Research Topics

1. A New Method for Calculating Rice Field Methane Emissions Adopted for the 2006 IPCC Guidelines

This February in its Fourth Assessment Report the Intergovernmental Panel on Climate Change (IPCC) more or less concluded that "global warming is already happening, and it is caused by the increased greenhouse gas (GHG) emissions by human activities." It is anticipated that humanity will enter a period of time warmer than any other in human history, and the measures must be addressed. For that purpose, worldwide initiatives are in progress, such as GHG emission reductions in the developed countries made under the Kyoto Protocol, which was adopted in 1997, and based on the UN Framework Convention on Climate Change (UNFCCC), which had been adopted at the 1992 Earth Summit. Also, the framework for the "post Kyoto-agreement" was debated at the Heiligendamm Summit this year.

The UNFCCC requires parties to estimate their GHG inventories of emissions and sinks, and to report them to the convention secretariat. Their estimation methods have been prepared in the form of the Guidelines by the IPCC. In the past, the parties did their estimates with the Guidelines released in 1996. However, in 2006 the Guidelines were revised, and from now on the parties are encouraged to use the revised Guidelines as the standard calculation methods for determining their GHG emissions. These revisions adopted a method for calculating rice field methane (CH_4) emissions which was developed by a team in which our institution played the leading role.

In the agricultural sector, rice fields are a significant anthropogenic source of methane, a major GHG. The measurement method in the 1996 IPCC Guidelines still had a great deal of uncertainty for reasons including determination of the emission factor[i] from a paucity of measured data. For that reason we built and analyzed a database which holds measurements of methane emitted from rice fields, and carried out research meant to propose a more precise calculation method.

This research involved collecting data on rice field methane emission measurements in Asian countries from the existing literature, and building a database comprising data on 868 growing periods at 103 locations in eight countries. These data were used in analyzing the relationship between average methane flux of entier growing periods and the factors controlling the flux. We then proposed a revised method for calculating the methane emitted from rice fields, which was adopted for the new IPCC guidelines.

Basically, the calculation method follows that in the previous IPCC guidelines by multiplying an emission factor (EF), which represents emission intensity, by the rice harvested area (A) and by the number of rice cultivation days (t) of all rice fields in the world for each of the categories such as irrigated rice fields and rainfed rice fields (Equation 1). Category-specific emission factors (EFi) are determined by multiplying the baseline emission factor (EFc) by correction factors, or scaling factors[ii], that takes into account influences on the emissions of methane, such as water management and organic amendment application (Equation 2).

Amount of methane emitted (Gg yr^{-1})

$$= \Sigma_{ijk} (EF_{ijk} \cdot t_{ijk} \cdot A_{ijk} \cdot 10^{-6}) \qquad \text{Equation 1}$$

$$EFi = EFc \cdot SFw \cdot SFp \cdot SFo \cdot SFs,r \qquad \text{Equation 2}$$

Where:

EF_{ijk} = emission factor for rice fields in each category (kg-CH_4 ha^{-1} day^{-1})

t_{ijk} = number of rice cultivation days in rice fields of each category (day)

A_{ijk} = harvest area of rice fields in each category (ha yr^{-1})

EFc = baseline emission factor (kg-CH_4 ha^{-1} day^{-1})

SFw = scaling factor used for rice field type and water management during growing period

SFp = scaling factor used for water management before growing period

SFo = scaling factor used for application of organic amendments

SFs,r = scaling factors used for soil type and rice cultivar (only when applicable)

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[i] Emission factor: Emission intensity per unit area, used in calculations.

[ii] Scaling factor: A coefficient that shows how methane emission is affected by rice field type, water management, organic amendment application, and other influences.

[iii] Uncertainty range: The IPCC guidelines assign an uncertainty range to emission factors and other values. Because in this case there are sufficient data as the basis for determining calculation factors, we have used the 95% confidence interval as the uncertainty range.

[iv] 2006 IPCC guidelines: http://www.ipcc-nggip.iges.or.jp/public/2006gl/index.htm (vol. 4, p. 5.44–53).

Table 1 Conversion factors of various organic amendments

Amendment	Conversion factors for organic amendment (CFOA)	Uncertainty range
Compost	0.05	0.01-0.08
Farmyard manure	0.14	0.07-0.20
Green manure	0.50	0.30-0.60
Straw on season (within 30 days before rice cultivation)	1	0.97-1.04
Straw off season (more than 30 days before rice cultivation)	0.29	0.20-0.40

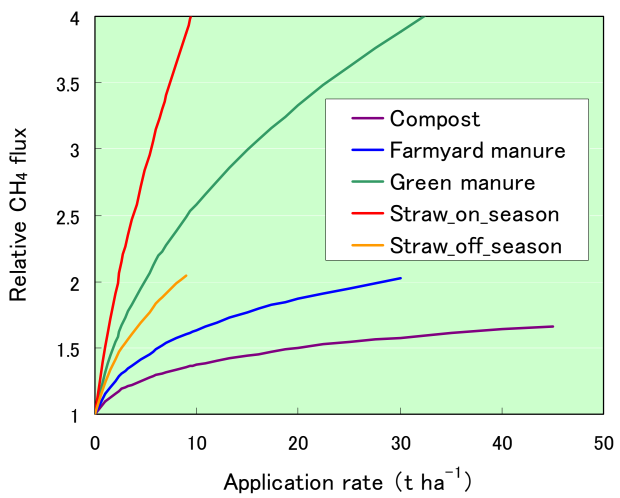

Fig. 1 Relationship between amounts of various organic amendments and methane emissions (compared with no organic amendments). See Table 1 for the categorizations of straw amendment.

Here, the baseline emission factor (EFc), which is the basis for calculation, is meant for rice fields which have a drainage period of less than 180 days before cultivation, which are continuously flooded during the growing season, and which are given no organic amendments. An analysis using the database yielded a value of 1.30 kg-CH_4 ha^{-1} day^{-1} (uncertainty range:[iii] 0.80 to 2.20). The 1996 IPCC guidelines used in the past set the baseline emission factor at a uniform 200 kg ha^{-1} per crop, which did not take into account differences in rice growing season length due to cultivar and climate. By contrast, the improved method can better calculate the actual amount of methane emitted. Additionally, it is now possible to correct the relationship of increased methane generation to water management and organic amendments prior to the growing season (Fig. 1, Table 1), whose significant influence on the amount of methane emitted is now known (Equation 3).

$$SFo = (1 + \Sigma_i ROA_i \cdot CFOA_i)^{0.59} \qquad \text{Equation 3}$$

Where:

SFo = scaling factor used for application of organic amendments

ROA_i = application rate of organic amendments in fresh weight (t ha^{-1})

$CFOA_i$ = conversion factor given in Table 1

See the 2006 IPCC guidelines[iv] for a detailed explanation of the calculation method outlined here.

Because these guidelines are used around the world for building GHG inventories, they make a considerable contribution to refining the calculation of the world's GHG emissions as called for by the UNFCCC. (K. Yagi and H. Akiyama)

2. Publication of the "Revised Agricultural Environment Monitoring Manual for Water Environment Conservation"

There is now broad awareness of the significant impact of agriculture on the water environment, and in many places people are implementing various measures for water environment conservation, such as the introduction of conservation agriculture techniques. The precise implementation of such measures requires first determining the situation at a locality and then implementing the most effective measures which suit that situation. To determine the situation at a locality, one must monitor the runoff of nitrogen, phosphorus, pesticides, and other substances from agricultural lands into rivers and groundwater. It is also necessary to estimate the load in the involved watershed of agriculture-derived nitrogen and other substances from statistical data. To determine the effectiveness of implemented measures, one must conduct a reevaluation based on monitoring, and then make further improvements to the measures if needed.

In 1999, the National Institute for Agro-Environmental Sciences (NIAES) published the “Agricultural Environment Monitoring Manual for Water Environment Conservation (in Japanese)” as a comprehensive and practical survey manual to perform such agriculture-related water environment monitoring. Relevant agencies in Japan have been using the manual.

Since 1999, NIAES has gained many new research findings related to water environment monitoring in our research projects. And because remarkable progress has been achieved in related research areas as well, we incorporated these latest research results for publication of this revised monitoring manual (Fig. 1, 2, 3).

The manual uses recent research to explain agricultural environment monitoring methods in plain language. Below are the revised manual’s and chapters (Table 1).

The PDF version of this revised manual in Japanese can be downloaded from NIAES website:
http://www.niaes.affrc.go.jp/techdoc/monitoring/index.html

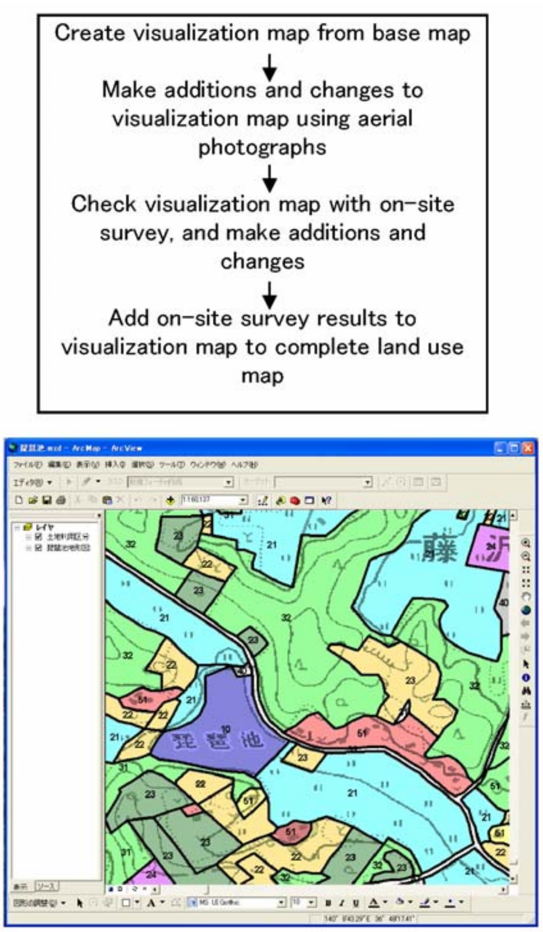

Fig. 1 Procedure to create maps showing the current state of land use (II-5)

Printed versions have been provided to mainly agriculture-related independent administrative institutions and public institutions, as well as to universities and environment-related institutions in Japan. (K. Banzai and K. Sugahara)

Fig. 2 Water quality monitoring using an automatic water sampler (IV-3)

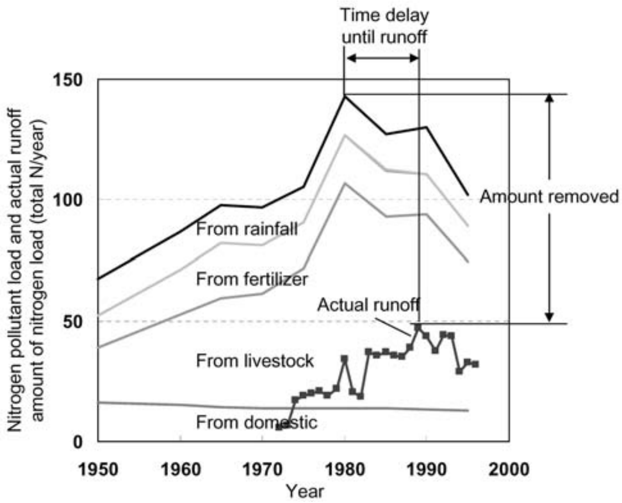

Fig. 3 Method for estimating nitrogen load according to pollutant load source in a catchment basin (VI-1)

Table 1 Makeup of Revised Manual

Ⅰ Basics of Agricultural Environment Monitoring
Ⅱ Watershed Environment Survey Methods
Ⅲ Survey Methods for Watershed Load Sources
Ⅳ Water Advection Survey Methods
Ⅴ Pollutant Matter Behavior Survey Methods
Ⅵ Watershed Water Quality Assessment Methods
Ⅶ Assessment of Water Environment Using Biota
Ⅷ Information for Water Environment Conservation

3. Simple Measurement of Cadmium Concentration in Unpolished Rice and Soil Using the Immunochromatographic Assay Method

Introduction

At its July 2005 session, the Codex Alimentarius Commission adopted international standards for cadmium (Cd) concentrations in foods including wheat (0.2 ppm), peeled potatoes (0.1 ppm), and vegetables (0.2 to 0.05 ppm). In July 2006, a new standard of 0.4 ppm was adopted for polished rice. It is anticipated that Japan's standards for domestic agricultural produce will be toughened. This creates a need to monitor pre-harvest Cd concentration to prevent the distribution of polluted foods, and otherwise manage risk at production sites, and that necessitates the development of fast and simple analysis methods that can yield results one or two days after sampling, and can be used by agricultural technicians on site. Under present circumstances, analyses are time-consuming and costly due to the need for complicated procedures such as acid digestion or organic solvent extraction, and expensive precision analytical instruments such as ICP spectrometry or atomic absorption equipment.

Recently the Kansai Electric Power Group developed a Cd detection kit for rice, which uses a technique for immunological measurement called the immunochromatographic assay method. This kit is a test-paper type that uses the antigen-antibody reaction of the Cd-EDTA complex and the anti-Cd-EDTA antibody, which reacts specifically with it, and can detect Cd in concentrations of at least 0.01 mg L^{-1} from the extent of coloration. The immunochromatographic assay method is mainly used as a qualitative analysis method to determine whether a target substance is above a certain level or not. A familiar example of it is the pregnancy test. Because antigen-antibody reactions themselves are quantitative, it is conceivably possible to a certain extent to estimate concentration by reading coloration with an instrument and comparing it with a reference liquid. In this research, we explored methods of estimating the Cd concentration in unpolished rice using this kit, and examined the possibility for its use in quick and simple determinations for contaminated rice, with the assumed users being agricultural extension agencies and others who do not have precision analytic instruments or other such equipment.

Measuring the Cd Concentration in Unpolished Rice Using the Immunochromatographic Assay Kit

The immunochromatographic assay kit (made by Kansai Electric Power) comprises a process for extracting and refining the Cd from unpolished rice, and an immunochromatographic assay component. Detection follows the procedure illustrated in Figure 1.

Preteatement (Cd extraction and purification): Add 20 mL of 0.05 mol L-1 hydrochloric acid solution to a 2-g sample of powdered and dried unpolished rice, shake for 1 h, and then filtrate. To remove heavy metals (such as Mn, Zn, and Cu) that exhibit cross-reactivity with Cd and interfere with the antigen-antibody reaction, allow 5 mL of this filtrate to pass through an interfering substance removal column and adsorb the Cd into the column. Wash interfering substances out of the column by adding 5 mL of 0.1 mol L-1 hydrochloric acid solution to the column, then add 5 mL of 0.05 mol L-1 nitric acid solution to elute the Cd. The interfering substance removal column contains silica gel coated with a chelating

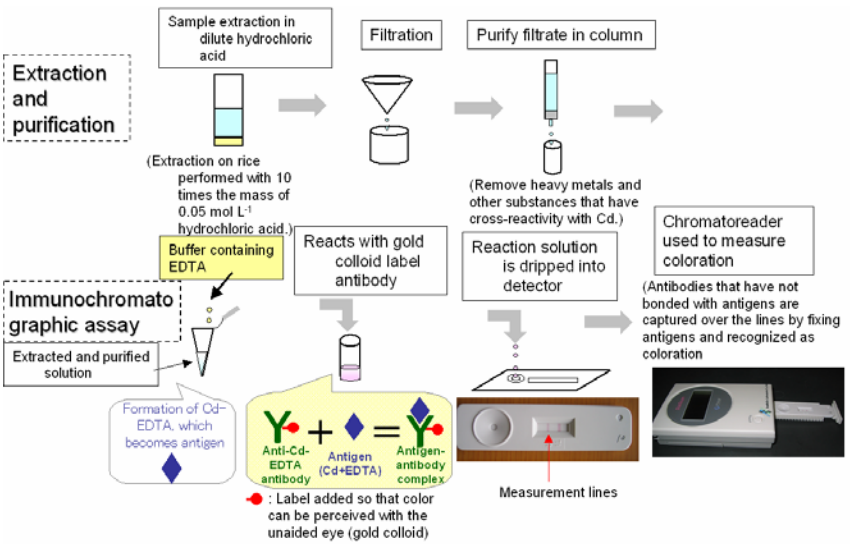

Fig. 1 Procedure to detect Cd in unpolished rice using the immunochromatographic assay kit

agent that specifically adsorbs Cd.

Detection by immunochromatographic assay: Mix 380 µL of EDTA buffer solution with 20 µL of the pretreated solution, and allow 100 µL of the mixture to react with a gold colloid label antibody. Drop 75 µL of that into the immunochromatography detection device, wait 40 to 50 min for coloration to stabilize, and measure the extent of coloration with a chromatoreader.

Figure 2 presents the results of coloration extent measurements made by performing immunochromatographic assays on pretreated solutions of unpolished rice with various concentrations of Cd, and on Cd reference solutions. Coloration readings corresponding to Cd concentrations assume a sigmoid curve, which is usual for the antigen-antibody reaction, and in the Cd concentration range from 0.01 to 0.1 mg L^{-1}, there was a good linear relationship between the logarithmic values of Cd concentrations and our readings. Thus, we found that it is possible to create a calibration curve (exponential equation) using 0.01 to 0.1 mg L^{-1} Cd reference solutions, and from the coloration readings calculate the Cd concentration of an extracted and purified solution.

Figure 3 plots the relationship between unpolished rice Cd concentrations quantified by a precision analysis (acid digestion and inductively coupled plasma mass spectroscopy (ICP-MS)) and Cd concentrations in unpolished rice calculated by the immunochromatographic assay method. We obtained a good regression with a slope of about 1.

Table 1 shows the spread in Cd concentrations obtained when using the immunochromatographic assay method to measure samples with concentrations near those of the international standard (0.4 mg kg^{-1}) and the Japanese standard (1.0 mg kg^{-1}), and reference samples (NIES CRM Nos. 10a and 10b). No. 10-a, which has an extremely low concentration, was higher in all runs than the certified value, but Sample 10-b and Sample I, for which runs were all near the international standard (0.4 mg kg^{-1}), were distributed in the range of 80 to 120% of the known concentration. Judging by these results, it is possible to use the Immunochromatographic Assay Kit for roughly determining Cd concentrations of unpolished rice in the field if measurements are repeated a number of times.

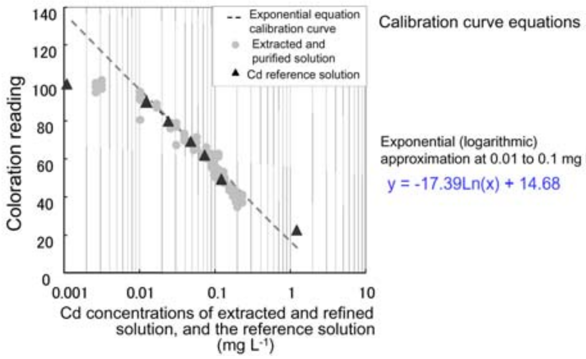

Fig. 2 Calibration curve for exponential equation approximations. Coloration readings are shown with an attenuation rate in which 0.001 mg L^{-1} is 100.

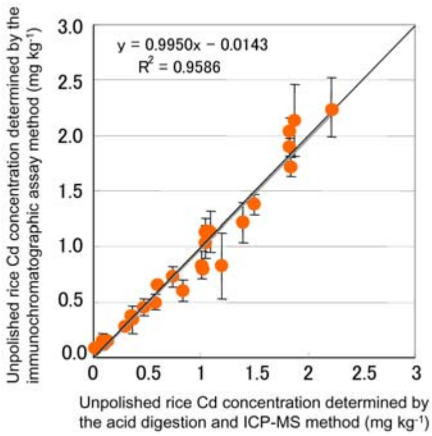

Fig. 3 Comparison of unpolished rice Cd concentrations determined by the immunochromatographic assay method (pretreatment + immunochromatographic assay) and the acid digestion and ICP-MS method.
Immunochromatographic assay values are the averages of three runs.

Table 1 Distribution of Cd concentration values measured in unpolished rice samples (reference samples and samples close to concentrations of standards) using the immunochromatographic assay method in comparison with certified or ICP-MS measured values. Average value ± standard deviation.

	Certified values or ICP-MS measured values (mg kg^{-1})	Immunochromatographic assay method (mg kg^{-1})
NIES CRM No.10-a	0.023±0.003*	0.065±0.017
NIES CRM No.10-b	0.32±0.02*	0.31±0.05
Sample Ⅰ	0.47	0.47±0.05
Sample Ⅱ	1.05	0.98±0.22

* certified values

Points to Keep in Mind When Measuring Cd Concentration with the Immunochromatographic Assay Kit

This method can be used as a simple and quick means of approximating Cd concentration but because of the average 14% (2 to 41%) coefficient of variance in measurements of unpolished rice Cd concentration, a close examination using precision analysis is needed to determine, for example, whether a sample near a standard value meets the standard.

Additionally, we are now investigating whether this immunochromatographic assay kit can be used for soil Cd concentration measurements as well. The Agricultural Land Soil Pollution Prevention Law requires that soil Cd concentration be determined by extracting Cd from the soil using 0.1 M hydrochloric acid, which means there will be problems with Cu and other interfering elements, but it appears that if a column is used for purification as with unpolished rice, comparatively precise measurements can be made. (K. Abe)

4. Decreasing Dieldrin Residue in Cucumbers by Using Low-Absorption Rootstock

Introduction

Dieldrin (Fig. 1) is one of the 12 substances regulated by the "Stockholm Convention on Persistent Organic Pollutants" (Stockholm POPs Convention) (adopted in 2001, entered into force in 2004), and in the past was widely used on agricultural land in Japan as an insecticide (registered in 1954, registration lapsed in 1975). Dieldrin is very stable in the environment, with a reported half-life in soil between 5 and 25 years. As such, once it is added to the soil, it does not easily decompose, and it remains in farmland even now, over 30 years since its use was discontinued in Japan. Dieldrin levels exceeding the residue standard were recently detected in cucumbers produced in several regions. This discovery attracted public concern as a matter that weakens confidence in “food safety;” producers were obliged to take urgent measures such as discarding cucumbers, and testing cucumbers and soil for the residues.

To avoid such problems, we compared the absorption of dieldrin in soil by various cultivars using cucumbers grafted with squash rootstock, and examined the effectiveness of reduction of residual dieldrin in cucumbers using various low-absorption cultivars.

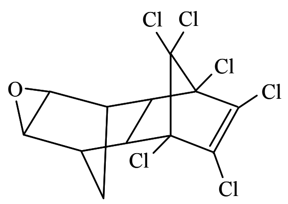

Fig. 1 Chemical structure of dieldrin

Differences Among Cultivars in the Absorption of Dieldrin by Grafted Cucumbers

Most cucumbers in Japan are grown with grafted vines using squash rootstock. Thus, when looking for cultivars that absorb less dieldrin, one must examine both the squash rootstock and the cucumber scion. Before the grafting test, therefore, we used soil contaminated with dieldrin to grow young plants of 10 squash cultivars commonly used as rootstocks for growing cucumbers, and of 23 cucumber cultivars used as scions. Dieldrin concentrations in their shoots were compared (Fig. 2). We found that for the squash plants used as rootstock and the cucumber plants used as scions, there were two- to three-fold differences in the absorption of soil dieldrin among cultivars. Judging from this result, taking advantage of inter-cultivar differences in absorption ability appears to offer hope for reducing contamination.

Which Controls Dieldrin Absorption — Rootstock or Scion?

To offer “low-absorption cultivars” for the production of grafted cucumbers using squash rootstock, one must determine whether the dieldrin absorption of the grafted plant is controlled by the squash rootstock or the cucumber scion. To test this, from among the tested cultivars (Fig. 2) we chose three squash rootstock cultivars (from largest to smallest absorption: Shintosa-1gou > Hikaripower-gold > Yuyuikki-black) and two cucumber scion cultivars (Sharp 1 > Natsubayashi), all with different dieldrin absorptions. These were used to make grafts with all six possible scion/rootstock combinations, which were cultivated in pots containing two types of soil with residual dieldrin. Dieldrin concentrations of the resulting cucumbers were compared (Fig. 3).

Dieldrin in the cucumbers of the grafted plants increased or decreased in both the Sharp 1 and Natsubayashi cucumber scion cultivars in accordance with the squash rootstock cultivar used. From largest to smallest concentrations, the squash cultivars were Shintosa-1gou > Hikaripower-gold > Yuyuikki-black. This order matches that of the dieldrin absorption ability of rootstock cultivars (see Fig. 2). The dieldrin absorption of grafted plants was influenced little by differences in scion cultivars but greatly by rootstock cultivars. Therefore, the selection of low-absorption rootstock cultivars is important for reducing the concentration of dieldrin in grafted cucumbers.

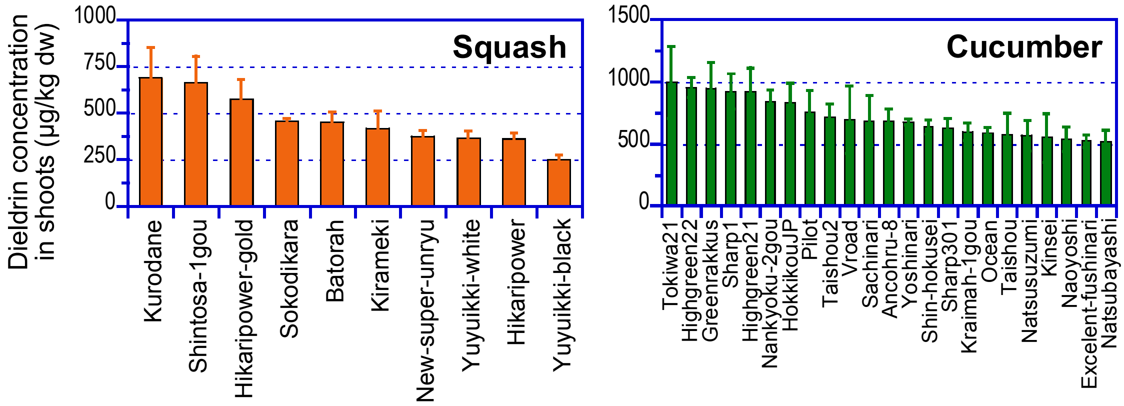

Fig. 2 Inter-cultivar differences in shoot dieldrin concentrations of squash (rootstock) cultivars and cucumber (scion) cultivars. Error bars indicate standard deviations (*n*=3).

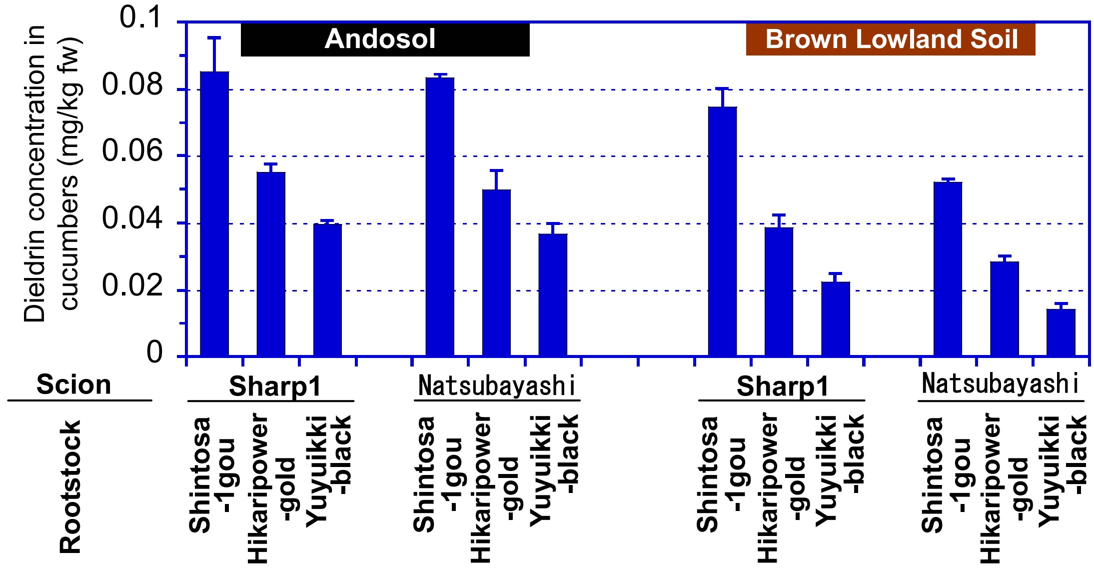

Fig. 3 Influence of rootstock cultivar difference on dieldrin concentration of grafted cucumbers. Error bars indicate standard errors (*n*=3).

Reducing the Concentration of Dieldrin in Cucumbers with Low-Absorption Rootstock Cultivars

Although the test results presented here are from cultivation in pots, when using low-absorption rootstock such as the Yuyuikki-black, the dieldrin concentration in cucumbers was about 30 to 50% lower than when using high-absorption rootstock, no matter which scion cultivar was used (Fig. 3). Currently, regional agricultural research agencies are conducting local demonstration testing on the effectiveness of reducing the concentration of dieldrin in cucumbers by using low-absorption rootstock cultivars. This is a promising technique that can reduce the dieldrin contamination of cucumbers without requiring extra cost or labor, and it is hoped that it will be included in prefectures' manuals of measures to cope with agricultural problems.

This work was supported in part by a Grant-in-aid (Hazardous Chemicals) from the Ministry of Agriculture, Forestry and Fisheries of Japan. (T. Otani and N. Seike)

5. "The Invasive Alien Species" Golden Mussel Has Broadened Its Distribution to About Half of the Lake Kasumigaura Shore

The Golden Mussel, a Invasive Alien Species

Exotic organisms are not originally present in a certain region, but invaded from another region mainly due to human activity. The Invasive Alien Species Act gives the designation of "Invasive Alien Species" to exotic organisms from other countries especially which have (or may have) a seriously harmful impact on ecosystems and human livelihoods. The Invasive Alien Species Act regulates importation, keeping, transport, and other actions for such organisms.

The golden mussel is a bivalve species designated as a Invasive Alien Species. It is a native to China, and is known for the heavy damage on natural environments and human livelihood (Fig. 1).

Fig. 1 Photograph of golden mussels attached to a rock from the western shore of Lake Kasumigaura

The Golden Mussel Has Invaded Lake Kasumigaura!

The invasion of the golden mussel into the Kiso-Nagara-Ibi River system and Lake Biwa-Yodo River System was confirmed in the 1990's, and reports began to emerge on damage to various water facilities. Starting in the second half of the 1980's, there were reports that golden mussels were mixed into shipments of live Asian Clams (*Corbicula fluminea*) imported from China; it is therefore likely that the golden mussel invaded Japan via these shipments. Since 2000, the presence of the golden mussel has been newly confirmed in prefectures outside of Western Honshu, such as Aichi, Gunma, and Shizuoka prefectures. In November 2005, someone discovered a large number of golden mussels attached to net pens in Lake Kasumigaura in Ibaraki Prefecture.

Lake Kasumigaura is Japan's second-largest lake after Lake Biwa, and its water is used mainly for tapwater and agricultural water supplies throughout the large Kanto region. It is possible that the golden mussel will have a serious impact on such water utilization facilities connected with Lake Kasumigaura. However, the discovery was recent, and almost nothing was known of the golden mussel's ecology in the lake prior to that. The National Institute for Agro-Environmental Sciences (NIAES) therefore surveyed the distribution of the golden mussel in Lake Kasumigaura and estimated the time of its invasion.

The Golden Mussel Has Already Broadened Its Distribution to Half of the Lake Kasumigaura Shore

From June to September 2006, we performed a habitat survey of the golden mussel in 90 locations along the shore of Lake Kasumigaura at depths of under 1 m; we used techniques such as looking for them visually under rocks, and searching on underwater embankments by manually feeling for them. This survey confirmed their presence at 41 locations, mainly along the shore in the lake's western part (Fig. 2). We found many golden mussels on concrete embankments and under rocks (Fig. 3), and the highest concentration was a survey site at Hasamado in Ami Town, Inashiki County. At this location, one survey member was able to collect 152 golden mussels in 10 min. Judging by the distribution and density data for the entire lake, the mussel's habitat range is expanding outward from the lake's western area. When considered in the light of golden mussel growth rate data obtained in the past, the present size of the golden mussels inhabiting the lake suggests that they invaded the lake no later than 2004.

This survey was conducted only in parts of Lake Kasumigaura that are shallower than 1 m, while the lake has an average depth of 4 m and a maximum depth of 7 m. Reports from past research say that golden mussels establish themselves in the greatest density at the depth of 6 m. The mussel's actual distribution may have a greater extent than indicated in Figure 2.

It is known that in part of the area belonging to the Lake Kasumigaura system, golden mussels that invaded irrigation water facilities have already caused damage such as plugging water distribution pipes. Areas where the golden mussel is distributed will require caution, for example when moving fishing equipment and operating irrigation facilities. Especially in the vicinity of areas where this species is found in high density, it will perhaps be necessary to take measures of some kind to pre-

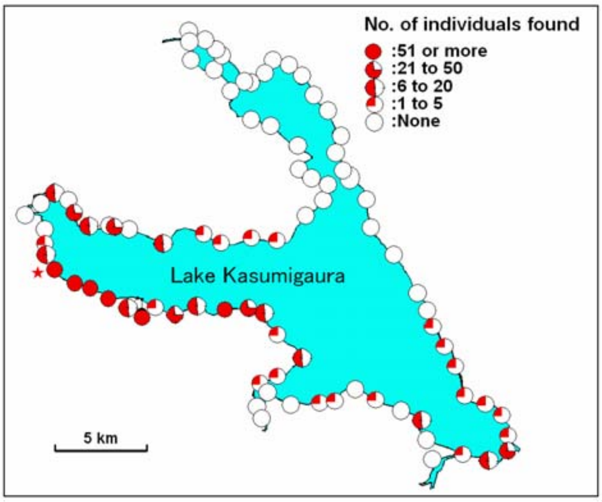

Fig. 2 Distribution of golden mussels in Lake Kasumigaura. The survey was performed by having one person look for mussels for 10 min at each survey location. Circles identify survey locations, and the more individuals found, the more each circle is filled in. The star identifies the location where the most individuals were found.

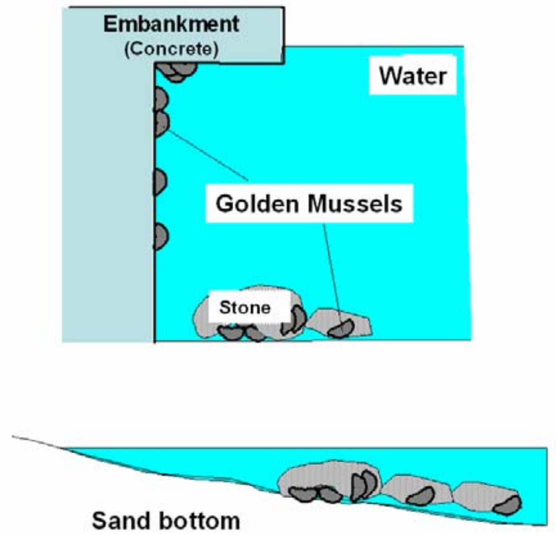

Fig. 3 Diagrams showing where golden mussels were found on concrete embankments (above) and sandy beaches (below)

vent the spread of golden mussels, such as restricting the movement of fishing gear and aquatic products. (K. Ito)

6. Low Possibility of Hybridization Between Genetically Modified Soybeans and Wild Soybeans in Cultivated Fields

Introduction

Genetically modified (GM) soybeans with tolerance to herbicides are being grown in more and more areas throughout the world for reduction in labor and cost in weed management and so on. Although GM soybeans are currently not commercially grown in Japan, an ancestor species, the wild soybean (*Glycine soja*), grows along rivers, in vacant lots, and other places in Asian countries such as China, South Korea and Japan (Fig. 1). It is concerned that they might hybridize with wild soybeans growing nearby if GM soybeans were grown. However, soybeans and wild soybeans are strongly disposed toward fertilization within the same flower (autogamy), making for a very low possibility of fertilization by pollen moving to another flower (allogamy). Further, because the plants flower at different times, it follows that natural hybridization of GM and wild soybeans would occur with difficulty.

Two Conditions Used to Facilitate Hybridization

In this experiment, we set up conditions in an experimental field under which hybridization between GM and wild soybeans could easily occur. (1) GM and wild soybeans were planted next to one another so that in the summer, the climbing wild soybeans entwined around the GM soybeans (Fig. 2). (2) To make the flowering periods overlap more, we planted GM soybeans three times. As a result, flowering overlapped 25 to 32 days. The combination in which GM soybeans were planted on July 20 brought both species' full-bloom periods closest together (Fig. 3).

Color and Size of Hybrid Seed

We harvested and inspected a total of 32,502 wild soybean seeds, and found one that was a hybrid with the GM soybean. This hybrid seed was found from among the 11,860 seeds yielded when GM soybeans were planted on July 20 (Table). We cultivated this hybrid seed, some characteristics such as size of pods/seeds and the color of seeds were between GM and wild species (Fig. 4).

Conclusion

There have been reports of natural hybridization between non-GM soybeans and wild soybeans, but this is the first instance that GM soybeans were used. These results show that if GM soybeans are cultivated in Japan, it is possible they would naturally hybridize with wild


Fig. 1 Wild soybeans entwined around tall goldenrod (Tsukuba, Ibaraki)

Fig. 2 A wild soybean entwined around a GM soybean (August 31, 2005)

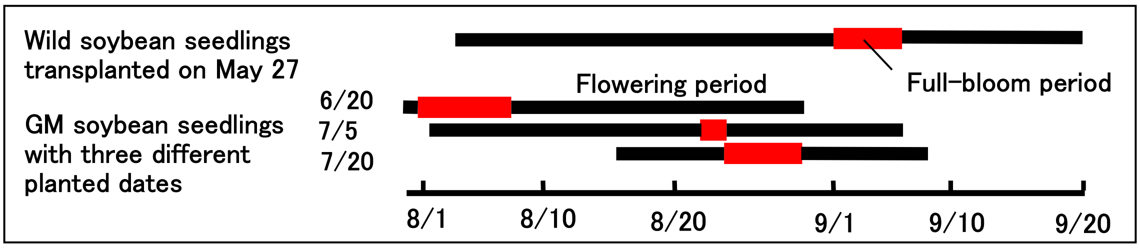

Fig. 3 Comparison of flowering periods of GM soybean planted at three different dates and wild soybeans transplanted on May 27

Table The number of total and hybrid seeds in wild soybean in hybridization test with GM soybean

GM soybean seeds planted dates	6/20	7/5	7/20	Totals
The number of seeds of wild soybean	7,814	12,828	11,860	32,502
The number of hybrid seeds in wild soybean	0	0	1	1

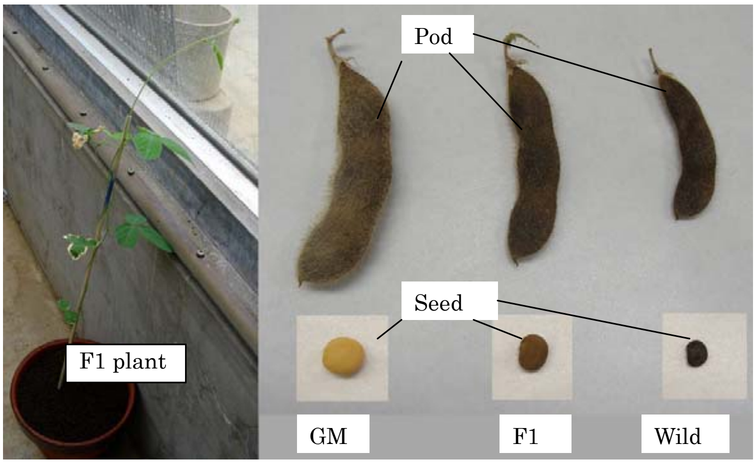

Fig. 4 Hybrid of GM and wild soybeans obtained in this test (left), and pods and seeds of GM, hybrid and wild soybeans

soybeans growing naturally nearby. However, considering that only one hybrid seed was obtained by artificially overlapping the two species' flowering periods and also growing them very close to one another, as in this experiment, this clearly shows that the possibility of hybridization in nature between wild soybeans and GM soybeans is extremely low. (Y. Yoshimura, K. Matsuo and A. Mizuguti)

7. Environmental Characteristics of Irrigation Ponds as Dragonfly Habitats

As alternatives to wetlands in the floodplains of rivers, paddy ecosystems are important habitats for aquatic organisms. In Japan many freshwater species inhabit irrigation ponds: for example, about half of the species of freshwater plants and of dragonflies depend on these ponds. The irrigation ponds that supply water to rice paddy fields were constructed mainly in the Edo era (from the 17th to 19th centuries), and in the 1950s there were about 300,000 ponds. However, because of agricultural development and urbanization the number of irrigation ponds has rapidly decreased and the environmental quality for the organisms inhabiting the ponds has declined. Consequently, the abundance of many pond-dwelling species has been reduced, and some species are endangered. To conserve the organisms living in the ponds we need to gain an understanding of the environments in which these organisms thrive. We therefore investigated the relationship between pond environment and the species composition of dragonflies, as a representative organism found in the ponds. The study sites were about 70 ponds located to the southeast of Mt. Tsukubasan in Ibaraki Prefecture. We surveyed the species and abundance of dragonflies in the study ponds, the environments within the ponds, and the land uses around the ponds. Land use was analyzed by a geographic information system (GIS). The data obtained were analyzed by multivariate analysis.

A total of 41 dragonfly species were recorded at the study sites. The number and composition of these species varied among the ponds. A cluster analysis was performed to classify ponds with similar species compositions into the same groups; the analysis produced six pond groups (Table 1). In addition, an indicator species analysis (INSPAN) was performed to determine the indicator species (species representative of each pond group), and four to nine indicator species were statistically selected for each of groups 1, 2, and 4 (Table 1). Groups 1, 2, and 4 had more species of dragonfly than groups 3, 5, and 6 (Table 1).

To characterize each pond group, the study ponds were ordinated by another statistical method, non-metric multidimensional scaling (NMDS) (Fig. 1). The ponds were plotted on a coordinate system the axes of which were correlated with certain environmental variables; thus, ponds with similar characteristics were plotted near each other. The ponds of each group were distributed near each other on the ordination space (Fig. 1), indicating that each group had specific environmental characteristics. Correlation analyses between the two axes and the environmental variables of the ponds gave the following results. The ponds in group 2 were surrounded by forests, and the bottoms of ponds were covered with debris such as dead leaves. The ponds in group 4 were relatively large in area and were surrounded by open space in the form of crop fields and wastelands. These characteristics were consistent with the habitat preferences of the indicator species. The ponds in group 1, however, were distributed around the intersection of the two axes, indicating no distinctive environmental characteristics. The indicator species of group 1 (Table 1) were common dragonflies found in many ponds, a fact that was consistent with this result. The ponds of groups 5 and 6 were characterized by concrete revetment and were considered to be bad habitats for dragonflies, because fewer dragonfly species were found there than in the other ponds and no indicator species were selected from these groups. In contrast, many species inhabited pond groups 1, 2, and 4, so these groups were good habitats for dragonflies. In particular, groups 2 and 4 had different environmental characteristics, suggesting that it is important to conserve the environments of different groups of ponds.

This study revealed the irrigation pond characteristics that provide good environments for dragonflies. To ensure the viability of species and populations of organisms, it is important to make a network of habitats as well as to conserve each habitat. The irrigation ponds sometimes dry up through drought or are drained for desilting; at these times aquatic organisms cannot survive in them. However, if the organisms are able to immigrate from other ponds after the dried ponds have refilled with water, their populations will recover. We are developing methods for analyzing the effect of the spatial distribution of ponds on dragonfly populations. (K. Tanaka, T. Yamanaka, N. Iwasaki, D. S. Sprague and Y. Nakatani)

Table 1 Classification of ponds and indicator dragonfly species representative for each pond group

Group	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
Mean no. of species	12.2	11.0	7.3	15.8	6.9	6.0
Indicator species	*Sympetrum infuscatum* *Macromia amphigena* *Orthetrum albistylum* *Pseudothemis zonata*	*Copera annulata* *Orthetrum triangulare* *Mnais pruinosa* *Anax nigrofasciatus*	No	*Cercion calamorum* *Cercion sieboldii* *Ischnura senegalensis* *Ischnura asiatica* *Deielia phaon* *Crocothemis servilia* *Sinictinogomphus clavatus* *Anax parthenope* *Epophthalmia elegans*	No	No

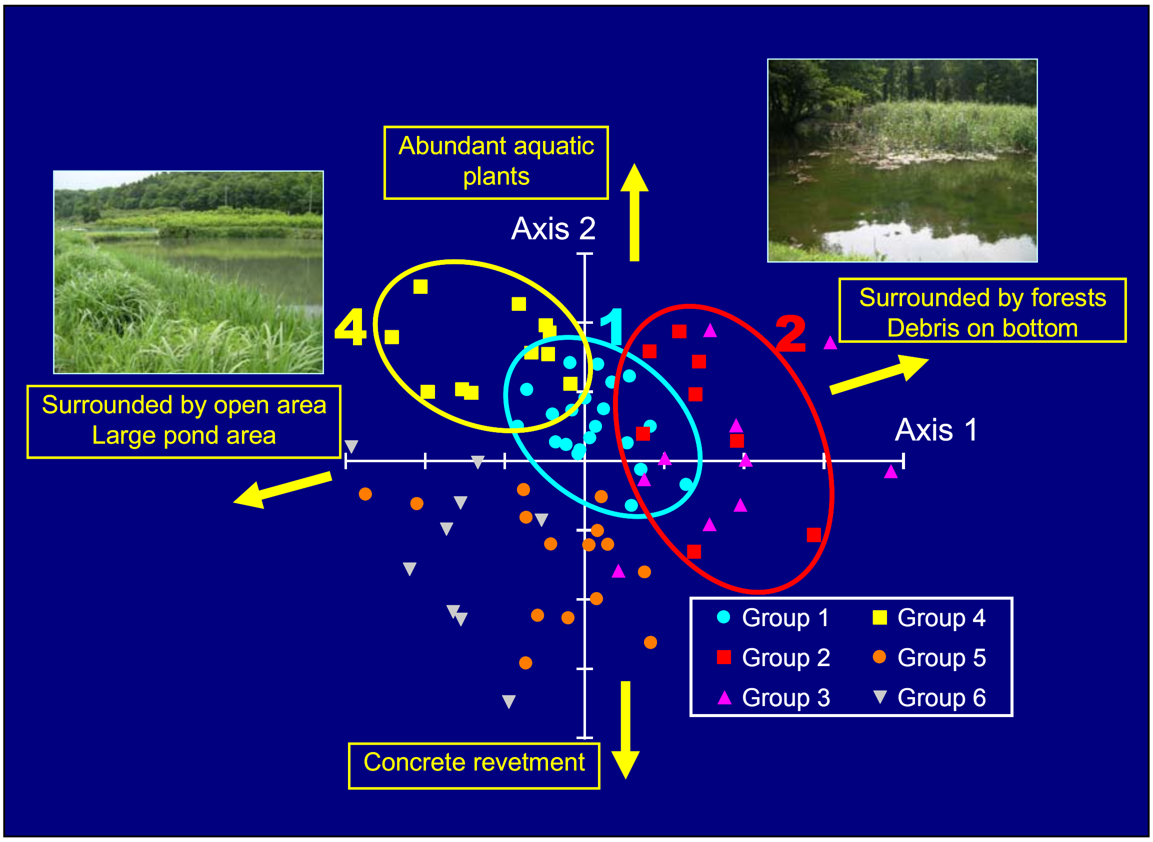

Fig. 1 Grouping of ponds on the basis of dragonfly habitat characteristics.
Circles show the pond groups for which indicator species were selected in Table 1.
Yellow arrows and boxes indicate environmental characteristics.
Photographs show typical ponds in groups 2 and 4.

8. The Smaller Tea Tortrix Moth Exhibits Resistance to Mating Disruptant (Sex Pheromone)

Introduction

Chemicals known as sex pheromones, which induce mating behavior in insects, are used in mating disruptants that serve as environmentally friendly pesticides. These pest control agents do not directly kill insects. Instead, sex pheromones wafting in the air make it difficult for adult male insects to recognize the sex pheromones emitted by adult female insects for mating, thereby preventing adult males from locating adult females of their own species. This effectively reduces opportunities for adult males to mate, and decreases the number of offspring. Because sex pheromones act differently on each insect, this technique can be used to block target insects alone, and therefore makes very environmentally benign insect pest control possible. Further, because mating disruptants using sex pheromones interfere with the species recognition process, developing resistance requires that the ways of species recognition should be changed, and it was therefore believed that the possibilities of such changes are small.

The effective ingredient of mating disruptant is (Z)-11-tetradecenyl acetate (Z11-14:Ac) has been used to control the smaller tea tortrix moth (*Adoxophyes honmai*) (Fig. 1), but its effectiveness has plummeted, and in 1996 this was reported as the world's first instance of mating disruptant resistance. To develop mating disruptants that do not cause resistance, we are conducting research to

Fig. 1 The smaller tea tortrix moth. Adult male on left, adult female on right.

Fig. 2 The mating disruptant formulation using sex pheromone, placed in tea bushes

determine what causes the expression of resistance.

The mating disruptant formulation is a tube-like object folded in two (Fig. 2). These are placed throughout a whole tea field at intervals of 1.5 to 2 m. The sex pheromone components are sealed into a polyethylene tube or other container so that they are slowly released over a period of several months.

Selection of Insect Strain with Resistance to Mating Disruptant

We collected many smaller tea tortrix moth from a tea field in Shizuoka Prefecture where resistance has been reported. Into a container that contained a mating disruptant (Z11-14:Ac) in high concentration, we put adult males and females, allowed them to mate, and collected the eggs. The insects born from these eggs would be descended from insects that can mate even in the presence of high-concentration of the mating disruptant. The offspring were raised on artificial food and became adults in about one month. We repeatedly raised insects in the same way and selected insects that can mate even under conditions of higher mating disruptant concentration. Our test was conducted by releasing 10 male and 10 female adult smaller tea tortrix moth into 1-L glass containers into which we had put mating disruptant (Z11-14:Ac), then checking to see what percentage of them were able to mate. The higher the mating rate at the same concentration, the greater the insects' resistance. Just after collecting moths from the field, about 60% of them were able to mate at a concentration of 0.001 mg/L, however, total interference occurred at 0.1 mg/L. The same held true until the 35th generation after selection. In the 46th generation, about 80% of the moths were able to mate at a concentration of 0.1 mg/L, and the mating rate did not show much decline even if the concentration was raised. Even at 1 mg/L, at least 60% of the moths could mate (Fig. 3). Because the strength of this resistance remained unchanged for 4 years after this selection, it was clear that the insects had gained resistance to the mating disruptant.

Attraction of Mating Disruptant-Resistant Insect Strain in an Indoor Wind Tunnel

Sex pheromone components needed to attract the smaller tea tortrix moth are the two substances, (Z)-9-tetradecenyl acetate (Z9-14:Ac) and Z11-14:Ac (mixed in a 7:3 ratio). The pheromone has no attractive power without one of these components. We used an indoor wind tunnel (30 cm in diameter and 2 m long) to investigate the reactivity to sex pheromone components of adult males from a standard strain having no resistance and from the resistant strain (R strain). Adult males from the standard strain (blue) reacted well to a sample of the binary mixture of Z9-14:Ac and Z11-14:Ac (Fig. 4, left), but did not react to a sample of only Z9-14:Ac without Z11-14:Ac (Fig. 4, right). However, adult males from the R strain (red) reacted strongly to the sample of only Z9-14:Ac without Z11-14:Ac (Fig. 4, right). This

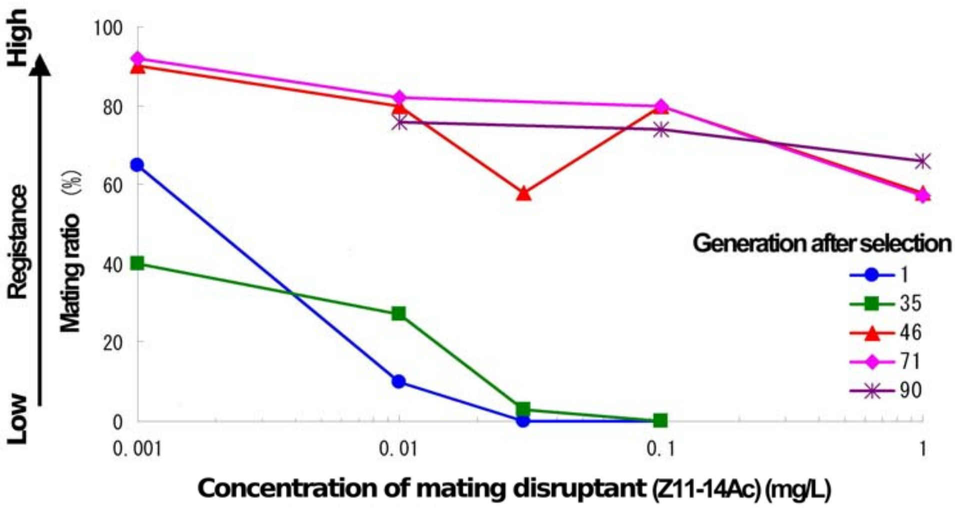

Fig. 3 Acquisition of strong resistance to the mating disruptant

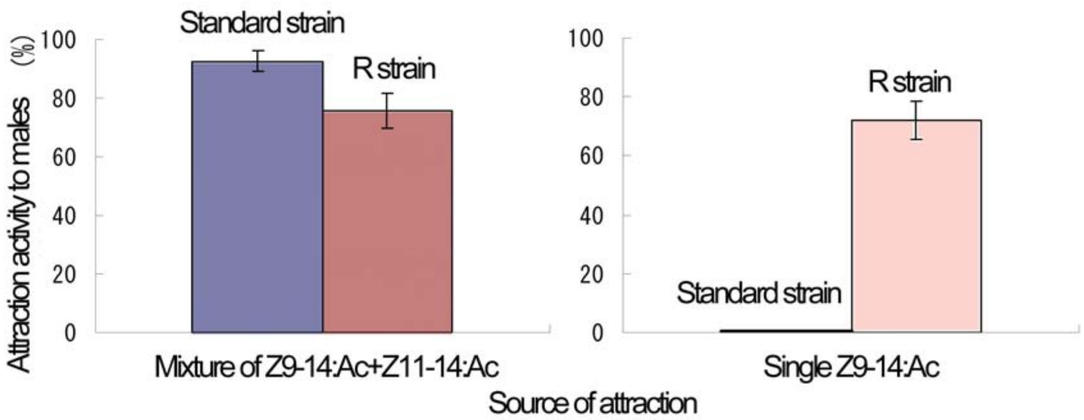

Fig. 4 Reactivity of adult males to sex pheromone components

means that R-strain adult males had gained an unexpected character.

Reason for Resistance to the Mating Disruptant

When the atmospheric concentration of the mating disruptant (Z11-14:Ac) is high, it interferes with the sex pheromone signal released by adult females, making it difficult for adult males to find adult females. However, because R-strain adult males do not need Z11-14:Ac as a sex pheromone signal, they are less susceptible to the mating disruptant. This appears to be one reason for the manifestation of resistance to the disruptant.

Conclusion

It is conceivable that there was in the field a very small number of adult male smaller tea tortrix moth that can locate adult females without Z11-14:Ac, and that owing to the continued use of Z11-14:Ac as a mating disruptant, the percentage of adult males with this character gradually increased, and resistance to mating disruptants appeared. Future use of sex pheromones for insect control through mating disruption will require periodic examination of resistance. (H. Sugie and J. Tabata)

9. Enhancement of Rice Plant Canopy Photosynthesis by Elevated Atmospheric CO_2 Concentration Is Dependent on Leaf Nitrogen Concentration

Introduction

Atmospheric CO_2 concentration is now 380 ppm and rising, and is predicted to reach between 540 and 970 ppm by the end of this century. Rising atmospheric CO_2 concentration will conceivably have a major influence on crop production. Especially the influence on production of rice, which is a staple food for about one-half of the world's population, is important from the perspective of a stable food supply. This makes it necessary to accurately predict the future amount of rice production.

It is known that rising CO_2 concentration promotes photosynthesis of a single-leaf in upper layer of rice canopies, but the extent gradually declines as plant growth proceeds. However, it is not clear whether enhancement of total photosynthesis of rice plant canopies, which consist of many individual leaves, declines in conjunction with growth. Because a simple integration of single-leaf measurements does not adequately explain the canopy responses and influences on canopy photosynthesis are directly linked to the growth rate of rice plant, they are one cause of uncertainty in predicting future rice plant production. This research was conducted to determine if the enhancement of canopy photosynthesis by elevated CO_2 concentration declines as it does in individual leaves, and if so, to find the causes.

Response of Canopy Photosynthesis to Elevated CO_2 Concentration

We used six controlled-environment chambers (Fig. 1) in the National Institute for Agro-Environmental Sciences to grow rice plants (the "Nipponbare" cultivar) for three years under two conditions: current atmospheric CO_2 concentration (380 ppm) and elevated CO_2 concentration (680 ppm). By continuously measuring the CO_2 balance in each of these controlled-environment chambers, we calculated the daytime photosynthetic rates and nighttime respiration rates of the rice plant canopies.

This experiment revealed, for the first time on the canopy level, that the canopy photosynthesis enhancement rate due to elevated CO_2 concentration was highest in the initial growth stage, and gradually declined as growth proceeded (Fig. 2). On the other hand, enhancement rate of canopy nighttime respiration was also highest in the initial stage but does not declined in parallel with that of canopy photosynthesis after then. Because

Fig. 1 Naturally sunlit controlled-environment chambers at the National Institute for Agro-Environmental Sciences. Here it is possible to investigate plant response, canopy photosynthesis and respiration rates, and other things under natural sunlight conditions with controlling the air temperature, humidity, and CO_2 concentration inside the chamber.

rice plant growth rate is determined by the balance of rice plant canopy photosynthesis and respiration, the enhancement rate of rice plant growth due to elevated CO_2 concentration declines as plant growth proceeds.

Reason for Declining Canopy Photosynthesis Enhancement Rate

Generally, the photosynthetic capacity of individual leaves is closely related to leaf nitrogen concentration. We therefore investigated the relationship between canopy photosynthetic capacity (photosynthetic rate per amount of light captured) and leaf nitrogen concentration, and found that there is a curvilinear relationship between them (Fig. 3). Further, we found that the relationship differs according to CO_2 concentration. It is evident from Fig. 3 that when leaf nitrogen concentration was high, the canopy photosynthesis enhancement rate was high, and that as leaf nitrogen concentration decreased, the enhancement rate declined. Because at both CO_2 concentrations, leaf nitrogen concentration declined as growth proceeded, the canopy photosynthesis enhancement rate fell off. Additionally, it was observed that leaf nitrogen concentration of rice plants grown at the high CO_2 concentration was lower than that of rice plants grown at the ambient CO_2 concentration. This phenomenon was also a factor that lowered the canopy photosynthesis enhancement rate. In other words, we found that change in the rice plant canopy photosynthesis enhancement rate can be explained by change in leaf nitrogen concentration, which is in turn governed by progress in plant growth and high CO_2 concentration.

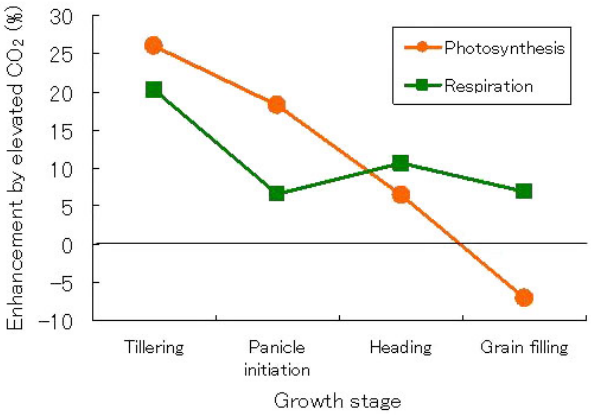

Fig. 2 Changes in the rate at which canopy photosynthesis and respiration are enhanced by elevated CO_2 concentration during rice growth period. Although the photosynthesis enhancement rate by elevated CO_2 concentration gradually declined as the rice plants grew, there was no decrease in the respiration enhancement rate.

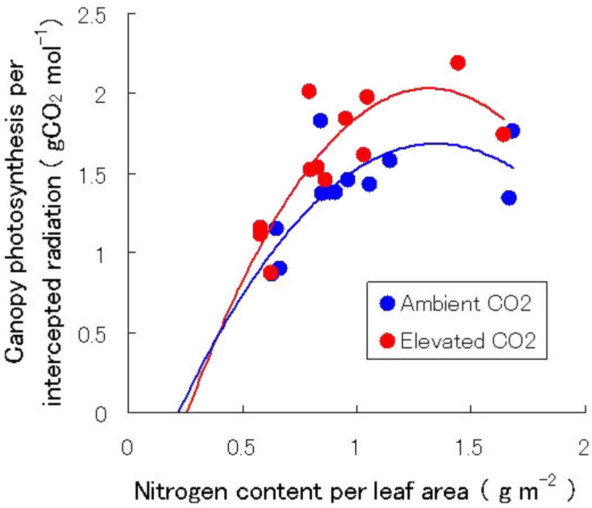

Fig. 3 Relationship between rice plant canopy photosynthetic efficiency and leaf nitrogen concentration, obtained from a 3-year experiment. As the nitrogen content per unit leaf area (leaf nitrogen concentration) dropped, the difference between the two CO_2 concentration groups became smaller. This result implies that the rate at which canopy photosynthesis is enhanced by increased CO_2 concentration declines in tandem with nitrogen concentration, when leaf nitrogen concentrations of rice plants grown under ambient and elevated CO_2 concentration are same.

Conclusion

These results indicate that accurately predicting rice production when CO_2 concentration has risen requires accurately predicting the nitrogen concentration in rice plants (especially leaves) under elevated CO_2 concentration. Also, we expect that nitrogen management is important to develop adaptive cultivation techniques, which take maximum advantage of the "fertilizer" effect of higher CO_2 concentration on rice plant growth. (H. Sakai)

10. Monitoring Spatio-Temporal Changes in Flooding and Rice Cultivation in the Mekong Delta Using Satellite Data

Introduction

Considerable concern exists regarding the effect that changes in climate and future water-resource development in the upper Mekong catchment will have on river stream-flow and how this will impact on agricultural production. In order to predict future Asian food production, it is important to observe both the extant water environment and agricultural land-use changes over a large region, and to understand their dynamic interrelationship. Various types of satellite data have been employed to observe agricultural area and to detect changes in inundated areas. However, the long period of monitoring cycles and low resolution of the former satellite sensor often affect the estimate accuracy of flood expansion and crop growth. We therefore tried using time-series satellite data acquired by MODIS/Terra, which provides high-resolution ground surface reflectance data almost daily. We developed a method for analyzing the spatio-temporal changes in the flooded areas as well as rice growth stage using water and vegetation indexes, which show the status of inundation and plant coverage of the ground surface. Here we describe the results obtained by this method to monitor 5-year flooding and rice-cropping patterns in the Mekong Delta.

The Mekong Delta in Vietnam

Since adopting a market-oriented economy, the production efficiency of Vietnamese agriculture has improved considerably, increasing about 2.3-times to 36.2 million ton in 2004 compared to the 15.9 million t harvested in 1985. The Mekong Delta is located on the southern end of the Indochina Peninsula and produces approximately half of the rice in Vietnam. According to agricultural statistics of UN Food and Agriculture Organization (FAO), Vietnam is the world's second-largest rice exporter after Thailand (3.8 million t in 2003, approximately 13% of the world's total rice exports). Nearly 90% of the rice exported from Vietnam is produced in the Mekong Delta. The Vietnamese rice is consumed in many Asian countries such as the Philippines and Indonesia and so forth. It indicates that the Mekong Delta is an important region for predicting future Asian rice supply.

Determination of Annual Flood Dynamics in the Mekong River

Figure 1 shows the estimated result of the first day, last day, and duration of flooding in the Mekong Delta for the years 2000 through 2004. The results indicate that the duration and the last day of large-scale floods (2000-2002) were longer and ended later than those of small- or middle- scale floods (2003-2004). It was also found that the spatio-temporal distribution of the floodwater, which restricts rice cropping from the rainy season to the early dry season, was uneven in upstream-regions for any given year.

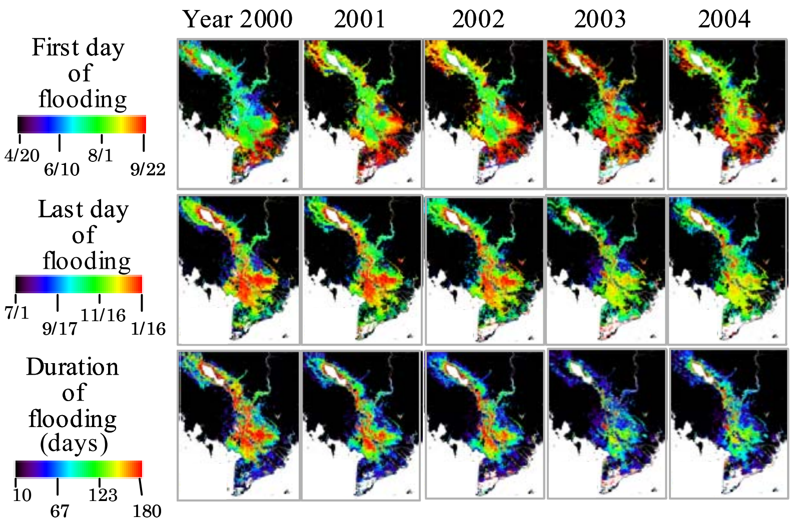

Fig. 1 Estimated first day of flooding, last day of flooding, and flooding period in the Mekong Delta for the years 2000 through 2004

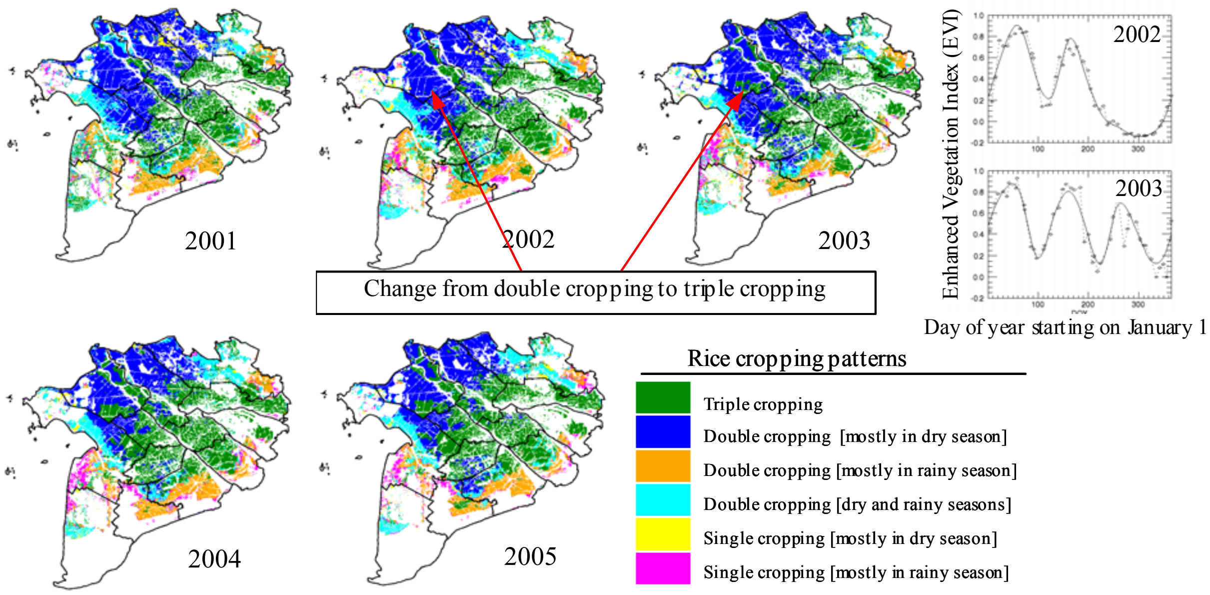

Fig. 2 Estimated rice-cropping patterns in the Mekong Delta in Vietnam. Graphs on the right show the changes in the vegetation index at the locations indicated by the red arrows.

Yearly Changes in Rice-Cropping Patterns

We classified rice-cropping patterns (such as triple cropping or double cropping [mostly in the dry season]), according to the number and timing of peaks in smoothed Enhanced Vegetation Index (EVI) data (Fig. 2, right).

In the upstream-regions (blue areas), the cropping pattern was classified as double rice cropping which is practiced so as to avoid flooding in the rainy season. In the coastal-zone (orange areas), the intrusion of saline water from the sea through the canal network occurs due to decreased river-water flow during the dry season. Since this has a negative effect on rice cultivation in the dry season, the farmers in these areas employ double rice cropping mostly during the rainy season. In the mid-stream region, where neither flooding nor salinity intrusion have serious impacts, there is an extensive triple-cropping region (green areas). These findings show that rice-cropping patterns in the Mekong Delta are very closely related to the amount of water resources, which changes both qualitatively and quantitatively due to the influence of the Asian monsoon. In addition, annual changes in the rice-cropping patterns observed from 2001 through 2005 indicate a pronounced increase in rice production, with midstream and upstream areas changing from double cropping to triple cropping. In the areas where on-site surveys were performed (see arrows in Fig. 2), we found that the construction of new dykes had made it possible to grow rice during the rainy season by protecting the areas from inundation due to flooding.

Conclusion

An annual increase in intensive agricultural production in the Mekong Delta has accompanied the introduction of market-oriented economy, relaxed controls on land use, and the mitigation of flooding events due to renewed development in flood-control infrastructure. The economy has had the effect of changing land use, particularly in coastal areas where rice fields are rapidly being converted into shrimp ponds (white areas in Fig. 1). Such transitions are not restricted to the Mekong Delta, and have also occurred elsewhere in Asia. Consequently, there are concerns that both agricultural production and regional environment will be affected by the rapid land-use changes that have occurred in response to rapid economic development. These changes in the water environment are particularly serious when considered within the context of global warming and the development of water resources. In order to deal with these problems effectively, it is necessary to accurately determine the state of land use through continuous monitoring using satellite data. (T. Sakamoto, N. Ishitsuka, H. Ohno and M. Yokozawa)

11. A Web-Based Illustrated Key to Japanese Species of the Tribe Pilophorini (Hemiptera, Miridae)

Introduction

Many species in plant bug tribe Pilophorini are believed to prey on mites and small insects, and play a role as a natural enemy of mites and other organisms in the natural environment. *Pilophorus setulosus*, the most common species of this tribe, is found in large numbers in agricultural ecosystems, chiefly rural farming districts, and it lives on various deciduous trees. This tribe, especially its largest genus *Pilophorus* Hahn, includes many species that are similar to one another in color and morphology (Fig. 1). Although they frequently turn up in biota surveys and other investigations, there are few illustrated references or other sources that can be used to identify them, and identification has been difficult for people who are not classification experts. For that reason, we prepared and made available an illustrated key, which graphically shows the characters used in identifying species. Thereby, we provided a means of easily identifying these insects.

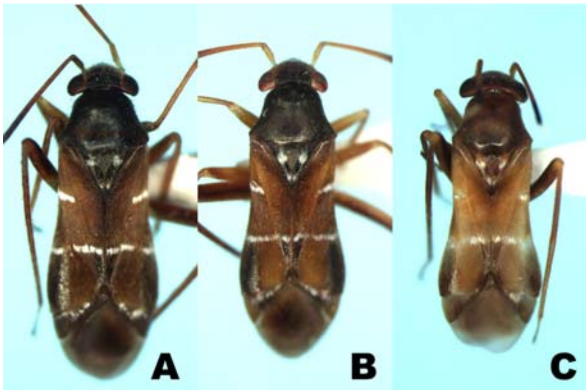

Fig. 1 Three species in the genus *Pilophorus* Hahn. A: *P. setulosus* Horváth, B: *P. erraticus* Linnavuori, *C: P. okamotoi* Miyamoto & Lee.

Tribe Pilophorini

The tribe Pilophorini belongs to family Miridae (plant bug), subfamily Phylinae, and is a comparatively small group with about 150 species known worldwide. Seventeen species have been found in Japan. They are very small bugs ranging in length from 2 to 4 mm. Members of the genus *Pilophorus* Hahn, found among this group, are known for their slim bodies, which are myrmecomorphic. These species have characteristic 2 rows of silvery scale-like setae on hemelytra, and look just like ants whose abdomens have been constricted (Fig. 2). It is said that nearly all the species of this tribe are predatory, but the details of their ecology have yet to be elucidated.

Web-Based Illustrated Key

I created a key to the Japanese species of the tribe Pilophorini which contains 17 species, and I prepared illustrations of useful characters to distinguish the species, like as the arrangement of the scale-like setae. I combined these into an illustrated key of the tribe Pilophorini (Fig. 3) and made it publicly available on the website (http://www.niaes.affrc.go.jp/inventry/insect/ illust_keys/pilophorini/key_pilophorini_e01.html). On the left appears the insect's entire body with arrows showing which part of the body is represented by enlarged images on the right. Users look at the indicated characters and choose which of two options matches the specimen they are trying to identify. Repeating this action leads them to the right species. We have also prepared an English-language version of this page. Presently, it is just a key for identifying species, but there are plans to enhance it with explanations and images of each species. (Y. Nakatani)

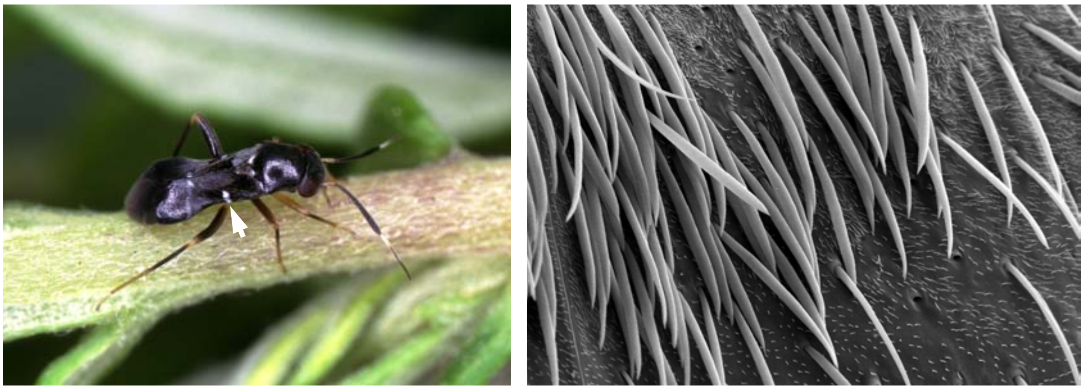

Fig. 2 Left: *Pilophorus typicus* adult (arrow shows a row of silvery scale-like setae). Right: SEM image of silvery scale-like setae.

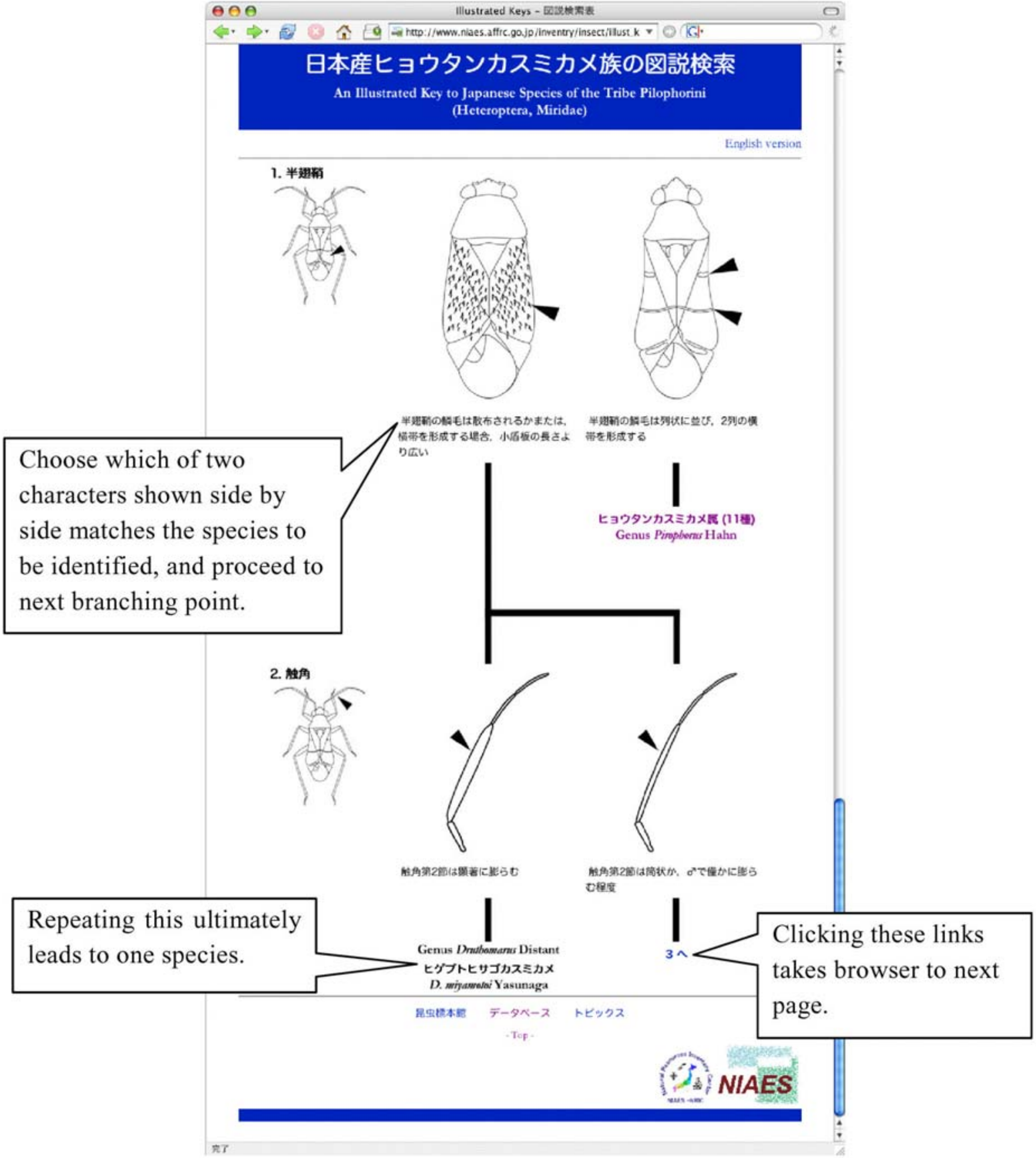

Fig. 3 Screen shot of tribe Pilophorini illustrated key. Users choose which of two characters shown side by side matches their species and proceed to the next branching point. Repeating this ultimately leads to one species.