Assessing the Potential of Groundhogs (*Marmota monax*) as Vectors for Black Rot (*Xanthomonas campestris pv. campestris*) at The Farm at Sunnyside in Washington, VA

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The farm biologist, Sam Quinn, at The Farm at Sunnyside in Washington, VA expressed to the Wildlife Ecology and Conservation students at the Smithsonian-Mason School of Conservation that he believed groundhogs were spreading a fatal disease, known as black rot, to among the *Brassica* crops on the farm. This monitoring study was developed to investigate if groundhogs were in fact spreading the disease. The study was conducted weekly for the duration of October 2016. Camera traps were used cooperatively with health surveys to determine if groundhogs were moving from nearby preserved wildlife habitat, into the *Brassica* crop field – crops known to be favored by this mammalian species. The camera traps were meant to confirm the presence of the groundhogs, as well as suggest the direction of their movement either towards or away from the crop field. The health surveys assessed disease and herbivory severity based on an 11-point scale.

The results of this study illustrated minimal signs of herbivory by groundhogs, aside from a localized feeding on a section of the *Brassica* variety kohlrabi. However, the entire crop field was impacted by the disease black rot. The most severe occurrence of this disease was observed in the rows containing the *Brassica* variety gai lan. These results suggest that some crops may be more susceptible to black rot, as only one *Brassica* variety of eight were severely impacted for the duration of the study. As a result of our findings, future investigative routes should be as followed: (1) Understand the role of groundhogs on farms in the occurrence and spread of black rot among *Brassica* crops, (2) Identify possible factors contributing to black rot preference of specific *Brassica* crops, (3) Understand the ecology of black rot on The Farm at Sunnyside, (4) Understand the Ecology of black rot across multiple farms, and (5) Educate farms on the resources available to prevent black rot occurrence and spread on their farms.

Introduction

Between 2011 and 2012, the demand for organic food in the United States nearly tripled, and has continued to rise since then due to an increased societal awareness of the environmental impacts and toxins associated with conventional farming methods (Greene, 2013). This demand promotes the development of new farms that exceed the expectations of traditional methods, incentivizing farm managers to explore innovative practices and techniques. Managers of successful organic farms acknowledge that crop production has complex implications on the surrounding ecosystems, and therefore concurrently work to limit environmental impacts and promote natural systems (Klonsky & Tourte, 1998). These farm managers often combine environmental and wildlife conservation with their ongoing organic production in an effort to maintain and balance sustainable practices.

The Farm at Sunnyside in Washington, Virginia, subsequently referred to as Sunnyside, is an example of an organic farm that attempts to create this balance to yield products for farmers markets, wholesale establishments, and their own Community Supported Agriculture program while enhancing wild biodiversity (Quinn & Lapham, 2014). This farm aims to work with the natural landscape in order to achieve continuous food production without depleting the land. Sunnyside has gone a step beyond seeking sustainable agricultural practices by signing a conservation easement (“The Farm,” 2016). This document ensures that the farmland will never be developed in order to protect the important conservation values, such as the ecosystem services provided by wetlands and the habitat provided by the surrounding forests.

Only approximately 40 acres of this 422-acre farm are actually used for organic fruit and vegetable production, while the remainder is managed to promote biodiversity and regulate important ecosystem services (Quinn & Lapham, 2011). Although Sunnyside has benefited from promoting native ecosystems throughout the farm, the managers still experience problems with native pests much like traditional farms. Due to the ample amounts of wildlife habitat available for use, it is possible that Sunnyside may even be experiencing higher rates of infiltration by native pests, particularly groundhogs (*Marmota monax*). This shortcoming has become apparent on the farm in the form of elevated herbivory, likely in association with known groundhog inhabitants. Additionally, the spread of the disease black rot (*Xanthomonas campestris* pv. *Campestris* or Xcc) has increased among *Brassica* crops possibly in correlation with groundhog movement.

Groundhogs, also known as whistle-pigs, marmots, and woodchucks, are classified as rodents and occupy the largest range of any other rodent in North America, from Eastern Alaska down to southern Georgia and parts of the Great Plains (Kwiecinski, 1998). The difficulties associated with these animals vary between farms, but largely pertain to agricultural yield of crops and financial loss due to crop destruction. These problems result from dense populations, large appetite and size of individuals, and extensive burrow systems of groundhogs on farm land (Grizzell, 1995). Burrows span anywhere from twenty to thirty feet wide, two to five feet deep, and normally have at least two entrances. The extensive tunnels in burrows can lead to unstable ground in certain areas, causing damage to livestock and creating difficulties in using farm equipment within affected fields (Weeks, 2011). Complications created by groundhogs often negatively affect farms by increasing financial loss, therefore influencing the classification of the species as a pest.

As a primarily herbivorous species, the diet of this animal is largely comprised of grass and forb species, but also includes a multitude of *Brassica* crops such as kohlrabi, kale, cabbage, broccoli, and many other leafy greens (Weeks, 2011). Groundhogs primarily eat the vegetation within a few hundred yards of their burrow creating large problems on working farms (Kwiecinski, 1998). Damage from groundhog herbivory is recognized by angled cuts on the stems of crops, due to their sharp incisor teeth, and often result in large open spaces within fields as a result of thinned leaf cover. This problem could be exacerbated at Sunnyside due to the close proximity of wildlife habitat to farm plots.

Another problem the farm managers at Sunnyside experience is a recurring infectious disease that appears in *Brassica* plots during warm, wet periods (for them, this is generally in October). The pathogen responsible for this disease, colloquially referred to as *Brassica* black rot, is a species of bacteria first described on cabbage in 1889 in Lexington, Kentucky, but it has since been found virtually worldwide (Garman, 1890). This species of *Xanthomonas* can also infect other economically or ethnobotanically important members of the *Brassica* genus, such as broccoli, cauliflower, kale, radishes, and Brussels sprouts.

Research on black rot is needed in order to mitigate the loss of *Brassica* vegetable production caused by its presence in agricultural areas worldwide. In the U.S., four species of *Brassica* (broccoli, cabbage, cauliflower, and collards) account for nearly ten percent of all vegetables produced by weight (“USDA Food Availability”, 2014). It is estimated that in 1976 black rot caused the loss of 1 million dollars worth of crops (Kennedy and Alcorn, 1980), more than 4.2 million by today’s standards, and its impact has only continued to increase.

The symptoms of black rot vary depending on the infected species and the surrounding environmental conditions, but are largely recognized by the presence of V-shaped, yellow or brown lesions originating from the edges of the leaves and, usually, terminating on a clogged leaf vein. It is this clogging of vascular tissue that causes the characteristic necrosis of the leaves, as well as what gives black rot its common name; the black substance causing it is a polysaccharide produced by the Xcc bacteria (Xanthan, a substance commonly used as a thickening agent in commercial food products) (Celetti and Callow, 2002).

As a result of ample groundhog habitat, Sunnyside has suffered crop damage and loss in their primary *Brassica* field. Furthermore, this plot of crops has suffered yearly outbreaks of black rot disease. Farm managers are unsure of the origin of the disease on the farm, but have reason to believe the groundhogs act as vectors when moving between various plants while feeding. Managers suspect the disease attaches to the fur on the groundhogs, where it is then deposited on new, healthy plants. According to the farm managers, historically, the outbreaks of black rot have occurred in mid-October. This study will investigate if there is a correlation between groundhog activity patterns and the occurrence of black rot, in the primary *Brassica* field at The Farm at Sunnyside for the duration of October 2016. The results of this study could be used by farm managers to make more informed decisions in regards to the management and prevention of black rot.

Methods

An initial plot assessment was conducted on Wednesday, October 5th, 2016 following a meeting with Sam Quinn, the farm biologist employed by The Farm at Sunnyside in Washington, Virginia. The decision was made to monitor the *Brassica* plot considered moderately impacted by black rot, surrounded on two sides by mixed crop fields, one side by preserved wildlife habitat, and on the final side by an open field. Additionally, is important to note that there was a *Brassica* crop field to the north that was minimally impacted by black rot and a *Brassica* crop field to the south that was severely impacted by black rot (Figure 1).

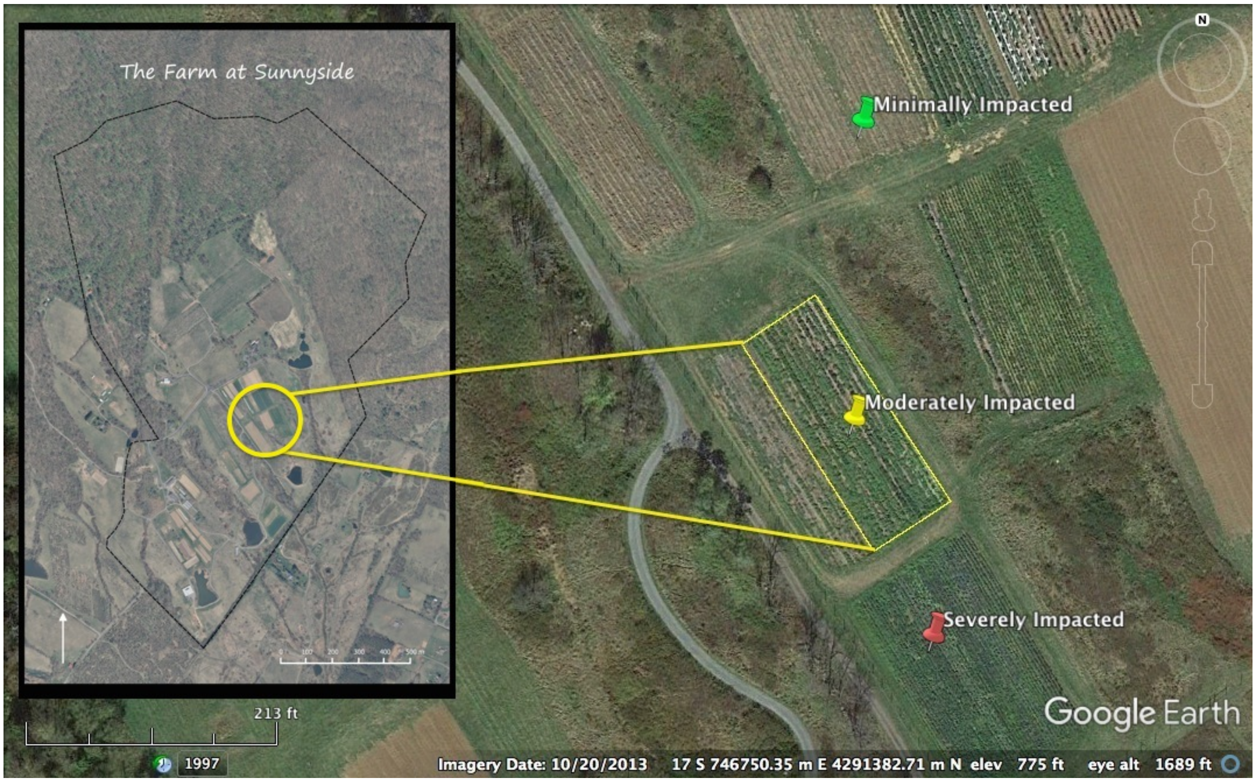

Figure 1. Map of The Farm at Sunnyside with a focus on the selected *Brassica* field to be monitored (moderately impacted by black rot) in relation to the northern *Brassica* field (minimally impacted by black rot) and the southern *Brassica* field (severely impacted by black rot).

The plot consisted of 10 crop rows, each 208 feet in length and variable in crop type distribution. For the purposes of the survey, each crop row contained sixteen, thirteen-foot long quadrats. The plot contained a daikon radish species (*Raphanus sativus*) and a morphologically variable species, (*Brassica oleracea*), which includes green cabbage, kohlrabi, gai lan, collards, black kale, curly kale, and red Russian kale. The types and amounts of *Brassica* crops varied in each row (Figure 2).

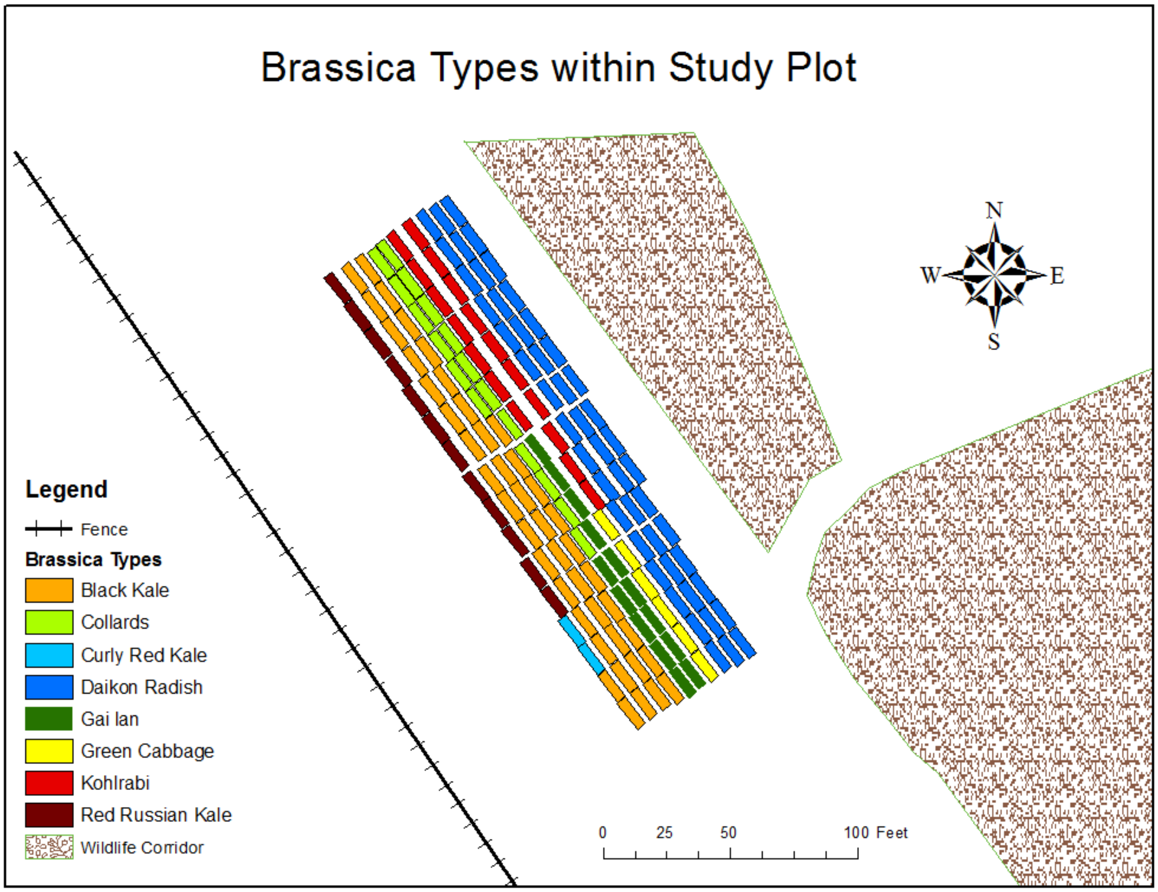

Figure 2. Digitization of *Brassica* plot depicting variation in *Brassica* type throughout plot.

In an attempt to manage the presence of groundhogs on the farm, the farm managers set four groundhogs traps – three along the preserved wildlife habitat and one along the fence to the southwest of the *Brassica* plot of interest.

To evaluate the targeted *Brassica* plot health, an initial survey was taken followed by 3 consecutive weekly surveys throughout October 2016. The surveys assessed the presence or absence and severity of black rot damage (i.e. disease), the severity of herbivory damage, and evidence of groundhogs (i.e. burrow entrances).

The initial survey was discarded in the final assessment due to the misidentification of black rot. While surveying, we remained cautious of contacting plant leaves, as to prevent further spread of disease to the plants. For the intended purpose of this study, the types of damage recorded were restricted to those with bacterial or mammal origins. Plant damage was classified using a 11 point ranking system whereby each successive number indicated an approximate ten percent increase in overall damage to the sample observed (i.e., 0 = 0% damage, 1 = 1-10% damage, 2 = 11-20% damage, etc.). The point-scale values for all transect rows were then compared across weeks 2, 3, and 4 for herbivory and disease damage, independently, using a digitization of each transect surveyed. Additionally, the difference in point-scale values between weeks 2 and 4 were calculated and displayed on a digitization of the *Brassica* plot. The damage caused by *Xanthomonas campestris* (the bacteria that causes black rot) is characterized by v-shaped lesions, wilt, necrosis, and blackening of veins (Figure 3). Mammal damage was identified by missing and torn vegetable tissue and other obvious signs of herbivory (e.g., tooth marks) (Figure 4).

Figure 3. Example of v-shaped lesions, wilting, necrosis, and blackening of veins due to black rot on a *Brassica* crop.

Figure 4. Example of herbivory rank 1 (1-10% damage) on a collard crop.

Groundhog activity was assessed primarily by presence of burrow entrances or holes, however observed scat was also noted on the data sheets (Figure 5).

Figure 5. Example of groundhog burrow entrance documented in survey.

GPS coordinates were recorded for the burrow entrances encountered during the visual survey, and were taken into consideration when allocating camera traps within the plot. Additionally, a one-time survey was performed on the fence surrounding the *Brassica* plots to determine areas where groundhogs or other mammals may be entering. Following the initial plot survey, the coordinate data for the plot perimeter, the groundhog traps set by the farm managers, and burrow entrances were compiled into a site map using the program ArcMap (Figure 6).

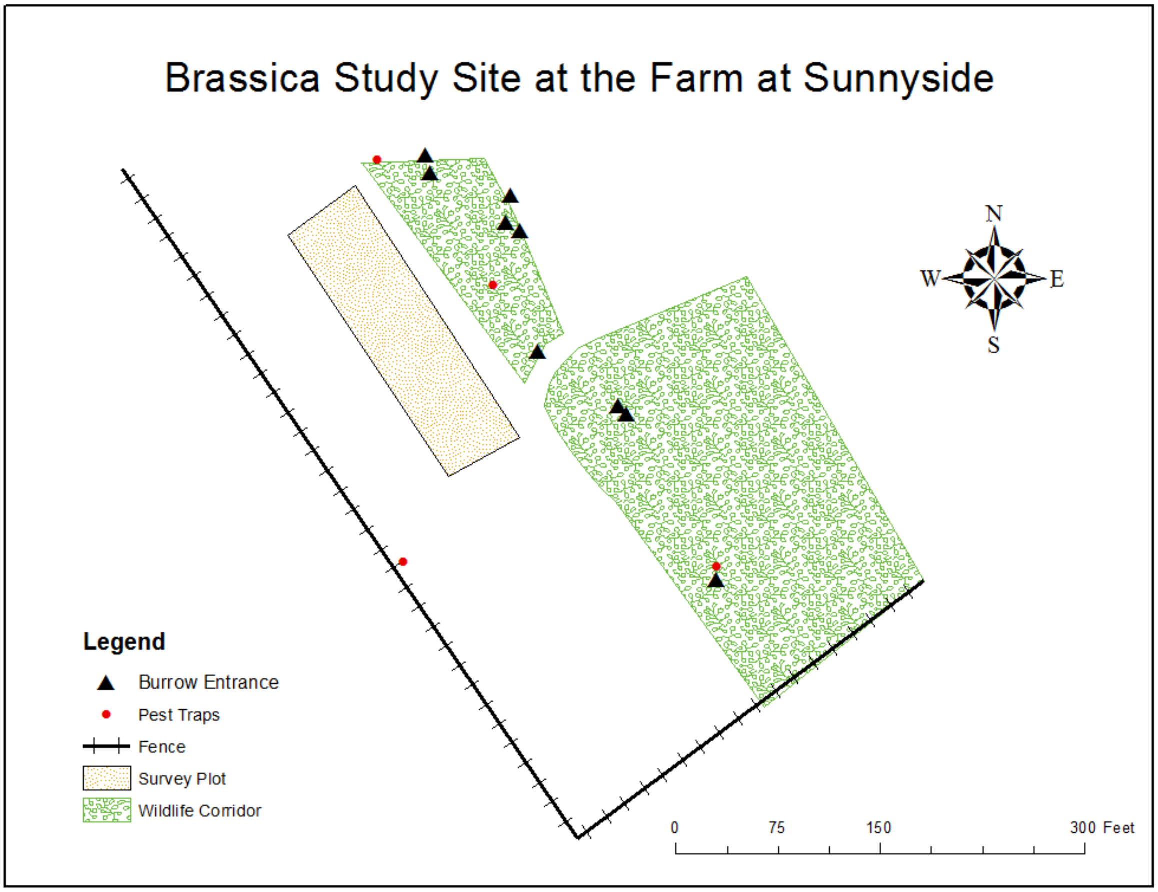

Figure 6. Site map of targeted *Brassica* plot considered moderately impacted by black rot at The Farm at Sunnyside.

Additionally, eight Spypoint BF-7 camera traps were placed in a row along the wildlife habitat area, parallel to edge of the observation plot. The goal was to place the camera traps in areas with a high probability of groundhog movement. Each trap was secured to a pair of steel T-posts approximately 1 inch above the vegetation layer and placed 26 feet apart, and were set to take photos in bursts of 3 without a resting period. Camera traps were checked each week to ensure the cameras had not been tampered with and were still functioning properly; when necessary, routine maintenance (i.e., battery replacement and memory card replacement) was also performed at these times.

The *Brassica* transect data was then converted into a risk map depicting the areas and crop types that exhibit a higher likelihood of contracting black rot. Additionally, several maps were made to show the concentration of black rot damage and the concentration of groundhog damage within the plot, which were then compared in order to either support or counter the hypothesis that groundhogs are spreading the disease.

Preliminary Results

For the duration of the study, 7 of the 8 camera traps did not record evidence of any groundhogs. However, during Week 4 on camera number 8 at the edge of our target plot facing the direction opposite the plot a single groundhog was captured in one photograph (Figure 7). In total, four species of mammals were observed on the camera traps for the duration of the study: humans (*Homo sapien*) (Figure 8), domestic dogs (*Canis lupis familiaris*) (Figure 9), an eastern cottontail rabbit (*Sylvilagus floridanus*), and the single groundhog (*Marmota monax*).

Figure 7. Photograph from Week 4, camera trap 8 of only observed groundhog for the duration of the study.

Figure 8. Photograph from camera trap during weekly SD card swaps of observed humans (*Homo sapiens*).

Figure 9. Photograph from camera trap of resident farm dog (*Canis lupus familiaris*).

Aside from one photograph of a groundhog, the only other evidence of groundhogs near the target *Brassica* plot was the presence of holes approximately 8-10 inches in diameter for a total of 11 entrances – 3 of which were within a $1m^2$ area (Figure 6).

Observed herbivory was minimal and only occurred in the kohlrabi variety of *Brassica oleracea* in all three weeks and in black kale on week 2 (Figure 10). Herbivory appeared to be most severe in a localized patch of kohlrabi in the upper portion of the fourth row closest to the wildlife habitat (Figures 10, 11, and 12). Any additional herbivory observations following week 2 remained only in the kohlrabi variety of the *Brassica* crops (Figure 13).

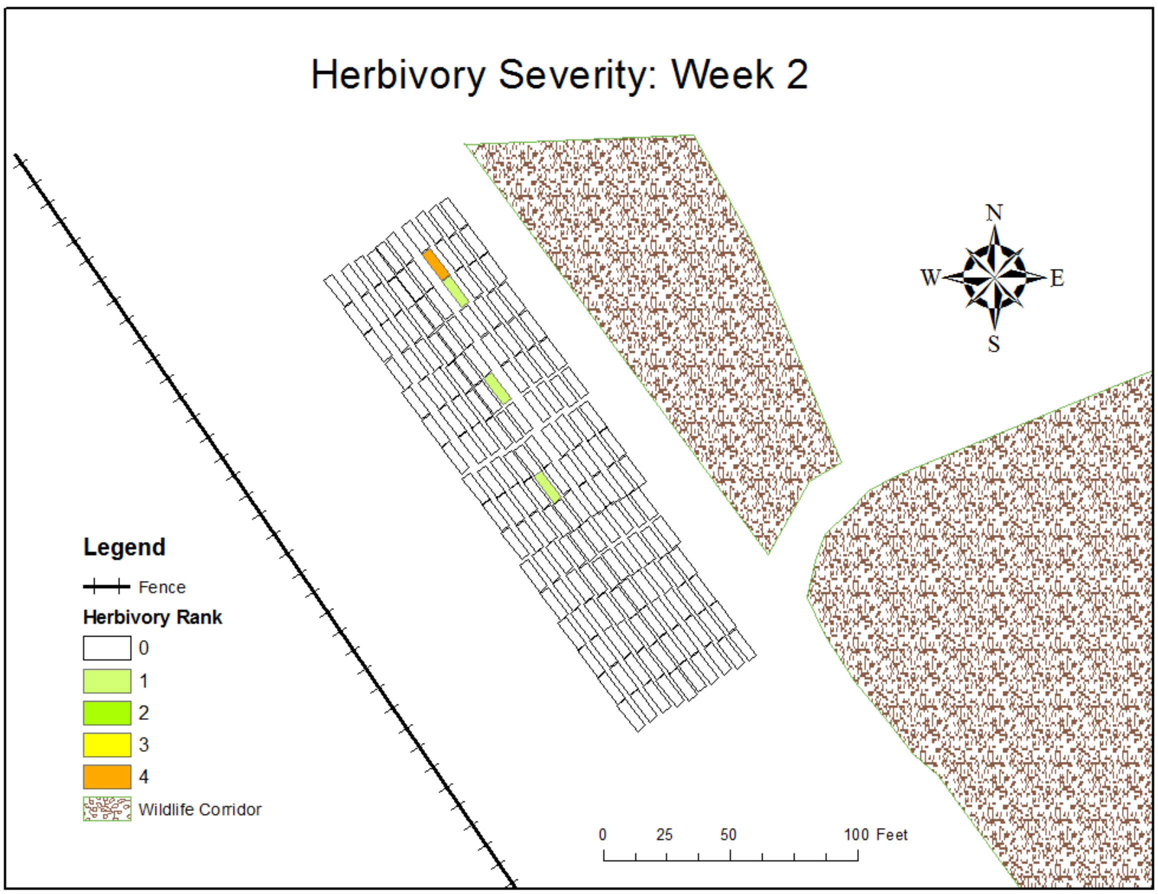

Figure 10. Digitization of herbivory severity recorded during week 2 survey.

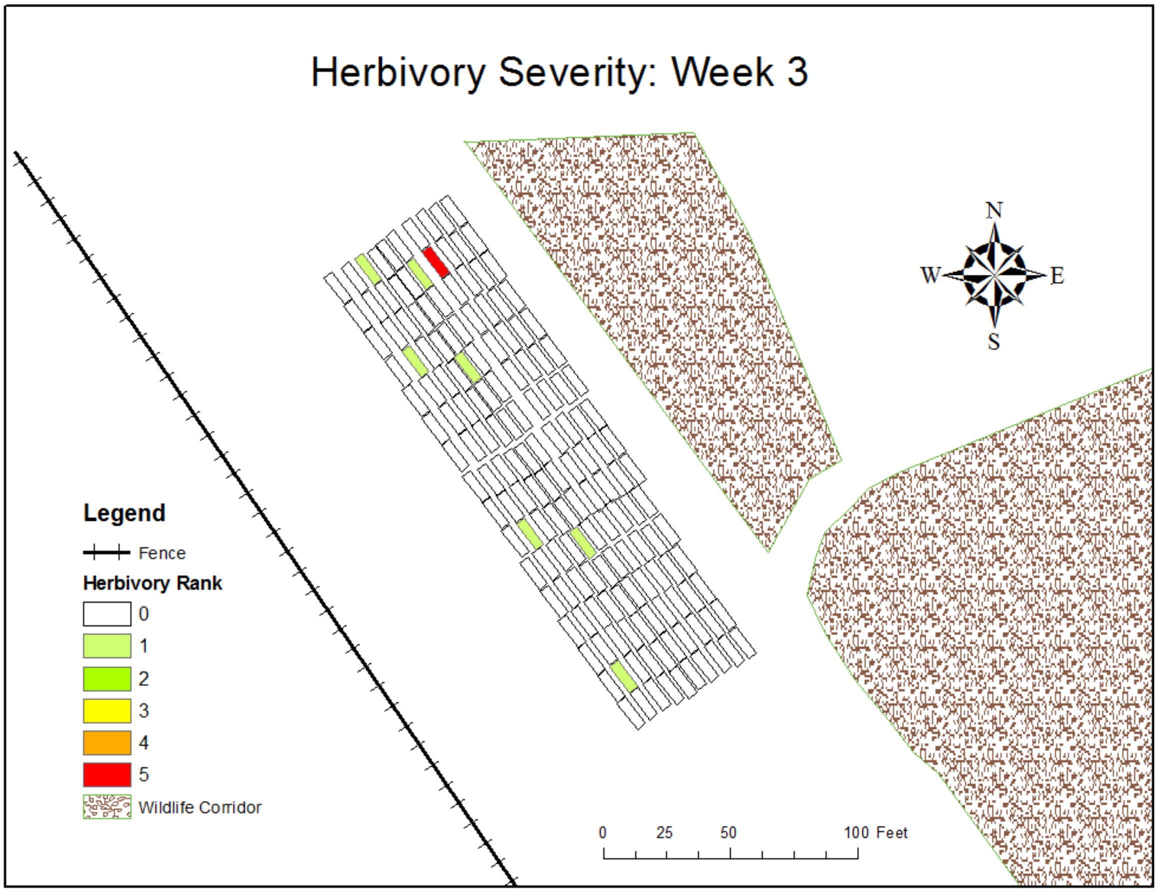

Figure 11. Digitization of herbivory severity recorded during week 3 survey.

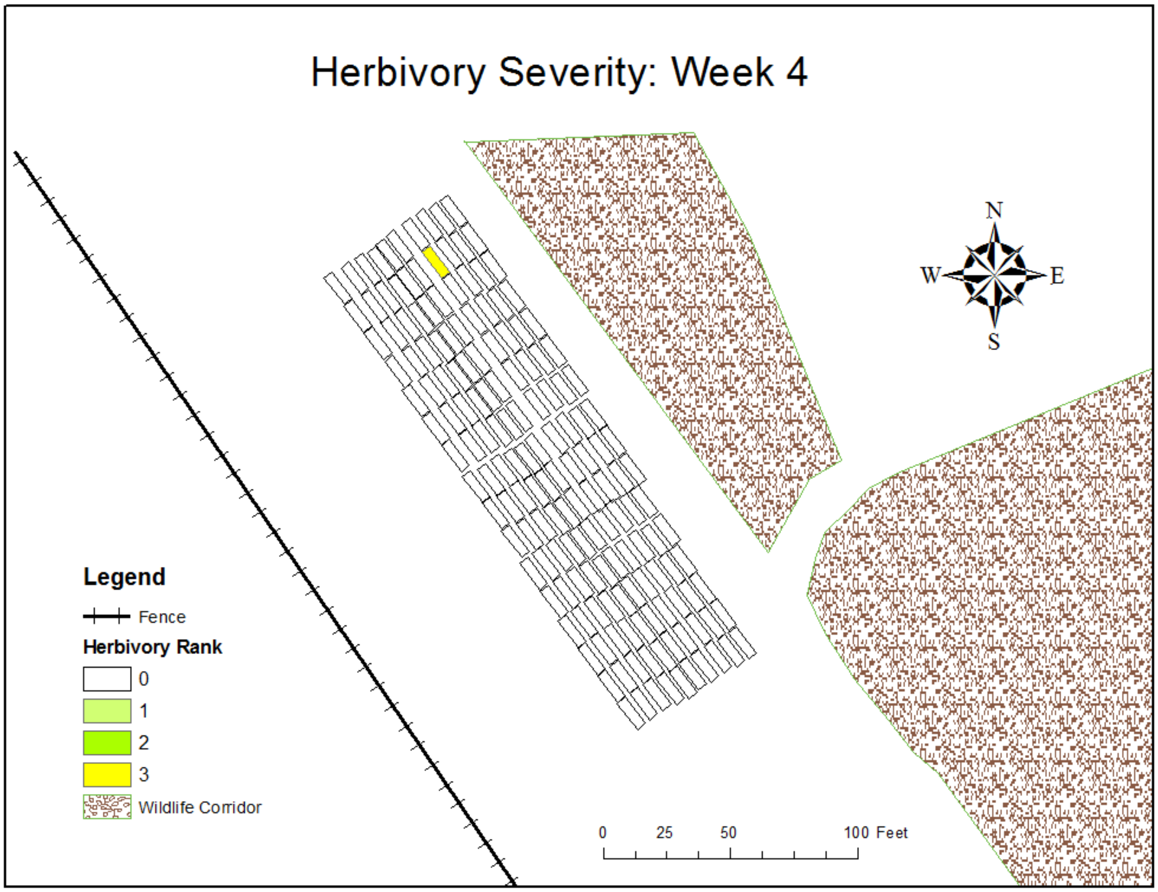

Figure 12. Digitization of herbivory severity recorded during week 4 survey.

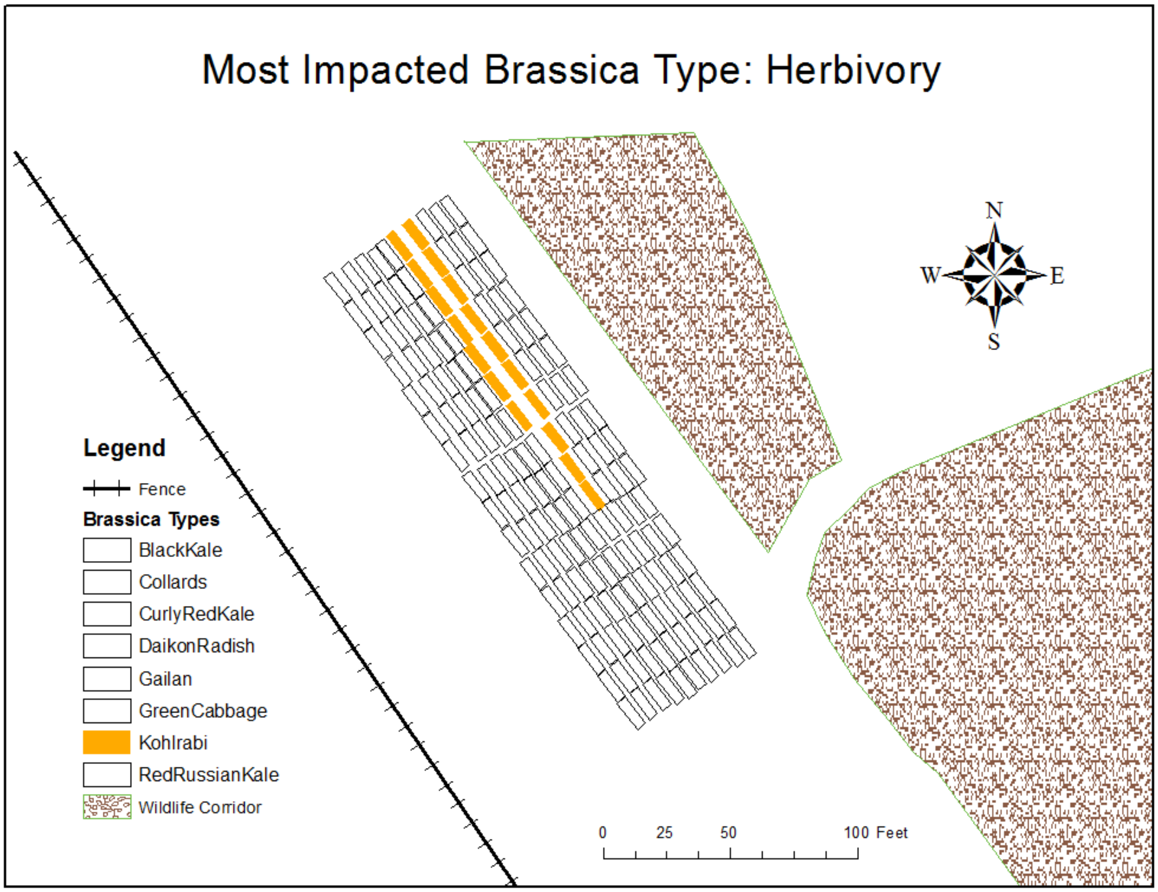

Figure 13. Digitization of the *Brassica* type most impacted by herbivory as observed during surveys for weeks 2, 3, and 4.

Disease (black rot) was present for the entire duration of the study and occurred in every crop type at least once (Figures 14, 15, and 16). The greatest disease severity, as indicated by higher rank values, was observed in the gai lan variety of *Brassica* crops (Figure 17). As the study progressed, the disease appeared to spread southeast towards the edge of the *Brassica* plot.

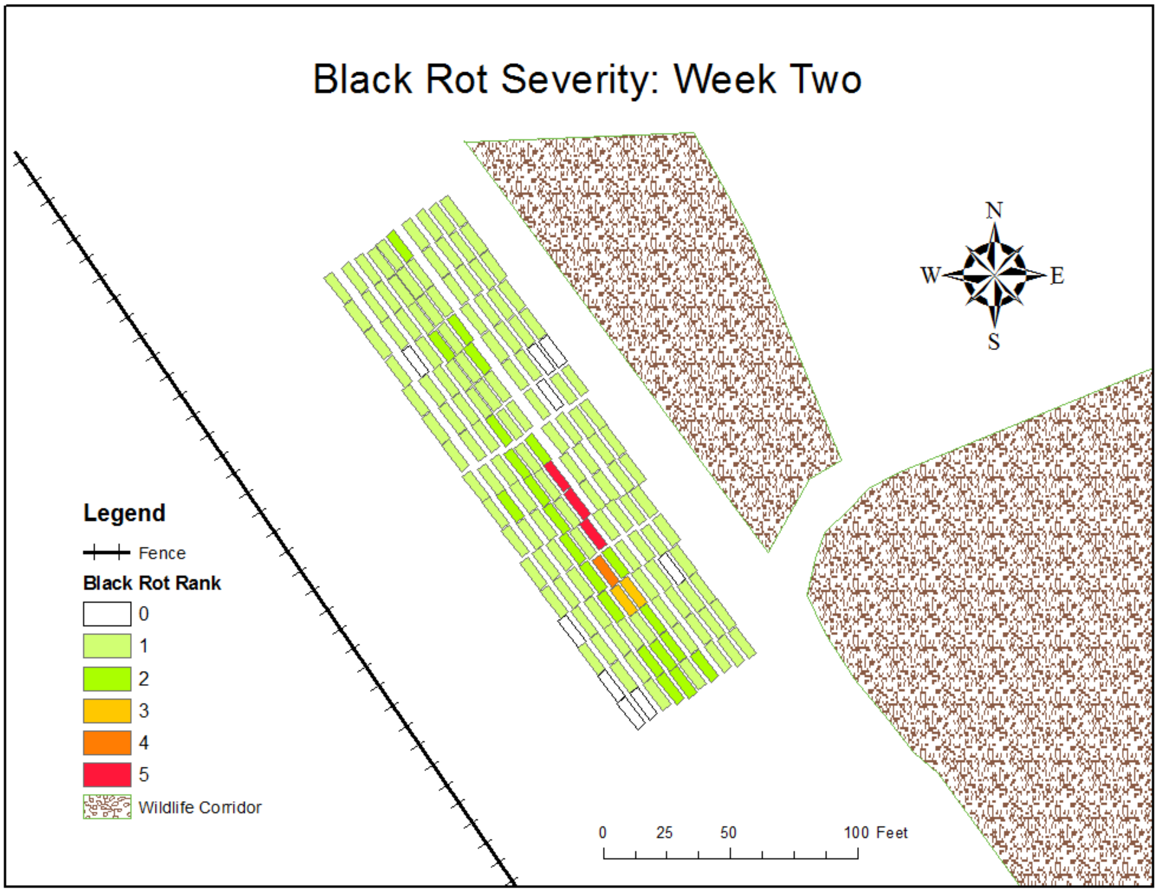

Figure 14. Digitization of black rot disease severity recorded during week 2 survey.

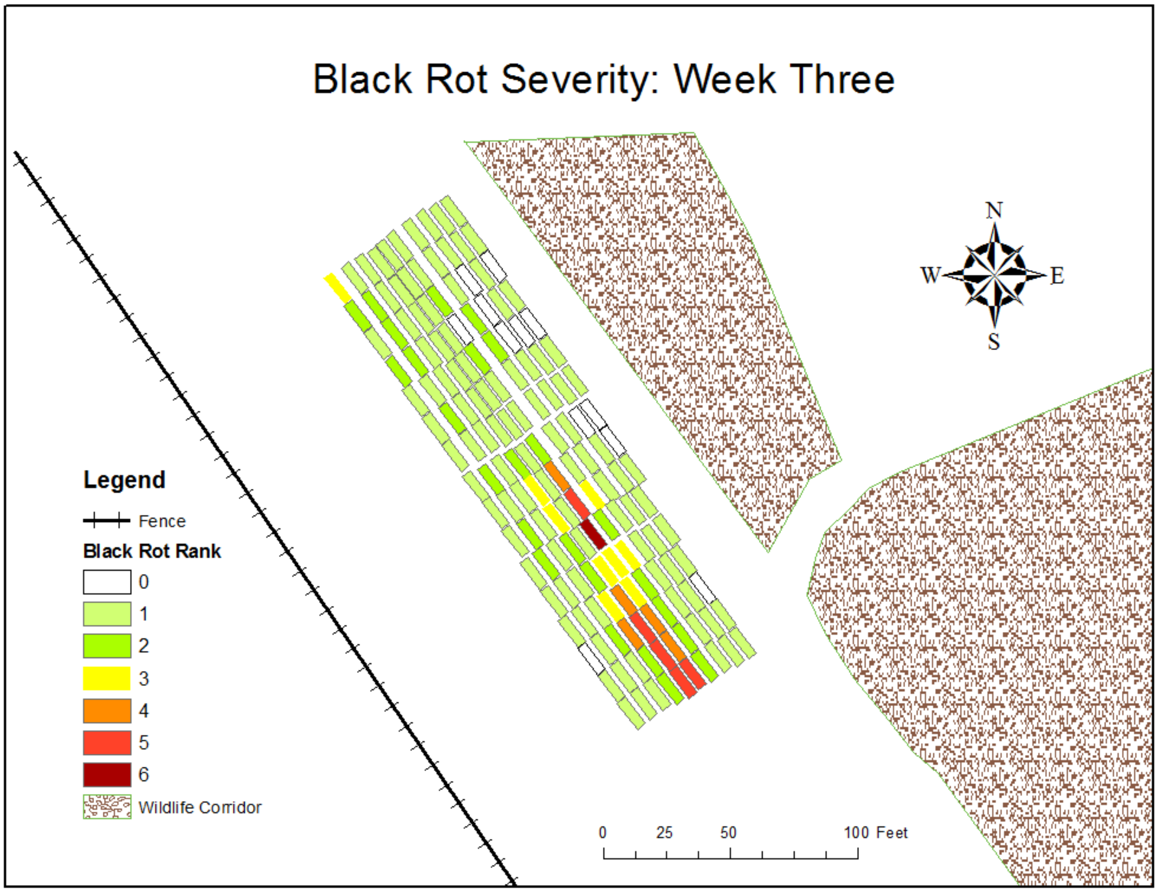

Figure 15. Digitization of black rot disease severity recorded during week 3 survey.

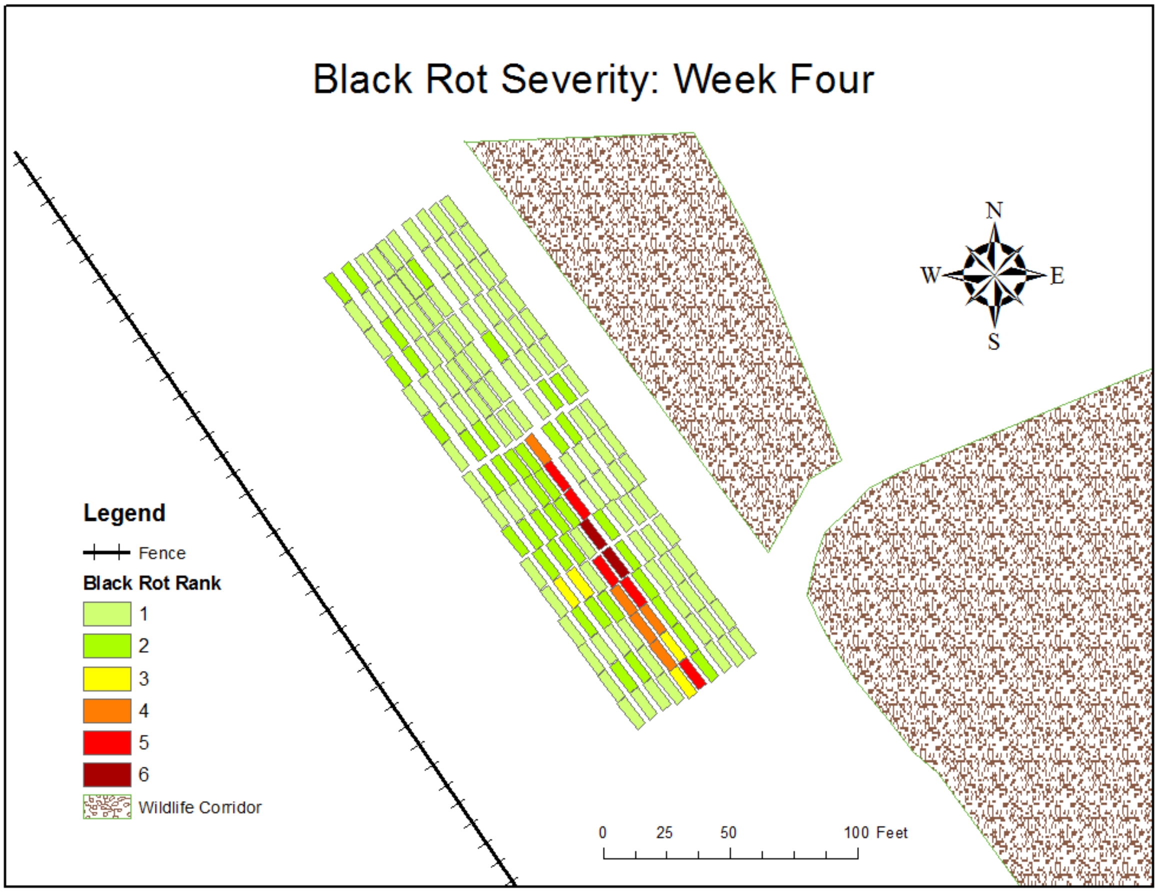

Figure 16. Digitization of black rot disease severity recorded during week 4 survey.

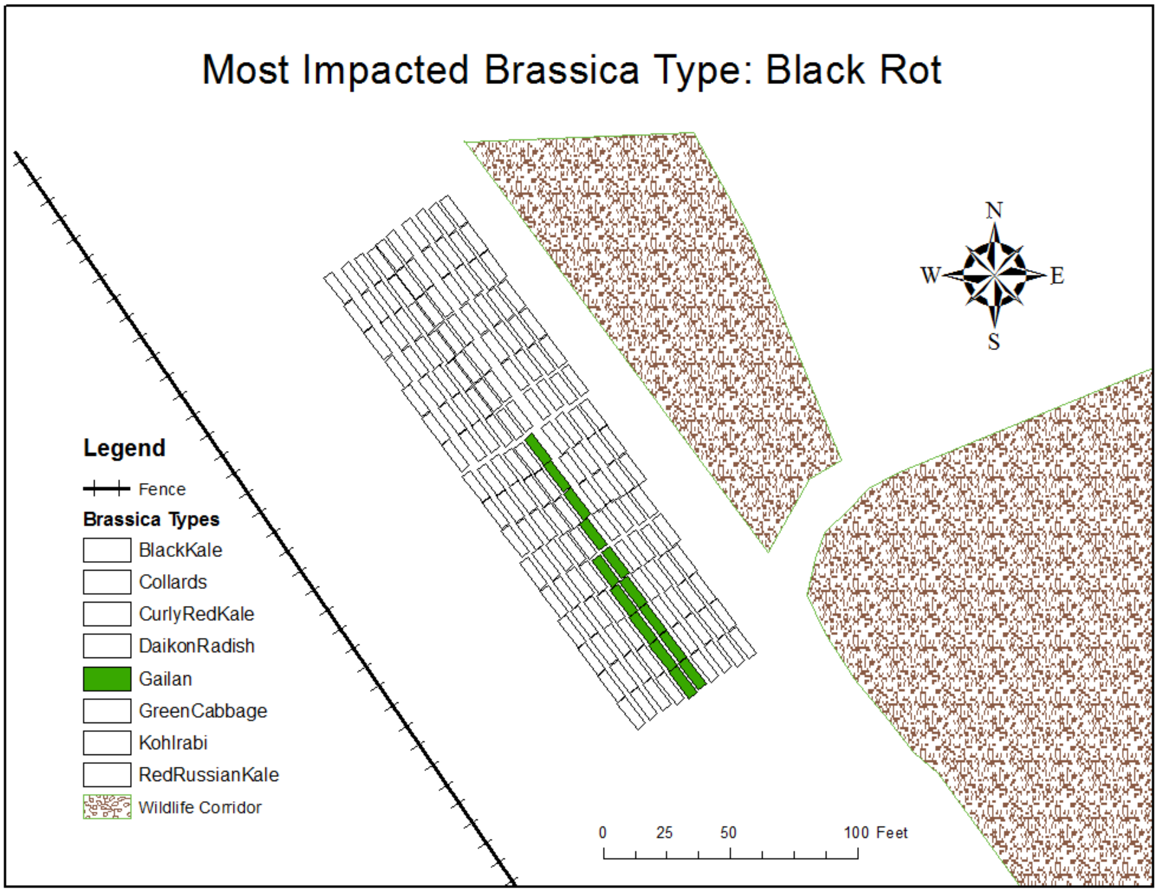

Figure 17. Digitization of the *Brassica* type most impacted by disease as observed during surveys for weeks 2, 3, and 4.

Action Plan

During the course of the four week monitoring period, kohlrabi, collards, and black kale were the only *Brassica* crops to exhibit evidence of herbivory, suggesting that herbivory was not an immediate threat to all *Brassica* species of crops. Additionally, the areas most severely impacted by black rot were on the opposite end (southeast) of the crop field relative to the areas impacted by herbivory (northwest). The spread of black rot was seemingly independent of any groundhog activity. The results of this study suggest one of two options, either there are very few groundhogs on The Farm at Sunnyside, or the groundhogs only prefer a specific variety of *Brassica* crops. In order to determine if groundhogs play a role in the spread of black rot, future studies on groundhog activity throughout the *Brassica* crops at The Farm at Sunnyside, as well as on other farms, should be conducted. Future studies will determine if our results were a product of the small-scale monitoring study, or if the groundhogs actually prefer certain *Brassica* crop types.

Since groundhog evidence was limited, the action plan will focus on the analysis of black rot disease. The disease was observed to occur more frequently and in higher concentrations throughout several specific crops (the gai lon and black kale varieties of *Brassica oleracea)*. Therefore, future studies should focus on investigating the possible causes of the discrepancies observed between Brassica crops.

Further research is needed to better understand the ecology of the black rot disease. Gaining a complete knowledge of the life history of black rot will aid farm managers in making decisions regarding crop selection, crop placement, and black rot prevention. Understanding the ecology of black rot on a small-scale, such as that of The Farm at Sunnyside, as well as a large-scale across many farms will contribute to future management strategies to limit the economic impact of black rot on small farms, as well as large industrial-scale farms.

In order to assist with this research, collected data needs to be made available to The Farm at Sunnyside and other farms within an easily accessible platform in the form of a website. This platform will focus on the overall ecology of black rot disease amongst *Brassica* crops, including effects, potential causes of spread, and suggested farm management strategies.

Goal 1: Understand the role of groundhogs on farms in the occurrence and spread of black rot among *Brassica* crops.

Objective: Experimentally evaluate if groundhogs are able to transmit black rot

In an effort to connect the spread of black rot disease to the presence of groundhogs at The Farm at Sunnyside, future research should include obtaining permission from the Institutional Animal Care and Use Committee (IACUC). Acquiring this permission will allow researchers to conduct experiments that involve direct contact with the groundhogs present at the study site.

By taking advantage of the trapping already taking place at Sunnyside, for instance, a sample of fur can be taken from a trapped groundhog and utilized in experiments where bacteria is hypothetically transferred via the groundhog fur from infected plants in the study plot to plants grown in a sterile environment. This type of physical experimentation is required to conclusively determine if groundhogs are capable of spreading the bacteria in question. If plants grown in a sterile environment following purposeful contact with black rot exhibit the characteristics of the disease as defined in our experiment, then that could suggest groundhogs are viable vectors for the disease.

Goal 2: Identify possible factors contributing to black rot preference of specific *Brassica* crops.

Objective: Determine if black rot prefers specific Brassica varieties over others, so that farm managers may make informed purchasing decisions when introducing new crops to fields.

Further experimentation should be carried out to determine possible factors that contribute to the susceptibility of various *Brassica* crops in order to add to the body of knowledge available to farmers and to better understand the impact of herbivory on crops. For instance, determining which crops are naturally resistant, or highly susceptible, to black rot would allow farmers to make informed decisions about the crops they choose to cultivate. The results from these studies, will allow researchers to determine if there is any variation in herbivory-black rot correlations between various *Brassica* varieties.

Although this monitoring plan sought to address this question, separate research is necessary to eliminate other factors as potential causes of the recorded patterns. For instance, it remains unknown whether the most highly affected crops were more prone to black rot infection because of inherent genetic or morphological characteristics, or whether they might have been sown from contaminated seed. This latter hypothesis is circumstantially supported by the geo-spatial pattern of black rot spread in the study plot. Since only one variety of *Brassica* crop was severely impacted for the duration of the study, it is possible that that batch of seeds were planted after already having been exposed to black rot disease. Future experimentation can eliminate some of this uncertainty by germinating a small portion of the same seeds used to sow the study plot in a sterile environment and observing which, if any, crops develop black rot.

Goal 3: Understand the ecology of black rot on The Farm at Sunnyside.

Objective: Expand The Farm at Sunnyside Brassica plot observations

Additional monitoring studies should be conducted on The Farm at Sunnyside that expands upon the original monitoring study. The new study will evaluate the health of all three *Brassica* crop fields (minimally, moderately, and severely impacted) in relation to black rot following the protocol established in the original single-plot study. These studies will be conducted for three consecutive years.

Focusing on a small-scale area of research allows datasets to be formed demonstrating a variety of trends between the *Brassica* crops and black rot disease. These trends could include the occurrence and persistence of black rot disease among crops utilized on The Farm at Sunnyside, the effectiveness of specific disease prevention strategies, and the influence of wildlife and pollinator areas within the farm setting. If black rot impacts one type of *Brassica* more than others, then the farm managers at Sunnyside may opt to eliminate the crops that are more susceptible. Over the course of the three-year period, it might become apparent that the crop plots closer to the pollinator areas, or wildlife habitat, may experience higher levels of disease spread suggesting that animals may influence the spread. Understanding black rot ecology on a small scale will help more directly address black rot issues that are negatively impacting individual farms, as is the case with The Farm at Sunnyside. Additionally, the data collected at The Farm at Sunnyside will contribute to a large dataset spanning multiple farms.

Goal 4: Understand the ecology of black rot across multiple farms.

Objective: Encourage black rot studies across multiple farms

Additional *Brassica* monitoring studies will be executed across various farms in the region and beyond. The new studies will follow the general protocol established by the original study conducted at The Farm at Sunnyside, but may be adjusted if needed on a case-by-case basis. Over the course of three-years, 15 organic farms in the region should be selected to conduct these studies, five new farms each year. If organic farms are not available to participate, conventional farms may contribute, however, their data will be kept separate from the rest of the data as their farming methods may influence their results. If these studies are a success, then there is a potential to expand this research network nationally. In the future, a database should be established to allow researchers and farm managers to contribute individual farm observations and survey information. This database will allow for future analysis of black rot ecological trends.

By expanding the scope of the research to include multiple farms, additional data may be obtained that could illustrate new trends that were not evident in the small-scale, short-term study conducted on The Farm at Sunnyside. Such trends could include the occurrence and persistence of black rot among all varieties of *Brassica* crops utilized for commercial farming (beyond those implemented at Sunnyside), the importance of wildlife corridors near the crop plots in relation to the spread of the disease, and effectiveness of various black rot management strategies. Understanding the large-scale trends in black rot ecology would allow universal black rot prevention strategies to be created and implemented by farm managers.

Goal 5: Educate farmers on the resources available to prevent black rot occurrence and spread on their farms.

Objective: Create an education resource and communication platform

For farm owners to reduce the impact of black rot on their crops, there must be effective communication transpiring between the farm managers and researchers. Therefore, it would benefit both farmers and researchers to establish a web-based, educational resource where agricultural managers can view the compiled research on effective management practices, as well as contribute their own obstacles and successes with other techniques. One of the many ways this will benefit research is by compiling data from diverse and spatially distant farms that would otherwise remain unstudied. The data gathered from these varied sources would illustrate differences in the impact of black rot on modern practices (e.g., conventional versus organic; large- versus small-scale).

To achieve this, an interactive web site was designed that focuses on the spread of black rot disease among *Brassica* crops, potential causes of the spread of the disease, and possible prevention strategies. This website details our results from the study we conducted at The Farm at Sunnyside, such that they me replicated on other farms. We suggest management strategies, such as selecting crops known to be less impacted by black rot (e.g. not gai lan). Additionally, this website has a forum that allows for farm managers, and anyone else who is interested, to ask questions regarding herbivory and black rot, as well as share solutions or trends they have observed on their own farms.

Resources

<u>Year One:</u>

Camera traps (SPYPOINT: FORCE - 10)………………………… $6,799.60 ($169.99 each)

Camera amounts would vary depending on study site. Assuming The Farm at Sunnyside is an average-sized organic farm, each of the 5 farms selected for the yearly study will receive 8 camera traps to be returned at the conclusion of the study (roughly one camera would be used for every 25 feet being monitored along a crop plot).

SD Card (8 GB) ……………………………………………………………$618.40 ($7.73 each)

Two would be needed for each camera - one for recording, one to act as replacement when collecting data. Each of the 5 farms selected for the yearly study will receive 16 SD cards to be returned at the conclusion of the study.

AA Batteries (Amazon, 48-Pack)…………………………………………$59.35 ($11.87 each)

Each camera trap requires 6 AA batteries. May need to be replaced throughout the study. Each of the 5 farms selected for the yearly study will receive one 48-pack.

Measuring Tape (Open Reel)……………………………………………$120.85 ($24.17 each)

One reel would be needed per study site to establish grid system for Brassica health surveys. Each of the 5 farms selected for the yearly study will receive one reel to be returned at the conclusion of the study.

<u>Year Two:</u>

AA Batteries (Amazon, 48-Pack)…………………………………………$59.35 ($11.87 each)

Each camera trap requires 6 AA batteries. May need to be replaced throughout the study. Each of the 5 farms selected for the yearly study will receive one 48-pack.

<u>Year Three:</u>

AA Batteries (Amazon, 48-Pack)……………………………………………$59.35 ($11.87 each)

Each camera trap requires 6 AA batteries. May need to be replaced throughout the study.

Each of the 5 farms selected for the yearly study will receive one 48-pack.

<u>Totals:</u>

Year One: <u>$7,598.20</u>

Year Two: <u>$59.35</u>

Year Three: <u>$59.35</u>

<u>3-Year Budget:</u> $7,716.90

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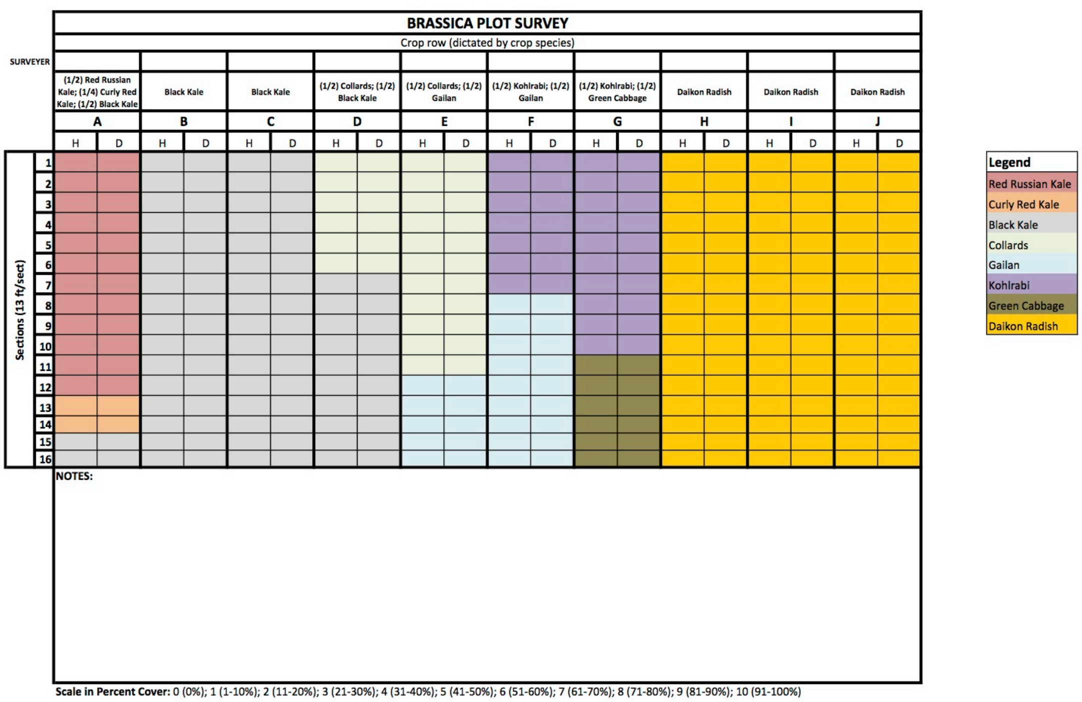
Appendices

Appendix A

Survey sheet used to conduct *Brassica* health surveys at the Farm at Sunnyside for the duration of October 2016.



Appendix B

Link to website communication strategy intended to bridge the gap between researchers and farm managers.

http://smscfall2016blackr.wixsite.com/blackrot