There was a large discrepancy of our calculations versus what standard practice indicated was being done in the industry with a nematode application. We conducted laboratory experiments to identify the actual amount of balls required per gram of soil as well as the population density of each ball. This included mixing pots of soil with varying amounts of balls and observing their distribution, and the nematodes durability. This was further complicated due to the two use cases we were attempting to establish. The first being a bag of soil sold to an end user with the balls already mixed in, the other was to agriculture applications where the balls might be mixed into an assortment of different soil types of varying moisture content. Lastly, we were concerned that our small-scale laboratory tests would not scale effectively to real world testing. Regardless, our observations indicated that we need 10 alginate balls with approximately 250 million nematodes per ball, mixed with 55 grams of dry soil minimum, and 500 alginate balls maximum at a ratio of 5 grams of balls per gram of soil.

Our findings were still in distinct contrast to industry standard for the application of nematodes, our numbers were much lower. Though we believed it to be due to the difference in applications. The standard approach is to mix the nematodes in water, and spray the mixture onto the soil surface, where UV light, dry air, and other environmental factors will quickly kill a portion of the nematode population. Whereas our approach of mixing our alginate balls into the soil allows the nematodes to escape suspension below the surface and be in direct contact with the fungus gnat larvae, enabling the inoculation process. With this data we developed a protocol to share with researchers at Penn State University and the University of Guelph who could investigate our hypothesis on a more realistic scale. By year end, the results were still inconclusive, but the preliminary data seemed to reveal that our alginate balls are so effective when mixed with soil that our formulation was wrong, and we were oversaturating the soil with nematode populations. This can be disruptive to the natural balance of the soil, as well as promote mold and bacterial growth which would be detrimental to the crop.

246
What scientific or technological advancements did you achieve as a result of the work described in Line 244?? (*Maximum 350 words*)

We concluded this year that our theory of mixing alginate balls directly into soil is an effective way to combat fungus gnats, and potentially other pests in the soil. Though the project was more complex than anticipated as there is a balance that needs to be attained where enough nematodes are introduced to start a natural reproduction cycle through inoculation of the larvae, but not enough where the excess biologicals introduce health challenges into the soil and impacting the crop. Technological advancement was achieved in the development of a protocol developed directly for the mushroom industry to be used in the testing at universities, which not only out performed standard practice in the application of nematodes, but initial observations indicated that our alginate ball calculations were wrong and therefore oversaturating the soil. Further examination and trials will be conducted in the next fiscal year.

242

What scientific or technological uncertainties ---could not be removed using standard practice? (*Maximum 350 words*)

The objective of this project was to develop an alginate ball product that would contain populations of nematodes for use in agriculture to combat fungus gnats. We had recently concluded a two-year plausibility study. It confirmed that our product was effective enough at inoculating the gnat larvae in the soil to a degree that it could maintain a perpetual population of nematodes for two years. With this knowledge we theorized we could create two similar new product offerings. The first being a bag of soil containing the proper ratio of alginate balls to soil to prevent the growth of fungus gnats. The second being a product for mushroom farms and similar agriculture to also prevent the growth of fungus gnats. The mushroom industry is incredibly large and has a continual challenge with combating fungus gnats as the high moisture growing conditions are also promote fungus gnat growth. We investigated public knowledge on how nematodes are used in these environments already to determine precisely how many nematodes were needed per ball, and the ratio of our balls per grams of soil. However, this is where we ran into technological uncertainty. As we compared our data against the what the literature calls for, we identified a discrepancy that indicated our initial hypothesized nematode to soil ratio was drastically off. Therefore, the data we have internally is drastically different, and we would need to conduct experiments to identify precisely how many nematodes would be required. If we introduced too few nematodes they may not be effective in combating the fungus gnats; too many will lead to carcasses in the soil as the nematode population quickly depletes, introducing mold and other health issues which upsets the balance of the environment. We did not have an adequate facility in house to identify the ideal alginate balls to soil ratio. Therefore, we hypothesized, we could establish protocols based on in house experiments and then partner with universities, Penn state and University of Guelph, to do the large-scale testing.

244

What work did you perform in the tax year to overcome the scientific or technological uncertainties described in Line 242?
(Summarize the systematic investigation or search) (*Maximum 700 words*)

We had observed after a two-year study, that our alginate balls could provide enough of a population mixed into soil to not only prevent the spread of fungus gnats, but our populations could continuously perpetuate healthily for upwards of two years after being introduced. This study was simply the start of a hypothesized idea to mix our alginate balls into soil to sell to both consumers and the agriculture industry. Though the soil was sourced from a natural location, there weren't any rigid controls being tested, it was just a proof of concept study.

The observation that our populations could thrive when mixed into soil lead us to begin investigating the specific parameters of how a product would work. The nematode population per alginate ball, the amount of balls per gram of soil, etc. We calculated approximations based on our previous knowledge and experience working with nematodes and sourced publicly available information on how nematodes are similarly used in the agricultural industry already. Specifically, we were looking at how nematodes are used both in the mushroom industry as well as greenhouses where fungus gnats are the most problematic due to ideal moist conditions intensifying their population growth.

WWA.

PROJECT #:8

PROJECT NAME

MUSHROOM PEST EXPERIMENT