The Effects of *Colpoda inflate* Predation on *Pseudomonas syringae* in Bean Phyllosphere

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Abstract *Pseudomonas syringae* has caused brown spot disease and frost damage to a variety of commercial crops, such as snap bean. Previous studies have indicated that abundant populations of *P. syringae* cause plants to be more susceptible to pathogenic harm. This exploratory study considered how protist predation may increase the fitness of bean leaves and thus the whole plant. The chosen predator species, *Colpoda inflata*, a common soil ciliate, is known to be capable of surviving on the phyllosphere. Bean leaves were inoculated with *P. syringae* and with or without *C. inflata*. The effects of predation were measured by enumerating bacteria and protist populations. *P. syringae* populations were counted with spiral plating. Results are showed that *P. syringae* populations are maintained at steady levels in the presence of protist predation. However, visual health and brown spot counts indicate protist predation does not eliminate brown spot disease from bean plants. While protozoa presence may not reduce disease risk, this study suggests that microbial communities containing protist and bacteria can coexist in the phyllopshere. Further studies are needed to understand the mechanisms of epiphytic microbial communities.

Introduction

The phyllosphere, or the leaf environment of plants, sustains microbial communities, some of which may be beneficial or detrimental to a plant's overall fitness (Ruinen 1961, Hirano and Upper 2000). While many microbes may inhabit the phyllosphere, *Pseudomonas syringae,* a well studied bacteria plant pathogen, is known to contribute to brown spot disease and frost damage of leaves (Gross and Devay 1977, Lindow *et al.* 1978, Lindemann *et al.* 1984, Hirano and Upper 2000). Studies have suggested large epiphytic populations of *P. syringae* species plant diseases (Lindemann *et al.* 1984, Rouse *et al.* 1985). If these large bacterial populations can be reduced, the severity of brown spot disease may also be alleviated.

Protozoa, a natural predator of bacteria, have been found to survive the daily flux of temperatures on leaf surfaces (Bamforth 1971, Tanner 1975). The protozoan predation of bacteria has been found to reduce bacterial numbers in freshwater environments. The study carried out by Drake and Tsuchiya (1978) suggested that *Colpoda steinii* helped decrease populations of *Escherichia coli.* Cox *et al.* (1999) demonstrated the potential of using protozoan predation as a means to reduce biomass accumulations on filters. Like filters, organisms generally adhere to leaf environments, unless the environment is disturbed. A protist likely to be found on leaf surfaces is *Colpoda inflata,* a soil ciliate (Hausmann and Hülsmann 1996). Though these studies opened further discussion on bacteria-protozoa interactions, bacteria and protozoa inhabiting on a leaf and their potential roles in plant pathology has not been documented before.

This experimental study scratches the surface of protozoan and bacteria interactions in the phyllosphere by studying the effects of a soil ciliate, *Colpoda inflata* on epiphytic populations of *P. syringae* on healthy bean leaves. The hypothesis that *Colpoda inflata* would significantly reduce populations of *P. syringae* was investigated.

Methods

C. inflata was grown in cerophyll medium (Sonneborn's *Paramecium*), prepared similar to the one used in Pratt *et al.*'s study (1997). Cerophyll leaves were boiled in 1 L of deionized, distilled water for 5 minutes and filtered through #1 Whatman filter paper. *Enterobacter aerogenes* ATTC 13048 was isolated from the initial protozoan culture and maintained on

Luria's agar plates to use as feed bacteria in subcultures. Subcultures were carried out ~7-10 days for about 2 months. In preparation for inoculating on bean leaves, about 100 mL of *C. inflata* was centrifuged for approximately 2 minutes at 2000 Gs. The supernatant was removed and about 80 mL of fresh grass medium was added for every 20 mL of protozoan pellet. Of the five or eight treatment samples, 20 ul of three leaf samples were scanned three times each with a light microscope at 16X for *C. inflata* presence.

P. syringae B728a was grown on King's medium B with rifampincine, benlate, and natamycin. The bacteria was suspended in KPO_4 buffer, whose concentration was determined with Spec 20. Appropriate amount of grass medium was added to give a concentration of 10^6 cells/mL.

Bean plants housed in a mist chamber were sprayed with either only *P. syringae* or *P. syringae* and *C. inflata*. Individual leaves were carefully placed in test tubes with deionized, sterile water and placed in a sonicater (Branson 8200) for 7 minutes. *P. syringae* counts were determined by spiral plating on the same King's medium B plates as above. For each of the two variation of treatment, 5 leaf samples were taken for Day 0 and 8 leaf samples subsequently for Day 1, 2 and 3.

Results

Day 1 and 3 were found to be significant by two-tailed t test ($p<0.05$). Day 0 was not found significant as expected. Populations were not significantly different on Day 2.

Brown spot counts were taken five days after Day 3 to determine the health of plants. Counts were not significantly different, also using two-tailed t test ($p<0.05$).

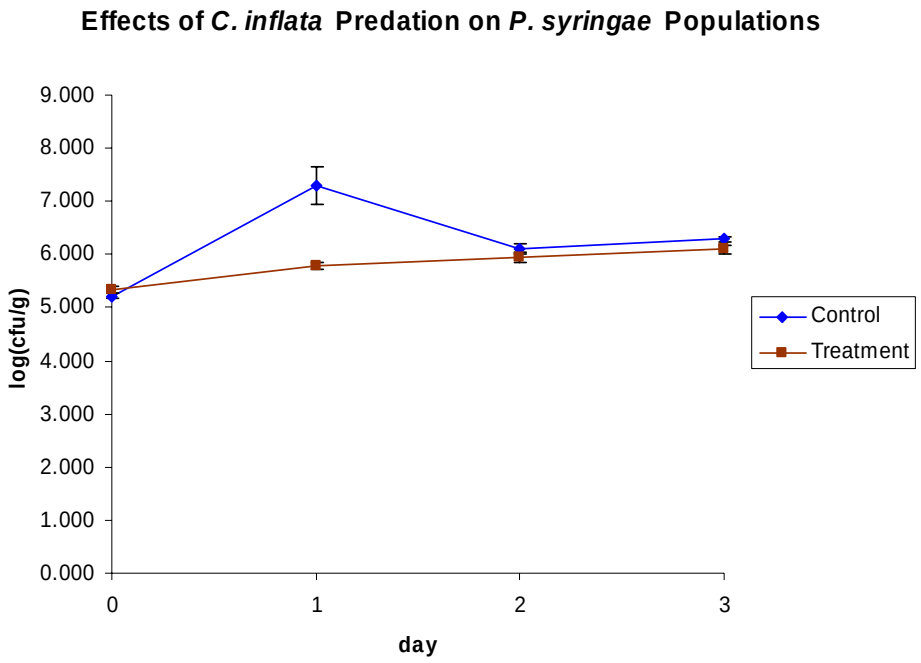

Figure 1: Control refers to plants sprayed only with *P. syringae.* Bean plants subjected to treatment were sprayed with both *P. syringae* and *C. inflata*.

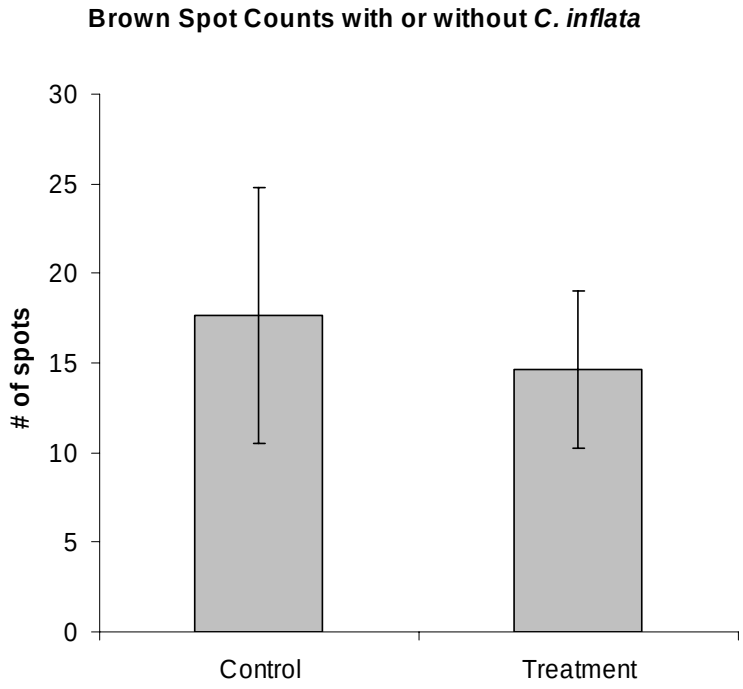

Figure 2: Control refers to plants sprayed only with *P. syringae.* Bean plants subjected

to treatment were sprayed with both *P. syringae* and *C. inflata*.

Discussion

Since samples of Day 0 were taken immediately after inoculation, the difference in populations should be explained by chance, as the results show (Figure 1). The inoculums of *P. syringae* with and without *C. inflata* were made on Day 0 so the protozoa did not have time to change bacterial numbers. By Day 1 on the control plants, it appeared *P. syringae* populations exploded without predation keeping them in check. Most plates of Day 1 were undistinguished because of high growth so populations were estimated to be 10^8 cfu/g. The 100-fold increase from Day 0 to Day 1 in the control samples is at best a general estimate. The subsequent 10-fold decrease suggested that the carrying capacity of the leaves decreased, so many bacteria could not survive. Nutrient levels can dictate the growth of bacteria populations rather than predation alone (Drake and Tsuchiya 1978).

The predator C. inflata may not have been active the entire length of the experiment. Day 3 Because protozoa require water to excyst and survive, the fact that the humidifier did not mist the mist chamber may have influenced predator behavior. While leaf samples were taken around the same hour of each day, leaf surfaces of Day 2 and 3 were noticeably dry and dew drops were not as prominent as of Day 0 and Day 1. Encystment of C. inflata occurs when environmental conditions are not suitable (Maguire 1963, Hausmann and Hülsmann 1996). Scanned solution samples of leaves indicate the presence of cysts in all days (Table 1).

Although the presence of predation may have surpassed *P. syringae* from growing out of control, the general trend of the treated leaves was increasing. The addition of *C. inflata* did not eliminate susceptibility to brown spot disease, as shown in figure 2. The possibility of treating brown spotted plants with protozoa may work more effectively when coupled with other brown spot disease controls, such as introducing mutants of the pathogen (Lindow *et. al* 1987). However, these treatments are not commercially available in the present time.

The subculture may have been contaminated as the cerophyll medium was kept in a single container, autoclaved once rather than divided into smaller bottles, like the method used in Pratt *et al.* study (1997). Since protozoa prey on yeasts as well as bacteria, the possible introduction of yeast may not have been significant (Cochran-Stafiira and Ende 1998). Fungi and mold

introduction may also have been possible. Since the King's medium B plates selected for *P. syringae,* fungi and molds were not detected in the leaf samples.

Since this study only investigated two inhabitants of phyllosphere, it reveals only part of the mechanics of epiphytic microbial communities. Cochran-Stafiira and Ende (1998) suggested that microbial communities may be as complex as communities in higher trophic levels. Future studies may give more insight to this growing field and attempt to replicate epiphytic microbial communities.

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Appendix

Time	Average # of Cysts
Day 0	62.7
Day 1	0
	2
	1
Day 2	0.3
	0
	0
Day 3	1.7
	0
	0

Table 1: Each cyst average is the mean of 3 separate scans of each sample.