MEETING

ABSTRACTS OF PAPERS PRESENTED AT

THE SECOND ISRAEL – JAPAN WORKSHOP
Ecologically Sound New Plant Protection Technologies

Organized by
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Introduction

Japan specializes in rainfall agriculture, while Israel excels in arid and semiarid agriculture. Thus these two countries have the potential to complement each other in solving agricultural problems in a diversity of climates and in developing countries. Glass- and plastic-house cultures adopted by Israel originated in Japan. These greenhouses enable Europe and other countries to grow fruits, vegetables and flowers all year round. Israel has become a promising market for Japanese chemical pesticides, and the experience obtained from Israel can be applied to all the Middle-East region. On the other hand, Israel has a prominent generic pesticide producer. Israel developed the drip irrigation system and there are many other aspects in Israeli technology, *e.g.* in biological control, biopesticides, integrated pest management, and biotechnology, which should be important subjects for fruitful cooperation among Japanese and Israeli scientists.

The agricultural technologies of Japan and Israel are highly developed. Extensive use of pesticides and chemical fertilizers leads to severe ecological hazards, which could be obviated by mutual cooperation. Special emphasis should be placed on developing novel pesticides causing minimum harm to man and the environment, along with the search for new approaches in controlling pest insects, diseases and weeds.

There has been a close relationship among Japan, Europe, the U.S.A. and developing countries, and similarly among Israel, Europe, the U.S.A. and developing countries. Due to the Arab boycott in the past, Japan and Israel were not so close as they should have been. However, since the visit of former Prime Minister Murayama to Israel as a result of the peace atmosphere in the region, and the signing of the Japan–Israel Science and Technology Cooperation Agreement, the door between the two countries was opened. For example, a research cooperation agreement was signed in 1996 between the Institute of Physical and Chemical Research (in Japan) and the Weizmann Institute of Science (in Israel). Thus the time became ripe for developing mutual cooperation in the field of agriculture and crop protection, the first Workshop on Plant Protection was held in Israel in 1997, and a bilateral relationship was established between Tokyo University of Agriculture and The Hebrew University of Jerusalem in 1998.

Israel is strong in research and development, especially in high-tech and agriculture, subjects which are highly valued by many institutions in the U.S.A. and Europe. In some sense Israel is most significant for Japan as a ‘feeler country’ for the most recent science and technology in the world.

One of the ways in which Japan can contribute to the Middle East peace is through agricultural cooperation among the neighboring countries. Cooperative relationships among Egypt, Jordan, the Palestinian Authority and Israel have already been initiated. In this, Israel is taking the leading role and may serve as a catalyst for cooperation between Japan and Middle Eastern countries.

For Abstracts of the First Workshop, see *Phytoparasitica* 25(4):345-366 (1997).

To promote Japan–Israel scientific and technological cooperation, it is of utmost importance that the parties involved invest considerable effort in research and development. Israeli scientists are enthusiastic and eager for such cooperation, whereas not many Japanese scientists are familiar with Israel. The present second Israel–Japan Workshop on Plant Protection will surely enhance cooperation between the two countries.

Prof. Emeritus Izuru Yamamoto
Chairman, Japan–Israel
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PLENARY SESSION

IPM in Japan

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The Japanese archipelago belongs to the temperate monsoon zone in East Asia, which involves a high pest risk to crop production. Pest control has been and will be a critical issue for stable production. On the other hand, the recent trend of advancing sustainable agriculture requires us to modify pest control practices in order to avoid adverse effects to the environment. Integrated pest management (IPM) is thus incorporated into pest control programs of national and local governments. First, introduction of economic injury level is encouraged for the basis of pest control, rather than for seeking complete freedom from pests. Furthermore, introduction of various pest control measures other than pesticides is recommended, together with the wise use of pesticides according to a pest forecast program operated by both national and local governments. On the field level, a demonstration program is carried out, to raise the farmers' understanding of IPM. Further efforts in enhancing the IPM program are required for effective execution of IPM in Japan.

Agricultural Science Cooperation with Southeast Asia

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In 1978 Tokyo University of Agriculture (TUA) was designated as the core university in the field of agricultural science by the Japan Society for the Promotion of Science (JSPS), a quasi-governmental organization under the auspices of the Ministry of Education, Science, Sports and Culture, which intended to promote academic exchange with developing countries. TUA, with the cooperation of the counterpart core universities in Indonesia (Bogor Agricultural University), Thailand (Khon Kaen University) and the Philippines (the University of the Philippines, Los Baños), is responsible for conducting programs of cooperative research, scientist exchange, and annual international seminars. Each core university also cooperates with many universities in each country. NODAI Research Institute of TUA and later NODAI Center for International Programs are in charge of the above programs. The above core university programs have been active for 20 years, with cooperative research topics numbering 55. A total of 1,192 scientists have been invited to Japan, and 1,396 were sent from Japan. International seminars – 23 all told – are held every year. The program also related to the dissertation Ph.D. program under the auspices of the JSPS, and as many as 106 scientists from three counterpart countries were granted a Ph.D. degree in agricultural sciences. Numerous institutional and individual networks were developed during the above programs. This led to the establishment of the International Society for Southeast Asian Agricultural Sciences (ISSAAS)

in 1994 and the sister relationship with some of the core universities. The activities of ISSAAS include publication of a journal and newsletters, and international seminars. All the above activities include plant protection topics. Growing interest in, and the need for plant protection led to the establishment of the International Association for the Plant Protection Sciences in 1999. Its regional centers will serve the eight geographical regions, among which centers covering East and Southeast Asian countries are being set up in Japan, making use of the network of the core universities and ISSAAS.

BIORATIONAL PESTICIDES

Biorational Insecticides – Mechanisms and Applications

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In recent years, insect control by broad-spectrum insecticides has come under assault and scrutiny because of their undesirable effects on human health and the environment. Furthermore, the rapidly developing resistance to conventional insecticides provides the impetus to study new alternatives and more ecologically acceptable methods as part of insecticide resistance management and integrated pest management programs. One of these approaches is the development of novel compounds affecting developmental processes in insects, such as chitin synthesis inhibitors, juvenile hormone mimics, and ecdysone agonists. In addition, extensive efforts have been made to develop compounds acting selectively on some groups of insects by inhibiting or enhancing biochemical sites such as respiration (diafenthiuron), the nicotinyl acetylcholine receptors (imidacloprid and acetamiprid), and salivary glands of sucking pests (pymetrozine). Among the most recent novel insecticides with selective properties are novaluron, thiamethoxam and spinosad. Novaluron is a benzoylphenyl urea that acts by both ingestion and contact. As such it is a powerful suppressor of Lepidoptera (by ingestion) and of whiteflies (by contact). Thiamethoxam is a novel neonicotinoid, which acts specifically on aphids and whiteflies. Emamectin, an avermectin derivative, acts on GABA receptors and affects a wide range of insect pests such as mites, lepidopterans and thrips. Spinosad acts on thrips and on many other insect species. The above compounds were discussed in relation to their modes of action, selectivity and importance in IPM programs.

GABA Antagonist Binding Site: A Promising Target for Selective Insecticidal Action

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δ-Aminobutyric acid (GABA) plays an important role as a major inhibitory neurotransmitter in the nervous system of animals. The rapid response of postsynaptic neurons to GABA is achieved mainly by ionotropic GABA receptors, which function as transmembrane Cl^- channels. Antagonists inhibit the action of GABA at the receptors, thereby causing toxicity to animals. Noncompetitive antagonists (NCAs), including diverse classes of compounds (*e.g.* picrotoxinin and related terpenoids, cyclodiene insecticides, bicyclophosphates and related convulsants, fipronil and related phenylheterocycles, and so on), are thought to allosterically block the channel by binding to a site (or sites) distant from the GABA binding site. Most studies using site-directed mutagenesis suggest that the binding site for NCAs is located within the channel, although some other study results are indicative of the presence of a second binding site. Even with the knowledge from molecular biology studies, the three-dimensional structure of the binding site(s) is still unknown. Under such circumstances, approaches using the NCAs as probes might be helpful for understanding the structure of the site(s) in three dimensions. We presented results from three-dimensional quantitative structure-activity relationship (3D QSAR) studies of NCAs, demonstrating the possibility that structurally diverse NCAs bind to an identical site in different orientations. Furthermore, the application of the 3D QSAR approach to housefly and rat GABA receptors reveals possible differences in the pharmacophore of the NCA binding site between animal species, which might provide opportunities for the design of safer insecticides.

Structure-Activity Studies of Neonicotinoid Insecticides on the Basis of Molecular Similarities

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Neonicotinoids represent a class of novel and attractive insecticides which act as agonists of nicotinic acetylcholine receptors (nAChR). Recent efforts to discover novel insecticides by agrochemical industries have succeeded in developing a number of neonicotinoids having various chemical structures such as imidacloprid, acetamiprid, nitenpyram, etc. During and after the process to develop acetamiprid at Nippon Soda, we were interested in the molecular similarity analysis that was successfully applied in understanding bioisosterism of drugs such as histamine H_2-antagonists. In order to rationalize the structure-activity profiles of neonicotinoids, the method of similarity indices introduced by Richards *et al.* was employed. The stereochemical aspects of acetamiprid by NMR (nuclear magnetic resonance) spectra and conformational analysis suggested that one of the stable conformations of acetamiprid resembled the structure of imidacloprid with respect to steric and electrostatic properties. The similarity of each molecule to the most active compound in a series of neonicotinoids was also significantly correlated with the binding activity to nAChR. This indicated that steric and electrostatic similarities of molecules were critically important for the activity of neonicotinoids. Based on such results, we extended the molecular similarity analysis to the three-dimensional quantitative structure-activity relationship (3D-QSAR). Two novel similarity indices representing steric and electrostatic properties of molecules in three-dimensional space were defined, respectively, and the partial least squares method was employed to analyze the correlation between the receptor-binding activity and the similarity indices of neonicotinoids. A significant QSAR model was obtained on the basis of similarity and dissimilarity of the molecules compared. The structural requirements of the molecules for the activity were visually displayed by highlighting the three-dimensional grid points that contributed significantly to the activity in terms of steric and electrostatic properties. The results of the 3D-QSAR presented a hypothetical binding model of neonicotinoids, which may help to illustrate the path of molecular recognition at nAChR.

Chitin Synthesis in Insects: Genes, Enzymes and Inhibitors

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Chitin, which is a large, water-insoluble carbohydrate homopolymer, is composed of β-(1,4)-N-acetyl-D-glucosamine. The biopolymers are coalesced extracellularly, forming microfibrillar crystallites which are associated with carbohydrates (in the cell wall of fungi) and proteins (in insect cuticles and peritrophic membranes). The dynamic processes that are involved in chitin synthesis are focused on the membrane-bound polymerizing enzyme, chitin synthase (CS). Such processes are involved in the formation of CS, its trafficking and integration into the plasma membrane at proper locations, catalysis and translocation of nascent chitin polymers across the plasma membrane barrier. The catalytic site of CS is inhibited by metabolic products (nucleoside peptides) of certain actinomycetes species which structurally resemble the enzyme substrate. The mechanism of chitin synthesis disruption by the synthetic acylurea compounds is poorly understood. The involvement of postcatalyic events, such as the translocation of chitin polymers across the plasma membrane, has been suggested. The recent isolation and sequencing of the insect CS genes may provide a breakthrough for understanding the exact biochemical lesion inflicted by acylureas. The deduced amino acid sequences indicate a remarkably large number of transmembrane segments (15–18). These extra transmembrane segments might be associated with translocation of polymers and with the disruption of such process by acylurea inhibitors.

Novaluron, a Novel Insect Growth Regulator: Its Biological Activity and Importance in IPM Programs

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Novaluron (Rimon EC-10), 1-[3-chloro-4(1,1,2-trifluoro-2-trifluoro-methoxy- ethoxy)phenyl]-3-(2,6-difluorobenzoyl)urea, is a novel benzoylphenyl urea that acts by both ingestion and contact. As such it is a powerful suppressor of lepidopteran, whitefly and leafminer pests. The compound is in the process of registration and commercialization worldwide by Makhteshim (Be'er Sheva, Israel) for the control of various agricultural pests. Our studies indicated that the LC_{50} value of Rimon on 3rd-instar *Spodoptera littoralis* fed on treated castor bean leaves is $\sim$0.1 mg a.i. l^{-1}. This value resembles that of Atabron (chlorfluazuron) and is approx. tenfold lower than that of Nomolt (teflubenzuron). An application of 25 g a.i. ha^{-1} in a cotton field resulted in 100% mortality of both *S. littoralis* and *Helicoverpa armigera* larvae upon their exposure to treated leaves up to day 8 after application, and $\sim$60% and 30% mortality, respectively, at day 15. Novaluron affects larvae of *Bemisia tabaci* to a much greater extent than does either chlorfluazuron or teflubenzuron. Two applications of 12.5 g a.i. ha^{-1} (125 g ha^{-1}) of Rimon in a cotton field resulted in total suppression of the *B. tabaci* population. Artificial rain at a rate of 40 mm h^{-1} applied 24 h after treatment of a cotton field, had no appreciable effect on the potency of Rimon on *S. littoralis* larvae. Hence, Rimon is considered a rain-fast compound and can be used in tropical areas and during a rainy season. Rimon has similar potency on susceptible and pyriproxyfen- and buprofezin-resistant *B. tabaci* strains, indicating that no cross-resistance occurs between Rimon and other leading compounds for controlling whiteflies. Rimon has no effect on parasitoids and phytoseiid mites and is considered to have a relatively mild effect on other natural enemies. As such, Rimon is an important component for use in integrated pest management and insecticide resistance management programs for controlling pests in field crops, vegetables and ornamentals.

Activated Fate in Organophosphorus Insecticides: Oxidative Activation of Non-AntiAChE Oxons and Their Glutathione Conjugates

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Organophosphorus (OP) compounds are one of the largest groups of insecticides used in the world. They are neutral esters, thioesters or amide derivatives of phosphorus acids or thiophosphorus acids. Most OP insecticides are converted into their oxon in insects or animals by microsomal mixed-function oxidases (MFO) to inhibit their acetylcholinesterase (AChE), and show an insecticidal activity. However, certain phosphorothiolates (RS-P(O)<) such as methamidophos, profenophos and prothiophos oxon are strongly insecticidal, but barely or never inhibit AchE *in vitro*. Their oxons are converted into the *S*-oxides by MFO, which either inhibit AChE or decompose, depending on the alkyl substituents on the sulfur atom. It is furthermore suggested in the case of the prothiophos oxon that its *S*-oxide not only inhibits AChE but also is conjugated with glutathione (GSH) by the action of glutathione *S*-transferase to inhibit AChE. Likewise, certain phosphoramidates (R_2N-P(O)<) such as isofenphos oxon, and schradan and propetamphos oxon are also weak *in vitro* AChE inhibitors, but strongly insecticidal. It is well known that isofenphos oxon is converted into the stable aminophosphate (H_2N-P(O)<) by oxidative dealkylation of the *N*-ethyl group to inhibit AChE. Schradan is oxidatively converted into nonactive *N*-desmethyl schradan *via* the metabolites of strong antiAChE activity. The unstable active intermediates are presumed to be the *N*-oxide (R_2N(O)-P(O)<) or *N*-methylol (R'CH(OH)RN-P(O)<) to inhibit AChE. The overall activation of phosphoramidates has been studied using 2,4-dichlorophenyl methyl *N*-alkylphosphoramidates as model compounds to elucidate the aminophosphate structure (R_2N-O-P(O)<) *via* *N*-oxide as one more activated form. This intermediate may lead to the GSH conjugate of antiAChE activity as well as the *S*-oxide intermediate of prothiophos oxon. The activation of chemicals by difference and combination of metabolic reaction, enzyme and isozyme may lead to different selective toxicity.

Organo-Clay Formulations of Herbicides for Reduced Leaching and Improved Efficacy

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New formulations of the widely used herbicides alachlor, metolachlor and acetochlor were designed and tested by adsorbing the herbicide to montmorillonite clay particles whose surfaces were modified from hydrophilic to hydrophobic by pre-adsorption of organic cations such as benzyltrimethyl ammonium (BTMA) and phenyltrimethyl ammonium (PTMA). Herbicide adsorption was measured by gas chromatography and its interactions with the organo-clay were studied by Fourier-Transform-Infra Red spectroscopy (FTIR). Adsorption of all herbicides on organo-clay complexes was significantly higher than that observed on untreated clay. Laboratory and field experiments demonstrated that herbicide leaching in soil is greatly reduced and weed control is improved without damage to crops. Slow release of the herbicide to the environment maintained the herbicidal activity in the topsoil, as determined by a bioassay using *Setaria viridis* and wheat. Organo-clay complexes pre-adsorbed with 0.5 mmole of BTMA or PTMA g^{-1} gave better formulations of alachlor than those pre-adsorbed with organic cation at the full cation exchange capacity (0.8 mmole g^{-1}). The environmental and agronomic implications of reduced leaching of these herbicides in the soil profile were discussed.

Plant-Specific Herbicidal Targets in the 21st Century

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Although the mechanisms of action of herbicides have not yet been fully explained, even for several of the commercially available materials, more than 60% of modern herbicides introduced during the last 40 years interfere with structures and functions of chloroplasts. Such herbicides, as well as those affecting auxin action or cell wall biosynthesis, should be plant-specific. Five important herbicidal targets in chloroplasts have been emphasized for both molecular design of herbicides and for genetic engineering to breed herbicide-resistant crops, namely: (i) photosynthetic electron transport system; (ii) photosynthetic membrane system; (iii) biosynthesis of photosynthetic pigments; (iv) ammonia metabolism and amino acid biosynthesis; and (v) lipid biosynthesis. Lately, many herbicides with biocidal side-effects are being phased out, because of lack of toxicological selectivity or due to environmental impacts. The high use rate of the herbicides often caused such side-effects. Even in herbicides targeting the plant-specific chloroplasts, some conventional ones require a high use rate (some kg/ha) to control weeds. Many herbicide scientists have concentrated their efforts on the molecular design of the low-use-rate of herbicides targeting chloroplasts and production of the herbicide-resistant crops, considering the mechanisms of action obtained from accurate biological and biochemical principles. Thus, we have found the most feasible two inhibitor-types targeting the chloroplasts: herbicides interfering with acetolactate synthase and the peroxidizing herbicides inhibiting protoporphyrinogen-IX oxidase. Both types of herbicides can control weeds by a low use rate (a few g/ha). Herbicide scientists have already started to search for additional plant-specific and biorational herbicidal targets to establish better weed control in the 21st Century. They are more fortunate than the older scientists, because they can seek any herbicidal targets, as long as those targets are plant-specific, asking their genetic engineers for selectivity (between crops and weeds) of inhibitors designed. Several possible herbicidal targets in plant cells were discussed.

Fatty Acids as Contact Herbicides

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It is well known that certain aliphatic fatty acids possess promising insecticidal properties. In addition, some aliphatic fatty acids, such as pelargonic acid, possess strong herbicidal properties. They are classic 'natural' pesticides, soap pesticides, but they are of great value as environmentally safe pesticides. The notable properties of fatty acid herbicides include: (i) contact-type efficacy, (ii) immediate effect, (iii) non-selectivity for weeds, and (iv) high biodegradability, *i.e.*, environmentally safe. They have a long history but little is known about their herbicidal mechanisms. We conducted research on the chemical changes in plant leaves following spraying with fatty acid herbicides. Using ion leakage tests, damage to cucumber cotyledon leaves was observed just after the treatment of fatty acids. In particular, C9 $\sim$ C11 fatty acids showed a strong effect. The amount of chlorophyll in the treated leaves also decreased and free fatty acids, which might be the degradation products of polar lipids, increased with time. Fatty acids such as pelargonic acid may kill plants primarily by membrane disruption.

The Parasitic Plant *Orobanche* in Israel: Agricultural Importance and Control

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Parasitic plants account for approximately 1% of extant angiosperm species and are represented in 22 plant families. Several of the parasitic species are important agricultural weeds, particularly in the closely related families Scrophulariaceae and Orobanchaceae. *Orobanche* species are obligate root holoparasites that germinate only in response to specific chemical germination stimulants exuded by host plant roots. The germinating parasite develops a haustorium that adheres to and penetrates the host root and ultimately establishes connections to the vascular system, depleting the host of nutrients, minerals and water. *Orobanche aegyptiaca, O. ramosa, O. crenata, O. cumana* and *O. cernua* are abundant in Israel, causing heavy losses in yield and quality to a wide spectrum of crops. These species parasitize vegetable, field, fodder, flower and spice crops from several botanical families (Fabaceae, Solanaceae, Compositae, Curcurbitaceae, Umbelliferae and Cruciferae). The *Orobanche* life cycle is highly specialized for parasitism and is extremely difficult to control, due to its underground and close association with host roots and complex mechanisms of seed dispersal, germination and longevity. Numerous cultural, mechanical, chemical and biological control measures have been attempted with limited success. Successful *Orobanche* management tools developed in Israel include soil solarization, novel selective herbicide application and resistant cultivars. *Arabidopsis thaliana* is widely used as an amenable model for the study of plant biology, including plant–pathogen interactions. Bringing *Arabidopsis* and *Orobanche* together in a controlled system enables the study of the molecular and genetic basis involved in host-parasitic plant interactions. We screened 13,000 *A. thaliana* M2 mutant plants and detected five M3 lines which had a reduced ability to induce germination. Low germination rates were found to be correlated with reduced distance from the roots at which germination occurred. While further studies are necessary to determine the segregation of low germination phenotypes, these lines might prove useful for detection of genes responsible for host root germination stimulants production.

Molecular Basis for Cross- and Multiple-Resistance to Acetolactate Synthase (ALS)-Inhibiting Herbicides and Atrazine in *Amaranthus blitoides* (Prostrate Pigweed)

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Two biotypes of *Amaranthus blitoides* (prostrate pigweed) that exhibit resistance to ALS-inhibiting herbicide (Su-R) and triazine (T-R) were found at sites (Ganot and Sorek) that have been treated for several years with sulfometuron + simazine. A sulfonylurea-sensitive and triazine-resistant (Su-S/T-R) population was collected in a corn field (Yavne). The sensitive (Su-S/T-S) population was collected from a nearby field (Kefar Shmuel) that has never been treated with herbicides. On a whole-plant basis (ED_{50}), the resistance ratio of Su-R/T-R and Su-S/T-S was 6 to 790 for sulfonylureas, 10 to 36 for imidazolinones, 8 for flumetsulam (triazolopyrimidines) and 18 for pyrithiobac-sodium (pyrimidinylthiobenzoates). *In vitro* studies have shown that the Su-R biotype was resistant at the enzyme level to all ALS inhibitors tested. The nucleotide sequences of two amplified regions of the *a/s* gene revealed two point mutations, one in domain A and the second in domain B, which conferred resistance RA and RB, respectively, denoting the different phenotypic symbols. The nucleotide sequences of domain A differed between the Su-S/T-S and the Su-RA/T-R in only one nucleotide 337 (GenBank Accession No. AAB67839.1, U55852); cytosine was substituted by thymine (<u>C</u>CT to <u>T</u>CT). This modification confers an exchange of the amino acid proline in Su-S/T-S and Su-S/T-R to serine in the Su-RA/T-R at position 188 of the ALS primary structure. In domain B one nucleotide substitution was observed at position 1721; guanine was substituted by thymine (T<u>G</u>G to T<u>T</u>G). This modification confers an exchange of the amino acid tryptophan in Su-S/T-S, Su-S/T-R and Su-RA/T-R to leucine in the Su-RB/T-R at position 569. Based on the whole-plant response, the resistance conferred in domain B to sulfometuron-methyl was higher than that conferred in domain A. Su-R/T-R and Su-RB/T-R are cross- and multiple-resistant biotypes that confer an additional single mutation in the chloroplastic *psb*A gene that changes serine to glycine at position 264 of the D1 protein. The biotype from Yavne was also atrazine resistant and had the same point mutation (Su-S/T-R). A mixture of three different mutations was found in the amino acid primary structure of the regions sequenced from the Ganot population; two mutations were responsible for the ALS-resistance and one for the PSII resistance. Comparing the sequences, the homozygote of each biotype was identified and revealed that some plants are heterozygotes that inherited two different mutant ALS-gene alleles. All of them were resistant to the ALS-inhibiting herbicides and the resistance seems to be due to a completely dominant, single nuclear gene mutation.

Herbicide-Resistant Weeds in Asian Paddy Fields

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Resistance to sulfonylureas developed in ten annual weeds and one perennial weed found in rice cultivation areas in Japan, in five in Korea, and three in Malaysia. Some resistant populations demonstrate high sulfonylurea resistance. Fifty percent lethal doses (LD_{50}) of some resistant biotypes were approximately 100 to 1000 times higher than those of susceptible biotypes. Crossing tests of *Monochoria korsakowii*, *Lindernia micrantha* and *Scirpus juncoides* suggested that a dominant nucleic gene controls the inheritance behavior of the sulfonylurea resistance. Genetic variations and gene mutation were investigated in the resistant weeds. The bee *Apis cerana japonica* is one of the main pollinators of *M. korsakowii*, and the female of *Lasiogloum secitulum* is the pollinator of *L. micrantha*. A control method was confirmed for sulfonylurea-resistant weeds in paddy fields in Japan. 2,4-D resistance in three tropical weeds was confirmed in Malaysia, Thailand and Philippines; a propanil-resistant *Echinochloa crus-galli* in Sri Lanka and Thailand; and a butachlor-resistant *E. crus-galli* in paddy fields in China.

PESTICIDES OF PLANT ORIGIN

Utilization of Natural Products for Development of Insect Control Agents

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Plants provide abundant sources of secondary metabolites possessing biological activities against insects. In recent years the most detailed studies of the effects of a natural product on insect behavior and physiology have been those conducted with azadirachtin from the neem tree, *Azadirachta indica.* Tropical countries have been shown to provide a rich source of plants containing various bioactive substances. The latter are the direct source of insect-regulating chemicals and are used as leading structures for the development of new insect control chemicals. Plant selection for screening was based on three criteria: (i) genera or family of plants which have been reported to be effective against insects, such as Annonaceae and Meliaceae; (ii) plants used in traditional medicine, such as Zingiberaceae; and (iii) random selection. Annonaceae have yielded many potent bioactive components with antifeedant and insecticidal activities against the diamondback moth *Plutella xylostella.* Squamocin and its related compound, acetogenin, were identified as insect-regulating compounds for *Annona glabra*, etc. The extracts of *Agraia harmsiana* and *Swietenia mahogani* (Meliaceae) showed very high antifeedant and mortality effects against the diamondback moth and the cabbage webworm *Crocidolomia binotalis.* Neem, with azadirachtin as its main active principle, is widely known for its insect-regulating activities. The active principle from *A. harmsiana* was identified as rocaglamide and its related compound. Plants of the Zingiberaceae are very well known for their medicinal properties. 1'- acetoxychavicol acetate was purified from *Alpinia galanga* as an insecticidal component. The efficacy of plant extracts was evaluated against larvae of the diamondback moth and the cabbage webworm, in cabbage cultivation in Indonesia. Treatment with *A. galanga* and *Gomphrena globosa* extracts at 0.5% against the diamondback moth significantly reduced larval density and the percentage of infested plants, but was not effective in reducing the cabbage webworm larval density or the intensity of cabbage damage.

Phyto-Chemicals for Controlling Insect Pests

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Methyl bromide and phosphine are the most widely used fumigants for controlling pest infestations in grain and dry food products, and quarantine insects in cut flowers. Some stored-product insects have developed resistance to phosphine and with the proposed phaseout of methyl bromide in the near future, there is an urgent need to search for suitable alternatives. In our laboratory, by screening a large number of essential oils extracted from local aromatic plants, we have succeeded to isolate two compounds which showed high potency similar to methyl bromide against the major stored-product insects. In addition, studies using these compounds against quarantine insects attacking cut flowers, such as the whitefly and thrips, showed effective mortality at low concentrations with no concomitant phytotoxic effects. The effect of these compounds on the enzyme acetylcholinesterase and the octopamine systems in insects was studied in order to elucidate their mode of action. Inhibition of acetylcholine-esterase activity *in vitro* was evident only at high concentrations, which indicates that this enzyme is not the main site of action for the essential oil. On the other hand, biologically active essential oils were found to cause a significant increase in the intracellular messenger, cyclic AMP of abdominal epidermal tissue. The effect was significant even at low, physiological concentrations. This intracellular response was found to resemble closely the significant increase in cyclic AMP of abdominal epidermal tissue due to treatment with the neurotransmitter/neuromodulator octopamine. Subsequent treatment with the octopaminergic antagonist phentolamine, effectively inhibited the cyclic AMP levels induced by the essential oil treatment, indicating possible competitive activation of octopaminergic receptors by the essential oil. Also volatiles extracted from oilseeds of crucifers were found to possess high activity against stored-product insects. These volatiles are much more potent than methyl bromide and the active essential oils. Their high activity makes them potential substitutes for methyl bromide.

Synthesis and Biological Activities of New Compounds Derived from *Alpinia speciosa* Components

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Alpinia speciosa is one of the Zingiberaceae species whose leaves emit a pleasant fragrance. The plant has been utilized traditionally in the packaging of rice cakes and for medicinal purposes for many years in Okinawa. Isothymol, thymol and eugenol in the essential oil of *A. speciosa* possess antifungal activity against plant pathogenic fungi. Since these active constituents are volatile in the natural environment, they have been changed into nonvolatile phosphorothionates by reaction with thiophosphoric agents using triethylamine to obtain more potent antifungal compounds. Many five- and six-membered cyclic phosphorus compounds were also prepared from these essential oils as exocyclic substituents. Cardamomin and alpinetin have been obtained from its seeds. Flavokawain B, dihydroflavokawain B, dihydro-5,6-dehydrokawain, and 5,6-dehydrokawain have been isolated from the rhizomes. Phosphorus derivatives synthesized from these natural compounds were examined for their insecticidal and antifungal activities. Dihydro-5,6-dehydrokawain, which is known to be non-toxic to mammals, had strong antifungal activity, indicating its possible use as a food antiseptic. Pyranyl-substituted cinnamates prepared from kawain analogs had an antioxidative activity.

Scale Insect Sex Pheromones in Israel

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Scale insects (Coccoidae) are important cosmopolitan pests of various crops. Pheromone-baited traps are useful for monitoring and may also contribute to their control. Three species were studied in Israel: the Israeli pine bast scale *Matsucoccus josephi*, the citrus mealybug *Planococcus citri* and the vine mealybug *P. ficus*. We identified and synthesized the racemic sex pheromone of *M. josephi*. Field tests confirmed the pheromonal activity to males and indicated strong kairomonal activity to the predator bug *Elatophilus hebraicus*. Pheromone traps were used to study the phenology of both insects. A series of analogs was prepared and tested. Results showed that either pheromonal or kairomonal activity can be induced by specific alteration in the structures. In addition, other *Matsucoccus* pheromones displayed kairomonal activity, attracting both predatory bugs and lacewings. The structure activity relationship of the *P. citri* pheromone has been studied and a convenient synthesis of the pheromone and analogs was developed. Field tests showed that some analogs display significant attractancy to males. However, no kairomonal activity was detected with the pheromone or with any of the analogs. The homolog analog may have practical importance because its synthesis is much more convenient than that of the pheromone. Pheromone traps were used to study the phenology of the insect in various parts of Israel. In an attempt to develop a monitoring system for *P. ficus*, independent research was conducted in California and Israel. The U.S. team identified an active pheromone component; we have identified an additional component. The attraction of *P. ficus* males to both components was demonstrated by bioassays in a petri dish arena as well as in flight assays in the mealybug rearing room. Indoors, both compounds displayed similar activity. However, trials in a vineyard indicated that the second compound was not active alone. Further tests are in progress in order to elucidate this phenomenon.

Mating Disruption of the Japanese Giant Looper in Tea Gardens Permeated with Synthetic Pheromone and Related Compounds

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In Japan, new pest management techniques without insecticides are a much desired goal, particularly for defoliators in tea gardens. In 1983, a product named Hamaki-con, a polyethylene tube containing (*Z*)-11-tetradecenyl acetate, was registered. This compound is a common component of the sex pheromones of both species. 'Hamaki-con" has been used to keep these species at a low population level mainly in the Shizuoka, Mie, and Kumamoto prefectures in Japan. The Japanese giant looper (mugwort looper, *Ascotis selenaria cretacea* Butler, Geometridae: Ennominae) is another lepidopterous species which seriously damages tea leaves. The females produce racemic (*Z,Z*)-6,9-*cis*-3,4-epoxynona-decadiene (epo3,*Z*6,*Z*9-19:H, main component) and (*Z,Z,Z*)-3,6,9-nonadecatriene (*Z*3,*Z*6,*Z*9-19:H, minor component). The orientation of the males to the synthetic pheromone placed in a trap was strongly disrupted by *Z*3,*Z*,*Z*9-19:H or a mixture of its monoepoxy derivatives (epoxydiene mixture, EDM) impregnated in septa and placed around the trap. Based on this result, polyethylene tubes containing *Z*3,*Z*6,*Z*9-19:H or EDM were prepared and the effect of these dispensers was examined in the field. Disruption of male orientation to synthetic pheromone traps was achieved in orchards permeated with *Z*3,*Z*6,*Z*9-19:H at a dispenser density of 3000 and 5000 tubes ha^{-1} (release rate: 0.55–0.61 mg day^{-1} $tube^{-1}$) and with EDM at every tested dose, 250–5000 tubes ha^{-1} (release rate: 0.25–0.39 mg day^{-1} $tube^{-1}$). Furthermore, disruption of mating in tethered females was observed in these orchards; mating was completely inhibited in the areas treated with EDM at 3000 and 5000 tubes ha^{-1}. This is the first formulation for the mating disruption of a geometrid pest.

Three-Dimensional Common-Feature Hypotheses of Novel Inhibitors of Calling and *in vitro* [^{14}C]Acetate Incorporation by Pheromone Glands of *Plodia interpunctella*

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Reproductive behavior in moths is dependent on chemical communication. In most species of moths, females produce and release an attractive blend of pheromone to which conspecific males orientate. Production of the pheromone blend is under the regulation of a neuropeptide termed pheromone biosynthesis activating neuropeptide (PBAN). The direct action of PBAN has been demonstrated by studies *in vitro* showing stimulation of pheromone production in the presence of synthetic PBAN by isolated pheromone-gland tissue. Biogenic amines, such as octopamine (OA), play a key role as neurotransmitters, neurohormones and neuromodulators in invertebrate systems with a physiological role analogous to adrenaline in vertebrates. In *Helicoverpa armigera*, it has been shown that OA and OA agonists significantly inhibit the pheromonotropic action due to PBAN in intact and decapitated moths as well as pheromone-gland incubations *in vitro*. The pheromonostatic receptor, playing a neuromodulatory role, represents a novel type of OA receptor. Some OA agonists were found to suppress *in vitro* biosynthesis of the calling pheromone of the Indian meal moth, *Plodia interpunctella*. Isolated pheromone-gland preparations incorporated [^{14}C]sodium acetate at a linear rate for 3 h when incubated with the PBAN. This incorporation was dependent on the dose of PBAN (up to 0.5 μM). Thin layer chromatography of a pheromone-gland extract revealed quantitative incorporation of radioactivity into a product exhibiting the same mobility as (*Z*,*E*)-9,12-tetradecadienyl acetate, the main component of the calling pheromone of *P. interpunctella*. OA agonists were initially screened using a calling behavior bioassay of female Indian meal moths. These compounds also showed *in vitro* inhibitory activity in intracellular *de novo* pheromone biosynthesis. The results of the present study indicate that these derivatives could provide useful information in the characterization and differentiation of OA receptor types and subtypes. Three-dimensional pharmacophore hypotheses were built from a set of 10 OA agonist 2-(Arylimino)imidazolidines (AIIs), 2-(Arylimino)thiazolidines (AITs) and 2-(Arylimino)oxazolidines (AIOs), etc. The active OA agonist agreed well with all the features of our hypothesis. On the other hand, less active compounds had difficulty in achieving the energetically favorable conformation which is found in the active molecules in order to fit the 3D common-feature pharmacophore models.

Towards Long-Term Use of Cry Proteins in Agriculture: Understanding Interactions of Separate Toxin Domains with Larval Gut Membrane Receptors

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The increasing use of insect-resistant transgenic crop plants expressing a variety of Cry proteins imposes a strong selective pressure towards the development of insect populations resistant to λ-endotoxins. One way to reduce this possibility is to include in the field a certain percentage of insect-sensitive (wild type) plants as refugia. Another way is to use other Cry proteins, which bind to different gut epithelium receptors. Thus, understanding the interactions of the three structural domains of the toxic part of Cry1 proteins may indicate which combinations of receptor recognition sites with the gut receptors may be developed for increasing toxicity, to expand the host range and serve as alternatives for λ-endotoxins used in transgenic plants to counteract the development of resistant insect populations. Many studies related to interactions of Cry1 proteins with gut membrane receptors are based on using mutated full length toxic parts resulting from domain swamping, or site-directed mutagenesis. In the present study we have tried to analyze net interactions of separate Cry1C structural domains with the gut epithelial membrane independent of neighboring structural effects of the two other domains.

Based on the common three-dimensional structure of Cry1 proteins, single structural domains and combinations of two consecutive domains of the toxic part of Cry1C were expressed in an *Escherichia coli* over-expression system. FPLC purified domain II as well as the 65 kDa toxic part were examined in BBMV binding assays. The Kcom value of domain II was 20-fold higher than that of the 65 kDa protein, indicating that the presence of the other structural domains is required for the high affinity interaction with the gut membrane. Indeed, when the BBMV interaction capacity with other domains was estimated, all three of the separate domains, as well as combinations of two adjacent domains, revealed significant interaction with the membrane vesicles.

The ability to express separate Cry1C structural domains as independent entities was used further to evaluate whether or not domains II and III interact with the same receptor and with identical or separate receptor sites. Competitive binding assays of the two domains indicated that domain II competes efficiently with domain III on binding to the BBMV. Whenever domain II was added, the overall binding of domain III was considerably reduced, suggesting a common interaction of the two domains with the same single site. This putative site is independently recognized by both domains, but reveals stronger affinity towards domain II. The reduced binding of domain III in the presence of domain II indicates indirectly that the primary interaction that occurs in the full length toxin (65 kDa) between domain III and the membrane receptor is probably dependent on a 3D native folding structure that exposes domain III first, while the interaction of domain II with the receptor requires a further change in the folding of the bound Cry1 protein.

Insect Pest Control Using the Sterile Insect Technique in Japan

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The Sterile Insect Technique (SIT) is one of the insect pest control methods developed in 1952 by Dr. Knipling for eradication of the screwworm in the USA. Since then, the SIT has been used throughout the world for control of fruit fly pests. In Japan, SIT was adopted as an eradication control method for the melon fly *Bactrocera cucurbitae* which invaded the Southwestern islands, and for the Oriental fruit fly *B. dorsalis* which invaded the Ogasawara islands; their eradication was accomplished a few years ago. On the other hand, the occurrence of the sweetpotato weevil *Cylas formicarius*, and the West Indian sweetpotato weevil *Euscepes postfasciatus*, was recorded in the last century on the Southwestern islands. They infested the storage root of sweetpotato severely and their distribution was expanding northward. Since they are also important plant quarantine pests, we have started a control project aiming at their eradication using the SIT. There has been no attempt to control pest weevils using SIT. Therefore, the success of the new project would mean that SIT will be applicable for control of other taxonomic insect pests. So far, SIT has been recognized as a control method only for special, economically important pests, because it is more expensive than other methods, such as insecticide application. However, as Dr. Salema said in his opening address at the International Conference on Area-Wide Control of Insect Pests held in Malaysia in 1998, “SIT is the most environment-friendly method of pest management . . . SIT is economically competitive or even superior to conventional methods when considered over longer time frames. . . .” To examine the feasibility of area-wide control using SIT for the sweetpotato weevils, we implemented the project on Kume Island over an area of *ca* 6,000 ha. The outline of the fruit flies projects and a report of our sweetpotato weevils project on Kume Island were presented.

New Trends in Chemical Structure and Mechanisms of Action of Fungicides

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In view of the rapid development and commercialization of genetically modified herbicide-tolerant and insect-resistant crops, the more classical domain of chemical fungicides prevails as the most dynamic agrochemical sector, with numerous new products and positive expectations for future growth. The relative market significance of fungicides will most likely increase in coming years also due to the rapid intensification of agriculture in developing countries. Three major current trends in the disease control arena were analyzed:
(i) The unprecedented rapid evolution of the strobilurin family as the dominant top-selling broad-spectrum fungicide group, with azoxystrobin exceeding $0.5 billion in global annual sales. This was accompanied by a corresponding shrinkage of the older triazole products that had controlled the market for 20 years. The competition in the strobilurin area generated in the last couple of years at least ten new commercially active compounds and scores of in-the-pipe-line products, all based on the rudimentary pharmacophores responsible for the unique mode of action: methoxyimino or methoxyetheno phenylacetic methyl esters or methylamides.
(ii) Concurrently, all major ag-chem companies are vigorously seeking new non-strobilurin fungicides with novel modes of action. Interestingly, practically all the recently announced active compounds embody the function of carboxamide or sulfonamide. Some of these amide-derived molecules (such as Du Pont's Famoxadone and Cyazofamide by Ishihara) are potent inhibitors of mitochondrial electron transport, blocking (analogously to strobilurins) the function of the ubiquinol: cytochrome-c-oxireductase complex. Others, *e.g.* Carpropamid of Nihon-Bayer and Fenoxanil by BASF, are classified as systemic melanin biosynthesis inhibitors. Other contemporary molecules are Fenamidone (Aventis), Fenhexamide and Iprovalicarb (Bayer), Ethaboxam (LG), Benthiavalicarb (Kumiai) and Silthiofam (Monsanto).
(iii) Emerging comprehension of natural plant defense strategies and mechanisms gave way to artificial induction of disease resistance in crops. The concept of systemic acquired resistance is now well established. Chemical activators such as acibenzolar-S-methyl and biological inducers such as the polypeptide Harpin are already available on the market. These compounds have no fungicidal activity *in vitro*, but when applied to a plant they initiate complex signaling pathways activating the plant's own defense system. Interestingly, enhanced tolerance to heat, chilling and drought stresses combined with increased nutrient uptake and faster growth are also observed.

Molecular Diagnostic Procedures for Production of Pathogen-Free Propagation Material

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Production of disease-free propagation material is considered a major means of controlling most bacterial diseases, particularly when neither resistant clones nor effective chemical treatment are available. For this purpose sensitive, specific, rapid and reliable detection methods are required. The advent of molecular biology and particularly the polymerase chain reaction (PCR) opened new ways for characterization and identification of plant pathogens and for devising disease management strategies. PCR-based detection methods are relied upon for the development of primers for the specific detection of the pathogen. The use of pathogenicity genes as targets for primers design is the preferred procedure for obtaining specific primers. However, other procedures may also be used for this purpose. The advantages and disadvantages of the available diagnostic methods were discussed by presenting several examples. In the case of *Erwinia herbicola* pv. *gypsophilae* causing gall forming in gypsophila plants, studies of the molecular basis of pathogenicity enabled the development of a specific PCR procedure based on the sequence of the cytokinin biosynthesis genes. In geranium, production of nuclear stock of mother plants free of *Xanthomonas campestris* pv. *pelargonii* is based on immunofluorescence combined with isolation on a plating medium. Since in this procedure false positives often occur, a specific PCR-based procedure for detection of the pathogen in geranium plants was developed. Detection of *Agrobacterium tumefaciens* in aster and rose is being carried out by plating on a semi-selective medium and confirming positive results by PCR. The use of this procedure for production of clean mother plants of the rootstock *Rosa indica* is currently being evaluated. In cabbage seeds, PCR-based protocols for detecting *Xanthomonas campestris* subsp. *campestris* and *Erwinia carotovora* subsp. *carotovora* have also been developed.

Development of Milbemectin as a Trunk Injection Agent against Pine Wilt Disease Caused by the Pine Wood Nematode *Bursaphelenchus xylophilus*

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A trunk injection of nematicidal compounds to pine trees has been recognized to be a practical and effective method to control pine wilt disease. The pine wood nematode, *Bursaphelenchus xylophilus* (Steiner et Buhrer) Nickle, is the causal agent of this disease of Japanese black and red pines. This nematode is transmitted to healthy pine trees by the Japanese pine sawyer, *Monochamus alternatus* Hope, during their maturation feeding on twigs. From the twigs the nematodes are known to spread down throughout the trunk of the pine tree within a couple of days, and to induce cavitation – which causes water deficit throughout the trunk and finally leads to death of the tree. Milbemectin, a mixture of milbemycin A_3 and milbemycin A_4, exerts potent anti-nematodal activity against the pine wood nematode. When it was injected into the trunk of a pine tree, it was found to be widely distributed inside the trunk and branches of the tree, and killed the nematodes before they caused fatal wilt of the tree. The site of transmission of the nematodes by the Japanese pine sawyer is a new twig of this year's growth. Therefore, milbemectin must be distributed not only throughout the inside of the trunk but also to the twigs, in order to control the nematode effectively.

Production of Anti-virus, Viroid Plants by Genetic Manipulations

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Most pathogenic plant viruses are RNA viruses, which make double-stranded RNA (dsRNA) forms when they replicate in their host plant cells. Introduction of dsRNA-specific ribonuclease such as *Shizosaccharomyces pombe*-derived pac I protein and animal cell-derived interferon-induced 2',5'-oligoadenylate synthetase (2-5Aase)/ribonuclease L (RNase L) system into diverse plants may make the plants resistant to various pathogenic viruses and viroids. It was demonstrated that the pac I or the 2-5Aase/RNase L transgenic tobacco plants are resistant to various viruses including tobacco mosaic virus (TMV), cucumber mosaic virus (CMV) and potato virus Y (PVY), and also that the pac I transgenic potato plants are resistant to potato spindle tuber viroid (PSVd). We established the transformation system of chrysanthemum plants, and recently developed the pac I transgenic chrysanthemum (*Dendranthemum grandiflora* cv. Reagan) resistant to chrysanthemum stunt viroid (CSVd).

Production of Patchouli Mild Mosaic Virus-Resistant Patchouli Plants by Genetic Engineering of Coat Protein Precursor Gene

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Patchouli (*Pogostemon cablin* Benth.) is an aromatic crop which yields an essential oil and is widely cultivated in South-East Asia. Patchouli mild mosaic virus (PaMMV) infects patchouli plants and causes a decrease in leaf biomass and essential oil yield. We succeeded in producing transgenic patchouli plants with PaMMV coat protein precursor (CP-P) gene by *Agrobacterium*-mediated transformation. PaMMV CP-P gene integration into the patchouli genome was confirmed by PCR and Southern blot analysis. The copy number of integrated genes in the transformants was estimated to be one to three by Southern blot analysis. The transformant with three copy genes was tested for resistance to PaMMV by artificial inoculation in an environmentally controlled cabinet, and this transformant was found to be highly resistant to PaMMV. The transgenic patchouli plant with PaMMV CP-P gene should provide valuable material for protection against PaMMV.

Transgenic Plants Tolerant to a Pathogenic Toxin Confer Enhanced Resistance to Plant Diseases

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A simple and useful strategy for molecular breeding of disease-resistant plants involves introducing a toxin-tolerant gene into plants, with typical examples being tobacco wildfire and kiwifruit canker diseases. Wildfire disease of tobacco plants is caused by *Pseudomonas syringae* pv. *tabaci* that produces tabtoxin, a potent inhibitor of glutamine synthetase. A tabtoxin resistance (ttr) gene encoding acetylation of the toxin was cloned from the pathogen itself and introduced into tobacco plants by *Agrobacterium*-mediated transformation. The transgenic tobacco harboring the gene became resistant not only to tabtoxin but also to wildfire disease. This was the first successful case of the transgenic plants evincing bacterial diseases using a genetic engineering technique, although numerous virus-resistant transgenic plants have been created. Another example is canker disease of kiwifruit plants caused by *P. syringae* pv. *actinidiae*, which produces the pathogenic toxin phaseolotoxin. This toxin inhibits ornithine carbamoyl transferase (OCTase) related to arginine biosynthesis in the chloroplasts of plant cells. The cloned phaseolotoxin-insensitive OCTase gene (*arg*K) from *P. syringae* pv. *phaseolicola* was introduced into kiwifruit plants by *Agrobacterium*-mediated transformation, in which the *arg*K gene was fused to the transit peptide gene of Rubisco SS from tobacco plants. An assay of disease resistance by spray inoculation of *P. syringae* pv. *actinidiae* showed that the transgenic kiwifruit plants had enhanced resistance to canker disease and suppressed bacterial propagation. Such progress will also contribute to a basic understanding of plant diseases on the relationship between symptom development and pathogenic toxins, and on the interaction between pathogens and plants.

Pesticide Degradation and Biochemical Activity of Solar Photocatalysis

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Titanium dioxide photocatalysis has been the focus of much water-purification research, which revealed its potential in degradation of hazardous organic substances, and recently also photocatalytically inactivated microbial cells have been reported. Both of these aspects were discussed in the present work, which includes results on the dye-enhancement of the TiO_2 photocatalytic process and on the photocatalytic degradation of proteins. Dye-photoenhancement of the TiO_2 photocatalytic process has been studied in herbicides of the bromouracil and triazine groups. Acceleration of the photocatalytic oxidation of bromacil and bromouracil was observed by a process of dye sensitization. Both UV and visible light were required for the enhanced decomposition. The mechanism of the reaction seems to involve a combination of oxidation by hydroxyl radicals, *via* the hole-electron semiconductor route, with oxidation by singlet oxygen of photo-oxidation intermediates of the OH process. In parallel, the photocatalytic degradation of proteins has been evaluated. The TiO_2 photocatalyzed oxidation of examined model proteins (albumin, ovalbumin and gamma globulin) shows the susceptibility of the protein to photocleavage and of the amino acids to photocatalytic degradation. Tyrosine was the most sensitive to photocatalytic degradation, while the degradation of the aliphatic amino acids glycine and asparagine was slow.

Photostabilization of Biocontrol Agents

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Biocontrol agents offer toxicologically safe and environmentally acceptable alternatives to synthetic pesticides. Invariably, the efficacy of biocontrol measures has been compromised by poor persistence and inadequate field performance. Photosensitivity to solar irradiation has been recognized as the major environmental factor responsible for such disappointing field performance. Clay surfaces were used for adsorption of cationic photoprotectants such as acriflavine, berberine and palmatine. Biocontrol agents like microbials (*Bacillus thuringiensis)* and entomopathogenic viruses and fungi were greatly photostabilized by clay–dye complexes. As the toxicological safety of the cationic dyes is uncertain, other potential photostabilizers, such as the negatively charged Naphthol Yellow S and Fast Green, were selected. In order to bind these anionic compounds successfully, the surface charge of clays such as montmorillonite, attapulgite, kaolinite and bentonite was reversed by adsorption to the polycation chitosan. The potency of the clay–chitosan–dye matrices to photostabilize the entomopathogenic fungus *Aschersonia* sp. was demonstrated.

INTEGRATED PEST MANAGEMENT

The Perspective of Ecological Weed Control

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Ecological weed control methods can be considered from the following three viewpoints: The first way is to create unfavorable conditions for weed plants, which enhance the competitive ability of target plants (crops) against the weed plants by modifying the seeding time, amount of sowing seed, management of water and fertilizer, and so on. As a result, unfavorable conditions for weed plants are created. The second method is to introduce companion crops, such as a cover crop and live mulch, which effectively cover the bare space on the ground surface and suppress the emergence of weed plants. Some of the companion crops may result in allelopathic depression. The third method is to suppress the formation and dispersion of the weed plant's reproductive organs – such as seeds, rhizomes, tubers, bulbs – by means of crop rotation and alteration of cropping sequence. This method also shuts out the troublesome weeds invading from outside. Weed plants are uninvited guests in the rural ecosystem, which are continuously spread by human intervention. In my opinion, the aim of weed control is not to suppress weed growth, but to provide an obstruction to the living space for weeds.

Nonfungicidal Strategies to Control Soilborne Plant Diseases

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Facing the possible explosion of the earth's population, maintenance of sustainable agro-production should be one of the most important aims of modern science. Diseases caused and developed by soilborne pathogens have been great obstacles to achieving that aim. Recently, environmental risks of applying biocidal soil-fumigants such as methyl bromide to control soilborne diseases have been targeted, and construction of integrated management systems which will be more in harmony with the environment is demanded. Non-fungicidal chemicals are expected to be less likely to lead to development of fungicide-resistant pathogens and to have less impact on the environment. Two examples of non-fungicidal chemicals capable of controlling soilborne diseases, and their possible mechanisms, are: (i) Epoxydon, with anti-auxinic activity and which reduces symptom development of clubroot disease (*Plasmodiophora brassicae*) on *Brassica* spp.; and (ii) Validamycin A, which induces resistance to Fusarium wilt disease (*Fusarium oxysporum* f.sp. *lycopersici*) by foliar spray on tomato.

Insecticide Resistance Management Programs: Their Importance in Controlling Insect Pests

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The Israeli insecticide resistance management (IRM) strategy, introduced in cotton in 1987 as part of an integrated pest management (IPM) program, is focused primarily on controlling cotton pests with novel insecticides along with alternative control measures such as sex disruption and natural enemy encouragement. Each insecticide is used once during a crop season, followed if required by another insecticide with a different mode of action. There has been extensive resistance monitoring throughout this period to detect any resistance arising in key cotton pests despite these IRM measures. The rational use of insecticides has led to substantial reductions in insecticide applications, and in general has maintained the susceptibility of the pests to the various groups of insecticides. However, after 13 years of the IPM–IRM strategy, some changes in resistance to conventional and novel insecticides have been observed. Modifications to the strategy, which include the use of BT-transgenic cotton, are being considered. At present, similar strategies are being developed and implemented area-wide for various crops in Israel. The history of resistance development to pyriproxyfen by the whitefly *Bemisia tabaci* (Gennadius) is a case in point. This provides a striking example of the genetic, ecological and operational factors that combine to promote resistance, even within a well-defined IRM strategy. Pyriproxyfen, a juvenile hormone mimic, normally shows outstanding effectiveness against *B. tabaci* field populations. The dynamics of pyriproxyfen resistance in *B. tabaci* have been studied most intensively in cotton fields and greenhouses in Israel. High resistance to pyriproxyfen evolved one year after its introduction in a greenhouse floriculture, after successive applications. After 9 years of pyriproxyfen use in cotton fields with only one application per season, a moderate to high level of resistance was observed in some localities. As a consequence, pyriproxyfen use in high-resistance localities has ceased, but in areas with low frequencies of resistance, cotton growers have continued to achieve reasonable control of *B. tabaci* with pyriproxyfen. The absence of pyriproxyfen applications in some cotton fields, led to lowered resistance levels in 1998–2000.

Enhancing Biological Control with Selective Pesticides: An Experimental and Practical Approach to Pest Management

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In most areas of the world, the identity of important natural enemies of major pests of vegetable crops is often unknown and thus the effects of pesticides on these natural enemies remain undetermined. In such areas, however, it is possible to determine the effects of pesticides on major pests and their natural enemies, and to find selective pesticides which enhance biological control. This can be done by recording seasonal changes in the abundance of all pests and their natural enemies in small field plots sprayed, repeatedly, with all available pesticides. We have developed this experimental and practical approach to pest management for eggplant and broccoli and obtained successful results in field experiments. On the basis of these results we suggested that enhancing biological control with the use of selective pesticides is a viable approach for vegetable pest management.

Need for IPM Paradigm Shift in Asian Agriculture

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The IPM philosophy has been the dominant paradigm in crop protection since it was established in the 1960s with a background of industrialized North American agriculture, although it has been diversified in the process of its promotion to multiform socioeconomic circumstances. Worldwide popularity of IPM has been attained because it is considered ideal for both reducing environmental risk and maximizing farmer’s profit. Nevertheless, the delay in IPM implementation has long been the source of dispute. The original concept of IPM, often referred to as strategic IPM, is to aim at maintaining the pest population below an acceptable level based upon thorough and extensive understanding of agro-ecosystems. To this end, systems approach has been considered fundamental. In practice, however, the most widely recommended and employed IPM technology has been to limit pesticide application only when the pest population density reaches a certain level. For this tactic IPM, the determination of economic injury level and corresponding action threshold, combined with monitoring of pest occurrence, plays a key role. I discussed the reason why neither form of IPM is difficult for Asian farmers with a small-size farm to adopt under economic globalization. On these bases I also proposed what I consider essential for appropriate IPM technology development in monsoon Asia.