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A: EPIDEMIOLOGY OF PLANT DISEASES

IMPROVED "SINGLE TILLERS" METHOD FOR LOSS ASSESSMENT IN WHEAT

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Common methods for loss assessment include establishment of experimentally derived damage functions expressing the relationships between disease level and yield; and estimates, based on the damage functions, of losses in other fields. There are drawbacks to these methods, such as (i) cost, preplanning and prospects for success of the field trials, and (ii) validity of the functions for other fields.

In the "single tillers" method the functions are based on hundreds of tillers. The main drawback to it is the validity of estimates derived from single tillers, for a whole crop. The advantages of the method are that (i) it can be applied simply in any desired situation without preplanning and without allocation of field experiments; (ii) the damage function is calculated for each field evaluated; and (iii) it is very inexpensive to operate.

Attempts to standardize procedures in order to improve the validity of the method were tested in field trials. Tagging only 300 single tillers was found to be the optimal sample size which provided small enough SE values. Disease severity on flat leaves at the early milk growth stage was the most important disease factor affecting yields. Different loss models were tested (the critical stage model, the multiple regression model, the stepwise regression model, and the area under disease progress curve model) and were found satisfactory for predicting yield losses. The critical stage model, which is simpler than the rest, was then chosen for use under Israeli conditions. By taking into account plant factors unaffected by the disease, percentage of yield variability explained by disease level was improved from previous analyses explaining only 3-8% of the variation, to new analyses explaining 53-78% of the variation. The losses determined by the improved method did not differ significantly from the values calculated from ordinary field trials.

"CRWD-1 AND -2" (DECISION MAKERS 1 AND 2): COMPUTERIZED RECOMMENDATION SYSTEMS FOR THE CONTROL OF FOLIAR WHEAT DISEASES

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Foliar wheat diseases may develop into epidemics and cause appreciable losses to yields. Epidemic development depends on climatological and agronomical conditions and it is impossible to forecast the specific disease development in each particular field. Decision making, based on the specific agrotechnical, epidemiological and meteorological conditions, is needed for each field.

The computerized method "CRWD-1" (Control Recommendations for Wheat Diseases)

was designed for use by the farmers. It combines general information from research (built-in information), with specific information for the particular field (information added by the farmer). It offers a strategy of control, based on disease thresholds, the conditions affecting disease development and specific economic conditions. The method was tested in 1985 in two regions (northern Negev and Bet She'an Valley). All recommendations to withhold control and 75% of the recommendations to apply control, proved to be beneficial.

Based on these findings, the method was then improved, to include weather forecasts in addition to the consideration of climatic conditions that had prevailed before decision making, which was used in the first version. The new version, "CRWD-2", will be tested in 1986 in the northern Negev.

DESCRIPTION OF EPIDEMICS IN NATURAL ECOSYSTEMS AND ANALYSIS OF FACTORS INVOLVED

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Populations of plants in natural ecosystems are inter- and intra-specifically heterogenous. Epidemics in these populations are caused by different pathogens on different and also on the same hosts. The detailed descriptions of epidemics may be complicated by the genetical and ecological heterogeneity of each species. Two model systems of a wild host and a pathogen were studied.

The wild barley – powdery mildew system: an annual host with a yearly regenerating pathogen. The host plants are usually crowded in groups. Onset of epidemics, rate of development and maximal disease level varied appreciably among geographical locations, between sites in each location, and between seasons at each site. In *ex situ* studies, conducted in shaded and non-shaded plots, disease situations were described by distribution graphs for representative individual plants, according to the disease level in each. Some of the graphs are normal, some are skewed one way or another, and some are flat. The populations were usually more susceptible when tested in the shade. Whereas the geographical origin of the pathogen did not seem to affect the disease, that of the plants did affect the disease level.

The Limonium – rust system: a perennial host with a dimorphic, continuously propagating pathogen. The hosts are discrete and therefore host plants were individually tagged and evaluated *in situ*. The stage of rust in the life cycle and the level of rust for each plant were recorded. Disease situations were described by distribution graphs for plants at each site in each location. The shape of the graph and the average disease level per plant differed between sites in the same location, and between dates at the same sites. Between-sites differences could be related to ecological conditions, since offspring of plants from a disease-free site were diseased when grown at the diseased site and *vice versa*. Within-site differences between seasons can be explained by differences in reactions of plants to two stages of the rust life cycle.

Several factors play a role in the welfare of plants in the wild: ecological niche, host genotype, host susceptibility – mediated hosts' competition, competition between pathogens, disease tolerance, beneficial effects of disease, stage of the host's life cycle affected, etc.

THE EPIDEMIOLOGY OF *LEVEILLULA* MILDEWS IS COMPLICATED BY THE ABUNDANCE OF THEIR GENOTYPES°

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Mildew diseases caused by the collective species *Leveillula taurica* and associated forms affect more than 700 host species in some 70 botanical families.

There is a great divergence in the choice of host species in *Leveillula* populations in various regions of the world. Crops such as pepper, cucumber, squash and cotton, and weeds such as *Prosopis* and *Chenopodium* spp., may be attacked in one region, but not in another. Moreover, the range of hosts that can be cross-infected with inoculum from a given host, differs greatly in various countries. Thus, in inoculation tests in Mediterranean countries, inoculum originating from artichoke infected pepper in France but not in Italy or Lebanon; inoculum from cucurbits infected solanaceous crops in Lebanon but not in Morocco. Inoculum that will infect only the host of origin is produced on numerous hosts: this is particularly common with hosts in the Compositae, much rarer in the Solanaceae. In many cases it has been found that inoculum derived from hosts on which cleistothecia tend to form, will not infect other host species, even if they are closely related to the original host. The reason for this is not clear.

The most likely explanation for the great divergence in host range and cross-infectivity found in populations of *Leveillula* is the existence of an extraordinarily large number of genotypes. These seem to undergo frequent changes and spread to additional regions, *e.g.* in North America.

From a practical point of view, the abundance of genotypes with widely varying host ranges makes it difficult to determine the likelihood of a crop being affected in any given region or season. Only where local cross-inoculation tests establish the danger of inoculum from each of the affected crops or weeds in the region contributing to the infection potential, can the essential epidemiological factor of effective inoculum load be assessed with any degree of accuracy.

HIGH pH ON THE LEAF SURFACE OF COTTON

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The leaf surface of cotton is the major site for the application of insect pathogens to this crop. Therefore, the chemical nature of the phylloplane, in addition to the ultra-violet irradiation from the sun, would determine the effectiveness of baculovirus and *Bacillus thuringiensis* preparations on this crop. The leaf surface pH of commercial cotton in Israel, *Gossypium hirsutum*, var. Acala SJ-2 and 16 other varieties bred in a greenhouse, was measured. The pH values ranged from 9.0 to 10.3 and did not differ between varieties or leaf sides. Washing the leaf surface reduced the pH from alkaline to neutral, but within 36 h the high pH was restored. pH values of leaf homogenates of washed and untreated plants were below neutral, due to the buffering capacity of the leaf tissues.

Scanning electron microscope observations of the epidermal glands responsible for the alkaline leaching showed that there are abundant glands. Topical reaction of the gland contents with pH reagents of basic range indicated that these glands were the source of the phyllosphere alkalinity.

°Lecture not delivered because no time was available.

By cation scanning of the leaf exudates, magnesium and potassium were detected, possibly as carbonates.

Protection of the microbial products from being inactivated by the high pH on cotton would be desirable for economical use of the bio-insecticides. This could be achieved by micro-encapsulation of the microbial agent. However, cloning of the entomopathogen into an epiphytic microorganism of the cotton leaf would improve the effectiveness of the present microbial formulations against the insect pest.

B: RESISTANCE OF PLANTS TO DISEASES

STUDIES OF RESISTANCE OF PERSIMMON ROOTSTOCKS TO DEMATOPHORA ROOT ROT

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The fungus *Dematophora necatrix* Hartig is the causal agent of the white root rot disease in various plants and fruit trees. In the last few years persimmon trees were killed by the fungus in En Zurim and Be'er Toviyya orchards, a new phenomenon not mentioned in the literature. In previous work we considered persimmon as a resistant crop to the disease due to phenol compounds in the roots. It was proven now that the fungus *D. necatrix* is the causal agent of white root rot disease in persimmon trees. A high percentage of the rootstocks *Diospyros kaki* and *D. virginiana* was found to be susceptible to the disease. Variability was observed in the resistance of seeded persimmon rootstock *D. virginiana* to *D. necatrix*. Of 468 plants 5.7% were resistant. Of 235 seeded persimmon plants of the *D. kaki* rootstock, 24.5% were resistant to *D. necatrix*. Vegetative propagation of resistant rootstocks is recommended to maintain resistance. Phenol extracts from persimmon roots growing in soil naturally infested with the Dematophora apple isolate inhibit growth in cultures of the apple but not the persimmon isolate. Cuttings of these same trees are resistant to the apple but not the persimmon isolate. There is no correlation between the level of phenols of *D. virginiana* and *D. kaki* and the rate of growth inhibition *in vitro* of *D. necatrix* apple isolates. Phenol extracts from roots of *D. virginiana* did not inhibit growth of the persimmon isolate of *D. necatrix*.

DETACHED ROOT INOCULATION: A NEW METHOD TO EVALUATE RESISTANCE TO ROOT ROT IN AVOCADO TREES

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A screening method was developed to differentiate between rootstocks of avocado *(Persea american)* resistant and susceptible to root rot disease; the method is applicable to fruit-bearing trees. Detached young (white) avocado root tips are suspended in double-distilled water and incubated with a zoospore suspension of *Phytophthora cinnamomi* at 24°C in the dark. Electrolyte leakage from inoculated root segments is followed by measuring electrical conductivity of the root-bathing solution 24, 48 and 72 h after inoculation. Variability between replicates was reduced by zoospre concentrations above $10^4/10$ root segments. The difference in electrolyte leakage between thick and thin roots was offset by expressing electrical conductivity as a function of root weight rather than of length. Root segments of the resistant avocado Duke 7 and G6 leaked significantly less 48 and 72 h after inoculation than those of six susceptible rootstocks. Large

numbers of horticulturally outstanding trees, or those which survive in infected groves, can be screened quickly and simply without sacrificing or damaging the tested trees. To stimulate the production of young feeder roots needed for the test, the soil surface around the trees should be covered with sawdust. After a few months the cover layer contains sufficient young roots for the assay.

POPULATION ANALYSIS OF EARLY BLIGHT OF POTATO IN THE NORTHERN NEGEV

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Alternaria solani is the causal agent of early blight of potato, an airborne disease of world-wide importance, which is characterized by the appearance of necrotic leaf-spots which grow to large blotches. In the Negev the blight is linked to sandstorms at the end of the growing season. Standard treatment, *i.e.*, spraying several times during the season with the usual protectant fungicides, is effective in other parts of the world, but in the Negev it causes only 20% reduction of the foliar infection and the effect on yield is not clear. The use of tolerant or resistant cultivars might be a good solution for areas where spraying is not feasible.

In northern Europe, screening for early blight tolerance is difficult because of the masking effect of late blight in unsprayed fields. Seed potatoes bred in Europe are sent to the Negev for screening for early blight resistance because in our hot, dry climate, using the differential fungicide metalaxyl (Ridomil), this can be accomplished without the interference of late blight.

In our experiments 200 potato varieties were heavily infected by a spray with *A. solani* spores; a control field was sprayed with a fungicide. Highly susceptible varieties grown in the infected field showed a yield reduction of 80-90%, whereas some varieties showed no reduction at all. The mean yield reduction in the infected population was 20%. The kinetics of the disease index throughout the growth period showed a minimal increase in symptom expression from day 40 to 70, but thereafter a rapid increase was observed in the percentage of leaf coverage by the disease, which we measured between days 85 and 110 as our main disease parameter. We found no correlation between this or any other parameter and yield reduction. The correlation coefficient (0.45) between maturity rate of the clones examined in the control field and the symptom expression in the infected field indicated that some of the symptoms observed may be due to the effects of natural senescence of individual cultivars.

We have shown that there may be factors other than the percentage of leaf coverage *per se*, responsible for the reduction in yield. Physiological parameters, such as stomatal opening, CO_2 fixation and chlorophyll content, were highly affected in the infected susceptible plant, even in green areas and in leaves which had no lesions; these parameters were less affected in resistant plants.

INHERITANCE OF RESISTANCE TO *SPHAEROTHECA FULIGINEA* RACE 2 AND *PSEUDOPERONOSPORA CUBENSIS* IN MUSKMELON

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Cucumis melo var. *reticulatus* cv. 'Ananas Yokne'am' is extremely susceptible to powdery mildew incited by races 1 and 2 of *Sphaerotheca fuliginea*, and to downy mildew incited by *Pseudoperonospora cubensis*. Among about 30 cultivars and lines of *C. melo* tested, PI 124111 was the only one which exhibited a high degree of resistance to the two pathogens. PI 124111 was

selfed for six generations and stabilized for resistance to both powdery and downy mildew by selecting the most resistant individuals.

In order to study the mode of inheritance of resistance of PI 124111, crosses were made between *Cucumis melo* line PI 124111 (resistant to both mildews) and *C. melo* var. *reticulatus* cv. 'Ananas Yokne'am' (susceptible to both mildews). Plants of the F_1 generation were selfed to produce the F_2, backcrossed to the resistant parent (CB_R), or backcrossed to the susceptible parent (BC_S). The F_1 plants were intermediate resistant to powdery mildew and downy mildew. The F_2 progenies segregated 1 resistant: 3 susceptible to powdery mildew and 1 resistant:15 susceptible to downy mildew. The BC_R progenies segregated 1 resistant:1 susceptible to powdery mildew and 1 resistant:3 susceptible to downy mildew. The BC_S progenies were all susceptible to both mildews. No linkage was observed between resistance to the two mildews. It is hypothesized that resistance to race 2 of *S. fuliginea* is conferred by a partially dominant gene, and that to *P. cubensis* by two partially dominant genes.

C: *PHYSIOLOGY OF THE DISEASED PLANT; PRODUCTION OF TOXINS AND MYCOTOXINS; PHYTOALEXINS*

ENZYME ACTIVITY AND SUGAR CONTENT IN TOBACCO PLANTS IMMUNIZED AGAINST *PERONOSPORA TABACINA*

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Tobacco plants (*Nicotiana tabacina* cv. Ky 16) were immunized against blue mold *(Peronospora tabacina)* by injecting conidia of *P. tabacina* into the lower part of the stem. Twenty-one days after stem injection, plants were protected against a challenge inoculation of the foliage with *P. tabacina.* Suckers developed in protected plants were found to be susceptible to the challenge inoculation, whereas foliage on the main stem was resistant. Cuttings taken from protected plants lost their resistance after being rooted (about 6-8 weeks). Leaf extracts were assayed for enzyme activity and sugars content. Protected, unchallenged plant extracts showed higher activity of peroxidase (x3-5), β-1,3-glucanase (x2-3) and lipoxygenase (x5-7) than extracts of unprotected (non-immunized), unchallenged control plants. No significant changes between protected–unchallenged and unprotected–unchallenged plants were detected in amylase or invertase activity. Invertase, β-1,3-glucanase and β-glucosidase activity increased greatly in lesions produced in unprotected–challenged plants compared with protected–challenged plants. Soluble sugars content (anthrone assay) was twice as high in leaves of protected as in unprotected control plants. Chromatographic studies revealed increased glucose and fructose contents in protected *vs* unprotected leaves. External application of 2% glucose to normal unprotected leaves protected them against a challenge inoculation with *P. tabacina.* It is assumed that interrupted sink-source relationships in *P. tabacina*-injected plants is one, and perhaps a major, reason for their resistance to blue mold.

THE ORIGIN OF PECTOLYTIC ENZYMES IN NORMAL AND IN NONRIPENING MUTANT TOMATO FRUITS INFECTED BY *RHIZOPUS STOLONIFER*

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Following infection of tomato fruits by *Rhizopus stolonifer*, polygalacturonase (PG) activity was recorded viscometrically in the mature-green normal fruit and in the nonripening *nor* mutant, at both the mature-green and the mature stage. None of these fruits showed any activity by the viscometric assay prior to infection. In the mature normal fruit, which exhibited PG activity prior to infection, fungal infection resulted in increased activity.

Analysis of PG enzymes by polyacrylamide gel electrophoresis showed that the infected mature-green fruits contained several PG isozymes, all of which corresponded with fungal isozymes, detected after *in vitro* growth on heat-treated tomato tissue. Rocket immunoelectrophoresis showed that fungal enzymes do not react with the specific antiserum to tomato PG and established that the active enzyme in the infected mature-green fruit, normal as well as mutant, is of fungal origin. Infected ripe fruit, on the other hand, contained both fruit and fungal enzymes. The immunological analysis showed that the amount of tomato PG in the ripe fruit increased due to *Rhizopus* infection. In the mature *nor* fruit, fungal infection did not induce fruit enzyme, and the small amount of PG antigen detected by the immunological assay prior to infection was not enhanced by *Rhizopus* infection.

PURIFICATION OF ENDO-POLYGALACTURONASES FROM *COLLETOTRICHUM GLOEOSPORIOIDES* AND FROM AVOCADO FRUITS BY MEANS OF AFFINITY CHROMATOGRAPHY

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Colletotrichum gloeosporioides, the causal agent of anthracnose disease in avocado, secretes endo-polygalacturonase (endo-PG) (E.C. 3.2.1.15) and pectinlyase (PL) (E.C. 4.2.99.3) into a medium containing polypectate. Polyacrylamide gel electrophoresis (PAGE) of the crude enzyme solution revealed three isozymes of PG when stained by negative staining with ruthenium red. One of the endo-PGs from the medium was purified to homogeneity by means of affinity chromatography on cross-linked polypectate and by PAGE. It possessed a molecular weight of 67,000 and an isoelectric point at pH 4.8; the optimal pH for the endo-PG was 5.5.

An endo-PG was purified from avocado fruit to homogeneity by means of affinity chromatography. The enzyme exhibited a molecular weight of 43,000 and an isoelectric point at pH 6.2.

Although both PL and endo-PGs of fungal origin were detected in the infected fruit, the endo-PG of avocado was responsible mainly for the softening of the fruit following infection. The latter conclusion is based on isoelectric focusing and the profile on affinity chromatography of the extract from infected tissue. Moreover, the induced softening of the infected fruits in distant uninfected regions suggests that the pathogen increased the activity of the fruit endopolygalacturonase.

EPICATECHIN, AN INHIBITOR OF AVOCADO LIPOXYGENASE, AND ITS POSSIBLE RELATIONSHIP WITH THE LATENCY OF *COLLETOTRICHUM GLOEOSPORIOIDES*

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A natural inhibitor of avocado lipoxygenase was isolated from peels of unripe avocado fruits and identified as epicatechin. The concentration of epicatechin in unripe fruits was 514 $\mu g\ g^{-1}$ fresh weight of peel; this decreased during ripening to 8 $\mu g\ g^{-1}$ fresh weight, before symptoms of *Colletotrichum gloeosporioides* infection were expressed. A comparison of two cultivars with differing susceptibility to *C. gloeosporioides* showed that the concentration of epicatechin decreased faster in the cultivar in which symptoms appeared first. An atmosphere containing 50 $\mu g\ l^{-1}$ ethylene enhanced the decrease of the lipoxygenase inhibitor in avocado fruits and shortened the period before disease symptoms were expressed. In overmature, firm and naturally infected fruits on the trees in the orchard, the concentration of epicatechin was 260 $\mu g\ g^{-1}$ in the area of the peel without symptoms and only 27 $\mu g\ g^{-1}$ in the area evincing symptoms of infection.

The results were considered in relation to the hypothesis that *(a)* the latency of the infection of avocado fruit by *C. gloeosporioides* can be accounted for by the degradation of the preformed antifungal compound, *cis,cis*-1-acetoxy-2-hydroxy-4-oxo-heneicosa-12,15-diene, which is catalysed by avocado lipoxygenase, and *(b)* the *in vivo* lipoxygenase activity may increase during ripening, owing to the decline in the level of its endogenous inhibitor, epicatechin.

BIOLOGICAL ACTIVITY OF A PROTEIN – LIPOPOLYSACCHARIDE COMPLEX PRODUCED BY *VERTICILLIUM DAHLIAE* ISOLATES

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Verticillium dahliae, during its growth in a synthetic liquid medium, secretes a toxic protein – lipopolysaccharide. This toxin can induce symptoms similar to those of the organism, *i.e.*, chlorosis and necrosis in detached leaves, at a concentration of 1.5×10^{-6}M.

In the present work the toxin was evaluated as to its effect on the growth of excised tomato roots in liquid culture and on the viability of protoplasts from four potato cultivars. Root elongation, secondary roots formation, uptake and incorporation of C^{14}-glutamic acid were examined in the presence of the toxin at a concentration of 1×10^{-6}M. A differential effect of the toxin was found between susceptible (cv. 'Hosen Eilon') and resistant (cv. VF-134) tomato roots. Damage was observed in the susceptible but not in the tolerant tissue. Protoplasts derived from tolerant potato cultivars were damaged less than those of the susceptible cultivars as measured by vital staining with fluoresceine diacetate. No difference was found among the cultivars in their susceptibility to the toxin at the callus level as measured by relative increases in wet and dry weights and in succulence.

ENVIRONMENTAL FACTORS INHIBITING PRODUCTION OF T-2, A MYCOTOXIN OF THE TRICHOTHECENE GROUP

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T-2 toxin (T-2), a trichothecene mycotoxin, is a secondary metabolite of several *Fusarium* spp. It has been implicated in diseases of humans and also as a causal agent in many cases of animal poisoning. T-2 toxicosis is characterized by feed refusal, lesions of the gastrointestinal tract, and emesis. The effect of controlled atmospheres (CA) and sulphur-containing amino acids on T-2 production by the fungus *Fusarium tricinctum* NRRL 3299 grown on PDA, was studied. Addition of methionine (L, D or DL) at a concentration of 10^{-2} M caused a significant reduction in T-2 production (8 μg/60 ml medium as compared with 20 μg/60 ml medium produced in the control). The reduction was accompanied by a decrease of 30% in both radial growth and dry weight of mycelium. L-cysteine and L-cystine at 10^{-2} M partially inhibited toxin production without affecting fungal growth.

In an atmosphere containing 50% CO_2 (given in combination with 20% O_2), the amount of the toxin produced was only 75% of that produced by colonies grown in air, while fungal growth – as measured by mycelial dry weight – was not affected. Increasing the CO_2 level up to 60% and 80% (with 20% O_2) caused a decrease of 75% and 90%, respectively, in T-2 production, but fungal growth was also inhibited – by 50% and 80%, respectively – under these conditions.

In colonies exposed to a combined treatment of 50% CO_2/20% O_2 + methionine 10^{-2} M supplemented medium, the amount of T-2 produced was only 85% of that produced by the control. The results indicate that T-2 production can be controlled using CA. The use of CA + methionine could have a synergistic effect in reducing the amounts of toxin produced.

MYCOTOXIN PRODUCTION BY *ASPERGILLUS FLAVUS, ASPERGILLUS OCHRACEUS* AND *FUSARIUM TRICINCTUM* GROWN IN A COMPETITIVE ENVIRONMENT

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The fungi *Aspergillus flavus* NRRL 5220, *Aspergillus ochraceus* NRRL 3174 and *Fusarium tricinctum* NRRL 3299 are capable of producing aflatoxin B_1, ochratoxin A and T-2, respectively. The production of these mycotoxins by the fungi growing on the same medium was studied using PDA. When *A. flavus* and *A. ochraceus* were grown together, the amount of ochratoxin produced was reduced by 80% (5 μg as compared with 25 μg produced in the control colonies); aflatoxin production was not affected. Similarly, a reduction of 50% was recorded in T-2 produced when *F. tricinctum* was grown with *A. flavus;* also in this case the amount of aflatoxin produced was the same as in the control. In a medium on which *A. ochraceus* + *F. tricinctum* were grown, the quantities of ochratoxin and T-2 produced were identical to the amount produced in the medium on which each of the fungi was grown alone.

In a series of studies *F. tricinctum* was grown for 3, 6 or 10 days on Czapek's broth supplemented with 1% peptone. Following that period the media were passed through millipore filters and subsequently inoculated with *A. ochraceus*. Following an additional 12 days, the amount of ochratoxin produced and the dry weight of mycelia were determined. The amount of ochratoxin detected in the media on which *F. tricinctum* was grown, was 400% higher than that produced on the control media. This was concomitant with a significant increase in dry weight of mycelia. The increase in both ochratoxin and dry weight of mycelium was recorded in all the cases when

A. ochraceus was grown on a medium previously inoculated with *F. tricinctum*, regardless of the period of *F. tricinctum* growth prior to *A. ochraceus* inoculation.

A CITRUS PHYTOALEXIN, 6,7-DIMETHOXYCOUMARIN, AS A DEFENSE MECHANISM AGAINST *PHYTOPHTHORA CITROPHTHORA*, AND THE INFLUENCE OF FOSETYL-AL AND PHOSPHOROUS ACID ON ITS PRODUCTION

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The fungus *Phytophthora citrophthora* is the causal agent of citrus collar rot disease in Israel. The citrus species sour orange *(Citrus aurantium)*, macrophylla *(C. macrophylla)* and trifoliata *(Poncirus trifoliata)* are resistant; rough lemon *(C. limon)*, sweet orange *(C. sinensis)*, and the cv. 'Niva' *(C. reticulata* x *C. sinensis)* are susceptible to this disease.

An antifungal compound was found in extracts of these species and its structure was identified as 6,7-dimethoxycoumarin (scoparone). It was produced in both resistant and susceptible citrus species, but the concentration 4 days following inoculation with *P. citrophthora* was 440 µg/g f.wt. in *C. macrophylla* (resistant), *vs* 31 µg/g f.wt. in *C. limon* (susceptible). The patterns of scoparone production in inoculated resistant and susceptible plants place this compound within the definition of phytoalexin. *In vitro*, scoparone inhibited a wide range of phytopathogenic fungi. The ED_{50} of scoparone against *P. citrophthora* was 97 ppm. In control (non-inoculated) bark, the concentration of this compound was 14-16 µg/g f.wt., and wounding had no effect on production.

Treatments of 3-month-old branches with fosetyl-Al (Aliette®) and phosphorous acid (H_3PO_3) caused in some cases an increase in the accumulation of scoparone in inoculated bark, whereas the level of scoparone in non-inoculated treated bark did not increase. The concentration of scoparone 4 days after inoculation was 850 µg/g f.wt. in *C. aurantium* treated with 250 ppm fosetyl-Al, compared with 220 µg/g f.wt. in untreated plants. In similar treatments with 100 ppm H_3PO_3, the scoparone concentration reached 912 µg/g f.wt. The length of the lesion caused by inoculation was inversely proportional to the increase in phytoalexin concentration caused by fosetyl-Al and H_3PO_3: 6 mm and 2.5 mm in untreated and treated plants, respectively. When branches of the very susceptible cultivar Niva were treated with up to 800 ppm fosetyl-Al and then inoculated, there was no increase over the normal scoparone concentration of 30 µg/g f.wt.; 400 ppm of fosetyl-Al was needed to stop the spread of the lesion. The ED_{50} of fosetyl-Al and H_3PO_3 *in vitro*, against *P. citrophthora*, was 55 and 6.9 ppm, respectively.

EFFECT OF THE GIBBERELLIN SYNTHESIS INHIBITOR PACLOBUTRAZOL (PP 333) AND RELATED COMPOUNDS ON FUSARIUM WILT IN MELON SEEDLINGS

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Previous studies had shown that induced resistance to *Fusarium* by dinitroaniline herbicides in melon seedlings is correlated with stunting. The ability of paclobutrazol (PP 333) – a gibberellin biosynthesis inhitor with fungicidal activity, which causes plant stunting – to induce resistance to *Fusarium oxysporum* f. sp. *melonis*, was tested. Melon seeds were sown in nontreated soil or in

soil treated with 0.3-1 μg paclobutrazol/g. Emerging seedlings were subsequently inoculated with the pathogen. Disease incidence in the pretreated seedlings was reduced by 90-95%. Soil application of paclobutrazol was more effective than foliar application in disease reduction.

Similarly, disease reduction of various extents was achieved by pretreating the seedlings with ergosterol biosynthesis-inhibiting fungicides, *e.g.* propiconazol (Tilt), triadimefon (Bayleton) and fenarimol (Rubigan), which also reduce plant growth. Plant colonization by the pathogen was tested by determination of *Fusarium* propagule density in the plant tissue. Since no significant effect of the tested compounds on tissue colonization by the pathogen was observed, it is assumed that disease reduction can not be attributed to fungitoxicity, but rather to the involvement of the compounds in physiological processes which induce resistance.

Ancymidol, a gibberellin-synthesis inhibitor that has very low fungitoxic activity, also reduced both plant growth and disease incidence. Various gibberellins (GAs) differed in their effect on plant susceptibility. Thus, foliar treatment with GA_3 had no effect on plant elongation and disease incidence, whereas GA_{4+7} caused plant elongation and increased its susceptibility to the pathogen.

EFFECT OF FOLIAR DISEASES ON GROWTH PROCESSES OF SPRING WHEAT IN ISRAEL

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The relationship between the severity of foliar diseases and wheat (*Triticum aestivum* L.) yield in Israel was found to change during the growing season. A linear relationship exists early in the season, when the healthier plants produce higher grain yields than diseased plants. A parabolic relationship between disease severity and yield was observed toward the end of the wheat growing season, when plants with high disease severity at this stage as well as disease-free plants, have lower yields than slightly diseased plants.

When the effect of Septoria leaf blotch on the grain yield accumulation was examined in cv. 'Barkai', it was found that the *rate* of dry weight accumulation in grains decreased in the diseased plants, but the *duration* of dry weight accumulation was extended in the diseased plants, as compared with the healthy ones.

The effect of yellow rust severity on photosynthesis and transpiration was examined in a commercial field (cv. 'Shapir') in the northern Negev. The effect of disease severity on the photosynthesis rate changed during the day throughout the growing season, and in some cases slightly diseased leaves had the maximum rate of photosynthesis. The transpiration rate decreased as disease severity rose.

The change during the season in relationships between foliar diseases and yield might be explained as due to the effect of the pathogens on the growth processes (photosynthesis and transpiration).

MYCOPLASMAS AS PLANT PATHOGENS

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Mycoplasmas are the smallest self-replicating prokaryotes. Their genome is only 500 to 1000 megadaltons in size and is extremely poor in guanine + cytosine. This imposes severe re-

strictions on coding capacity, explaining the low number of cell proteins synthesized by mycoplasmas and, consequently, their limited biosynthetic abilities, exacting nutritional requirements, lack of a cell wall, and parasitic mode of life. Mycoplasmas are widespread in nature and cause disease in man, animals, plants and insects. At least two large groups of mycoplasmas are known in plants and insects. One group, consisting of helical mycoplasmas named spiroplasmas, appears to be mainly parasitic in insects; only a few members of this group cause disease in plants, including citrus stubborn and corn stunt. The other group, consisting of pleomorphic cocci and filaments, is named mycoplasma-like organisms (MLOs) and is responsible for a large number of plant diseases, known collectively as yellows diseases. All the plant mycoplasmas are located in the phloem sieve tubes and are transmitted by leafhoppers.

While many spiroplasmas have been cultivated, none of the MLOs has so far been grown *in vitro* and, consequently, MLOs are poorly characterized. The great difficulty encountered in cultivating mycoplasmas impedes laboratory diagnosis of mycoplasma infections. To overcome this difficulty DNA segments or genes specific for a particular group of infectious agents or for a single species have been selected, cloned, labeled and used as probes in hybridization tests with DNA of the infected tissue. Positive hybridization tests indicate the presence of the agent. The probes developed in our laboratory include ribosomal RNA (rRNA) genes, protein genes, and spiroplasma plasmid DNA. Since rRNA genes are highly conserved, the recombinant plasmid pMC5 constructed from pBR325 and an insert containing rRNA genes of *Mycoplasma capricolum* hybridizes with rRNA genes of other mycoplasmas, other prokaryotes, and even with chloroplast rDNA. Nevertheless, differences in restriction sites within the rRNA operons and their flanking sequences yield Southern hybridization patterns specific for the mycoplasmas, enabling identification of mycoplasmas infecting cell cultures. The test is capable of detecting less than 1 ng DNA, equivalent to approximately 10^5 mycoplasmas. Application of the dot blot technique simplifies the test considerably. It is not only much faster but also much more sensitive, enabling detection of 10 pg DNA. However, pMC5 will be incapable of identifying the mycoplasmas in this test. Moreover, application of pMC5 as a probe for detecting spiroplasmas and MLOs in DNAs of infected periwinkle plants was hampered by the positive hybridization reaction of the prokaryotic rRNA gene probe with the homologous chloroplast rRNA genes. However, the use of a cloned *Spiroplasma citri* plasmid as a probe enabled detection of *S. citri* in infected plant material and in hemolymph of infected leafhoppers at a high sensitivity level, detecting down to 10^3 spiroplasmas per sample.

D: MYCOLOGY, AND THE ETIOLOGY OF PLANT DISEASES

WOODY PLANTS AS HOSTS OF DOWNY MILDEWS (PERONOSPORACEAE)

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Although downy mildews are common among herbaceous plants, they are relatively rare among woody plants (trees, shrubs and vines). In a preliminary search of the literature and herbaria, plants of only 15 out of 330 families are listed as hosts of four genera: *Peronospora* (P), *Pseudoperonospora* (Ps), *Plasmopara* (Pl) and *Peronophythora* (Ph), as follows. Acanthaceae: *Asystasia* (Pl); Bignoniaceae: *Catalpa* (P); Cannabidaceae: *Humulus* (Ps); Caprifoliaceae: *Viburnum* (Pl); Leguminosae: *Cercis* (Pl), *Cassia* (Ps); Meliaceae: *Melia* (Pl); Myricaceae: *Myrica* (P); Myrtaceae: *Syzygium* (P); Passifloraceae: *Passiflora* (P); Rosaceae: *Rosa, Rubus* (P); Sapindaceae: *Litchi* (Ph); Saxifragaceae: *Ribes* (Pl); *Whipplea* (P); Tiliaceae: *Heliocarpus, Triumfetta* (P); Ulmaceae: *Celtis* (Ps); Vitaceae: *Vitis, Parthenocissus, Ampelopsis* (Pl).

Analysis showed: *(a)* The fact that more than one of the mere 21 genera of known susceptible woody hosts (comprising only 0.2% of all plant genera) belong to a particular family, *e.g.* three host genera in Vitaceae, two in Saxifragaceae, etc., points to special affinity of downy mildews in woody plants of certain families. *(b)* Although most families with susceptible woody hosts are scattered throughout the dicots without order, there is also clustering in some, because three families belong to the Rosales and another three to the Urticales, orders which are phylogenetically rather close. *(c)* Two downy mildew genera may be found in a single family, as in herbaceous hosts. *(d)* Plants of families considered relatively advanced phylogenetically, *e.g. Viburnum* and *Asystasia,* may be hosts of sporangium-forming downy mildews; likewise less-advanced plants, such as *Celtis. (e)* In some hosts oospores form, *e.g. Celtis, Humulus, Rosa* and *Vitis,* whereas in others they have not been found, *e.g.* in *Cassia. (f)* There seem to be no easily discernible morphological or physiological characters in the woody hosts that would help explain their susceptibility, when compared with hundreds of woody or herbaceous genera which resemble them but are immune to downy mildew. *(g)* Although downy mildews will certainly be found on woody plants of other genera, some present records have been shown to be in error, *e.g.* on *Acer* and *Eleagnus.*

POSSIBLE PHYLOGENETIC IMPLICATIONS OF CAPILLICONIDIATION FROM HYPHAE OF *ERYNIA RADICANS* (SUBGENUS *ZOOPHTHORA:* ENTOMOPHTHORALES)

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Capilliconidia are a special form (Type II) of secondary conidia produced on long, narrow capilliconidiophores arising from primary and secondary conidia and from other capilliconidia, exclusively in the Entomophthorales (Zygomycetes). Unlike most primary conidia and Type I secondary conidia of this order, they are not ejected actively. The term "secondary conidium" in fungi defines conidia produced from pre-existing ones, by resporulation.

Manipulation, *in vitro,* of *Erynia radicans* isolates, yielded capilliconidia on small numbers of capilliconidiophores produced directly on hyphae rather than on other conidia. Hyphal capilliconidiation, first reported here, was therefore primary.

Capilliconidiation occurs in all three accepted families of Entomophthorales, being present in some species or subgenera of eight genera, *i.e.,* the subgenus *Zoophthora* of *Erynia,* and absent in others. As it is highly unlikely that these morphologically very specialized structures could have been created *de novo* in each of these genera of the only order in which they are known, by convergent or parallel evolution, we consider them to be an ancestral character of the Entomophthorales, lost over time in some members of the order and preserved in others. Capilliconidia occasionally arising from hyphae might possibly be a reversal to an ancestral condition in which capilliconidiation could have been, along with forcible ejection, one of two coexisting types of primary conidiation, or actually the only type at that time.

In the genus *Stylopage* (Zoopagaceae: Zoopagales), all species produce primary conidia on conidiophores on hyphae, usually markedly similar to capilliconidiophores and capilliconidia of the Entomophthorales. Resporulation in *Stylopage* is by secondary conidia on long, narrow conidiophores, strikingly similar to Type II secondary conidia of the Entomophthorales. These two characters, along with the evacuation of cytoplasm and nuclei from the parent conidium through the capilliconidiophore into the capilliconidium, indicate strongly that this type of resporulation in the Entomophthorales is homologous to that in *Stylopage,* implying either a closer relationship between the two orders, or that *Stylopage* belongs in the Entomophthorales.

GERMINATION TRIALS AND STRUCTURE OF MICROSCLEROTIA OF *DEMATOPHORA NECATRIX*°

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The pathogen fungus *Dematophora necatrix* Hartig produces in culture and in soil black microsclerotia forming sclerotial sheets. We tried to study the role of the microsclerotia in the fungal life cycle and in the epidemiology of the disease. No germination trials of these microsclerotia were reported in the literature. Microsclerotia produced in culture and sclerotial sheets that were collected from avocado diseased roots, were treated as follows: *(a)* immersion in 1% sodium hypochlorite for different lengths of time; *(b)* heat treatment at different temperatures and lengths of time; *(c)* freezing at temperatures below 0°C; *(d)* washing and extraction of possible inhibitor compounds by water and chemicals; and *(e)* treatment with fumes of acetaldehyde, methanol, and volatile products of plant tissues from almond, apple, avocado and persimmon. No germination of microsclerotia was found after these treatments. Microsclerotia from the above treatments were buried in natural non-infested heavy soil of Hanita, sterilized soil of Hanita, and sandy soil of Rehovot. No germination was found by these treatments and no colonization of *D. necatrix* on *Morus alba* stems was obtained that were previously buried in the soil. In naturally infested soil of Hanita the *M. alba* stems were colonized by *D. necatrix* after 10 days. In microscope slides of avocado microsclerotial sheets it was found that the microsclerotium is built of an outer layer of mycelium rich in melanin which protects the inside vegetative mycelium. It seems that the structure is a pseudosclerotial body that is involved in the penetration stages and in the survival of the fungus, which is protected by the melanin layer, in the soil.

MICROSCOPICAL STUDIES OF INITIAL DEVELOPMENT OF VIRULENT AND AVIRULENT ISOLATES OF *GEOTRICHUM CANDIDUM* ON LEMONS

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Mature, fully colored lemons were inoculated by injecting spore suspensions of virulent and avirulent isolates of *Geotrichum candidum* into the lemon peel. Inoculation sites injected with spores of virulent isolates produced either actively developing soft rot, or dry, small, non-expanding, arrested lesions of 2 mm diam. The nonvirulent isolates produced only arrested lesions. The rate of germination and hyphae development were followed by examining samples of inoculated lemon peel up to 72 h after inoculation. The samples were examined by light microscope, transmission electron microscope and scanning electron microscope. Spore germination and incipient maceration were observed after inoculation with both types of isolates. Disorganization and maceration of the exocarp tissue preceded the penetration of fungal hyphae at all inoculation sites. Degradation of pectic substances progressed with the maceration, as observed by ruthenium red staining. Ultrastructural examination of lemon peel inoculated with the virulent isolate, showed different stages of cell disintegration. In fragments of tissue taken from arrested lesions, no specific changes of the middle lamella or the cell wall could be observed in response to inoculation with any of the isolates. However, frequent occurrence of projections extending from the plastid membrane produced cytoplasmic inclusions.

°Lecture not delivered because no time was available.

FACTORS INVOLVED IN PATHOGENICITY OF *GEOTRICHUM CANDIDUM*, THE CAUSAL AGENT OF SOUR ROT ON CITRUS FRUITS

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A comparative study between virulent and avirulent isolates of *Geotrichum candidum* was undertaken in order to identify mechanisms for virulence of this pathogen on citrus fruits. The initial development of virulent and avirulent isolates during 48 h following inoculation, as measured by number of propagules, was similar, although only the virulent isolates produced symptoms. Production of endopolygalacturonase (endo-PG) under *in vitro* conditions was generally higher in virulent than in nonvirulent isolates. However, when lemon fruits were treated at 80°C for 2 min, the symptom development of avirulent isolates was similar to that of virulent isolates. The possible involvement of active defense mechanisms was examined by searching for differences in various parameters such as levels of phenylalaline ammonialyase and phenols, and challenge inoculation with a virulent isolate following pre-inoculation with an avirulent one. The negative results obtained suggest that active defense might not be involved. On the other hand, endo-PGs from both virulent and avirulent isolates caused macerations of lemon albedo tissue. The enzyme from the virulent isolate was more effective than that from the avirulent in causing maceration, whereas the heat treatment of lemon fruit mentioned above increased the rate of maceration with both enzyme preparations.

These results suggest that the initial amount of endo-PG produced under *in vivo* conditions, and the accessibility of the pectin *in situ* to these enzymes, are the main factors that govern virulence of *G. candidum* on citrus fruit.

SUDDEN WILT IN MUSKMELONS: A CONTINUING CHALLENGE

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Sudden wilt in muskmelons (*Cucumis melo* var. *reticulatus* cv. 'Galia') continues to cause severe crop losses in Israel. Fruit-bearing plants may wilt and become desiccated within one week, even in fields treated with methyl bromide. During 1985, affected plants were collected from fields in Netofa, Ben Shemen, Kefar Shalem and on the University campus, surface-sterilized and plated on PDA + chloramphenicol medium. Symptoms in affected plants and in the fungi isolated from them varied in the different locations, as follows: Netofa and campus – corkiness of basal stem part: *Fusarium solani* and *F. equiseti;* Ben Shemen – corkiness as above, and occasionally reddish resin secreted and microsclerotia seen on basal stem: *F. solani, F. equiseti, Macromina phaseolina;* Kefar Shalem – corkiness, and occasionally root rot: *F. solani, F. equiseti, Monosporascus eutypoides.* Typical symptoms of sudden wilt were caused neither by artificial inoculation with one of these fungi nor in plants grown in soil infested with both *F. solani* and *M. phaseolina.* Plants grown in Ben Shemen soil in the greenhouse developed corkiness on the basal part of stems, from which *F. solani* and *F. equiseti* were isolated, but produced no wilt at 90 days after sowing. More studies are needed to elucidate the etiology of the disease.

MONITORING OF SEED-BORNE DISEASES IN POTATO TUBERS

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Since the potato tuber is anatomically a stem, viral bacterial and fungal pathogens are carried by the tuber. In order to improve potato cultivation it is essential to examine the seed lots for the relevant diseases. The sources of potato seed tubers for planting in the spring season in the Negev area are as follows: imported seed tubers from northern Europe (Holland, Scotland and Ireland); seed tubers produced in the Golan Heights (∿600 m elevation); and seeds produced in isolated areas in the Negev, under intensive pest management in the previous spring and stored for 8 months at 2°C.

During the spring the seed tubers are produced for planting in autumn, and efforts are made to use the best lots for our seed industry. Therefore, routine monitoring of each lot entering the Negev area is done for the following diseases: powdery scab; Fusarium complex; late and early blight; black and silver scurf; Pythium; common scab; and black-leg (Erwinia complex). These diseases are examined in a random sample of 200 tubers per lot, using visual and microscopic observtions and serological and isolation techniques. In cases of seed produced in Israel, we monitor – in addition to the diseases mentioned above – also the *Verticillium dahliae* latent infection level in the seed tubers. We fully agree with the growers that one of the key factors in improving potato yields and reducing soil infestation, is to use low-disease-level seeds. This can be achieved only by laboratory analysis of the seeds.

FIRE BLIGHT – A NEW DISEASE IN ISRAEL°

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Fire blight, caused by *Erwinia amylovora*, is an indigenous bacterial disease in North America. It is a serious disease of pears and apples in the eastern and western USA. During the past 20 years the disease has spread to northwest Europe subsequent to its first detection in England in 1957, and recently outbreaks have occurred in the eastern Mediterranean, Egypt and Cyprus. In spite of extensive cultivation of its host plants, fire blight was not found in Israel until May 1985. During May–August 1985 *E. amylovora* was isolated from eight pear orchards and two apple orchards. In three of the eight pear orchards, 5–10% of the trees showed some symptoms whereas in the five others, only a few trees were affected. Of the more highly infected pear orchards, two were ∿25 years old and one was 6 years old. The affected pear orchards are located in two regions, 200 km apart (Galilee and the southern coastal plain). On apple the disease was found in a few trees in Galilee.

Although fire blight symptoms were first noticed in May 1985, the primary infection most likely had occurred during the preceding rainy days in March 1985, at which time the pear trees were in bloom. The existence of withered flowers on diseased branches strongly favors this supposition, as the only subsequent rainfall was a month later, by which time pear fruit set was complete. Considering the temperature and rainfall records during the years 1977–1985 at meteorological stations close to the affected orchards, it is assumed that fire blight could become endemic in Israel. The simultaneous occurrence of rain and suitable temperatures (daily mean 15°–25°C) during the bloom periods of pears and apples is likely to facilitate the development of primary

°Lecture not delivered because no time was available.

infections of fire blight in the blossoms. Later in the spring, with favorable temperatures and humidity, the disease can spread, resulting in secondary infections of the young shoots. In some years autumn blooming, in October–November, occurs in many pear orchards in Israel. During these months climatic conditions favorable for development of fire blight could result in additional disease buildup and provide bacterial inoculum in the following spring.

YIELD LOSSES IN SWEET CORN INFECTED BY THE FUNGUS *PENICILLIUM OXALICUM*

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During the growing season of 1985, severe epidemics incited by the fungus *Penicillium oxalicum* Currie and Thom occurred in sweet corn (cv. 'Jubilee') in all the growing areas in Israel. A series of field experiments was conducted in order to study the relationships between disease occurrence and yield losses. Experiments were carried out in commercial corn fields (cv. 'Jubilee') spontaneously infected in the coastal plain.

Growth retardation was observed in 70% of the infected leaves, with height and stem diameter being ∿25% lower and yield 30% lower than in the uninfected plants. No growth retardation, yield losses or any other symptoms were observed in the remaining 30% of the infected plants. In spite of disease occurrence in the seeds and slight growth retardation during the early growth stages, these plants recover rapidly during the growing period.

Experiments conducted in growth chambers showed that at 20°C, growth retardation of infected plants occurred only if plants were inoculated before the three-leaf stage with an inoculum suspension of at least 50,000 spores/ml. Otherwise, in spite of plant infection, no growth retardation occurred. Inoculation of seeds with low inoculum concentrations (< 50,000 spores) resulted in growth retardation only at the temperature range of 25°–35°C.

Results obtained in this study show that *P. oxalicum* may cause severe yield losses. When plants are infected during an early growth period, damages caused by the fungus are dependent on inoculum concentration and temperature.

E: BIOLOGICAL CONTROL AND OTHER NONCONVENTIONAL METHODS TO CONTROL PLANT DISEASES

MICROBES ASSOCIATED WITH HIGHER PLANTS

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As plant pathologists we are in the unique professional position of being familiar with both higher plants and the pathogens associated with them. New opportunities have arisen in the new field of biotechnology in which we can play an important role. In order to be most effective, we should extend our role and broaden our outlook to include an enlarged view of *all* microbes associated with plants, whether they be pathogens, symbionts or quite loose in their relationship to the host. One must view these relationships in terms of how they may be exploited for eventual usefulness to mankind. For instance, some data are available on the potential use of *Agrobacterium rhizogenes* in promoting root growth in bare root stock, vegetable transplants, and cuttings of ornamental species. The growth arising from the genetic transformation of the plant *via* this bacterium may encourage better establishment, higher yield, and greater drought tolerance of the

plant. Specific data on some of these points have been reported for olives, almonds, cabbage and violets.

Pathogens of weedy plant species and their toxins need to be isolated, studied and examined for their potential use in weed control. In particular, toxins recently isolated from *Drechslera sorghicola (Bipolaris sorghicola)* and characterized by X-ray analysis were shown to be a series of unique isomers of ophiobolin A. These compounds not only kill Johnson grass, but also are present in *D. maydis* (race T & O). This presents some interesting aspects as to the importance to the pathology of these compounds in the corn blight epidemic which occurred in the USA in 1970. A study of weed pathogen biochemistry may provide important leads in understanding the chemistry of pathogens of crop plants.

THE EFFECT OF FLUORESCING PSEUDOMONADS ON SYMPTOM EXPRESSION BY *MYCOSPHAERELLA GRAMINICOLA* AND *PUCCINIA RECONDITA* ON WHEAT SEEDLING LEAVES

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Several isolates of fluorescing pseudomonads which originated from soil applied onto wheat seedlings prior to inoculation with spores of *Mycosphaerella graminicola* (anamorph, *Septoria tritici*), *Erysiphe graminis* or *Puccinia recondita*, markedly reduced symptom expression. These isolates reduced growth of several fungi *(Geotrichum candidum, Rhizoctonia solani* and *Sclerotium rolfsii)* and bacteria *(Bacillus subtilis, Escherichia coli, Aerobacter aerogenes, Micrococcus* spp. and *Proteus vulgaris) in vitro*. They were ineffective against *Serratia marcescens* on a defined medium. Inhibition of *S. tritici* development was manifested on silica gel precoated thin layer chromatography (TLC) plates by several fractions soluble in organic solvents. A melanin-producing *S. tritici* isolate was used in this bioassay. Some of the fractions were inhibitory *in vitro* to *S. tritici* and other wheat pathogens. Inhibition of *S. tritici* growth on a defined medium was also expressed by ammonia-like gaseous compounds produced on King's B medium plates. The growth of the antagonistic pseudomonads on defined medium was not affected by the following commercial fungicides: Captafol, chlorthalonil (Daconil 2787), maneb, mancozeb (Mancozan), triadimefon (Bayleton), benomyl (Benlate), metalaxyl (Ridomil), fenarimol (Rubigan), prochloraz (Sportak), propiconazole (Tilt); and the herbicides: 2,4-D and diclofop-methyl (Illoxan) applied at the recommended concentrations.

BIOLOGICAL CONTROL OF *SCLEROTIUM ROLFSII* SACC. BY *SERRATIA MARCESCENS*

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Serratia marcescens isolated from the rhizosphere of *Sclerotium rolfsii*-infested plants was found to be the most effective biocontrol agent of this pathogen under greenhouse conditions when compared with 200 different bacterial colonies isolated from the same rhizosphere. *S. marcescens* significantly reduced the incidence of damping off in radish caused by *Rhizoctonia solani* but was not effective against *Pythium aphanidermatum* on cucumber.

Application of a suspension of *S. marcescens* at a concentration of 10^7 colony forming units (CFU)/g soil to *S. rolfsii*-infested soil as a drench at planting and subsequently at 10^4 CFU/g every 24 h for 19 days, reduced disease incidence in beans by 70%. This method was the most effective when compared with spraying or mixing of inoculum with soil or with seed coating.

Bacterial concentrations of 10^5 and 10^6 CFU/g soil were optimal for reducing disease damage in pots and the treatment's effect lasted for at least three growth cycles, with both lower and higher bacterial concentrations being less effective for controlling *S. rolfsii.*

The establishment of *S. marcescens* in the rhizosphere was followed by using a natural mutant resistant to both rifamycin and nalidixic acid. After application of the inoculum to the soil, 10^7 CFU of *S. marcescens*/g of dry roots could be detected after 7 days in the rhizosphere of bean roots.

Serratia marcescens secreted volatile substances which inhibited the linear mycelial growth of *S. rolfsii* to petri dishes. The bacterium produced chitinolytic enzymes that caused lysis of *S. rolfsii* mycelium, as observed by enzymatic assay and by electron microscopy.

PLANT GROWTH-PROMOTING EFFECTS OF *AZOSPIRILLUM BRASILENSE* ON BEAN, MELON, CUCUMBER AND TOMATO SEEDLINGS

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Azospirillum sp. contributes to increased yield of cereal and forage grasses by improving root development in properly colonized roots, increasing the rate of water and mineral uptake from the soil, and by biological nitrogen fixation. The effect of *Azospirillum* as a biocontrol agent capable of reducing damping-off disease caused by soil-borne fungal plant pathogens was investigated. The possibility of a direct inhibitory effect on the pathogens, or competition for colonization sites and nutrients on the roots of the host plants, was considered. Disease incidence was determined in pot experiments under greenhouse conditions with different combinations of pathogen–*Azospirillum* inoculum levels. The pathogens tested in combination with *A. brasilense* were *Rhizoctonia Solani* and *Sclerotium rolfsii* for bean, *Pythium aphanidermatum* for cucumber, and *Fusarium oxysporum* for melon. In most cases no significant reduction in disease incidence could be detected in systems treated with *Azospirillum*, as compared with those treated with the pathogen only. In the model system, melon – *Fusarium* – *Azospirillum*, a consistent reduction in disease could be observed; however, the level of reduction was not high enough to determine the mechanism(s) involved in the interaction. The possibility of a direct plant growth-promoting effect following inoculation with *Azospirillum* (10^8 colony forming units/ml), was investigated. There was a significant increase (above nontreated controls) in leaf dry weight (30-50%), root dry weight (20-30%), total leaf area (30-50%) and plant height (10-15%) of tomato cvs. M-82 and 'Marmande' and melon cv. 'Ananas Yokne'am'.

CONTROL OF SOIL-BORNE DISEASES BY SOIL SOLARIZATION AND FUMIGATION, SEPARATELY OR COMBINED

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Experiments were carried out at three locations to test the effectiveness of two soil disinfestation methods, employed separately or in combination, in controlling soil-borne diseases. At Bene Darom, the corky root disease in processing tomatoes was effectively controlled by soil solarization (Ssol) or fumigation with methyl bromide (MB) at 800 kg/ha. The respective increase in yield over the untreated check was 63% and 38%, respectively; the yield increase by the combined treatments was 57%.

In another experiment, with eggplants in the south of the country, MB at 250 or 500 kg/ha was effective in controlling root knot nematodes and increased the yield by 36-47% in the first crop cycle and by 430% in the second cycle, in which root knot incidence was more severe. Ssol was less effective than MB in controlling root knot nematodes and increased the yield by 36% and 217% in the first and second crop cycles, respectively; combining the treatments did not improve the results.

Ssol either by strips or by a broad application and MB by a broad application and at 50 g/m^2, were effective in controlling the pathogen and the wilt disease in watermelons caused by *Fusarium oxysporum* f.sp. *niveum* and increased the yield by 890-1070% over the untreated check. MB at 50 g/m^2 by strip application was not effective in controlling the pathogen or the disease, or in increasing the yield.

BIOLOGICAL CONTROL OF SOIL-BORNE PATHOGENS IN SOLARIZED SOILS

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Control of soil-borne diseases by solarization may have a long-term effect, which is expressed in the second and even third years following treatment. This phenomenon is derived partly from biological control processes induced in the soil following solarization, and was found with certain pathogens, *e.g. Sclerotium rolfsii, Fusarium oxysporum* f. sp. *lycopersici, Verticillium dahliae* and *Rosellinia necatrix.* Frequently, soil solarization (Ssol) has produced the effect of "induced suppressiveness", *i.e.,* disease incidence in soil following solarization and reinoculation was lower than that in untreated, reinoculated soil. Solarized soils have shown typical biocontrol activity: inhibited chlamydospore formation, decreased fungistasis, increased lytic bacterial populations, increased microflora producing soluble antibiotic and volatile substances, and also pathogen growth inhibition with population decline. Sublethal temperatures have a weakening effect on the pathogen, which exhibits increased sensitivity to antagonistic microorganisms. The phenomenon of induced suppressiveness in soil against the fungus *R. necatrix* was found to be still present at Kibbutz En Zurim 9 months following Ssol. The increased biocontrol capacity and the formation of detrimental conditions for pathogen survival in the soil following Ssol are widespread phenomena found with different pathogens in soils of various textures and compositions in different parts of the world.

SOIL SOLARIZATION – A MEANS TO CONTROL *PHYTOPHTHORA CINNAMOMI* IN AVOCADO GROVES

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Phytophthora cinnamomi, the causal agent of avocado root rot, was detected for the first time in Israel in September 1982. Since then the pathogen was identified in an additional 23 avocado groves located mainly in the Sharon area. With but one exception, all infected sites had only a few diseased trees. In the search for efficient control measures, by which further spread of the pathogen to neighboring healthy trees would be prevented, the efficacy of soil solarization (Ssol) was explored. Highly infected avocado trees were cut back to the ground before the soil was wetted and covered for 42 days with transparent polyethylene sheets. The effect of Ssol on the inoculum potential in the soil was studied by analyzing soil samples taken from various depths. *Persea americana* and *Persea indica* seedlings were used in the experiments as host and trap plants, respectively. The effect of Ssol on disease rate was unequivocal: in avocado seedlings planted in the solarized soil samples, disease percentage was 3% only, as opposed to 70% in the control. Efficacy was equal in the upper and lower soil layers. In tests in naturally infested soil, the pathogen was found to be extremely heat-sensitive. A 4-h exposure at 36°C eradicated the pathogen almost totally. Based on these data it seems clear that the effectiveness of Ssol in the field derived from the high sensitivity of the pathogen to heat. However, there is evidence that additional factors are involved in the killing of the pathogen. The population dynamics of the pathogen was followed in reinfested solarized soil. Mycelial growth was suppressed and the pathogen population declined, as opposed to its growth and survival in untreated soil.

F: CHEMICAL CONTROL OF PLANT DISEASE, RESISTANCE TO FUNGICIDES, AND PROBLEM SOILS

REDUCTION OF CAVITY SPOT IN CARROTS BY METALAXYL APPLICATIONS

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Cavity spot in carrots is a major world-wide problem which often causes rejection of a significant percentage of the crop. A number of factors, such as calcium deficiency or anaerobic bacterial, have been suggested as the cause of the problem. Recently, several works have been published showing good control of cavity spot by metalaxyl applications. Various species of *Pythium* were found to be the cause of the disease.

In the winter of 1985, two field trials were performed in commercial carrot fields of Kibbutz Sa'ad, in the northern Negev of Israel. In the first trial carrots sown in mid-October were treated at 11 weeks of age with granular Ridomil 5G at the rate of 1120 g metalaxyl/ha. The treatment reduced disease index by 75% as compared with the control, infected roots by 62%, and damaged roots by 67%. In the second trial carrots sown in the beginning of October were treated at the age of 14 weeks with Ridomil–Mancozeb WP at the rate of 1200 g metalaxyl/ha. The treatment achieved a reduction of 83% in disease index, 75% in infected roots and 92% in damaged roots.

A *Pythium* species was isolated several times from cavity spot lesions but has not yet been identified.

This is the first report from Israel confirming the results of European work as to the efficiency of metalaxyl in controlling cavity spot disease in carrots.

EPIDEMIOLOGY AND CHEMICAL CONTROL OF COMMON SMUT ON SWEET CORN

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In a series of field trials, disease development and disease incidence caused by the fungus *Ustilago maydis* on sweet corn plants (cv. 'Jubilee') were investigated. Disease was initiated by inoculating five plants in the field. Results showed that the disease developed slowly during the early stages of plant growth (apparent infection rate 0.04, *sensu* Van der Plank). After tasseling, disease development was accelerated (apparent infection rate = 0.15). All above-ground parts of the plants are susceptible to the disease, with kernels being the most sensitive. The percentage of plants evincing disease symptoms on the leaves, stems and kernels was 0.17, 0.4 and 9.5, respectively. The antifungal activity of the systemic fungicides benomyl (Ridomil) and propiconazole (Tilt) against common smut, was tested. Under greenhouse conditions both benomyl and propiconazole provided better control of the disease than in the field. Both fungicides retained their activity for more than 10 days and were resistant to being washed off. In field experiments, propiconazole gave the best control of the disease. Propiconazole at 50 ppm, applied in soil one day before inoculation, completely controlled the disease. Under the same conditions, only partial control was achieved with benomyl even at high concentrations (>200 ppm). The efficacy of control by the two fungicides decreased linearly with time, to zero when the fungicides were applied 12 days before inoculation.

THE EFFICACY OF ALLYL ALCOHOL AS A MOLD INHIBITOR IN WHEAT GRAIN°

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The fungicidal activity of allyl alcohol applied as a fumigant to wheat grain (local harvest) was studied using fumigation chambers of 3 liters each. Grains were inoculated separately with spore suspensions of the fungi *Aspergillus ochraceus* and *A. flavus;* following 2, 7 and 30 days of incubation at 25°C, grains were exposed to allyl alcohol. Uninoculated grains were also fumigated, to study the effect on the natural microflora. In all cases, fungi were destroyed from the outer surface and the inner parts of the grains, when allyl alcohol was given for 24 h at a concentration ff 50 mg/l or for 48 h at a concentration of 15 mg/l. Following these treatments, fumigated grains planted on PDA, Czapek or malt-salt media did not yield any fungi after one month of incubation at 27°C. Growth of mycelium of *A. ochraceus* exposed for 6 h to a concentration of 2.5 mg/l allyl alcohol was completely inhibited whereas a concentration of 15 mg/l was needed to inhibit growth of *A. flavus.*

The concentrations which were effective in controlling molds in the grains also caused mortality of *Tribolium castaneum, Sitophilus oryzae* and *Rhyzopertha dominica,* three common insects of stored grains. These data suggest that allyl alcohol could be used for integrated control of both fungi and insects in stored grains.

°Lecture not delivered because no time was available.

EVALUATION OF BIOLOGICAL INTERACTION TYPES BETWEEN FUNGICIDES IN A MIXTURE

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The use of fungicide mixtures for controlling fungal plant diseases has increased markedly during the last decade. The main cause of this development is the evolution of phytopathogenic fungal genotypes, resistant to systemic fungicides. Theoretically, when fungicides are applied jointly in mixtures, the biological effect may be equal to (additive), greater than (synergistic) or less than (antagonistic) that to be expected from the sum of the toxicant activity when administered separately. The use of different methods has led to a different interpretation of the mixture's biological activity. Two approaches are commonly used: (i) Wadley's formula, based on the assumption that the components comprising the mixture act independently, but have a similar mode of action. Thus, one component can substitute at a constant ratio for the other. The interaction is expressed by the ratio between a theoretical dose and the actual one needed to control a constant proportion of the fungal population. In using this formula, a somewhat complex mathematical transformation of the experimental data is needed. (ii) The Abbott formula, based on the assumption that the components of the mixture have independent and different modes of action. The interaction is expressed by the ratio between the observed toxicity of the mixture and a theoretical one calculated on the assumption described above. The accuracy of this method is doubtful at relatively high effective doses of the components. The use of the two methods side by side may cause confusion. In choosing the appropriate approach to be taken in analyzing results of a given experiment, one should consider the advantages and disadvantages of each method, as well as the theoretical hypothesis on which the method is based. At present, there is no direct way to formulate the interaction in a given fungicide mixture.

CHEMICAL CONTROL OF OOMYCETE PLANT DISEASES IN ISRAEL: FAILURES AND PROSPECTS

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Metalaxyl (25% Ridomil WP) provided excellent control of downy mildew in cucurbits *(Pseudoperonospora cubensis)* during 1977-79. Loss of efficacy occurred early in 1980 due to the appearance of isolates resistant *(R)* to the chemical. During 1980-85, 152 isolates of *P. cubensis* were collected throughout the country; 60% of them were *R*. In 1985, one out of 12 isolates was sensitive *(S)* to metalaxyl. Ridomil + mancozeb mixtures were registered for control of potato late blight *(Phytophthora infestans)* in 1982. The first occurrence of *R* isolates of this fungus was reported in March 1982. In 1983, 1984, 1985 and 1986 (April), 41, 71, 30 and 24 isolates were collected, and 61, 51, 47 and 79% of them, respectively, were *R*. Resistant isolates of *P. cubensis* exhibited higher fitness to cucumbers than *S* isolates. Preliminary results with *P. infestans* showed that *R* isolates varied in fitness and some of them possessed higher fitness to potatoes than did *S* isolates.

Ridomil + mancozeb has provided inadequate control of *P. infestans* in the past 2-3 years. Curzate® (cymoxanil) 50% WP at 250-500 μg a.i./ml was effective in controlling both *S* and *R* isolates of *P. infestans* and *P. cubensis* in the greenhouse. Mancozeb + cymoxanil mixtures (4:1 = Mancur) were highly effective in the field against either pathogen when applied once a week, even when *R* isolates predominated. A mancozeb + cymoxanil + oxadixyl (Sandofan) mixture

(7:2:1 = Sandocur-M) was effective in field plots treated once in 2 weeks. Propamocarb (Banol; Previcur-N) was effective, although slightly phytotoxic, as a foliage spray against late blight in potatoes when applied once in 10 days.

GENETICS OF RESISTANCE TO BENOMYL IN *VENTURIA PIRINA* FROM PEAR ORCHARDS IN ISRAEL

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Fourteen single-spore cultures of benomyl-resistant *Venturia pirina* were isolated from pear scab lesions at four sites in Israel. According to the ability of the isolates to germinate and grow at varying benomyl concentrations, four levels of resistance were determined *in vitro:* three isolates with low resistance *(LR)* grew at 0.5 but not at 5 μg/ml benomyl; five moderately resistant *(MR)* isolates grew at 5 but not at 50 μg/ml benomyl; five highly resistant *(HR)* isolates grew at 50 μg/ml but their hyphae were curled; and one isolate with very high resistance *(VHR)* grew unaffected at 50 μg/ml benomyl. The differential response to 50 μg/ml benomyl of the *HR* and the *VHR* strains could be observed only by microscopic observation. However, the difference between the *HR* and the *VHR* phenotypes was clear on a medium amended with *N*-(3,5-dichlorophenyl) carbamate (MDPC): only the *VHR* isolate exhibited negative cross-resistance, being unable to grow at 1 μg/ml MDPC, whereas the *HR* and all other isolates grew unaffected.

Crosses between resistant isolates and sensitive wild types, as well as between different resistant isolates, showed that the various levels of resistance are conferred by four allelic mutations that constitute a polymorphic series at a single locus. All the isolates were obtained from orchards that had not been treated with benzimidazole fungicides since 1977; thus, the persistence of resistant strains in the absence of selection pressure is indicative of their good fitness.

ACCELERATED DEGRADATION OF CARBENDAZIM IN ISRAELI SOILS

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The fungicide benomyl is applied as a foliar spray or soil drench treatment to a variety of vegetable and ornamental crops. In four Israeli soils collected from agricultural fields in Talme Yafe, Bet haGaddi, Sharsheret and Gilat, all with a history of benomyl application, accelerated degradation of carbendazim (MBC) was observed, in comparison with soils from adjacent fields with no benomyl application history. The half-life of MBC in the "history-soils" was several days, whereas in "nonhistory-soils" it was approximately 3 weeks. Concomitant with rapid degradation, a loss of fungitoxic activity was observed.

Accelerated degradation of MBC in soil was induced by a single pre-application of the compound at doses as low as 0.5 μg/g soil. MBC preapplication is not a prerequisite for induction of accelerated degradation of this fungicide, since amending "MBC-nonhistory" soil with 2% (w/w) of "MBC-history" soil, induced accelerated degradation of MBC throughout the soil volume.

Antimicrobial treatments such as soil disinfestation in the field by fumigation with methyl bromide (500 kg/ha) or soil solarization, curbed accelerated degradation of MBC. MBC persistence in disinfested "MBC-history" soil was even greater than in nondisinfested, "nonhistory" soils. Amending "MBC-history" soil with the fungicides TMTD or fentin acetate at 20 μg/g soil, extended MBC persistence in the treated soils, although to a lesser extent than observed with the more drastic, disinfestation treatments.

ADDITIONAL PAPERS PRESENTED AT THE CONGRESS

Genetic engineering and plant diseases
M. Bar-Joseph

Bacterial diseases in plants
G. Kritzman