Department of Biological Safety

The Department of Biological Safety includes the 3 research groups of Plant Ecology, Entomology, and Microbiology, as well as the Genetically Modified Organism (GMO) Assessment Team, as described below. The GMO Assessment Team was formed with the aim of using recent advances in biotechnology and bioindustry for environmental impact assessment of GMOs. The mission of the Department as a whole is to assess the environmental impact of GMOs as well as of alien invasive and introduced living organisms, and to investigate the interaction of biodiversity and agriculture in terms of biosafety and sustainable agriculture. The Department is developing advanced methods to use in these assessments.

The major research domains of the Department are: 1) evaluation of the influence of agricultural activities on agro-ecosystems and biodiversity; 2) environmental impact assessment of introduced natural enemies and alien invasive organisms; 3) identification of biologically active chemicals and their effects on organisms in agro-ecosystems; and 4) risk assessment of GMOs within agro-ecosystems. Research is performed in collaboration with other research groups within and outside NIAES, and the approaches cover a number of research fields, such as molecular, chemical, population, and landscape ecology.

The research of the **Plant Ecology Group** is focused on vegetation dynamics and conservation of vegetation, assessment of invasive and introduced plant species in agro-ecosystems, and plant diversity in relation to agricultural production. Current research topics are: 1) effects of sulfonylurea herbicides on plant species, including aquatic plants, in agro-ecosystems; 2) landscape ecological approaches to the prediction of vegetation dynamics in relation to farmland use; and 3) the search for allelochemicals and elucidation of allelopathic mechanisms for maintaining agro-ecological vegetation.

The **Entomology Group** focuses on the following 3 major targets: 1) ecological risk assessment of alien insects such as natural enemies of insect pests; 2) analysis of the population dynamics of insect herbivores responding to the spatial distribution patterns of plants; and 3) identification of semiochemicals and analysis of the mechanisms of sex pheromone resistance.

The **Microbiology Group** aims to characterize microbial communities and to develop technologies for effective management of microbial resources in agro-ecosystems. Current research activities are: 1) investigation of microbial diversity and interactions in the soil under different agro-ecosystems; 2) analysis of the effects of environmental factors, including microbial secondary metabolites, on the survival and diversity of microbes; and 3) determination of the taxonomy, biology, and ecology of nematode communities.

Photo Participants from 8 Asian countries at the international workshop held at the Agricultural Research Institute, Taichung, Taiwan, in November 2004 to discuss the development of a biological invasion database.

The research objective of the **GMO Assessment Team** is to investigate the effects of the release of GMOs on the environment. The principal fields of interest are: 1) clarification of the dispersal and transfer mechanisms of genes from GMOs to other organisms; 2) assessment of the impact of *Bacillus thuringiensis* (Bt) toxin in corn pollen on *Lepidoptera*; and 3) monitoring of changes in the composition of weeds, insects, and soil microorganisms caused by the cultivation of genetically modified crops.

The major activities of the department in FY 2003 were: 1) publication of 11 main research results in the *NIAES Major Research Topics Annual* (these results are described below as topics in the introduction of research groups and the team); 2) organization of an international workshop on "Development of a database on biological invasion in the Asian-Pacific region" in Taiwan in November 2004, in collaboration with Taiwanese agricultural organizations, as well as the combined national meeting of the 24th Symposium on Agro-Environmental Science and 7th Seminar on Vegetation Science on 'Agricultural Use of Biological Functions for the Conservation of Agro-ecosystems; Co-actions through the Natural and Bioactive Substances'; 3) implementation and coordination of a research project on 'Assurance of the Safe Use of Genetically Modified Organisms' and participation in several projects organized by MAFF and the Ministry of Education, Culture, Sports, Science and Technology (MEXT); and 4) attendance of departmental staff at many international symposia and workshops.

Furthermore, Dr. Y. Fujii, a research unit leader in the Chemical Ecology Unit, won the Awards for his research on allelochemicals from the Japanese Society of Soil Science and Plant Nutrition in April 2004 (see Highlights).

1) Plant Ecology Group

The Plant Ecology Group consists of the **Vegetation, Landscape**, and **Chemical Ecology Units**. These units individually carry out research on the impact of agriculture on plant diversity, methods for monitoring vegetation change in agro-ecosystems, and interactions among agricultural organisms through biologically active chemicals. The major results obtained in 2004 are described below in research topics 1 to 5.

The Plant Ecology Group organized a domestic seminar on "Agricultural use of biological functions for the conservation of agro-ecosystems: co-actions of natural and active substances", held at NIAES on 10 December 2004. More than 120 people participated in the seminar, discussing the potential use and commercialization of some of the substances produced by organisms.

Topic 1: cis-Cinnamoyl glucosides as major plant growth inhibitors in *Spiraea thunbergii*

Spiraea thunbergii Sieb., a widespread ornamental plant originally from China, is used as a hedge or garden plant in Japan. This plant shows high allelopathic potential (i.e. it can inhibit the growth of other plants by the production of plant-growth inhibitory chemicals). We therefore conducted a bioassay-directed purification to isolate these plant-growth inhibitory chemicals. Two compounds were isolated as major plant-growth inhibitors, and their chemical structures were elucidated as novel *cis*-cinnamoyl glucosides, *cis*-CG and *cis*-BCG (Fig. 1, Hiradate et al., 2004b; *see Appendix for full reference*). The plant-growth inhibitory activities of *cis*-CG and *cis*-BCG were of the same strength and 2 to 4 times stronger than that of *cis*-abscisic acid (a plant hormone, Fig. 2A), indicating that the inhibitory potential of *cis*-CG and *cis*-BCG could be among the highest yet reported for natural products. *cis*-Cinnamic acid (*cis*-CA, Fig. 1), which is a component of *cis*-CG and *cis*-BCG,

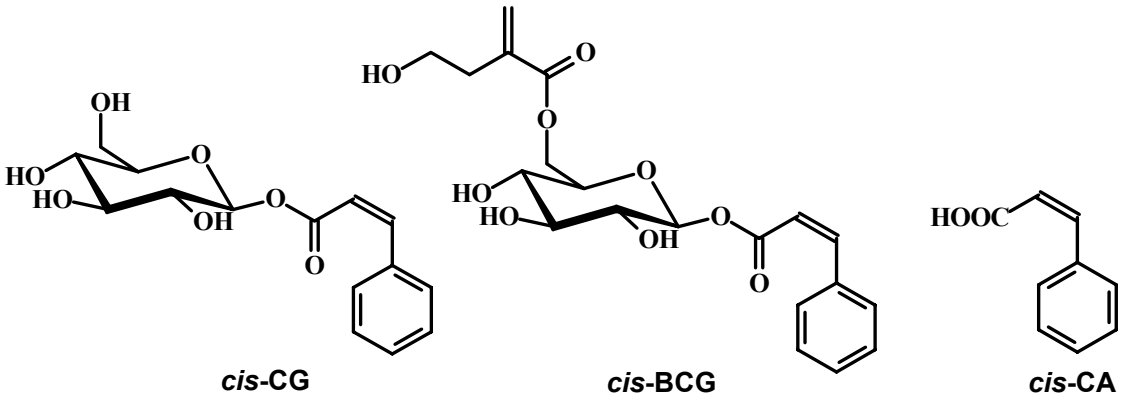

Fig. 1. Chemical structures of *cis*-CG, *cis*-BCG, and *cis*-CA.

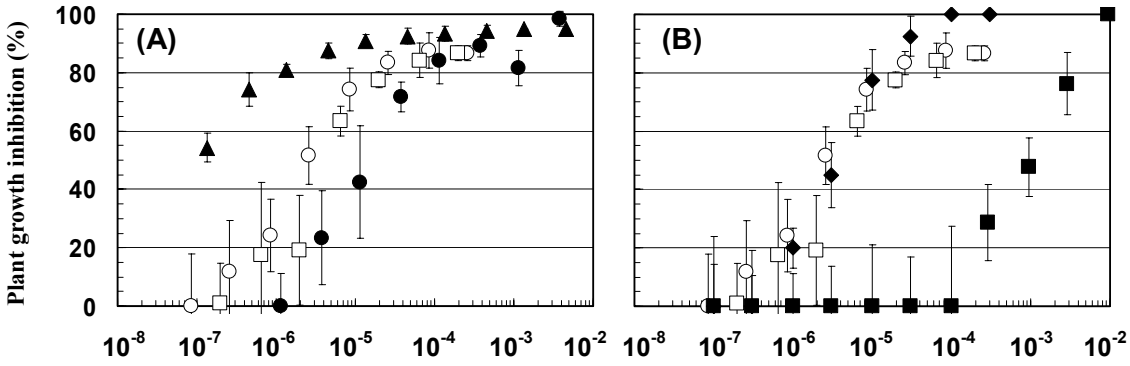

Fig. 2. Comparison of plant-growth inhibitory activities of *cis*-CG (○) and *cis*-BCG (□) with those of commercial compounds (A; 2,4-D (▲), *cis*-abscisic acid (●)) and with those of cinnamic acids (B; *cis*-cinnamic acid (◆), *trans*-cinnamic acid (■)). Test plant: lettuce (*Lactuca sativa* cv. Great Lakes 366). Bars indicate standard deviation (n = 5 or 6).

was found to possess almost the same inhibitory activity as *cis*-CG and *cis*-BCG (Fig. 2B), indicating that the chemical structure essential to the high inhibitory activity of *cis*-CG and *cis*-BCG was *cis*-CA (Hiradate et al., 2005). These findings indicate the potential roles of *cis*-CA and its glucosides as allelochemicals and their possible use as plant growth regulators in agricultural fields (Hiradate et al., 2004a). (S. Hiradate, H. Araya, Y. Fujii, H. Sugie, S. Morita, A. Furubayashi, J. Harada)

***Topic 2:* Isolation of allelochemicals from *Ophiopogon japonicus, Robinia pseudoacacia*, and essential oils from 35 domestic tree species**

We conducted laboratory and greenhouse experiments to evaluate the allelopathic potential of dwarf lily-turf (*Ophiopogon japonicus* Ker-Gawler) on lettuce, alfalfa, timothy, and rape. We investigated the effects of dry leaf debris, an aqueous extract of fresh leaves, and soil in which *O. japonicus* had been grown. The emergence, dry weight, and root and shoot length of all bioassay species were inhibited in a concentration-dependent fashion when grown in soil to which we had added oven-dried leaves of *O. japonicus*. However, the degree of inhibition varied among the test plant species. The aqueous leaf extract was highly phytotoxic and significantly reduced the germination, seedling growth, and fresh weight of all test species. The active chemicals in *O. japonicus* were isolated as β-sitosterol, *p*-hydroxybenzoic acid, and salicylic acid (*o*-hydroxybenzoic acid; Fig 3a). Of these compounds, salicylic acid was the most active and was present at a concentration of about 0.03% in the leaves; we concluded that this compound is responsible for the allelopathy.

Robinia pseudo-acacia L. known as black locust is a useful tree in temperate and subtemperate zones, but now become invasive alien plant in the central part of Japan. Vicinities dominated by this tree shows reduced growth of weeds nearby and underneath, presumably because of allelopathic interactions. Growth of both the radicles and hypocotyls of weeds (barnyard grass and white clover) and of edible plants (lettuce and Chinese cabbage) was significantly reduced when these species were grown in soil mixed with a leaf powder of *R. pseudo-acacia* at various concentrations. Aqueous leaf extracts caused significant suppression of radicle growth of lettuce and other weed species. Compounds identified from the extracts included robinetin (Fig. 3b), myricetin, and quercetin. Robinetin was the major growth inhibitor, and at 100 ppm it caused 50% growth suppression of the

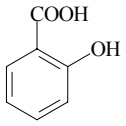

Fig. 3a Salicylic acid

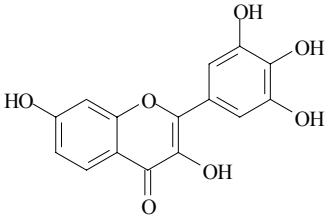

Fig. 3b Robinetin

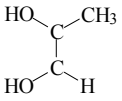

Fig. 3c 1,2-Propanediol

roots and shoots of tested plants. Myricetin and quercetin inhibited plant growth to a lesser extent. We conclude that weed decline underneath stands of *R. pseudo-acacia* and its spread into adjacent grassland vegetation results from an allelopathic interaction.

PCS (a commercial air freshener product of Field Science Co, Japan) is composed of essential oils from 35 plant species, including essential oils of Hiba (*Thujopsis dolabrata* Sieb. et Zucc.), Hinoki cypress (*Chamaecyparis obtusa* Sieb. et Zucc.), and Kumazasa (*Sasa albo-marginata* Makino. et Shibata). Chromatographic separation of a methanolic solution of PCS resulted in the isolation of a plant-growth promotive substance, which was identified by gas chromatography-mass spectrometry and nuclear magnetic resonance spectroscopy as 1,2-propanediol (Fig. 3c). Seedling growth bioassay using lettuce as a test plant revealed that 1,2-propanediol acts as a plant growth promoter, and at a concentration of 0.01 mg/L it enhanced the growth of lettuce seedlings. The concentration of 1,2-propanediol in PCS was estimated as 4 g/L. These studies suggest that 1,2-propanediol is an important plant growth-promoting agent in PCS. (Y. Fujii, S. Hiradate, H. Araya, Z. Iqbal, H. Nasir, E. Nakajima)

Topic 3: **Clone distribution of hybrid dandelions on the Kanto Plain**

An examination of the plants collected in the Environmental Indicator Species Survey (Survey of Common Wildlife) by the Ministry of Environment (NIAES Annual Report, 2002) revealed that 85% of the plants identified morphologically as introduced dandelions were hybrids that had originated from crosses between native and introduced dandelions. From the viewpoint of environmental indicators, we used nuclear DNA micro-satellite markers to survey the genetic structures of hybrid dandelions collected from the Kanto Plain. Of the 263 tetraploid hybrids, 246 (93%) were classified as genetically identical clones. This dominant clone was widely distributed on the Kanto Plain. (H. Shibaike, Y. Kusumoto, T. Ohkuro, M. Ide)

2) Entomology Group

The mission of the Entomology Group is to prevent the disturbance of agro-ecosystems by native and exotic insect species, and to assess the non-target effects of introduced insects. In FY 2004, the 3 units of the division studied 3 major subjects covering 8 practical research subjects. Furthermore, the Entomology Group conducted cooperative studies with the Food Production Prediction Team, the GMO Assessment Team, and the Plant Ecology Group.

The **Introduced Insect Assessment Unit** compared the host range, sex ratio, and fertility of *Dacnusa sasakawai*, an endoparasitic wasp of the leafminer, with those of an introduced endoparasitic wasp, *Dacnusa sibirica*. We recognized no significant difference between them, so we could not obtain ecological proof as to why the introduced parasitoid prevailed over the native parasitoid. We also examined in detail the hybridization rates among introduced and native parasitoids of the chestnut gall wasps *Torymus sinensis* (introduced parasitoid) and *Torymus beneficus* (native one) by using mitochondrial and nuclear DNA markers. Hybrid posterity between *T. sinensis* and *T. beneficus* was detected within 5% of total offspring. A laboratory experiment for assessment of the non-target impact of the introduced green lacewing *Chrysoperla carnea* on the native species *Chrysoperla nipponensis* was conducted to determine rates of larval interspecific predation. (Topic 1). To accumulate and share data on invasive alien species in the Asia–Pacific region, we developed the Asian-Pacific Alien Species Database and made it available on the Internet (http://apasd-niaes.dc.affrc.go.jp/). Furthermore, a mechanistic model for describing the dispersal distance of organisms was developed by generalization of the Brownian motion model, allowing stochastic fluctuations of step length (Topic 2).

The **Population Ecology Unit** studied the effects of the spatial distribution of host plants on the population dynamics of the ragweed beetle *Ophraella communa.* We measured the food consumption of this beetle and revealed that ovipositing females consumed the greatest amounts of food. We also investigated the effect of food conditions on the dispersal of beetles from the hosts by flight. We developed a simulation model for analyzing the population dynamics of this insect, incorporating factors such as the spatial distribution of host patches and the rate of dispersal of beetles between host patches. The results of the simulation fitted the occurrence of the insects on host patches in the field.

By selection of individuals, the **Insect Semiochemical Unit** studied and established a strain of the smaller tea tortrix moth *Adoxophyes honmai* resistant to communication disruptants containing the sex pheromone (Z)-11-tetradecenyl acetate. The cause of the resistance was investigated in males of both strains (the resistant and susceptible strains) that were set in the stream of the smell of (*Z*)-11-tetradecenyl acetate; their antennae were then sprayed with synthetic pheromone containing 4 components. The antennae of males of the resistant strain showed significantly higher response (as measured by electroantennogram) than those of the susceptible strain. Furthermore, we studied the chemical structure of

the substance that is released from the stink bug *Eysarcoris lewisi* which causes pecky rice; we found that the substance was an alcohol with the chemical formula $C_{15}H_{24}O$.

The **Insect Gene Bank Project** was implemented by the above-mentioned 2 Units and the Insect Systematics Laboratory. This project started in 2000. The purposes of this project are to collect and rear successively insect species or strains, such as natural enemies or other insects, for uses such as bioassays, and to supply these insects to laboratories requesting them for research. In 2004, two aphid species, *Rhopalosiphum padi* and *Acyrthosiphon pisum*, were added to the collection. The brown planthopper *Nilaparvata lugens* (a strain virulent to a resistant rice carrying the *bph-4* gene) was added to the active collection (i.e. the collection of insects that are available for supply). The physiological and ecological characteristics of about 42 items in the collection were evaluated.

Topic 1: **Laboratory experiment to assess the non-target impact of the introduced green lacewing *Chrysoperla carnea* (Stephens) (Neuroptera: Chrysopidae) on the native species *C. nipponensis* (Okamoto): larval interspecific predation**

The green lacewing *Chrysoperla carnea* (Stephens) is frequently used for biological control. It has long been assumed to be a single species that is morphologically identical throughout a Holarctic distribution range. However, more recent evidence suggests that it is not a single species, but instead a complex of several or many biological species characterized by different male courtship songs (Henry et al., 1993; 2001). In Japan, the native green lacewing is widely distributed and has been classified as *C. carnea* (Tsukaguchi, 1985). However, the name was revised to *C. nipponensis* (Okamoto) by Brooks (1994) on the basis of external morphological differences such as the color of the gradate crossveins, which are black in *C. nipponensis* and green in *C. carnea*. Its courtship song also differs from those of other species of the *carnea* group (Henry and Wells, 2004; Taki et al., 2005). In 1996, a green lacewing designated as *C. carnea* was imported from Germany on a test basis. It was registered as a biological pesticide in 2001 and is now on the market in Japan. Its gradate crossveins are primarily green.

The 2 species can now encounter each other in the same habitat. Serious concerns over the non-target impact of introduced exotic natural enemies in native ecosystems have been raised by a number of prominent ecologists and conservation biologists (Follett and Duan, 2000; Wajnberg et al., 2001; Louda et al., 2003). As part of a risk assessment of the non-target effects of the introduced green lacewing in native ecosystems, I performed a laboratory experiment to characterize the symmetry of interspecific predation between the introduced green lacewing *C. carnea* and the native closely related species *C. nipponensis* at different life stages. Older and larger larvae always ate younger and smaller individuals, regardless of species. When larvae of the same instar and similar size were paired, almost equal predation rates between the two species were observed (Fig. 1). These results suggest that size was the most important determinant of the symmetry of interspecific predation between *C. carnea* and *C. nipponensis*. Populations of *C. nipponensis* will not be decreased by inter-

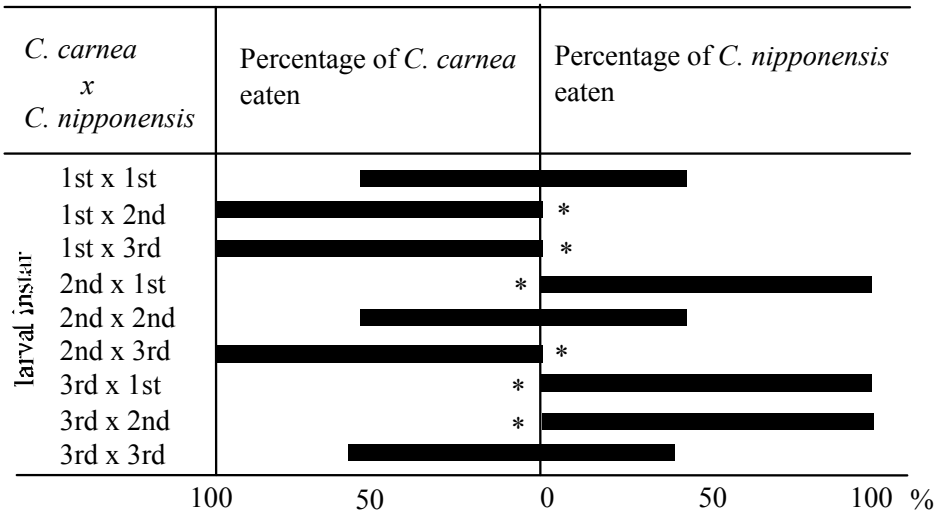

Fig. 1 Interspecific predation between introduced *Chrysoperla carnea* and native *C. nipponensis* larvae. * represents significant difference from 50% predation in each pair by X^2 test ($P < 0.01$).

specific predation with *C. carnea* unless there are extreme mass releases of *C. carnea* over a small arena (A. Mochizuki).

***Topic 2:* A new mechanistic model for describing dispersal distances of organisms by a generalization of the Brownian motion model**

The United Nations declared 2005 to be the International Year of Physics, because this year is the centenary of the seminal scientific discoveries by Albert Einstein that form the basis of modern physics. Einstein published 3 breakthrough papers in 1905: (1) special theory of relativity, (2) photoelectric theory, and (3) Brownian motion theory. The former two theories have been subsequently developed mostly within the field of physics, whereas the Brownian motion model has been applied to wider fields, including biology and economics. The Black-Scholes model, which predicts option prices, was developed by applying the Brownian motion model to the fluctuation of stock prices, and Myron Scholes won the Nobel Prize in 1997 for this work. However, it is now widely recognized that the Black-Scholes model cannot describe the fluctuation of stock prices in the real world. Stock prices fluctuate more sharply than are predicted by the Brownian motion model. A similar problem is also recognized in the field of biology. Real plants increase their ranges of distribution at speeds much faster than expected from the Brownian motion model. This problem is traditionally called "Reid's paradox".

The model developed by Einstein cannot describe actual fluctuations, so what is the difference between the Brownian motion model and the real world? The Brownian motion model seems quite comprehensive, and it includes the spatial heterogeneity of dispersing particles. However, probably for mathematical reasons, Einstein did not consider the temporal heterogeneity of dispersing particles. Yamamura (2004; Population Ecology 46: 87–101) improved the Brownian motion model by including temporal heterogeneity and derived a solution in an explicit form. This model is called the Gamma model, because a generalized gamma distribution was used in describing temporal heterogeneity.

The Gamma model can describe the real world quite well. An example of its application is shown in Figure 2,

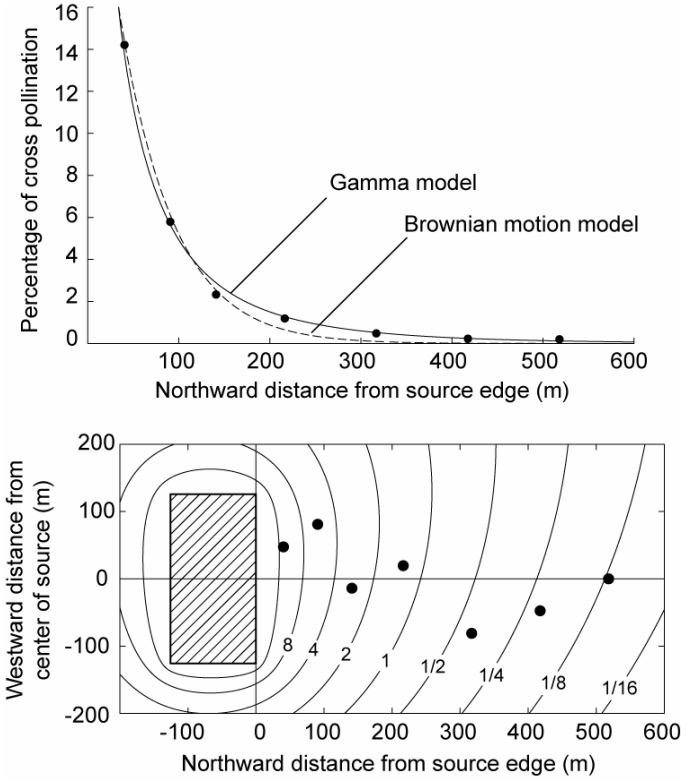

Fig. 2 Diffusion of corn pollen. Data are from Jones and Brooks (1950; Okla. Agr. Exp. Stn. Tech. Bull. T-38:1–18). *Upper panel:* Black dots indicate observed percentages of cross-pollination. Solid curve is from Gamma model. Dotted curve is from Brownian motion model. *Lower panel:* Estimated contours of percentages of cross-pollination. Black dots indicate spatial positions of samples used to estimate parameters. Hatched rectangle indicates the pollen source. (Copyright: Society of Population Ecology and Springer-Verlag).

where the spatial diffusion of corn pollen is described. The solid curve indicates the curve of the Gamma model, whereas the dotted curve indicates the curve of the Brownian motion model. The model parameters were estimated by a quasi maximum likelihood method in both cases. The Gamma model can describe long-range dispersal where the Brownian motion model fails. Thus, the Gamma model can explain Reid's paradox of plant dispersal. (K. Yamamura)

3) Microbiology Group

The Microbiology Group aims to characterize microbial communities in agro-ecosystems and develop technologies for effective management of microbiological resources. The group consists of a Microbiology Research Coordinator and 3 research units: Microbial Ecology, Microbial Genetics and Physiology, and Nematology and Soil Zoology.

Work is conducted on the following research themes: 1) investigation of the effects of soil microorganisms on the population dynamics of sclerotium-forming fungi; 2) investigation of the effect of secondary metabolites from microorganisms and plants on the multiplication of microorganisms; and 3) genus- or species-level analysis of soil nematode communities in and around upland fields, and investigation of the biological characteristics of entomopathogenic nematodes. The following activities were completed in FY 2004

The Microbiology Research Coordinator coordinated research and registration of microorganism genetic resources for related laboratories, which act as sub-banks for the MAFF Genebank system.

The **Microbial Ecology Unit** is investigating the effects of soil microorganisms on the population dynamics of sclerotium-forming fungi. *Trichoderma* spp. are associated with, and often antagonize, *Rosellinia necatrix* and *Helicobasidium mompa*, which are root pathogens of fruit trees. The concomitant presence of *Gliocladium catenulatum* is known to suppress the antagonism of *Trichoderma* spp. to *R. necatrix*, but microscopic observations failed to reveal the process of suppression. *G. catenulatum* did not antagonize *R. necatrix* on potato dextrose agar plates. Populations of fungi and bacteria in soil infested with *H. mompa* recovered soon after fumigation with chloropicrin, but addition of fluazinum diminished the fungal population. Use of the soil cover culture method revealed that treatment with these fumigants nullified fungistasis in the soil. PCR-DGGE of soil DNA enabled us to monitor changes in the microbial community in a field treated by soil fumigation. These methods are useful to evaluate the effects of artificial disturbance on soil microflora (see Topic 1). Use of a fluorescence *in situ* hybridization technique with specific probes that we developed revealed the presence of a bacterium, *Pantoea* sp., on the sclerotia of *Sclerotium rolfsii*, in masses on the hyphae.

The **Microbial Genetics and Physiology Unit** found that alginate gelation of wheat seeds, together with treatment with antagonistic bacterial strains (fluorescent pseudomonads) against *Gaeumannomyces graminis* var. *tritici*, suppressed take-all of wheat more effectively than direct treatment of seeds with antagonistic strains alone. The method was suggested to be useful in increasing the fixation of antagonistic strains to the plant's roots. To understand the colonization and its relationship to disease suppression, we engineered the antagonistic strain to express a green fluorescent protein (Gfp) constitutively, and we then used the green fluorescent strain to analyze the behavior of the antagonistic strain on wheat seeds and roots. The antagonistic strain moved along the germinating root(s) from the seed and colonized them. Such green fluorescent strains should be powerful tools for analyzing the behavior of microorganisms introduced into the rhizosphere.

We investigated the genotypic identification and characterization of *Burkholderia cepacia* complex (Bcc) strains recovered from clinical and environmental sources. On the basis of 16S rDNA RFLP analysis, Bcc strains derived from clinical sources were assigned to *B. cepacia* genomovar 1, *B. cenocepacia, B. stabilis,* and *B. vietnamiensis*. In contrast, the majority of Bcc strains from environmental sources belonged to *B. cepacia* genomovar 1, whereas the rest belonged to *B. cenocepacia*.

We are developing a PCR method for use in quantitative analysis of a recombinant soybean root nodule bacterium (*Bradyrhizobium japonicum*) in soil. First, we designed primers/TaqMan probe on the basis of the sequence of the marker gene integrated onto the chromosome of the target strain; this sequence proved to be highly strain-specific. Furthermore, by using a bead-beating method with the addition of skim milk, we succeeded in extracting from the soil DNA that was suitable as a template for quantitative PCR.

To clarify the relationships among the *Agrobacterium/Rhizobium* complex and their relatives, we tried to select the indices required for molecular phylogenetic analysis. We selected 4 single-copy, indispensable genes, *recA*, *atpD*, *dnaK*, and *rpoD*, as index candidates. Using the respective genes, we preliminarily constructed phylogenetic trees, all of which showed similar results in regard to topology at a family level.

The **Nematology and Soil Zoology Unit** identified free-living soil nematodes in a no-till manure-amended field. Identification of bacteriophagous nematodes be-

longing to the order Rhabditida has been almost completed, with the detection of the genera *Deontolaimus* and *Oigolaimella*. Identification of fungivorous nematodes belonging to the order Tylenchida bore the genera *Filenchus* (Tylenchina, Tylenchidae) and *Ditylenchus* and *Safianema* (Anguinidae). A nematode belonging to the genus *Aphelenchoides*, which has a unique tail-end shape, had presumably never previously been described.

From our investigation of the biological characteristics of fungivorous nematodes, we ascertained that *Tylencholaimus parvus*, belonging to the order Dorylaimida, is a real fungivore that replicates in some fungal cultures. Its growth rate in the culture of 9 species of fungi was significantly lower than that of *Aphelenchus avenae*.

To find indices suitable for evaluating the diversity or function of soil nematodes, we analyzed nematode biodiversity data from a no-till manure-amended field and the results of an experiment to determine the effects of fumigants on nematodes.

***Topic 1:* Impact of soil fumigation on microbial communities, as revealed by a culture-independent molecular method**

Soil microorganisms have important roles in agriculture. They are responsible for the mineralization of organic matter, whereby nutrients are recycled and pollutants are degraded, as well as for the suppression of soil-borne diseases. The impact of soil fumigation on microbial communities has been an issue of concern to soil microbiologists. Traditionally, investigations of soil microbial community have been based mainly on culture experiments. These methods are both time- and labor-consuming and are applicable to the fewer than 1% of microorganisms present in the soil that can readily be cultured. Recent improvements in methods of extraction of DNA from the soil have facilitated molecular analyses of microbial communities, including of unculturable microorganisms.

We used a culture-independent, molecular method to study the impact of fumigants such as chloropicrin and 1,3-dichloropropene (D-D) on the soil microbial community in an experimental plot at NIAES over 2 years. DNA was directly extracted from the soil, and the bacterial community structure was studied by 16S rDNA PCR-DGGE. Prominent DGGE bands were excised and sequenced to gain insight into the identities of the predominant microbes. Bacterial community analysis revealed that the majority of band sequences from unfumigated soil samples were most closely related to the sequences of unculturable bacteria (Fig. 1). After chloropicrin treatment, these bands became undetectable, and other bands, which showed high sequence homology to those of culturable bacteria, increased in intensity (Fig. 1). These results indicated that unculturable bacteria are more drastically affected by soil fumigation.

The DGGE patterns from unfumigated, control soil showed no temporal fluctuation. In contrast, 1 month after the first fumigation the DNA level in chloropicrin-treated soil decreased and the DGGE pattern changed dramatically. Even 1 year after fumigation, neither the

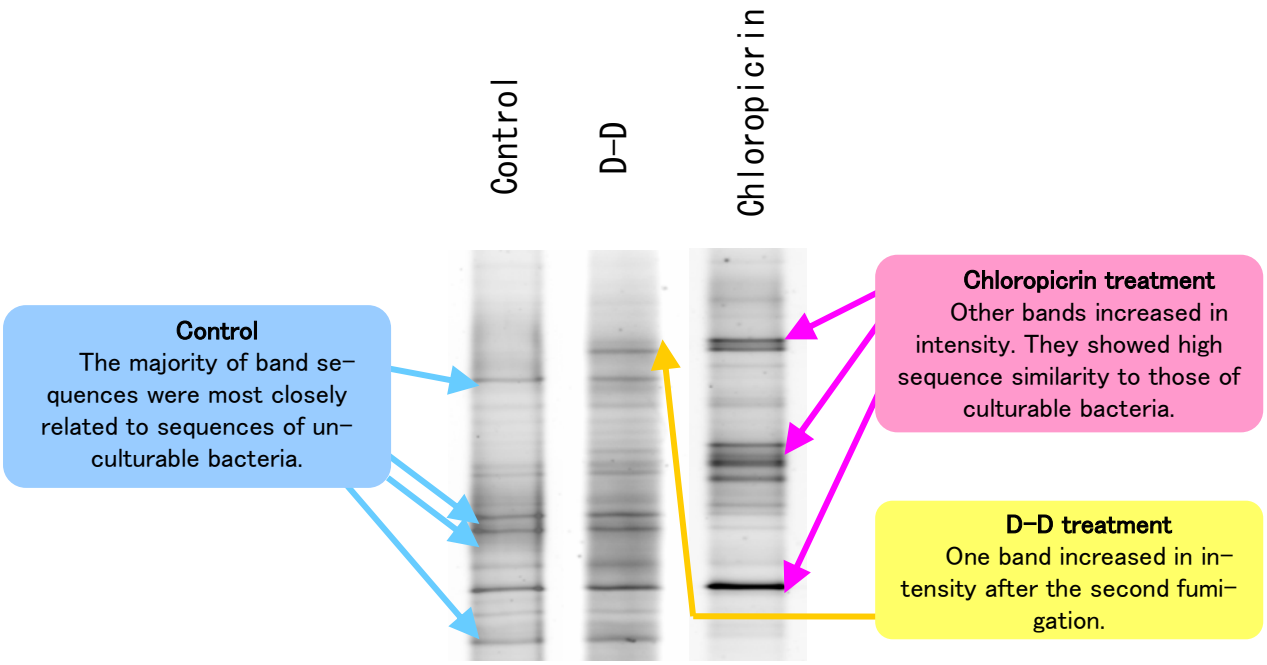

Fig. 1 16S rDNA PCR-DGGE patterns after fumigation with D-D and chloropicrin

DNA level nor the DGGE pattern had recovered. In soil treated with D-D to eradicate nematodes, the DNA level and DGGE pattern were the same as those in unfumigated, control soil, except that 1 band increased in intensity after the second fumigation (Fig. 1). The results imply that D-D has less impact on the microbial community than does chloropicrin.

Direct soil DNA extraction and subsequent PCR-DGGE are useful for assessing the impact of fumigation on bacterial communities. We can also analyze fungal communities by using the same DNA samples as used for bacterial communities, because soil DNA theoretically contains DNA from all organisms in the soil. We expect that this culture-independent method will be applicable to other impacts, such as the effects of chemical pollution and of organic matter application on soil microbial communities. (Y. T. Hoshino)

Topic 2: Influences of soil fumigation with agrochemicals on free-living soil nematode populations and their recovery.

Of all the activities performed on farms, fumigation with agrochemicals such as chloropicrin has the greatest influence on soil-inhabiting organisms. It is well known that these fumigants reduce the numbers of target soil-borne microorganisms or plant-parasitic nematodes, but there have been few reports on the effects that non-target soil-inhabiting animals suffer from fumigation. No reports have shown the long-term effects of fumigant application on soil animals or the periods needed for free-living nematode populations to recover their density before the next application. We designed an experiment to determine the effects of chloropicrin, D-D, and methyl bromide on free-living nematodes in microplots at NIAES in 2001 and 2002. Soil fumigated with chrolopicrin or D-D, together with untreated control soils, were arranged in nine 5 × 6 m microplots in a 3 × 3 Latin square design. Soil fumigation was done late in September in both years. In 2002, fumigation of the surrounding space with methyl bromide was added. Two weeks after treatment, each plot was cultivated to volatilize the fumigants and then seeded with spinach. Spinach cultivation was repeated to give 2 crops a year, with the products turned over. Four soil samples were taken from each plot before fumigation, just after fumigation, and 1, 6, 9, and 12 months after fumigation. Nematodes in 20 g soil were extracted by the Baerman-funnel method and counted to each distinguishable taxon under a compound microscope with a magnification of ×100. Figure 2 shows the changes in numbers of species after each fumigation treatment. The number of species in the control plots was about 20 and stable, whereas numbers in the fumigated plots were drastically diminished to below 5. Species numbers in the fumigated plots showed a gradual recovery to about 15, less than in the control plots. In the fumigated plots the total numbers

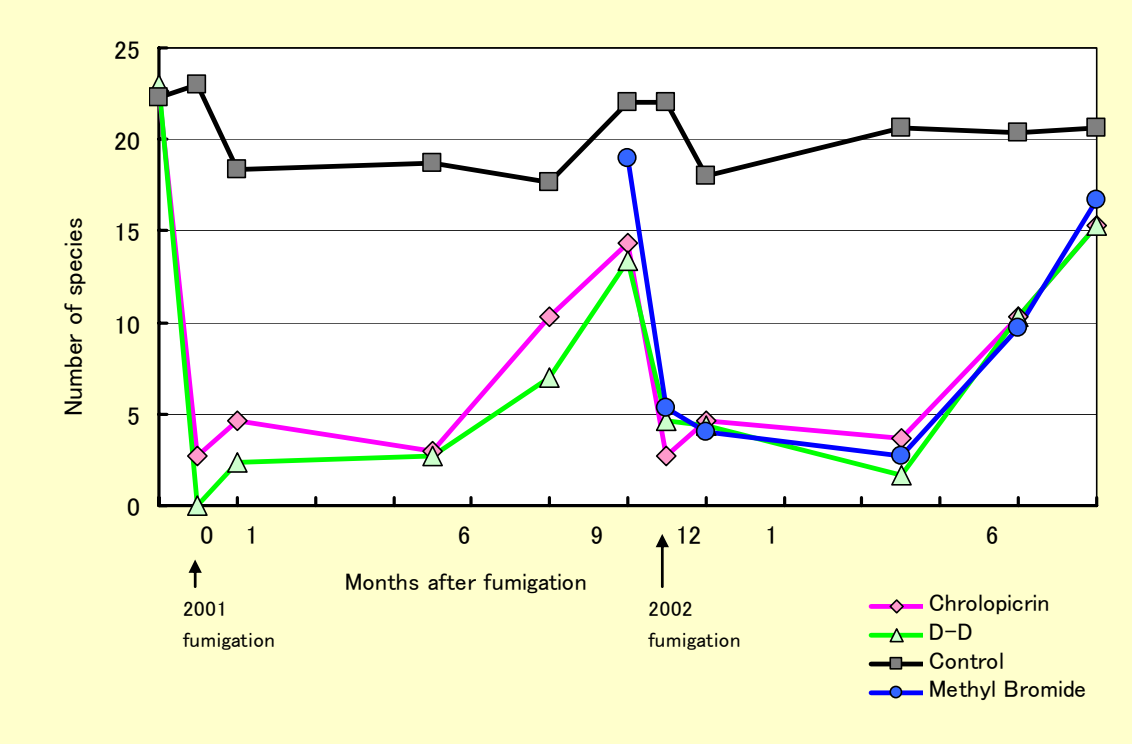

Fig. 2 Changes in numbers of species after each fumigation treatment.

of nematodes detected were also reduced, to 13/100 g wet soil, compared with more than 1000 in the control plots. However, total number of nematodes in the fumigated plots sometimes surpassed that in the control plots 9 months after treatment and was quicker to recover than number of species (data not shown). Nematodes belonging to the genus *Aphelenchoides* (fungivores), the order Rhabditidae and the genus *Acrobeloides* (bacteriovores) were the main components of the nematode population after recovery. On the other hand, nematodes belonging to the genera *Filenchus* (fungivore), *Heterocephalobus*, *Cerevidellus* (bacteriovores), and *Opisthodorylaimus* (omnivore) decreased in number after recovery. The drastic influence of fumigant application on free-living nematodes in the soil was clearly shown here, indicating that nematode community structure changes after fumigant application. (M. Araki)

4) GMO Assessment Team

The missions of the GMO Assessment Team are: 1) to clarify the ecological impact of GMOs (genetically modified organisms); 2) to develop standards of risk assessment for GMOs; and 3) to collect basic information and documents relating to GM and conventional crops. The major results of our research projects are described as follows.

A field experiment was conducted to monitor the changes in composition of weeds, insects, and soil microorganisms in response to long-term GM crop cultivation. The aim of this research project is to evaluate the environmental impact of continuous cropping of GM crops for 3 to 5 years. We conducted monitoring experiments on GM crops of maize, rice, canola, and soybean at 4 national agricultural research institutes and centers to clarify the effects of these GM crops on organisms. As part of these experiments, we have cultivated glyphosate-tolerant (GMO) and conventional (non-GMO) soybean cultivars in summer and glyphosate-tolerant and conventional canola cultivars in winter in a 0.2-ha experimental field since 2001. Weeds are controlled by glyphosate application in the GM experimental plots and by intertillage in the control plot. We have investigated changes in vegetation and in the composition of insect and soil microorganism populations over the past 4 years. The cultivation of GM soybean and canola will be followed by that of conventional wheat and soybean on the same experimental fields next winter and summer, respectively, to evaluate the environmental effects of long-term GM crop cultivation.

Pollen flow and outcrossing rate were investigated in 2 commercial sweet corn cultivars with yellow and white grains, which were pollen donors and pollen recipients. The outcrossing rate was determined by the xenia phenomenon, which appeared on the ears of the white recipient corns. We have examined the outcrossing rate within 50 m of the pollen donor plants for 5 years since 2001, and in 2002 we also initiated collaborative research work between NIAES and the Tsumagoi Station of the National Center for Seeds and Seedlings (NCSS) to conduct large-scale experiments in a 4.5-ha (100 m × 450 m) field. Outcrossing rates and distributions of hybrid plants have been different each year. However, recipient plants within a distance of 1 m from the yellow donor plants showed a mean outcrossing rate of 49.6% over 4 years at Tsukuba, and 43.6% over 3 years at Tsumagoi. There were no large differences between the 2 experimental sites in terms of the mean outcrossing rate of recipient plants neighboring the donor plants. These rates decreased sharply with increasing distance from the donor plants. In the small-scale field at Tsukuba, the mean outcrossing rate over 4 years decreased to 0.2% at a distance of 50 m. On the other hand, the mean rates over 3 years decreased to 2.3% at 50 m, 1.3% at 100 m, 0.7% at 200 m, and 0.1% at 400 m in the large-scale field at Tsumagoi. At the Tsumagoi Station we also performed a field experiment to see if we could decrease the outcrossing rate of corn by using a windbreak net.

We have been monitoring the distribution and weediness of GM canola (*Brassica napus*) around the Kashima Seaport, which is one of the ports in Japan where canola seeds are unloaded (Fig. 1). We have also conducted further investigations into the transfer of introduced herbicide-tolerant genes to relatives of *B. napus* growing in the area. (K. Matsuo)

Fig. 1 Feral canola (*Brassica napus*) growing by the side of a road.