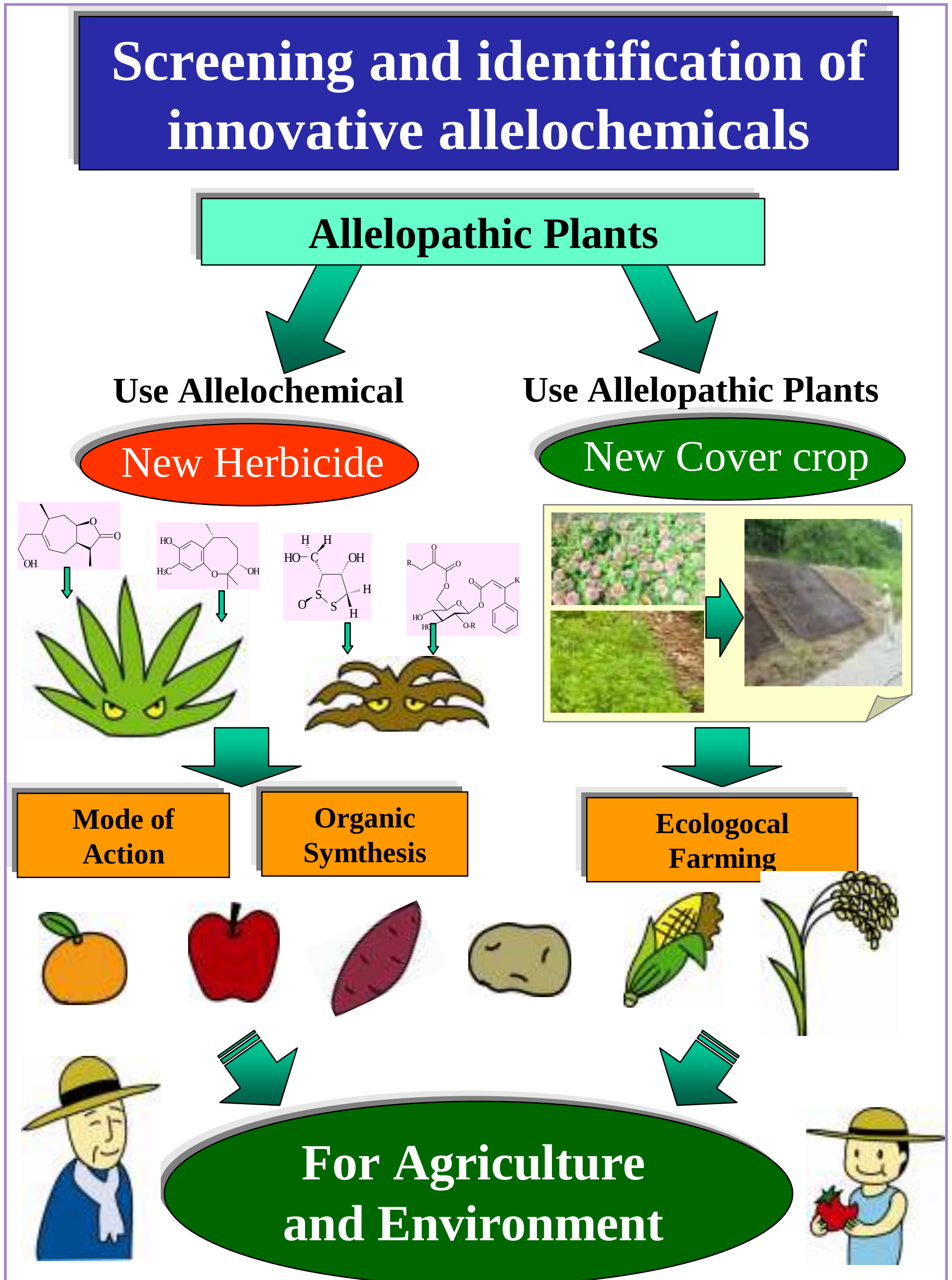
Allelopathy Group in NIAES

20 members including 7 foreign students

“BRAIN” Project



BRAIN:**B**asic **R**esearch **A**ctivitiesfor **IN**novative Biosciences

"Screening of Allelochemicals and Development of Innovative Bioactive Substances"

2008 to 2013

* Supported by Ministry of Agriculture, Forestry and Fisheries, Japan

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2) What is "Allelopathy"

Alelopatia

Interaccion
(inhibitoria o estimulatoria)
entre plantas o plantas y
otras formas de vida
a traves de quimicos
naturales (aleloquimicos)

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Red Pine Tree
(*Pinus densiflora*)
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3) Allelopathy Research in Japan

Our novel techniques

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1. Screening by Bioassay

(Specific Bioassay)

Metodos para la Evaluacion de Alelopatia

Bioassay especifico

Rutas de la Alelopatia y Bioassay

1)Exudacion (desde raices)

→ Metodo Plant Box

2)Lixiviacion (desde hojas)

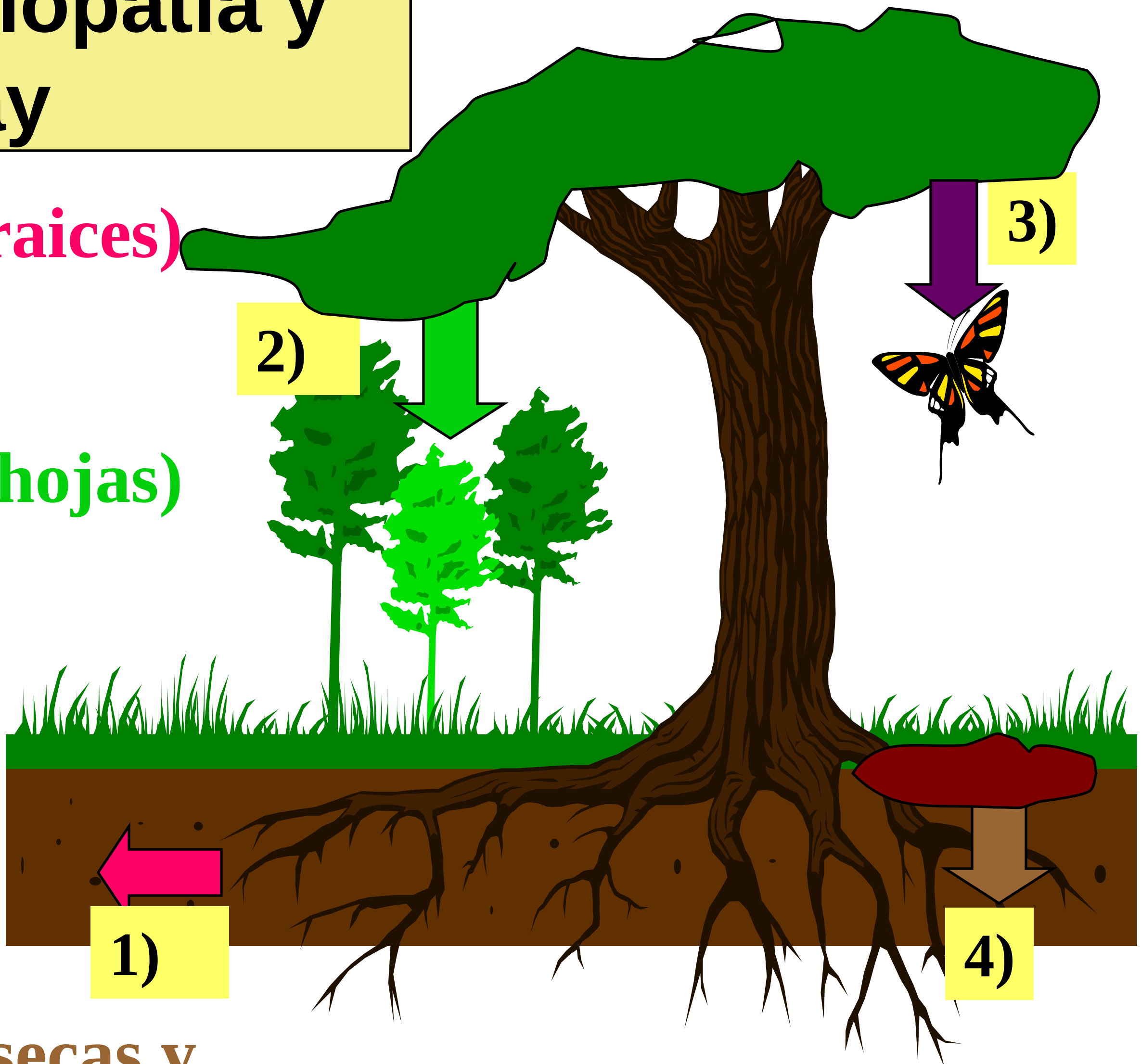
→Metodo Sandwich

3)Volatilizacion (desde hojas)

→ Metodo Dish Pack

4)Lixiviacion (hojas secas y restos vegetales)

→ Metodo Sandwich



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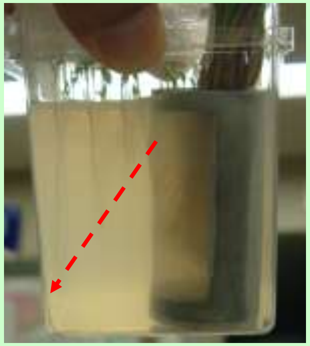
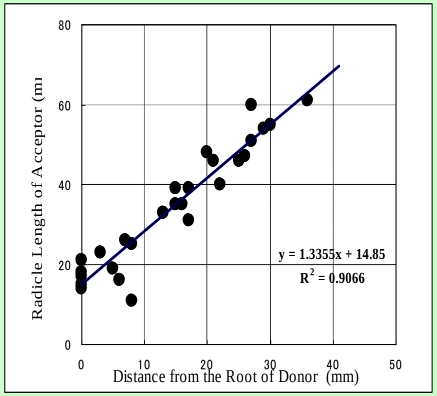
1. Plant Box Method

for root exudates (Mixed planting)



Root zone separating tube



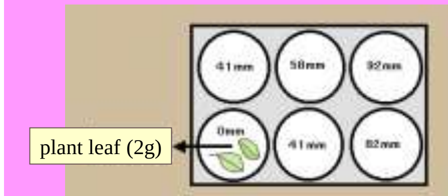
- Sand Culture for 1-2 month
- **Plant Box (for tissue culture)**
- Agar Medium (no nutrients)

Dr. Fujii, 1991

Fujii, Y. et al., Plant-Box Method: A Specific Bioassay to Evaluate Allelopathy through Root Exudates. Allelopathy, New Concepts and Methodology, Science Publisher, 39-56 (2007).

3. Dish Pack Method

for volatile allelochemicals

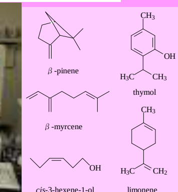


6 well multi-dish

sealed by tape

→ measure after 3 days

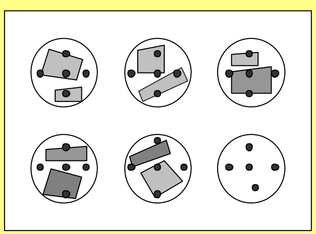
Volatile chemicals were analyzed by GC-MS

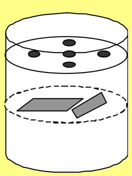


Fujii, Y. et al., Dish pack method: a new bioassay for volatile allelopathy. In Proceedings of the 4th World Congress on Allelopathy, August 21-26, 2005, Wagga Wagga, Australia, 493-497 (2005).

2. Sandwich Method

for leaf leachates (Mulching, Litter)





6 well multi-dish

make sandwich by agar

→10 or 50 mg d.w./10 cm^2

For alleloptphy by fallen leaves and litters

fallen leaves are constant

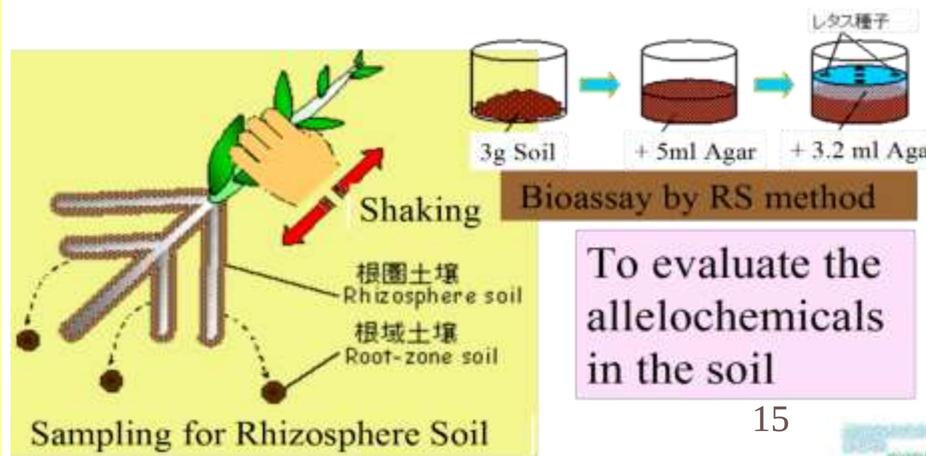
(3 ton / ha / year) = 30 mg d.w./10 cm^2

Dr. Fujii, 1991

Fujii, Y. et al., Assessment method for allelopathic effect from leaf litter leachates. Weed Biology and Management, 4(1), 19 – 23 (2004).

4. Rhizosphere Soil Method

for allelochemicals in Rhizosphere



To evaluate the allelochemicals in the soil

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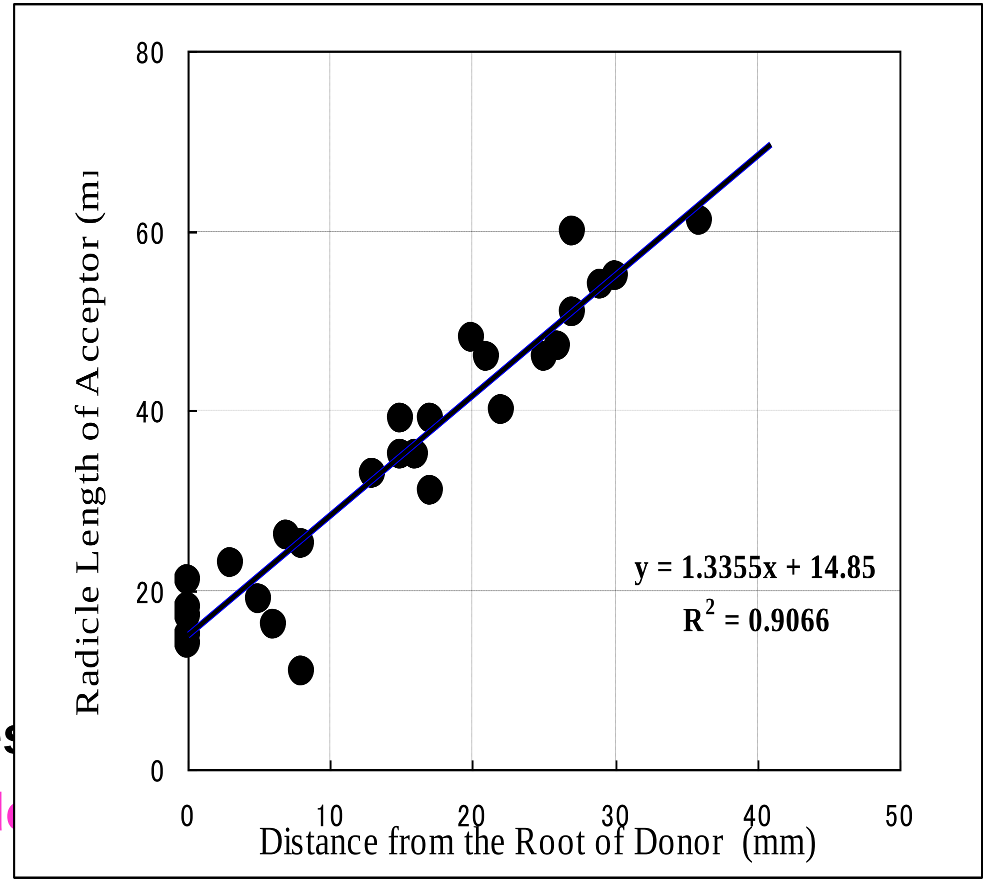
1) Metodo Plant Box

exudados radiculares (cultivos asociados)



- **Cultivo en arena de 1 a 2 meses**
- **Plant Box (para cultivo de tejid**
- **Medio de agar (sin nutrientes)**
- **Zona radicular separada por una gasa de nylon**

Dr. Fujii, 1991

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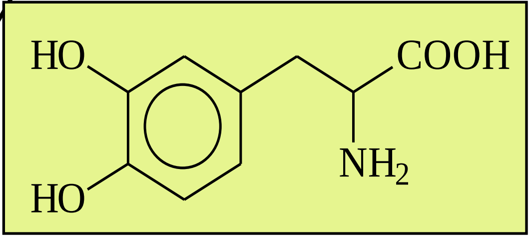
Table 1-a. Continued

Scientific name (English name, and/or *Japanese name*)	Radicle length (%)	*n*	Root dry wt. (mg)
Lablab purpurea (=Dolicos lablab) (Lablab Bean)	41 *	5	135
Latyrus sativus (Grass Pea)	41 *	7	169
Lens esculenta (Lentil)	33 **	2	176
Lespedeza striata (Japan Clover)	60	2	125
Leucaena leucosephala (Leucaena, *Gin-nemu*)	22 ***	2	542
Lotus corniculatus var. *japonicus* (*Miyako-gusa*)	62	3	107
Lupinus albus (White Lupine)	60	10	140
Medicago polymorpha (Bur Clover)	23 ***	3	218
Medicago lupulina (Hop Clover)	19 ****	9	120
Medicago sativa cv. *dupy* (Alfalfa)	34 **	6	324
Medicago sativa cv. *natsuwakaba* (Alfalfa)	19 ****	3	491
Melilotus albus (White Sweet Clover)	23 ***	2	440
Melilotus officinalis (Yellow Sweet Clover)	23 ***	2	440
Mimosa putica (Mimosa)	57	3	425
Mucuna pruriens var. *utilis* (Velvetbean, av. of 5 cv)	7 *****	18	201
Mucuna pruriens var. *utilis* cv. *Hassjo*	4 *****	3	203
Mucuna pruriens var. *utilis* cv. *rajada*	8 *****	2	145
Mucuna pruriens var. *utilis* cv. *capitata*	10 *****	2	197
Mucuna pruriens var. *utilis* cv. *ana*	11 ****	8	315
Mucuna pruriens var. *utilis* cv. *florida*	12 ****	3	146
Pachyrhizus erosus (Yam Bean)	37 **	4	67
Phaseolus vulgaris (Kidney Bean)	43 *	4	101
Phaseolus coccineus (Flower Bean)	43 *	4	366

Mucuna pruriens



L-3,4-Dihydroxyphenylalanine (L-DOPA)

Fujii, et al., L-3,4-Dihydroxyphenylalanine as an allelochemical candidate from *Mucuna pruriens*(L.) DC.var.*utilis*. Agric.Biol.Chem. 55(2), 617-618 (1991)

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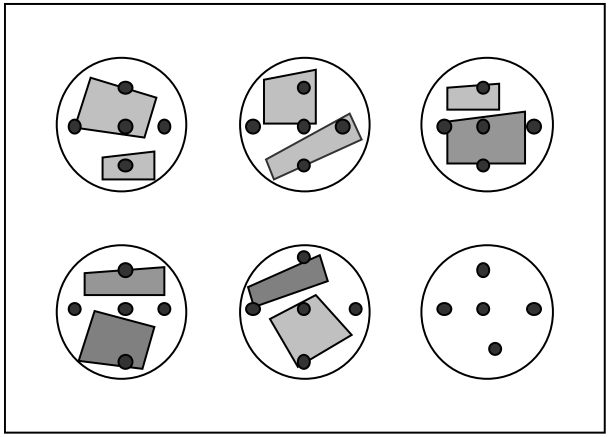
Velvetbean: *Mucuna pruriens* var. *utilis*

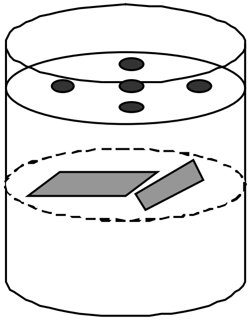
Good food rich in protein

Old Japanese people used Mucuna as food in many ways

Allelopathic and drought resistant

2) Metodo Sandwich
lixiviacion de hojas (mulch, restos vegetales)





- **Multi-recipiente de 6 hoyos**
- **Hojas（10 mg o 50 mg peso seco/10 cm^2）**
- **Preparacion de sandwich con agar**

Evaluacion basada en hojarasca y restos vegetales
La cantidad de hojas se basa en la cantidad de hojas caidas por año（3tn/ha/año）

Dr. Fujii, 1991

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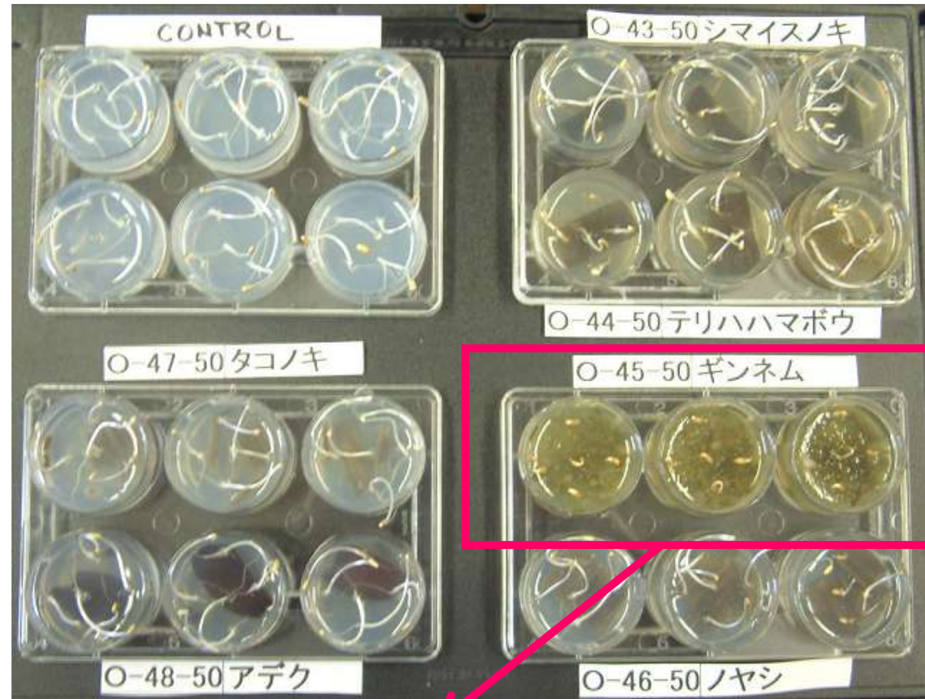
Metodo Sandwich



Leucaena leucocephala **(origen sudamericano)**

<u>Y. Fujii</u>, S. S. Parvez, M. M. Parvez, Y. Ohmae and O. Iida, Screening of 239 medicinal plant species for allelopathic activity using the sandwich method. Weed Biol. Manage., 3. 233-241. 2003.

表１ 落葉を寒天培地に包埋したときのレタスの生育 サンドイッチ法に		
学名 日本での呼び名）	幼根	下胚軸
Eucalyptus citriodora (ﾚﾓﾝﾕｰｶﾘ)	0	0
Cola nitida (ｺｰﾗﾉｷ)	8	60
Eucalyptus cinerea (ｷﾞﾝﾖｳﾏﾙﾊﾞﾕｰｶﾘ)	9	9
Aleurites cordata (ｱﾌﾞﾗｷﾞﾘ)	10	38
Cercidiphyllum japonicum (ｶﾂﾗ)	11	18
Bixa orellana (ﾍﾞﾆﾉｷ)	11	20
Enkianthus perulatus (ﾄﾞｳﾀﾞﾝﾂﾂｼﾞ)	12	46
Bougainvillea spectabilis (ｲｶﾀﾞｶｽﾞﾗ)	14	45
Quercus variabilis (ｱﾍﾞﾏｷ)	15	59
Leucaena leucocephala (ｷﾞﾝﾈﾑ)	16	64
Passiflora quadrangularis (ｵｵﾐﾉﾄｹｲｿｳ)	16	58
Cedrus deodara (ﾋﾏﾗﾔｽｷﾞ)	16	39
Sequoia sempervirens (ｾｺｲｱ)	16	34
Bougainvillea glabra (ﾃﾘﾊｲｶﾀﾞｶｽﾞﾗ)	17	55
Phytolacca di		
Erythria indica		
Magnifra indic		
Hura crepitans		
Ficus religiosa (ｲﾝﾄﾞﾎﾞﾀﾞｲｼﾞｭ)	20	120
Cam		
Datu		
Acer		
Hibis		
Ginkg		
offea		
Artocarpus heterophylla (ﾊﾟﾗﾐﾂ)	26	69
Pinus palustris (ﾀﾞｲｵｳｼｮｳ)	26	52
Cupressus sempervirens (ｲﾄｽｷﾞ)	26	77
Mucuna pruriens var.utilis cv. capitata (ﾑｸﾅ)	28	86
Hevea brasiliensis (ﾊﾟﾗｺﾞﾑﾉｷ)	28	88
ornus florida (ﾊﾅﾐｽﾞｷ)	30	56
Pinus virginicus (ﾊﾞｰｼﾞﾆｱﾏﾂ)	30	75
Cassia surattensis (ﾓｸｾﾝﾅ)	31	126
Jatropha multifida (ﾓﾐｼﾞﾊﾞﾔﾄﾛﾌｧ)	32	110
Strophanthus caudatus (ｷﾝﾘｭｳｶ)	32	91
Hibiscus rosa-sinensis (ﾌﾞｯｿｳｹﾞ)	33	89
Sophora japonica (ｴﾝｼﾞｭ)	34	112

表１ 落葉を寒天培地に包埋したときのレタスの生育 サンドイッチ法に		
学名 日本での呼び名）	幼根	下胚軸
Picea jezoensis (ｴｿﾞﾏﾂ)	34	91
Pinus banksiana (ﾊﾞﾝｸｽﾏﾂ)	35	69
Annona muricata (ﾄｹﾞﾊﾞﾝﾚｲｼ)	37	109
Macadamia ternifolia (ﾏｶﾀﾞﾐｱ)	37	80
Platanus orientalis (ｽｽﾞｶｹﾉｷ)	38	84
Strongylondon macrobotrys (ｼﾞｪｲﾄﾞ･ﾊﾞｲﾝ)	38	138
Chrysophyllum cainite (ｽｲｼｮｳｶﾞｷ)	39	98
Metasequoia glyptostroboides (ﾒﾀｾｺｲｱ)	40	97
Ilex latifolia (ﾀﾗﾖｳ)	41	82
Psidium gujava (ﾊﾞﾝｼﾞﾛｳ)	41	107
Alnus japonica (ﾊﾝﾉｷ)	42	72
hododendron mucronulatum var. cilliatum	43	103
Taxodium distichum (ﾗｸｳｼｮｳ)	43	60
Phyllostachys heterocycla f. pubescens (ﾓｳｿｳﾁｸ)	43	102
	44	57
	44	113
	44	116
	45	86
Pseudosasa japonica (ﾔﾀﾞｹ)	45	96
		67
		06
		54
		98
		97
		75
Cinnamomum japonicum (ﾔﾌﾞﾆｯｹｲ)	49	93
Caesalpinia echinatum (ﾌﾞﾗｼﾞﾙﾉｷ)	49	100
Eugenia javanica (ｵｵﾌﾄﾓﾓ)	49	108
Quercus serrata (ｺﾅﾗ)	50	112
Aesculus turbinata (ﾄﾁﾉｷ)	50	126
Cinnamomum camphora (ｸｽﾉｷ)	50	95
Ceiba pentandra (ｶﾎﾟｯｸ)	50	162
Aphananthe aspera (ﾑｸﾉｷ)	52	99
Aecr palmatum (ｲﾛﾊｶｴﾃﾞ)	54	80
Forsythia suspensa (ﾚﾝｷﾞｮｳ)	54	78
Aesculus hippocastanum (ﾏﾛﾆｴ)	54	120
Cytisus scoparius (ｴﾆｼﾀﾞ)	54	

Evaluo 4000 especies en 15 años

Plantas Tropicales y Medicinales muestran una fuerte actividad alelopatica

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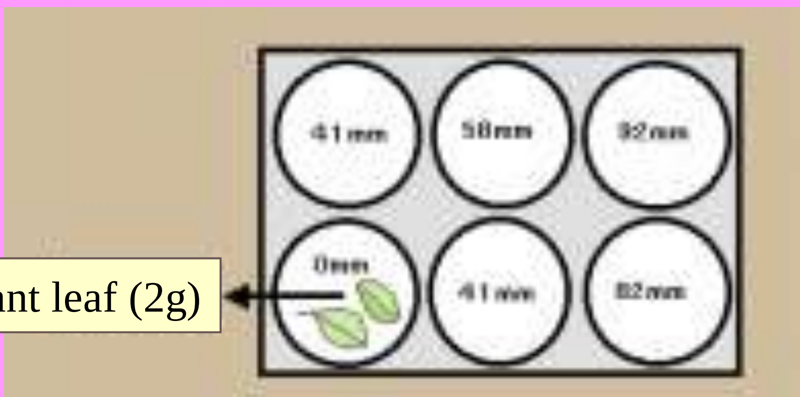
3. Dish Pack Method

for volatile allelochemicals



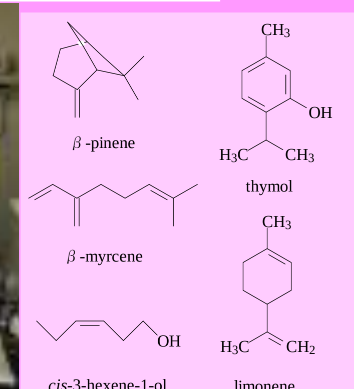
plant leaf (2g)

6 well multi-dish

sealed by tape

→ measure after 3 days

Volatile chemicals were analyzed by GC-MS



Fujii, Y. et al., Dish pack method: a new bioassay for volatile allelopathy. In Proceedings of the 4th World Congress on Allelopathy, August 21-26, 2005, Wagga Wagga, Australia, 493-497 (2005).

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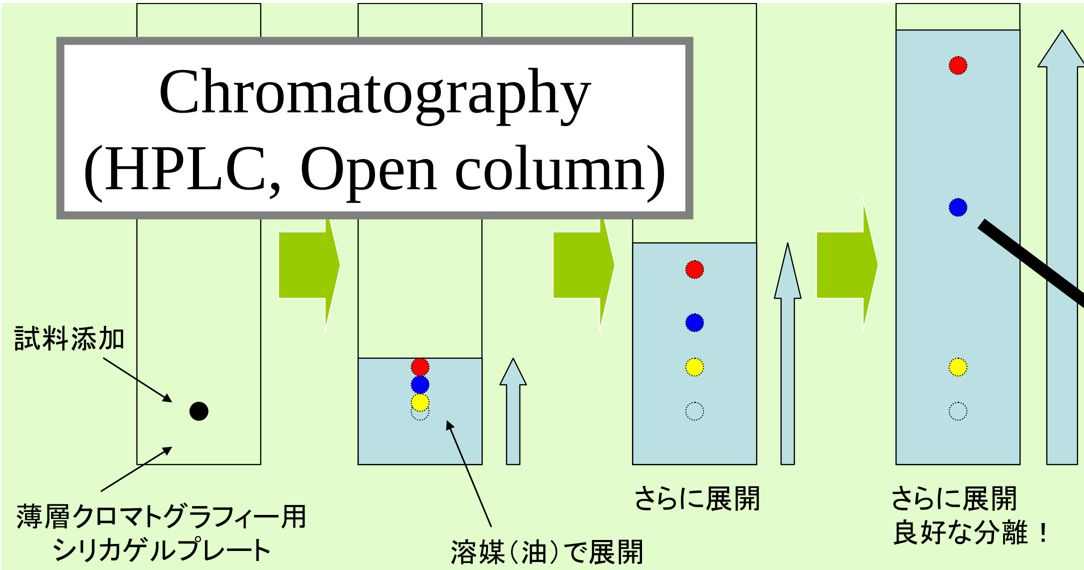
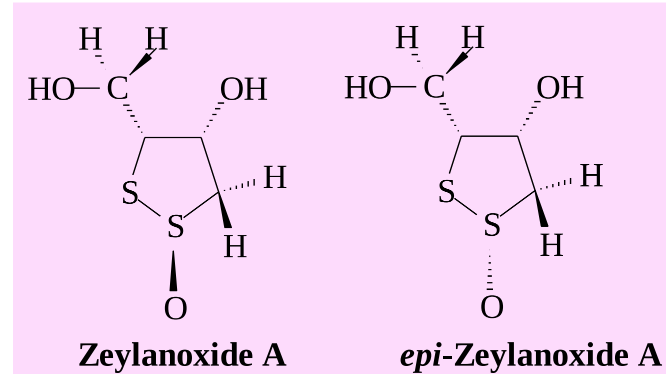
2. Isolation and Identification of Allelochemicals

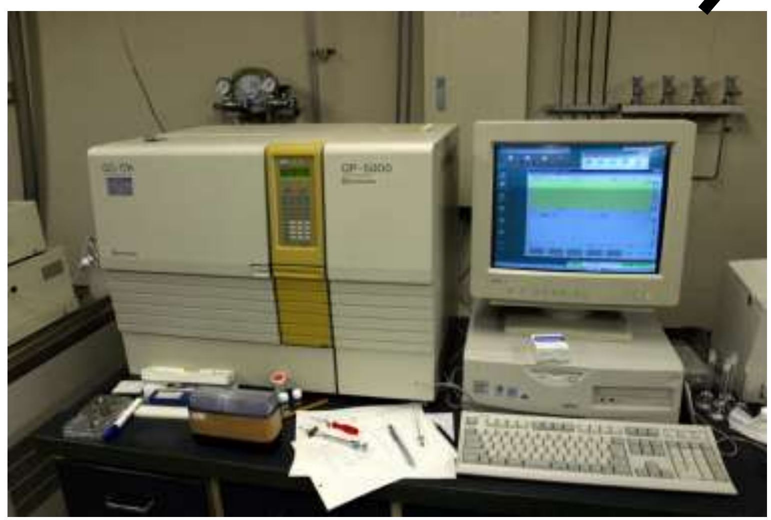
Chromatography (HPLC, Open column)





Structure

Solvent Extraction



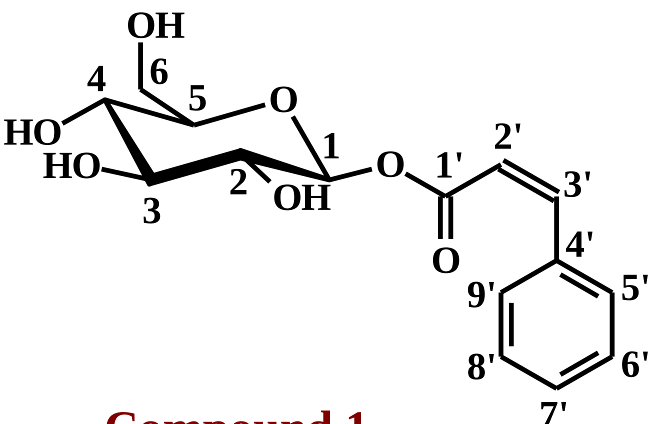
GC-MS
（150,000 $）

600 MHz NMR
（1.800,000 $）

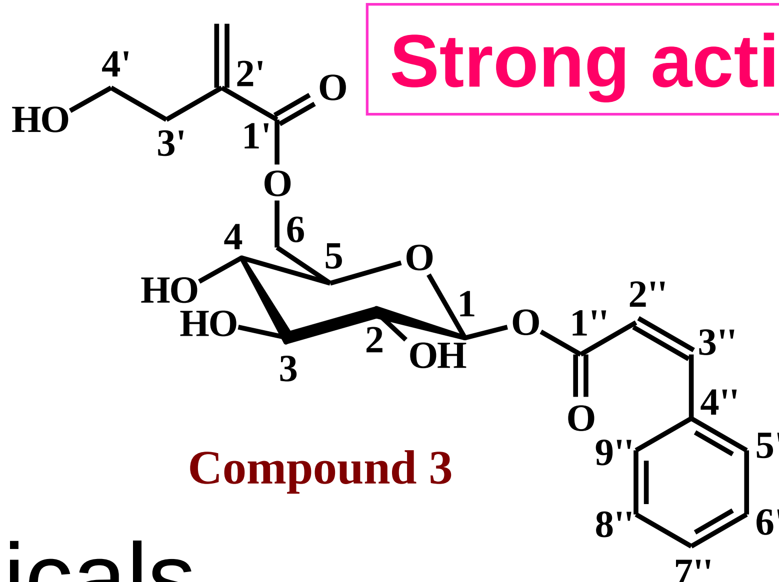
IR, ESR, Raman, X-ray analysis, etc

Isolation of *cis*-cinnamic acid from *Spiraea*

Yuki-yanagi *(Spiraea thunbergii)*



Compound 1

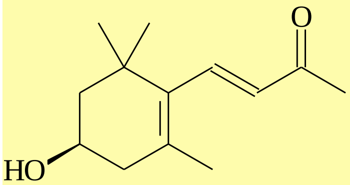


Compound 3

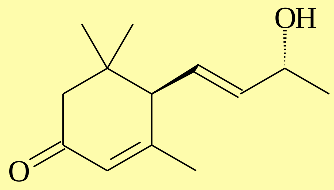
Strong activity !

→ New Agrochemicals

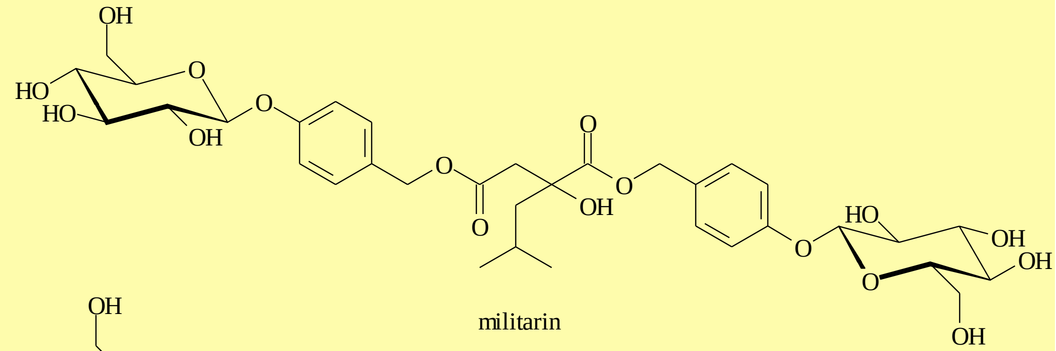
S. Hiradate, S. Morita, H. Sugie, <u>Y. Fujii,</u> and J. Harada, Phytotoxic *cis*-cinnamoyl glucosides from *Spiraea thunbergii*. Phytochemistry, 65(6), 731-739, 2004



(-)-3-hydroxy-beta-ionone



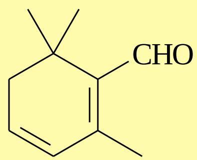
(+)-3-oxo-alpha-ionol



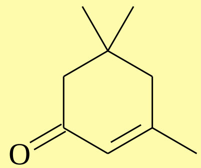
militarin

ナギナタガヤ*Vulpia myuros*

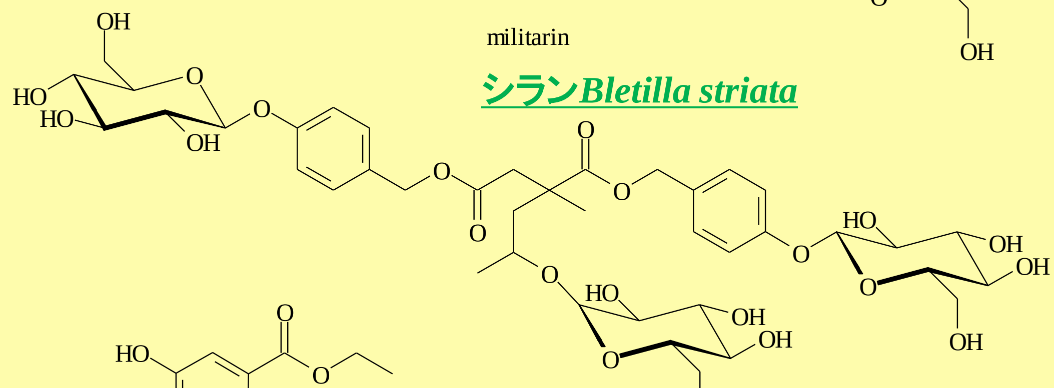
シラン*Bletilla striata*



safranal



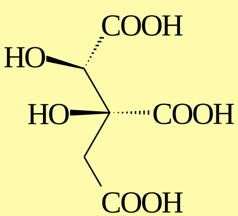
isophorone



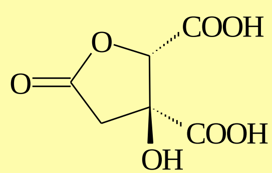
dactylorhin A

サフランCrocus sativus

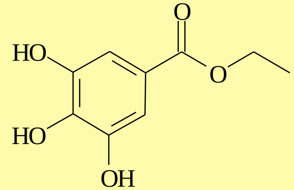
シラン*Bletilla striata*



(-)-(2*S*,3*S*)-hydroxycitric acid



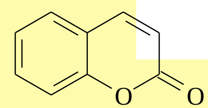
(-)-(2*S*,3*S*)-hydroxycitric acid lactone



ethyl gallate

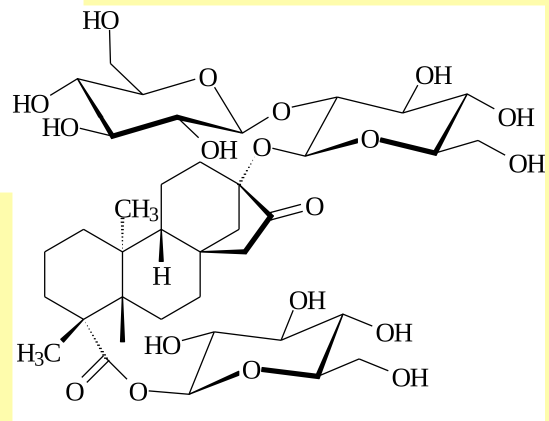
Geranium carolinianum

ガルシニア*Garcinia cambogia*

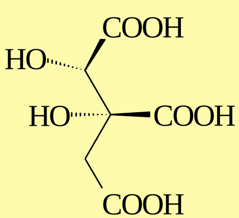


coumarin

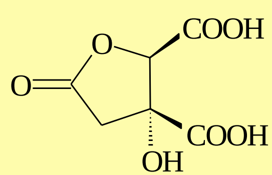
Gliricidia sepium



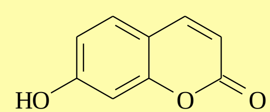
stevioside



(+)-(2*R*,3*R*)-hydroxycitric acid



(+)-(2*R*,3*R*)-hydroxycitric acid lactone



umbelliferone

ローゼル*Hibiscus sabdariffa*

Diplostephium foliosissimum

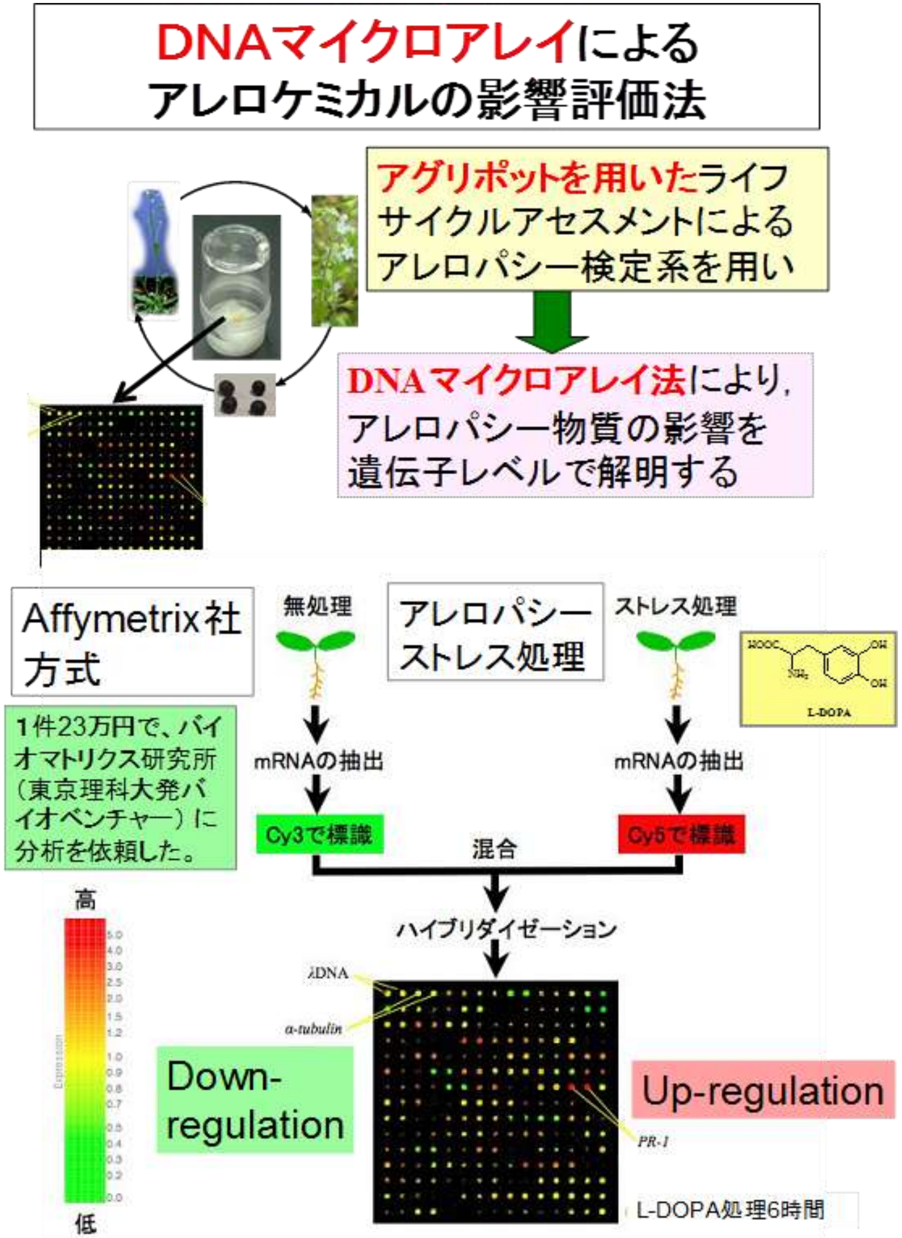
Stevia rebaudiana

Major Allelochemicals isolated by our group

NIAES

3. Mode of action of Allelochemicals

NIAES

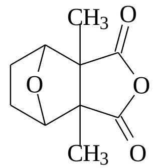


Evaluation of Mode of action of Allelochemicals by DNA Microarray in combination with Life Cycle Assessment of Allelopathy

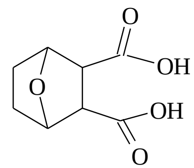
Golisz, Sugano, Hiradate, Fujii (2010) Microarray analysis of Arabidopsis plants in response to allelochemical L-DOPA , Planta, 233(2), 231-240.

4. Use Allelochemicals as new Bioactive Substance

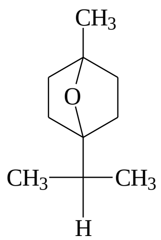
Allelochemicals　　New herbicide



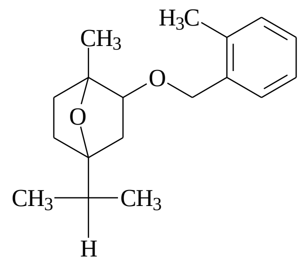
Cantharidin



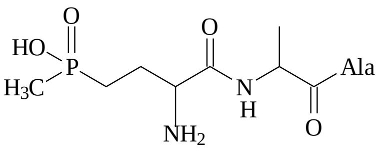
Endothall



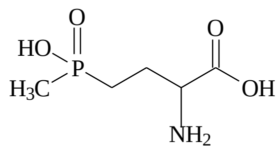
1,4-Cineole



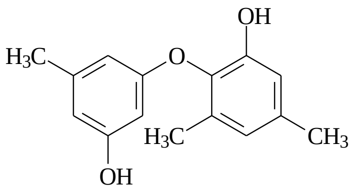
Cinmethylin



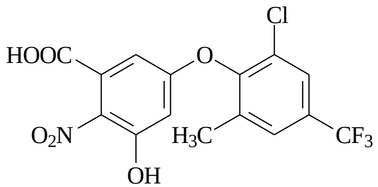
Bialaphos



Gulfosinate

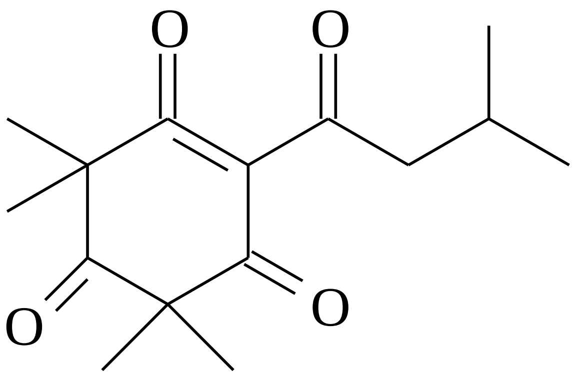


Cyperine

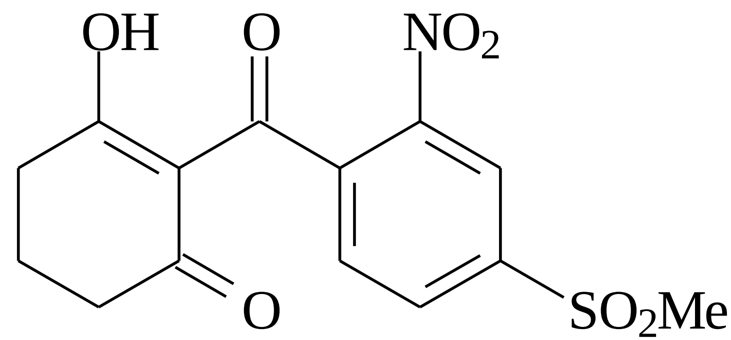


Acifluorfen

Allelochemical



New herbicide



Organic Synthesis

Leptospermone

Allelochemical (natural)

LD_{90}= 5 kg/ha

Mesotrione

Herbicide (modified)

LD_{90}= 0.001 kg/ha

Inhibition of Photosynthesis

Syngenta (Swiss) developed a new Herbicide, Mesotrione from allelochemical (reinforced 5000 times)

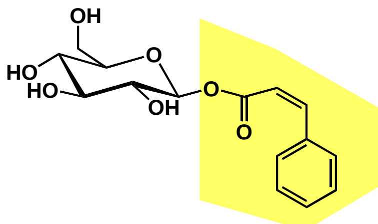

New mode of action

HPPD inhibitor

lemon bottlebrush (*Callistemon citrinus*)

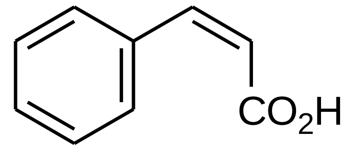
Organic synthesis of allelochemicals

Cooperation with Kyusyu Univ. and Herbicide Company



1-O-*cis*-cinnamoyl-β-D-gulcopyranose

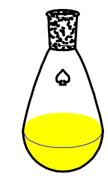
cis-CA derivative
identified in NIAES



cis-CA

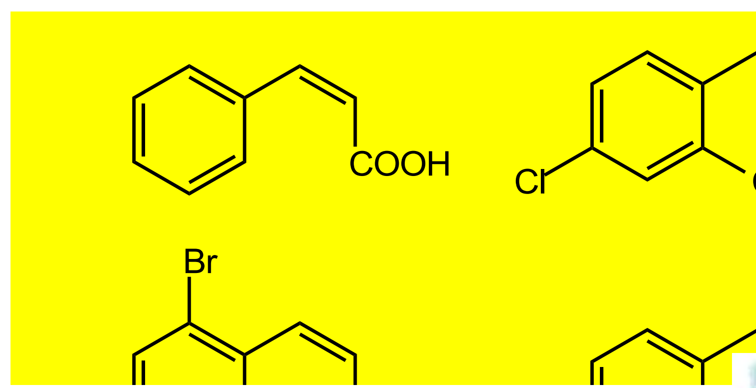
Organic Synthesis

Library of derivatives



NIAES,
Company

Evaluation



Specific Bioassay as agrochemicals
水田、畑作用除草剤スクリーニング
殺菌剤スクリーニング
殺虫剤スクリーニング

5. Use Allelopathic plants for Sustainable Agriculture

My Work help Japanese Farmers

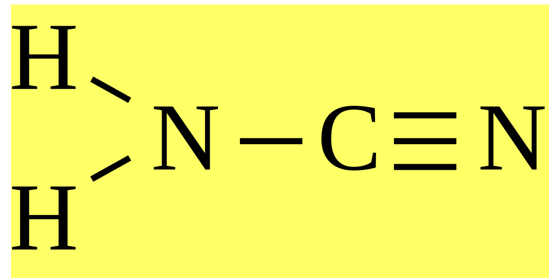
NIAES

Hairy vetch（*Vicia villosa*）

Fujii,Y :Screening and future exploitation of allelopathic plants as alternative herbicides with special reference to hairy vetch. *Journal of Crop Production*, 4(2), 257～275 (2001)

Identification of Cyanamide as Allelochemical from Hairy vetch

★ **Cyanamide**



★ known as active constituents of synthetic fertilizer (Calcium cyanamide)

First finding from plants (as natural producs)

Kamo, T., Hiradate, S. and Fujii, Y.: First isolation of natural cyanamide as a possible allelochemical from hairy vetch *Vicia villosa* . J. Chemical Ecol. 29(2), 275-283 (2003)

Help organic farming

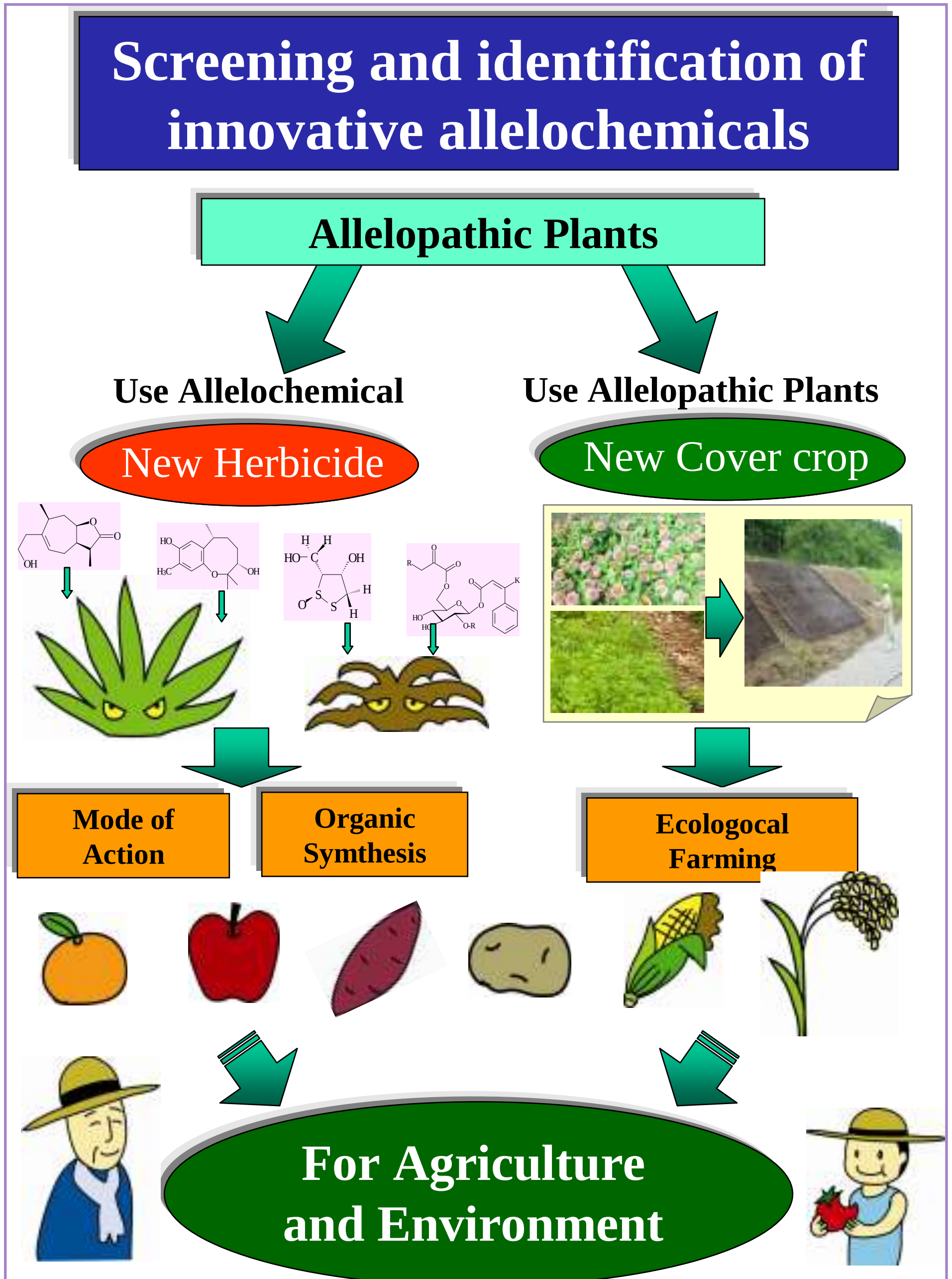
Hairy vetch is now planted 10,000 ha in Japan

(No 2 as cover crop, next to traditional Milk vetch

成21年度 科学技術分野の文部科学大臣表彰
Scientific Achievement Award 2009
From the Ministry of
Education, Culture, Sports,
Science and Technology
賞状

4) Cooperative Research under MOU

“BRAIN” Project



BRAIN:**B**asic **R**esearch **A**ctivitiesfor **IN**novative Biosciences

"Screening of Allelochemicals and Development of Innovative Bioactive Substances"

2008 to 2013

* Supported by Ministry of Agriculture, Forestry and Fisheries, Japan

NIAES

MOU between IIAP(Peru) and NIAES(Japan)

2009.0209-11, our 1st visit

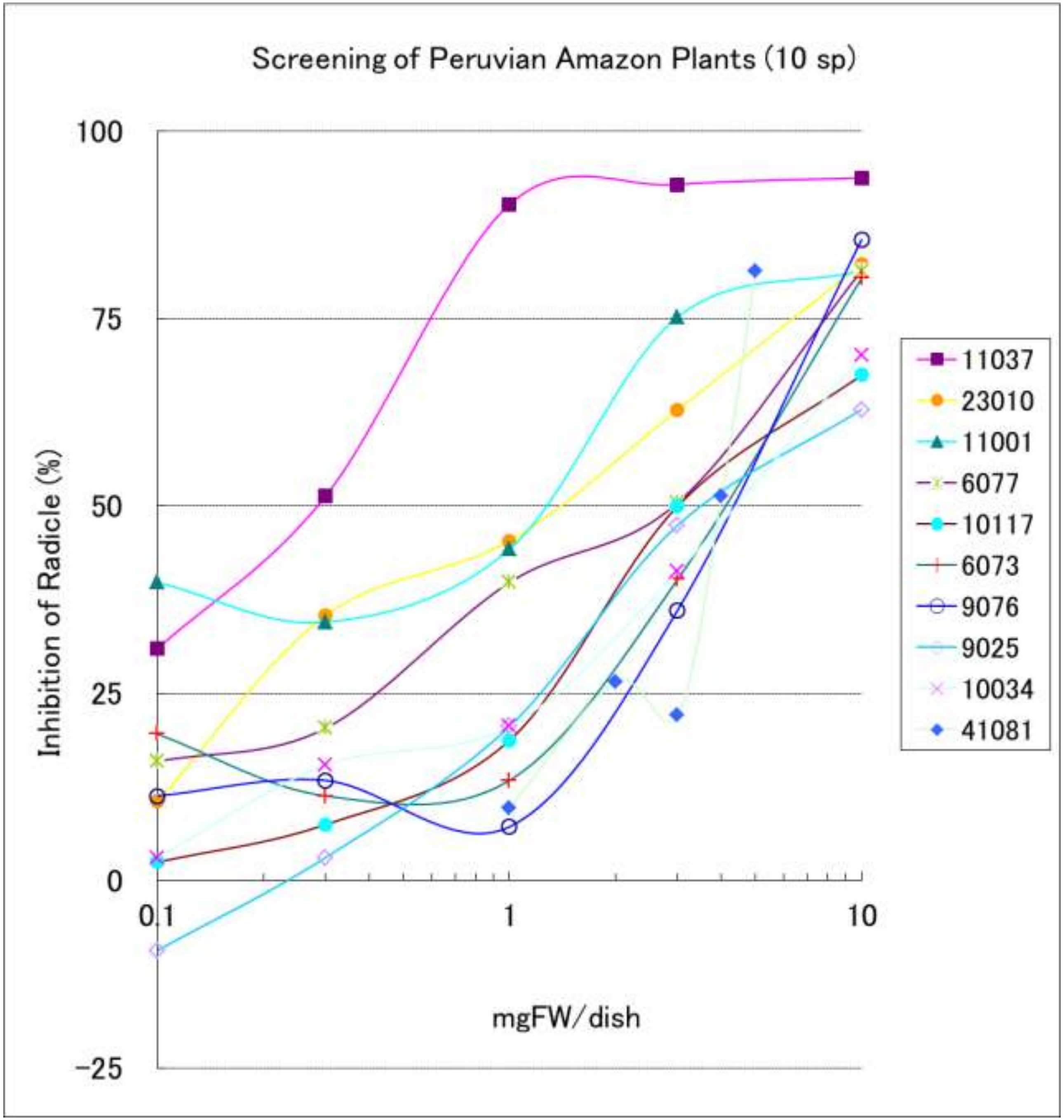
2010.0118, 2nd visit

2011.0205-7, 3rd visit

NIAES

<u>Dr. Sotero</u> (IIAP) visited NIAES(Japan) for the Cooperative Research **2010.0902-25**

Screening by Bioassay



Top 10 Allelopathic Plants from the cooperative screening at 2010

As a result of cooperative research (unpublished)

NIAES

Duroia hirsuta (supay chacra)

NIAES

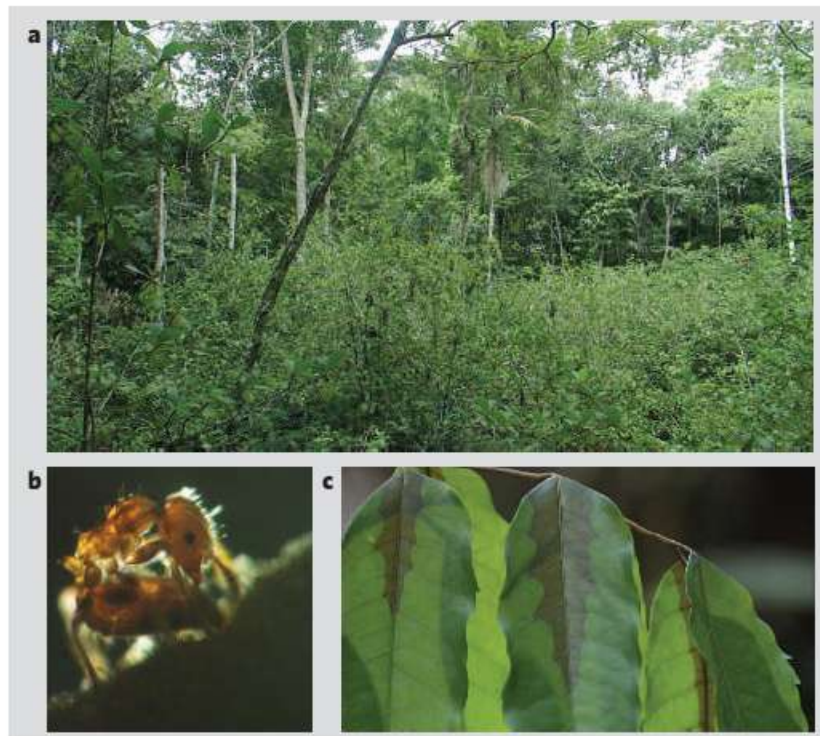
Megan Frederickson, Michael Greene, Deborah Gordon
(Stanford University, USA)

'Devil's gardens' bedevilled by ants

An ant species uses herbicidal weaponry to secure its own niche in the Amazonian rainforest.

'Devil's gardens' are large stands of trees in the Amazonian rainforest that consist almost entirely of a single species, *Duroia hirsuta*[1–5], and, according to local legend, are cultivated by an evil forest spirit. Here we show that the ant *Myrmelachista schumanni*, which nests in *D. hirsuta* stems, creates devil's gardens by poisoning all plants except its host plants with formic acid. By killing these other plants, *M. schumanni* provides its colonies with abundant nest sites — a long-lasting benefit as colonies can live for 800 years.

M. schumanni lives in the hollow, swollen stems (domatia) of *D. hirsuta*, the tree species that dominates devil's gardens (Fig. 1a). Previous studies of the mutualism between *D. hirsuta* and *M. schumanni* indicated that devil's gardens result from allelopathy, which is the local inhibition of plant growth by another plant, by *D. hirsuta*[2–5]. However, studies of a different ant–plant mutualism — between an unidentified species of *Myrmelachista* and the ant-plants *Tococa guianensis* and *Clidemia heterophylla* — indicated that *Myrmelachista* may create stands comprising only its host plants by using herbicide[6,7].



M. DOHRN © BBC

Figure 1 | The ant *M. schumanni* creates devil's gardens by killing all plants other than its host tree, *D. hirsuta*. a, A devil's garden, or monospecific stand of *D. hirsuta*, in the foreground contrasts with the species-rich rainforest in the background. **b,** A worker *M. schumanni* ant attacking a plant: the ant bites a small hole in the leaf tissue, inserts the tip of its abdomen into the hole and releases formic acid. **c,** Leaves develop necrosis along primary veins within hours of the attack.

promptly attacked the saplings in devil's gardens from which ants had not been excluded, injecting a poison into their leaves (Fig. 1b),

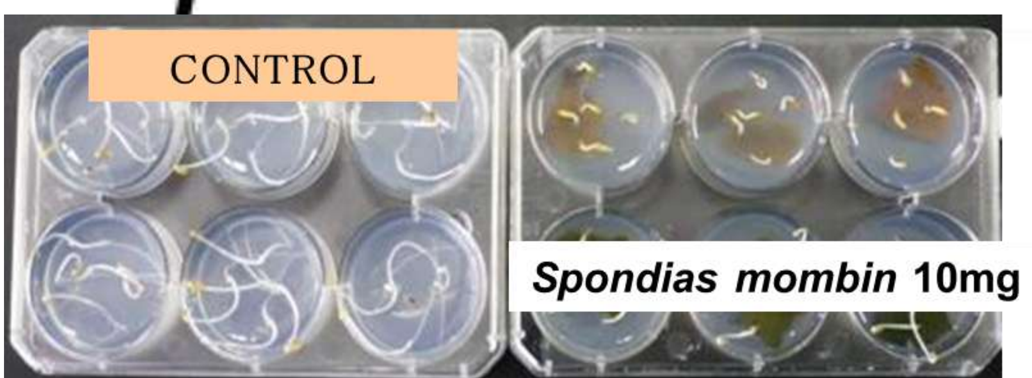
whether *M. schumanni* attacks only plants that are not its host plants and whether the ant uses domatia to recognize its host. We planted

Screening by **<u>Cecilia ONO</u>** → **Ubos** is promising

Familia	*NC*	R	H
Anacardiaceae	***Spondias mombin*** **L. (UBOS)**	3	11
Bombacaceae	*Matisia cordata* Bonpl.	18	85
Fabaceae	*Arachis pintoi* Krapov. & W.C. Greg.	29	84
Malvaceae	*Malachra alceifolia* Jacq. [a]	32	76
Rubiaceae	*Uncaria tomentosa* (Wild. Ex Schult) DC.	33	88
Sterculiaceae	*Theobroma bicolor* Bonpl.	35	96
Sterculiaceae	*Theobroma cacao* L.	35	89
Sterculiaceae	*Theobroma grandiflorum* (Willd. ex Spreng.) K. Schum.	37	93
Rubiaceae	*Uncaria guianensis* (Aubl.) J.F. Gmel.)	39	80
Fabaceae	*Desmodium axillare* (Sw.) DC.	42	106
Euphorbiaceae	*Caryodendron orinocense* H. Karst.	45	82
Solanaceae	*Inga edulis* Mart.	45	111
Lauraceae	*Aniba rosaeodora* Ducke	46	88
Dioscoraceae	*Dioscorea* sp.	46	97
Solanaceae	*Nicotiana tabacum* L.	47	42
Oxalidaceae	*Averrhoa* carambola L.	48	107
Pasifloraceae	*Passiflora quadrangularis* L.	48	69
Fabaceae	*Tachigali paniculata* Aubl.	49	112
Piperaceae	*Piper* sp.	51	85
Amaranthaceae	*Alternanthera halimifolia* (Lam.)Standley.	51	98

Tested 80 plants in Amazon

Spondias mombin = Ubos

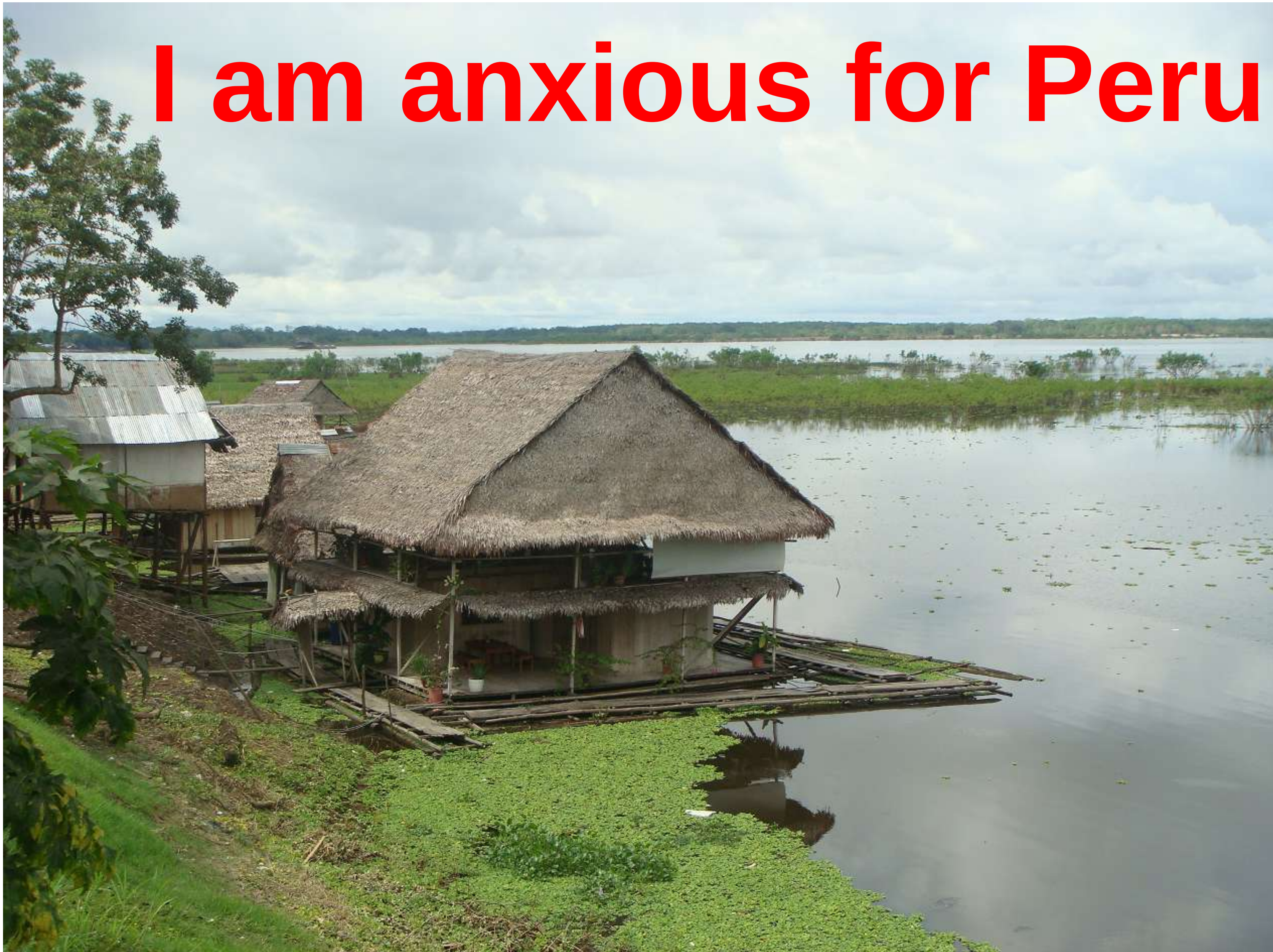


Promising

as allelopathy

Teach us Allelopathy Phenomena for Cooperative Work

I am anxious for Peru



Stop Invasion of Alien Plants

To keep your Biodiversity

Gracias

Usemos nuestra preciada BIODIVERSIDAD para la existencia sustentable de la humanidad en el mundo

NIAES