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Pseudomonas *Syringae* Pathovars and Related Pathogens

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ALTERNATIVE STRATEGIES FOR CONTROLLING PLANT DISEASES CAUSED BY *PSEUDOMONAS SYRINGAE*

ABSTRACT

The most common strategy for controlling diseases caused by *Pseudomonas syringae* is, as it has been for more than 5 decades, to spray bactericides. These mainly include a variety of copper compounds or other heavy metals, with or without various combinations of fungicides or other pest-control chemicals. Spraying with antibiotics or other organic bactericides have also been used, but to a lesser extent. Unfortunately, these strategies have never been satisfactory, resulting in heavy crop losses during severe epidemics. Over the last years, pathogenic strains that are resistant to copper spraying have been detected globally and are threatening the continuation of this strategy. Three different strategies are slowly being introduced into the field, (i) seed disinfection by heat or a combination of heat and bactericides, (ii) biological control by antagonistic microorganisms, and (iii) soil solarization especially against pathogens that spend part of their life cycle in the soil. Additional minor strategies include: (i) spraying infested plants with natural-occuring antibacterial compounds, and (ii) various techniques of sustainable agriculture without the use of chemicals (organic farming).

INTRODUCTION

As long as the global agricultural production of fruits and vegetables increases annually and uses current agro-technical procedures, the need for controlling pathovars of *Pseudomonas syringae* will be *a* major agricultural issue since these bacterial pathogens are among the most destructive known. For many bacterial diseases, current management techniques are often ineffective. Phytopathogenic bacteria can be symptomless for prolonged periods (8). Slight changes in the environment which favor the bacteria may cause a rapid outbreak, creating severe epidemics that can destroy the crop. The available means of controlling field epidemics are few, decades-old, rarely effective and too expensive for low value crops. Thus, the major approach is to prevent the development of epidemics, e.g. contain the pathogen when its level is low.

Attempts to control outbreaks and epidemics caused by *Pseudomonas syringae* pathovars have evolved little over the last century. Unfortunately, most of our knowledge on the control of bacterial disease is in the "gray zone" of science, being the domain of pesticide companies and popular agricultural magazines rather than peer-reviewed scientific journals. This makes it difficult to evaluate the field reliability of each strategy. Nevertheless this mini-review will try to sum up the major, minor, current, and future options for the researcher today, and hopefully, for the farmer in the future.

MAJOR CONTROL STRATEGIES

(i) Chemical control

The most common strategy for controlling diseases caused by *Pseudomonas syringae* pathovars is, as it has been for more than a century, to spray bactericides. These mainly include a variety of copper compounds (like the traditional "Bordeaux mixture", cupric hydroxide, copper sulfate, ammoniacal copper and copper salts of fatty acids) (21,46-48,64,67) or other heavy metals, with or without various combinations of fungicides or other pest-control chemicals. These compounds were the first biocides used for disease control and are the only bactericides registered and allowed for use on most crops. They were, and sometimes are, especially effective if applied in the proper manner (good sprayers, precise spraying schedules, good coverage of both sides of the leaf etc.) and with the proper timing (mainly prophylactic) (30,31). These factors are crucial to avoiding a wide-spread epidemic, a disease form which can hardly be controlled. Since the incidence of diseases caused by some *P. syringae* pathovars is correlated with the epiphytic population of this species on plants before infection (28,45), such sprays are more effective if the pathogen has epiphytic survival ability since they can delay the establishment of high epiphytic pathogenic populations required for an epidemic (43,55,66). Spraying with antibiotics such as streptomycin or tetracycline alone, or combined with copper or other organic bactericides (12), is used (15) on a smaller field scale and mainly in greenhouse applications. Antibiotic treatments had limited success in tomatoes (31), but greater success in controlling pear blossom blast (11) and bacterial blight of coffee (32). The major difficulties of using antibiotics for agriculture are that they are difficult to register, or even prohibited (like in Australia) because of human health concerns (66) and the potential for disease resistance as occurred long ago in *Xanthomonas campestris* pv. *vesicatoria* (58).

Unfortunately, and despite of its wide usage, the chemical approach has not been very satisfactory in the last decade. It provides protection only when the environmental conditions for disease development are limiting and results in heavy crop losses during severe epidemics (32). Most spray schedules almost never completely controlled any disease caused by *P. syringae* pathovars. Increasing copper dosages are undesirable in many crops because of phytotoxicity (32,33,51), especially since dosages are already in their upper limits for most crops.

The most crucial factor against this strategy is the development of bactericide resistance by the pathogens (60). Although they probably evolved undetected over the last 30 years , pathogenic strains that are resistant to copper spraying have been detected globally over the last decade (5,17,18,50). Development of bactericides resistance in bacterial communities does not require the independent evolution of resistance by each strain or species, but, rather, the community can evolve cooperatively by exchanging genetic information between strains, pathovars, species and genera (17,18). The way that cells of *P. syringae* protect themselves against copper toxicity is probably by accumulating copper outside of the cells with periplasmatic copper biding proteins (18,19). Currently, copper-tolerant strains are claimed to be poorly controlled in the field by standard applications of copper compounds and therefore, threaten the future of the chemical strategies.

(ii) Seed disinfection by heat or a combination of heat and bactericides

In short season crops such as vegetables, an epidemic in the field might be irreversible since even if the disease is reduced by other means later, it will not give the plant sufficient time to recover and compensate for the lost yield. Thus, seed disinfection might be a good preventive alternative to foliage spraying. Surprisingly, and despite plant protection laws of many countries (like Israel [59]) requiring seed disinfection or at least the sale of pathogen-free seeds, what little has been published on *P.syringae* pathovars in seeds is decades old (6,13,56,62). Furthermore, many seed lots are regularly contaminated by bacterial pathogens despite the regulations, e.g., most of the commercial seed lots in Israel (imported or produced locally) were contaminated with one or more phytopathogenic bacteria (40). Common seed treatments are not always reliable since they appear to eliminate only bacteria that reside on the seed surface, but not bacteria inside the seeds. Even when their survival number is meager, the latter can survive for prolonged periods, are able to multiply under favorable environmental conditions and cause disease outbreaks in the field which may develop into epidemics later in the season (9,28,57). Therefore, an effective seed disinfection method must eradicate these few endophytic bacteria as well. Treatment of seed-borne *P. syringae* pv. *tomato* with streptomycin that eliminates the pathogen (52) can not be considered applicable since such antibiotic treatment is not allowed for use in many countries as explained earlier in this review. Seed disinfection is done usually by heat treatments (thermotherapy). In essence, the seeds are suspended in hot water for various periods of time according to the tolerance of the pathogen and the tolerance of the seed to high temperatures. This approach represents an easy and preventive way of eradicating of bacterial pathogens, yet thermotherapy of highly infected seed lots is usually ineffective, especially with dry heat (27). Furthermore, these treatments are somewhat tricky since the long exposures to the high temperatures required to eradicate the pathogen are very close to what might damage the seed embryo (27). Thus, seed manufacturers tend to reduce the disinfection treatments, thereby reducing their effectiveness.

Alternative methods to toxic chemicals or high temperature disinfection methods have been developed. Aqueous suspensions of non-human toxic chemicals (calcium propionate, tartaric acid) or acidic cupric acetate at only 50^{o}C controlled *P. syringae* pv. *lachrymans.* The heat is probably essential to the procedure since it increases solvent uptake (42). A combination of several chemicals and warm temperatures (25-45^{o}C) which form an organic copper complex in the seeds bathing solution, almost completely eradicated a broad spectrum of tomato bacterial pathogens including *P. syringae* pv. *tomato*. Most of the tomato seeds produced or imported by Israel are now treated using this method (41).

In sum, there is no apparent general method for eradicating bacterial pathogens from seeds. Each pathogen and plant species might need modification of the methods described above. Currently, it appears that a combination of temperature and inhibitory chemicals are most effective under realistic seed manufacturing and plant growth conditions.

(iii) Biological control by antagonistic microorganisms

Biological control by antagonistic microorganisms is a well developed concept, mainly for soil-borne pathogens and almost exclusively against fungal diseases (38). The first commercial products of this approach are already in the market, but only on a small scale (7,61). Foliar biological control (apart from prevention of ice nucleation, [65]) receives minimal attention today, and very few studies relate to pathovars of *P. syringae* (4,53). For example, in the last conference of Plant Growth-promoting Rhizobacteria of 1994 in Australia, most of the papers concerned biocontrol agents, yet none out of over 80 studies presented referred to foliar biocontrol. Nevertheless, since this management approach is gaining considerable momentum against soil-borne pathogens, its potential should be pointed out. Two general approaches may be the key for developing biocontrol foliar agents; (i) searching for natural microorganisms that have antagonistic characteristics to the pathogen, and (ii) increasing the efficiency of a strain through a better formulation or genetic manipulation (39). A few examples of each approach can be found in the literature.

A strain of *P. putida* isolated from pepper fruits was able to inhibit a wide spectrum of pathogens including 5 pathovars of *P. syringae* in *in vitro* studies. However, its field potential was tested only against *Erwinia carotovora* subsp. carotovora on potatoes (44). A nonpathogenic, copper resistant Tn5 mutant of *P. syringae* pv. *tomato* when co-inoculated with a pathogenic strain significantly reduced the incidence of bacterial speck of tomato in the greenhouse. But, when inoculated with a copper sensitive pathogen on plants treated with copper, the control was even better (16). Saprophytic *P. syringae* provided complete protection against gray mold caused by Botrytis cinerea in wounded pear fruits (29).

The potential of foliar biological control of *P. syringae* can be better evaluated from studies on the control of other bacterial pathogens. Crown gall caused by *Agrobacterium tumefaciens* has been successfully controlled on a commercial scale for over 15 years by the use of *A. radiobacter* (producing mainly a specific antibiotic, "agrocin 84", and less by the antibiotic "agrocin 434" against agrobacteria). Unfortunately, Agrocin-84 is coded on a plasmid which can be transferred to pathogenic agrobacteria, a fact which makes the latter resistant to biocontrol by *A. radiobacter.* Thus, a mutant was constructed which no longer can transfer the agrocin plasmid to pathogenic agrobacteria retaining the biocontrol capacity (23,49,54).

At the moment, we have to accept the fact that foliar biocontrol is a long term prospect for the development of a comprehensive biocontrol strategy for *P. syringae*.

(iv) Soil solarization, especially against pathogens that spend part of their life cycle in the soil.

Soil solarization is a non-chemical method for soil disinfection which captures solar irradiation under a clear plastic mulch (26,35). Soil solarization was invented in Israel (36) where the agro-technical and climatic conditions are optimal for such a technology. In principle, soil solarization affects pathogens by heating wet soil to temperatures up to 50^{o}C in the upper layer and to about 40^{o}C at 20 cm deep (depending on the geographical area and soil type). The eradication of pathogens is achieved by the prolonged exposure to these temperatures (a few weeks to a few months (34). Excellent eradication of numerous fungal soil-borne plant pathogens and subsequent yield enhancement was achieved during the years that this method was applied in Israeli fields. Nonetheless, it is not widely used there because it is highly crop-dependent and is inversely correlated with the availability of other soil disinfection methods available to the farmer (26). For unestablished reasons, soil solarization in Israel is considered less reliable than common fumigants and a slightly risky procedure, especially for cash crops. However, soil solarization is the only practical solution for soil disinfection when chemical fumigants (like methyl bromide) are phytotoxic to the next crop like onions or in organic farming where chemicals are banned. Soil solarization is more popular in crops that require no additional expense such as some winter vegetables which already grow in mulched soil. The only difference for the farmer between common commercial growth and solarized growth is the longer period of soil coverage, starting in mid-summer and leaving the mulch in its place until the end of the growing season in the next spring. This method is also accepted for container cultivation where the high organic matter content of the growing substrate prevents efficient disinfection by fumigants. Despite its efficiency in various areas of plant pathology (increasing the yield in 50-100%), soil solarization is currently used to control soil fungal diseases (like *Fusarium*, *Verticillium* , *Rhizoctonia, Sclerotium rolfsii*) and nematodes (24,25,37).

To the best of my knowledge, soil solarization has not been methodically tested as an effective method to control any foliar bacterial pathogen, despite the verbal statements of Israeli Agricultural Extension Service personal who claim that no severe incidence of bacterial epidemics have occurred in solarized soil. However, it is known for example, that *P*. *syringae* pv. *tomato* can survive in the soil and infested soil can inoculate successfully tomato plants (10). This bacterium is very susceptible to medium temperatures (around 50ºC) (20) which soil solarization provides. Thus theoretically, soil solarization should control this pathogen. However, as stated by Greenstein (26) regarding winter cash crops like tomatoes, farmers prefer proven fumigant disinfection of the soil rather than novel methods such as solarization. In his 15 years-old review, Katan (34) pointed out the need to study soil solarization in relation to phytopathogenic bacteria. This need is as important today as it was 15 years ago.

MINOR CONTROL STRATEGIES FOR THE FAR FUTURE

None of the following methods ever passed the experimental stage for the control of bacterial pathogens. They are suggested in this mini-review as theoretical ideas for future evaluation with respect to *P. syringae*.

(i) **Spraying infested plants with natural-occurring antibacterial compounds**

The antibacterial activity of tea and coffee wastes against *P*. *s*. pv, *phaseolicola* and *P. s*. pv. *pisi* was evaluated in laboratory and greenhouse trials. It was suggested that different components in these wastes may control these two pathogens (2).

(ii) Various techniques of sustainable agriculture without the use of chemicals (organic farming).

In organic farming magazines, there are many statements that the incidence of bacterial disease, in general, in organic farming is much lower compared to large scale commercial agriculture. No scientific explanation for this has been provided.

It can be only proposed that these claims should be subjected to rigorous testing under accepted plant pathology procedures.

Several agro-technical practices are reported to reduce the incidence of bacterial diseases in general, especially in semi-arid lands under drip irrigation, (14). It is well known that many pathovars of *P. syringae* require free-water on the leaves for multiplication (28). Thus, methods which keep the leaves dry should be advantageous. However, I found meager information on this aspect in the scientific literature.

Common decontamination procedures like the burning of crop residues together with tillage eliminate many bacterial pathogens, including *P. syringae*. These treatments were even more efficient than spraying with the common copper substances or antibiotics (Donegan et al. 1992).

(iii) Induced systemic resistance

Induced systemic resistance is the phenomenon of activating the defense mechanisms of the plants prior to disease development, either by environmental factors, by microorganisms or by both. Systemic resistance can be induced by pathogens, non-pathogens, seed treatments with Plant Growth-Promoting Rhizobacteria (PGPR) and microbial metabolites (63). Most studies were carried out against fungal pathogens. However, few studies on pathovars of *P. syringae* showed that this approach may have potential in the future control of these diseases. Inoculation with two PGPR strains protected cucumber plants against *P. s*. pv. *lachrymans* (Liu, unpublished cited in 63) and inoculation with *P. fluorescens* induced protection against *P. s.* pv. *phaseolicola* (1,3)

CONCLUSIONS AND FUTURE PROSPECTS

The control of pathovars of *P. syringae* is one of the most neglected research areas of phytobacteriology. Unfortunately, it has attracted little attention despite the heavy crop losses that these pathogens are able to induce. Ironically, the most common strategy, both in use and in registration, is the least efficient of all, chemical spraying. Resistant mutants which are appearing in the fields in alarming numbers will someday render this approach useless, leaving growers with no efficient means of protecting their crops. More advanced methods such as biological control, soil solarization and induced systemic resistance are in their infancy. It is my opinion that it will be difficult or perhaps impossible to control diseases caused by *P. syringae* in the future. Clearly more research effort should be done in this field.

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