Isaac Barash

How Way Leads on to Way*

Isaac Barash

Department of Molecular Biology and Ecology of Plants, Tel Aviv University, Tel Aviv 69978, Israel; email: isaaci@post.tau.ac.il

Annu. Rev. Phytopathol. 2014. 52:1–17

The *Annual Review of Phytopathology* is online at phyto.annualreviews.org

This article's doi: 10.1146/annurev-phyto-102313-045953

*Quoted from "The Road Not Taken" by Robert Frost.

Keywords

pectic enzymes, phytotoxins, ammonium toxicity, type III effectors, evolution of pathogenicity, host specificity

Abstract

In this article, I briefly recount the historical events in my native country that led me to become a plant pathologist. I started as a field pathologist specializing in fungal diseases of legumes, moved to biochemical research on virulence factors, and then on to molecular plant-microbe interactions. I describe the impact my graduate studies at the University of California (UC)-Davis had on my career. My life's work and teaching can be said to reflect the development in plant pathology during the past 40 years. I have included a concise review of the development of plant pathology in Israel and the ways it is funded. Dealing with administrative duties while conducting research has contributed to my belief in the importance of multidisciplinary approaches and of preserving the applied approach in the teaching of plant pathology.

HOW I BECAME A PLANT PATHOLOGIST

The early choices that led to my career in plant pathology were strongly affected by historical events that took place in my native country. I was born in Tel Aviv, Israel (then Palestine), just prior to World War II. My parents arrived from the Ukraine in the 1920s. Like many other Jewish pioneers who immigrated to Palestine from Eastern Europe during the late nineteenth and early twentieth centuries, they came in search of a new life.

Following World War I (1918), Palestine came under the control of Great Britain through a mandate issued by The League of Nations, later to become the United Nations. This period was characterized by the establishment of the kibbutz, a voluntary collective community, mainly agricultural, in which all wealth is held in common and profits are reinvested in the settlement. The people who started the kibbutzim (plural for kibbutz) were idealists who believed in adopting agriculture as the basis of their new life. Their members came mainly from pioneering youth movements both in Palestine and abroad, and played a significant part in the development of Israel as a state. The formation of these kibbutzim, together with the development of underground combat units that were to become the regular army, were crucial to the foundation of the State of Israel in 1948 on a portion of Palestine, which was supported by a decision of the United Nations. Like many other children and teenagers of my generation, I joined a pioneer youth movement focused on the kibbutz as the ideal way of life. Summer vacations from school were devoted to working in the kibbutzim, where I was exposed to agriculture.

After high school I joined the NAHAL (the Hebrew acronym for Fighting Pioneer Youth) Brigade for two and a half years of compulsory military service. NAHAL had two aims: to produce first-class soldiers and to prepare the group for establishing new kibbutzim or joining existing ones. Israel, prior to the Six Day War in 1967, stretched 263 miles from north to south and 9.3–71 miles from east to west. Our group established a NAHAL settlement on the border at the narrowest point, which later became Kibbutz Magal. I was assigned to work in field crops, where I gained some practical and theoretical knowledge in their cultivation. Almost all the members of my group were high school graduates interested in furthering their education. After completing my military service, the conflict between my desire for higher education and continuation as a kibbutz member was resolved when I decided to enroll in the Faculty of Agriculture at the Hebrew University of Jerusalem.

Studies in the Faculty during the late fifties were divided into two parts. The first year and a half was devoted to basic courses in biology, chemistry, and physics on the Jerusalem campus. The second part consisted of three additional years that, together with a research thesis, led to an MS degree in agronomy. The latter courses were taught in the Faculty of Agriculture on the Rehovot campus, and most were oriented toward applied agriculture. The courses in the last three semesters were electives aimed at specialization.

During the third year of my undergraduate studies, I began looking for a part-time job. Priman, at that time a major manufacturer of canned peas in Israel, contracted with farmers to cultivate high-quality peas under factory supervision. When the pea fields became heavily infected with diseases that resulted in low seed quality, reduced yield, and plant death, the company offered a grant to Professor Isaac Wahl, a plant pathologist at the Faculty of Agriculture and one of the founders of plant pathology in Israel, to find solutions. He hired me. I learned through symptoms and classical Koch tests that the disease was caused by *Ascochyta* spp. The *Ascochyta* blight disease complex of peas is inflicted by three fungal pathogens: *Ascochyta pinodes* (with a sexual stage: *Mycosphaerella pinodes*), known as *Mycosphaerella* blight, *Ascochyta pinodella* (which causes *Ascochyta* foot rot), and *Ascochyta pisi* (which causes *Ascochyta* blight and pod spot). The causal agent of the disease in Israel had previously been recorded at the Volcani Institute [Israel's Agricultural

Research Organization (ARO)] as *A. pisi*. However, I soon discovered that the incubation period following inoculation of young pea plants with infected pea straw was two to four days, which fits *A. pinodes* or *A. pinodella*, whereas the incubation period for *A. pisi* was six to eight days. Isolation of numerous perithecia that contained two-celled ascospores from dry pea plants were identified as *Mycosphaerella*, and the appearance of *A. pinodes* symptoms following inoculation with isolated ascospores confirmed the identification. The epidemiological significance was that while *A. pisi* and *A. pinodella* lack a sexual stage and disseminate by water splashing of the pycniospores a short distance only, the ascospores of *A. pinodes* are discharged from the perithecia by a mechanism that allows them to become airborne and be carried for considerable distances. Their long-distance dissemination and their genetic diversity caused by the sexual stage may account in large part for their aggressive nature as compared with the other two pathogens. *A. pinodes* was found to be the predominant pathogen in all regions of the country. This study was extended to include a search for initial sources of inoculum as well as resistance in wild pea species in Israel (none was found) and was submitted as an MS thesis and a publication in Hebrew (26). The work on *Ascochyta* diseases inspired me to continue my career in plant pathology research. Meanwhile, I continued my studies toward an MS degree in plant protection.

The first Department of Plant Pathology was established in 1921 by Professor Israel Reichert, the father of phytopathology in Israel (55), at the Agricultural Experiment Station, which later became the Volcani Institute of Agricultural Research, or the ARO. Additional researchers, many of whom had received their educations in Europe, joined Reichert at Volcani. In 1943, the Department of Plant Pathology was established at the Hebrew University Faculty of Agriculture, and Reichert was appointed the first lecturer of phytopathology. He was later joined by I. Wahl. Many of the courses in plant pathology during that time were taught by staff of the ARO. The Israeli Phytopathological Society (IPS) was founded in 1970. The development of phytopathology in Israel is described in detail by Katan (55).

STUDIES AT UC DAVIS AND A STINT IN MEDICAL MYCOLOGY AT UCLA

During my MS studies, which were dedicated mainly to applied plant pathology, I became interested in biochemical factors that govern host-pathogen interactions. Because this subject was neither taught nor researched in Israel at that time, I decided to continue my studies abroad. Armed by recommendations from Professors Reichert and Wahl, I applied to the Departments of Plant Pathology at the Universities of Minnesota and California (UC Davis) and was accepted to both. I chose Davis because of its excellent reputation in agricultural and biological studies and its similar climate to Israel. The chair of the Department of Plant Pathology, Dr. L.D. Leach, offered me a USDA assistantship to help Dr. John M. Klisiewicz, a USDA researcher, in his work on safflower diseases, provided my PhD thesis would be related to safflower pathogens. In January 1961, I arrived at Davis with my wife Debora, who had just completed her MS degree in genetics at the Hebrew University Medical School. Davis was a small, pleasant, and friendly university city, which we enjoyed tremendously throughout our stay. Dr. B.R. Houston, professor of plant pathology and dean of the graduate school, was appointed my academic advisor. With the exception of some compulsory departmental courses, he gave me a free hand in selecting my course program.

At that time Dr. Tsune Kosuge, who had a PhD in comparative biochemistry from UC Berkeley, joined the department as an assistant professor. His extensive knowledge of biochemistry, along with his interest in biochemical mechanisms of pathogenicity and his pleasant personality, motivated me to work with him. He became my informal mentor, advisor, and

eventual collaborator. Acting on his advice, I took advanced courses in biochemistry with an emphasis on plant metabolism taught by Eric E. Conn and Paul K. Stumph, two distinguished professors who arrived from UC Berkeley and established the Department of Biochemistry at UC Davis. Additional courses were plant physiology and enzymology as well as an advanced laboratory course in biochemistry that taught me most of the biochemical methodologies known at the time and was instrumental in my early research. My first research project was to investigate biochemical factors that affect pathogenicity of *Botrytis cinerea* on safflower. This pathogen commonly occurs on safflower seed heads, causing them to become readily detached from the plant. I demonstrated that glucose, fructose, and pectic substances present in a blossom's leachates induced spore germination and polygalacturonase formation by germinated spores and were responsible for the observed symptoms (11). Another study dealt with the dissemination of *Phytophthora drechleri*, the causal agent of root rot in safflower, via zoospores (14). By employing C^{14}-labeled biochemicals present in the exudates, it was shown that motile as well as germinating zoospores can differentially utilize a variety of carbon and amino acid compounds. Results suggested that motile zoospores, although capable of metabolizing external compounds, utilize endogenous substrates as their main source of energy and their motility is independent of exogenous biochemicals. However, the possible presence of various physical factors and stresses in situ may affect the duration of motility.

Toward the end of my studies I began looking for a postdoctorate position in fungal physiology. While searching, I received an attractive offer to be acting assistant professor in the Department of Microbiology and Immunology at the UCLA Medical School. It was agreed that in addition to my research, the first year would be devoted to studying human pathogenic fungi, and in the second year I would replace Dr. D.H. Howard, their medical mycologist, who was due for a sabbatical year. I was to teach human fungal pathogens to medical students as part of their medical microbiology course. It was a challenging and interesting proposal that I recognized would increase my chances of obtaining an academic position in Israel. In the summer of 1965, I left with my wife and young daughter Ronit, who was born in Davis, for UCLA, where I continued my research in the biochemistry of spore germination.

I began research there on the biochemistry of spore germination of dermatophytes. Dermatophytes are superficial mycoses, as opposed to deep mycoses that cause internal diseases and even death. Both are found in humans and in animals. The species *Microsporum gypseum* was chosen as a representative of dermatophytes because of its abundant macroconidia production. Germination of macroconidia was stimulated and synchronized by catalytic concentrations of either D- or L-leucine or unsaturated long-chain fatty acids, whereas short-chain fatty acids were inhibitory, suggesting that spore reserves are adequate to supply the energy and carbon needed for germination (12). A shift from lipid utilization to carbohydrate and biosyntheses of proteins and nucleic acids during germination was the subject of this investigation. Teaching about and working with human pathogenic fungi revealed the unique adaptation of pathogenic fungi to a wide spectrum of microenvironments in living organisms.

During the mid-1960s, Professor Wahl moved from the Hebrew University to Tel Aviv University (TAU), where he established a division of plant pathology in the Department of Botany. Although I could have continued my work at UCLA, where I was deeply involved in medical mycology, his attractive proposal that I come to TAU, coupled with my desire to live in Israel, led to my decision to return. So at the beginning of 1967, I returned to Israel as an assistant professor at TAU.

EARLY YEARS AT TEL AVIV UNIVERSITY

Tel Aviv University was founded in 1956 by the Tel Aviv municipality by combining several academic institutions, one being the Institute for Biological Studies. In 1962, TAU became an

autonomous body supported by the government, the municipality, and the friends of TAU in Israel and abroad. During the late 1960s and early 1970s a new campus was built in north Tel Aviv, called Ramat Aviv, and many young staff members who had completed their postdoctorate training abroad were recruited. TAU rapidly grew from approximately 1,650 students in 1962–63 to some 30,000 today. It is Israel's largest university with an academic staff of around 1,200 spread over nine faculties, 90 research institutes, and 3 supercenters. Upon my arrival, the Faculty of Life Sciences was located on the old campus at Abu Kabir in the southern part of Tel Aviv. Lacking adequate equipment for basic biochemical work, it made the transfer from UCLA to TAU quite traumatic. However, funds were made available to purchase necessary basic equipment, and during the early 1970s the Life Science faculty moved to the new campus in Ramat Aviv. I was assigned to teach the compulsory mycology course for undergraduates and an elective in fungal physiology for seniors and graduate students. During the following years my research was concerned with several projects, briefly described below. In addition to my scientific interest in the biochemical factors of pathogenicity, the work on these projects was strongly affected by the ability to obtain the funding essential for keeping the laboratory active.

The Role of Pectic Enzymes in Soft-Rot Decays of Citrus

Upon my return to Israel, I received grants from the Citrus Marketing Board and later, along with E. Butler of UC Davis, from the Binational Agricultural Research and Development Fund (BARD) to work on the control of sour rot and other soft-rot decays of citrus (oranges and lemons) in packing houses. Among the three common soft rot–inducing fungi, the green mold (*Penicillium digitatum*), blue mold (*P. italicum*), and sour rot (*Geotrichum candidum*), the watery soft rot of the citrus fruit peel caused by the sour-rot pathogen was unparalleled compared with the rot caused by the other pathogens. Because soft rots were already known to result from pectin degradation, attention was focused on comparative properties of the pectic enzymes of the three citrus rot pathogens.

Pectic enzyme liberation by *G. candidum* was detected before germ tube appearance but increased significantly thereafter (4). A considerable amount of polygalacturonase was located on the surface of nongerminating spores, and its release was found to be pH dependent but independent of energy supply (10). The pectic enzyme was bound to spore components outside the cell membrane by salt and disulfide linkages. This publication was the first report demonstrating surface localization of pectic enzyme on nongerminating fungal spores. A single polygalacturonase was produced by *G. candidum* (8, 27). The enzyme was classified as endo-polygalacturonase (endo-PG), and its kinetics and other properties were elucidated (27). No traces of exo-polygalacturonase (exo-PG) could be detected because the enzyme preparation could not hydrolyze digalacturonic acid. A purified homogenous enzyme preparation could trigger sour-rot symptom when injected into lemon albedo tissue. The mode of sour-rot formation was inferred from comparative studies with a virulent (citrus race) (34) and noncitrus avirulent race of *G. candidum* (51). Results suggested that the higher initial amount of enzyme produced in vivo by the citrus race and the higher sensitivity of the pectin in situ to this enzyme were the two main factors governing the unique virulence of the citrus race on citrus fruits (51). A later manuscript by Nakamura et al. (73) showed 68% identity at the amino acid level between endo-PG from citrus and noncitrus races, suggesting that the two enzymes are similar but not identical. It could be hypothesized that the endo-PG of the citrus race can efficiently degrade the protopectin in citrus fruits (water-insoluble pectic sustenance present in intact tissue), whereas the noncitrus race cannot.

In contrast to the endo-PG produced by *G. candidum*, only a single exo-PG could be isolated from lemon peel infected by *P. digitatum* (6). Exo-PG was also the predominant enzyme in orange peel infected by the blue mold (52). Results of these studies indicated that exo-PGs were the

predominant enzymes in decays induced by the green and blue molds compared with endo-PG in sour rot. These results may explain the rapid maceration during sour-rot development compared with the mold pathogens. Further studies, in collaboration with microbiologists at TAU, were aimed at screening inhibitors of the sour-rot endo-PG from bacteria (2), and a patent was registered (1). Fungicides for the control of sour rot on lemon fruits in the packing house were evaluated for recommendation (81).

Ammonium Toxicity

As part of my interest in fungal physiology, I studied ammonium transport and assimilation in fungi (5, 13, 19, 20, 30–32, 82). Within this project I concentrated also on ammonium toxicity, with attention to glutamate dehydrogenase (GDH), one of the key enzymes in the assimilation of ammonium into organic nitrogen (20, 31, 44). During analysis of GDH in oat leaves infected with the crown rust *Puccinia coronata*, we noticed the appearance of a new isozyme that could not be detected in healthy plants. The premise that the new isozyme is induced by free ammonium that accumulates because of infection was examined. Substrate-controlled induction of GDH at that time was demonstrated in microorganisms but not in higher plants. Further studies showed that the level of GDH activity associated with the new isozyme substantially increased within a few hours when excised oat leaves were floated on a 15 mM ammonium solution under controlled conditions (25). The new GDH isozyme was also detected in leaves of intact plants grown in nutrient solution supplemented with ammonium. The stimulatory effect of ammonium on GDH appearance was rapid, specific, and proportional to ammonium concentration, and could be prevented by cycloheximide. Both density labeling and labeling with a radioactive amino acid demonstrated de novo biosynthesis of the new isozyme (21, 22). The results suggested that this process could be a host defense mechanism for preventing ammonium toxicity.

Phytotoxins, Iron, and Siderophores

Phytotoxins can be classified as either host selective or nonselective. The host-selective toxins exhibit exclusive and generally extreme toxicity to hosts of the producing pathogen and in most cases act as primary disease determinants. Although most of the phytotoxins that have been identified in cultures of microbial pathogens are nonselective, they could act as secondary disease determinants. In most cases, the lack of suitable genetic systems, which would provide unequivocal proof of their involvement in disease, prevented a precise evaluation of their function in the infection process. Nevertheless, the potential of phytopathogens to produce phytotoxic compounds in culture, their structural identification, and their ability to trigger disease symptoms remained of scientific interest and merited investigation.

In 1973 and 1974, I spent a sabbatical year with Dr. Gary Strobel at Montana State University (MSU) in Bozeman. Gary and I had become friends at UC Davis during our graduate studies. Similar to Davis, Bozeman was then a small, pleasant university town in the northwest whose cold climate was completely foreign to me and my family. It has expanded and developed over the years, as has my friendship with Gary, bringing me to visit him and Bozeman frequently.

Following my stay in Bozeman working on the isolation of a phytotoxin from *Stemphylium botryosum* (9), Gary and I decided to continue collaborating on phytotoxins research, focusing on *Phoma tracheiphila*, the causal agent of mal secco disease of citrus. Mal secco is a severe tracheomycotic disease that results in substantial reduction of the quality and quantity of the crop. Lemon, being the most susceptible and damaged citrus species, served as the host for our studies. We were joined on this project by Dr. A. Nachmias, who was then my doctoral student, and Dr. Z. Solel

of the ARO Volcani Center, an expert on the disease. An extracellular phytotoxic glycopeptide capable of inciting symptoms similar to mal secco disease in lemon shoots was isolated and characterized from culture filtrate and lemon infected with *P. tracheiphila* (71). Correlative evidence for the participation of the toxin, designated as malseccin, in inciting disease symptoms was obtained (23, 70, 72).

S. botryosum f. sp. *lycopersici*, the causal agent of leaf spot and foliage blight of tomato, results in severe losses to tomato fields in Israel. In a preliminary study we found that the pathogen secretes into culture filtrates several phytotoxic compounds capable of producing disease symptoms on tomatoes. One of these phytotoxins, stemphyloxin I, was characterized as an enolic β-ketoaldehyde with an empirical formula of $C_{21}H_{32}O_5$ (24). The functional groups of stemphyloxin I were initially identified by infrared and nuclear magnetic resonance spectrometry, but the final structure was determined by X-ray crystallography (18). Stemphyloxin I is a highly functionalized β-ketoaldehyde *trans*-Decalin tricyclic compound. Cyclic β-ketoaldehydes are quite rare among natural products. The presence of the enolic β-ketoaldehyde group and the adjacent hydroxyl group on carbon 13 suggested that stemphyloxin I may act as a chelating agent (18). Injection of the toxin into intact leaves produced a differential phytotoxicity toward various plants, with tomato being the most sensitive (24). In further studies, Dr. Shulamit Manulis, a research assistant working with me on her doctorate, identified an additional tricyclic phytotoxin that resembled stemphyloxin I and was designated as stemphyloxin II (63). The secretion of stemphyloxins was iron regulated, and both I and II showed high affinity for ferric, but not for ferrous, iron (64). The β-ketoaldehyde group appears to be crucial for toxicity and chelation. The structure of stemphyloxin II differed from stemphyloxin I in two major ways: the alteration in the position of the β-ketoaldehyde group and the disappearance of a saturated ketone, which was present in stemphyloxin I (17). The phytotoxicity of stemphyloxins is independent of the presence of iron, although iron increases the toxicity of stemphyloxin I in plants (7).

The possibility that stemphyloxins are also involved in iron transport was rejected because their iron-binding constant was considerably lower than siderophores. Dimerum acid and coprogen B, two common hydroxamate siderophores, were identified and their transport systems characterized in *S. botryosum* f. sp. *lycopersici* (62, 65) as well as in *Gaeumannomyces graminis* var. *tritici*, the pathogen of take-all disease in wheat (43). In contrast, a high-affinity nonsiderophores system was characterized in *G. candidum* (69), although *G. candidum* could utilize iron from various hydroxamate siderophores produced by other microbes (67). Enhancement of disease expression through iron limitation was observed during infection of peanut and other plants by *Verticillium dahlia* (7, 28). Our studies on phytotoxins, siderophores, and iron were supported by three cooperative grants from the US-Israel Binational Science Foundation (BSF); they were carried out with G. Strobel and R.A. Olsen, both from MSU.

MOVING TO MOLECULAR BIOLOGY

The development of molecular biology and recombinant DNA technologies during the 1970s and 1980s greatly influenced my teaching and research. In the late 1970s, I modified the Mycology and Fungal Physiology courses into Biology of Phytopathogenic Fungi and Bacteria for undergraduates and Molecular Plant-Microbe Interactions for seniors and graduate students. During 1981 and 1982, I divided a sabbatical year between MSU with Dr. Strobel and UC Davis with Dr. Kosuge. At MSU, I worked on crystallization of stemphyloxin I and at UC Davis, I worked on *Pseudomonas savastanoi*, the causal agent of olive and oleander knot. The sabbatical in Davis came soon after IAA synthesizing genes had been identified on a plasmid and cloned by Dr. Kosuge's group (40, 41). I began to practice recombinant DNA techniques, which became essential to my research.

My first project involving molecular genetics techniques dealt with hypovirulence in *Rhizoctonia solani*. After returning from sabbatical, I began to work on this project together with Dr. Baruch Sneh from my department. We found that approximately 30% of 107 *Rhizoctonia* isolates, representing 6 different anastomosis groups, could suppress pathogenicity (damping off) of virulent strains in various plant hosts (53, 54). The *Rhizoctonia* system exhibited some similarities to the unique chestnut blight pathogen *Cryphonectria* (*Endothia*) *parasitica*, in which double-stranded RNA (dsRNA) is associated with the hypovirulence phenomenon, a property that was developed for biocontrol of the disease (84). We obtained financial support from the Joint BMFT [Bundesministerium für Forschung und Technologie (German Ministry of Research)] Germany-Israel program and from BARD, in cooperation with T. Kosuge, and extended this research with Dr. Yigal Koltin from the TAU Department of Microbiology. Virus particles containing two or three major segments of dsRNA were characterized in *R. solani* (48). RNA-dependent RNA polymerase activity was associated with the viral capsids. However, unlike *C. parasitica*, hypovirulent strains of *R. solani* were devoid of dsRNA viruses, whereas dsDNA viruses could be found in the wild-type strains. Attenuation of virulent strains to hypovirulence was correlated with the loss of some or all of the segments of dsRNA (48). In addition, transmission of dsRNA was associated with virulence. Transcription and in vitro translation of the dsRNA virus isolated from *R. solani* were demonstrated (47). Comparative purification and characterization of pectolytic enzymes in *R. solani* disclosed the presence of endopectinlyase activity in virulent strains, whereas none or only traces of this enzyme were detected in hypovirulent strains (66).

COLLABORATION WITH ARO, THE VOLCANI CENTER

In 1987, I was asked by the director of the Plant Protection Institute at ARO, The Volcani Center, to chair the Department of Plant Pathology. I accepted this position while maintaining my professorship and teaching obligations at TAU. My experience during this period is described in the section "Academic Administrative Activities."

The work at the Volcani Center exposed me to applied phytopathology research and the opportunity to collaborate with other scientists on various subjects. One of the projects that attracted my attention was carried out by Dr. Hillela Vigodsky-Haas on the gall-forming bacterium *Erwinia herbicola* pv. *gypsophilae* (later known as *Pantoea agglomerans* pv. *gypsophilae* or *Pag*) (16). *Pag* prevents the development of roots in cuttings of *Gypsophila paniculata* (baby's breath)—an ornamental plant used for commercial cut-flower production and an important Israeli export—limiting its propagation. Neither resistant clones nor effective chemical control measures are available, and the only way to control the disease is to produce pathogen-free cuttings through culture indexing, followed by eradication of infected nurseries. The similarity of *Pag*'s symptoms to *P. savastanoi* piqued my interest in the molecular mechanism of gall formation by this pathogen, and I realized it was an ideal system on which to collaborate with Dr. Kosuge. Shortly before my arrival at the Volcani Center, Dr. Yedidia Gafni of the Plant Genetics and Breeding department began to study this system. We worked together for two years until he chose to focus his efforts on his other projects. In 1988, Dr. Shulamit Manulis-Sasson was recruited to the Department of Plant Pathology following her postdoctorate with Noel Keen at UC Riverside. We collaborated on the *Pag* project, sharing students, grants, and papers, and together with T. Kosuge submitted a grant proposal to BARD on the "Molecular Basis for Virulence by *Pag* and *P. savastanoi*." To my great sorrow, Kosuge died of cancer in 1988, just before the grant was approved. Tsune had been universally respected not only for his contribution to the field of molecular plant pathology but for his enthusiasm and generosity as well. He was replaced on the BARD grant by Steve Lindow of UC Berkeley, with whom we had a most beneficial collaboration. During the next two decades

Manulis-Sasson and I received financial support for this project from several BARD grants in cooperation with Dr. Lindow and later with David Coplin of Ohio State University; from the Israeli Science Foundation (ISF); and from the DFG [Deutsch Forschungsgemeinschaft (the German Research Foundation)] program for Trilateral Cooperation between Israel, the Palestinian Authority, and Germany. Dr. Guido Sessa of my department at TAU joined our team in 2005.

P. agglomerans, a widespread epiphyte and commensal bacterium, has evolved into an Hrp-dependent and host-specific tumorigenic pathogen on various hosts by acquiring a plasmid that contains a pathogenicity island (PAI) (16, 59). The PAI evolved on an iteron plasmid of the IncN family, which is distributed among genetically diverse populations of *P. agglomerans* (86). *Pag* elicits galls on *Gypsophila* (42) and hypersensitive response (HR) on beet (45); *P. agglomerans* pv. *betae* (*Pab*) causes galls on both beet and *Gypsophila* (33). The pathogenicity plasmids of *Pag* and *Pab*, designated $pPATH_{Pag}$ and $pPATH_{Pab,}$ respectively, may vary in size (60), and expulsion of these plasmids results in loss of pathogenicity (86). The gall-forming *P. agglomerans* is a unique system that offers an opportunity to study how a commensal bacterium evolved into a plant pathogen. This system has been reviewed (15, 16, 59), and some of its highlights are presented here.

1. The structure and plasmid location of the PAI suggests a recent evolution of pathogenesis. The $pPATH_{Pag}$ of strain 824-1 was sequenced and extensively investigated. Its 135 kilobases contain a PAI of approximately 75 kb that carries a functional *hrp-hrc* gene cluster (68, 78); six experimentally confirmed and active type III effectors that reduce or abolish virulence upon mutagenesis (15, 45, 57, 68, 83); and a cluster of genes encoding indole-3-acetic acid (IAA) and cytokinin (CK) biosynthetic enzymes (39, 49, 57). $pPATH_{Pag}$ and $pPATH_{Pab}$ share an identical replicon (86) and multiple diverse insertion sequences (ISs) (50, 58), suggesting a common origin.
2. The type III secretion system (T3SS) and its effectors are crucial for gall formation. Mutations in any of the *hrp-hrc* genes encoding the T3SS or the *hrp* regulatory cascade completely abolished gall initiation (68, 77), indicating that gall formation by *Pag* and *Pab* is absolutely dependent on the T3SS.
3. Plant-synthesized rather than pathogen-secreted IAA is mandatory for tumorogenicity. Simultaneous inactivation of IAA and CK biosynthetic genes of *Pag* substantially reduced gall size (by ~50%) but did not eliminate gall initiation (61). In contrast, disruption of polar auxin transport (PAT) in *Gypsophila* by specific inhibitors completely abolished gall initiation (37), suggesting that plant-derived IAA is crucial for gall initiation, whereas bacterial IAA and CK exert a significant effect on gall size but do not prevent gall initiation.
4. Host specificity is determined by effectors, two of which act as transcription factors. Three of the six active effectors found on the $pPATH_{Pag}$ have been identified in *Pseudomonas syringae* pathovars and most likely were acquired by horizontal gene transfer (15). In contrast, PthG, HsvG, and HsvB are unique to *Pag* or *Pab* and presumably evolved by a pathoadaptive mechanism (16). PthG acts as a virulence effector in *Gypsophila* but as an avirulence gene in beet and is responsible for the HR elicitation of *Pag* on beet (45, 46). Mutation in *pthG* allows *Pag* to also elicit galls on beet. An exciting outcome of this study was the characterization of HsvG and HsvB as transcription activators that determine host specificity on *Gypsophila* and beet, respectively. HsvG and HsvB are two paralogous effectors present in *Pag* and *Pab* (75). Their structure includes a T3SS recognition motif, a region of predicted α helices, two nuclear localization signals (NLSs), and a transcription activation domain in yeast (75, 85). The activation domain of HsvG has two direct repeats (71 and 74 amino acids), whereas that of HsvB has only one repeat. Exchanging the activation domains between HsvG and HsvB resulted in a switch in host specificity (75) (**Figure 1**). Each of the repeats was sufficient

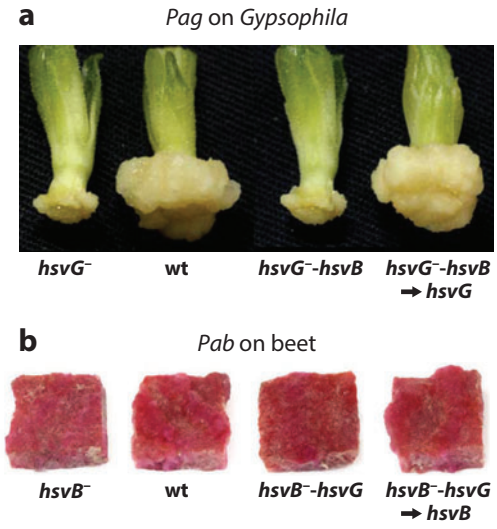

Figure 1

Domain switching and host recognition. HsvG and HsvB are type III secretion system effectors that determine host specificity on *Pag* and *Pab*, respectively. Exchanging the activation domains of these effectors resulted in interchange in host specificity of the two pathovars. (*a*) Modifying the host specificity function of HsvB to HsvG expressed by pathogenicity on *Gypsophila* cuttings. Abbreviations: *hsvG*$^{-}$, marker exchange mutant of *hsvG*; *hsvG*$^{-}$-*hsvB*, marker exchange mutant of *hsvG* complemented with *hsvB*; *hsvG*$^{-}$-*hsvB*→*hsvG*, marker exchange mutant of *hsvG* complemented with *hsvB*, which was converted to *hsvG*; wt, *Pag* wild type. (*b*) Modifying the host-specificity function of HsvG to HsvB expressed by pathogenicity on beet cubes. Abbreviations: *hsvB*$^{-}$, marker exchange mutant of *hsvB*; *hsvB*$^{-}$-*hsvG*, marker exchange mutant of *hsvB* complemented with *hsvG*; *hsvB*$^{-}$-hsvG→hsvB, marker exchange mutant of *hsvB* complemented with *hsvG*, which was converted to *hsvB*; wt, *Pab* wild type. Adapted from Reference 75.

to activate transcription in yeast (75). Transient expression of green fluorescence protein showed that the effector proteins were localized to the cell nucleus in host and nonhost plants (75). HsvG and HsvB were demonstrated as DNA-binding proteins (74). Taken together, the above characteristics suggest that HsvG and HsvB may also act as transcription factors in their respective host plants, a suggestion supported by the isolation of a target gene of HsvG, *HSVGT*, in *Gypsophila* (74). HSVGT is a predicted acidic protein of the DnaJ family that harbors typical conserved motifs of a eukaryotic transcription factor. Quantitative real-time polymerase chain reaction (qRT-PCR) demonstrated that *HSVGT* transcription is specifically induced in planta by HsvG, reaching a peak of sixfold within 4 h, whereas *hsvG* mutant or HsvG converted to HsvB were inactive (74). Gel-shift assays demonstrated that HsvG binds to *HSVGT* promoter, indicating that HSVGT is a direct target of HsvG. With the exception of TAL effectors (29), HsvG and HsvB are the only effectors in plant bacterial pathogens that have been found to act as transcription factors.

5. *hrp* box promoters exhibit differential strength that may play an important role in coordinating effectors' expression and optimal virulence. The regulatory cascade that activates the HrpL regulon in *Pag* has been characterized (77). HrpL, an alternative sigma factor, is generally known to activate the transcription of the Hrp regulon by its binding to a common *hrp* box promoter. The *hrp* box was previously defined by computational techniques as a consensus bipartite *cis* element (87). Mutational analysis indicated that the *hrp* box consensus can be divided into crucial and noncrucial nucleotides, whereas some adjacent nonconsensus

nucleotides exert a significant effect on the promoter's strength. The degeneracy in *hrp* box promoters of T3SS effectors in *Pag* indicated significant differences in the promoter activity (76). Transcriptional regulation is influenced mainly by the efficiency of the promoter and the steady-state transcript levels. The degeneracy observed in *hrp* promoters may represent a mechanism of fine tuning for expressing effectors in *Pag* (76). Results emphasized the importance of the promoter's strength in coordinating disease expression.

6. Global regulatory networks control the Hrp regulon of *Pag*. The quorum sensing (QS) regulatory system of *Pag* was found to affect gall formation via the Hrp regulon (35). Moreover, IAA and cytokinins produced by the bacterium significantly affected the transcription of the QS regulatory genes (i.e., *pagI* and *pagR*) (35). The interactions between the plasmid-born HrpL regulon and the chromosomal global regulatory systems, namely, the QS system and the Gac-Rsm pathway, were elucidated (79). Gel-shift experiments demonstrated that the transcription factor PagR of the QS system directly activates the genes of the HrpL regulatory cascade and the *gacA* of the Gac-Rsm pathway by a signal-dependent process. The Gac-Rsm pathway also manipulates the transcription of the *hrp* regulatory cascade by controlling RsmA activity via rigorous regulation of the small RNA *rsmB* (79).

In 2005, we completed a DFG-Trilateral cooperative research project with Professor Rudolf Eichenlaub (Bielefeld University, Germany), who worked on the molecular basis of *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) while we worked on the *Pag* system. The DFG agreed to continue the support for our studies but only on a single pathogen, and *Cmm*, which had become a very important pathogen in Israel as well, was selected. The collaborators in Israel were S. Manulis-Sasson and G. Sessa. The *Cmm* research was further supported by two consecutive BARD grants with Dr. Christine Smart of Cornell University in Geneva, New York. Dr. Laura Chalupowicz, who had just completed her doctorate on the QS of *Pag*, spent a postdoctorate with Professor Eichenlaub and transferred molecular methodologies developed for *Cmm* in Bielefeld to Israel. After initial analysis of *Cmm* strains present in Israel (56), colonization and movement of *Cmm* in infected tomato plants and sequential expression of bacterial virulence and plant defense genes were investigated (36, 38). The interaction between *Cmm* and tomato was also explored by analyzing gene expression and protein profiles of *Cmm*-infected tomato stems and of *Cmm* samples generated in vivo (3, 80). These analyses led to our finding that during infection *Cmm* senses the plant environment, transmits signals, and induces and then secretes multiple hydrolytic enzymes and other cell wall–degrading enzymes.

ACADEMIC ADMINISTRATIVE ACTIVITIES

Over my career, I have been involved in various university, national, and international committees, including grant evaluation committees of the Israel Science Foundation (ISF) and BARD. I served at BARD as a member or chair of the plant health committee and later as a member of the technical advisory committee and the 20-year evaluation committee. My close association with BARD brought me to appreciate its major contribution to agricultural research and to the beneficial interactions between Israeli and US scientists. In 1980, I was appointed Chairman of the Plant Science Department at TAU for a period of four years.

As mentioned above, in 1987 I accepted the offer to chair the Department of Plant Pathology at ARO, The Volcani Center. Researchers at Volcani are generally assigned to their area of specialization but have to obtain their own financial support through competitive grant applications. Their proposals are submitted to professional committees appointed by the Chief Agricultural Scientist of Israel and funded by the Ministry of Agriculture and the growers.

Both the contribution to Israeli agriculture and the scientific quality of the project are taken into consideration. The Chief Scientist can also channel funds to specific urgent problems on which several researchers cooperate. Funds for basic research relevant to agriculture in Israel come mainly from BARD, but may also come from the ISF, BSF, German-Israeli Foundation (GIF), the trilateral program of DFG, and European Community programs. My job as Chair included the recruitment and promotion of researchers, the fostering of an academic atmosphere, and the organizing of meetings between experts from Volcani and the universities on major phytopathology issues. In 1993, I left my position as Chair but continued to be active on committees that evaluated promotions and reviewed grant proposals.

In 1995, I was elected Dean of the Faculty of Life Sciences at TAU for a period of five years. The Faculty of Life Sciences encompasses six departments: Biochemistry, Cell Research and Immunology, Microbiology and Biotechnology, Neurobiochemistry, Plant Sciences, and Zoology. When I took over as Dean, each department was responsible for the MS and PhD degree programs in its field. My main goals as Dean were to introduce into the teaching program different disciplines vital to the study of modern biology, to establish a graduate school, and to play an instrumental role in the recruitment of new academic staff to the Department of Plant Sciences, many of whose members were due for retirement.

The first goal was achieved by proposing the double major that combined biology and a major from any other faculty that offered a similar program. This required a 50% reduction in biology courses. After two years of discussions the double major program was approved by the faculty council. The combinations with chemistry, psychology, and computer science were the most popular, but other combinations, such as biology and philosophy, were used. The program prompted the development of biology curricula that enriched the interdisciplinary nature of the faculty. Ten BS programs were developed over the next ten years, including bioinformatics (computer science and biology), cell biology and immunology, ecology and environmental quality, plant sciences (including plant pathology), genetics, neurobiology, biotechnology, and others. The double major program also produced a substantial increase in the enrollment of first-rate students and the number of fellowships to support outstanding students.

My second goal, to establish the graduate school, was meant to improve, expand, and enhance the graduate programs in the Faculty of Life Sciences, ensure a common unifying structure for those programs, and relieve the individual departments of the burden of sole responsibility for both undergraduate and graduate studies. Although students continued to receive administrative services from department secretaries, their academic programs were supervised, upgraded, and regulated by the graduate school. Years later, in 2004, the graduate school received a generous donation and was officially named The Smolarz Family Graduate School of Life Sciences. The graduate school continues to develop and today offers ten MS programs and four PhD programs. Of the more than 1,800 students studying at the Faculty of Life Sciences, 500 of them are in the graduate school.

Finally, my third goal was realized when, as Dean, I was instrumental in recruiting molecular plant biologists to the Department of Plant Sciences. This change in staff led to the change in the name of the department from the Department of Plant Sciences to the Department of Molecular Biology and Ecology of Plants. Although it considerably improved the competitive ability of the department to recruit graduate students, the reduction of scientists specializing in organism biology and ecology was an adverse side effect that has yet to be corrected.

CLOSING THOUGHTS

Since the late twentieth century we have witnessed revolutionary developments in biology expressed by recombinant DNA, the polymerase chain reaction, and the sequencing of whole

genomes, to name only a few. Unparalleled opportunities now exist that depend on the participation of researchers from chemistry, physics, computational science, and other fields. The advent of new techniques and concepts has already had an enormous impact on the practice and research of plant pathology. Mechanisms of virulence and host defense responses are being unraveled, and this might lead to new methods of disease control. Transformative science that brings scientists with different specialties together in a collaborative team may lead to new insights into ways to prevent plant diseases. Efficient manipulation of systemic plant defense responses and development of genome-guided breeding for developing resistant plants are only two approaches to avoiding infection that come to mind. Research funding is the fuel needed to set up such large interdisciplinary teams with a focus on important problems in plant pathology. However, team science is not appropriate for every aspect of phytopathology research. Researchers sitting in a lab with a couple of postdocs and graduate students, perhaps collaborating with a few colleagues down the hall or in another part of the world, may provide the chemistry and synergy needed to pave the way to solving critical problems in plant pathology. Guidelines for funding agencies can promote both scenarios—the large interdisciplinary teams and the few select scientists.

The response of plant pathology departments to the biological revolution has been progressive. Staff members representing disciplines other than the traditional areas of plant pathology have been recruited, resulting in a substantial reduction in courses on plant diseases in exchange for subjects such as molecular genetics of host-pathogen interactions. In my view, university departments should retain their phytopathology identity and flexibility and follow a dual course: to provide a foundation for training field practitioners and to equip the students with the tools needed for today's world of plant pathology.

DISCLOSURE STATEMENT

The author is not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

LITERATURE CITED

1. Aaronovitz Y, Bauer S, Loya R, Schreiber R, Barash I, Gutnick DL. 1987. Specific inhibitors of tissue degrading enzymes for control of post-harvest diseases of fruit and vegetables. Isr. Patent Appl. No. 8422
2. Aaronovitz Y, Bauer S, Loya R, Schreiber R, Barash I, Gutnick DL. 1989. A specific bacterial inhibitor of the extracellular polygalacturonase of *Geotrichum candidum*. In *Novel Microbial Products for Medicine and Agriculture. Society for Industrial Microbiology*, ed. AL Demain, GA Somkuti, JC Hunter-Cevera, HW Rosmoor, pp. 151–59. Amsterdam, Neth.: Elsevier Sci.
3. Balaji V, Jacob-Hirsh J, Sherf O, Eichenlaub R, Iraki N, et al. 2008. Tomato transcripton changes in response to *Clavibacter michiganensis* subsp. *michiganensis* reveal a role for ethylene in disease development. *Plant Physiol.* 146:1797–809
4. Barash I. 1968. Liberation of polygalacturonase during spore germination by *Geotrichum candidum*. *Phytopathology* 58:1364–71
5. Barash I. 1972. Accumulation of urea and allantoin during purine utilization by germinating spores of *Geotrichum candidum*. *J. Gen. Microbiol.* 72:539–42
6. Barash I, Angel E. 1970. Isolation and properties of an exopolygalacturonase produced by *Penicillium digitatum* during infection of lemon fruits. *Isr. J. Bot.* 19:599–608
7. Barash I, Dori S, Mor H, Manulis S. 1993. Role of iron in fungal phytopathologies. In *Iron Chelation in Plants and Soil Microorganisms*, ed. LL Barton, BC Hemming, pp. 251–67. San Diego, CA: Academic, Inc.
8. Barash I, Eyal Z. 1970. Properties of a polygalacturonase produced by *Geotrichum candidum*. *Phytopathology* 60:27–30
9. Barash I, Karr JA, Strobel GA. 1975. Isolation and characterization of stemphylin, a chromone glucoside from *Stemphylium botryosum*. *Plant Physiol.* 55:646–51

10. Barash I, Klein L. 1969. The surface localization of polygalacturonase in *Geotrichum candidum*. *Phytopathology* 59:319–24
11. Barash I, Klisiewicz JM, Kosuge T. 1964. Biochemical factors affecting pathogenicity of *Botrytis cinerea* on safflower. *Phytopathology* 54:923–27
12. Barash I, Conway ML, Howard DH. 1967. Carbon catabolism and synthesis of macromolecules during spore germination of *Microsporum gypseum*. *J. Bacteriol.* 93:656–62
13. Barash I, Lowy I, Pomerantz S. 1972. Effect of urea on ammonium-dependent synthesis of carbamyl phosphate during spore germination of *Geotrichum candidum*. *Plant Physiol.* 50:642–44
14. Barash I, Klisiewicz JM, Kosuge T. 1965. Utilization of carbon compounds in zoospores of *Phytophthora dreschsleri* and their effect on motility and germination. *Phytopathology* 55:1257–61
15. Barash I, Manulis-Sasson S. 2007. Virulence mechanisms and host specificity of gall-forming *Pantoea agglomerans*. *Trends Microbiol.* 15:538–45
16. Barash I, Manulis-Sasson S. 2009. Recent evolution of bacterial pathogens: the gall-forming *Pantoea agglomerans* case. *Annu. Rev. Phytopathol.* 47:133–52
17. Barash I, Manulis S. 1986. Iron and phytotoxins as exemplified by stemphyloxins and other toxins. In *Iron, Siderophores and Plant disease*, ed. TR Swinburne, pp. 273–81. New York: Springer
18. Barash I, Manulis S, Kashman Y, Springer JP, Chen MHM, et al. 1983. Crystalization and X-ray analysis of Stemphyloxin I, a phytotoxin from *Stemphylium botryosum*. *Science* 220:1065–66
19. Barash I, Mor H. 1972. Studies on the control and properties of ornithine transcarbamylase in germinated spores of *Geotrichum candidum*. *Plant Cell Physiol.* 13:119–30
20. Barash I, Mor H. 1973. Regulation of nicotinamide adenine dinucleotide phosphate-specific glutamate dehydrogenase in germinated spores of *Geotrichum candidum*. *Plant Physiol.* 51:852–58
21. Barash I, Mor H, Sadon T. 1975. Evidence for ammonium-dependent de novo synthesis of glutamate dehydrogenase in detached oat leaves. *Plant Physiol.* 56:856–58
22. Barash I, Mor H, Sadon T. 1976. Isozymes of glutamate dehydrogenase from oat leaves: properties and light effect on synthesis. *Plant Cell Physiol.* 17:493–500
23. Barash I, Pupkin G, Koren L, Ben-Hayyim G, Strobel GA. 1981. A low molecular weight phytotoxin produced by *Phoma tracheiphila*, the cause of mal secco disease in citrus. *Physiol. Plant Pathol.* 19:17–19
24. Barash I, Pupkin G, Netzer D, Kashman Y. 1982. A novel enolic β-ketoaldehyde phytotoxin produced by *Stemphylium botryosum* f. sp. *lycopersici*: partial chemical and biological characterization. *Plant Physiol.* 69:23–27
25. Barash I, Sadon T, Mor H. 1973. Induction of a specific isoenzyme of glutamate dehydrogenase by ammonia in oat leaves. *Nat. New Biol.* 244:150–52
26. Barash I, Wahl I. 1960. Studies of *Ascochyta* diseaeses of peas in Israel. *Hassadeh* 41:262–67 (In Hebrew)
27. Barash I, Zilberman E, Marcus L. 1984. Purification of *Geotrichum candidum* endopolygalacturonase from culture and from host tissue by affinity chromatography on cross-linked polypectate. *Physiol. Plant Pathol.* 25:161–69
28. Barash I, Zion R, Krikun J, Nachmias A. 1988. Effect of iron status on verticillium wilt disease and on in vitro production of siderophores by *Verticillium dahliae*. *J. Plant Nutr.* 11:893–905
29. Bogdanove AJ, Schornack S, Lahaye T. 2010. TAL effectors: finding plant genes for disease and defense. *Curr. Opin. Plant Biol.* 13:394–401
30. Breiman A, Barash I. 1976. Characterization of L-asparagine transport systems in *Stemphylium botryosum*. *J. Bacteriol.* 127:1127–35
31. Breiman A, Barash I. 1978. In vivo regulation of NADP-specific glutamate dehydrogenase by L-amides in *Stemphylium botryosum*. *J. Gen. Microbiol.* 106:343–52
32. Breiman A, Barash I. 1980. Methylamine and ammonia transport in *Stemphylium botryosum*. *J. Gen. Microbiol.* 116:201–6
33. Burr TJ, Katz BH, Abawi GS, Crosier DC. 1991. Comparison of tumorigenic strains of *Erwinia herbicola* isolated from table beet with *E. h. gypsophilae*. *Plant Dis.* 75:855–58
34. Butler EE, Webster RK, Eckert JW. 1965. Taxonomy, pathogenicity and physiological properties of the fungus causing sour rot in citrus. *Phytopathology* 55:1262–68

35. Chalupowicz L, Barash I, Panijel M, Sessa G, Manulis-Sasson S. 2009. Regulatory interactions between quorum-sensing, auxin, cytokinin and *hrp*-regulatory genes in relation to gall formation and epiphytic fitness of *Pantoea agglomerans* pv. *gypsophilae*. *Mol. Plant-Microbe Interact.* 22:849–56
36. Chalupowicz L, Cohen-Kandli M, Dror O, Eichenlaub R, Gartemann K-H, et al. 2010. Sequential expression of bacterial virulence and plant defense genes during infection of tomato with *Clavibacter michiganensis* subsp. *michiganensis*. *Phytopathology* 100:252–61
37. Chalupowicz L, Weinthal D, Gaba V, Sessa G, Barash I, Manulis-Sasson S. 2013. Polar auxin transport is essential for gall formation by *Pantoea agglomerans* on *Gypsophila*. *Mol. Plant Pathol.* 14:185–90
38. Chalupowicz L, Zellermann E-M, Fluegel O, Dror O, Eichenlaub R, et al. 2012. Colonization and movement of GFP-labeled *Clavibacter michiganensis* subsp. *michiganensis* during tomato infection. *Phytopathology* 102:23–31
39. Clark E, Manulis S, Ophir Y, Barash I, Gafni Y. 1993. Cloning and characterization of *iaaM* and *iaaH* from *Erwinia herbicola* pathovar *gypsophilae*. *Phytopathology* 83:234–40
40. Comai L, Kosuge T. 1980. Involvement of plasmid deoxyribonucleic acid in indoleacetic synthesis in *Pseudomonas savastanoi*. *J. Bacteriol.* 143:950–57
41. Comai L, Kosuge T. 1982. Cloning and characterization of *iaa*M, a virulence determinant of *Pseudomonas savastanoi*. *J. Bacteriol.* 149:40–46
42. Cooksey DA. 1986. Galls of *Gypsophila paniculata* caused by *Erwinia herbicola*. *Plant Dis.* 70:464–68
43. Dori S, Solel Z, Kashman Y, Barash I. 1990. Characterization of hydroxamate siderophores and siderophore-mediated iron uptake in *Gaeumannomyces graminis* var. *tritici*. *Physiol. Mol. Plant Pathol.* 37:95–106
44. Errel A, Mor H, Barash I. 1973. The isozymic nature and kinetic properties of glutamate dehydrogenase from safflower seedlings. *Plant Cell Physiol.* 14:39–50
45. Ezra D, Barash I, Valinsky L, Manulis S. 2000. The dual function in virulence and host range restriction of a gene isolated from the $pPATH_{Ehg}$ plasmid of *Erwinia herbicola* pv. *gypsophilae*. *Mol. Plant-Microbe Interact.* 13:693–98
46. Ezra D, Barash I, Weinthal DM, Gaba V, Manulis S. 2004. *pthG* from *Pantoea agglomerans* pv. *gypsophilae* encodes an avirulence effector that determines incompatibility in multiple beet species. *Mol. Plant Pathol.* 5:105–14
47. Finkler A, Ben-Zvi B, Koltin Y, Barash I. 1988. Transcription and in vitro translation of the dsRNA virus isolated from *Rhizoctonia solani*. *Virus* 1:205–19
48. Finkler A, Koltin Y, Barash I, Sneh B, Pozniak D. 1985. Isolation of a virus from virulent strains of *Rhizoctonia solani*. *J. Gen. Virol.* 66:1221–32
49. Gafni Y, Manulis S, Kunik T, Lichter A, Barash I, Ophir Y. 1997. Characterization of the auxin synthesis genes of *Erwinia herbicola* pv. *gypsophilae*. *Israel J. Plant Sci.* 45:279–84
50. Guo M, Manulis S, Mor H, Barash I. 2002. The presence of diverse IS elements and an *avrPphD* homologue that acts as a virulence factor on the pathogenicity plasmid of *Erwinia herbicola* pv. *gypsophilae*. *Mol. Plant-Microbe Interact.* 15:709–16
51. Hershenhorn J, Bher L, Barash I, Arzee T. 1989. Mode of sour rot formation as inferred from comparative studies with virulent and avirulent strains of *Geotrichum candidum*. *J. Phytopathol.* 126:257–71
52. Hershenhorn J, Manulis S, Barash I. 1990. Polygalacturonase associated with infection of Valencia orange by *Penicillium italicum*. *Phytopathology* 80:1374–76
53. Ichielevich-Auster M, Sneh B, Koltin Y, Barash I. 1985. Pathogenicity, host specificity and anastomosis groups of *Rhizoctonia* spp. isolated from soils in Israel. *Phytoparasitica* 13:103–22
54. Ichielevich-Auster M, Sneh B, Koltin Y, Barash I. 1985. Suppression of damping-off caused by *Rhizoctonia* species by a nonpathogenic isolate of *R. solani*. *Phytopathology* 75:1080–84
55. Katan J. 2009. Phytopathology in Israel: nine decades of research. *Phytoparasitica* 37:333–36
56. Kleitman F, Barash I, Burger A, Iraki N, Falah Y, et al. 2008. Characterization of a *Clavibacter michiganensis* subsp. *michiganensis* population in Israel. *Eur. J. Plant Pathol.* 121:463–75
57. Lichter A, Barash I, Valinsky L, Manulis S. 1995. The genes involved in cytokinin biosynthesis in *Erwinia herbicola* pv. *gypsophilae*: characterization and role in gall formation. *J. Bacteriol.* 177:4457–65
58. Lichter A, Manulis S, Valinsky L, Karniol B, Barash I. 1996. IS1327, a new insertion-like element in the pathogenicity-associated plasmid of *Erwinia herbicola* pv. *gypsophilae*. *Mol. Plant-Microbe Interact.* 9:98–104

59. Manulis S, Barash I. 2003. The molecular basis for transformation of an epiphyte into a gall-forming pathogen as exemplified by *Erwinia herbicola* pv. *gypsophilae*. In *Plant-Microbe Interactions*, ed. G Stacey, N Keen, 6:19–52. St. Paul, Minnesota: American Phytopathological Society
60. Manulis S, Gafni Y, Clark E, Zutra D, Ophir Y, Barash I. 1991. Identification of a plasmid DNA probe for detection of *Erwinia herbicola* pathogenic on *Gypsophila paniculata*. *Phytopathology* 81:54–57
61. Manulis S, Haviv-Chesner A, Brandl MT, Lindow SE, Barash I. 1998. Differential involvement of indole-3-acetic acid biosynthetic pathways in pathogenicity and epiphytic fitness of *Erwinia herbicola* pv. *gypsophilae*. *Mol. Plant-Microbe Interact.* 11:634–42
62. Manulis S, Kashman Y, Barash I. 1987. Identification of siderophores and siderophore-mediated uptake of iron in *Stemphylium botryosum*. *Phytochemistry* 26:1317–20
63. Manulis S, Kashman Y, Netzer D, Barash I. 1984. Phytotoxins from *Stemphylium botryosum*: structural determination of stemphyloxin II, production in culture and interaction with iron. *Phytochemistry* 23:2193–98
64. Manulis S, Netzer D, Barash I. 1986. Structure-activity relationships as inferred from comparative phytotoxicity of stemphyloxins and betaenones. *J. Phytopathol.* 115:283–87
65. Manulis S, Netzer D, Barash I. 1987. Acquisition of iron by *Stemphylium botryosum* under iron-replete conditions. *Can. J. Microbiol.* 33:652–59
66. Marcus L, Barash I, Sneh B, Koltin Y, Finkler A. 1986. Purification and characterization of pectolytic enzymes produced by virulent and hypovirulent isolates of *Rhizoctonia solani* Kuhn. *Physiol. Mol. Plant Pathol.* 29:325–36
67. Mor H, Barash I. 1990. Characterization of siderophore-mediated iron transport in *Geotrichum candidum*, a non-siderophore producer. *Biol. Met.* 2:209–13
68. Mor H, Manulis S, Zuc M, Nizan R, Coplin DL, Barash I. 2001. Genetic organization of the *hrp* gene cluster and *dspAE/BF* operon in *Erwinia herbicola* pv. *gypsophilae*. *Mol. Plant-Microbe Interact.* 14:431–36
69. Mor H, Pasternak M, Barash I. 1988. Uptake of iron by *Geotrichum candidum*, a nonsiderophore producer. *Biol. Met.* 1:99–105
70. Nachmias A, Bar-Joseph M, Solel Z, Barash I. 1979. Diagnosis of mal secco disease in lemon by enzyme-linked immunosorbent assay. *Phyopathology* 69:559–61
71. Nachmias A, Barash I, Solel Z, Strobel GA. 1977. Purification and characterization of a phytotoxin produced by *Phoma tracheiphila*, the causal agent of mal secco disease of citrus. *Physiol. Plant Pathol.* 10:147–57
72. Nachmias A, Barash I, Solel Z, Strobel GA. 1977. Translocation of mal secco toxin in lemons and its effect on electrolyte leakage, transpiration and citrus callus growth. *Phytoparasitica* 5:94–103
73. Nakamura M, Suprapta DN, Iwai H, Arai K. 2001. Comparison of endo-polygalacturonase activities of citrus and non-citrus races of *Geotrichum candidum*, and cloning and expression of the corresponding genes. *Mol. Plant Pathol.* 2:265–74
74. Nissan G, Manulis-Sasson S, Chalupowicz L, Teper D, Yeheskel A, et al. 2012. The type III effector HsvG of the gall-forming *Pantoea agglomerans* mediates expression of the host gene *HSVGT*. *Mol. Plant-Microbe Interact.* 25:231–40
75. Nissan G, Manulis-Sasson S, Weinthal DM, Mor H, Sessa G, Barash I. 2006. The type III effectors HsvG and HsvB of gall-forming *Pantoea* determine host specificity and function as transcriptional activators. *Mol. Microbiol.* 61:1118–31
76. Nissan G, Manulis S, Weinthal D, Sessa G, Barash I. 2005. Analysis of promoters recognized by HrpL, an alternative sigma-factor protein from *Pantoea agglomerans* pv. *gypsophilae*. *Mol. Plant-Microbe Interact.* 18:634–43
77. Nizan-Koren R, Manulis S, Mor H, Iraki NM, Barash I. 2003. The regulatory cascade that activates the Hrp regulon in *Erwinia herbicola* pv. *gypsophilae*. *Mol. Plant-Microbe Interact.* 15:1249–60
78. Nizan R, Barash I, Valinsky L, Lichter A, Manulis S. 1997. The presence of *hrp* genes on the pathogenicity-associated plasmid of the tumorigenic bacterium *Erwinia herbicola* pv. *gypsophilae*. *Mol. Plant-Microbe Interact.* 10:677–82
79. Panijel M, Chalupowicz L, Sessa G, Manulis-Sasson S, Barash I. 2013. Global regulatory networks control the Hrp regulon of the gall-forming bacterium *Pantoea agglomerans* pv. *gypsophilae*. *Mol. Plant-Microbe Interact.* 26:1031–43

80. Savidor A, Teper D, Gartemann K-H, Eichenlaub R, Chalupowicz L, et al. 2012. The *Clavibacter michiganensis* subsp. *michiganensis* – tomato interactome reveals the perception of pathogen by the host and suggests mechanisms of infection. *J. Proteome Res.* 11:736–50
81. Schachnai A, Barash I. 1982. Evaluation of the fungicides CGA 64251, guazatine, sodium o-phenylphenate, and imazalil for control of sour rot on lemon fruits. *Plant Dis.* 66:733–35
82. Shorer J, Zelmanowicz I, Barash I. 1972. Utilization and metabolism of urea during spore germination by *Geotrichum candidum*. *Phytochemistry* 11:595–608
83. Valinsky L, Manulis S, Nizan R, Ezra D, Barash I. 1998. A pathogenicity gene isolated from the pPATH of *Erwinia herbicola* pv. *gypsophilae* determines host specificity. *Mol. Plant-Microbe Interact.* 11:753–62
84. Van Alfen NK. 1982. Biology and potential for disease control of hypovirulence of *Endothia parasitica*. *Annu. Rev. Phytopathol.* 20:349–62
85. Weinthal D, Barash I, Tzfira T, Gaba V, Teper D, et al. 2011. Characterization of nuclear localization signals in the type III effectors HsvG and HsvB of the gall-forming bacterium *Pantoea agglomerans*. *Microbiology* 157:1500–8
86. Weinthal DM, Barash I, Panijel M, Valinsky L, Gaba V, Manulis-Sasson S. 2007. Distribution and replication of the pathogenicity plasmid pPATH in diverse populations of the gall-forming *Pantoea agglomerans*. *Appl. Environ. Microbiol.* 73:7552–61
87. Xiao Y, Hutcheson S. 1994. A single promoter sequence recognized by newly identified alternate sigma factor direct expression of pathogenicity and host range determinants in *Pseudomonas syringae*. *J. Bacteriol.* 176:3089–91