MEETING

ABSTRACTS OF PRESENTATIONS ON PLANT PROTECTION ISSUES AT

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A: PEST CONTROL

An Analysis of the Pest Management Situation in Mango Agroecosystems

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Mango, like most fruit tree crops, is usually attacked by two or three pests, several secondary pests, and a large number of occasional pests in localized areas where it is grown. Of approximately 260 species of insects and mites that have been recorded as minor and major pests of mango, 87 are fruit feeders, 127 are foliage feeders, 36 feed on the inflorescence, 33 inhabit buds, and 25 feed on branches and trunk. Attempts to develop integrated pest management programs for mango must be based on sampling, economic thresholds, and take into account the effects of cultural control practices, horticultural sprays and disease control on pest and natural enemy interactions.

In general, mango pest management is largely dependent on the use of pesticides. Pesticides that are used in integrated pest management programs must have selective toxicity. The current trend is the development of chemicals that are highly effective for a limited group of insects. For example, cyromazine has been suggested as a means to reduce fertility of *Anastrepha obliqua*, and oils for control of scales in mango; however, most of the recommendations from other countries are based on highly toxic or illegal, nonregistered persistent chemicals. Control of mango fruit pests by chemicals alone has been complicated by development of insect resistance and resurgence and elevation of minor pests to major pest status.

Biological control has great potential as a tactic for regulating pest populations in integrated pest management programs in mango orchards; however, it will be difficult for biological control alone to

reduce a pest attacking fruit from an economic to a completely noneconomic status. A combination of augmentative releases of parasitoids and the use of sterile insects, has been considered to be more effective for fruit flies than either method applied alone, at least theoretically. Biological control should be highly effective for indirect pests, and numerous studies have been conducted in many mango-producing countries to promote the use of parasites and predators for this type of pest.

Tolerance of mango to pests is mentioned for *Saissetia mangiferae, Noorda* sp. and *Idioscopus* sp., resistance to *Milviscutulus mangiferae* has been recorded, and also different degrees of susceptibility of mango cultivars to *A. obliqua.* Most of the research, however, needs to be assessed further. *Mangifera altissima* does not seem to be affected by mango pests, *i.e.*, leafhoppers, tip borers and seed borers, in the Philippines. There is little doubt that wild mangoes have potential value in breeding.

Recent developments in the identification and synthesis of sex pheromones have resulted in their possible use for pest management in mango orchards. Food attractants, however, are the most common monitoring tools. Trapping techniques can be utilized to reduce pesticide use by improving timing of sprays as a result of better monitoring of pest populations. It remains uncertain if trapping techniques can be used to predict infestations by fruit feeding pests and if they can be used for direct control (by mass trapping) over several years.

The evolution of research concerning pest control in mango during the past few decades, from the unilateral use of pesticides to integrated control, has developed out of necessity. Greatly increased regulation of pesticides, heightened public awareness of environmental contamination, pesticide resistance problems in pests, and the high cost of chemical pest control have resulted in increasing reliance on integrated pest management control as an important strategy in sustainable agriculture. Integrated insect control programs on mango are unsatisfactory for many pests, due to inadequate sampling techniques (the inability to relate the results of sampling to levels of damage in many cases) and the absence of sound economic injury levels. *(L)**

The Present Status of Mango Pests in Israel

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Since the first mango tree was planted in Israel in 1929 and the first fruits were picked in 1935, several surveys of mango pests have been conducted. The number of pests has increased, but the number of economically important pests has remained low. The status of three economically significant pests: the mango shield scale, *Milviscutulus mangiferae* (Green); the mango bud mite, *Eriophyes mangiferae* (Sayed); and the mango thrips, *Scirtothrips mangiferae* (Priesner), is the same today as in 1993. The new developments in the pest situation are related to the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann); the oriental red scale, *Aonidiella orientalis* (Newstead); and the western flower thrips, *Frankliniella occidentalis* (Pergande).

Limitation of aerial sprays against *C. capitata*, large-scale experimental use of newly designed traps for monitoring and mass trapping, and additional use of mineral oils to control the oriental red scale, led to the restoration of the biological equilibrium regarding this pest and to the appearance of many natural enemies. Those activities were conducive as well to an increase of the population of natural enemies of the mango shield scale. *Compariella lemniscata* Compere and Annecke (Hymenoptera: Encyrtidae) – a parasitoid of *A. orientalis*, and two predatory coccinelid beetles – *Chilocorus circumdatus* Gyllenhil and *C. baileyii* (Blackburn), were introduced into Israel from Australia. These natural enemies are reared commercially in Australia for control of the oriental red scale on papaya. Their present status of acclimatization is not known and research is needed to evaluate the establishment of these natural enemies in Israel.

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* *L* = lecture sessions; *P* = poster (market place) sessions.

Integrated Management of *Bactrocera (Dacus) dorsalis* in Mangoes

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Studies conducted at Pantnagar to develop an integrated management schedule for control of the oriental fruit fly, *Bactrocera dorsalis* Hendel, indicate that harvesting of fruits at physiological maturity reduced damage to 4.6% as compared with 10% in fully ripe, dropped fruits of 'Dashehari' on 22 June, and from 8% to 4% in 'Bombay Green'. Four methyl eugenol (0.2%)-baited yellow traps/acre run for 18 weeks (2 April to 30 July) reduced damage by 83.3% in physiologically mature fruits of Dashehari and by 37.5% in fully ripe, dropped fruits. The highest trap catch of 233 male flies/week was between 18 June and 25 June. Adult emergence was zero when fully mature larvae were allowed to pupate at 30 cm soil depth, followed by 20%, 65%, 5%, 45% and 30% at 20, 10, 5, 1 and 0 cm depths, respectively, indicating the suitability of 30-cm depth for burying the damaged fruits. Mango juice 5% was the most effective bait in reducing the damage to fruits (36.6% in physiologically mature and 17% in dropped fruits). 0.002% Deltamethrin gave 83.3% reduction in damage of physiologically mature and 78.8% reduction in dropped fruits; this was at a par with 0.01% fenvalerate. *Biosteres dacussii* (Cameron) was found to be the most important parasite of *B. dorsalis*; the parasitization was, however, very low. *(P)*

Effect of Height of Traps in the Capture of Fruit Flies of *Anastrepha* and *Ceratitis* spp. in Mango Trees in Venezuela

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With the purpose of determining the optimal height for placement of traps on mango trees to capture the fruits flies *Anastrepha* spp. and *Ceratitis* sp., a test was conducted in a mango orchard located at Maracay, Aragua State. The trees used were divided into three imaginary heights: lower, middle and upper. A randomized block design was applied with six repetitions and three treatments for lower, middle and upper height. The trap used was the McPhail glass type. The lure used was Torula Yeast Borax (TYB), five pellets in 500 cc of water. The traps were checked weekly for 10 weeks, with renewal of the liquid lure every week. The data on the capture of flies were analyzed after being uniformized through the expression $\sqrt{X + 05}$; afterwards an analysis of variance was made. A significant difference between captures at the three positions was found. To determine which position was best, a Tukey test was conducted showing that the best one was the upper height of the tree. In order to confirm there results, an analysis of regression was made, indicating that at the upper height the capture of flies increased significantly. These results suggest that McPhail traps for the capture of fruit flies of the *Anastrepha* and *Ceratitis* spp. should be placed in the highest part of the trees. *(L)*

Field Evaluation of Various Wrapping Materials against the Mango Fruit Fly, *Bactrocera dorsalis*

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Four wrapping materials, namely nonwoven fabric; standard newsprint; sugar bag wrappers; and sandwich bags, were used as wrapping materials for the pullet-egg-size developing fruits of 'Manila Super Mango'.

Complete protection from insect pests was observed in the nonwoven-fabric-wrapped fruits in addition to its being reusable in the succeeding season. With standard newsprint there was some incidence of ant and mealybug penetration of the wrapper.

The nonwoven fabric bag proved to be the best wrapping material, followed by standard newsprint. The sugar bag wrapper and the sandwich bag ranked third and fourth, respectively. The unwrapped or control treatment showed the highest insect pest infestation and disease infection.

Considering all the attributes of the different bagging materials, the nonwoven fabric utilized in conjunction with presprayings of insecticides and fungicides was the best wrapping material. *(P)*

Resistance against Hoppers in Mango

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More than 100 varieties of mango were screened against hoppers, under both natural infestation and artificial release conditions. Nylon screen bags and polystyrene bags sleeved at both ends were used for releasing the hoppers on panicles. Observations were recorded on the hopper population inside the bags and hopper survival/development and multiplication, and fruit set were recorded. Observations were also taken on hopper population and fruit set outside the bags on panicles as well as on the trunk.

Results of the trials conducted during 1992/93 and 1993/94 indicated that very few varieties could tolerate hopper infestation. Varieties Pulhora, Amin, Kakaria Amin, Kala Hapus, Ramkela, Pau, Annanas, Kalank Goa and Kesher Basti were resistant to hoppers. Tolerant varieties included Dudha peda, Goff of Havasari, Padiri, Azwain, NX Humayuddin, Tamancha, Eruvadi Banglora, Malihabadi, Neelum, Mallika, Banglora, Panja Pasand, Langra Rampur, etc., whereas the varieties N x Chausa, Hapus, Himsagar, Rahiri, Panchadrakalsa, Eruvadi Romani, Kodurchinnarson, Gulab khas, Green, Vanraj, Kalapaddy, Mithua Malda, Sonakullu, Nilyora, Hathizhool, Kazalio, N x Panchadrakalsa, Cecil, Asadio, Baramalda, Ladavio, Rajapuri, Pathar, Naspati, Bijoragarh, Maharaja of Mysore, Popatpari, Fakira, Keshar, Chinnaswaranarekha, Vellakachi, Cherumani, Malgoa, Hazoor Pasand, etc., were susceptible. *(P)*

B: MANGO DISEASES

Mango Breeding for Resistance to Diseases and Pests

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The mango breeding program for resistance to disease and pests of the Instituto Agronômico de Campinas (IAC) has two main objectives: (i) To obtain polyembryonic rootstocks resistant to the fungus *Ceratocystis fimbriata*, which causes the mango wilt; the goal is to distribute at least ten resistant rootstocks in order to avoid genetic vulnerability. (ii) To obtain mono- or polyembryonic varieties for utilization as crown, with good qualities, mainly productivity and fruit taste, plus resistance to the mango wilt, anthracnose and fruit fly. The first cultivar derived from this program, named IAC 100 Bourbon, was distributed to farmers in December 1989. It is a resistant mutant of the popular table variety Bourbon, which had been almost eliminated by the mango wilt. Two rootstocks resistant to the mango wilt, IAC 101 Coquinho and IAC 102 Touro, were distributed in January 1994. Four new varieties, two crowns – IAC 103 Mococa and IAC 105 Campinas, and two rootstocks – IAC 104 Dura and IAC 106 Jasmim, are scheduled for distribution in January 1998.

Three other crown varieties, IAC 107 Tietê, IAC 108 Pindorama and IAC 109 Votuporanga, are being prepared for distribution. It has been relatively easy to obtain mutants with resistance to mango wilt from susceptible polyembryonic varieties. The distributed varieties are not resistant to all diseases and pests. The precocious table varieties (IAC 103 Mococa and IAC 107 Tietê) have sufficient natural resistance against fruit fly damage, but the late varieties (IAC 105 Campinas and IAC 109 Votuporanga) require control measures against this pest. This research was supported by FAPESP and CNPq. *(P)*

Mango Decline: Research in Florida on an Apparently Widespread Disease Complex

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Decline syndromes are recognized in virtually all mango-production regions. Symptoms are diverse and include all or some of the following: dieback, defoliation, gummosis, vascular discoloration, marginal chlorosis and necrosis of leaves, foliar nutritional deficiencies, and root degeneration. Fungi are the most commonly described agents, and in most situations a single species is indicated. Abiotic factors, such as poor host nutrition or water stress, are also reported to predispose the host.

In Florida, the etiology of mango decline is confused. Prior to the present study, fungi (*Botryosphaeria ribis* and *Diplodia* sp.), a nematode (*Hemicriconemoides mangiferae*), and nutritional deficiencies (especially of Mn and Fe) had been suggested to be causes. To clarify this issue, we addressed the following objectives: (i) identify fungi associated with declining trees in south Florida; (ii) determine the virulence and symptoms caused by the isolated fungi; and (iii) investigate the role played by host nutrition.

During survey work in 1994 and 1995, internal colonists recovered from symptomatic tissue included, alphabetically: *Alternaria alternata, Cladosporium* sp., *Colletotrichum gloeosporioides, Dothiorella dominicana, Fusarium* spp., *Lasiodiplodia theobromae, Penicillium* sp., *Pestalotiopsis* sp. and *Phomopsis* spp. The relative abundance of the isolated species varied by sample source and date. On 'Keitt', *A. alternata, C. gloeosporioides, D. dominicana, L. theobromae* and two species of *Phomopsis* caused all or some of the following decline symptoms: bud necrosis, tip dieback, gummosis and vascular discoloration. Thus, mango decline appears to be a disease complex in Florida involving several different fungi. Although plants inoculated with *D. dominicana* developed significantly greater vascular discoloration when they were deficient in Mn and Fe, other nutrition X pathogen interactions were not evident. *(L)*

Mango Malformation – Visualization of *Fusarium subglutinans* in Infected Flowers and Branches by GUS Transformants

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Although more than 100 years have elapsed since mango malformation was first described, the development of *Fusarium subglutinans* – the causal agent of the disease – within malformed tissues is still obscure. The reason for such lack of information is due partly to the dispute among researchers concerning the identity of the causal agent. The unusual nature of the disease, characterized by a unique disease syndrome, reflects drastic hormone-like changes, which contribute to the dispute. In order to trace and follow the fungus in mango tissues, the GUS (β-glucoronidase) reporter gene was used. Virulent wild-type isolates of *F. subglutinans* were transformed with a plasmid containing both GUS and the hygromycin reporter genes. The transformants did not lose their virulence and after

artificial inoculations typical disease symptoms developed. The fungus was viewed microscopically in flowers and in developing vegetative buds, cleared by chloral hydrate. The presence of the pathogen in different plant organs, and its preference for colonization around certain sites, were demonstrated and discussed. *(L)*

Mango Malformation in Egypt

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Mango 'malformation' has become a limiting factor in mango production. Three main symptoms of this phenomenon were recorded in Egypt: malformed and stunted growth of seedlings in the nursery stage, vegetative growth malformation and inflorescence malformation in the bearing trees. Fundamental changes in the morphological structure and sex ratio occur in the malformed panicles. This phenomenon has not been reported to be translocated by grafting.

The commercial mango cultivars were classified into three groups according to their susceptibility to this abnormal phenomenon: highly susceptible, moderately susceptible and nonsusceptible cultivars. However, the incidence of the phenomenon can differ from tree to tree of the same variety within an individual orchard. Zonal variation has been reported for individual varieties.

In the light of the previously mentioned facts about mango malformation, it could be suggested that other factors may be responsible for the disorder. It might be due to rootstocks used for mango grafting in other cultivars. However, there have not yet been any convincing reports on the effect of mango rootstocks on this phenomenon.

The various constituents, minerals and biochemical components such as carbohydrates, amino acids, phenolic compounds and nucleic acids of the malformed panicles, were shown to vary in comparative studies using malformed and healthy inflorescences and other mango tree tissues. However, these differences might be a consequence of malformation and not a direct cause of it.

Cultural practices like pruning, fertilization program, irrigation, spray with some micronutrients and growth regulators, may be minimizing factors of the mango malformation disorder. However, no cultural practices succeeded to prevent absolutely mango malformation. *(L)*

Infection Pathway of the Stem End Rot Fungus, *Dothiorella dominicana*, in 'Kensington' Mango

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Dothiorella dominicana (Dd) is one of several pathogens causing stem end rot of mangoes in Australia and other countries. *Dd* is also recovered after triple sterilization from symptomless seedlings grown from infected fruit, suggesting endophytism. This experiment aimed to trace the infection pathway of the pathogen from the inoculum placed onto the cut stem end of the fruit to the seedling. 'Kensington' mangoes obtained from the Northern Territory were inoculated with *Dd.* Fruits were surface-sterilized and isolations made from 18 points in the fruit and seed at 0, 1, 2, 4, 6, 8 and 11 days after inoculation. Uninoculated controls were assayed on days 0, 4, 8 and 11. The fungi grew down the vascular tissue into the peduncle (day 2) and pedicel (day 4), and under the skin (day 6). Colonization preceded lesion development, which began on day 8. The seed was infected first through the funiculus (day 6) and seed coats (day 8) and then into the seed (day 8-11),

where it caused decay. *Dd* was not detected in the control fruit. Seedlings grown from inoculated fruit showed highest recovery of *Dd* in the connective tissue between the seed and seedling and the hypocotyl zone. These results have implications for the control of the disease and are part of a study into the endophytic infection of mango by *Dd*. *(P)*

Biology and Control of *Nattrassia mangiferae*

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Nattrassia mangiferae has been associated with various diseases in a number of different crops. In South Africa it is the causal organism of several diseases occurring in mango: blossom blight; and the postharvest diseases, soft brown rot and stem-end rot. It is thought that *N. mangiferae* infects the inflorescence at an early stage during flowering. After infection, it spreads systemically through the inflorescence into the fruit, where it remains latent until the fruit begins to ripen. The aim of the present study was to formulate effective control measures. In order to do so it was necessary to confirm that *N. mangiferae* was the causal organism of the above mentioned diseases, and to determine the sources of inoculum and the time of infection. Commercially, blossom blight is currently controlled effectively by sprays of the systemic fungicides flusilazol or pyrazophos. In the current study various other chemicals were also tested for effectiveness in controlling soft brown rot and stem-end rot occurring on the fruit after harvest. These included both pre- and postharvest applications. Of all the fungicides tested, monthly preharvest applications of copper oxychloride during the period from fruit set until harvest resulted in significantly improved disease control. *(L)*

Semi-Selective Medium for Isolation of *Nattrassia mangiferae*

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Nattrassia mangiferae is the causal organism of various mango diseases in South Africa. These are blossom blight and the postharvest diseases soft brown rot and stem-end rot. In epidemiological studies it is necessary to make numerous isolations from tissue which may be infected by the specific organisms. The isolation of *N. mangiferae* from branches, fruit and panicles by plating onto agar media, is severely hampered by the presence of saprophytic fungi. The development of a selective medium was therefore necessary. Ten chemicals were screened for their effect on the radial growth of *N. mangiferae*. Growth of *N. mangiferae* was the least inhibited by benodanil (100 ppm), rose bengal (50 ppm) and tannic acid (3000 ppm). These three chemicals were then tested at different concentrations and combinations for their effect on the radial growth of seven saprophytic fungi isolated commonly from mango tissue, as well as on *N. mangiferae*. A selective medium consisting of tannic acid (4000 ppm) and benodanil (100 ppm) provided a superior medium for the detection of *N. mangiferae* in isolations made from mango branches, blossoms and fruit. *(P)*

Bacterial Black Blight of Mango in Israel

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Bacterial black blight is one of the mango diseases unknown in the optimal mango-growing regions. The disease appears in Israel during late winter – early spring, causing a black branch and leaf blight. The necrosis in the branch is confined to the bark tissues. Leaf petioles and the basal

portion of leaf blades turn black as well. After unusually cold winters, all the young growth (one-year-old branches) is necrotic in affected trees. Pure cultures of *Pseudomonas syringae* pv. *syringae* can be isolated from branches and leaves with black blight symptoms. When mango branches are inoculated with those bacteria during summer months, disease is not incited. However, symptoms appear following inoculations performed during the winter months. Koch's postulates were fully established. The disease is highly damaging during cold winters accompanied by frequent wind and hail storms. Most commercial varieties grown in Israel are susceptible to the disease; sources of resistance do, however, exist. In years with mild winters, good disease control can be achieved by preventive sprays of Bordeaux mixture; in years with severe winters, the control is unsatisfactory. To achieve better control, studies to identify the initial inoculum sources were conducted and were discussed. *(L)*

Mango Disease Losses: Balancing Economy and Ecology

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The damage and losses caused by mango pathogens are unacceptable to orchardists, marketeers and consumers. But what is the evolutionary significance of the magnitude of the losses? In nature, and in terms of tree survival, the losses are *not* of major significance.

Pathogen biology and disease ingress are influenced by host/ecosystem traits resulting from natural selection operating on an evolutionary time scale in regions to which *Mangifera indica* (and related spp.) was endemic. However, natural (loss-minimizing) balances have also been compromised (and damage tolerance thresholds lowered) by the artefacts of orchard cultivation and exotic/depauperate ecosystems, as well as the demands of harvesting, transport and storage.

What facets of host genome, environment, and micro- and macroflora and fauna could be manipulated, improved or reassembled to adjust the balance, so that disease losses are minimal and product quality is maintained at the level dictated by commerce?

What do we know about these factors? What more do we need to know? How can the information be exploited?

How can nature's gifts be resurrected, enhanced or supplemented? *(L)*

C: POSTHARVEST PATHOGENS AND THEIR CONTROL; QUARANTINE MEASURES TO PREVENT DISEASE AND OTHER BLEMISHES

Hot Water Brush: A New Method for the Control of Postharvest Disease Caused by Alternaria Rot in Mango Fruits

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Alternaria alternata is the main postharvest pathogen attacking mango fruits in Israel. The pathogen penetrates the fruit through lenticels during fruit growth and remains quiescent until harvest. After harvest, while the fruit ripens, the pathogen renews its development and black spot symptoms of decay appear on the fruit, resulting in a reduction of fruit marketability. No preharvest treatments are applied in Israel to prevent postharvest diseases. However, postharvest treatments are applied depending on the relative humidity conditions at the grove location. Fruits from groves

located in relatively dry regions, where the postharvest incidence of decay is low, are only water-washed. However, fruits from groves located in humid regions, where the incidence of decay is very high, are treated with 900 μg/ml prochloraz. A simple postharvest treatment that involves hot water spray at temperatures of 50–60° C while the fruits are brushed (hot water brush), was developed. The treatment significantly reduced decay development by *A. alternata* to levels similar to the prochloraz treatment in several mango cultivars stored for 3 weeks at 14°C. When fruits were stored for longer periods of time, the fungicide was more effective than the hot water brush. The possible use of this simple treatment in the postharvest mango industry, to reduce the use of fungicides, was discussed. *(P)*

New Options for the Prevention of Postharvest Disease in Mango Fruits

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Postharvest diseases caused by *Colletotrichum gloeosporioides* and *Alternaria alternata* attack mango fruits during fruit growth and remain quiescent until the fruit is harvested. Susceptibility of mango fruits to postharvest diseases increases after harvest and prolonged storage as a result of physiological changes occurring in the fruits that enable pathogen development. In the past, resistance of unripe mango fruits was suggested to be related to a decrease in concentration of the preformed 5-substituted resorcinols in the peel of unripe fruits. New antifungal compounds of similar structure have been identified in the peel of unripe fruits and their relation to fruit susceptibility has been determined. The possible relation between levels of antifungal compounds and new biological and physical treatments that prevent postharvest diseases was investigated. Postharvest decay development was delayed by: (i) Co-inoculation of *C. gloeosporioides* with a strain of *Colletotrichum magna* (nonpathogenic on mango); (ii) exposure of some mango cultivars to a CO_2-enriched atmosphere; (iii) hot water dip at 55°C; and (iv) hot water brush. Hot water brush represents a new, simple and efficient method for the control of postharvest decay development in mango. The importance of each method as a postharvest treatment was discussed. *(L)*

Hot Water Quarantine Treatment and Water Cooling of 'Haden' Mangoes

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A part of Mexican-grown mangoes must be subjected to a quarantine treatment by hot water immersion (46°C, 90 min) before shipment to export markets (Work Plan for Mexican Mango Preclearance Program, USDA-APHIS-IS/SARH-DGSV, 1994). The effects of this heat shock stress can be attenuated by cooling. The purpose of this study was to determine the impact of water heating on respiration, histological structure and conservation of the quality of 'Haden' mangoes as well as the effect of water cooling (21°C, 30 min) and refrigeration on the recovery of the fruits. Physiologically mature Haden mangoes produced in Mexico (18°32′ N; 99°52′ W) were used. The treatments were applied in the packinghouse and the fruits were stored at 25° or 13°C, at 85–90% relative humidity. The parameters evaluated were: respiratory activity, appearance and thickness of the cuticle, SSC, titratable acidity, flesh firmness, flesh color, % physiological weight

loss, and sensory evaluation tests. Statistical analyses (ANOVA α=0.05) indicated that water heating increased respiratory metabolism, expanded the cuticle, and induced the formation of small fissures and widening of pores, thereby causing a decrease of the storage life. Water cooling partially reversed the effects of the water heating: respiratory activity tended to return to normal, and the thickness and appearance of the cuticle recovered partially. Refrigeration also reversed the effects of water heating, but to a lesser degree. Neither treatment had a conspicuous effect on quality and sensorial parameters. It was concluded that water cooling should be recommended for hot-water-treated mangoes.

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The Response of Mango Stem End Rot Pathogens to Heat

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In Australia, stem end rot of mango is caused by *Dothiorella* spp., *Lasiodiplodia theobromae, Phomopsis mangiferae* and other fungi. *D. dominicana* is the major cause of mango stem end rot in Queensland. Previous studies have shown that in cv. 'Kensington Pride', stem end rot is less effectively controlled by heat treatments (*e.g.* hot water and vapor heat) than is anthracnose (*Colletotrichum gloeosporioides* and *C. acutatum*). To investigate the reasons for this, the *in vitro* heat tolerance of selected *D. dominicana, L. theobromae* and *Phomopsis* sp. was studied. Mycelial suspensions of each isolate were immersed in hot water (at temperatures ranging from 46–55°C for periods of up to 60 min), rapidly cooled and then plated onto potato dextrose agar. Isolates of *D. dominicana* were found to be the most heat-resistant of the three species, and *Phomopsis* sp. the least heat-resistant. In mango fruit (cv. Kensington Pride) inoculated with *D. dominicana* or *L. theobromae*, however, vapor heat treatment (fruit seed surface temperature of 46.5°C for 10 min) controlled stem end rot caused by *D. dominicana* more effectively than that caused by *L. theobromae*. The significance of these findings was discussed. *(L)*

Heat Transference in Mangoes by Hot Water Dip and Forced Hot Air

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Quarantine treatments involving heat have been used to treat fruits destined for export. Knowledge about transference of heat in the pulp of fruit is basic information needed for the fast and rational development of new treatments.

We compared the heat gain in different depths of unripe 'Tommy Atkins' mangoes of 400 and 650 g weight submitted to two treatments: hot water dip and forced hot air at 46°C for 75 min. The temperatures were recorded from the start of the experiment at 5-min intervals at depths of 1, 2 and 3 cm. The heat transference in both treatments can be described by an asymptotic regression, $y = a + b.exp(-cx)$.

The data were submitted to null hypothesis test and resulted in significant differences between mangoes of the same weight treated with either hot water or hot air [$15.0 < F > 94.5/F_c(3;174)$ to 0.001 + 5.7] and between mangoes with different weights submitted to the same treatment [$11.2 < F > 73.6/F_c(3;174)$ to $0.001 + 5.7$].

The final temperature in mangoes of different weights did not differ in relation to treatment, because there was heat saturation in the pulp of the mangoes. *(L)*

Use of Hot Water Treatment to Enhance Natural Resistance of 'Carabao' Mango against Anthracnose Disease

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'Carabao' mango fruits were inoculated with spores of *Colletotrichum gloeosporioides* (10^6/ml spore suspension) at various time intervals after hot water treatment (HWT). HWT did not appear to inhibit disease development in inoculated fruits when disease severity was plotted against time in days. However, plotting severity against peel color index (PCI) gave lower rates of development of anthracnose in fruits subjected to HWT. This can be attributed to the acceleration of ripening by HWT. In one trial, the inhibition was most pronounced in fruits inoculated 48 h after HWT. These results indicate the possibility of an antifungal compound formed in response to HWT.

To relate the above response to recommendations for the application of HWT, naturally infected fruits were subjected to HWT at various times after harvest. Results showed that treatment at 12 h after harvest delayed the onset of disease such that, on the average, fruits exhibited symptoms only when almost fully yellow (PCI 6). In contrast, those treated at 6 h or more than 12 h after harvest, showed symptoms of anthracnose at the yellow-with-green-tip stage (PCI 5). However, all treated fruits showed slight disease development. Mango peel was sampled for extraction with ethanol and subsequent analysis for antifungal compounds. *(P)*

Application of Shortwave Infra-Red Radiation to Control Postharvest Decay of Mangoes

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A postharvest treatment of mangoes consisting of a 5-min hot water dip (50°C) followed by a 20-sec ambient temperature prochloraz dip is the conventional method used to ensure adequate control of postharvest diseases. This treatment is time consuming and represents a bottleneck in the packinghouse. Shortwave infra-red (IR) radiation has long been recognized as being a highly efficient form of heating. The effect of IR radiation was therefore compared with the commercially used hot water treatment, for the control of postharvest diseases on mango. A 3-min exposure to IR was found to be as effective as the commercially used 5-min hot water treatment, in controlling anthracnose (*Colletotrichum gloeosporioides)* and soft brown rot (*Nattrassia mangiferae*) on seven mango cultivars. There were no significant differences between IR and hot water treatments as far as fruit quality was concerned. The IR treatment is, however, cheaper and faster than the hot water treatment. *(P)*

Evaluation of Phosphonate for Control of Postharvest Mango Pathogens

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Field trials were carried out over two seasons in an orchard in southern Queensland, Australia, to investigate the effect of preharvest trunk injection and soil drenches of phosphonate on postharvest

disease levels in mango cv. 'Kensington Pride'. In the first trial, trees were injected when fruits were 'hen egg' size. Injection of a 400 *g/l* formulation of potassium phosphonate (15 ml/m canopy diameter) significantly reduced stem end rot levels in fruit after storage at 22°C for 20 days. There was also a reduction in the isolation frequency of endophytic stem end rot pathogens from inflorescences, ~6 weeks after injection. In the second trial, injections and soil drenches were applied earlier in fruit development (flowering/fruit set stage). There was no reduction in fruit stem end rot levels.

Results of the first trial suggest that further trials should be undertaken to investigate the potential of phosphonate for the control of postharvest mango pathogens. *(L)*

Effect of Hydrocooling and Bavistin (Carbendazim) Dip on the Shelf Life and Quality of Mango during Storage under Various Environmental Conditions

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The present investigation was undertaken to study the effect of hydrocooling and Bavistin (carbendazim) dip on the shelf life and quality of mango during storage under various environmental conditions. For this purpose, the fruits of three mango varieties, 'Kesar', 'Totapuri' and 'Vanraj', were harvested with stalk (2.5 cm), hydrocooled at 12°C, and given a postharvest dip in Bavistin (1000 ppm) and stored under two conditions: room temperature 24° to 35°C, and 48 to 80% r.h., in a low-cost, low-energy-input, easily installable cool chamber (22 to 26°C and 92 to 95% r.h.). It was found that the shelf life of Kesar, Totapuri and Vanraj could be extended up to 25, 36 and 31 days, respectively, when stored in the cool chamber. In contrast, the shelf life of these varieties was barely 17, 21 and 19 days, respectively, when stored at room temperature. It was observed, too, that in the hydrocooled and Bavistin-treated fruits lower physiological weight loss was recorded and higher organoleptic score when stored in the cool chamber, compared with at room temperature. The cool-chamber-stored fruits looked fresh, firm and attractive. The untreated fruits were found to be infected with *Colletotrichum gloeosporioides* and *Diplodia natalensis*. *(P)*

Effect of Method of Harvesting on Postharvest Loss Reduction and Extension of Shelf Life of Mango

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The present investigation was undertaken to study the effect of the method of harvesting on postharvest loss reduction and extension of shelf life of mango grown in an arid zone. For this purpose, the mango fruits of cv. 'Kesar' were harvested with stalk (2.5 cm), using various harvesters such as Dapoli, CIHNP, IIHR, and IARI mango harvesters, along with manual harvesting without stalk. The fruits were then graded and packed in corrugated fiber board boxes and stored at ambient conditions (24 to 38°C and 42 to 81% r.h.). It was found that with the help of the Dapoli harvester, the maximum number of fruits could be harvested (182 fruits/h), followed by CIHNP, IIHR, IARI harvesters, and manual harvesting. Kesar mango with stalks intact had exhibited delayed ripening as well as delayed shrivelling. The fruits harvested by any of the harvesters had a shelf life of 15 days, as against 11 days following manual harvesting. The fruits harvested by using these harvesters had a minimum physiological weight loss of 12–13%, as against 17% with manual harvesting. The spoilage of manually harvested fruits was 13% after 16 days of storage. The mango fruits were found to be infected with *Colletotrichum gloeosporioides* and *Diplodia natalensis*, when harvested manually. *(P)*

Detrimental Effects of Detergent in the Development of Mango Skin Browning

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The term mango skin browning covers a range of postharvest blemishes affecting mango fruit. Skin browning is a major fruit quality problem, causing the downgrading and rejection of the product on Australian markets. Etching and lenticel spotting are two of the more prevalent forms of browning. Etching consists of numerous small dark flecks and lenticel spotting is a dark halo of tissue around the lenticels.

To determine which points in the harvesting and postharvest handling operations contribute most to these blemishes, mango handling systems were investigated on several Australian farms during the 1994 and 1995 seasons. The operations that contributed most to etching and lenticel spotting were those associated with wetting of the fruit by detergent, whether by harvest aid or on desapping racks in the packinghouse. Fruit wetted with detergent showed greater levels of skin browning, lenticel spotting and postharvest diseases.

Laboratory investigations into the effect of detergent on mango skin, showed that detergent alone at commercial rates could cause etching and lenticel spotting. Etching appeared to be caused by the aided entry of aqueous solutions into the epidermis and underlying cells through small fissures in the cuticle. These fissures usually occur in the center of cuticular platelets as they subdivide, or at the junctions between platelets.

It seems that detergents can be a major contributor to lenticel spotting and forms of browning such as etching. The presence of detergent in the harvest/postharvest handling system may be causing as much harm as benefit to mango fruit quality. *(L)*

MEETING

ABSTRACTS OF PRESENTATIONS ON PLANT PROTECTION ISSUES AT THE XTH INTERNATIONAL CONGRESS OF VIROLOGY

August 11–16, 1996
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Part 1
Plenary Lectures

Virus Movement in Plants

J.G. Atabekov *A.N. Belozersky Inst., Moscow State University, Moscow, Russia*

The cell-to-cell translocation of a plant virus genome is a function of both the viral and the host genomes, in which virus movement protein(s) (MP) and host-coded components are involved. Progress in understanding the phenomenon of the virus-coded intercellular transport of a viral genome was summarized. The molecular biology of cell-to-cell and long distance movement relevant to the resistance of plants to viruses was surveyed. Specific topics included: (i) examples of strategies of movement function and expression by different plant viruses, with an emphasis on some single-MP-coding and triple-gene-block-containing viruses; (ii) functional compatibility between the MPs of unrelated viruses examined by three experimental approaches (double infection, expression of the helper-virus MP in a transgenic plant, and construction of the recombinant virus genomes with the MP gene from a foreign virus) and (iii) MP-derived resistance in transgenic plants to viruses. *(L)*

Emergence, Quantitative Taxonomy and Evolution of Geminiviruses and Potyviruses

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Like animal viruses, plant viruses are emerging and several examples among the Geminiviridae and Potyviridae families were reported and commented upon. Geminiviruses are in expansion worldwide and three examples are provided to illustrate different types of emergence. For the African cassava mosaic virus, Man was the major actor until the whitefly vector became the major player in the emergence of another geminivirus, the East African cassava mosaic virus, which has been spreading in Uganda since 1985. Whiteflies are booming in several places in the world, where they carry new viruses such as the cotton leaf curl virus in Pakistan and several tomato viruses in Central America and in the Mediterranean Basin. Man, with the increasing world trade, transports whiteflies and new epidemics occur because vectors encounter new hosts and new viruses. From these examples it is clear that two major actors are influential in the emergence of plant viruses: insect vectors and humans.

To understand and track emergence of plant viruses, we attempted to use the molecular diversity of viral genomes to quantify the virus taxonomy in the Potyviridae and Geminiviridae families. The distribution of the molecular diversity of the genomes of their members is organized. It is possible to quantify strain, species and genus taxa. In addition, because of the great homogeneity of the molecular diversity, it is possible in each of these two cases to identify very small sequences that are representative of the entire viral genome, allowing rapid taxonomy and diagnostics. From these studies it is proposed that plant viral evolution is a fairly slow process over millions of years, but with rare and dramatic bursts of diversity. *(L)*

Africa: The Verdant Pasture for Newly Emerging and Re-emerging Viral Diseases

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In Africa, virus diseases which still affect man, animals and plants with significant human toll and economic devastation, have been known for a considerable time. Poliomyelitis, yellow fever, and measles infections of man; rinderpest, foot and mouth disease, and Newcastle disease of animals and birds are some examples of these re-emerging diseases. New viral infections described in Africa within the last 20–30 years include Lassa and Ebola hemorrhagic fevers and HIV infection of man, and plant diseases of economic importance caused by rice yellow mottle and maize streak viruses.

Two major inter-related factors, social and scientific, are responsible for the uncontrolled devastation caused by emerging and re-emerging viral infections in Africa. Poor standards of living and preventive health care delivery, resulting not from poverty *per se*, but from misdirected and misplaced priority on public and private spending, form the core of the social factors. An inappropriate science education system, coupled with flawed training programs, has rendered many African scientists unsuitable for, and incapable of solving the majority of Africa's disease problems. If Africa is not to remain a flourishing pasture for new emerging and re-emerging viral pathogens, priorities in public and private expenditures must be reordered, while African scientists need to be more concerned with finding solutions to Africa's human, animal and plant problems. *(L)*

Engineering Hypoviruses to Probe and Modify Signal Transduction Underlying Fungal Virulence

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Members of the newly established RNA virus family Hypoviridae persistently and stably alter a number of interesting phenotypic traits in their pathogenic fungal host, the chestnut blight fungus *Cryphonectria parasitica*. Alterations can range from reduced pigment production (a convenient experimental marker), to reduced asexual and sexual reproduction (vital host functions), to reduced fungal virulence (a complex process of practical importance). Consequently, hypoviruses can be used

as biocontrol agents and serve as unique tools for examining regulation of fungal gene expression and pathogenesis. An understanding of how a hypovirus modifies such a diverse range of host traits, functions and processes has been a long-standing research goal. We reviewed a growing body of evidence linking hypovirus-mediated reduction in the accumulation of a specific cellular G-protein alpha subunit with altered host phenotype, including virulence attenuation. Given the conserved nature of signal transduction processes among eukaryotes, it is likely that the information derived from a detailed examination of hypovirus-mediated disruption of cellular G-protein signaling will find relevance for RNA viruses of plant and animal hosts. *(L)*

Virus and Host Functions in Bromovirus RNA Replication and Gene Expression

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Brome mosaic virus (BMV), a member of the alphavirus-like superfamily of positive strand RNA viruses, encodes two mutually-interacting RNA replication factors: 1a contains domains implicated in helicase and capping functions, while 2a contains a polymerase-like domain. The RNA-dependent RNA polymerase (RdRp) involved in BMV RNA replication and subgenomic mRNA transcription is a membrane-associated complex of 1a, 2a, and host proteins. Specific viral RNA sequences also appear to play a non-template role in assembling the replication complex.

Initiation of BMV (-) strand synthesis involves RdRp interaction with two widely separated *cis*-acting regions on the (+) strand RNA template, one at the 3'-terminal, tRNA-like (-) strand initiation site, and another approximately 1 kb away. A variety of observations suggest that these sites perform different functions in initiation, which may be a multi-stage process. Additional *cis*-acting sequences encoded at the 5' end of the (+) strand are required for full RNA replication, including (+) strand RNA synthesis.

In addition to other host cells, engineered BMV RNA replicons can replicate and express foreign genes in yeast (*Saccharomyces cerevisiae*), creating new possibilities to identify and study host functions in RNA replication. Using these approaches, yeast mutants have been identified with altered abilities to support BMV RNA replication and transcription. Results indicating that BMV-directed RNA replication and gene expression can be affected by changes in multiple yeast genes were discussed. *(L)*

I: POSITIVE STRAND RNA VIRUSES

cis-Acting Coding Sequences Required for the Accumulation of Alfalfa Mosaic Virus (AlMV) RNAs

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AlMV RNAs 1 and 2 encode the replicase proteins P1 and P2, respectively. RNA 3 encodes the movement protein P3 and coat protein (CP). CP is expressed from the subgenomic RNA 4. Tobacco has been transformed with cDNA of the P1 gene (P1 plants), P2 gene (P2 plants), or both replicase genes (P12 plants). It was observed that P1 expressed by P1 plants could be used in *trans* for the replication of RNAs 2 and 3 but not for the replication of RNA 1. Similarly, P2 expressed by P2 plants could be used in *trans* for replication of RNAs 1 and 3, but not for replication of RNA 2. Studies with P12 protoplasts confirmed that the transgenic P1/P2-replicase accepted inoculum RNA 3 as a template but not RNAs 1 or 2.

Mutations in the P3 gene have little effect on RNA 3 accumulation in P12 protoplasts but mutations in the CP gene reduce (+)RNA 3 accumulation 100-fold. The defect in (+)RNA accumulation of a CP mutant could not be complemented in *trans* by CP of a P3 mutant in doubly infected P12 protoplasts. It is concluded that each of the AlMV RNAs requires an encoded protein in *cis*, which can be used in *trans* for the replication of other AlMV RNAs. Apparently, only P3 functions exclusively in *trans*. *(L)*

Analysis of Coat Protein and Replicase Interaction Sites at the 3'-Terminus of Alfala Mosaic Virus (AlMV) RNA 3

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AlMV is a plant virus with a tripartite single-stranded RNA genome of plus-strand polarity. The formation of specific RNA-coat protein complexes is required for both virion assembly and initiation of infection. The 3'-termini of the three genomic RNAs of AlMV contain a common sequence of 145 nucleotides (nt) with a specific binding site for coat protein (CP). This sequence consists of several stem/loop structures interspersed with single-stranded AUGC-motifs. Using band-shift assays, at least two specific binding sites for CP were identified near the 3'-end of RNA 3. Deletion studies revealed that both sites could bind CP independently of each other and sequence elements that are essential for binding of CP to both sites were identified.

Structural requirements for the binding of CP to the 3'-terminus of RNA 3 were studied in more detail by assaying the ability of short RNA fragments to bind CP. These short fragments were transcribed *in vitro* by T7 RNA polymerase from synthetic partially double-stranded DNA templates containing a 24-base double-stranded T7 promoter and single-stranded sequences corresponding to the 3'-terminal 39 nt of RNA 3. By transcribing synthetic mutant cDNAs, mutations were introduced into the hairpins or AUGC-boxes of the 39 nt RNA fragment. The effect of these mutations on binding of CP to the RNA fragments was discussed.

AlMV encodes two subunits of the viral replicase: P1, with putative methyl transferase and helicase activity, and P2, with putative polymerase activity. The interaction of these replicase proteins with the 3'-terminus of RNA 3 has been studied by *in vitro* replicase and band-shift assays with replicase preparations from plants and by north-western assays with purified maltrose-binding-domain/P1 or P2 fusion proteins. *(P)*

Host Effects on Cucumber Mosaic Virus (CMV) RNA-Dependent RNA Polymerase Activity *In Vitro*

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The CMV2a protein was tagged with hexahistidine at the C-terminus and the CMV RNA-dependent RNA polymerase (RdRp) was isolated from infected tobacco and squash plants by affinity chromatography on nickel nitrilotriacetate (Ni-NTA). The isolated CMV RdRp showed template specificity for CMV and CMV satellite RNAs. However, the RdRp isolated from tobacco only synthesized (-) RNA from added (+) RNA template, producing double-stranded RNA, whereas the RdRp isolated from squash synthesized multiple products, including some co-migrating with the single-stranded CMV RNAs on two gel systems.

The squash RdRp also synthesized both (+) and (-) satellite RNAs. Prior to affinity chromatography on Ni-NTA, RdRp from both hosts could only synthesize (-) RNA. These data suggest that a host factor may be involved in the inhibition of (+) RNA synthesis from (-) RNA templates. *(L)*

Template and Protein Functions in the Temperature-Sensitive Replication of Cucumber Mosaic Cucumovirus (CMV) in *Cucumis melo*

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CMV is a tripartite (+) sense RNA plant virus. RNAs 1 and 2 encode the 1a and 2a proteins, with replication-related functions. The 1a protein encodes the putative methyl transferase domain and helicase domains. Previously a temperature-sensitive replication phenotype (ts) of the Sny-CMV was identified in muskmelon (*Cucumis melo*, cv. 'Iroquois') and mapped to RNA 1. Using recombinants between cDNA clones of RNA 1 of Sny-CMV and a temperature-resistant strain, Fny-CMV, we further delineated the ts domain. We found that more than one region of the RNA 1 is involved in the ts phenotype. The two domains are capable of complementation, suggesting *trans* functions. In addition, approximately 2% of plants inoculated with some ts recombinant strains generate stable mutations that allow the virus to overcome the ts phenotype. The details of the ts domains, and the role of the 1a protein and the RNA 1 template in the ts phenotype, were presented. *(P)*

Subcellular Localization of Brome Mosaic Virus RNA Replication Factors

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The helicase-like 1a and polymerase-like 2a proteins of brome mosaic virus (BMV) are required for viral RNA replication *in vivo*, present in membrane-bound viral RNA polymerase extracts, and share conservation with many other viruses in the alphavirus superfamily. To complement ongoing molecular and genetic studies of viral and host factors required for BMV RNA replication and to produce a more complete understanding of the RNA replication process, it is essential also to define the location and ultrastructure of the replication complex. The subcellular distributions of 1a and 2a were studied by indirect immunofluorescence and confocal microscopy. In double-labeling of BMV-infected protoplasts, 1a and 2a co-localized at all time-points in which they were detected. By 4 hpi, 1a and 2a localized in defined spots that by 16 hpi appeared as vesicle-like structures mostly near the periphery of the nucleus. Labeled nascent RNA co-localized with 1a and 2a, indicating that 1a and 2a accumulate at sites of viral RNA synthesis. Double immunofluorescence using antibodies to plant cellular structures and to 1a and 2a, shows that BMV replication complexes occur in a subset of the endoplasmic reticulum. *(P)*

The *Pol*-Like Core of Brome Mosaic Virus (BMV) 2a Protein Maintains Activity and 1a-Selectivity in RNA Replication

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BMV encodes two proteins required for viral RNA replication: 1a and 2a. Gene exchanges between BMV and the related cowpea chlorotic mottle virus (CCMV) show that 1a and 2a function interdependently in RNA synthesis. The N-terminal 2a segment preceding the *pol*-like core interacts with 1a *in vitro* and prior deletion studies had suggested that this 2a segment was essential for RNA replication *in vivo*. We have now used protein fusions and deletions to explore possible parallels between noncovalent 1a-2a interaction and covalent fusion of similar protein domains in tobacco mosaic virus (TMV), and to see whether the N-terminal 2a-1a interaction was the primary basis for 1a-2a interaction and compatibility *in vivo*. We found that 2a can function as part of a TMV-like 1a-2a fusion and that the *pol*-like core was sufficient to provide 2a functions in such a fusion. Unexpectedly, the unfused 2a *pol*-like core also supported efficient RNA replication when it and wt 1a were expressed as separate proteins. Moreover, like wt BMV 2a, the separately expressed, *pol*-like 2a core supported RNA replication with BMV 1a but not CCMV 1a, and therefore depends on compatibility with 1a for function. Thus, the *pol*-like core of 2a must interact with 1a in a way that is essential for viral RNA synthesis, and 1a-2a interactions are more complex than the single, previously mapped interaction of the N-terminal 2a segment with 1a. *(P)*

Sequences and Proteins Involved in Genetic Recombination of Brome Mosaic Bromovirus

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Brome mosaic bromovirus (BMV), a tripartite positive sense RNA virus of plants, supports both homologous and nonhomologous recombination among its RNA segments. Homologous crossovers are supported by regions of homology and nonhomologous crossovers by complementary regions. Studying the sequence requirements of recombination we have found that homologous recombination can occur at locations which represent a combination of AU-rich and GC-rich regions. This suggests that homologous recombination occurs *via* a template switching mechanism during plus-strand synthesis. While the Au-rich regions might facilitate the detachment of the nascent strands on the primary RNA template, the GC-rich regions can anchor the nascent strands on the acceptor template.

For nonhomologous recombination we have demonstrated that antisense regions as long as 20 nt can direct recombination crosses and that the frequency of recombination is proportional to the length of the antisense regions. Apparently, the formation of local double-stranded regions brings together the recombining RNAs and thus facilitates the crossover events. The role of heteroduplexes was confirmed for both the 3' noncoding and for the intercistronic regions in BMV RNAs.

To determine whether virally encoded proteins participate in recombination, viable mutants of 1a and 2a proteins, the two replicase proteins of BMV, were examined for recombination activity. Mutations in the helicase domain of 1a protein shifted the locations of crossovers for the heteroduplex-mediated nonhomologous recombination. On the other hand, mutations in the N-terminal and the core domains of 2a protein affected both homologous and nonhomologous recombination. Specifically, one 2a mutant eliminated only the heteroduplex-mediated crossovers, suggesting that the two types of recombination can be separated not only from each other but also from BMV RNA replication.

In summary, RNA-RNA recombination is a process which requires both proper nucleotide sequences on the template and viral replicase proteins to occur. This confirms a model that involves the viral replicase switches between the two recombining

RNA substrates. Further studies are required to unravel the molecular mechanism of the crossovers. *(P)*

Expression and Analysis of the Genes Involved in Bean Yellow Mosaic Virus (BYMV) Replication

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BYMV, a member of the family Potyviridae, has a positive sense ssRNA genome, which is translated as a single polyprotein and subsequently cleaved by viral encoded proteases into its mature proteins. To investigate the role of BYMV encoded proteins in viral replication, we are currently expressing BYMV genes in *Spodoptera frugiperda* (Sf9) insect cells using a baculovirus vector. Recombinant baculoviruses have been engineered to express the P3, 6K1, CI, 6K2, NIa, NIb genes and combinations thereof. Many of the properties of the proteins, previously described in plant cells, appear to be maintained in the Sf9 cells. Not only is the NIa protease functional in insect cells, but electron microscopic studies have revealed the presence of cytoplasmic and nuclear inclusion bodies similar to those characteristically formed in BYMV-infected plant cells. The NIb protein is synthesized at high levels and has been purified by nickel ion affinity chromatography along with the CI protein to raise specific antisera. The recombinant NIb protein has been detected in both a soluble and insoluble form. With the demonstration that several of the properties of the BYMV proteins are maintained in Sf9 cells, work is under way to investigate possible polymerase activity associated with the NIb protein with an exogenous template and oligo (U) primer. *(P)*

cis-Acting Elements in the Potato Virus X (PVX) 5' Nontranslated Region that Regulate Genomic and Subgenomic RNA Synthesis

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To understand the mechanisms of PVX replication, we are studying *cis*-acting sequences and/or structures required for RNA synthesis. S1 nuclease protection and primer extension were used to measure synthesis of minus- and plus-strand PVX RNAs in tobacco protoplasts inoculated with wild-type and modified infectious transcripts. Minus-strand, genomic-length RNA accumulated in protoplasts inoculated with transcripts containing a variety of deletions in the 5' nontranslated region (5' NTR). In contrast, deletion of more than 12 nt from the 5' end and internal deletions in the 5' NTR inhibited both genomic and subgenomic plus-strand RNA synthesis. Some of the deletion mutations may impact a hairpin structure that we have mapped to this region by chemical modification *in vitro*. Additional experiments are under way to understand how specific sequences and structures in the 5' NTR may co-regulate both genomic and subgenomic RNA synthesis and to define *cis*-acting elements elsewhere in the PVX genome that regulate these processes. *(P)*

The Mechanism of Generation of Cymbidium Ringspot Virus Defective Interfering RNA

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Defective interfering (DI) RNAs are deletion mutants of viral genomes which have lost all essential genes required for vital functions. They are heterogeneous in size, ranging from *ca* 0.4 to 0.7 kb, and require the presence of the helper virus for replication. DI RNAs replicate at the expense of the helper genome from which they derived as a result of a series of progressive deletions. To study the mechanism of DI RNA generation, *Nicotiana clevelandii* plants were inoculated with wild type and mutant *in vitro* transcripts of DI RNAs (*ca* 0.7 kb). It was shown that new smaller DI RNAs (*ca* 0.4 kb) were formed from the larger precursor molecules *in planta*. Evidence will be provided that a highly base-paired structure in the longer DI RNA is responsible for the generation of the smaller DI RNA. Mutations increasing the stability of the highly base-paired structure enhanced the frequency of the generation of the smaller DI RNA. Moreover, the recombination sites can be positioned into another region of molecule (where they never occurred normally) by transposition of the highly base-paired structure along the molecule. The results obtained suggest that the viral replicase is not always able to unwind the highly base-paired region and copies it, but circumvents it without releasing and producing co-linear deletion mutants. This recombination event is not sequence- but rather structure-specific and can be reproduced if the appropriate DI RNA sequence is substituted by a foreign sequence carrying a highly base-paired secondary structure. *(P)*

II: VIRUS–HOST INTERACTIONS

Sequence Stability of a Clonal Viral Population after Long-Term Passaging in Divergent Plant Hosts

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A clonal population of tobacco mosaic tobamovirus (TMV) was made which was preadapted for tobacco. This population was produced from *in vitro* transcripts from a bacterially cloned TMV sequence. The sequence had been cloned from a TMV culture passaged in tobacco for decades. The *in vitro* transcripts were inoculated onto tobacco (*Nicotiana tabacum* cv. 'Xanthi') for an initial passage to build up the inoculum. This population was then inoculated onto seven different plant hosts (representing six disparate plant families), with four replicate plants per host, for a total of 24 populations (four later died out during passaging) derived from the initial clonal tobacco population. All 24 populations were passaged approximately once monthly for 11 or 12 passages, totalling 413 to 515 days. At this time, RT-PCR-amplified sequence of the tobamovirus variable region at the 3' end of the movement protein gene (nts 5500–5690) was bacterially cloned. These individual clones were sequenced to assay the populations for evolution and heterogeneity. Of 28 individual clones analyzed so far (5510 bp total), representing all seven hosts, only one mutation was found. This clonal population under natural selection pressures did not evolve significantly, suggesting that TMV may evolve in the field more by competition of pre-existing variants than by *de novo* sequence evolution. *(P)*

Phe^{366} in the 126 kDa Protein of Tobacco Mosaic Virus (TMV) Impacts Viral Replication, Cell-to-Cell Movement and Symptom Induction

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It has been shown previously that Ser^{361} and Lys^{368} within the 126 kDa protein of TMV are important for symptom induction. These amino acids are in a nonconserved region of the 126 kDa protein and its analogues from sindbis-like plant viruses. The function of this region is unknown. A database search and sequence alignment showed that a WFP motif between Ser^{361} and Lys^{368} is present in membrane-associated and actin-binding proteins. The WFP motif, or a portion of it, is also

present in the analogous positions in some other, similar viruses. To investigate the importance of the WFP motif, two mutants were made within a clone of TMV, where Ala and Tyr replace Phe^{366} in WFP, yielding progeny viruses WAP and WYP. WAP accumulated to levels less than 0.9% of the parental virus in tobacco protoplasts, indicating that F in the WFP motif is important for viral replication. WYP replicated as well as the parental virus in protoplasts, but induced small necrotic lesions on a hypersensitive host. The necrotic lesion size did not increase when the hypersensitive response was eliminated by growing plants at 32°C. This result showed that the small lesions were caused by the inability of WYP to move from cell to cell and not by a superinduction of the hypersensitive response. We are using an *in vivo* pulse labeling method to determine whether this is a direct effect of the mutation or an indirect effect through the viral movement protein. Although WYP can move through the vasculature, symptoms are not so severe as those induced by the parental virus. Therefore, Phe^{366} impacts symptom induction. *(P)*

A Movement Role for the Tobacco Mosaic Virus (TMV) 126 kDa Protein in Systemic Invasion of Tobacco

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The masked (M) strain of TMV causes very mild symptoms on tobacco and invades tissues *via* phloem more slowly than the U1 strain. The differential symptom and systemic invasion phenotypes have been mapped to eight amino acids within the 126 kDa protein open-reading frame (ORF). Single or multiple mutations within the codons encoding these eight amino acids yielded progeny viruses that were impaired further in systemic invasion than the M strain or intermediate of the M or U1 strain. These mutants and the M and U1 strains accumulated to similar levels in tobacco protoplasts, indicating that the 126 kDa proteins of the M strain and its mutants were not impaired in replicative functions. Possible effects of mutation of the 126 kDa ORF on expression of movement protein (MP) was investigated by detection of MP in infected leaf extracts and in protoplasts. The parental strains and all the tested mutants synthesized MP over a similar time scale and to similar amounts. The TMV 126 kDa protein appears, therefore, necessary for efficient systemic invasion of tobacco in addition to its role in replication. *(P)*

Immunocytolocalization of Host Translation and Cytoskeletal Factors to Cytoplasmic Bodies Containing Viral Replication or Coat Proteins

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Previous research from many laboratories has identified cytoplasmic bodies induced by infection of tobacco with the U1 strain of tobacco mosaic virus. The virally-encoded 126 kDa protein and 183 kDa protein which, respectively, modulates or is necessary for virus replication, have been localized to these structures. These structures, referred to as viroplasms in their immature stage and as X-bodies in their mature stage, have no confining membrane but contain electron-dense rope-like structures in a ribozyme-rich and membrane-containing matrix. The diameter of the rope-like structures was similar to that of microtubules. Because of this and for other reasons, we initiated a study to determine whether cytoskeletal and host translation factors could be immunolocalized in abundance to these structures. We have determined that X-bodies contain β-tubulin, a monomer necessary for microtubule formation, and elongation factor 1α (EF-1α), a host factor necessary for translation which has been shown to bind to the tRNA-like structure of TYMV and to microtubules. We have also observed β-tubulin within coat protein (CP) bodies adjacent to X-bodies formed in cells infected by a virion-defective, but CP-producing, mutant TMV. The possible relationships of X-bodies to viral replication, translation, and intracellular movement to the plasmodesmata were presented. *(L)*

Ultrastructure of Cells from Samsun Tobacco Leaves Infected by a New Heat-Resistant Strain of Tobacco Mosaic Virus (TMV)

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Ultrathin sections of Samsun tobacco leaves infected by a newly discovered heat-resistant strain of TMV isolated from Menoufia (Egyptian Nile Delta) showed two types of inclusions: in the form of needles or fibrous structures; and an X-body containing viral protein in the form of bands arranged singly or in groups. Virus particles were numerous in the cytoplasm of infected cells, accumulating within evaginations of the tonoplast protruding into the vacuole. Virus particles were also found in the vacuole. Cell wall thickenings were observed frequently. The infected nucleus did not seem to contain virus particles, but sometimes developed an irregular boundary. Mitochonodria were sometimes elongated and deformed. Some crystals in mitochondria-like structures were also found in the cytoplasm. The chloroplasts were reduced in size, grana were not well differentiated, thylakoids mostly of long profile, and a number of osmiophilic globules were distributed along the stroma. The chloroplasts did not contain virus particles. Viral proteins probably accumulated in sieve elements. *(P)*

Determinants of Host Range in Tobacco Mosaic Virus (TMV) Rakkyo Strain

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The rakkyo strain of TMV (TMV-R) infects rakkyo (*Allium chinense* G. Don), a monocot host which the common strain of TMV (TMV-U1) is unable to infect. However, TMV-R causes only latent infection in the inoculated leaves of *Nicotiana tabacum* cv. Bright Yellow (BY), whereas TMV-U1 infects BY systemically and induces mosaic symptoms. The determinants of those differences in host range have been studied by constructing chimeric viruses between the two strains. An examination of the infections in BY and rakkyo by several constructed chimeras showed that determinants defining the above differences reside on the 130K/180K replicases regions. *(P)*

Atomic Force Microscopy (AFM) Imaging of Three Plant Viral RNAs: Direct Evidence for the Conformational Versatility of RNAs

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The overall structural organization of three plant viral genomic RNAs has been investigated by *in situ* AFM. Tobacco mosaic virus RNA (TMV RNA), turnip yellow mosaic virus RNA (TYMV RNA) and satellite panicum mosaic virus RNA (SPMV RNA) were selected as model systems because (*a*) these viruses possess different dimensions and shapes, and (*b*) the sizes of their genomes vary from hundreds to several thousands of nucleotides. Stable and reproducible images have been obtained in air,

water, and propanol, and both lengths and heights have been measured. For the TMV RNA, several large structural domains have been identified, and the length of the extracted genomic RNA, 140 nm, is about twofold smaller than that of the virus particle. Moreover, several degradation states of its RNA, as well as a strong influence of the presence or the absence of magnesium ions on the TYMV RNA conformation, have been observed. For the SPMV RNA, three different aggregation states ranging from 38 nm to 200 nm in size were observed, and separated RNA particles of *ca* 4 nm in length are suggested to be the genomic RNA. This is the first attempt at imaging single-stranded RNAs by AFM, a technique which should, in future, be considered as a powerful tool to study, under various experimental conditions, the conformational versatility of RNAs. *(P)*

Characterization of Host Plant Proteins Interacting with Tobamovirus Movement Proteins (MP)

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Plant virus-encoded MPs are required for active transport of viral RNA from infected cells to adjacent healthy ones. An increasing amount of experimental data suggest that MPs may interact with plasmodesmal proteins to effect an increase in plasmodesmal permeability. Our recent results show that recombinant 29 kDa MP of cruciferous tobamovirus specifically binds *in vitro* to several proteins from cell walls of tobacco plants. Results of further comparative study of cruciferous tobamovirus MP and TMV MP interaction with cell wall proteins from tobacco and turnip plants were presented. *(P)*

The Hypersensitive Response to Cucumber Mosaic Virus (CMV) in *Vigna unguiculata* Affects Viral RNA Accumulation in Protoplasts

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The hypersensitive response (HR) induced by most strains of CMV in cowpea (*Vigna unguiculata*) was mapped to specific nucleotide changes at positions 1978 and 2007 of Fny-CMV RNA 2. Both nucleotide changes in Fny-CMV RNA 2 to those seen in systemically infecting B-CMV, resulted in systemic infection of the mutated Fny-CMV. The double mutant showed increased accumulation of viral RNA in cowpea protoplasts *vs* Fny-CMV, after 20 h post-inoculation (p.i.), due to a lag in accumulation of Fny-CMV RNAs at 15–18 h p.i. There was also a specific decrease in the Fny-2a protein level after 6 h p.i. Mutation of only Fny-CMV RNA 2 position 1978 yielded a virus that induced an HR that did not inhibit virus spread, leading to systemic necrosis. This mutant showed no lag in viral RNA accumulation in protoplasts. This indicates that there are two separate mechanisms for HR and the inhibition of virus movement. *(L)*

Host Range and Symptom Determinants of Cucumber Mosaic Virus (CMV) and Tomato Aspermy Virus (TAV)

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CMV and TAV belong to the cucumovirus group, but their host range and symptomatology are clearly different. In our study we used cucumber and *Nicotiana glutinosa* plants. Cucumber is a natural host of CMV, but our P-TAV isolate does not infect cucumber. On *N. glutinosa* plants the R-CMV has very strong symptoms, there is strong leaf curling and severe stunting, whereas on the P-TAV-infected plants there is blotchy mosaic but no stunting. Both protoplasts and plants were used to study the background of these features by employing pseudorecombinant and artificial recombinant viruses. We introduced an NdeI restriction site in exactly the same position, just preceding the coat protein (CP) gene, in the cDNA clones of P-TAV RNA 3 and R-CMV RNA 3. Using these restriction sites we created two recombinant clones: TR3 contains the 5' non-coding region, the 3a gene and the intergenic region of the T3 clone, and the CP gene and the 3' non-coding region derived from the R3 clone. The RT3 clone was the converse of TR3, with the 5' part of R3 and the 3' part of T3. Using these clones we synthesized transcripts *in vitro*. In tobacco and cucumber protoplasts all the viruses, pseudorecombinants and recombinants replicated. Only CMV caused local and systemic symptoms on cucumber plants; R1R2T3 and R1R2RT caused local infection; and TlT2R3 and TAV did not infect cucumber at all. This confirms that some of the host range determinants are located on RNA1 and/or RNA2. On *N. glutinosa* plants. R1R2T3 and R1R2RT caused symptoms similar to those of TAV, whereas T1T2R3 caused symptoms more similar to those of CMV. This suggests that the CP is a major determinant of symptom expression in this host. *(L)*

Altered Local and Systemic Spread of Movement-Deficient Virus in Transgenic Tobacco Plants Expressing the Cucumber Mosaic Virus (CMV) 3a Protein

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The 3a protein encoded by RNA 3 of CMV has been identified as the cell-to-cell movement viral protein. The constitutive expression of 3a protein in transgenic tobacco plants is able to complement in *trans* the short-distance movement of 3a defective CMV mutants. This ability depends upon the accumulation levels of 3a protein in transgenic tobacco plants. However, an initial delay in viral accumulation and spread of the defective virus when compared with the wild-type virus was determined in complementation tests. Furthermore, a reduction in disease symptoms as well as a different pattern of long-distance movement from that of the wild-type virus was determined. These results show that the early events in viral infection affect the long-distance spread of the virus. *(P)*

Molecular and Biological Variability of Lettuce Mosaic Potyvirus (LMV)

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A collection of ten LMV isolates was characterized by their ability to overcome the three resistance genes described in lettuce cultivars (*Lactuca sativa*). Several pathotypes could be defined in our study. An immunocapture-RT-PCR assay was developed in conjunction with direct sequencing of the amplified fragments to study the molecular variability of LMV. The N and C terminal regions of the coat protein gene, localized in the 3' part of the genome, and the 5' end region were amplified and sequenced for the ten LMV isolates. Phylogenetic analysis performed using these data showed the existence of two major groups of LMV isolates with limited intra-group variability. Our results indicate that there is little sequence variability among LMV isolates but that biological variability is important. In addition, there is no obvious correlation between molecular and biological

variabilities. *(P)*

Pathogenic Interactions during Infection of *Arabidopsis* by Cauliflower Mosaic Virus (CaMV)

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We have been investigating the pathogenic interactions of the plant pararetrovirus, CaMV, with *Arabidopsis thaliana* in an attempt to understand the molecular genetic factors controlling symptom expression. A broad range of symptom characters is expressed in *Arabidopsis* ecotype Columbia when infected by different CaMV isolates obtained worldwide. Some isolates which produce symptoms in other susceptible hosts such as turnip were found to be asymptomatic in *Arabidopsis*. CaMV isolate Bari-1 produces mild symptoms in turnip and *Arabidopsis* ecotype Landsberg, but is asymptomatic in ecotype Columbia. However, differences in the pathogenic character of the interactions are dependent upon environmental factors. For instance, high light intensity can suppress symptom development and viral replication. We have also isolated *Arabidopsis* mutants with altered responses to CaMV infection. One such mutant (dv1) has a variant phenotype and shows delayed symptom development upon infection with CaMV. During propagation of dv1, a phenotypic revertant (dv1R) arose with wild-type plant characteristics but an altered response to CaMV. The significance of these observations in understanding compatible host/virus interactions was discussed. *(P)*

Symptom-like Phenotype of Transgenic *Arabidopsis* Expressing the Cauliflower Mosaic Virus (CaMV) Gene VI Protein

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Gene VI of the plant pararetrovirus CaMV, has been shown to be an important determinant of symptom response in a variety of infected host species. We have introduced into *Arabidopsis*, Gene VI from mild (Bari 1) and a severe (B-JI) isolate of CaMV, plus a recombinant virus (Baji 31) containing a hybrid Gene VI. The construct used a highly efficient promoter to maximize expression of the transgene product. As a background, two *Arabidopsis* ecotypes which show CaMV isolate-dependent differences in symptom expression were used. Approximately one-third of plants containing one or more copies of the transgene exhibited obvious symptom-like phenotypes. These included chlorosis, stunting and, in several plants, vein clearing together with dark green interveinal islands, similar to the symptoms induced by severe strains. Each of the three Gene VI transgenes was capable of inducing these phenotypes. Levels of Gene VI protein, determined serologically, varied by more than an order of magnitude between transgenic lines. The severity of the phenotype was strongly correlated to both the level of Gene VI protein expressed and the origin of the transgene. The correlation between phenotype severity and Gene VI origin was in the order Bali 31 > B-J1 > Bari 1; this order parallels that of severity of symptom expression in plants infected by the three strains. The importance of both level of expression and the nature of the Gene VI polypeptide, in modulating symptoms expression, was discussed. *(P)*

Petunia Vein-Clearing Virus (PVCV), A Pararetrovirus that Also Exists as a Retroelement in the Chromosome of Its Host

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The PVCV, a tentative caulimovirus, was confirmed to be graft-transmissible and also to be transmitted through a large portion of petunia seed. Infected petunia contains a 7–8 kilobase pair (kbp) DNA unique to diseased plants. Inferred amino acid sequences revealed protein domains typical of caulimo- and badnaviruses when cloned segments of PVCV DNA were sequenced. Further experiments with virus-specific clones, used as hybridization probes, showed that seemingly healthy petunia DNA released fragments of the virus genome when subjected to restriction endonuclease digestion. Further analyses revealed that diseased but not healthy petunia contain a rapidly migrating component in agarose gels of 7–8 kbp that reacted with the PVCV probe. In addition, higher molecular weight, more slowly migrating cellular DNA of both healthy and infected petunia gave positive hybridization signals with the PVCV probe. However, this was not true of the DNA from *Nicotiana glutinosa* which had been infected by graft transmission of PVCV. The DNA of this host contained only the 7–8 kbp component that reacted with the PVCV probe. These and other observations indicate that the PVCV genome exists in petunia in an integrated form, or retroelement, which is propagated from generation to generation through the germ cells of petunia. *(L)*

Does the Exchange of the Coat Protein Genes between Potyviruses Affect Their Ability to Infect Hosts?

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The role of the coat protein (CP) of potyviruses in infection of different hosts was examined by exchanging the CP gene between zucchini yellow mosaic virus (ZYMV) and tobacco vein mottling virus (TVMV). ZYMV shows systemic infection on cucurbits, whereas TVMV is limited to tobacco and a few other Solanaceae. A common systemic host for both viruses is *Nicotiana benthamiana*. Almost all the CP gene of ZYMV was replaced by the respective region in the CP gene of TVMV (leaving the ZYMV cleavage site at the N' terminus), thus creating a hybrid full length clone. The integrity of the hybrid cDNA was ascertained by restriction analysis, sequencing of the chimeric CP region and *in vitro* translation of transcripts. The chimeric full length clone was tested either by transcribing the cDNA under the T7 promoter or by direct use of the cDNA under the 35S promoter. The transcripts and clones were inoculated to squash, *N. benthamiana* and *Chenopodium amaranticolor*. All the attempts to infect either squash or *N. benthamiana* were negative even when bombarded using highly efficient infection methods. Occasional infections were recorded on the local lesion host *C. amaranticolor*. The cell-to-cell movement function that was recently assigned to the CP, may provide an explanation for the lack of systemic infectivity of the chimeric virus. This issue is being investigated by inoculating *N. benthamiana* protoplasts with RNA transcripts of the hybrid virus, to prove replication. *(P)*

Formation and Immunogold Localization of Inclusion Bodies in Maize Plants Infected by Maize Dwarf Mosaic Virus (MDMV)

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The development of cylindrical inclusion (CI) in leaf cells of sweet corn (*Zea mays* L.) leaf tissue systemically infected with MDMV strain A was monitored. The principal viral genome-encoded nonstructural protein associated with these bodies was purified and a polyclonal antiserum was prepared. The purified MDMV-CI '70 KD' protein was reacted in Western blots with this antiserum, and also reacted by cross-reactivity with TEV-CIP and TMV-126 KDP. Ultrathin sections of MDMV-infected leaves were treated with goat anti-rabbit antibodies conjugated to 15-nm gold particles after the sections had been exposed to rabbit antisera specific to MDMV particles, MDMV capsid or 70 KD CIP. Anti-MDMV or anti-MDMV capsid serum specifically detected virus particles and capsid protein in the cytoplasm. The 70 KD CIP was found in the pinwheel inclusions and laminated aggregates. *(P)*

Facilitated Amplification of Infectious Full-Length Clones and Their Use in Analysis of Potyvirus Pathogenesis

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Investigation of virus–host plant interactions often starts with analysis of the virus genome in order to identify the viral determinants of various pathogenic characters. Full-length infectious clones are important tools for these studies since they allow generation of hybrids of viruses differing in specific characters and introduction of specific mutations into the virus genome. However, generation of full-length clones of some viruses has proven difficult due to instability or toxicity of the viral sequences in *Escherichia coli* in which the full-length clones are amplified. Using two isolates, P-1 DPD1 and P-4 NY, of pea seedborne mosaic potyvirus (PSbMV), a strategy was developed which allowed assembly of an infectious full-length clone of P-4 NY which was refractory to cloning using conventional methods. This strategy also greatly facilitated amplification of full-length clones of P-1 DPD1. Several hybrid, mutant, and reporter labeled viruses have now been generated and the identification of determinants of seed transmission, pathotype and long-distance movement of PSbMV is in progress. *(P)*

Long-Distance Movement of Pea Seedborne Mosaic Potyvirus (PSbMV) in *Chenopodium quinoa*

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PSbMV isolates differ in their ability to systemically infect *Chenopodium quinoa*. The isolates P-1 DPD1 and P-4 NY both move from cell to cell in inoculated leaves. P-1 DPD1 also moves long distance to establish systemic infection whereas P-4 was never detected beyond the petiole of the inoculated leaves. To identify the viral gene product(s) which determines long distance movement, a number of hybrids between P-1 DPD1 and P-4 NY were generated and analyzed for their ability to move to uninoculated leaves of *C. quinoa*. The P-1 DPD1 coat protein (CP) coding region was found to be both necessary and sufficient for long-distance movement since all hybrids carrying the P-4 NY CP were restricted to inoculated leaves whereas hybrids carrying the P-1 DPD1 CP moved to uninoculated leaves. To identify the tissues in which CP host-factor interactions determine further progress of PSbMV in *C. quinoa*, the distribution and spread of P-1 DPD1 and P-4 NY were visualized by tagging the viruses with reporter genes. The results of these experiments were presented. *(P)*

Profile of Seedborne Viruses of *Pisum* and *Vigna* Crops in Northern India

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Some 1400 seed lots of pea, cowpea, mungbean and urdbean were collected from different parts of northern India and tested in the laboratory for the presence of seedborne viruses on the basis of transmission, serology and reactions on diagnostic hosts. Three seedborne viruses, *viz.*, pea seedborne mosaic, bean yellow mosaic and cucumber mosaic, were identified in pea seeds. Similarly, alfalfa mosaic virus (AMV), cowpea aphid-borne mosaic virus (CABMV), black eye cowpea mosaic virus (BECMV), southern bean mosaic virus-cowpea strain (SBMV-CS), cowpea mosaic virus (CPMV) and cowpea mild mottle virus (CMMV) belonging to five taxonomic groups were found to be transmissible in cowpea. The rate of seed transmission based on virus and cultivar interaction varied from 7.04% to 32.05% of AMV, 8.45% to 42.10% of CABMV, 2.35% to 7.77% of SBMV-CS, 8.10% to 20.93% of BECMV, 1.14% to 4.70% of CPMV and 1.00% to 3.00% of CMMV. Mungbean and urdbean mosaic virus and urdbean leaf crinkle virus have been found seed-transmissible in mungbean and urdbean, respectively. Transmission of viruses through seeds tends to assure viral perennation and long distance dissemination with the movement of contaminated seeds. The currently used methods of monitoring the presence of seedborne viruses for the production of foundation and certified seed are only *via* visual inspection: once before flowering and a second time at the flowering and fruiting stage. These methods are not adequate for detecting and identifying the presence of viruses in a seed crop. For strengthening seed certification programs it is, therefore, essential to implement improved methods of detecting and identifying viruses along with more realistic sampling procedures. *(P)*

Identification and Characterization of Blackeye Cowpea Mosaic Virus (BLCMV) and Cowpea Aphid-Borne Mosaic Virus (CABMV) (Potyviruses) Serotypes

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Surveys were conducted in various cowpea-producing areas (both cultivated fields and experimental plots) of Togo (West Africa) to detect CABMV and BLCMV serotypes and to identify cowpea varieties resistant to these viruses. Serological tests (ACP and DAS-ELISA) with biotin-labeled monoclonal and polyclonal antibodies were used to identify BLCMV and CABMV serotypes of both potyviruses; BLCMV (serotypes A and B) and CABMV (serotypes C to G) were identified in the major cowpea-producing areas. Serotypes of one or both viruses were detected in cowpea but also in the following weeds: *Acanthosperum hispidum* (Compositae), *Cassia occidentalis* (Caesalpinaceae), *Centrosema pubescens, Desmodium tortuosum* (Fabaceae), *Pupalia lappacea* and *Passiflora foetida* (Passifloraceae). Four cowpea cultivars of the 21 screened using the CABMV serotype C (the most virulent of the serotypes) were resistant to this serotype. *(P)*

Studies of the Movement of Cowpea Mosaic Virus (CPMV) Using the Jellyfish Green-Fluorescent Protein

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The gene encoding the jellyfish green-fluorescent protein (GFP) was used to study the movement of CPMV in plants. In initial experiments the gene was used to replace the coat protein (CP) genes in a full-length cDNA clone of CPMV RNA 2. Transcripts of this construct were replicated in the presence of wild type (wt) RNA 1 in protoplasts, and GFP expression could be readily detected by fluorescent microscopy. It was not possible to infect cowpea plants with these transcripts; however, when combined with a mutant RNA 2, in which the movement protein (MP) gene has been deleted, an infection did occur. After 3 days green fluorescent spots were visible under UV light on the inoculated leaf. In some experiments a systemic infection occurred but green fluorescence was never observed in these tissues. Presumably these systemic infections were the result of a recombination event between the two mutant RNA 2's. Subsequently the GFP gene was introduced between the MP and CP genes of RNA 2, utilizing artificial proteolytic processing sites for the viral proteinase. This CPMV-GFP was highly infectious on cowpea plants and the green fluorescent spots that developed on the inoculated leaves were larger and brighter than those produced by the three RNAs described above. Also in systemically infected tissues green fluorescence was now evident. This makes this virus a useful tool for further studies of the accumulation and spread of CPMV in the plant. When GFP was fused to the C-terminus of the MP, this MP fusion still was able to form tubular structures in protoplasts, which now could be visualized directly by fluorescence microscopy. This MP-GFP fusion will be used to study the intracellular localization of the MP in living cells. *(P)*

Identification of Distinct Functional Domains of the Cowpea Mosaic Virus (CPMV) Movement Protein by Mutational Analysis

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The genome of CPMV is divided over two positive-sense RNA molecules. The RNA 2-encoded 48K movement protein (MP; consists of 342 amino acids) is capable of forming tubules on protoplasts. These tubules contain virus particles and are also found in infected plants, where they are located in presumably modified plasmodesmata. To determine whether distinct domains of the MP are involved in different steps in the cell-to-cell movement process, mutations were induced in the MP coding region within a full-length cDNA clone of RNA 2. The mutant proteins were analyzed for their targeting properties and their ability to form tubules on protoplasts. To date, we have identified two distinct domains, one necessary for tubule formation (encompassing the first 313 amino acids), and a second probably involved in the incorporation of virus particles in the tubule, located in the C-terminal part of the protein (between amino acids 314 and 331). Further research will focus on a more detailed analysis of these and other domains. To identify a domain of the MP involved in targeting to the plasma membrane and/or plasmodesmata, mutant MPs will be fused with the jellyfish green-fluorescent protein. The subcellular localization and distribution of these proteins will be studied in inoculated protoplasts by fluorescence microscopy of living cells. *(P)*

A Coat Protein Determinant of Cowpea Mosaic Virus (CPMV) Movement

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CPMV particles contain 60 copies of a large (L) and a small (S) coat protein. Two forms of virions (fast and slow) can be isolated on the basis of their differing electrophoretic mobilities. The slow form can be converted to the fast form by the loss of 24 amino acids from the carboxy-terminus (C-terminus) of the S protein. These two forms have identical specific infectivities and particle stability and thus the C-terminus of the S protein appears to play no role in the structure of the particles. When stop codons were introduced to give S proteins truncated after the cleavage site, the mutant viruses – although infectious – had reduced cell-to-cell spread and did not produce systemic symptoms. After a single passage, reversions occurred which restored the S protein to its wild-type length and led to the development of systemic infections. To prevent this reversion, a series of deletion mutants were constructed. Providing the deletions did not extend beyond the point where cleavage occurs, infectious, stable particles were produced. However, cell-to-cell spread was slower than wild-type. In addition, systemic spread was delayed or abolished. These results indicate that the cleavable C-terminus of the S protein plays a role in virus movement in infected plants *(P)*

Cell-to-Cell Transport of the Barley Stripe Mosaic Virus (BSMV) Hybrids with Replacement of the Triple Gene Block for the Foreign Movement Protein Genes

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Hordeiviruses, tobamoviruses and dianthoviruses are the groups of positive-strand RNA plant viruses. The type members of these groups – BSMV, tobacco mosaic virus (TMV), and red clover necrotic mosaic virus (RCNMV) – can infect systemically *Nicotiana benthamiana* plants. The natural host of BSMV is cereals, whereas RCNMV and TMV infect dicotyledonous plants. To investigate the influence of the movement protein (MP) genes of BSMV and TMV on the host range determination and symptoms of infection, we constructed chimeric BSMV cDNAs with replacement of the triple gene block for the MP genes of TMV and RCNMV. These recombinant genomes were used for inoculation of *Chenopodium amaranticolor* and *N. benthamiana.* It was found that artificial viruses gave rise to local lesions in *C. amaranticolor* and move from cell-to-cell in inoculated leaves in *N. benthamiana.* Nevertheless, specific hosts (barley and tobacco) were resistant to hybrid viruses. *(L)*

Comparative Studies of the Gene Structure and Functions between Different Hordeiviruses

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The tripartite genome of barley stripe mosaic virus (BSMV), the type hordeivirus, comprises RNAs designated α, β and γ. We have cloned and sequenced the genomic RNAs of two other members of the hordeivirus group, poa semilatent virus (PSLV) and lychnis ringspot virus (LRSV). Deduced genetic organization of PSLV and LRSV genome components is similar to the analogous BSMV RNAs. The analogous BSMV, PSLV and LRSV proteins exhibit considerable similarity in their primary structure, confirming a close relation with the hordeivirus. The portion of BSMV triple gene block has been replaced by respective regions of PSLV and LRSV RNAs and the chimeric RNA transcripts have been used together with infectious BSMV RNAα and

RNAγ transcripts for inoculations on different host species. Some of the hybrids were infectious to *Chenopodium amaranticolor* and produced symptoms different from BSMV in phenotype and time of appearance, indicating that determinants located in the triple gene block can affect hordeivirus symptom expression. *(P)*

Characterization of a Brome Mosaic Virus (BMV) Movement Protein Expressed in *Escherichia coli*

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The movement protein (MP) gene of BMV was cloned into a pT7-7-vector and expressed in strain BL21 (DE3) of *Escherichia coli*. Using the purification protocol of Citovsky *et al.*, milligram quantities could be obtained easily from 1 *l* cultures. The protein was renatured in 10 mM Tris buffered with HCl at pH 8.0 containing 200 mM NaCl, 1 mM EDTA, 10% (v/v) glycerol, 1 mM dithiothreitol, 1 mM phenylmethylsulfonyl fluoride and 0.4% (v/v) Nonidet P40 detergent and appeared to be stable for months at 4°C. Culturing at room temperature prevented the accumulation of the protein in inclusion bodies and allowed purification without denaturing agents. Binding studies of the protein with nucleic acid showed preferred binding to single-stranded over double-stranded DNA. Gel retardation studies with oligonucleotides of 100 and 500 bases also indicated preferred binding to single-stranded DNA. The results of the nucleic acid binding were discussed in view of the observation that also MP of BMV formed tubules *in vivo* when cowpea protoplasts were infected by BMV. *(P)*

Electron Microscopy of Plants Infected by Viruses

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Ultrathin sections of leaves of wheat, ryegrass, oat, clover and radish plants infected by viruses were studied. The investigation revealed a slight destruction of cell organelles of diseased plants. The cylindrical inclusions and the flexuous and spherical virions were observed in the cytoplasm of cells of wheat plants infected by wheat streak mosaic virus and brome mosaic virus. The bacilliform virions, the tubular structures of nucleocapsids and the viroplasm were observed in the cells of oat plants infected by the cereal mosaic and cereal pseudorosette viruses. Ellipsoid single-membrane-bounded inclusions, containing finely filamentous (4–6 nm) material, were observed in the cytoplasm of cells of ryegrass plants affected by virus-like spot mosaic. It is possible that the filaments are a tenuivirus. The spherical virions were observed in cells of radish infected by radish mosaic virus and in cells of red clover infected by red clover mosaic virus. The inclusions of the tubular structures were revealed in the cytoplasm of cells of the diseased clover. *(P)*

Subcellular Localization of Bamboo Mosaic Potexvirus (BaMV) 28-kDa Protein

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The 28-kDa protein of BaMV, encoded by the open reading frame 2 of BaMV genome, is the first gene product of the overlapping triple gene block. This protein is thought to play a role in the cell-to-cell movement between cells. We have produced a polyclonal antibody to the 28-kDa protein to investigate its synthesis and subcellular location. Immunoblot analyses of infected tissue extracts revealed that this protein was mainly in the insoluble fractions (cell wall and P30). This correlates with immuno-electron microscope observations. The 28-kDa protein was shown to be associated with the electron-dense crystalline bodies in both cytoplasm and nuclei of the infected tissue. Virion aggregates were often detected in the periphery of the cytoplasmic inclusions associated with the 28-kDa protein. *(P)*

Chromatin Structure and Gene Regulation of Abutilon Mosaic Virus

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Abutilon mosaic virus is a phloem-limited biparite geminivirus and replicates *via* a double-stranded DNA intermediate packed in minichromosomes. Most frequently, 12 nucleosomes cover the genomic DNA circle, leaving a space of *ca* 200 basepairs free for the interaction with host factors. This nucleosome-free gap was mapped with a combination of nuclease sensitivity assays and ligation mediated polymerase chain reactions (LMPCR). With these methods it was possible to localize the gaps *in situ, e.g.* in purified nuclei of infected plants, and to represent the interaction of host proteins and virus in phloem cells. Nuclease-sensitive sites were found in DNA A as well as in DNA B in the common region which harbors promoter elements for viral and complementary transcription and at a second location in front of the second complementary transcript for AC2/AC3 and BC1. We propose a sliding nucleosome model for gene regulation with three alternative states of the minichromosomes: completely packed in nucleosomes, open in the common region, and open in the second promoter structure. *(L)*

Role of the Triple Gene Block Proteins in Potato Virus X (PVX) Intercellular Transport

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Three proteins, termed triple gene block proteins, encoded by the PVX genome, are required for intercellular transport of the virus. Whereas the 25-kDa protein is required for plasmodesmata gating, the separate contributions of this protein, the 12-kDa and 8-kDa proteins to the transport process have not been characterized. To understand the interactions of these proteins with each other and with the host, we fused the coding region of the green fluorescent protein (GFP) separately to the 3' end of each of the triple gene block open reading frames (ORFs). The subcellular location of the GFP fusion proteins was examined by confocal microscopy. Fusion of the GFP coding region to the 12-kDa protein and 25-kDa protein coding regions resulted in deletion of either the downstream 8-kDa ORFs or 12- and 8-kDa ORFs, respectively. To determine whether the specific subcellular location of the 25-kDa and 12-kDa proteins is altered in the absence of the remaining triple gene block proteins, PVS viruses expressing these fusion proteins were used to inoculate wild-type and transgenic tobacco plants expressing the triple gene block proteins. Finally, to determine whether the site of subcellular accumulation of these proteins is essential for their function, a series of random mutations was introduced into the triple gene block coding region of these viruses. The effects of these mutations on protein function and subcellular localization were discussed. *(P)*

Understanding Homology-Dependent Virus Resistance in Transgenic Plants

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We have investigated the behavior of potato virus X (PVX) on plants exhibiting homology-dependent resistance (HDR).

Initial experiments involved expression of the PVX RdRp open reading frame under control of a strong constitutive promoter. A surprising link was found between virus resistance and low accumulation of the transgene mRNA. These and other observations suggested that virus resistance and low mRNA accumulation were consequences of the same post-transcriptional gene silencing mechanism. To investigate this phenomenon further, we used PVX as a vector to insert marker genes of nonviral origin. Transgenic plants exhibiting post-transcriptional silencing of homologous marker genes did not support infection by these PVX derivatives. A PVX derivative with an inserted β-glucuronidase (GUS) gene has been used to localize the RNA target for HDR in two transgenic tobacco lines to the 3' region of the GUS coding sequence. Curiously, methylation of the GUS transgene in these lines is also localized in the 3' region. This concurrence suggests an intimate relationship between DNA methylation and the RNA target of HDR. A model to explain this relationship and other aspects of HDR was presented. *(P)*

Localization of a Virulence Determinant(s) in a Group 1 Strain of Potato Virus X (PVX)

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Resistance to PVX is commonly found in potato cultivars. There are two types of resistance: extreme resistance (immunity), provided by the Rx genes; and hypersensitive resistance (HR), controlled by the two N genes, Nx and Nb. Phenotypically, the resistance provided by the N genes is a localization of the virus with formation of necrotic lesions around the site of infection. HR resistance depends on a highly specific interaction between the product of a resistance gene in the host (Nx, Nb) and a determinant present in the avirulent pathogen: hypersensitive necrosis is induced in the plant as a consequence of this recognition event. PVX group 1 strains elicit HR resistance in cultivars carrying the Nb gene, whereas group 3 strains infect Nb cultivars systemically. Both viruses elicit HR resistance in cultivars carrying the Nx gene. The complete nucleotide sequence of a full-length cDNA of PVX-Roth 1, a group 1 strain, was determined and was found to be 95% identical at the nucleotide level to the group 3 strain PVX-UK3. Infectious cDNA clones were constructed and used to generate viral hybrids between Roth 1 and UK3. The avirulence phenotype of the hybrids was determined by inoculation onto potato cultivars carrying different combinations of the genes Nx and Nb. This analysis of the hybrids revealed that as for UK3, the Nx determinant is localized in the coat protein gene of Roth 1. The Nb determinant has been localized within a region of Roth 1 which encompasses the C-terminal end of the replicase open reading frame (ORF) and part of the 25K ORF. Both these regions encode a single amino acid difference when compared with UK3. We are currently analyzing the avirulence phenotype of Roth 1-UK3 hybrids which differ with respect to these single amino acids. *(P)*

Studies of Potato A Potyvirus (PVA) Using Infectious Transcripts from a Full-length cDNA: Elicitation of Hypersensitive Resistance Responses in Potato, and Aphid-Transmissibility

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A full-length cDNA clone, from which infectious transcripts can be produced, was obtained from the B11 isolate of PVA (PVA-B11). The coat protein (CP) genes of two additional PVA isolates (PVA-U and PVA-M) were cloned and sequenced. PVA-U elicits a hypersensitive response (HR) whereas PVA-B11 and PVA-M cause mottle symptoms with no HR in potato cv. 'King Edward' carrying the resistance gene Na_{KE}. The significance of the CP gene for elicitation of HR was studied by replacing the CP gene of PVA-B11 with the CP gene of PVA-U. The recombinant virus caused no HR in cv. 'King Edward', indicating that the CP gene was not the elicitor of Na_{KE}. PVA-B11 has a DAS amino acid motif at the CP N'-terminus and is not aphid-transmissible, whereas PVA-U has a DAG motif and is aphid-transmissible. A point mutation changing the DAS motif to DAG converted PVA-B11 to aphid-transmissible. Similarly, when the CP gene of PVA-B11 was replaced by the PVA-U CP gene, the recombinant virus was aphid-transmissible. *(P)*

Nucleic Acid-Binding Properties of the Potato Leafroll Virus (PLRV) ORF-3min Protein

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The genome of the PLRV consists of a positive-sense RNA of ~5.8 kb and encodes six major open reading frames (ORFs; 1, 2a, 2b, 3, 4 and 5). In the C-terminal sequence of ORF 2a coding for a protein with helicase motifs, there is a small 5.5 kDa protein, namely, ORF-3min. The *in vivo* function of this putative minimal protein is not known. We have studied the capacity of the ORF-3min protein expressed in *Escherichia coli* as a glutathione-S-transferase fusion protein to bind nucleic acids *in vitro* by UV-crosslinking and electrophoretic mobility shift assays (EMSA). The 3min protein consisting of 45 amino acids bound both RNA and DNA in a cooperative manner and the binding was not sequence-specific under the experimental conditions employed. ORF-3min bound single-stranded as well as double-stranded nucleic acids. The nucleic acid-binding properties of ORF-3min strongly suggest that this protein may play an important role *in vivo* during virus replication. *(P)*

Mutational Analysis of Virion Sense Genes of Tomato Yellow Leaf Curl Virus (TYLCV)

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Deletion, frameshift, and single amino acid mutations were inserted into open reading frames (ORFs) V1 and V2 (capsid protein) of TYLCV. The ability of these mutants to replicate, to spread and to induce symptoms was tested both in leaf discs and in intact plants. A deletion mutant that lacked the carboxy half of the coat protein (CP) gene and a frameshift mutant, in which an early termination resulted in removal of 13 amino acids from the carboxy terminus of the virus CP and changing of an additional nine amino acids, were found non-infectious. Two other double mutants, in which a single amino acid was changed in the overlapping part of V1 and V2, and one mutant in which a deletion was introduced exclusively to V1, were able to spread systemically, but infections remained symptomless; the synthesis of ssDNA was significantly lower than in an unmutated clone. The latter three mutants were acquired and transmitted by *Bemisia tabaci*. *(L)*

Directed Adaptation of Tomato Spotted Wilt Tospovirus (TSWV) by Reassortment on Resistant Plants

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The biological and genetic diversity of TSWV may be shaped by a combination of reassortment of tripartite genomic segments (L, M, S) and selective host genetics in addition to mutation and recombination. In this study we demonstrated that transgenic resistance is a selective force which drives adaptive reassortment of genomic segments, resulting in the generation of a new strain capable of overcoming resistance. In order to determine whether a viral population could be directed by resistant plants to form a resistance-breaking strain through reassortment, two parental isolates, TSWV-D and TSWV-10, were co-inoculated on normal *Nicotiana tabacum* cv. Burley 21 (susceptible) and transgenic Burley 21 (resistant). Neither of the parental strains was capable of overcoming the resistance. The genomic segments from each strain were monitored following each sequential transfer. The viral population remained a mixture on the susceptible tobacco after seven transfers. However, the mixed population was driven to form a pure reassortant, $L_{10}M_{10}S_D$, on the resistant line. Our experiments revealed that genomic reassortment is a mechanism utilized by TSWV to overcome host resistance. *(P)*

Serological Comparison of Tospoviruses with Polyclonal Antibodies Against the Main Structural Proteins of Tomato Spotted Wilt Virus (TSWV)

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A new purification procedure for tospoviruses of serogroups II and III was developed. SDS-PAGE of purified TSWV (serogroup I); tomato chlorotic spot virus (TCSV; serogroup II); groundnut ringspot virus (GRSV; serogroup II); and Impatiens necrotic spot virus (INSV; serogroup III) showed that G2 from members of serogroups II and III differs significantly in size from that of serogroup I. Western immunoblots analysis using polyclonal antibodies against purified TSWV-G1 and G2 (native) and denatured hydrophilic parts of TSWV-G1 and G2, demonstrated a higher homology among G1 of different serogroups than for G2. These results are supported by homology comparisons for the nucleoprotein and glycoprotein gene sequences of different serogroups. *(P)*

Localization of Tomato Bushy Stunt Virus (TBSV) Genes Responsible for Host Specificity

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Systemic infection occurs if the virus has the ability to replicate, to move from cell-to-cell, and to move long distances through the vascular system of a host plant. In many cases the inability of a virus to infect a particular plant can be explained by the disruption of one of these functions. In the present study TBSV pepper isolate (TBSV-P) and cymbidium ringspot virus (CymRSV) were used to determine viral genes involved in host specificity and symptom development. Both viruses were assigned to the genus *Tombusvirus* as a definitive member. TBSV-P and CymRSV have very similar primary structure and genome organization. However, we have found that pepper plants can be infected systemically by TBSV-P, whereas CymRSV is not able to infect this plant. These observations and the availability of biologically active cDNA clones for both viruses prompted us to construct chimeric viruses. To test the role of factors contributing to host specificity, we analyzed the biological activity of hybrid viral RNAs constructed by exchanging the genes between TBSV-P and CymRSV. The resulting chimeras were able to direct systemic infection of *Nicotiana benthamiana*, indicating that they retain basic competence for infection. However, their failure or success to infect pepper demonstrated that, for successful infection of this selective host, the virus gene(s) must be specifically adapted to the host. *(P)*

Differences in Biological and Physical Properties and Nucleotide Sequences Among Isolates of Prunus Necrotic Ringspot Virus (PNRSV)

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PNRSV, like the other members of the Ilarvirus group, is a divided genome virus, consisting of at least three particle types. Each of the particles contains an encapsidated RNA molecule of different size: RNA1, RNA2 and RNA3. It is presumed that, as in the case of other members of the Bromoviridae family, RNA1 and RNA2 are monocistronic, and proteins coding by them are components of viral replicase. RNA3 contains two open reading frames (ORFs). Protein coded by an ORF at the 5' end of the molecule shares close homology to cell-to-cell movement proteins (MP) of other known Ilarviruses. The ORF of the 3' end of the RNA3 is the coat protein (CP) gene. 3' UTR of RNA3 contains many AUGC repeats which are presumed to be involved in the initiation of the replication of viral RNA.

We have collected several isolates of PNRSV differing in their biological and physical properties. These isolates were tested for such features as: reaction of test plants to inoculation (deformation, chlorosis, necrotic spots, etc.), thermal inactivation point, longevity *in vitro* at room temperature, etc. Genomic RNAs from each of the PNRSV isolates were purified and partial cDNAs of RNA3 were synthesized using reverse transcription-polymerase chain reaction (RT-PCR). Fragments including 3'-end of MP, interval non-coding sequence and 5'-end of CP were sequenced and analyzed using MACAW software (NCB1). *(P)*

Nucleotide Sequence of the 3'-Terminal Region of the Sour Cherry Strain of Plum Pox Potyvirus (PPV): Evidence for a New Group of PPV Strains

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The sequence of the 3'-terminal 1360 nucleotides of the sour cherry strain of PPV (PPV-SoC) from Moldova has been determined and compared with that of several PPV isolates. The predicted amino acid sequence included 48 C-terminal amino acids of the NIb protein and 332 amino acids of the coat protein (CP) gene. At the nucleotide level, a surprisingly low (74.2% to 78.3%) degree of similarity has been found between PPV-SoC and other isolates of PPV. This is due to the high nucleotide divergence between the N-terminal region of the CP gene of PPV-SoC and that of other isolates. The sequence of the C-terminal region of PPV-SoC CP and the adjacent 3' non-coding region revealed up to 94% similarity to that of other PPV isolates. The

C-terminal region of PPV-SoC CP contains neither the RsaI restriction site – common for all isolates of the PPV-D group – nor the conserved AluI site detected in all PPV isolates. The N-terminal region of PPV-SoC CP contains the recognizable conserved motifs DAG, possibly determining aphid transmissibility. The predicted M_w of PPV-SoC CP was 36.8 kDa, which is in accordance with that determined by Western blot analysis. Based on our findings, we propose to consider PPV-SoC as a unique strain of PPV, a prototype of a new group termed PPV-Cherry (PPV-C). The described properties of PPV-SoC, along with its unusual natural host (*Prunus cerasus* L.), may suggest that PPV-SoC went through evolutionary changes which made it capable of systemically infecting sour cherry plants, a host that is highly resistant to infection by other PPV strains. *(P)*

Cloning and Integration of the Plum Pox Potyvirus (PPV) Cytoplasmic Inclusion Gene into Higher Plants

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The cytoplasmic inclusion (CI) protein gene from a Hungarian isolate of PPV (PPV-SK68) was integrated into *Nicotiana benthamiana* plants. The CI protein of PPV is an RNA helicase and involved in the replication of the virus genome. The amino acid sequence of CI protein contains conserved nucleotide binding motif (NTBM) in common with the protein family of putative RNA helicases. Using the recombinant DNA techniques we have developed two gene constructions for plant transformation. The first contains the original coding sequence of CI gene under the control of 35S promoter of CaMV and no terminator signal; the second was mutated in the NTBM region. The recombinant plasmids with kanamycin resistance marker were mobilized into *Agrobacterium tumefaciens* and leaf disks of *N. benthamiana* were transformed. The transgenic plant lines were selected on kanamycin-containing medium. The integrated viral genes were confirmed using polymerase chain reaction and the transcription was detected by Northern hybridization. The effects of integrated CI genes on the replication of plum pox virus and the development of inclusion bodies are under investigation. *(P)*

Nucleotide Sequence of the 3-Terminal Region of the Sweet Cherry Isolate of Plum Pox Potyvirus (PPV)

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A 1246-nucleotide-long fragment, containing the coat protein gene (CP-gene) and part of the NIb gene from the sweet cherry isolate of PPV (PPV-SwC), was amplified using the immunocapture reverse transcription–polymerase chain reaction (IC-RT-PCR), from naturally infected leaves. The amplification product was directly cloned into a pCR^{TM} II vector and then sequenced. The nucleotide sequence of the CP-gene of PPV-SwC shared a high level of homology with the correspondent region of the sour cherry isolate (PPV-SoC); however, the two isolates diverged significantly from other known isolates of PPV in the N-terminal region of the CP-gene. *(P)*

Contribution of Tombusvirus Genes to Host Specificity and Symptom Phenotype

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A series of hybrid viral genomes was constructed from infectious cDNA clones of three different tombusviruses (cymbidium ringspot virus, CymRSV; carnation Italian ringspot virus, CIRV; and tomato bushy stunt virus pepper strain, TBSV-P). All individual genes were exchanged between the three viral genomes and the viability of each hybrid was tested for its ability to infect *Nicotiana benthamiana* plants. All hybrids were able to elicit systemic infection of *N. benthamiana*, indicating that they retain all basic viral functions for infection. However, there were significant differences in severity of the symptoms produced. Hybrids carrying the 5' coding sequences of CIRV and 3' nested genes of CymRSV were not able to induce systemic necrosis, suggesting that more than one gene contributes to the host necrotic response. It was also demonstrated that specific interference between different viral genes is essential for systemic invasion of hosts other than *N. benthamiana*, such as for instance *N. clevelandii*. The role of coat proteins and other non-structural genes in the host specificity was discussed. *(P)*

Molecular Analysis of Watermelon Chlorotic Stunt Virus (WCSV) from Sudan

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Watermelon chlorotic stunt virus (WCSV) is a geminivirus transmitted by *Bemisia tabaci*. The disease caused by the virus results in major crop losses of watermelon in Sudan and Yemen. The virus may be present also in adjacent countries. A full-length genomic DNA of WCSV from Sudan has been cloned and sequenced. The genome organization classifies it among the Old World geminiviruses with a high degree of similarity to the tomato yellow leaf curl viruses from the Near East and the Mediterranean Basin, as well as to African cassava mosaic virus. Using molecular hybridization WCSV has been detected also in plant samples of melon and snake cucumber originating from different regions of Sudan, whereas it was absent in squash and zucchini samples from the same areas. The molecular evolution and the epidemiology of WCSV were discussed in view of the recent spread of the tomato yellow leaf curl viruses in similar geographic and climatic habitats. *(P)*

Long-Distance Transport of Red Clover Mottle Virus (RCMV) in a Systemic Host

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The RCMV content in different parts of host plants in relation to the time after inoculation was determined by enzyme-linked immunosorbent assay (ELISA). One day after inoculation the virus could be detected only in inoculated leaves. From 2 to 4 days after inoculation RCMV was found in the stems. Virus content was high in segments situated directly over inoculated leaves and decreased towards the top of plants. Five days after inoculation, viruses were found in the top of host plants. In roots, in non-inoculated leaves and in stem segments under inoculated leaves, RCMV could not be detected. If host plants were infected with *Rhizobium leguminosarum* before virus inoculation, RCMV appeared in segments of stems under inoculated leaves and in roots. These results show that the distribution of virus in host plants is dependent on the host metabolism, and especially on the source/sink relation. Transport within the phloem is possible but in darkened source-leaves, which become sink-leaves, no RCMV can be detected by ELISA. Possibly a lack of light and the metabolism of these leaves are the reason for the absence of viruses.

These investigations were supported by the German Research Association. *(P)*

Investigations of Long-Distance Movement of Red Clover Mottle Virus (RCMV) in *Pisum sativum*

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Specific sequences of the two RCMV RNAs present in total-RNA extracts of infected pea plants were detected following amplification by polymerase chain reaction (PCR) or Northern blot hybridization. The critical step for virus detection is the extraction of total-RNA from different parts of the plant. Various methods were tested. To achieve quantification and to compare several RNA extractions, we used the plant RNA kit (QIAGEN). Inclusion of an internal control of each RNA sample is essential to identify false-negative results. We used cytosolic glycerinaldehyde-3-phosphate-dehydrogenase (GAP-DH) from *Pisum sativum* to evaluate an internal standard for PCR and for hybridization experiments. The sensitivity is several orders of magnitude higher than serological methods and enabled the detection of early phenomena of the long-distance transport. Preliminary experiments showed acropetal transport of the virus depending on the host plant's metabolism. Already after 3 days, nucleic acids can be detected in the upper part of the stem and in the youngest leaves.

These investigations were supported by the German Research Association. *(P)*

Nuclear Inclusion Proteins?

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Potyviruses were characterized on the basis of the types of inclusions formed. One type of inclusion characteristic of potyviruses is nuclear inclusion (NI). It was determined to be composed of two virally encoded proteins subsequently named NI protein a (NIa) and b (NIb). We determined that peanut stripe virus (PStV) produces NIa and NIb but does not form NIs. The fate of these two proteins was investigated in infected *Nicotiana benthamiana* by Western analysis and immuno-electron microscopy. Polyclonal antibodies to PStV NIa-VPg, NIa-proteinase and NIb were produced and used to measure the accumulation of NIa and NIb in time-course experiments. Western analysis revealed that each protein was being synthesized and was accumulating to similar levels in infected tissue. Infected tissues were fixed and embedded for immunogold localization with the antisera to NIa and NIb. It was found that the NIa protein accumulated to high levels in the nuclei of infected cells. Sequence alignment comparisons confirmed the presence of an NIa protein nuclear localization signal similar to that described for tobacco etch potyvirus. NIb could also be found in the nucleus but at a much lower concentration. The proteins appeared to be randomly distributed throughout the nucleus, with no indication of the formation of a NI. *(P)*

Promoter Activity Associated with Banana Bunchy Top Virus (BBTV) Components 1 to 6

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BBTV is a phloem-limited, monocot-infecting virus with a genome consisting of six circular ssDNA components. We have cloned the non-coding regions of BBTV components 1 to 6 upstream of the β-glucuronidase (GUS) reporter gene. The subsequent fusions were transiently introduced into *Nicotiana tabacum* cell suspensions, *via* micro-projectile bombardment. Histochemical staining and quantitative fluorometric assays indicate all components can function as promoters to varying levels within undifferentiated tobacco cells. The strongest of the BBTV promoters were those of components 2 and 6, which compared favorably with the cauliflower mosaic virus 35S promoter. Tobacco plants, transformed with the BBTV-GUS fusions, were characterized by Southern hybridization and histochemically stained for GUS expression. All transformants displayed weak phloem-limited GUS activity. This tissue specificity may be analogous to the site of BBTV replication within its natural host. *(P)*

Extra Copies of an AU-rich Sequence Motif in the 3' Noncoding Region of a Potyvirus Affect Disease Expression but not Replication or Movement of Virus in Infected Plants

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A mutant of the potyvirus tobacco vein mottling virus (TVMV) that contains four extra, tandemly arranged sets of a 14-nt AU-rich sequence motif that is present in the 3' noncoding region of TVMV RNA, has been characterized. The mutant virus spreads and accumulates in infected plants to the same extent as wild-type virus, but induces only mild or no disease symptoms. Cross-protection experiments demonstrated a delay or masking of symptom production in wild-type virus-inoculated plants that were previously inoculated with the mutant virus. Removal of two of the 14-nt motifs results in the disease-producing phenotype. Alteration of computer-derived secondary structure in the region of the sequence motif does not result in a change in phenotype. Current efforts include the introduction of multiple copies of mRNA instability motifs into the 3' noncoding region of TVMV RNA to determine whether the 14-nt motif may be playing such a role. *(P)*

Tobacco Necrosis Virus Strain D^H Genes Required for RNA Replication and Viral Movement

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The complete sequence of the genome of the tobacco necrosis virus strain D Hungarian isolate (TNV-D^H) was determined. The sequence analysis of the 3760-nt long TNV-D^H genome revealed the presence of six open reading frames which had the potential to encode proteins of 22, 82, 7a, 7b, 7c, and 29 (coat) kDa. Highly infectious synthetic transcripts from full-length TNV-D^H cDNA clones have been prepared and used for reverse genetic study to map viral genes required for movement and replication. Protoplast inoculation with A22 and A82 mutants revealed that both gene products are required for RNA replication. No viral RNA was detected in the inoculated or systemic leaves of *Nicotiana benthamiana* infected by the synthetic transcript of the 7a, 7b, and 7c initiation codon mutants. In contrast, viral RNA accumulation was observed in the inoculated, but not the systemic, leaves of plants inoculated with viral RNA lacking the intact coat protein (CP) gene. The CP mutants were unable to elicit symptoms on upper non-inoculated leaves, but produced large local lesions on the inoculated leaves. *(P)*

Spatial Distribution of Acidic Chitinases and Their Messenger RNAs in Tobacco Plants Systemically Infected by Cherry Leaf Roll Virus

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In a hypersensitive reaction (HR), a virus-infected plant induces necrotic lesions that contribute to the viral limitation at the initial infection site. Concomitant with the HR, there is an induction of proteins, commonly referred to as pathogenesis-related (PR) proteins. The PR-P and PR-Q chitinases are the major acidic PR proteins induced in TMV-tobacco plants. Cherry leaf roll nepovirus (CLRV) is capable of invading systemically *Nicotiana tabacum* cv. 'Xanthi nc' plants, forming necrotic ringspots in non-inoculated leaves. The time-course induction of chitinases in infected plants was studied by Western blot analysis. In inoculated and systemic leaves the acidic chitinase proteins appeared at 3 and 8 days post-inoculation, respectively. By using the tissue-printing technique, the spatial localization of both the chitinase protein and its messenger RNA (mRNA) was visualized. Both protein and mRNA appeared to be localized preferentially in the symptomatic areas of the infected tissue. Northern and Western blot analyses revealed that in symptomatic areas there was an increment of 2.5-fold respective to the chitinase protein or its mRNA accumulation in asymptomatic areas. *(P)*

Identification of Viral Regions Involved in Photosynthesis Inhibition in Infected Plants

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In *Nicotiana benthamiana* plants, infection by tobamovirus pepper mild mottle virus, strains S and I, or by paprika mild mottle virus, induces different disease syndromes. In both symptomatic and asymptomatic leaves, the viral infection is associated with an inhibition of the photosynthetic rate. Alteration of the chlorophyll fluorescence induction, as well as oxygen evolution, were determined. In chloroplasts isolated from the infected plants, the polypeptide composition of the oxygen-evolving complex was altered with respect to the control plants. The viral regions involved in the photosynthetic inhibition are being investigated by use of chimeric viral genomes among the three viruses. *(P)*

III: VIROIDS

RNA Structural Features Responsible for Potato Spindle Tuber Viroid (PSTVd) Pathogenicity

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We have carried out a combination of quantitative bioassays, temperature gradient gel electrophoresis analyses of circularized RNA transcripts, and theoretical calculations with 12 representative sequence variants of PSTVd in an effort to determine how nucleotides within the pathogenicity domain modulate symptom expression. No consistent correlation between denaturation temperature and symptom severity could be identified. Thermodynamic calculations do indicate, however, that severe variants have access to a wider range of suboptimal secondary-tertiary structures than do milder variants. Major differences were also identified in the overall geometry of the pathogenicity domain; *e.g.* variants producing intermediate symptoms contain a linear arrangement of three consecutive helices, whereas this domain is 'bent' in opposing directions for mild or severe variants. Differences in secondary or tertiary structure, together with concomitant alterations in RNA-protein interactions, may be the primary cause of viroid pathogenicity. *(L)*

Structure of Potato Spindle Tuber Viroid (PSTVd) Multimeric (-) RNA

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Viroids are replicated *via* a rolling-circle mechanism. All replication steps are catalyzed by the host enzyme polymerase II. The circular (+) viroid is transcribed into oligomeric (-) RNA. This strand acts as template for the synthesis of an oligomeric (+) strand RNA which is cleaved and ligated to mature viroid circles. Biologically important structural elements within the oligomeric (-) strand RNAs must be responsible for (+) strand synthesis. From site-directed mutagenesis it had been concluded that the formation of a thermodynamically metastable (-) strand RNA structure containing a characteristic hairpin (HP II) is critical for (+) strand synthesis. Because structure investigations of synthesized (-) strand replication intermediates *in vivo* are not possible due to their low intracellular concentration, we analyzed the structure of (-) strand *in vitro* transcripts using temperature gradient gel electrophoresis (TGGE), optical melting curves, oligonucleotide mapping, chemical modification and secondary structure calculations. We have shown that (-) strand transcripts can assume different coexisting structures and chemical modification was applied to identify structural motifs within coexisting structures which are essential for (+) strand synthesis. *(P)*

Pedigree Analysis of Potato Spindle Tuber Viroid (PSTVd) Genome

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It is generally assumed that RNA genomes propagate as a population of related sequence variants (quasi-species). To understand the mechanism of generation and subsistence of such quasi-species, tomato plants were inoculated with several different PSTVd variants in an infectious cDNA form. The progenies from such inoculations were analyzed after the first and sixth consecutive passages in tomato Six different PSTVd variants, with different levels of pathogenicity, were analyzed in this way. The progeny of intermediate 12, 13, 14, mild M and severe S27 variants diverged both at the sequence and symptoms expression levels. Whereas the 12 and M variants were maintained in their progeny, the 13, 14 and S27 variants completely disappeared from the progeny, sometimes after as little as one passage. These data, when interpreted within the conceptual framework of the Eigen theory, show that PSTVd sequences, regardless of the severity of the disease they induce, comprise a unique quasi-species with distinct possibilities of phenotype conversion. *(P)*

Viroid Processing: Pathway from Cleavage to Ligation is Driven by a Switch from a Tetraloop to a Loop E Conformation

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Viroids are replicated in a rolling circle-like mechanism. In the final step the linear oligomeric replication intermediates are processed to mature circles of unit length. Using temperature gradient gel electrophoresis (TGGE) combined with oligonucleotide

mapping it could be shown for potato spindle tuber viroid (PSTVd) that particular structural motifs of the so-called central conserved region are essential for correct processing in a nuclear extract from potato cells. Host cleavage and ligation enzymes specifically recognize local RNA structural elements within the active processing structure [Baumstark and Riesner (1995)]. We have now identified the cleavage and ligation sites within the RNA substrate by primer extension and S1-mapping of the linear processing intermediates. We found them close to a region which was described earlier as very similar to the loop E of 5S rRNA and whose spatial arrangement of several non-canonical basepairs can be tested by a UV-induced interstrand crosslinking [Branch *et al.* (1985)]. Using this specific UV crosslink we could confirm the presence of loop E in the linear and circular products of processing, but not in the active conformation of the full-length substrate. Refined secondary structure calculations revealed that two stems with GNRA tetraloops can form an alternative to the loop E element. The most stable of these tetraloops is strictly conserved among all viroids of the PSTVd-class, and it is exactly this tetraloop which bears the cleavage site. In a detailed mechanistic model of viroid processing, the tetraloop substructure specifies the first cleavage; a subsequent switch into the loop E conformation specifies the second cleavage and drives the process towards ligation. Implications of this mechanism for the *in vivo* structure formation of replication intermediates, as well as the possible involvement of tertiary structure, were discussed. *(L)*

Enzymatic Processing of Potato Spindle Tuber Viroid (PSTVd)

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We have isolated from nucleoli of potato suspension cells an endoribonuclease (M_r *ca* 55 kD) which is capable of cleaving out from a 1073-nts-long precursor-like linear PSTVd (+) RNA, linear monomeric molecules of unit length. At their 3'- and 5'-termini this RNase generates a 2',3'-cyclophosphate and a free 5'-OH group, respectively. It cleaves in single-stranded regions at the 3'-site of pyrimidines (Py), preferring the sequence 5'-Py, Py/Purine-3'.

We have also isolated from potato cells a RNA ligase (M_r *ca* 100 kD) which is able to convert the RNase-generated linear monomers to circular molecules. This reaction is stimulated by ATP and by $MgCl_2$. The ligation site exhibits a 2'-phosphomonoester-3',5'-phosphodiester bond which indicates that during circularization the 2',3'-cyclophosphate has been phosphorylated. The corresponding cyclo-phosphatediesterase-activity and the poly-nucleotidekinase-activity could not yet be separated from the RNA ligase activity.

These results indicate that most probably the monomerization of the PSTVd and the related viroids proper is performed by a specific nucleolar host cell RNase also *in vivo*. Further, that the subsequent circularization of the resulting monomeric linear RNA is catalyzed by an evidently trifunctional host cell RNA ligase, which exhibits properties similar to those of the RNA ligase that mediates the splicing of tRNA precursor molecules in yeast. *(P)*

Transgenic Resistance to Potato Spindle Tuber Viroid (PSTVd) Conferred by Ribozyme

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A hammer-head ribozyme (R-) and a mutated ribozyme (mR-) were designed to target the PSTVd (-) RNA. The results of cleavage activity showed that the R(-) functioned accurately *in vitro*, whereas the mR(-) had no activity. In order to study the *in vivo* inhibition of PSTVd replication by the ribozymes, the R(-) or mR(-) dimers were cloned into a plant expression vector, pROK2. Transgenic potato plants were obtained *via Agrobacterium tumefaciens*. Thirty-four transgenic plants were generated with the pROK2-R(-)D. Analysis of RNA from the transgenic plants by a return PAGE showed that 23 lines were free of PSTVd accumulation 30 days after PSTVd inoculation. The remaining 11 plants showed variable accumulations of the PSTVd, but had considerably less than the control plants transformed with pROK2 alone. Sixty transgenic plants were regenerated with pROK2-mR(-)D. RNA analysis indicated that 59 lines had a level of PSTVd accumulation similar to the control, but only one line had less accumulation than the control. These results were confirmed by Northern hybridization analysis of total RNA from the transgenic lines using a PSTVd full-length cDNA probe. All the transgenic lines of R(-) and mR(-) were individually propagated vegetatively. RNA analyses showed that the vegetative progeny plants from the primary R(-) transgenic plants showing no PSTVd accumulation after infection, had no detectable PSTVd. These results suggest that the resistance against PSTVd infection was transmitted stably to the vegetative progenies. *(P)*

Viroid Sequence Variation in Grapevines

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Viroids are the smallest known pathogens infecting only higher plants. They exist as circular, single-stranded RNA molecules with a length of 240 to 600 nucleotides. Viroids of grapevine are distributed worldwide. In extensive epidemiological studies using the most modern approaches of molecular biology, our group could show the extraordinary diversity of viroid populations in single grapevines and also between different plants within a vineyard. We employed Northern blot analysis, PCR and Single Strand Conformation Polymorphism Analysis (SSCP) to detect and characterize viroids in Franconian vineyards and in an Italian breeding facility for grapevine rootstocks. The search with probes against viroids of the potato spindle tuber viroid (PSTVd) and apple scar skin viroid (ASSVd) groups, respectively, revealed only two of the five known grapevine viroids, namely, grapevine yellow speckle viroid 1 (GYSVd1) and the grapevine isolate of hop stunt viroid (HSVdg). GYSVd1 appeared always together with HSVdg and the overall infection rate varies between 50% and 100%. But the most important outcome was that the viroids in grapevines represent quasi-species. Even in neighboring plants in a vineyard, different GYSVd1 and HSVdg main-variants were isolated. These variants seem to be randomly spread in the vineyard. This, together with our finding that rootstocks used comonly in Franconia very rarely show HSVdg, and in no case GYSVd1 infections, makes it probable that the viroid variant pattern had been established during planting of the vineyard. The graft is the source of the infection. It is unlikely that the viroids are spread by regular pruning, because in most cases uninfected plants were found next to infected plants. Within single plants, both viroids (GYSVd1 and HSVdg) appeared in certain sequence sub-variants. The main-variant was always accompanied by one or more sub-variants. We described 18 new GYSVd1 variants and six previously unknown HSVdg variants. Half of the new GYSVd1 variants show alterations of hairpin I, a structure which is important during the replication cycle. Whether this feature has an impact on the symptom expression of GYSVd1 remains unclear. Reintroduction experiments with cloned viroids into viroid-free grapevine seedlings are under way. *(L)*

Chimeric Analysis of Viroids: Infectivity and Pathogenicity of HSVd-CEVd Chimeras

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To investigate the structural determinants of replication, pathogenicity and host specificity in viroid molecules, we have produced four chimeric cDNA constructs between hop stunt viroid (HSVd) and citrus exocortis viroid (CEVd). Viability of these four chimeric viroid constructs: CEHS (CEVd left half + HSVd right half), CEHS-TR (CEVd-TL, -P, -CCR and -V + HSVd-TR), HSCE (HSVd left half + CEVd right half) and HSCE-TR (HSVd-TL, -P, -CCR and -V + CEVd-TR) [TR = terminal right; TL = terminal left domains], was assayed by inoculating their dimeric plus strand RNA transcripts onto cucumber and tomato seedlings. The result showed that CEHS-TR and HSCE-TR replicated stably and showed disease symptoms on tomato and cucumber, respectively, but CEHS and HSCE were not infectious. Amounts of CEHS-TR accumulated in tomato and HSCE-TR in cucumber were reduced to 1/10 to 1/25 of that of CEVd in tomato and of HSVd in cucumber, respectively. The results indicated that the TR domain was exchangeable between viroid subgroups such as CEVd and HSVd, which strongly suggested the possible involvement of the TR domain in viroid recombination events. The effect of the TR domain on viroid pathogenicity was replication (or accumulation) efficiency, rather than symptom expression such as leaf curling, stunting or necrosis. *(P)*

Citrus Exocortis Viroid (CEVd) Infection and Ethephon Inhibit Synthesis of Proteinase Inhibitors in Tomato Leaves Induced by Wounding and Methyl Jasmonate

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Citrus exocortis viroid (CEVd) infection and ethephon (2-chloroethylphosphonic acid, an ethylene-releasing compound) inhibit the synthesis of proteinase inhibitors I and II in tomato leaves induced by wounding and methyl jasmonate (MJ). According to Northern analysis, this inhibitory action occurs at the transcriptional level. It was also found that salicylic acid (SA) increases at least tenfold in infected leaves after viroid infection and fivefold in ethephon-treated plants. Since it has been demonstrated by S H. Doares *et al.* that SA is a potent inhibitor of systemin-induced and jasmonic acid-induced synthesis of proteinase inhibitors, the elevated levels of SA in viroid-infected and ethephon-treated plants could explain the inhibition of proteinase inhibitors synthesis found. All this suggests strongly that ethylene and SA are sequential mediators of this pathogen-induced inhibition. *(L)*

Ultrastructural and Histological Localization of Viroids by Transmission Electron Microscopy and Confocal Laser Scanning Microscopy

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We have used *in situ* hybridization and other molecular methods such as PRINS (PRimed IN Situ hybridization) and *in situ* PCR in conjunction with transmission electron microscopy and confocal laser scanning microscopy to study the localization of viroids at the ultrastructural and histological levels. Avocado sunblotch viroid (ASBVd), citrus exocortis viroid (CEVd), and coconut cadang cadang viroid (CCCVd) were localized using these techniques, and the results of all three localization studies are different. ASBVd is localized to the chloroplastic thylakoid membranes, CEVd is spread across the entire nucleus, whereas CCCVd is spread throughout the nucleus, but with a marked concentration within the dense fibrillar peripheral region of the nucleolus. While the results of these studies provide some further evidence for the taxonomic differentiation between ASBVd and the potato spindle tuber group of viroids, they also raise important questions as to the involvement of particular RNA polymerases in the replication of these viroids. Initial results were published in 1994 (Bonfiglioli *et al.*). In studies initiated recently, the ultrastructural localization of hop stunt viroid and also viroids in the apple scar skin viroid group are being investigated. *(L)*

A Viroid Infects Mandarin and Navel Orange

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Nucleic acid extracts from leaves, stems and the cork of citrus trees (mandarin and navel orange) showing symptoms resembling those of cachexia viroid disease, were analyzed by non-denaturing PAGE. Data show a slight difference between the electrophoretic migration of the two trees' extracts. Symptoms produced on *Cucumis sativus, Chrysanthemum monifollum, Gynura arantiaca* and *Lycopersicon esculentum* plants were nearly the same. Ultrathin sections of infected cells examined by electron microscopy revealed the presence of paramural bodies known as plasmalemmasomes and a type of membrane structure which resembled a coiled watchspring which can be correlated with the presence of viroid-RNA. Also changes in the chloroplast, mitochondria and cell wall were observed. *(P)*

Sequence Analysis of Infectious cDNA Clones of Peach Latent Mosaic Viroid (PLMVd): A Tool for the Study of Structure–Function Relationships

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Structure–function relationships in PLMVd and avocado sunblotch viroid, two atypical viroids with hammerhead ribozymes, are poorly understood. As an approach to this question a set of cDNA clones, obtained from a severe PLMVd isolate by RT-PCR, were inoculated into GF305 peach seedlings. Monomeric inserts resulted in infections and could be used directly. Sequencing showed a high variability, with an average of 10–20 changes per insert with respect to the reference isolate. Changes were concentrated in a region comprising 2/3 of the molecule which included the self-cleaving domains. Most infected plants developed symptoms but some remained symptomless. This observation parallels the frequent reversion of PLMVd severe isolates to a latent condition observed in natural infections. Sequencing a second series of clones from two latent PLMVd isolates showed changes also concentrated in the same region. However, the lower variability observed indicates that the phenotypic stability of the latent isolates - which do not revert to a symptomatic condition - can be correlated, in the frame of a quasi-species distribution, with a more limited spectrum of sequence variants. *(L)*

Infectivity and Structure Analysis of Carnation Stunt Associated Viroids

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Two small RNAs were found associated with the stunting disease of carnations, but were unable to cause the stunting disorder. These RNAs are designated carnation stunt associated RNAs (CarSAVd) 1 and 2. CarSAVd could be transmitted to healthy carnation plants. Both LiCl-soluble RNAs extracted from infected carnations as well as gel-purified CarSAVd RNAs were infectious. The two CarSAVd RNAs are similar in sequence to each other as determined by RNA fingerprint analysis, and the sequence of RNA 1 is nearly identical to that of a Spanish isolate of CarSAVd RNA. Structural analysis by thermal-gradient gel electrophoresis showed that CarSAVd RNA1 had an unusual structure for a viroid, containing several independent melting transitions, whereas CarSAVd RNA 2 had a melting profile typical of other viroids. These data suggest that the two RNAs have different secondary structures. *(L)*

The DNA of a Plant Retroviroid-Like Element Integrates at Different Sites in the Genome of a Caulimovirus and Shows Multiple Forms with Sequence Deletions

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CarSV RNA (*C*arnation *S*mall *V*iroid-like RNA) and its homologous DNA, are the two forms of a retroviroid-like system present in certain carnation plants. CarSV RNA is a 275-nt non-infectious viroid-like RNA which contains hammerhead ribozymes in both polarity strands. Multiple variants of this RNA with sequence deletions and/or repetitions have been characterized. On the other hand, CarSV DNA is organized as a series of head-to-tail multimers forming part of extrachromosomal elements. These elements contain, aside from CarSV DNA, sequences of CERV (*C*arnation *E*tched *R*ing *V*irus), a plant pararetrovirus of the caulimovirus family. Here we report that CarSV DNA sequences were found interrupting diverse open reading frames of CERV DNA. A small number of nucleotides common to CarSV and CERV occur at all junctions between both sequences. Moreover, a series of CarSV DNA forms with the same deletions previously found at the RNA level, have also been characterized. These results suggest that CarSV DNA forms emerge from inter- and intramolecular recombination events probably mediated by the CERV-coded reverse transcriptase. *(P)*

RNA-Recombination Between Two Parental Viroids is Still Taking Place in Individual Coleus Plants Today and Generates a Novel Viroid Offspring

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In various *Coleus blumei* cultivars three new viroids were found, which are named CbVd 1, CbVd 2 and CbVd 3 and whose prototypes are 250, 302 and 362 nucleotides long, respectively. They all contain an identical central region (CR) but its sequence differs strikingly from the CRs of all other presently known viroids. Except for their CR, CbVd 1 and CbVd 3 are completely unrelated, whereas CbVd 2 is a recombinant viroid that arises by fusion of the right-hand part and the CR of CbVd 1 with the left-hand part of CbVd 3. The distribution pattern of these three viroids in two sets of 50 individual plants propagated from a single mother plant, respectively, by cuttings, was analyzed over a period of 4 years. At the beginning, CbVd 1 was present in all plants, *ca* 90% of which contained CbVd 3, 20% harbored CbVd 2, and in 13% of the plants only CbVd 1 and CbVd 2 were found. This pattern changed during the following years of culture but finally still 100% of the plants contained CbVd 1 whereas 65% carried the recombinant CbVd 2. However, in 50% of the latter plants CbVd 3 had been evidently defeated by the novel CbVd 2 offspring. Thus the Coleus viroid system is a naturally occurring viroid family which represents a kind of missing link in support of the repeatedly proposed RNA recombination as an important factor in viroid evolution. *(L)*

IV: VIRUS POPULATIONS

The Helper Virus Controls the Generation and Maintenance of Quasi-Species in Cucumber Mosaic Cucumovirus Satellite RNAs

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Cucumber mosaic cucumoviruses can harbor a molecular parasite known as a satellite RNA (CMV sat RNA). CMV sat RNAs exist as numerous related strains that have from 85% to 98% sequence similarity. The CMV sat RNAs are supported by both subgroups of CMV as well as the related tomato aspermy cucumovirus (TAV). The generation of variation in CMV sat RNAs has been monitored by using cDNA clones of sat RNAs. Recently it was found that the helper virus plays a significant role in the generation and subsequent maintenance of stable populations of variants (or quasi-species). Different helper viruses vary both in the time required for sat RNA variation to occur, and in the size of the sat RNA quasi-species cloud tolerated by the helper virus. Analysis of the helper virus functions involved in this process as well as the implications of quasi-species cloud size on virus variation and evolution were discussed. *(L)*

Sequence Comparisons of Zucchini Yellow Mosaic Virus (ZYMV): Implications for Developing Transgenic Resistance in Cucurbits

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In Australia, ZYMV and papaya ringspot virus type W (PRSV-W) are the most important viruses infecting cucurbit crops. Genetic engineering of cucurbits offers the possibility of developing virus-resistant germplasm and expression of virus-derived genes such as coat protein (CP) genes has, in many cases, resulted in virus-resistant transgenic plants. These resistance genes are usually derived from one isolate of the virus. Tennant *et al.* showed that expression of the CP gene of a Hawaiian isolate of PRSV-P in transgenic papaya plants did not confer resistance against all isolates of this virus. A study was undertaken to determine the sequence variability of field isolates of ZYMV within Australia to predict the effect on transgenic plants in the field. Sequence comparisons of Australian and one South African isolate of ZYMV with published sequences indicate that there is significant variability in ZYMV, worldwide. In comparison with PRSV in Australia (less than 2% variability in the CP gene), ZYMV isolates from Australia show up to 16% variability in the CP gene and up to 17% variability with overseas isolates, suggesting more than one introduction of ZYMV into Australia. In addition, while expression of viral genes for one local isolate should confer

resistance to all local isolates of PRSV, ZYMV-derived resistance genes derived from one isolate may not be effective against all isolates. *(P)*

Plant Viruses Isolated in Egypt

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Potato virus Y belonging to the Y^0 group was isolated from *Solanum nigrum* var. *judaicum*; it induced mosaic, mottling, vein clearing, malformation, asymmetry of laminae, curling and puckering of leaves. Tomato mosaic virus (TMV) on tomato cv. 'Marmande' caused mosaic, mottling and abnormalities in summer and necrotic spots and patches in winter. A mosaic virus isolated from *Raphanus sativus* var. *aegyptiacus* which has long white roots induced mosaic, mottling, vein banding, blistering, leaf variegation, and vein clearing. A strain of TMV with some similarities to tobacco type and tomato mosaic type isolated from *Nicotiana glauca*, induced mosaic, mottling, vein clearing and various abnormalities. Eggplant mosaic, pepper mosaic leaf curl and petunia distortion mosaic are probable isolates of TMV. A new, highly heat-resistant strain of TMV that resists boiling at 100°C for 31 min was isolated from tomato cv. 'Money Maker'; it induced mosaic, mottling, severe abnormalities and enations. *(P)*

A Possible Nepovirus in Cucumber in Southern Italy

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Cucumber plants showing chlorotic ringspots on fruits and leaves were observed in a greenhouse crop in southern Italy. A virus isolated from infected fruits was mechanically transmitted to a number of herbaceous hosts belonging to different families. Virions had a spherical shape and contained two ss-RNAs species, of *ca* 8300 and 7400 nucleotides. Purified virions migrated in SDS-PAGE as two bands of *ca* 14.5 and 50 kDa. Ultrastructure investigations showed cytopathic inclusions typical for nepovirus infection, but virus particles did not react against antisera to several nepoviruses in an immunodiffusion test. *(P)*

Variability within Three Papaya Ringspot Potyvirus (PRSV) Genes

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In a previous study, we sequenced the coat protein (CP) gene of Australian and Asian isolates of PRSV. Comparison of these sequences with reported sequences of PRSV revealed up to 12% nucleotide sequence variability. Furthermore, no stable amino acid or nucleotide difference was identified between Australian PRSV-P and PRSV-W isolates, which may account for their host range difference; these isolates were found to be more closely related to each other than to any other isolate. We have proposed that in Australia, PRSV-P has arisen from PRSV-W rather than by introduction. More recently, we have extended our investigations to include the replicase (NIb) and P1 genes or PRSV. Interestingly, the NIb gene was found to have a similar level of nucleotide sequence variability to the CP gene. The P1 gene, known to be the most variable of the potyviral genes, had up to 30% nucleotide and amino acid sequence variability among Asian, US and Australian isolates of PRSV. Although the function of the P1 gene is unknown, these data suggest that it can tolerate extreme variability at both the amino acid and nucleotide levels. *(P)*

Sequence Variability of Papaya Ringspot Virus (PRSV) in Thailand

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PRSV, a potyvirus, is classified into two types which can be distinguished only by host range. PRSV-P infects papaya and cucurbits whereas PRSV-W infects cucurbits only. We have previously reported up to 12% nucleotide sequence variability in the coat protein gene of PRSV-P from different geographic regions but less than 2% variability between P and W within Australia. Since PRSV-P was recently reported in Australia and PRSV-W had been present for at least 20 years, it was suggested that PRSV-P arose as a mutation from PRSV-W. We subsequently extended this study to include Thailand, where both strains of PRSV have been present for considerably longer. Significant variability (7–10%) was found within PRSV-W in Thailand but only 1–3% variability within PRSV-P. Thai isolates of PRSV-P and -W had 85–92% similarity with PRSV-P from other geographic regions. This provides further support to the hypothesis that PRSV moves internationally in cucurbits and moves into papaya following a mutation from the W strain. Interestingly, a survey in Thailand failed to detect PRSV-P in cucurbits in the field, a phenomenon previously noted in other countries. However, an uncharacterized cucurbit isolate of PRSV was 96–99% and 90–92% similar to Thai isolates of PRSV-P and -W, respectively, suggesting either a low frequency of PRSV-P in field cucurbits, or, alternatively, it may represent the W isolate from which the original P isolate arose in Thailand. *(P)*

Are European Luteovirus Isolates from Oilseed Rape and Sugar Beet Similar or Different Viruses?

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In Europe, beet western yellows luteovirus (BWYV) and beet mild yellowing luteovirus (BMYV) cause economically important diseases in oilseed rape (*Brassica napus* L.) and sugar beet (*Beta vulgaris* L.), respectively. The acronym BMYV is invariably described in the literature as a synonym of BWYV. However, our studies have shown that all luteovirus isolates from oilseed rape differed from those from sugar beet in host range, transmissibility by aphid vectors, and serological reactivity with monoclonal antibodies. BWYV and BMYV have different sequence data, and this has been exploited in using RT-PCR to differentiate between them. We have concluded that European luteovirus isolates pathogenic to the genus *Brassica* represent a separate virus, which, because it is non-pathogenic to the genus *Beta*, should no longer be designated 'beet' western yellows virus. We propose that the *Brassica*-infecting luteovirus should be renamed turnip yellows virus (TuYV), the name by which it was originally describved. 'BMYV' should be retained for luteovirus isolates pathogenic to sugar beet. *(P)*

Epidemic Infestation of Winter Oilseed Rape Crops by Turnip Yellows Luteovirus (TuYV) in Germany

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During the period from 1991 to 1995, pronounced differences were detected in the infestation of winter oilseed rape crops (*Brassica napus* L.) by TuYV (syn. beet western yellows luteovirus) in different regions of Germany. Whereas in 1993/94 the degree of TuYV infections in most of the crops ranged from 0 to 6%, in 1992 and 1995 the levels were 42% to 100% in many crops. In the 1994/95 growing season, 21 out of 64 oilseed rape crops tested suffered 52% to 100% infection by TuYV. The cause of the high degree of TuYV infections in oilseed rape crops is probably its wide host range, including a number of cultivated plants and common arable weed species. Furthermore, in our studies 17 out of 23 aphid species were able to transmit TuYV on oilseed rape plants. The differences in the infection degree in various years can be traced to an interruption of the infection cycle caused by the hot and dry summers in 1992 and 1993, respectively. In 3-year experiments, yield losses from 12% to 34% were caused by TuYV infections on two cultivars tested. Together with the high degree of infection of oilseed rape, this leads us to the conclusion that it is necessary to prevent yield losses by the breeding of new cultivars resistant to TuYV. *(P)*

Infectious Transcripts of Potato Leafroll Virus (PLRV)

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Infectious transcripts play a key role in the research of plant viruses at the molecular level. *In vitro* 'run-off' transcripts synthesized on the template of a full cDNA copy of the viral genome are used for infection of protoplasts or whole plants. Mutagenesis of cDNA clone enables studies of the functions of particular open-reading frames. PLRV belongs to group II of the luteoviruses. Its genome consists of a single 5.9kb (+)RNA molecule. A number of cDNA clones covering the whole genome of the Polish isolate of PLRV were constructed. Four overlapping clones were selected and assembled using restriction sites. The full copy was positioned between the T7 RNA polymerase promoter and the unique *ScaI* site. The full-length capped transcripts of the sequence of the viral genome were able to replicate in protoplasts and to produce the viral coat protein. The next step in the work will be construction of an agroinfectious clone in order to infect the host plant. Cloning the cDNA copy of the PLRV genome under a 35S CaMV promoter in a binary vector is now in progress. *(P)*

Sequencing of the Coat Protein Gene of a Potato Virus Y (PVY) Isolate Infecting Petunia

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Formerly petunias were propagated by seeds, but the new varieties like 'Surfinia' are propagated vegetatively. This kind of propagation, producing large numbers of plants, has resulted in a deterioration in quality during the last 2 years. Most of the problems were caused by virus infections, PVY being one of the most serious viruses. The coat protein gene and the 3' non-coding region of PVY isolates from petunia were amplified with PVY-specific primers and cloned. The sequence data obtained from these clones were compared with corresponding sequences of other potyviruses, thus enabling a further characterization of the PVY isolate from petunia. *(P)*

Some Properties of a Potyvirus Isolated from Peanut

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During a survey of viruses of peanuts (*Arachis hypogaea*) in South Africa, a mechanically transmissible virus was isolated from a plant exhibiting yellow ringspots with dark green borders on the leaves. Typical potyvirus-like flexuous particles were detected during electron microscopic examination. Observation of pinwheel-shaped inclusions in ultrathin sections, reaction with the potyvirus common epitope antisera, and aphid transmission using *Myzus persicae* confirmed that the virus was a potyvirus. Purified virus contained a 33kD coat protein (CP). Host range studies suggested that the virus was none of the potyviuruses reported on peanuts. The isolate did not react with antisera to available legume potyviruses. Nucleotide sequence data of a 625bp DNA product were obtained following immunocapture with potyvirus common epitope antiserum, cDNA synthesis using reverse transcriptase, and PCR amplification with published potyvirus specific primers which amplify the 3' untranslated region and a part of the CP gene. A blast search using nucleotide sequence revealed that the isolate is distantly related to a number of potyviruses, sharing the highest identity (74%) with the sequence of the same genome segment of plum pox virus. These data suggest that the isolate may be a previously unreported potyvirus. This will be confirmed following extensive serological tests. *(P)*

Sequence Variability in Sugarcane Bacilliform Virus (SCBV)

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SCBV, a recently recognized pathogen of sugarcane, is widely distributed throughout the major sugarcane-growing regions and infects noble canes (*Saccharum officinarum* L.), commercial cultivars and species within the '*Saccharum* complex'. The virus is serologically highly variable, and hence a PCR-based test targeting conserved regions in the putative reverse transcriptase coding region was developed. The DNA sequence of the 221 bp region amplified by the primer pair F5/R5 from different samples also shows variation. Amplified DNA has been analyzed by restriction fragment length polymorphism (RFLP) with six different enzymes, and by hybridization studies where different amplified DNAs were used sequentially as probes. SCBV sequences have been assigned to RFLP and hybridization groups by comparing the similarity of restriction profiles and the intensity of Southern hybridization signals among the amplified DNAs. Characterization and definition of the extent and type of variability among the amplified sequences may lead to the identification and description of strains of SCBV. *(P)*

Differentiation of Isolates of Rice Tungro Viruses from the Indian Subcontinent and Southeast Asia

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One of the most devastating rice diseases, tungro, is caused by two viruses: rice tungro spherical virus (RTSV), which is responsible for transmission of itself and of the second virus, rice tungro bacilliform virus (RTBV). RTBV cannot be transmitted alone, but causes most of the disease symptoms. Plants infected with RTSV alone have mild or no symptoms. Previous studies of the variation of tungro viruses were based mainly on symptomatology. Recently, RFLP mapping and sequence analysis of

whole genomes suggested two distinct strains of RTBV, one from South-East Asia and the other from India. RTSV variation was studied using cross-hybridization of RT-PCR products which correspond to the coat protein (CP) region. These results indicated differences between RTSV isolates, with the Indian isolate differing the most. However, the geographic distribution of variation of RTSV does not correlate with that of RTBV. We obtained further evidence of the existence of two RTBV strains and possibly several RTSV strains. Isolates from Nepal, Myanmar, Bangladesh, Vietnam and Thailand (northwest), have been analyzed by PCR, RT-PCR and Western blots. Partial nucleotide and corresponding amino acid sequences for some of them have been determined and compared. Based on these variation studies it was possible to delimit more precisely the two strains of RTBV. The epidemiological significance of the distribution of RTBV strains and of differences in distribution of variants of the two viruses was discussed. *(P)*

The Characteristics of Viral Isolates from Volatile-Oil Plants

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Studies of such volatile-oil plants as rose, mint and lavender showed a wide spectrum of viral infection symptoms characteristic for southern Ukraine. Using histological methods, virus inclusions were discovered in the leaves of infected plants: oblong-shaped in filaments, oval-shaped in the epidermis. In purified viral isolates from different parts of infected plants, rod-shaped (280 × 350 nm), thread-shaped (680 × 875 nm) and spherical (28 × 32 nm) virus particles were found. Purified viral samples had the typical ultraviolet-absorbing spectrum of viral nucleoproteids. The viruses in question are RNA viruses. They are found mainly in the cytoplasm. To study antigen similarity of these viruses, the indirect ELISA test was used. Viral isolates extracted with the help of the above-mentioned methods are believed to be representatives of Tobamo-, Poty- and Carlavirus groups. A biotechnological method was proposed for obtaining virus-free plants. *(P)*

Identification of a New Tombusvirus Infecting Statice

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Statice decline virus (StaDV) was isolated from commercially grown statice (*Goniolimon tataricum*) in southern Germany, in which crop the flower damage is severe. The virus was identified as a tombusvirus closely related to tomato bushy stunt virus strain BS3, on the basis of host range, symptomatology, physical properties and serology. PCR-derived techniques (RACE, ligation-anchored/PCR) were used to clone both the 3' and 5' ends of the StaDV genome. The sequences obtained from these clones, corresponding to the 5' non-coding region and part of the 3' non-coding region of the StaDV genome, showed high homology with the corresponding sequences of other tombusviruses. Specific primers corresponding to the 3' end and the 5' end of the genome were designed, and used to amplify the entire genome. *(P)*

V: VIRAL BIOINFORMATICS

Progress Towards a Universal Virus Database: ICTVdB On-Line

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Recognizing that all aspects of the generation and maintenance of the International Committee on Taxonomy of Viruses Data Base (ICTVdB) will be greatly facilitated using the World Wide Web, over the last year efforts have been directed to making the database available on the WWW. Descriptions of all virus families and genera, and type species treated thus far in the ICTVdB, are available on the Web. Current work, to complete the standardized character list for virus proteins, replication and biological properties, will be incorporated into comprehensive electronic data submission forms available on the Web. These and other features of the ICTVdB on the Web were demonstrated. In the near future the virological community will be invited to submit data in their areas of expertise. Subsequent data management in Canberra will coordinate this expert input and present it for approval by the ICTV Study Groups. The objective is to facilitate the speedy completion of ICTVdB with descriptions of all viruses from the family level down to strains and isolates.

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Identifying Viruses with Intkey

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Intkey is a MS-Windows program for interactive identification and information retrieval. Its features include: entry and deletion of attributes in any order during an identification; calculation of the 'best' characters for use in identification; the ability to allow for errors (whether made by the user or in the data); the ability to express variability or uncertainty in attributes; optional display of notes on characters; automatic handling of characters that become inapplicable when other characters take certain values; restricting operations to subsets of characters or taxa; obtaining lists of taxa possessing or lacking particular attributes or combinations of attributes; listing similarities or differences between taxa; describing taxa in terms of nominated sets of characters; generating diagnostic descriptions for specimens or taxa, to specified degrees of redundancy; user-definable toolbar; selective output of results to files; screen display of illustrations of characters and taxa; and complete on-line help. Examples of the use of Intkey with virus data were presented. *(L)*

Probabilistic Identification of Viruses on the World Wide Web

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Software to identify viruses using the Wilcox's implementation of Baye's theorem was developed and implemented; it is available on the WWW site *http://bioinfo.ernet.in/www/prob.html.* The server software is written for the Solaris operating system in C and the Unix shell scripts. This is activated by NCSA httpd for the WWW. The software consists of three main programs: (i) online forms to gather data for analysis; (ii) generation of identification matrix; and (iii) identification of organisms by the probabilistic approach. An additional utility which links to the WWW version of the Animal Virus Information System (AVIS) has also been developed. The present software not only carries out probabilistic identification of an unknown virus, but also helps to choose additional tests which have the capacity to increase confidence limits of identification. Standard probability matrices

which include data on host range, tissue tropism, experimental viremia, etc., are provided as data to allow the user to identify the unknown organism. A shell is provided to generate a probability matrix using raw data from users' tests and standard weighing schemes. Users can also feed their probability matrices directly. Details of this interactive user-friendly intelligent online data analysis software were discussed. *(L)*

Confidence Levels in Structural Analyses of Large Viral RNA Genomes

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The optimal, suboptimal and phylogenetically conserved structures of complete viral RNA genomes were calculated with the programs mfold and mfold-phylo (Zuker, 1989). Single run calculations were performed on over 50 continuous (+) sense viral RNA genomes including picornaviruses (five genera), retroviruses (HIV1, SIV), nodaviruses, bromoviruses, bacteriophages, reoviruses (ten segments), flaviviruses (hep. C), and potexviruses. In addition, calculations were done on a few (-) sense genomes and as a control on randomized (+) and (-) sequences (same base composition). No statistical distinction could be found in the amount or quality of calculated secondary structures for the optimal predicted structures of genomic *vs* randomized sequences (% base pairing, number of helices, loops, GC content, etc.). However, the systematic computation of the sum of all pairing partners for each base in the suboptimal structures showed a distinct pattern of alternating low and high values when plotted against the sequence length, observed clearly only for genomic sequences. Some known secondary elements (*e.g.* IRES) match well with low value segments. Phylogenetic foldings of aligned sequences seem to corroborate that known biological motifs seem best distinguished by low pairing values. Such motifs can be visualized by coloring a secondary structure plot of the optimal fold as a function of the number of pairing partners. *(L)*

VI: VIRUS RECEPTORS

Study of the Nuclear Localization Signal of the Cauliflower Mosaic Virus (CaMV) Coat Protein Gene

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Caulimoviruses are icosahedral, dsDNA viruses whose invasion of the nucleus by the viral DNA is critical for viral infection. However, the DNA alone cannot penetrate the double membrane layer of the nucleus because of its restricting size (8 kbp). The gene product of ORF (open reading frame) IV [encoding for the viral coat protein (CP)] and its proteolyzed forms are the major components of the virions. We hypothesized that the major CP could participate in the entry of the viral DNA into the nucleus. Different deleted and mutant forms of the CaMV CP were detected by immunofluorescence after transient expression in *Brassica* or *Nicotiana* protoplasts. Constructs that contained the lysine-rich domain (nucleic acid binding domain of the CaMV CP) showed a fluorescent signal in a sub-region of the nucleus (possibly the nucleolus). The signal covered the entire surface of the nucleus when this domain was deleted. Mutation in the basic amino acid sequence . . RKRK . . found at the N-terminal of the CaMV CP abolished nuclear targeting. This shows the importance of this region in nuclear targeting. We have shown that the phosphorylation of the CP does not influence nuclear targeting. An antibody raised against the . . RKRK . . region helped us to show that, by immunoprecipitation of the purified virus, this region is exposed outside the virus and available for interaction with putative plant host factors involved in nuclear transport. We have also mutated the virus in the . . RKRK . . region to determine the importance of this region *in vivo* and on the virus viability. We are presently investigating the influence of the acidic region of the nonprocessed forms of the CP located in the N-terminus in nuclear targeting of the CP. *(L)*

VII: EFFORTS TOWARD ERADICATION OF VIRUS DISEASES

Studies of the Epidemics of Aphid-Transmitted Viruses Infecting Melon in Spain

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Insect-transmitted viruses are a major problem for vegetable cultivation worldwide. Melon (*Cucumis melo* L.) is an important vegetable crop in Spain, where it is in first place by acreage and in third place by total production. Virus-like symptoms are frequently associated with melon cultivated in the open in the various production areas. Most of the infections are due to aphid-transmitted viruses but little information is available about virus predominance, population structure and epidemiology. Such data are critical for the establishment of control measures in order to eradicate the viruses or minimize the losses that they cause. A 3-year survey (1994–96) was conducted in different melon-growing areas of Spain in an attempt to characterize the epidemics of aphid-transmitted viruses. The results indicate that cucumber mosaic cucumovirus and watermelon mosaic potyvirus-2 are the predominant viruses. Zucchini yellow mosaic potyvirus and papaya ringspot potyvirus were also present, but of minor importance. Data were presented on the prevalence of the different viruses, along with data on the temporal and spatial evolution of virus infection in plots from three different growing areas in Spain. *(P)*

The Occurrence and Eradication of Raspberry Bushy Dwarf Virus (RBDV) in the Czech Republic

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RBDV was found for the first time in the Czech Republic in 1994. During 1995 the virus was detected by ELISA in 16 cultivars of raspberry and blackberry at four localities. No uniform or typical symptoms were observed on infected plants. The virus was transmitted by mechanical inoculation from four cultivars to *Chenopodium quinoa* plants. Successful graft transmission of the virus to four plants of cv. 'Malling Jewel' revealed that the resistance-breaking isolate of RBDV is present. The presence of the S isolate and RBDV occurrence in wild raspberry and blackberry plants remain to be proved. Meristem tip cultures were used successfully for virus elimination; however, one of ten plants tested was found positive in RBDV ELISA and reinfection of virus-free plants was recorded in the field. As a result, RBDV ELISA was added to the existing certification scheme for the production of virus-free raspberry seedlings. Establishment of new isolated nurseries and removal of cane before flowering were recommended to nurserymen to prevent pollen transmission of the virus. *(P)*

VIII: BACTERIOPHAGE EXPRESSION

A New DNA Integrase from Filamentous Temperate Bacteriophage Cflt

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Cflt is a single-stranded DNA filamentous phage isolated from *Xanthomonas campestris* pv. *citri*. Southern blot analysis revealed that the replication form of the Cflt phage genome can integrate into the host chromosome at a specific site. Integrative recombination of most temperate bacteriophages is catalyzed by site-specific recombinases or integrase. However, despite a few reports on integration in *Xanthomonas*, data on whether filamentous phage produces integrase or another factor responsible for site-specific integration have not yet been published. A 1.9-kb segment of DNA from Cflt was found to be responsible for site-specific integration into *X.c.* pv. *citri* in the absence of any *Xanthomonas* origin replication. Deletion analysis and introduction of amber stop codons into this fragment from Cflt revealed an open reading frame (ORF 344) which was involved in the integration function. The predicted amino acid sequence of ORF 344 bears no homology with conserved sequences of the integrase family. Cflt forms stable lysogens in *X.c.* pv. *citri via* site-specific integration of the phage genome. Recombination occurs within specific phage and bacterial attachment sites and is catalyzed by the phage-encoded integrase protein *in vivo*. We described the overexpression and purification of Cflt integrase and its ability to mediate integrative recombination *in vitro*. We found that Cflt integrase-mediated recombination is greatly stimulated by extracts of *X.c.* pv. *citri* but not by *Escherichia coli* extracts, purified *E. coli* integration host factor, or purified non-specific DNA binding protein, indicating the presence of a novel *X.c.* pv. *citri* integration host factor. *(L)*

Host-Dependent Modification/Restriction and Therapeutic Potential of *Pseudomonas* Phage

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The recently isolated phages ϕST3 and ϕST1 were studied as to their lysis behavior and growth adaptation in *ca* 100 different *P. aeruginosa* strains. The replication of ϕST3 varies greatly in the different host strains. A case of 'non-classical' modification and restriction was demonstrated which is caused by altered virus absorption to cells due to reversible virus protein modification by the former host strain. ϕST1 displays stable lysis properties in most of the host strains. No case of non-classical (virus protein) or classical (virus DNA) modification/restriction was found. The latter can be explained by a selection against recognition sites of hosts' restriction enzymes in the viral genome. This makes ϕST1 a candidate for therapeutic phage preparations. *(L)*

Regulation by One Repressor at Different Operators in 16-3 Phage of *Rhizobium meliloti*

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Phage 16-3 of the nitrogen-fixing bacterium is a typical temperate phage, as a lambdoids of *Escherichia coli*. Repressor 16-3 binds to cognate operator regions, rightward and leftward. Both regions contain two operator units: rightward, O_{R-1} and O_{R-2}; leftward, O_{L-1} and O_{L-2}. Several constructs containing different operator units and their combinations were created to prove that all four operator units are active *in vivo*, and to study the cooperative binding of the repressor molecules. For *in vitro* studies the repressor coding region was cloned in expression vector and the protein was overproduced in *E. coli*. The minimal sequences required for the repressor to bind DNA were determined by testing the ability of the purified protein to bind DNA fragments containing chemically synthesized sequences with different length O_R and O_L operator units. The results of these *in vivo* and *in vitro* experiments showed that the structure of the operator units in the different operator regions differs significantly. The repressor is able to recognize the 5'-ACA.....TTGT-3' inverted repeat as its binding site when the palindrome sequences are separated by either a 6-base pair spacer (O_R units) or a 4-base pair spacer (O_L units). From these topologies and steric relations, at least two questions of general importance arise: (i) How can the confirmations of protein and/or DNA accommodate the spatial and rotational differences in sequence specific binding? (ii) How does the overall specificity of repression build up over O_L *vs* O_R? We expect that our ongoing footprinting analyses can provide some clues to answer these questions. *(P)*

Analysis of Phage MS2 Replicase Gene Translation Initiation Region in a Natural Selection Evolution System

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In the present work we studied the regulation of phage MS2 replicase gene translation. Briefly, a partially random stretch of 15 nucleotides was introduced into the phage RNA through its cDNA. A number of viable mutant phages were obtained, used to infect *Escherichia coli* F^+ cells for many further phage replication cycles. RNAs of resulting phages were sequenced over the region(s) of interest to monitor whether and what compensatory mutations had appeared in response to the initially introduced base changes. Our analysis of several descendant phage populations at early infection cycles revealed that: compensations had appeared in RNA of all phage populations under study; in most instances these base compensations represent either pyrimidine to purine transversions or A to G transitions. As one of the main determinants of translation initiation regions on prokaryotic mRNA is the polypurine sequence or Shine-Dalgarno (SD) box, the appearance of observed secondary mutations may be interpreted as rebuilding of the SD region. However, some of the compensations seem not to be related only to optimization of the structure of a ribosome binding site in a simple way. The replicase gene translation start hairpin is the binding site of translational repressor – phage coat protein and base changes might indicate evolutionary events leading toward restoration of a functionally active regulatory structure. *(P)*

IX: PHYLOGENETIC VIRUS TAXONOMY

Endosymbiotic and Monophyletic Early Origin of Viruses

Carlos A. Sariol-Carbelo

Many theories have been proposed to explain the origin of viruses. All of them consider how viruses originated, and some propose ideas about when viruses arose. In none are the time and manner in which viruses originated analyzed as a dynamic, continuous, and progressive process closely linked to the origin and evolution of cells. The strong function and structural links between virus and cell firmly support that virus evolution and host cells' evolution have been unique processes with a predominance of interchange and complementation modulated by competition and exclusion. In this hypothesis, the virus–cell relationship is explained while proposing an endosymbiotic and early monophyletic origin of virus as an integral part of the cell. An origin for all viruses from an ancestor of -RNA is proposed. Through their endosymbiotic origin, viruses are identified as

well organized genetic elements that arose inside the same medium and, at the same time, in a specific cooperative state with their cell ancestors. These evolved cells are presently their host, where the cell provides the necessary mechanism to maintain the virus–cell specificity, and the viruses guarantee the genetic information exchange from cell to cell, accelerating and enriching cell evolution. *(P)*

Biodiversity of Viruses in the Guanacaste Conservation Area of Costa Rica

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Numerous studies of biodiversity have been initiated in several parts of the world in the last decade. Recently these studies have begun to include microorganisms, but viruses have been largely ignored. An All Taxa Biodiversity Inventory (ATBI) has recently been initiated in the northwestern portion of Costa Rica, in a 120,000-ha region known as the Guanacaste Conservation Area. This region includes both primary and recovering secondary dry forest, as well as rain forest, cloud forest, and land used for agriculture. This study is the first comprehensive biodiversity inventory to include viruses. A basic outline of the proposed project was presented, as well as a pilot project for plant viruses. In addition, implications of this study for the understanding of virus evolution and emerging virus diseases were discussed. Participation by the international community of virologists is sought in accomplishing this enormous task, which should contribute significantly to our understanding of basic virology and of virus diseases. *(L)*

Phylogenetic Relationships of the Polymerases of DNA Viruses and Retroviruses

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RNA polymerases have been used by several laboratories, to establish phylogenetic relationships among the families of positive ssRNA viruses, dsRNA viruses and negative ssRNA viruses. These studies have suggested that the RNA viruses examined can be placed in a single phylum of four classes which contain positive ssRNA viruses only, a fifth class that contains the ssRNA bacteriophages and the dsRNA totiviruses, four additional classes of dsRNA viruses, and a single class of negative-strand RNA viruses. The relationships among the polymerases of the DNA virus families have not received the same attention. A preliminary analysis was done of the relationships among ten families of DNA viruses, while another study established the relationship between the retroviruses, and caulimoviruses, and revealed unexpected differences among members of the three families of tailed phages. The sequence relationships of the DNA polymerases of the five families of ssDNA viruses and the 17 families or groups of dsDNA viruses were examined and compared with the reverse transcriptases of the hepadnaviruses, badnaviruses, caulimoviruses and retroviruses. *(L)*

A Reappraisal of the Taxonomy of the Genus *Ilarvirus* and an Examination at the Molecular Level of the Relationship between Members of this Genus and Alfalfa Mosaic Virus

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Sequencing of a number of ilarviruses has revealed that some reorganization within the genus is possible. Five viruses (AV-2, CVV, EMoV, SpLV and PMoV) currently given distinct names are probably best considered as strains of the same virus. These five viruses form a separate subgroup that is serologically related to, but distinct from, the remaining members (CiLRV and TAMV) of the currently accepted subgroup 2 of the ilarviruses. Phylogenetic analysis confirmed this separation and further indicated that these two groups are distinct from tobacco streak virus (TSV), the type member of the genus *Ilarvirus*. This analysis also confirmed that alfalfa mosaic virus (AMV) is more closely related to members of the genus *Ilarvirus* than to either of the other genera (*Cucumovirus* and *Bromovirus*) that are included in the family Bromoviridae. Despite increasing evidence at the molecular level of similarities between AMV and the ilarviruses, it is still insufficient to support the inclusion of AMV within the genus *Ilarvirus*. *(P)*

Nucleotide Sequences of Apple Mosaic Ilarvirus RNAs 1 and 2 and Their Homology to Other Viruses

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Apple mosaic ilarvirus genomic RNAs 1 and 2, which purportedly code for the viral RNA polymerase, have been cloned and identified. RNA 1 encodes a large polypeptide whose amino acid sequence shares homology to the putative helicases of the tripartite viruses citrus leaf rugose, alfalfa mosaic, and cucumber mosaic, and also to several tobamoviruses, pea early browning tobravirus, and lettuce infectious yellows closterovirus. RNA 1 nucleotide sequence shares homology with the RNA 1 of cowpea chlorotic mottle bromovirus. RNA 2 nucleic acid or amino acid sequence shares homology with the putative polymerase domains to most of the previously mentioned viruses as well as a number of viruses that infect vertebrates. Independent clones of both RNA molecules confirm that the 3' nontranslated region is highly homologous, but not identical, to the previously published apple mosaic virus RNAs 3 and 4 sequences. *(P)*

Four Potential Taxonomic Levels in the Family Potyviridae

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Taxonomy of Potyviridae has long been confused due to the large size of the group and their complex serological relationships. Comparisons among nucleic acid and protein sequences have already been used successfully to solve this problem, but within the limits of the 3'-terminus genome (*e.g.* 10% of the genome). With the increasing amount of molecular data available, it is now possible to extend this approach to other regions of the genome. In this study we compared at the nt and aa levels complete or partial sequences encoding for the different potyviral proteins or for some specific regions of these proteins. All percentage pairwise identities were plotted as frequency distribution and alignment data were used as inputs to generate phylogenetic trees. Surprisingly, despite the heterogeneous level of homology among the different proteins, we observed a conserved pattern of frequency distribution all along the potyviral genome. Thus, four clusters are invariably found regardless of the chosen protein: one corresponding to the genus level, one to the species level and one to the strain level, as previously reported for the 3' end of the genome. However, in addition to these clusters, we noted a fourth cluster intermediary to the strain and the species levels, which could be interpreted as another taxonomic level in the Potyviridae family. This fact is also supported by the strength of the phylogenetic relationships among members of this fourth cluster and may explain a number of situations

that do not conform with the species or strain levels. The significant differences between each cluster suggest that it is possible to quantify the taxonomic status of a given isolate. Short sequences, representative of the entire viral genome, have been selected, then primers based on conserved sequences will be designed to allow rapid diagnostic and classification of a virus isolate. In the light of these recent data, a possible evolution scheme of Potyviridae was proposed. *(L)*

Sweet and Sour Cherry Isolates of Plum Pox Potyvirus (PPV): Prototypes of a New Group of PPV

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PPV isolates have been classified conventionally into two groups, M and D. This classification is based mainly on serological reactions and RFLP analysis of 3'-terminal region of the coat protein gene (CP-gene). Members of the D and M groups share an *Alu* I restriction site. Isolates of the M group are differentiated from those of the D group by the lack of a *Rsa* I restriction site. A 243 bp fragment from the C-terminal region of CP-gene of PPV – sweet cherry isolate (PPV-SwC), from Italy, and one of PPV – sour cherry isolate (PPV-SoC), from Moldava, were amplified from total nucleic acid extracts of infected tissue, using the RT-PCR technique. RFLP and nucleotide sequence analyses of the amplified products revealed the absence of *Alu* I and *Rsa* I restriction sites in both PPV-SwC and PPV-SoC. Nucleotide sequences of the two isolates shared 98% similarity, but showed a lower level of homology when compared with the corresponding region of other known isolates of PPV. In addition, PPV-SwC and PPV-SoC are able to infect systemically cherry trees, whereas the isolates from D and M groups cannot. These findings suggest that the cherry isolates are prototypes of a new group of PPV, for which the name PPV-Cherry (PPV-C) is proposed. *(P)*

Phylogenetic Justification for Splitting the Rymovirus Genus of the Family Potyviridae

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The Potyviridae family is currently divided into four genera on the basis of vector transmission – *Potyvirus* (aphid-transmission), *Rymovirus* (mite-transmission), *Bymovirus* (fungal-transmission) and *Ipomovirus* (whitefly-transmission). We have cloned and sequenced the 3'-terminus of a South African isolate of the rymovirus ryegrass mosaic virus (RGMV-SA) and have reassessed the phylogeny of the Potyviridae taking into account the new sequence data of RGMV-SA and other putative members of the *Rymovirus* genus. The analysis revealed that rymoviruses RGMV-SA, Agropyron mosaic virus (AgMV) and Hordeum mosaic virus (HoMV) cluster together basal to a well-defined cluster of generic potyviruses rather than to putative rymoviruses wheat streak mosaic virus (WSMV) and brome streak mosaic virus (BsMV). We suggest that the RGMV – AgMV – HoMV group be reclassified as a mite-transmitted subgenus of the genus *Potyvirus*. *(P)*

Phylogenetic Analysis of Badnaviruses: Taxonomic Implications

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Since Brunt *et al.* (1964) first described cacao swollen shoot virus (CSSV), the group of viruses to which it belongs has grown steadily. Subsequently named the badnaviruses, this genus of plant viruses consists of small (30×130 nm), non-enveloped bacilliform viruses with genomes of open circular, double-stranded DNA. The sequences of four different badnavirus species have been published; CSSV, commelina yellow mottle virus (CoYMV), sugarcane bacilliform virus (SCBV) and three rice tungro bacilliform virus (RTBV) sequences. These sequences, together with the recently completed nucleotide sequences of a rice tungro isolate originating from India, banana streak virus (BSV) and *Dioscorea alata* bacilliform virus (DABV), have been used to analyze the relationships among the badnaviruses by the construction of phylogenetic dendrograms. Based on the results obtained, as well as biological evidence, a subdivision of the genus *Badnavirus* into two sub-genera is proposed. *(P)*

Analysis of the Sequences of Two Badnaviruses, Banana Streak Virus (BSV) and *Dioscorea alata* Badnavirus (DABV)

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Badnaviruses are characteristically small, non-enveloped, bacilliform particles containing circular dsDNA genomes. Features of the genome, and of the putative protein products it encodes, suggest they are pararetroviruses. There are nine definitive members of the genus, four of which have been cloned and sequenced. Here we present the sequences for two definitive members, BSV and DABV. BSV was purified from infected Nigerian banana plants. A full-length PCR product of the circular DNA cloned as restriction fragments was sequenced in both directions. DABV, one of two badnaviruses identified in *Dioscorea* spp., was purified from infected Nigerian *D. alata*. The genomes of both viruses have an organization characteristic of one of the proposed sub-groups of the badnaviruses. The sequences of the two viruses will be compared with those of other badnaviruses. *(P)*

Sequence Analysis of Rice Tungro Bacilliform Virus Indian Isolate (RTBV)

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Badnaviruses are one of the two families of plant pararetroviruses. Nucleotide sequences for several badnaviruses are available but little is known about variation within each one. RTBV, which is transmission-dependent on rice tungro spherical virus (a plant picornavirus), is one of the best studied badnaviruses in terms of variation. RFLP mapping and cross-hybridization of different geographical isolates of RTBV have shown that isolates from the Indian subcontinent differ significantly from others originating from South-East Asian countries. In this study the sequence of an Indian isolate of RTBV was compared with that of a Philippines isolate. The overall nucleotide sequence homology between Indian and Philippines isolates is *ca* 70%. The Indian isolate is 68 bp shorter, with deletion of 64 bp. Amino acid sequence similarities (familial) for individual open reading frame(s) (ORFs) range from 79% (ORF IV) to 94% (ORF II). ORF III has highly conserved regions for protease, reverse transcriptase and RNaseH. Like the Philippines isolate, the Indian isolate has ORF IV, which has not been found in other badnaviruses. Phylogenetically, the Indian isolate differs significantly from other badnaviruses as well as from the Philippines isolate. Based

on the homologies with other badnaviruses, the possible functions of ORFs were discussed. *(P)*

Taxonomic Relationship between Animal and Plant Circoviruses

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Porcine circovirus (PCV) is a small isometric virus 17 nm in diameter with a monopartite circular ssDNA genome. Similar viruses have been identified in a range of animal and plant species but their unrelatedness to other ssDNA viruses has led to their classification in a new virus family, the Circoviridae. Although limited protein homology exists among plant circoviruses, no significant homology has yet been demonstrated within the animal circoviruses or between the animal and plant circoviruses. The only characteristics that the multipartite plant circoviruses – banana bunchy top virus (BBTV), subterranean clover stunt virus (SCSV) and coconut foliar decay virus (CFDV) - share with the animal circoviruses are that all have small isometric virions and a ssDNA genome. This has led to the proposal that they should be classified separately. Nucleotide sequence analysis of the genome of PCV detected no significant homology with any of the animal circoviruses but, surprisingly, the greatest protein homology was detected with the plant circovirus BBTV, with high protein homology also being detected with the other plant circoviruses, SCSV and CFDV. This finding shows that PCV bridges the gap between animal and plant circoviruses and provides the necessary proof that they should remain within the same virus family. *(P)*

Olive Latent Virus 2, Representative of a Putative New Genus in the Family Bromoviridae

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Olive latent virus 2 (OLV2), a virus with quasi spherical to bacilliform particles resembling those of alfalfa mosaic alfamovirus (AMV), has a non-polyadenylated, tripartite, positive-sense ssRNA genome. OLV2 is serologically unrelated to AMV and to all other tested viruses with a tripartite genome. Virions encapsidate four major RNA species, all of which were sequenced. RNA1 (3126 nt) and RNA2 (2734 nt) are both monocistronic molecules coding for replication-related proteins, with conserved motifs of helicase, methyltransferase (RNA1) and RNA polymerase (RNA2). RNA3 (2438 nt) is a bi-cistronic molecule homologous to the third genomic RNA of tripartite genome viruses. RNA3 codes for a 36.5 kDa polypeptide with conserved motifs for the 30 kDa superfamily movement proteins, and the 20 kDa viral coat protein (CP). RNA4 (2078 nt) is co-terminal with RNA3 and lacks coding capacity. Its biological function, if any, was not determined. A subgenomic RNA (*ca* 1042 nt) with strong homology with RNA3, probably responsible for CP production, is formed in infected plants but it may not be encapsidated. Comparative computer-assisted analysis of virus-coded protein sequences disclosed a relationship in diverging directions with members of the family Bromoviridae, thus qualifying OLV2 as the possible representative of a new genus in the same family. *(P)*

Variation of Genomic Structure in *Trichovirus*

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Trichovirus, a recently established plant virus genus with a polyadenylated monopartite ssRNA genome, has two definitive [apple chlorotic leaf spot (ACLSV) and potato T (PVT) viruses] and three tentative [grapevine A (GVA), grapevine B (GVB) and heracleum latent (HLV) viruses] species. ACLSV, GVA and GVB have been completely, and PVT partially, sequenced. ACLSV and PVT have three open reading frames (ORFs) encoding, respectively, a 216K polypeptide with conserved motifs of viral replicases (ORF 1), a 40-50K polypeptide identified as the movement protein (ORF 2), and the 21-28K coat protein (CP; ORF 3). GVA and GVB have five ORFs. ORF 1 (195K) is the replicase cistron; ORF 2 codes for a 20K polypeptide with unknown function, for which no significant homology was found in several data base protein sequences; ORF 3 encodes a 31-36K polypeptide possessing the conserved motifs of the 30K superfamily movement proteins; ORF 4 (21.5K) is the CP cistron; and ORF 5 codes for a small (10-14K) polypeptide sharing some homology with the 3'-terminal polypeptides of different plant viruses. Computer-assisted comparative analysis of virus-coded proteins has shown that the four viruses are phylogenetically related but, in most cases, they cluster in couples, *e.g.* ACLSV/PVT and GVA/GVB. ACLSV and PVT are both readily transmitted by sap inoculation, replicate in parenchymas and have unknown vectors. By contrast, GVA and GVB are transmitted mechanically with great difficulty, are phloem-limited in the natural host, and have pseudococcid mealybug vectors. These molecular and biological differences warrant a re-definition of the taxonomic position of GVA and GVB. *(P)*

X: ANTIVIRAL AGENTS FROM PLANT SOURCES (PLANT EXTRACTS; PROTEINS)

Inhibition of Sensitive and Acyclovir-Resistant HSV-1 Strains by an Elderberry Extract *In Vitro*

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A standardized plant extract (Sambucol, SAM), based on the fruit of the black elder (*Sambucus nigra* L.), was tested against HSV-1 in MS 9 (human diploid fibroblasts) and BGM cells. The replication of HSV-1 strains was completely inhibited: (*a*) by pre-incubation with SAM before infection of the cells; (*b*) when SAM and the virus were added simultaneously to the cells; and (*c*) when SAM was added 30 min after viral adsorption on the cells. No cytopathic effect was observed during 10 days. The four strains of HSV-1 tested, *viz.*, a reference strain (ATCC), a strain isolated from a patient, and two acyclovir-resistant isolates, gave similar results. The inhibition of acyclovir-resistant strains by this elderberry extract may be of significance and deserves further *in vitro* and *in vivo* testing. *(P)*

Beneficial Effect of an Elderberry Extract in the Treatment of HTLV-1 Associated Myelopathy (HAM/TSP): A Preliminary Study

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A standardized plant extract, Sambucol (SAM), based on the fruit of the black elder (*Sambucus nigra* L.), was found previously to inhibit the replication of influenza virus type A and B and of HIV. In the present study SAM was given *per os* on a daily basis to six HAM/TSP Israeli patients of Iranian origin for a period of one month. The titer of HTLV-1 antibodies as

well as the percentage and number of CD-3, CD-4 and CD-8 lymphocytes were measured in these patients, before and after SAM treatment. In three of the six patients tested, the HTLV-1 antibody titer decreased by 50% after SAM treatment. In two of the six patients, the numbers of CD-3 and CD-4 lymphocytes – which were high before treatment – decreased by 30% after treatment. These patients also reported a feeling of well being following treatment. These preliminary results seem to justify a controlled study in the future on HAM-TSP patients. *(P)*

Anti-HIV-1 Effects of Extracts from *Calendula officinalis* Flowers

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Calendula officinalis (Asteraceae) is an annual herbaceous plant, used in Bulgarian folk medicine as an anti-inflammatory, antipyretic, antitumorogenic, and cicatrizing remedy. Recent pharmacological studies of extracts of the aerial parts of this plant have confirmed the presence of flavonol glycosides and triterpenoids. Organic (lipophilic) and aqueous (hydrophilic) extracts of dried *C. officinalis* flowers were examined for their ability to inhibit HIV-1 replication *in vitro*. Uninfected and acutely infected human lymphocytic Molt-4 cells were maintained in the absence and in the presence of increasing concentrations (0.001–10 mg/ml) of test extracts and monitored up to 96 h post-infection. The results showed that both extracts were potent anti-HIV drugs, but were relatively non-toxic to Molt-4 cells. The anti-HIV action was found to be dose-dependent, with an ID 50% of 0.031 mg/ml and 0.119 mg/ml for the organic and aqueous extracts, respectively. Furthermore, in the presence of the lipophilic extract (0.05 mg/ml) the uninfected Molt-4 cells were completely protected for up to 24 h from fusion and subsequent death, caused by persistently infected U-937/HIV-1 cells. Also, an equivalent concentration of the organic extract caused a significant and time-dependent reduction of HIV-1 reverse transcriptase activity: 68% inhibition was achieved after a 30-min treatment of partially purified enzyme in a cell-free system. These results suggest that extracts of flowers from *C. officinalis* possess anti-HIV properties of therapeutic interest. *(P)*

Evaluation of the Antiviral Potential of *Sanicula europaea* Extracts in the Bacteria-Bacteriophage System

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The antiviral activity of the *Sanicula europaea* L. extract in a bacteria-bacteriophage system was studied. We observed that the water-soluble fraction of *S. europaea* extract has a viricidal effect on phage T2 hr^+ and that it inhibits T2 infection on *Escherichia coli* B2 cultures without any side effects on host cells. The viricidal activity of the extract increased with increasing incubation time and incubation temperature. In the presence of mono- and divalent ions in the incubation medium, the viricidal activity of the extract disappeared completely. The fractions separated from the crude extract through Sephadex gel filtration chromatography were also tested separately on the growth of phage T2; only fraction I was found to be active. *(P)*

In Vitro Synergistic Effect of Absinthe, Rose and Jadwar on Vero and HEp2 Cells against Herpes Simplex Virus (HSV) Type 1 and Type 2 Infection

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It was reported that various combinations of *Artemesia absinthium* (absinthe=Ab), *Rose centifolia* (rose=Ro), *Resin pistacia lenticus* (mastic=Ma), and *Delphenum denodatum* (jadwar=Ja), when used to treat mentally depressed patients, also helped them to recover from the symptoms of HSV infection. *In vitro* tests were conducted to evaluate the toxicity of these plant extracts in water, hexane, methylene chloride and methanol using separate monolayers of African green monkey kidney (Vero) and Human epithelial type 2 (HEp2) cells. These cells were grown in minimum essential medium (MEM) supplemented with 5% fetal calf serum and 1% pen/strep at 37°C with 5% carbon dioxide tension. The noncytotoxic level of combination extract in water was 1:4 and in all others was 1:32 at 48 h against control. Anti-HSV type-1 and type-2 activities, using noncytotoxic dilution of the extracts, were tested in Vero and HEp2 cells. The test plates were observed for 96 h for cell protection as well as cytopathic and cytotoxic effects. Complete protection of Vero and HEp2 cells against both HSV types was observed only with combination (3Ab:2Ro:1Ja) water extracts at 1:4, 1:8, 1:16 and 1:32 dilution. No protection was observed with other extracts alone or in combination. The protein patterns of HSV-1 and HSV-2 infected cell cultures treated with 3Ab:2Ro:1Ja were different from those of untreated infected cells and treated control cells when analyzed by SDS-PAGE. With thin layer chromatography and protein assays it was possible to determine that no lipid and a negligible amount of protein are present in these plant extracts. Further work is in progress to purify and identify active compounds in plant extracts for the treatment of HSV infection. *(P)*

Antimicrobial Activity of Some Essential Oils Extracted from Aromatic, Condiment and Medicinal Plants of Cameroon

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This paper concerns the antibacterial activity of 43 essential oils (E.O.s) extracted from different parts of 30 plants. The oils were extracted by the hydrodistillation technique. Antibacterial assays were performed by the micro-atmosphere technique for the determination of the minimal inhibitory quantity, and by the liquid-dilution technique for the minimal inhibitory concentration. Several E.O.s evinced important antibacterial activity. We have also done these assays on fungi and are considering conducting the same work on viruses. *(P)*

Constitutive Expression and Inclusion Body Formation of Pokeweed Antiviral Protein in *Escherichia coli*

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Pokeweed antiviral protein (PAP) is a specific glycosidase which inactivates ribosomes. PAP cDNA was synthesized by RT-PCR. PAP was expressed in *E. coli* with a constitutive promoter. The yield of PAP was calculated to be *ca* 4 mg/*l* of culture medium. The effect of endogenous expression of PAP on *E. coli* growth rate was analyzed. *E. coli* cells harboring the PAP gene in the sense orientation when compared with the antisense one, had a noticeable delay in growth. However, the growth rates of subcultures made were similar to those of cells which do not express PAP. Ultrastructural immuno-gold labelling of *E. coli*

cells harboring the expression vector showed that PAP was found in inclusion bodies. These results demonstrate that PAP can be expressed in *E. coli* from not only an inducible promoter, but also from a constitutive promoter, and the expressed PAP may have an initial inhibitory effect. *(P)*

Antiviral and Ribosome-Inactivating Activity Analysis of Pokeweed Antiviral Protein cDNA Deletion Mutants

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Pokeweed antiviral protein (PAP) is an enzyme of 29 kDa known to inactivate a wide variety of eukaryotic ribosomes. It has potent antiviral activity against many plant and animal viruses. Here we report the effects of C- and N-terminal deletions on antiviral and ribosome-inactivating activities of PAP. Progressive deletions of PAP cDNA from both 3' and 5' ends were conducted and expressed in *Escherichia coli*. Truncated forms of protein expressed were quantified by an immuno-blot assay. Analysis of antiviral and ribosome-inactivating activity was performed using tobacco mosaic virus local lesion assay and a rabbit reticulocyte lysate *in vitro* translation system. The biological relevance of these two activities was determined. Results show that: (i) two mutants, with 55 and 80 amino acids removed from the C- and N-termini, werre found to have their antiviral activity reduced by 8.3% and 81.2%, respectively; (ii) N-terminus sequence is required for the activity of PAP; and (iii) the antiviral activity of the mutants is correlated with their ribosome-inactivating activity. *(P)*