Bacterial Growth in Milpa Polyculture and Monoculture Soils

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INTRODUCTION

- Polycultures, or multicrops, are groupings of plants that grow more prolifically when planted together as compared to when planted alone.
  - One of the best known and widely utilized polycultures is the “three sisters” of *milpa* agriculture - the cultivation of maize, beans, and squash together.
- Though the physiological basis for this crop system has been explored[1], the role of soil microbes in this synergy is still unclear.
- In this study, bacterial strains extracted from the squash bug (*Anasa tristis*), the endosymbiont *Burkholderia* SQ4A and the plant pathogen *Serratia* ZO1, were exposed to soil utilized for milpa cultivation and were incubated over a 1-week period to determine whether the multicropping system influences soil bacterial growth.
- Since polycultures have been shown to decrease susceptibility to pests compared to monocultures[2], we hypothesize that cultivating maize, beans, and squash together as a polyculture will reduce growth in both bacterial strains.
- Highlighting this ecological link is not only important for economic security in the agricultural industry, but also for providing further insight into the microbe-environment relationship that includes us.

ESSENTIAL QUESTIONS

- Why milpa?
  - Milpa is a prevalent agricultural system in Central America; it has been utilized for centuries, and many rely on its abundant harvests for survival.
- Why *Burkholderia* SQ4A?
  - This strain is an endosymbiont of the squash bug, a pest of the squash plant. It has been shown to decrease both the mortality and maturation time of its host while also increasing its resistance to insecticides[3,4].
- Why *Serratia* ZO1?
  - This strain is known to be the primary causative agent of cucurbit yellow vine disease (CYVD) in squash that has afflicted southern states[5]. It uses the squash bug as a vector.

BACKGROUND

M=maize [*Zea mays* subsp. *mays*] B=beans [*Phaseolus vulgaris*] S=squash [*Cucurbita pepo*]

Fig. 1 Inoculated soil with 0.9% saline solution; an aqueous layer (supernatant) is formed containing the bacteria.

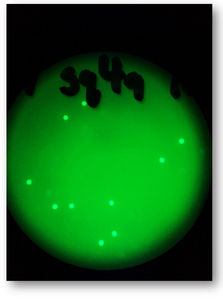


Fig. 2 The endosymbiont *Burkholderia* SQ4A after immediate inoculation in soil (initial period).

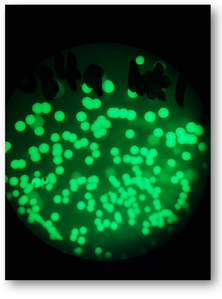
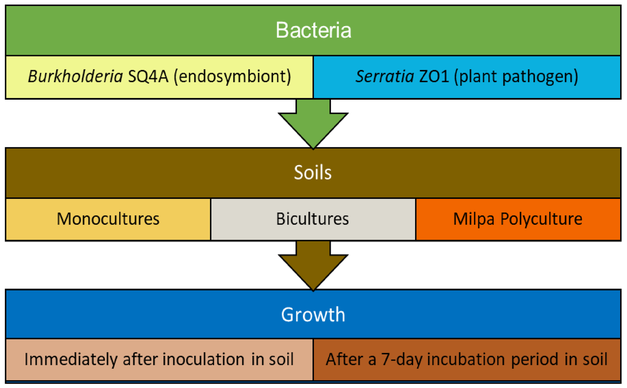


Fig. 3 The endosymbiont *Burkholderia* SQ4A after 1 week in soil; bright fluorescent colonies are SQ4A while dim ones are competing species.

METHODS



RESULTS

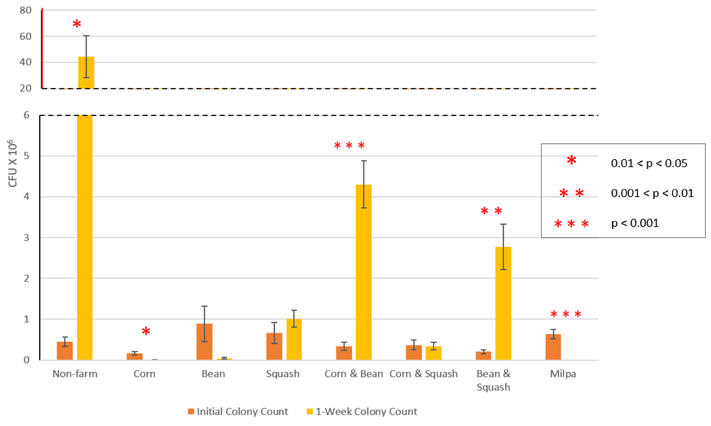


Fig. 4 Growth of *Burkholderia* SQ4A, the endosymbiont, after immediate soil inoculation and after one week in soil. Each bar represents the average number of colony forming units (CFUs) present per period from aliquots of three dilution factors (10^4, 10^5, 10^6). Error bars denote standard error. Note the break in scale.

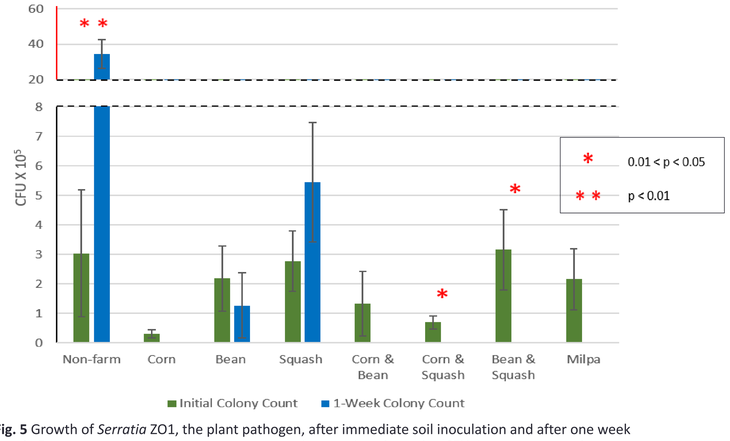


Fig. 5 Growth of *Serratia* ZO1, the plant pathogen, after immediate soil inoculation and after one week in soil. Each bar represents the average number of colony forming units (CFUs) present per period from aliquots of three dilution factors (10^4, 10^5, 10^6). Error bars denote standard error. Note the break in scale.

DATA ANALYSIS

- A paired samples *t*-test was conducted for each soil per bacterial strain to discern significant differences between the means of CFUs in the initial growth period and the 7-day incubation period.
  - With the endosymbiont (SQ4A), a significant difference was reported among periods in the following soils:
    - Non-farm (p=0.0251)
    - Corn monoculture (p=0.0104)
    - Corn & bean biculture (p=0.0001)
    - Bean & squash biculture (p=0.0016)
    - Milpa polyculture (p=0.0004)
  - With the plant pathogen (ZO1), a significant difference was reported among periods in the following soils:
    - Non-farm (p=0.0049)
    - Corn & squash biculture (p=0.0136)
    - Bean & squash biculture (p=0.0488)
- With both strains, the most significant increase was reported in the non-farm soil.
- With the endosymbiont (SQ4A), two bicultures reported the most significant increases among farm soils.
- With the plant pathogen (ZO1), the corn monoculture, all bicultures, and the milpa polyculture did not exhibit any bacterial growth after one week.
  - Only non-farm as well as bean and squash monocultures remained moist over one week; this may have affected microbial growth.

CONCLUSIONS

- Analyzing the growth of both strains, the abundance of organic matter and overall nutrient richness of the non-farm soil may have contributed to the dramatic increases reported.
- The cultivation of milpa in its polyculture configuration demonstrates antibiotic activity towards the *Burkholderia* strain SQ4A since no growth was reported after one week in soil.
  - Our hypothesis was partially supported. If growth of the endosymbiont is limited, decreased pest activity and maintenance of crop health are likely to occur.

AUTHOR CONTRIBUTIONS

- Kino Maravillas
  - Performed bulk of experiments and data analysis, from liquid culture incubation to quantification.
- Erika Diaz-Almeyda
  - Formalized study and protocols, revived bacteria strains, assisted in inoculation and plating.
- Nicole Gerardo
  - Provided materials, facilities, support, and supervision.

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