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FINAL REPORT

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Epidemiology and Control of Bacterial Leaf Spot Diseases in Vegetables: *Pseudomonas Lachrymans* — Angular Leaf Spot of Cucumbers, *Xanthomonas Vesicatoria* — Bacterial Scab of Pepper and Tomato, *Pseudomonas Tomato* — Bacterial Speck of Tomato

Y. Okon, R. Goodman, G. Kritzman,
Y. Henis, D. Zutrah

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Names of Investigators:

Principal Investigator: Dr. Yaacov Okon

Cooperating Investigator(s): Prof. R.N. Goodman Dr. Giora Kritzman Prof. Y. Henis
Mr. D. Zutra

Name and Address of Affiliated Institutions:

Principal Institution: Faculty of Agriculture, The Hebrew University of
Jerusalem

Cooperating Institution(s): Department of Plant Pathology, University of
Missouri Columbia. Vulcani Centre

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Principal Investigator

Institution's Authorizing Official

Prof. Yaacov Okon

Yaacov Okon

ד"ר א. ברק
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- 1) Tomatoes
- 2) Peppers
- 3) Cucumbers
- 4) Bacterial diseases of plants

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C) Abstract

Fundamental information was obtained concerning growth and development of Pseudomonas syringae pv. tomato (PST) and Xanthomonas campestris pv vesicatoria (XCV) and Pseudomonas syringae pv lachrymans (PSL) in host tissue. Infection of tomato leaves by PST was monitored by scanning electron and light microscopy. The primary infection sites were the stomata and the leaf trichome. Necrosis was initially observed 100 hours after inoculation. Soil infested with PST significantly reduced germination. Tomato plants grown in infested soil that remained symptomless from sowing to flowering had 20-30% less foliage than plants grown in uninfested soil. Differences in relative humidity profoundly influenced disease development, particularly in pepper plants inoculated with XCV. It appeared that modification of disease intensity is possible with judicious water management in PST, XCV and PSL.

Difficulty in detecting small numbers of pathogenic bacteria in and on seed because of contaminating saprophytic bacteria was encountered. However, culturing the mixture inside detached leaves on water agar provided an ideal environment for growth of the pathogen. Thionin-like compounds from both tomato and melon that have both intense agglutinating and antibiotic activity (cylinder-agar plate antibiotic inhibition technique) against PST, PSL, *Erwinia amylovora* and XCV were isolated and characterized.

Physiological process in diseased susceptible tomato plants were compared also infected resistant plants. Studies included detection of activity of proteases, deaminases, Pectic enzymes, cutinases, Polyphenol oxydase and Peroxidase during disease development.

D) WORK CARRIED OUT DURING SUPPORT BY BARD NO. 1-214-80 IN ISRAEL 1980-1983

Yaacov Okon, Principal Investigator Yigal Henis, Faculty of Agriculture Rehovot.
Glora Kritzman, Dan Zutrah, Vulcani Center Beth Dagan.

Studies of bacterial population dynamics and epidemiology under controlled and field conditions

Pseudomonas syringae pv. tomato (PST) infection of tomato leaves was followed by scanning electron and light microscopy. The primary infection sites were the stomata and the bases of the leaf trichomes from which bacterial masses burst out at later stages of disease development. During incubation the bacteria disappeared from the surface of the leaves of the susceptible variety and became located in the intercellular spaces. Necrosis was first observed microscopically 100 h after inoculation. Bacteria were detected in infected tissue but not in necrotic tissue. Lesions increased in numbers and in size until the leaves were totally infected with bacteria. Bacteria disappeared from the leaves of the resistant tomato cultivar within 140 h of inoculation.

These phenomena led us to the conclusion that the resistant cultivar inhibited in a yet unknown way, the multiplication of PST both inside and outside the leaves, whereas the susceptible cultivar favoured the multiplication of the pathogen (Bashan et al. 1981 b).

Xanthomonas campestris pv. vesicatoria (XCV) infection of pepper leaves was monitored by scanning electron and light microscopy. During incubation the bacteria became located in the intercellular spaces between the mesophyll cells. The primary infection sites were the vein area. XCV reach high population levels on the leaf surface but usually not in the stomata. Necrosis was first observed microscopically 120 h after inoculation near the leaf veins. Bacteria were detected in infected tissue but not in necrotic tissue. It seems that the leaf population of the pathogen can multiply and survive on the leaf surface without further supply of bacterial cells from the inside of the infected leaf in spite of the fact that the pathogen has the ability to multiply inside the leaf tissue. These findings fill the gap in our knowledge, which existed, about events between inoculation by PST and XCV and the appearance of visible symptoms on the tomato leaf and upper leaves (Sharon et al. 1982a).

To date, no comprehensive study has been reported of the factors affecting infection by this disease although a few studies of artificial inoculation under different conditions are available.

The purpose of the greenhouse experiments was to explore the factors affecting infection of pepper by XCV in order to establish a system that will enable further studies.

XCV, the causal agent of bacterial scab of pepper, was isolated in several regions in Israel. When artificial inoculation was practiced, pathogen growth was enhanced by high temperatures (30-36°C), and an inoculum concentration of 10^4 colony forming units (CFU) per ml was optimal for symptoms to develop on plants. Pre-inoculation treatments such as wounding the leaves by rubbing them with carborundum powder or spraying them with diluted wax solvents, markedly increased disease severity, but were not essential. Pre-inoculation conditions at two different relative humidity levels (R.H. 100% or R.H. < 40%) did not affect disease severity. Young leaves were more severely affected following infection than older leaves. Disease severity was similar with several isolates of XCV (Diab et al. 1982b).

It is known that Israeli isolates of the causal agent of bacterial scab of pepper, prefer relatively high temperatures (optimum) 30-32 (under R.H. 100%). It can be found in late spring and summer pepper fields in semi-arid regions and is more abundant in fields with sprinkle irrigation than with drip irrigation.

The purpose of this work was to determine the precise relationship between humidity and development of bacterial scab (leaf spot) of pepper.

Differences in relative humidity (R.H.) significantly affected bacterial scab (leaf spot) development in pepper plants. High R.H. with free moisture on the leaves for long periods favoured infection. However, XCV had modest requirements for high R.H. in order to cause disease. When inoculated plants were exposed to high RH (> 85%) for a few hours during 1 or 2 days, the pathogen could cause disease symptoms.

Short periods of unfavourably low R.H. after inoculation with the pathogen temporarily prevented disease development, but it continued later when high R.H. conditions were provided. Long periods at low R. H. irreversibly prevented the pathogen from initiating disease, even if high R.H. was provided later.

Thus, there is a potential for disease development in sprinkle irrigated pepper crops in arid areas (Diab et al. 1982a).

The epidemiology of the bacterial scab disease of pepper (Capsicum annum) was studied in the field. Under the climate condition where the trials took place, it seems that disease development was favoured by a relative humidity level above 85%, by free water on the leaves for several hours and by temperatures above 30°C. Decreasing humidity and/or temperature inhibited disease development. Bacterial scab developed and caused damage to all cultivars of pepper for fresh market, industrial and export that are used in Israel. Cultivars that were tested in the late spring, summer and hot autumns were susceptible. It was found that pepper cv. Maor which is the most widely used cultivar for export in Israel was also the most susceptible one to bacterial scab.

XCV caused damage of up to 45% of the crop, mainly when plants were infected at early stages of growth and disease developed throughout the growing season. Infection caused defoliation and the fruits that remained on the plants were damaged due to sun exposure. These fruits could not be marketed.

The most susceptible physiological age to infection was at early stage. Susceptibility decreased in plants that reached the flowering and fruit developing period.

The most efficient control of bacterial scab was by spraying copper hydroxide (Kocide-101) at a concentration of more than 0.4% once a week during plant development. Prophylactic treatments before symptoms appearance were the most successful. (Azaizeh, 1983).

Systemic invasions of cucumber plants, fruits and seeds by *Pseudomonas syringae* pv. *lachrymans* (PSL).

Greenhouse studies showed systemic invasion of cucumber after inoculation with PSL. Plants were inoculated by stabbing the basal stem, by immersing roots or by spraying flowers with PSL. Several tests were made in order to demonstrate the systemic mobility of the bacteria: Stem cutting and collection of the roots pressure water. Sampling of tissues at a distance from the inoculum site. Fruit and seed examination. All the experiments were done in comparison with PST. and *P. fluorescens* which are non pathogenic bacteria to cucumber and with a PSL strain

resistant to streptomycin. Our studies show that the cucumber can be systemically invaded by PSL and not by the other tested bacteria through the roots or the stem. Only under humid conditions the plant shows symptoms: The pathogen could also be carried into the fruit through the stem as well as directly from the flower into the placenta area. About 60% of the seeds were found to be contaminated on the surface by systemically moving PSL. The bacteria were found inside the seeds on about 16% of the seeds (Kritzman and Zutra 1983a).

Detection of small numbers of phytopathogenic bacteria in seed and leaves

A simple differential staining procedure for demonstrating infection within the leaf tissue by PST, PSL and XCV has been developed. It is based on (1) clearing of plant tissue with a mixture of glycerol, lactic acid, phenol and water; (2) treating the leaf tissue with boiling KOH; and (3) staining with aniline blue-chloralhydrate. When observed under a light microscope, the bacteria appear dark blue, whereas the leaf tissue appears transparent and colourless.

Microcolonies of PST inside tomato leaves, XCV inside pepper leaves and PSL in cucumbers and muskmelon leaves were successfully detected. This staining procedure is simple and may be used successfully for observing the development of phytopathogenic bacteria inside the leaves of their hosts (Bashan et al. 1981a).

The commonly used media for growing of PST are undefined complex growth media which enhanced difficulties in biochemical, physiological, nutritional and genetic studies of the pathogen.

A synthetic medium based on D-galactose as a single carbon source and either L-asparagine, L-glutamine or L-threonine as a single nitrogen source has been developed for PST.

In comparing the newly defined medium to yeast-peptone broth, nutrient broth, or with a basal medium supplemented with glucose and KNO_3 it was found that the new synthetic medium could replace the complex media for growth of PST.

Other PST isolates such as ATCC 10852, D-1 and 15 local PST isolated during the last five years from all over Israel were grown on this medium as well as PST WT-1 (Bashan et al. 1982a).

To date, the detection of small numbers of phytopathogenic bacteria in and on seed has been difficult because of the relatively large numbers of saprophytic bacteria, taxonomically related to the pathogens, which accompany the pathogens and interfere with their growth on selective media. A method for detecting 10^1 - 10^2 cells of phytopathogenic bacteria in either tomato or pepper seed was developed. The method is based on the enhancement of the compatible pathogen inside a detached leaf of its host when placed on a water agar medium. It was found to be superior to the diagnostic growth media method commonly used and to permit the detection of the pathogens in symptomless plants.

The simplicity and its high sensitivity makes it suitable for use by seed producers for the following purposes:-

- (i) preliminary screening for the presence of the pathogen on leaves taken from plants growing in fields designed for seed production, ruling out positive fields unless appropriate preventive treatment is applied;
- (ii) routine seed checks throughout seed production to determine whether or not the expensive disinfection process is needed;
- (iii) quality control of seed disinfestation;
- (iv) early detection of bacterial speck or scab in fields and greenhouses made possible because the method detects disease in symptomless leaves. Chemical control can then be given only when necessary, saving costly bacteriocides and lessening the environmental pollution caused by frequent sprays. Finally, the method perhaps could be extended to detect bacterial pathogens other than PST and XVC (Sharon et al. 1983b).

Interaction between the pathogen and seeds and soil

It was demonstrated for the first time that soil infested with PST significantly affects tomato seeds at the pre-emergence stage.

Infestation of sterilized or natural soil with PST at inoculum concentrations of 10^2 to 10^9 propagules/ml inhibited germination of seeds and caused damping-off of tomato cv. VF-198, susceptible to bacterial speck. Infestation with saprophytic *P. fluorescens* at an inoculum concentration of 10^9 propagules/ml did not cause any damage. Germination of seeds of tomato cv. Rehovot 13, resistant to PST was not affected in PST-infested natural soil, but was inhibited when tested in PST-infested sterilized soil. Tomato plants which were symptomless from sowing to the flowering

stage when growing in infested soil had 20-30% less foliage than plants growing in uninfested soil.

At present pre-emergence damping-off of tomato seedlings is probably of relatively little economical importance, because tomato seeds are sown in excess. However, the decrease in germination and emergence demonstrated in our experiments may have greater economical significance in the future if more expensive seeds of F_1 cultivars are to be used (Bashan and Okon, 1981).

The purpose of the survival studies was to study the survival of an isolate of XCV and PST and to assess its potential as a source of inoculum in the next growing season.

A population of XCV developed as endophytes in the leaves and rhizosphere of apparently symptomless plants grown under mist but not under dry conditions. The pathogen survived for long periods on, and could be isolated from the surface of infested dried seeds, inoculated sandy loam soil, dried leaves and the rhizosphere of pepper and of other non-host plants. In addition, small numbers of the pathogen survived for 18 months in a field previously cropped with pepper diseased with bacterial scab. Healthy nursery or mature plants developed symptoms while growing in soil containing infested leaves, either buried or placed on the soil surface.

The great versatility of the means by which XCV survives from one season to the other and within the same season, ensures possible disease outbreaks under normal environmental conditions, at any time and place in pepper production fields.

Seed treatment, crop rotation etc., may decrease the number of inoculum sources and the inoculum size. But to fully prevent outbreaks of the disease appears to be a very difficult task (Bashan et al 1982d).

PST, XCV, the causal agents of bacterial speck of tomato and bacterial scab of pepper, survived on dried seeds for 20 and 10 yr. respectively. Seedlings from old infested seeds either developed visible disease symptoms or no symptoms. Symptomless plants grown under high relative humidity contained massive populations of the pathogen.

PST was first detected in Israel in 1971. Its presence in 20 yr. old seeds

suggested that it is a local pathogen. The increase in bacterial speck severity during the last 10 yr. may have resulted from a change from resistant cultivars to susceptible cultivars.

Results of this study suggest that bacteria in "dried" form can survive for long periods of time. Thus, old seed stocks maintained for breeding programs and cultivar collections may be sources of dormant "latent" infestations that can be "awakened" and initiate disease in susceptible plants. They should be tested for presence of bacterial pathogens before being used (Bashan et al. 1982b).

Survival of PSL in the soil, plant debris, and the rhizosphere of non-host plants.

The ability of the pathogen PSL to survive in soil, plant debris and the rhizosphere of non-host plants was studied under controlled conditions for 92 weeks. The pathogen was a poor survivor in the soil, with its population declining from 5.6×10^5 bacteria per gram soil to zero after 8 weeks in inoculated soil which kept dry, without irrigation. On the other hand, the organism persisted for over 90 weeks wetted soil containing diseased cucumber debris. The pathogen disappeared in soil which was kept wet for 72 weeks followed by 10 weeks incubation without any irrigation. The pathogen survived in the rhizosphere of non-host plants for four growth cycles in the same soil, kept its pathogenicity to cucumber seedlings, and increased its population over this period (Krtizman and Zutra 1983b).

Physiological of host and non-host interactions with bacterial pathogens

The relationships between phytopathogenic bacteria and non-host plants are problematical and controversial. On the one hand it seems that the hypersensitive reaction (HR) to incompatible bacteria in plant tissue is a general phytopathologic event. On the other hand there are, however, only a few reports available dealing with the isolation of substances from bacterial cells which induced cell collapse of non-host tissues.

A necrosis-inducing factor (NIF) was extracted from two virulent strains of PST sonicated cells. The factor had an approximate molecular weight of 108,000, contained 90% protein and 10% polysaccharide, and was partially purified by affinity chromatography, gel chromatography, gel electrophoresis and electrophoresis on cellulose acetate strips. The factor caused loss of electrolytes within 48 h and necrosis

on bean, pepper and cucumber leaves but not on tomato leaves of different cultivars. Similar fractions extracted from cells of P. fluorescens did not show any activity. Activity was destroyed by pronase or by hydrolysis with HCl indicating that the activity is due to the protein component. The factor did not show any pectolytic or cellulolytic activity. The factor was produced at a maximum after 24 h of growth of PST and was more abundant when extracted from cells grown in rich medium.

Therefore, it can be proposed that the extraction of a high molecular weight component from PST capable of producing necrosis in bean, pepper and cucumber leaves at low concentrations cause a different interaction between materials from that of pathogenic bacteria and plants. Thus, the necrosis-inducing factor might play an unknown role in starting events which finally lead to necrosis (Bashan et al. 1982c).

The involvement of morphological characteristics of tomato leaves in preventing or enhancing the infection process of the pathogen PST was studied. Natural openings such as stomata, broken trichomes and cuticle cracks were counted in susceptible and resistant cultivars. It was found that susceptibility increased proportionally to the increase in the number of these potential penetration sites. However, their role in the defence mechanism of the plant is probably limited because even in the highly resistant cultivars there are enough natural openings to account for a successful penetration (Bashan, 1982).

The possibility of active penetration through the cuticle and the cell wall was studied by measuring enzymatic activity in cell free extracts obtained from the pathogen and from healthy or diseased plants. Only moderate activity of bacterial pectinases and cutin-esterase could be detected during the penetration process. Whereas cellulase and protease activities were not detected during this period. It was concluded that PST is capable of active penetration but only to some limited extent (Bashan, 1982).

The main disease symptoms observed was the formation of necrotic areas in the leaves. The possible presence of toxins in the development of speck symptoms in tomato was studied by using charcoal adsorption, ion exchange and gel filtration column chromatography, electrophoresis and chemical tests. Ammonia in culture filtrates of PST and in homogenized diseased leaves caused necrosis of healthy tomato and bean leaves. In addition electrolyte leakage and symptoms formation

in tomato leaves infected with PST were preceded by the production of toxic quantities of ammonia. By contrast, non-pathogenic pseudomonads produced ammonia only in culture. No chlorosis or any other necrosis inducing substance could be obtained from filtrates of any of the two isolates of PST tested. Macromolecules in the culture filtrates, cell free extracts and cell debris of P. tomato did not visibly affect tomato leaves.

The ammonia metabolism of the pathogen and of the infected plants was studied in relation to pathogenesis (Bashan, 1982).

PST produced a constitutive protease during the logarithmic phase with an optimum activity at pH 7.0 and 30°C. Addition of proteinaceous compounds increased specific activity. No correlation between pathogenicity and proteolytic activity in vitro by several pseudomonads was found. Proteolytic activity was higher in diseased susceptible plants than in resistant ones and reached a maximum at later stages of infection. Total proteolytic activity consisted of 6 isozymes originating from the pathogen, its host, and of new isozymes produced during the interaction between them. Proteolytic activity was not affected by inoculum concentration. Direct correlation between disease severity and proteolytic activity in infected tissue was demonstrated. Proteolytic activity was located around developing necrotic zones and was related to leaf age at infection time. This activity was followed by decrease in soluble leaf proteins and increase in asparagine. It is suggested that proteolytic activity may play a role in the development of symptoms of bacterial speck of tomato (Bashan, 1982).

Increase in free amino acids in the plant tissue were observed following inoculation of susceptible plants. It appears that amino acids induced the production of deaminative enzymes capable of degrading asparagine and glutamine to ammonia and carbon skeleton. As a result, both the ammonium ion content and the pH increased in the tissue. When the pH reached a level of about 8.0 the ion was converted into gaseous ammonia, which evaporated from the tissue, thus causing death of cells (necrosis). Furthermore, by adding to the leaves low concentrations of amino acids it was possible to induce much more severe disease (Bashan, 1982).

The phenol content, peroxidase and polyphenoloxidase activities were tested in leaves of susceptible and resistant tomato cultivars. The plants reacted to either

disease or injury by enhancing peroxidase activity. However, no correlation between resistance and increase in peroxidase activity could be detected. Correlation between resistance, polyphenoloxidase activities and accumulation of phenols was found when testing 20 different tomato cultivars ranging from highly susceptible to highly resistant (Bashan, 1982).

The production of ethylene by pepper leaves infected with XCV was investigated. It was found that:-

- (1) Ethylene caused defoliation of pepper leaves. Its production during development of bacterial scab of pepper was unique to this bacterial leaf spot disease, and could not be detected during development of either bacterial speck of tomato or angular leaf spot of cucumber. Ethylene was produced during development of powdery mildews. Wounding and environmental stresses did not induce production of ethylene.
- (2) The pathogen did not produce ethylene in aerobic culture. XCV produced ethylene in cultures with low dissolved oxygen, but the same was true for other organisms tested.
- (3) A positive correlation was found between: the inoculum level and the amount of ethylene that was produced in the infected leaves; the number of bacteria in the tissue and ethylene production; the disease index and ethylene production; days after inoculation and ethylene production.
- (4) Younger leaves were found to be more sensitive to the disease and produced more ethylene. Placing of the inoculum on the petiole caused increased ethylene production in this site, chlorosis and defoliation.
- (5) Application of methionine to the leaves enhanced ethylene production in severely infected leaves. Application of exogenous auxin even in low concentration (5×10^{-4} M) decreased significantly disease index, percent defoliation and production of ethylene. Addition of ethylene synthesis inhibitor aminooxyacetic acid (AOA) caused a significant decrease in ethylene production by the infected leaves and reduced the disease index. There was a negative linear correlation between IAA-peroxidase activity and the disease index (Ben David, 1983).

Preliminary studies on the physiology of bacterial scab of pepper revealed that:-

- (6) Among the hydrolytic enzymes tested, only polygalacturonase was active in cul-

ture and in diseased leaves. Protease activity increased proportionally with time after inoculation and with disease index. Protease activity was detected in both cell free medium and in cell free extracts of XCV. Polyphenoloxylase and peroxidase activities increased during disease development but no changes in phenol content could be detected.

- (7) It was observed that the pathogen could infest the seeds inside the pepper fruit, probably by penetrating through the knob (Ben David, 1983).

Resistance to bacterial speck of tomato

The acquisition of additional genetic factors for resistance and an understanding of the mode of action of their resistance genes are desirable and even essential for the success of future breeding programmes.

The purpose of the genetic study was to investigate the inheritance of resistance to bacterial speck in cv. Rehovot 13 (an old commercial variety of the Marmande type) which was found to possess a high degree of field resistance to the disease. The purpose was also to test the susceptibility of tomato cultivars, species and lines to bacterial speck of tomato, and to explore new sources of resistance to the disease.

Inheritance of resistance to bacterial speck of tomato was determined by analysing F_1 , F_2 and backcross progenies of crosses involving a susceptible (VF-198) and a resistant cultivar (Rehovot 13). The results fit the hypothesis that resistance is controlled by a single dominant gene in interaction with minor genes. Cultivar susceptibility to PST was tested under greenhouse conditions under high inoculum pressure using infested tomato seeds together with infested soils and spray-inoculated wounded plants. Of 21 species, cultivars and lines, Rehovot 13, Ontario 7710 and Lycopersicon pimpinellifolium P.I. 126927 were found to be resistant to the pathogen. VF-198 and Tropic-VF were the most susceptible. Extra Marmande, Saladette, Acc. 339944-3 and wild type Lycopersicon esculentum var. cerasiforme were moderately resistant (Fallik et al 1983a).

In an additional trial the allelic of resistance of Rehovot 13 and Ontario 7710 (Canadian resistant cultivar) was tested. The results support the hypothesis that the resistance factors are non-allelic and controlled by two different genes. These findings should encourage further work on genetical improvement of tomato cultivars for resistance to bacterial speck of tomato (Fallik et al 1983b).

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Work carried out during support by BARD No 1-214-80 In USA 1980-1983
Professor R.N. Goodman Department of Plant Pathology University of Missouri
Columbia, Missouri

Our studies and others suggest that some plant cells are capable of synthesizing or releasing substances that localize invading pathogens at or very near the host's cell wall surface. The precise biochemical nature of these sequestering substances has not been established. However, they may be representations of a class of proteins distinct from lectins in plants and antibodies in man and animal that are capable of suppressing plant pathogens. It appears that plants under stress are capable of mobilizing small protein molecules, such as the agglutinin malin that we have isolated from apple and that others have detected in wheat barley, and rice that have antimicrobial properties. These proteins belong to a group known as thionins, the most well known is purothionin isolated from wheat which is capable of suppressing yeast growth and hence seriously interferes with the rising of dough in bread making. Approximately 25% of the amino acid residues of thionins are lysine and arginine and they also have a high proportion of disulfide bridges (10/150 residues). It is the latter characteristic from which they derive their name.

We have recently isolated thionin-like compounds from both tomato and melon that have both intense agglutinating and antibiotic activity (cylinder-agar plate antibiotic inhibition technique) against PST, PL, EA, XV (see Fig. 1).

Crystallized malin appears to be a mixture of eleven closely related polypeptides in SDS gel electrophoresis. It is possible that the granules we have observed localizing bacteria in apple petiole xylem vessels are in fact aggregates of this mixture of polypeptides. The evidence from our studies with the apple agglutinin (malin) suggests that although apple seed, stem and leaf tissue contains an endogenous level of the substance is inducible and results in the synthesis or release of larger amounts following challenge inoculation. We designed experiments to examine the contention that malin was in fact serologically related to the substances we observed electromicroscopically to be agglutinating EA in xylem vessels.

Antisera raised to non-crystalline malin was linked to protein-A and then to colloidal gold applied to bacterial cells (EA) became uniformly adsorbed in significant amounts to the surfaces that were treated with malin, but not to untreated controls (Fig. 5)

1. Inhibition of Erwinia amylovora (acapsular E_8 mutant) by partially purified thionin-like proteins extracted from a) tomato seed (Early-Pak 7) 200 $\mu\text{g/ml}$, b) apple seed (Malus ranetka) 50 $\mu\text{g/ml}$, c) rice seed (TN-1) 200 $\mu\text{g/ml}$, d) rice seed (IRRI-8) 100 $\mu\text{g/ml}$, e) melon seed (Planters jumbo) 100 $\mu\text{g/ml}$.

2. Rocket immunoelectrophoresis of a 2 fold serial dilution of 10 mg/ml partially purified tomato agglutinin (TA). Precipitin peaks reflect the activity of TA in wells moving into upper agarose band containing 75 μl crude E. amylovora (E_8) LPS/5 ml agarose under a constant voltage of 28v/cm at 25 C for one hour (arrow shows reaction in 8th dilution).

Fig. 5. Cells of Erwinia amylovora acapsular mutant E₈ from a 12-hour liquid culture, a) glutaraldehyde-fixed cells exposed initially to apple agglutinin, malin, subsequently to malin antisera and then to protein A (pA) gold complex, X35,000. b) Same as (a) except not exposed initially to malin X35,000.

Preliminary experiments with the protein extracted tomato seed revealed that on a μg basis it is almost as active an agglutinin as malin and is inhibitory to the growth of E₃ strain of EA, our target organism (Fig. 1). At concentrations of 200 $\mu\text{g}/\text{ml}$ (non-crystalline form) a zone of inhibition produced by the tomato protein (TA) was on average 16.5 mm in diameter which approximates the activity of 2.0 $\mu\text{g}/\text{ml}$ streptomycin against the E₃ strain of EA in a cylinder-agar-plant assay.

- a) Romeiro, R., A. Karr and R. Goodman. 1981. Isolation of a factor from apple that agglutinated Erwinia amylovora. Plant Physiol. 68:772-777.
- b) Romeiro, R., A. Karr and R. Goodman. 1981. Erwinia amylovora cell wall receptor for apple agglutinin. Physiological Plant Pathol. 19:383-390.

E) Description of cooperation.

There was a constant and fruitfull exchange of ideas, plant material, bacterial strains, visits to field experiments between the research groups in Israel and extensive correspondence was maintained with Dr. Goodman in Missouri. The groups of Israel supplied Dr. Goodman with local plant material and pathogen isolates. Dr. Goodman taught the Israeli groups purification methods such as rocket electrophoresis, ion exchange and gel filtration, and kindly helped in the preparation of manuscripts related to this work.

Dr. Okon in 1981, Drs. Kritzman and Bashan (Ph.D. student in the project) in October 1982 visited Dr. Goodman's laboratory in Missouri held discussions and surveyed experiments with his group. Dr. Goodman has a very fruitfull visit to Israel in June 1983.

F) Benefit to Agriculture.

Substantial progress was made in achieving the objectives of this research. Procedures for detecting and eliminating extremely small numbers of bacteria in and on seeds in symptomless tissue and in soil (PST, XCV, PSL) were developed and recommended for their use to the seed Industry.

The information obtained on population dynamics of the bacterial pathogens, should help develop strategies for control in the field. For example we recommend to spray with copper compounds as a profilactic measure, even before symptoms appearance and following periods of high relative humidity. It was found that PST XCV and PSL survived on seeds and on the rhizosphere of non-host plants, therefore their eradication will be almost impossible and the only means of control are by developing resistant cultivars (Such as those found in this project). Basic understanding of the physiology of the diseased plant, as achieved in this project may help develop new-non-conventional approaches for disease control. For example deaminating enzymes aspartase and glutaminase were found to be involved in causing necrosis in bacterial speck of tomato. If this enzymes could be inhibited with a specific inhibitor that may arrest damage. The production of agglutinins by the plant, that arrest infection, can be enhanced thus preventing infection.

PUBLICATIONS OF BARD No. 1214-80

- Azaizeh Mahmoud 1983. Epidemiology and control in the field of Xanthomonas campestris pv. vesicatoria (Didge 1920) Dye 1978b causal agent of bacterial scab of pepper. M.Sc. Thesis, Faculty of Agriculture, The Hebrew University of Jerusalem, p. 48. (In Hebrew).
- Bashan, Y. 1982. Characterization of host-pathogen relationships between the tomato and Pseudomonas syringae pv. tomato, the causal agent of bacterial speck of tomato. Ph.D. Thesis. The Hebrew University of Jerusalem, 128 pp. (In Hebrew) supervised by Prof. Y. Henis and Dr. Y. Okon.
- Bashan, Y., Kritzman, G., Sharon, E., Okon, Y. and Henis, Y. 1981a. A note on the detection of phytopathogenic bacteria within the leaf by a differential staining procedure. Journal of Applied Bacteriology, 50:315-317.
- Bashan, Y., Sharon, E., Okon, Y. and Henis, Y. 1981b. Scanning electron and light microscopy of infection and symptom development in tomato leaves infected with Pseudomonas tomato. Physiological Plant Pathology, 19:139-144.
- Bashan, Y. and Okon, Y. 1981. Inhibition of seed germination and development of tomato plants in soil infested with Pseudomonas tomato. Annals of Applied Biology, 98:413-417.
- Bashan, Y., Okon, Y. and Henis, Y. 1982a. A note on a new define medium for Pseudomonas tomato. Journal of Applied Bacteriology, 52:297-298.
- Bashan, Y., Okon, Y. and Henis, Y. 1982b. Long-term survival of Pseudomonas syringae pv. tomato and Xanthomonas campestris pv. vesicatoria in tomato and pepper seeds. Phytopathology 72:1143-1144.
- Bashan, Y., Okon, Y. and Henis, Y. 1982c. Detection of a necrosis-inducing factor of non host plant leaves produced by Pseudomonas syringae pv. tomato. Canadian Journal of Botany 60:2453-2460.
- Bashan, Y., Diab, S. and Okon, Y. 1982d. Survival of Xanthomonas campestris pv. vesicatoria in pepper seeds in symptomless and dry leaves and in soil. Plant and Soil, 68:161-170.
- Ben David, Anat. 1983. Ethylene production in pepper leaves infected with Xanthomonas campestris pv. vesicatoria. M.Sc. thesis, Faculty of Agriculture, The Hebrew University of Jerusalem, p. 76.
- Diab, Shahr. 1981. Studies on the bacterial scab disease of pepper caused by Xanthomonas vesicatoria (Doidge) Dawson. M.Sc. Thesis, Faculty of Agriculture, The Hebrew Univeristy of Jerusalem, p. 71.
- Diab, S., Bashan, Y., Okon, Y. and Henis, Y. 1982a. Effect of relative humidity on bacterial scab caused by Xanthomonas campestris pv. vesicatoria on pepper. Phytopathology 72:1257-1260.
- Diab, S., Bashan, Y. and Okon, Y. 1982b. Studies of infection with Xanthomonas campestris pv. vesicatoria, causal agent of bacterial scab of pepper in Israel. Phytoparasitica 10:183-191.

Fallik, Elazar. 1981. Genetics of resistance of the tomato Lycopersicon esculentum (Mill) to bacterial speck of tomato caused by Pseudomonas tomato (Okabe) Alstatt and the relation between total phenol content in the plant and its resistance to the disease. M.Sc. Thesis, Faculty of Agriculture, The Hebrew University of Jerusalem, p. 45.

Fallik, E., Bashan, Y., Okon, Y., Cahaner, A. and Keder, N. 1983a. Inheritance and sources of resistance to bacterial speck of tomato caused by Pseudomonas syringae pv. tomato. Annals of Applied Biology, 102:365-371.

Fallik, E., Bashan, Y., Okon, Y. and Kedar, N. 1983b. Genetics of resistance to bacterial speck of tomato caused by Pseudomonas syringae pv. tomato. Annals of Applied Biology (accepted for publication). 1984

Kritzman, G. and Zutra, D. 1983a. Systemic invasion of cucumber plants, fruits and seeds by Pseudomonas syringae pv. lachrymans. Canadian Journal of Plant Pathology, In press.

Kritzman, G. and Zutra, D. 1983b. Survival of Pseudomonas syringae pv. lachrymans in soil, plant debris, and the rhizosphere of non-host plants. Phytoparasitica, 11:99-108.

Sharon, Edna. 1981. Detection methods of Pseudomonas tomato (Okabe) Alstatt. and Xanthomonas vesicatoria (Doidge) Dawson in infected tomato and pepper seeds. M.Sc. Thesis, Faculty of Agriculture, The Hebrew University of Jerusalem, p. 61.

Sharon, E., Bashan, Y., Okon, Y. and Henis, Y. 1982a. Pre-symptomatic multiplication of Xanthomonas campestris pv. vesicatoria on the surface of pepper leaves. Canadian Journal of Botany, 60:1041-1045.

Sharon, E., Okon, Y., Bashan, Y. and Henis, Y. 1982b. Detached leaf enrichment: A method for detecting small numbers of Pseudomonas syringae pv. tomato and Xanthomonas campestris pv. vesicatoria in seeds and symptomless leaves of tomato and pepper. Journal of Applied Bacteriology, 53:371-377.

There are 5 manuscripts based on Y. Bashan, Ph.D. Thesis;

1 manuscript based on M. Azaizeh, M.Sc. Thesis;

and 1 manuscript based on A. Ben David M.Sc. Thesis

which are in preparation and are to be sent for publication in scientific Journals.

There were 10 publications in Hebrew in "Hassade" Journal and six abstracts in Congresses. ^{and}

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