Considerations for using bacteriophages for plant disease control

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The use of bacteriophages as an effective phage therapy strategy faces significant challenges for controlling plant diseases in the phyllosphere. A number of factors must be taken into account when considering phage therapy for bacterial plant pathogens. Given that effective mitigation requires high populations of phage be present in close proximity to the pathogen at critical times in the disease cycle, the single biggest impediment that affects the efficacy of bacteriophages is their inability to persist on plant surfaces over time due to environmental factors. Inactivation by UV light is the biggest factor reducing bacteriophage persistence on plant surfaces. Therefore, designing strategies that minimize this effect are critical. For instance, application timing can be altered: instead of morning or afternoon application, phages can be applied late in the day to minimize the adverse effects of UV and extend the time high populations of phage persist on leaf surfaces. Protective formulations have been identified which prolong phage viability on the leaf surface; however, UV inactivation continues to be the major limiting factor in developing more effective bacteriophage treatments for bacterial plant pathogens. Other strategies, which have been developed to potentially increase persistence of phages on leaf surfaces, rely on establishing non-pathogenic or attenuated bacterial strains in the phyllosphere that are sensitive to the phage(s) specific to the target bacterium. We have also learned that selecting the correct phages for disease control is critical. This requires careful monitoring of bacterial strains in the field to minimize development of bacterial strains with resistance to the deployed bacteriophages. We also have data that indicate that selecting the phages based on in vivo assays may also be important when developing use for field application. Although bacteriophages have potential in biological control for plant disease control, there are major obstacles, which must be considered.

Bacterial Diseases in Agriculture

Bacterial pathogens are associated with plant diseases in temperate, sub-tropical and tropical environments and can account for major economic losses to agricultural production. Disease control for many bacterial-incited diseases is challenging.[1] Major challenges associated with control of members of the proteobacteria include: pathogen diversity; the inability to identify durable resistance in the host plant to the target pathogen; the pathogen's ability to reach high populations in a relatively short period of time when conditions are conducive for disease development; and lack of effective chemical control. For most plant diseases, including bacterial incited diseases, an integrated management strategy is essential, combining proper cultural practices, biological control, bactericides or plant activators, where applicable, and plant resistance.[2,3]

Chemical control has been a major component of plant disease management, especially for diseases caused by bacteria. Unfortunately, bacterial plant pathogens have been more recalcitrant to chemical treatments than their fungal counterparts. Those disease management approaches that have relied heavily on chemicals alone have had limited success. Chemical control of bacterial diseases has traditionally consisted of bactericides such as antibiotics and copper-based compounds. For many years, copper has been used as a chemoprotectant more extensively than any other chemical for the control of bacterial plant diseases; however, copper resistance has been identified and characterized in many plant pathogenic bacteria and is primarily associated with plasmids although there is chromosomal associated copper resistance.[4–10] Antibiotics, although used less extensively than copper, have also been used as part of a management strategy for various bacterial diseases. The aminoglycoside antibiotic, streptomycin, has been in use since the 1950s.[11] As a result of overuse, streptomycin-resistant strains became prevalent in a very short period of time (i.e., within several years), which in turn limited its efficacy for managing bacterial spot of tomato and pepper.[11] Streptomycin has also been used for many years for the management of fire blight of apple and pear,[12] and a number of other bacterial plant pathogens.[13] The efficacy of streptomycin for control of fire blight lasted much longer than for bacterial spot of tomato and pepper because the streptomycin resistance was associated with a plasmid which required acquisition by sensitive strains. Recent advances in the development of chemical compounds that stimulate plant defenses have offered another promising approach

for disease control. Often referred to as plant defense activators, these chemical compounds mimic phytohormones that in turn induce systemic acquired resistance (SAR) in the plant. These materials have integrated well into existing management strategies because of their unique mode of action, and have shown success for managing several bacterial diseases, including bacterial speck and spot of tomato and pepper,[2,14,15] *Xanthomonas* leaf blight on onion[16] and fire blight on apple.[17] Due to their effect on plant physiology, some negative effects on yield have been associated with plant activators in certain plant species,[15,16] while with other pathosystems they have been relatively ineffective.[18] Although improved performance has been possible in some cases through the optimization of rates and application intervals,[19] or making applications directly to plant roots rather than a traditional foliar spray.[20] Regardless of their efficacy, plant activators alone have not provided sufficient control of bacterial diseases and clearly require integration with other effective compounds.

Biological Control

Biological control has been a desirable strategy for controlling plant disease in which nonpathogenic microorganisms are applied to foliar or root tissues resulting in disease suppression. Strategies for using biological control for bacterial diseases include using nonpathogenic or pathogenically-attenuated strains of the pathogen species,[21–24] saprophytic bacteria,[25] non-pathogenic bacteriocin-producing *Agrobacterium radiobacter* strains that inhibit closely related pathogenic strains,[26,27] and plant growth-promoting rhizobacteria (PGPR)[25] to suppress pathogen populations or induce SAR or a similar response in the plant that reduces the ability of the pathogen to colonize the plant and cause disease. These biological control approaches achieved varying levels of success, and clearly require additional research to improve their reliability under field conditions.

Early Use of Bacteriophages in Agriculture

Just as soon as they were discovered by Twort[28] and by d'Herelle[29] at the beginning of the 20th century, bacteriophages were envisioned as disease fighting agents of humans and animals.[30,31] Soon afterwards they were found in association with bacterial plant pathogens as well, and proposed as plant disease control agents.[32] The first pioneers were Mallman and Hemstreet, who in 1924 observed that filtrate of the liquid collected from the decomposing cabbage inhibited the growth of the bacterium that caused the rot, *Xanthomonas campestris* pv *campestris*.[33] In 1925 Kotila and Coons[34] demonstrated that bacteriophages isolated from the soil suppressed growth of *Pectobacterium carotovorum* subsp *atrosepticum, the* causal agent of blackleg disease of potato. They performed bioassays and successfully prevented rotting of potato tubers by co-inoculating the phage with the phytobacterium.[34] Additionally, they isolated phages active against *Pectobacterium carotovorum* subsp *carotovorum* and *Agrobacterium tumefaciens* from a number of environmental sources, such as river water and soil.[35]

The first field trials were conducted by Thomas[36] against Stewart's wilt of corn. He treated corn seeds infested with the pathogen, *Pantoea stewartii*, with phages isolated from diseased plant material. This seed treatment was quite effective and resulted in a reduction of disease incidence from 18% (untreated) to 1.4% (phage). Almost half a century later (1969), Civerolo and Keil,[37] used various foliar phage-treatments and reduced bacterial spot (*Xanthomonas pruni)* severity on peach seedlings by 86% to 100%.

Challenges Using Phage Therapy

Even though there were successes at the early stages of bacteriophage application to control bacterial plant diseases, phage-therapy did not live up to its promise, and did not turn into a practical antibacterial strategy for controlling plant pathogenic bacteria due to problems with efficacy and reliability. One leader in the field, Okabe, concluded in 1963 that phages, in general, appeared to be ineffective as a control strategy.[38] Furthermore, their narrow spectrum of activity against specific bacterial species put phages at a disadvantage against other antibacterial materials, such as antibiotics, which had more broad spectrum activity.[39]

Resistance to phages. The probability that bacteria mutate and become resistant to individual phages is a real concern when considering phages for use as biological control agents. This concern arose in early studies by Katznelson[40] and was expressed later in review articles by Okabe and Goto[38] and Vidaver[41] as a major limiting factor for use as a control strategy. In the classical experiment by Luria and Delbrück (1943) the mutation rate of *E. coli* to resistance to phage T1 was determined to be 3.4×10^{-9}.[42]

Criteria for selecting suitable phage. Identifying phages to use as part of a biological control strategy has been considered by researchers with different criteria for selection. In experiments with *Xanthomonas campestris* pv *pruni,* a pathogen associated with a bacterial spot of plum and peach, a lytic phage with the broadest host range was selected from a collection of eight phages for biological control experiments.[43,44] In a study with citrus bacterial canker, Balogh (2006)[45] observed that phages vary in their ability to interact with and multiply on their host bacterium, *X. citri,* on the surface of grapefruit leaves, an ability that correlated with their disease control efficacy against citrus canker. However, he found no in vitro characteristics, such as plaque size, ability to reduce bacterial populations in liquid culture or ability to multiply in liquid culture that predicted disease control ability against bacterial spot on tomato (Balogh, unpublished results). Svircev et al.[46] employed a unique strategy for controlling fire blight of pear, by selecting phages that lysed the target organism, *Erwinia amylovora* and also the phyllosphere bacterium, *Pantoea agglomerans*, that is antagonistic to *E. amylovora*. Clearly, pre-screening phages before application for their potential value as biocontrol agents rather than arbitrarily selecting them based on lytic activity alone is recommended for disease control studies.

Strain variation. Studies of bacterial strains associated with particular plant species have shown that significantly greater genetic diversity may exist than considered previously and this could be a major factor when identifying suitable phages for

biocontrol. For instance, a bacterium associated with bacterial spot of tomato and pepper, *Xanthomonas campestris* pv *vesicatoria,* what had been formerly considered one bacterial species, has now been separated into at least four distinct species.[47] Since phages are generally limited to certain strains within one bacterial species, each of the four species requires the use of different phages for disease control. Furthermore, there can be considerable variation in terms of phage specificity to bacterial strains within a species. Bouzar et al. (1999) used 26 bacteriophages to type approximately 100 *X. euvesicatoria* strains isolated from various countries in the Caribbean including Central America, and identified at least 26 different phage lysis patterns.[48]

Persistence of phages in the phyllosphere and rhizosphere. Maintaining biocontrol agents at high populations in close proximity to the target bacterium is of critical importance, given that biocontrol efficacy is influenced by the densities of the biocontrol agent and the target pathogen.[49] Phage therapy necessitates that high densities of phage exist.[50] Clearly, for good control, phages must exist above a certain threshold titer relative to the target bacterium; below that threshold phages will have a minor effect on bacterial populations and subsequent disease control. In support of this threshold effect hypothesis, Balogh (2002) observed that phage mixtures applied at 10^6 or 10^8 PFU/ml concentration provided similar levels of control of bacterial spot to tomatoes inoculated with 10^8 cfu/ml of *Xantomonas perforans*, but at 10^4 PFU/ml was ineffective.[51] One other factor relating to the phyllosphere that should be considered beyond densities of the phage and target bacterium is physical accessibility of the target bacterium to phage infection. As Gill and Abedon (2003) pointed out, an important component of this model is the possibility of the target pathogen residing in spatial refuges that are inaccessible to the biological control agent or in this case the bacteriophage.[50]

The phyllosphere environment is extremely deleterious to phage leading to sharp declines in the administered bacteriophage population over time.[37,51–54] This short-lived persistence on plant leaf surfaces is the major limiting factor for phage therapy in the phyllosphere. In field and laboratory studies looking at viruses selected for use as biological control agents for insects, the viruses were inactivated by high temperatures, high and low pH and sunlight, and were readily dislodged by rain.[55,56] Of all the environmental factors associated with virus survival, the UV-A and UV-B spectra of sunlight (wavelength range 280–400 nm) were the most destructive to viruses.[55]

Bacteriophages as viruses encounter similar deleterious factors in the phyllosphere, with their populations plummeting to non-detectable levels for effective biological control. These factors include sunlight irradiation, especially in the UV-A and B regions, ambient temperature, desiccation, and exposure to certain chemical pesticides, such as copper-based bactericides that are commonly used for bacterial disease management.[54] Of these various factors, sunlight irradiation was found to be the most deleterious factor affecting phage survival.[54] During the early afternoon hours when UV radiation was highest, phage populations plummeted over a 6 h period on tomato leaf surfaces from approximately 10^9 PFU/g of tissue to non-detectable levels.[54] However, bacteriophages applied in the late afternoon or early evening close to sunset improved the persistence of phage overnight, allowing for the carryover of higher phage populations to interact with bacterial strains on the leaf surface.

In experiments looking at survival of phages in the rhizosphere and translocation into stems, the phages were applied to soil surrounding tomato plants. Translocation of the phages was assessed over a two-week period. Phages were detected in foliar plant tissues at levels as high as 10^6–10^7 PFU/g of plant tissue in the upper leaves and stems 2 d after initial application. Phage populations plummeted below the limit of detection by the 7th day in plants with damaged roots and by the 15th day in plants with undamaged roots. In general phages persisted in the rhizosphere and roots of treated plants, although they did decline between ten and a hundred-fold over a 14-d period.[24]

Approaches for Optimizing Bacteriophages in Plant Systems

Application strategies. Bacteriophage applications must take into account biological and environmental factors and appear to depend on the pathosystem. For foliar pathogens the harshness of the leaf surface environment greatly limits bacteriophage survival.[50,54] Therefore, studies have shown that the timing of bacteriophage applications is essential to extend the persistence of high bacteriophage populations in close proximity to the target bacterium to encourage biological control. Civerolo and Keil[37] achieved a significant reduction of peach bacterial spot only if phage treatment was applied one hour or one day before inoculation with the pathogen. They observed a slight disease reduction when phage was applied one hour after inoculation and no effect if applied one day later. Civerolo[57] conjectured that once bacteria reached the intercellular spaces, they were inaccessible to phage. Schnabel et al.[58] achieved a significant reduction of fire blight on apple blossoms when the phage mixture was applied at the same time as the pathogen, *Erwinia amylovora*. In contrast, disease reduction was not significant when phages were applied a day before inoculation. Bergamin Filho[59] investigated the effect of timing on the efficacy of phage treatment in greenhouse trials with two pathosystems: black rot of cabbage, caused by *Xanthomonas campestris* pv *campestris* and bacterial spot of pepper, caused by *X. campestris* pv *vesicatoria*. They applied phages once, but varied the applications time relative to the time of inoculation: 7 d before inoculation (DBI), 6 DBI, 5 DBI, 4 DBI, 3 DBI, 2 DBI, 1 DBI, the day of inoculation, 1 d after inoculation (DAI), 2 DAI, 3 DAI or 4 DAI. On cabbage significant disease suppression resulted from treatments applied at 3 DBI, 2 DBI, 1 DBI, day of inoculation and 1 DAI, whereas on pepper treatments applied at 3 DBI, 2 DBI, 1 DBI and day of inoculation resulted in significantly lower disease. The greatest disease reduction occurred when phages were applied at the day of inoculation in both pathosytems.

The time of day when phages are applied also may affect efficacy. With sunlight irradiation being the single most detrimental factor reducing phage persistence in the tomato canopy,[54] application of phages when they are not exposed immediately to direct sunlight (before dawn or after sunset) prolonged their

Figure 1. Effect of *Ralstonia solacacearum* specific phage following inoculation with *R. solacacearum* on bacterial wilt. Plant on left was inoculated with bacterium only while plant on right was inoculated with bacterium and then bacteriophage.

residual activity.[54] Balogh et al.[53] achieved more effective control of tomato bacterial spot when applying bacteriophages in the early evening, immediately preceding sunset, in comparison with morning applications.[53] There was minimal reduction in viable phage on tomato leaf surfaces during the evening and thus the concentrations remained high on the leaf surfaces. We speculated that this allowed a longer period of time for phage to interact with the bacterial host on the leaf surface.

Overall, given that it is impossible to time bacteriophage applications with infection events under field conditions, and that phage persistence on leaf surfaces beyond a 24 h period is limited, frequent applications of high phage levels in the evening hours have been used to maximize exposure of the target bacterium to the phage populations necessary to maximize disease control. Another factor that one might consider when choosing when to apply phages, (and what application volume to use), is the availability of free moisture on the leaves. Phages will only interact with their target bacterium if the leaf surface is wet, and consequently it seems advisable to apply them when free moisture is expected to stay on the leaves for an extended time period in order to ensure longer exposure time with the target bacterium.

Bacteriophages as discussed earlier were shown to persist and be translocated in tomato plants. It was speculated that if phage can be translocated systemically in the plant then they could possibly be used therapeutically following infection by a bacterial pathogen.[24] Several experiments were conducted using phage specific to *Ralstonia solanacearum,* in which the phage suspensions were applied to soil surrounding tomato plants pre- and post-inoculation with the bacterial wilt pathogen.[24] Disease

control experiments for bacterial wilt showed that maximum disease control occurred if the phage were applied 3 d before inoculation and at the time of inoculation (**Fig. 1**), but was ineffective if applied 3 d after inoculation. Bacteriophages were unsuccessful as a therapeutant for control of the bacterial wilt pathogen.[24]

Strategies for increasing persistence in the phyllosphere and rhizosphere. Although, application timing has been shown to be an important factor in improving efficacy of bacteriophage, persistence on leaf surfaces is drastically reduced over a 24 h period. In order to reduce detrimental effects of environmental factors, formulations (discussed later) have been identified which improve bacteriophage persistence on leaf surfaces.[53,54] However, over a 24 h period phage levels still plummeted below detectable levels on leaves that were free of the target bacterium. Therefore, long-term survival of phage on leaf surfaces is a major challenge that requires different strategies for maintaining high phage concentrations.

A second approach for improving persistence is based on the unique advantage of bacteriophages over chemical pesticides in the ability to increase their populations by multiplying on the target bacterial host. This ability could potentially be used if phages are applied into an environment where a phage-sensitive bacterium is present, or where the phages and bacterial host are delivered together. On leaf surfaces, where high host populations persist, phages persist at significantly higher levels than on surfaces without the host.[45]

This approach was investigated for the soilborne bacterium, *Ralstonia solanacearum* in which Tanaka et al.[60] used an avirulent strain of *Ralstonia solanacearum* and its phage that was active against both the virulent and avirulent strains to reduce tobacco bacterial wilt incidence. Although application of the avirulent strain alone caused significant disease control, co-application of phage with the avirulent strain significantly improved disease control beyond the avirulent strain alone. Svircev et al.[46] employed a similar strategy for controlling fire blight of pear, by selecting phages based on the ability to lyse both the target organism, the pathogen *Erwinia amylovora* and also an antagonistic phyllosphere bacterium, *Pantoea agglomerans*. *P. agglomerans*, a biological control agent for *E. amylovora*, served as a phage carrier and as a propagator of phage on the inoculation sites. While *P. agglomerans* significantly reduced disease, combining it with phage resulted in significantly better disease control; the combination resulted in disease control comparable to streptomycin treatment. A similar approach is to develop an attenuated strain of the bacterial pathogen that would occupy the same niche as the pathogen, but would cause minimal disease and still serve as a host for the bacteriophage. This approach has been successful for improving bacteriophage persistence in greenhouse studies, extending phage persistence by at least a week.[45] In field studies, the addition of an attenuated strain of *X. perforans* with a disrupted *OpgH* gene dramatically improved phage persistence on the tomato leaves[24] (**Fig. 1**).

Resistance to phages. Jackson (1989)[61] developed a strategy to minimize the occurrence of phage-resistant mutants. By preparing mixtures of wild-type phages and including host range mutant phages (h-mutants), bacterial strains resistant to the parent phage

are lysed.[62] In field trials, tomato bacterial spot control with the mixture of four phages including wild-type and h-mutant phages when applied twice weekly to plants provided significantly better disease control and produced greater yield of extra large tomato fruits than the standard copper-mancozeb treatment.[63] Bacterial strains within a species may vary in their sensitivity to bacteriophage. As previously mentioned, considerable diversity in a collection of *X. euvesicatoria* strains in the Caribbean was observed;[48] therefore, phage selection for field use requires careful monitoring of bacterial strains in the field for their natural resistance to deployed phages. Ideally, deployed phages should be a mixture of phages known to lyse the bacterial strain(s) present in the field. Monitoring for resistance of bacterial strains can be done periodically by isolating bacteria present in the field and testing for sensitivity to phages that were used in the field as described by Balogh et al.[53] Lack of phage plaques in the plates will indicate development or presence of resistant bacterial strains and a need for deployment of phages virulent on those strains.

Future Considerations for Improving Phage Efficacy

As stated earlier, biological control using bacteriophages is dependent on the ability of the biological control agent to persist at high levels in close proximity to the target bacterium.[49] Furthermore, the phages must reach and attach to their hosts before environmental factors reduce phage populations below levels effective for biological control.[64] Several considerations exist with regard to improving phage-bacterium interactions: population density and accessibility of the target bacterium; timing of phage application to optimize efficacy; the ability of the phage to infect and replicate in the target environment; phage density at the site of interaction (i.e., phyllosphere or rhizosphere); rates of virion degradation (phage vary in degradative properties); and the presence of adequate moisture to promote phage diffusion.[50] Below, we will address key issues, which must be considered for improving phage efficacy.

Phage selection. The proper assay for phage selection is a critical and often overlooked factor in ensuring success of phage therapy in agriculture. Although in vitro assays are frequently used for selecting phages, these may not be adequate predictors of biological control ability. These assays provide optimal conditions for phage infections—such as exponentially growing susceptible bacterial culture, controlled constant temperature, constant level of free moisture, more or less constant pH, the availability of a wide range of nutrients, and protection from sunlight exposure—and as such, does not represent the "reality of life" in the plant environment, where nutrients and water are scarce and the environmental conditions are in a constant limbo. Balogh[45] found that two of three phages, which were active on *Xanthomonas citri* pv *citri* in plate assays, were unable to lyse the bacterium on the leaves of grapefruit. These two phages, not surprisingly, were not able to suppress citrus canker in greenhouse trials. Balogh evaluated eight bacteriophages that were active against *Xanthomonas perforans* for a number of in vitro characteristics, such as plaque size, antibacterial activity or phage multiplication rate, and found no correlations between these attributes

and actual disease control efficacy (unpublished results). Based on these results, actual plant bioassays are unavoidable in order to gauge biocontrol activity.

Persistence of phages on leaf surfaces. Persistence on leaf surfaces is a major limiting factor in using phage therapy for disease control in the phyllosphere. Several strategies have been evaluated for increasing phage persistence, including the use of protective formulations, application scheduling for sunlight avoidance and co-application of bacterial hosts for in vivo phage propagation. In several studies solar protectants were identified that increased biocontrol efficacy not only for bacteriophages, but also for entomopathogenic viruses and proteinacous biopesticides.[53,65–67] Balogh[51] identified compounds that, when mixed with phage, extended the persistence of phage on the phyllosphere. Balogh et al.[53] enhanced the efficacy of phage treatment with protective formulations that increased phage persistence on tomato foliage. Balogh et al.[53] determined that phage populations persisted at significantly higher concentrations on leaf surfaces when the phage suspensions were applied in combination with skim milk alone or in combination with sucrose. Iriarte et al.[54] corroborated that the combination of phages with skim milk was important for improved persistence on leaf surfaces, even under intense UV irradiation, although phage populations dropped to extremely low levels even with the formulated phage. In other field studies treatment of plants with formulated phages resulted in reduced disease and increased yield.[2,3] Although Balogh et al.[53] and Iriarte et al.[54] demonstrated that the addition of skim milk to phage suspensions improved the ability of phages to persist on leaf surfaces, there is considerable need for identifying formulations that are superior to skim milk.

Phages as Part of Integrated Management Strategy

In contrast to the long list of antifungal products commercially available for the control of fungal and fungal-like pathogens, the search for bactericides suitable for crop protection have resulted in only a few chemicals with limited efficacy. Therefore, continuous efforts have been made to identify complimentary strategies that could be used to enhance the control of plant pathogenic bacteria. The use of pathogen-free seed or planting material, the deployment of genetic host resistance, and the adoption of appropriate cultural and sanitation practices are widely accepted practices that improve disease control. In practice, none of these practices may provide efficient control on their own, but when integrated together provide the basis of an integrated pest management program that should be more effective, reliable and sustainable.

Several approaches have been explored to integrate phage therapy with other disease control strategies. Tanaka et al.[60] reduced tobacco bacterial wilt by co-application of an avirulent strain of the pathogen, *R. solanacearum*, with a phage that was active against both virulent and avirulent strains. Using a similar approach, Svircev et al.[46] reduced fire blight of pear with co-application of the antagonistic epiphyte, *Pantoea agglomerans* and a phage. The selected phage lysed both the antagonist and the pathogen, *Erwinia amylovora; P. agglomerans* also had biological activity against the pathogen on pear blossoms.

Lang et al.[68] evaluated phage treatment in combination with ASM or with copper-mancozeb for the control of *Xanthomonas* leaf blight of onion and found that both combinations resulted in enhanced disease control. To the contrary, Balogh[69] observed no improvement in the control of citrus canker or citrus bacterial spot with the combination of bacteriophages with copper-mancozeb.

However, several experiments demonstrated the benefit of using phages as a component of an integrated strategy against bacterial spot on tomato. Obradovic et al.[2,3] compared efficacy of various combinations of unformulated phages, biocontrol agents, including strains of PGPR, bacterial antagonists, SAR inducers (harpin, ASM) and copper hydroxide in greenhouse experiments. The intention was to integrate some of these practices, optimizing their benefits in control of tomato bacterial spot in the greenhouse, aiming at developing a comprehensive phage-based integrated management strategy for disease control in commercial tomato fields. Several combinations of treatments that effectively controlled tomato bacterial spot in the greenhouse were tested in field trials performed in north and central Florida during three consecutive seasons. Although copper-sensitive strains were used, the application of formulated phages twice a week was either more effective or equally as effective as the standard copper-mancozeb treatment. Phage-treated plants produced significantly more marketable fruit than plants not receiving phage. However, integration of phage treatments and ASM provided an additional reduction in disease pressure and resulted in more efficient foliar disease control than ASM, phage, or copper-mancozeb alone.[2]

Conclusions

Phages have the potential to control plant pathogens in the phyllosphere. Success requires that high populations of phage exist at critical times in order to ensure interaction with the target bacterium. Given that the single most important physical factor that limits bacteriophage persistence in natural environments is UV, designing strategies for reducing exposure of bacteriophages to UV is critical to the use of phage as biological control agents. The timing of application can be altered to maintain high concentrations of the phage on the leaf surface. Protective formulations have been used to prolong phage activity in the field; however, more work is needed in this area to identify compounds that can extend persistence on leaf surfaces compared with skim milk. Another strategy for maintaining high populations of phages has been to use non-pathogenic or attenuated bacterial strains that are sensitive to the phage(s) or a closely related organism that does not cause disease on the plant host. Selecting the correct phages for disease control is critical and ways to better screen phage to predict in planta activity is needed. This requires careful monitoring of bacterial strains in the field to minimize development of bacterial strains with resistance to the deployed bacteriophages or the proliferation of wild-type strains that are not sensitive to the bacteriophages that are used for field application. Bacteriophages have shown the potential to confer effective disease control as part of an integrated management strategy.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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