Research Project

1. Development of technologies to utilize the natural circulation functions of forests and farmlands through to water bodies

Agriculture, forestry, and fisheries are industries that depend fundamentally on nature and are maintained by carbon and nutrient cycles through chains of various natural organisms. However, we have not yet thoroughly elucidated either the natural circulation functions of these industries or the ways in which they maintain the resources of the natural environment, and we do not know how to utilize these resources in a sustainable way. A project entitled “Development of technologies to utilize the natural circulation functions of forests and farmlands through to water bodies” was therefore initiated in FY 2000 by the Agriculture, Forestry and Fisheries Research Council Secretariat.

The aim of the project was to clarify the natural purification and circulation functions of forests and farmlands through to water bodies. By developing technologies to control environmental burdens such as nitrate-nitrogen from agriculture, forestry, and fisheries, our objective was to enhance the ability of primary production, particularly in water bodies. From FY 2001 onward, the project proceeded through grants at a time of transition of the research institutes affiliated with the Ministry of Agriculture, Forestry and Fisheries into independent administrative institutions. For a total of 6 years (a first 3-year term from FY 2000 to FY 2002 and a second term from FY 2003 to FY 2005), researchers with different areas of expertise in the ecosystems of forests, farmlands, and water bodies worked together to tackle the following two major challenges:

1. Elucidation of the natural circulation functions of a topographical sequence, and development of technologies to enhance this function

We elucidated the nitrogen balance in a forest ecosystem, and then the nitrogen loading in a topographical sequence (a catchment area with vegetable or tea farms uphill and spring-fed paddy fields on the lowlands) that varied depending on the region. From this, we determined where to expect nitrogen purification functions to occur.

2. Elucidation of the natural circulation functions of ecosystems in forests, farmlands, and water bodies, and preparation of guidelines for managing them

We studied several watersheds, including the Yahagi River basin, and elucidated the natural circulation functions of the forests and farmlands through to water bodies. We then tested a model for the management of farmland and aquatic ecosystems.

Our major achievements can be summarized as follows:

1. We clarified the nitrogen balance in a forest ecosystem and elucidated the impacts of tree thinning on the quality of water released from the forest.
2. We developed a technology for precisely evaluating nitrogen runoff potential from farmland, and we clarified how nitrogen is eliminated through a topographical sequence.
3. We found that nutrients carried by rivers support the production of organisms such as shellfish in river mouths and coastal areas.
4. Research on the flow of nitrogen and phosphorus from forests in upstream regions through farmland to the mouth of the Yahagi River indicated that flow control of the river is vital for maintaining the water quality and biological production in Chita Bay.

2. Development of Techniques for Predicting Food Production Changes Resulting from Global Warming

Global warming caused by changes in land use and by anthropogenic releases of greenhouse gases such as carbon dioxide and methane has become a topic of discussion worldwide. The Third IPCC Report on Global Warming, published in 2002, points out that the rising temperature trend over the last 50 years is mainly the result of human activity. At the annual Conference of the Parties to the UN Framework Convention on Climate Change, politicians and government officers discuss ways of mitigating these problems. Because global warming is expected have substantial effects on agriculture through rises in temperature, increases in atmospheric carbon dioxide concentrations, spatial and temporal changes in rainfall patterns, and changes in soil characteristics, there is considerable concern about potential changes to food production.

NIAES ran a research project on this problem, commencing in FY 2001 and ending in FY 2005. The project examined the effects of global warming on agricultural production in northeast and East Asia from the points of view of the natural environment, crop physiology, and socioeconomics. Among the topics covered by the project were the development of methods for predicting precipitation at the local scale, the development of methods for detecting cropland and crop phenology by using satellite data, the elucidation of changes in water and soil resources and their effects on the physiology of crop growth, and changes in the occurrence of insect pests as temperatures rise. The project also included the modification of an existing model for worldwide food supply-and-demand to permit the prediction of changes in food production arising from environmental changes.

We concluded that dry matter and its efficiency in the water use of rice will increase as a result of the elevation of atmospheric carbon dioxide levels, but that spikelet fertility will also increase as a result of the higher temperatures caused by global warming. Moreover, although the amount of cultivable land is expected to increase, we are concerned that rice production will become unstable as a result of temporal and spatial maldistributions in precipitation and increased levels of insect damage.

As part of this project, we also held an international workshop on "Prediction of Food Production Variation in East Asia under Global Warming" at the Tsukuba Center for Institute, on 17 to 19 March 2004; there were 20 oral presentations and 62 participants.

3. Assurance of Safe Use of Genetically Modified Organisms

Recent advances in molecular biology have led to the production of many genetically modified organisms (GMOs) and their release into the environment for practical uses. Most of these GM crops have helped improve agricultural productivity in harmony with the agro-environment. On the other hand, commercialization of GMOs has raised public concern about food safety. As a result, extensive environmental testing is required before GMOs are released into the environment.

A comprehensive research project entitled "Assurance of Safe Use of Genetically Modified Organisms" was conducted from FY 2001 to FY 2005 under the auspices of the Agriculture, Forestry, and Fisheries, Research Council (AFFRC) of MAFF. This project aimed to promote safe utilization and improve public acceptance of transgenic products by developing appropriate methods of safety evaluation and safety management for emerging types of transgenic products. For example, the research subjects included new traits, pollen dispersal and crossing, detection methods, and public acceptance.

The main research results obtained were as follows:

In response to public concern about transgene dispersal into the agro-ecosystem, outcrossing rates estimated in conventional corn are available for gene flow studies. We conducted small-scale experiments in a field (0.14 ha) at Tsukuba and larger scale (4.5 ha) field experiments at Tsumagoi, in Gunma Prefecture. The outcrossing rate and distribution of hybrid plants in the field, detected by xenia in conventional corn (pollen donor: yellow grain; pollen recipient: white grain), differed by year. However, in both fields, plants that grew within 1 m of the donor exhibited the highest mean outcrossing rates (22.6% to 56.8% over 3 years in Tsukuba, and 32.0% to 43.2% over 2 years at Tsumagoi). No remarkable difference between the two experimental fields in terms of the main crossing rates of plants adjacent to donor plants was observed. Values decreased sharply with distance from the donor plants. These results are useful as fundamental data to establish end points for risk assessment and separation distances from GM crops in risk management in Japan, where most farmers cultivate small or variably sized fields.

A specific method for assessing allelopathy was developed and named the "plant box method". It uses intact plants growing in an agar medium without nutrients (Photo 1). It deals with the transfer of allelochemicals through root exudates and was adopted to assess the allelopathy exhibited by newly developed genetically modified crops (GM crops) (Fig. 1). To elucidate the allelopathic effect of fresh leaf and leaf litter leachates under laboratory conditions, a "sandwich method", in which leaves are placed between two layers of agar, was employed and modified. This bioassay seems to be a reliable method for screening for allelopathic activity in leaf litter leachates. A "dish pack method" was developed for analyzing volatile allelochemicals. In this method, leaves are put into one of the holes in a six-well multi-dish. It is possible to analyze the internal volatile gas collected from the hole with a gas-tight syringe through the septum set up on the holes. By using this method, it is possible to determine the chemical structures of volatile allelochemicals in action.

A "rhizosphere soil method" was developed to evaluate allelopathy in the soil. Rhizosphere soil was brushed off the root surfaces after removal of the surface soil by the "air shaking method". The texture and type of soil did not affect the results. We used this method to evaluate the allelopathic activity of ground-cover plants and weeds.

A key element of environmental risk assessment is evaluation of the potential long-term effects of GM crops. In particular, it is necessary to test whether GM crop cultivation influences farmland biota differently from the management of conventional cultivars, and subsequently to determine whether any of the effects detected are detrimental to the environment. We cultivated glyphosate-tolerant (GM) and conventional (non-GM) soybeans over 4 years and ordinary wheat as a succeeding crop for 1 year (Photo 2). There were no differences in the numbers of weed species in GM and non-GM soybean fields before weeding. The number of insect species found on stems and leaves was generally low, and no specific species were found throughout the research period. The numbers of microbes in the soil were consistent

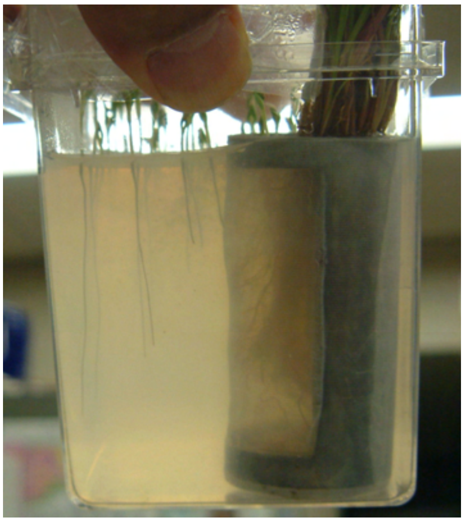

Photo 1 The plant box method

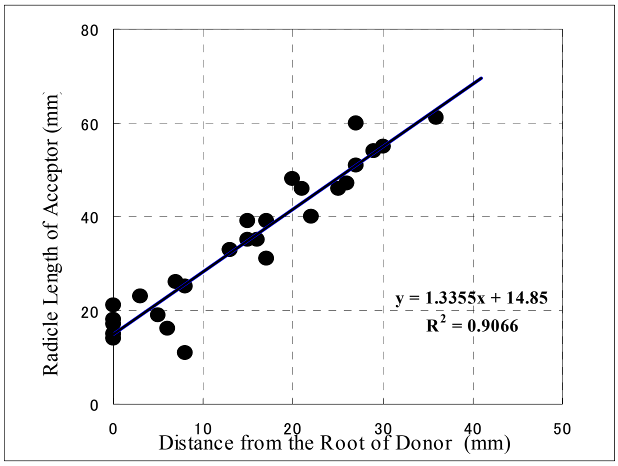

Fig. 1 Inhibitory action of allelochemicals in root exudates of a plant

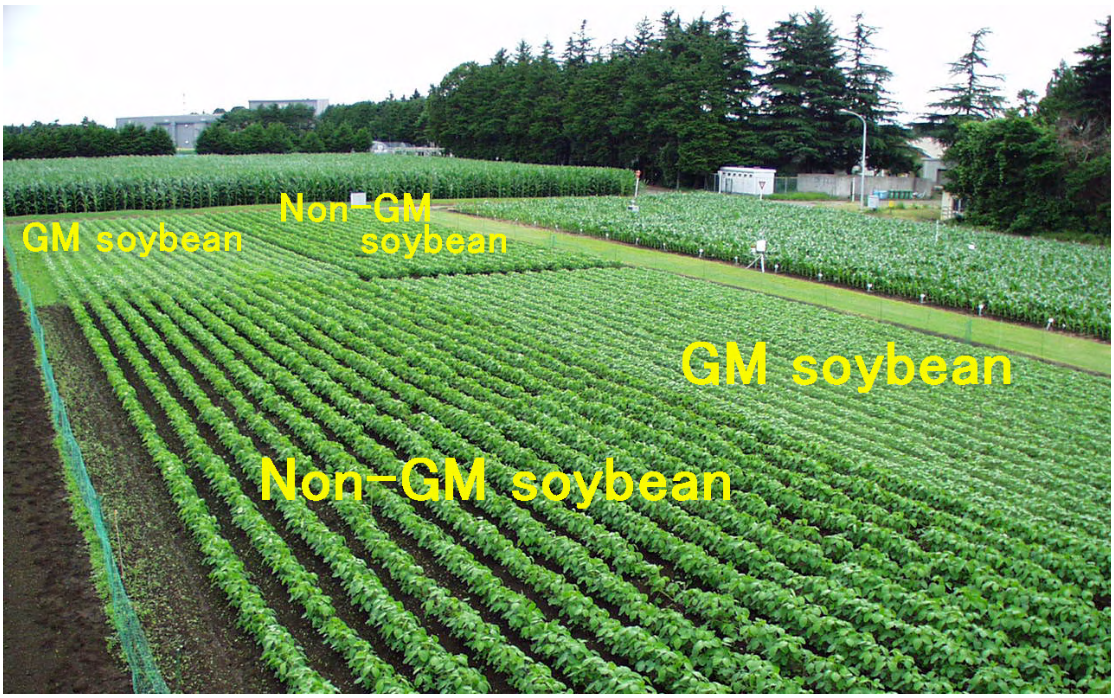

Photo 2 Monitoring a field of GM soybean at NIAES

between GM and non-GM crops. There were no differences in the wheat crops cultivated in the two fields. From these 5-year results, we concluded that there were no differences in the vegetation, composition of insects, or soil microorganisms in fields grown with GM soybean as compared with conventional soybeans.

This comprehensive research project was coordinated by NIAES over the last 5 years in collaboration with 34 institutions, including six universities. The project is now being conducted for a second 5-year period.

4. Grouping of minor crops for assessment of pesticide residues, and development of a method for rapid detection of residues in these crops

Grouping of minor crops for assessment of pesticide residues: Pesticides are indispensable for the production of minor crops (defined in Japan as crops produced at 30 000 t/year or less), but only a few chemicals are currently permitted for use as pesticides. We therefore need to group minor crops if we are to efficiently use these pesticides. In this project, we examined several minor crops, including cucurbits, cereals, Laminaceae, and Umbelliferae. In cucurbit crops, initial rates of pesticide deposition were almost the same among cucumber, bitter gourd, and zucchini. The rate of decline in pesticide concentrations in cucumber did not differ from that in zucchini, but pesticides persisted longer in bitter gourd than in either cucumber or zucchini. Among the cereal crops, the persistence of pesticides in Awa (foxtail millet, *Setaria italica*) was greater than that in Hie (Japanese barnyard millet, *Echinochloa esculenta*) or Kibi (broomcorn millet, *Panicum miliaceum*). We were thus able to evaluate the persistence of pesticides in the two latter millets relative to that in Awa. We evaluated the persistence of pesticides on 16 Laminaceae crops, including lemon balm, spearmint, apple mint, sage, sweet marjoram, thyme, rosemary, basil, and perilla. We found that the patterns of persistence of pesticides in all of these crops were very similar, except in sweet marjoram. We evaluated the persistence of pesticides on 13 Umbelliferae crops, including coriander, mitsuba, caraway, soup celery, parsley, dill, chervil, and celery. We found that the persistence of pesticides on dill and celery was lower than that on the other crops.

Development of a method for prompt detection of pesticide residues: Haptens of tebufenozide and chlorothalonil were synthesized to develop a method for prompt detection of these pesticides in crops. The haptens were conjugated to keyhole limpet hemocyanin, and the hapten conjugate was used to immunize mice. Then a monoclonal antibody was made from the hybridoma. A hybridoma was made by hybridizing an antibody-producing cell with an immortal cell line. Finally, we developed an enzyme-linked immunosorbent assay (ELISA) kit for these pesticides. We also developed several ELISA kits for the detection of these pesticide residues in rice, tomato, spinach, broccoli, and apple. We compared the pesticide concentrations determined by ELISA with those determined by high-performance liquid chromatography and found that they were almost the same. Thus the ELISA kits had a high level of performance.

5. Evaluation of pesticide residues in minor crops

In Japan, crops produced at annual rates of 30,000 t or less are called minor crops. Minor crops include many regional and traditional crops. Because various pests infest these crops, many kinds of pesticides are needed for their stable production. However, when we register a pesticide for a crop, we need to perform analyses of the levels of pesticide residue in the crop, the efficacy of the pesticide for pest control, and the phytotoxicity of the pesticide to the crop. In particular, the cost of analyzing crops for pesticide residues is high. Therefore, to date, only a few pesticides have been registered for minor crops. Prompt registration of more pesticides for use on these crops is important if we are to maintain crop production.

During the period 2005–2007, we will characterize and classify pesticide residues in minor crops and will develop methods of analyzing pesticide residues in fruit and leafy vegetables.

6. Study and surveillance of farmer and environmental safety in tank mixing of pesticides

On-site tank mixing of different pesticide formulations just before application is a popular practice because it reduces the workload in applying pesticides. Farmers currently use the “Case Study Data on Tank Mixing of Pesticide Combinations” as a guide to the crop safety and the activity of fungicide and insecticide combinations. This publication includes information provided by the Japan Plant Protection Association, Japan Agricultural Cooperatives, and pesticide companies. However, we still have very limited human and environmental safety data on tank-mixed pesticides.

The purposes of this project are to survey the important combinations of pesticides, especially in vegetables and fruits; to elucidate the safety of pesticide combinations in humans and insects; and to determine an appropriate risk management protocol for tank mixing. The project has the following four study components: (1) surveillance of tank mixing of various pesticide combinations and selection of the most commonly used combinations; (2) design of methods for testing effects on humans, effects on insect activity, and pesticide drift; (3) conducting of these tests; and (4) risk management of tank mixing.

The project began in FY 2005 and is scheduled to end in FY 2007. It is supported by a research project for utilizing advanced technologies in agriculture, forestry, and fisheries. And not only NIAES but also Chiba University, the Institute of Environmental Toxicology, and the Japan Agricultural Cooperatives are participants. In the first year, we selected 64 out of 184 tank mixing pesticide combinations used on vegetables in Japan. The most common combinations were wettable powder plus emulsifiable concentrate, followed by suspension concentrate plus emulsifiable concentrate. We developed an in vitro skin irritation test method that uses a three-dimensional cultured human skin model. This method is appropriate for Tier 1 tests, which is Screening (Priority setting for testing), and was rated highly by the public in terms of both animal protection and ethics. We established a laboratory test system to evaluate the effects of the abovementioned combinations on the house fly (*Musca domestica* L.). The test effectively detects additive, antagonistic, and synergistic actions in terms of acute toxicity. We also developed a pesticide droplet-detection device that uses a wind tunnel and laser particle spectrometer. It enables us to measure very small droplets (<30 μm), which are commonly observed in pesticide drift. With these newly developed methods we could achieve most of the results with selected tank mixing pesticide combinations.

7. Research on hazardous substances contained in fertilizers and suspected of affecting human health

In light of recent directions in public health and rising demand for improved food safety, in this study we are taking up the hazards associated with those substances in fertilizers that are suspected of affecting human health but about which we know little, unlike the case of well-known toxic metals such as cadmium. We aim to accumulate data on the movement of these substances in the soil, their degree of uptake by plant bodies, and their influence on human health. The study will run for 3 years (2005–2007) and covers the following sub-topics:

1. Specification of the hazardous substances that will be studied except the substances on sub-topic 2 and 3.
2. Clarification of the movements of natural radioactive materials that are inevitably mixed in with the raw materials (rock phosphates) used to make fertilizers.
3. Clarification of the rise and fall in the levels of these substances in the composting process, the shift of these substances into crops, and their remaining in crops.

NIAES is in charge of the second of these sub-topics. The different organization is respectively in charge of other two sub-topics. Although the dangers of radioactive materials have been reviewed internationally, because the uranium concentrations in the phosphate fertilizers produced from some deposits is comparatively high, Japan's Radiation Council examined whether the levels of uranium in fertilizers should be restricted. The restriction was decided not to be done in 2004. But the possibility that the restriction is done in the future was not denied. And in the process of this discussion, it was understood that the data obtained on the influence of fertilizer uranium on Japan's agricultural environment were insufficient. We therefore aimed to obtain more data so that the issue could finally be clarified.

This sub-topic can be divided into the following subjects:

① Clarification of the movement of uranium in farmland soil environments.
② Calculation of the migration coefficients of uranium in major farm products.

As part of subject 1, the concentration of uranium in the soil of a field with a history of long-term fertilizer application is being investigated by inductively coupled plasma – mass spectrometry (ICP-MS). The realities of the accumulation of uranium in the farmland soil will then be clarified in terms of such factors as differences in land and fertilizer use, year-to-year changes in the concentration of uranium, and the vertical distribution of uranium in the soil. In subject 2, after the establishment of a method for the microanalysis of uranium in plants, the rate at which the uranium in the soil shifts into the edible parts of the crops (the migration coefficient) will be calculated for various commonly cultivated crops. (N. Kihou)

8. Risk assessment and control of alien plants

A large number of alien species have been introduced into Japan over a long period of time. Although some of them have been able to coexist with native species over time, others affect the local biological diversity. Recently, the movement of people and substances has become more active as human mobility has increased. Living organisms are being introduced, through intentional or unintentional human activity, into new regions from other countries or other regions in a manner that exceeds the living organisms' natural ability to migrate. Among these living organisms are those that are being used for a variety of purposes, including cultivation, landscaping, home gardens, and aquarium. These organisms have penetrated, and coexisted with, our daily lives and culture over a long period of time, and they have come to serve a variety of active roles in our primary industries. In contrast, there are cases where, if living organisms not previously present in a region are brought in through human intervention, they can drastically alter the biodiversity of the region by feeding on, or driving out, living native organisms that do not have appropriate defenses. Such occurrences are being reported in all parts of the world, including Japan, and have sometimes caused harm to humans or to agriculture, forestry, or fisheries. Preventing the introduction and establishment of invasive alien species is therefore a very desirable measure in terms of both environmental preservation and cost-effectiveness. If these species do invade, a rapid and appropriate response will give the greatest chance of eradicating them. Japan has therefore enacted the Invasive Alien Species Act (Law No. 78, 2004; (http://www.env.go.jp/

en/nature/as/040427.pdf) to prevent potential adverse effects on Japanese ecosystems, human safety, and agriculture, forestry, and fisheries from those alien species that have been intentionally or unintentionally introduced into Japan from overseas through human activity and that exist outside their original habitats. A person who raises IAS (Invasive Alien Species; see below) with the aim of selling or distributing them in violation of the provisions of the Act shall be punished by imprisonment for up to 3 years or by a fine of up to 3 million yen. This is the strictest environment-related Act in Japan.

To support this Act, a new National Project, "Risk Assessment and Control of Alien Plants" has just started in Japan. The first designation of species as "IAS" (Invasive Alien Species, which pose a risk of damage to biodiversity, human safety, or agriculture in Japan); UAS (Uncategorized Alien Species, which may be categorized as IAS after detailed investigation and thus require detailed investigation before they may be imported into Japan); and LORCA (Living Organisms Required to have a Certificate Attached during their importation in order to verify their designation) was proposed by academic experts in October 2004. The second designation was completed in July 2005. The aim of this project is to survey the distributions of, and hazards posed by, alien invasive plants; to prepare scientific background data for the classification of harmful alien species; and to develop practical methods for mitigation of the damage caused by alien invasive plants. These aims will be achieved as follows:

1) Determine the distribution of alien invasive plants in Japan and their invasive potential by: a) investigating their true invasion status; b) determining their mechanism of invasion; and c) analyzing their allelopathic or toxic principles. Allelopathy and the presence of toxic chemicals play important roles in the success of invasion.
2) Develop a method of risk assessment of alien invasive plants by: a) determining their route of invasion; b) determining their mechanism of settlement, development, and expansion; c) preparing a database for their risk assessment; and d) using risk assessment methods to determine which plants will be hazardous. The WRA (Weed Risk Assessment) introduced in 1997 in Australia is a good model for us.
3) Develop a method of mitigating the damage caused by hazardous alien plants and for controlling or eliminating these plants by: a) chemical control with herbicides; b) mechanical control; c) biological control with cover plants; and d) development of comprehensive mitigation schemes. (Y. Fujii)