The Influence of Microbial Life on Tomato Plant Growth, Fruit Quality, and the Overall Balance of an Ecosystem

Master Thesis

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Abstract: The interest in microorganism for agricultural purposes has steadily grown over the last few decades. As technology has improved, many people have begun to realize the significance of these organisms for agricultural purposes. Biofertilizers and biostimulants have emerged as possible means to introduce beneficial microorganisms to degraded soils in order to improve plant growth and yield. This paper takes an agroecological approach to analyzing the effectiveness of three such biostimulants— "Unleash" by Aquabella, a collection from Micosat and an on-farm developed compost tea—when used on tomato plants. The products were tested on two different varieties of tomatoes *(Solanum lycopersicum, L.),* one local ("Costoluto Genovese"), and the other a popular hybrid ("San Marzano"). Data was collected to understand plant growth as well as yield and quality. A significant part of this trial was affected by an infection of *Didymella lycopersici*, so the plants were also observed to understand what helps build resistance to this specific fungal pathogen. This paper concludes by exploring the greater impact of the use of biostimulants on an agroecosystem.

Key Words: Microorganisms, Symbiosis, Biostimulants, Tomatoes, Agroecology, Soil microbial activity

A Note of Thanks

My interest in this topic began in the very first week of the masters. To kick off the program our professors organized a condensed version of the community phase in order to get a feel for the action learning approach. For two days a group of us worked on a small horticulture farm in Costigliole d'Asti by the name of Duipuvrun. During the first morning I found myself scattering these strange pellets across a freshly prepared rows of soil. Stefano, the farmer begged me to use it sparingly as the product was quite expensive. This was the first time I had ever seen a mycorrhizal inoculant. In fact, before that morning I had never even heard of the stuff, and it wasn't until many months later that I actually understood what it was.

As I was preparing to go home for the winter holidays, I decided to peruse the library in search of a good read to occupy me over the break. On the shelf I saw Merlin Sheldrake's *Entangled Life,* and immediately my mind went back to that curious substance I had used at Duipuvrun. I rented the book and unlocked a whole new world. Not only did I finally understand what mycorrhizal fungi were, I also learned about so many other fascinating and beneficial fungi unrecognizable to the naked eye. From that winter forward, my excitement for fungi has only grown as has my knowledge of the microscopic world.

Though I was enthusiastic, the topic of mycorrhizal fungi and other microorganisms useful to an agroecologist was still quite challenging. I lacked many of the necessary skills, tools and resources for such a subject. However, we found a way to make it work and for that I have many people to thank. First and foremost, I must thank my professors and mentors who offered their knowledge and support throughout this process: Paola Migliorini, Alex Taran, Charlotte Prelorentzos, and Nicholas Panayi. These people helped me find a way to incorporate my ideas into a thesis and made connections that were crucial for its success.

Next, I would like to thank Fabio Costantini for welcoming me onto his farm for the Spring and Summer months and allowing me to take over a whole terrace with my tomatoes.

From him I have learned so much about farming, philosophy and community. I would also like to thank all the people of U Giancu for making every morning such a joy and for making me feel like a part of the team.

I must also give a very special thank you to Giorgio Masoero, without whom there would be no results section. Thank you for making litterbags, taking samples, running tests and talking me through statistics. This project was a challenge and without this particular collaboration, it would have been nearly impossible to achieve.

Finally, I would like to thank all of my friends and family who supported me in this process. To Lasse Kay, who labored and puzzled over the experiment along with me. And to my classmates who asked and answered many questions and who always motivated me to put forward my best work. I hope this thesis can spur more conversations of similar quality.

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1. Introduction

Whether in their roots or shoots, plants have relied on fungi for nutrition and defense for as long as there have been plants.

- Merlin Sheldrake (2020)

The symbiotic relationship between plants and the microbes with which they live is a fascinating world to explore, even more so when the gravity of this relationship is understood. The movement of the green revolution did a lot to undermine this important connection, as the world attempted to revolutionize how plants are grown. At the time, those involved were not aware of the extent of the damage that would be done by the end of the century. Not everyone was on board with this form of agriculture, and many movements were happening simultaneously, such as Masanobu Fukuoka's "natural farming," which remained focused on working in cooperation with nature rather than struggling against it. There were quite a lot of others who warned against the use of synthetic fertilizers and potent pesticides. One such voice was Albert Howard, who warned in the 1940s how chemical fertilizers would disrupt the mycorrhizal relationships on which our world depends (Howard, 1943). Today, the number of voices has only grown and the microorganisms that were once dismissed are now thrust into the spotlight.

The (relatively) recent appreciation for microorganisms, combined with the demand for a sustainable substitute to replace other, more damaging products, has resulted in an explosive new industry of biofertilizers and biocontrol products. The FAO (2006) defined biofertilizers as "products containing living or dormant micro-organisms, such as bacteria, fungi, actinomycetes and algae alone or in combination, which on application help to fix atmospheric N or solubilize/mobilize soil nutrients in addition to secreting growth-promoting substances."

Biocontrol products act similarly by introducing a consortium of microorganisms intended to provide plants with a defense system against pests and diseases. The employment of microorganisms for such purposes is an old trick, however what makes todays movement more interesting is the fact that microorganisms are now being purposefully shipped and distributed around the globe and introduced to various soils and environments.

Since 1992, 74% of the plant protection products on the European market have been removed (Singh et al., 2016). With this withdrawal comes a need for alternative solutions, and many are turning to bioproducts as the best option. The market is reflecting this enthusiasm as it is said that "plant microbiota is expected to contribute to 60% of biocontrol products by 2025, for a $11 billion market globally (Sessitsch et al., 2018)." (Deguine et al., 2023). This burgeoning industry offers many hopes, raises a lot of questions, and is most generally simply a curious change taking place in the world.

This thesis attempts to enter into the conversation about microorganisms and their potential contributions to the field of agroecology. It considers two different microbial products on the market, as well as on-site methods for capturing native microorganisms for a self-produced inoculation. It also touches on the importance of a plants genetic characteristics and how this impacts its growth and interaction with the different treatments. Finally, it does its best to consider the broader impacts the use of these organisms might have on an ecosystem, by addressing other studies that has been conducted on this topic thus far. It is a humble attempt, though the hope is that this thesis can shed some light on the topic and offer its support to the community of researchers concerned with the interactions between the worlds above and below ground, large and microscopic.

1.1 Research Question

The primary question I seek to answer is:

How can various microorganisms influence the growth and quality of tomato plants?

More specifically, this thesis attempts to understand and compare how the use of three different biostimulants effect each tomato plant individually. The three products include: a self-produced compost tea using local material, a commercially available mycorrhizal fungi inoculant, and a commercial bacteria-based biostimulant. These three products are compared across two different varieties of tomatoes: one local, and one hybrid. Consequently, the second question that this thesis addresses must be:

how does the genetic make-up of a tomato plant influence its ability to grow in a low input environment and how does it influence the compatibility with each biostimulant?

We go on to ask, what effect does the history between a variety and the land have on the relationship between the plants and the microorganisms, consequently leading to the development of the plant itself? The last question of this thesis addresses the agroecosystem:

How does the introduction of external or internal microorganisms influence the soil ecosystem?

How do the native and introduced microorganisms interact with one another?

1.2 Hypothesis

It is clear that a plant cannot easily survive on its own. It is reliant on the microscopic networks of life that run along its leaves and stems, around its roots and throughout the soil. Humans have dedicated a lot of work to eliminate this dependency, however, this can only increase the plant's reliance on other, often artificially created, factors. Modern plant breeding practices tend to ignore the role of microorganisms, as they focus on creating varieties that provide a high yield, or a sweeter flavor, or an easier harvest. The plants are designed to live in an environment that is enriched with fertilizer and protected with pesticides, fungicides and insecticides. In this bubble plants no longer rely on their microscopic partners and the ability to form crucial symbiotic relationships fades away. Mycologists speculate that the trajectory of plant breeding has led to a suppression of mycorrhizal colonization and dependency among modern crop varieties (An et al., 2010). I think it could be taken one step further to say that there is a possibility that these crops are unable to perform symbiosis with a range of soil microbes, beyond just mycorrhizae.

In his new book, Merlin Sheldrake eloquently explains how plants came to be with the help of mycorrhizal fungi (Sheldrake, 2020). The earliest plants didn't even have roots, but instead relied on the fungal hyphae to transport nutrients from soil to stalk. Over the millennia this relationship has changed and though plants now have their own root systems, the symbiotic relationship has remained. Plants, fungi, bacteria and other organisms have survived in harmony for centuries, giving, taking, communicating and destroying. There are vast, complex economies undetectable to the naked eye, and a cooperation that lends to the survival of the very substance that gives us life. By harnessing the natural tendency of cooperation, we have the opportunity to grow plants that are more adaptable and resilient. Local varieties should have a connection with the land encoded in their genes. They have a history in that particular environment and carry with them an inherent knowledge of the climate and life that surrounds them. Modern hybrid varieties have lost this connection, which could make them more adaptable to relocation, but simultaneously making them more reliant on the creation of a hospitable, nutrient-rich environment.

It is therefore my hypothesis, that a local landrace variety of tomato will perform better than a hybrid variety, when given a dose of microbial products, as it will be more open to symbiosis. I suspect that the local variety will form the strongest relationships with the local microorganisms and therefore the combination of the compost tea and the costoluto Genovese will outperform the other combinations. Aquabella's "Unleash" will also be interesting, as it contains no mycorrhizal fungi. Instead, it aims to create a strong root system that will encourage the plant to connect with native mycorrhizal species present in the soil. This combination of introducing exotic microorganisms to work in unison with the local microbes, can be very interesting and potentially even more potent than using one hundred percent local organisms.

That leads me to the second part of my thesis: what happens when these exogenous microorganisms are introduced to an agroecosystem? In a heavily degraded soil, the introduction of microorganisms could prove useful for regenerating the land. However, in a soil that is already rich in life, there is the possibility that these new microorganisms could outcompete the native ones, and take over similar to an invasive species. The incoming organisms might not form symbiotic relationships with the native species, but may instead aid other nondomestic plants. My hypothesis is that the promotion and preservation of the endogenous microorganisms will lead to a more balanced and resilient agroecosystem.

2. Previous Work on the Topic of Microbial Inoculants

2.1 The Creation and use of Mycorrhizae Inoculants in Agriculture

The involvement of mycorrhizal fungi in agriculture has grown significantly over the last few decades. Commercially available inoculants are readily available on the market, and many recipes and techniques have begun circulating for self-propagation. Whether one is buying or creating an AMF inoculant, it is important to be aware of the environment in which the fungi is being introduced. Mycorrhizal fungi are living microorganisms, and as such, they have preferred conditions, and their survival is subject to the relationships they form with a host plant. This gets more complicated when creating artificial networks using exotic inoculums and plant species. A symbiotic relationship stems from each participant relying on the other for survival. The benefit is mutual and positive. Consequently, if that mutual reliance is destroyed, colonization will most-likely not occur. That assumption has led some researchers to conclude that the likelihood of successful root colonization is increased in an environment that is deprived of phosphorus (McCoy, 2016).

It is, however, also important to consider the role of other microorganisms and their interaction with the symbiotic plants and fungi. Peter McCoy recommends that AMF inoculation is accompanied by a dose of compost tea as "AMF spore germination is decreased in sterile soil and increased in the presence of microbes." He goes on to suggest that "part of the ingredients used to make the tea should include soil sourced from the natural habitat of the AM species being worked with. This will help bring in the nitrogen-fixing and phosphorus-solubilizing bacteria that are intimately linked to the AM symbiosis" (McCoy, 2016). There are hundreds of recipes for compost teas, but the most important thing that links them all is the focus on using local ingredients and aeration and agitation (Darwish, 2013).

The ability for compost tea to protect plants from soilborne pathogens has been addressed to an extent. Liroa Shaltiel-Harpaz et al. (2016) studied the success of compost tea in protecting plants against fusarium wilt and other studies have been conducted with *Pythium* spp., *Rhizoctonia solani*, and *Fusarium* spp. (Erhart et al. 1999; Hoitink et al. 1997; Borrero et al. 2006). These studies show the disease-suppressing potential of compost tea.

Many propagation techniques have emerged, allowing farmers to introduce these symbiotic fungi to their soils similar to how one adds fertilizer. Some of the most significant methods include single or monosporic (Fracchia et al., 2001; Selvakumar et al., 2016), hairy root (de Souza & Declerck, 2003), solid substrate (Douds Jr. et al., 2010; Millner & Kitt, 1992), aeroponic (Mohammad et al., 2000) and hydroponic (Tajini et al., 2009). Though there are many ways to propagate AMF spores, the ability to do so often remains inaccessible to farmers on a practical level. Douds has been aware of this barrier and has done quite a lot to break it down through his work with the Rodel Institute in the United States (Douds et al., 2016; Douds Jr. et al., 2010). There is also an issue of storage and maintaining an AMF population, though there are some techniques such as the trap culture technique which focus on minimizing these challenges (Selvakumar et al., 2016). Despite efforts to make on-farm inoculants an option for farmers, limits still remain be it time, space, or awareness. Commercial inoculants have proven successful, though the extent of success varies (Hart et al., 2018).

Research has been done evaluating the success of each of these inoculation methods, but there is a significant gap in comparing across the various options. It is important to understand how an on-farm produced inoculum compares to a commercially produced one, and to what degree. This paper makes an effort to fill this gap to a certain extent, by comparing an on-farm produced inoculant with purchased ones.

2.2 Plant Varieties: Hybrid versus Local

The emergence of hybrid plant varieties came with the wave of synthetic fertilizers. In general plants were bred to produce high yields within a well fertilized, high input system. Little attention was given to the consequences of such a set up until the most recent decades. In the 1990s plant geneticists and mycologists began exploring the implications of modern plant breeding for the soil food web. G. -H. An et al. (2010) summarizes that "Mycorrhiza scientists and plant geneticists speculate that the breeding programs on fertilized soils lead to selection for suppressed AM colonization (formation) and dependency in crops". This suggests that a hybrid variety that is typically bred in this manner, will be less dependent and open to colonization. However, this speculation is not fully supported by the research conducted thus far and the results vary significantly. Modern plant breeding programs have proved to suppress AM colonization in some experiments (Tawaraya, 2003; Kaeppler et al., 2000; Rao et al., 1990) but others have shown the hybrid varieties to have similar colonization levels as landrace varieties (An et al., 2010; Bryla and Koide, 1990; Koide et al., 1988). This inconsistency shows that further research must be done to understand the links between plant genotypes and its role within the soil food web.

This paper also intends to look beyond mycorrhizal fungi to include all beneficial microorganisms present in the soil and harnessed in modern biostimulants. Little is known about the links between plant genotype and the terrestrial ecosystem (Schweitzer et al., 2008), though what has been conducted so far reveals a need for further exploration of the subject.

2.3 Introducing Exogenous Microorganisms to an Agroecosystem

The opinions on the effects of introducing non-native mycorrhizal species to farm ecosystems is inconclusive and at times contradicting. Miranda M. Hart et al. published a paper which summarized all the research done on AMF inoculants in agriculture so far and concluded that "the current practice of AMF inoculation is at best a gamble, and at worst an ecological threat" (Hart et al., 2018). They pointed out the flaws in the research that has been conducted thus far and identified topics that must be explored further before any decisive conclusions can be made about the use of AMF inoculants. The paper highlights how context-specific each farm ecosystem is, and how introducing foreign substances may imbalance the situation and create competition for local AMF. They warrant the use of AMF inoculum for horticulture practiced in a closed system such as a greenhouse or hydroponics, but insist that, when possible, use of natural inoculum from local soils is preferred. They also note the benefits of AMF inoculants in restoring heavily degraded soils. In conclusion, Hart's research explains that not enough research has been done to understand the true effects of mycorrhizal inoculants on a farm ecosystem.

The debate on AMF inoculants continues into the world of restoration, where the majority seem to settle on a middle ground, claiming that inoculation with native microorganisms proves to be the most successful. One such study used an inoculation of local

AMF which resulted in a greater diversity of native plants and a suppression of non-native species in grasslands (Koziol et al., 2023). In this particular case, the inoculant was cultured in a laboratory, but many other studies have experienced similar results by adding a scoop of soil from a donor ecosystem (Duell et al., 2022, 2023; Koziol et al., 2022; Vahter et al., 2020; Wubs et al. 2019). This method is possibly even more effective when successful as it could bring in a greater diversity of beneficial microbes. In 2022 an analysis of 80 experiments was done on this exact collection method. It showed that adding a small scoop of soil from intact ecosystems helps to boost plant biomass production by 64% on average, across ecosystems (Averill et al., 2022). This study is particularly significant as it acts as a strong argument for the use of local microorganisms, and illustrates the power they have to restore a degraded ecosystem.

There is certainly a strong case for the benefits of microbial inoculations, especially in a degraded ecosystem. However, such actions must be taken with careful planning and it is strongly encouraged to use native species. A strong voice behind these warnings is Jessica Duchicela. In her work on the Galapagos, her and her team experienced that negatives that can come from non-native AMF inoculations, where the non-native species benefited more from the inoculation than the native species (Duchicela et al., 2020). Other studies support this finding within the world of agriculture (Schwartz et al., 2006). However, this is not always the case, as some studies report AMF inoculations to have no significant impact on the native soil communities (Antunes et al., 2009).

Though a lot of research has been done on the subject, it is still unclear the true impacts of microbial inoculations. What is clear is that the soil microbiome is delicate and can be influenced by even the most subtle changes within an ecosystem (Schweitzer et al., 2008). It is clear that prescribing and inoculum should be done with intention and care, and it seems as though the use of local soil as an inoculant is the least risky, least costly, and yet still effective method. This paper attempts to further the research done comparing across these options of inoculation to determine their influence on plant growth within an agroecological context.

3. The Research

3.1 The Context: A Case Study

The creation of this thesis is situated within the context of a small farm located on the Italian coast. The geographical location as well as the cultural context are significant factors to keep in mind when reading through this thesis. I have therefore provided a brief summary of these two points in the following section.

3.1.1 Fescion Farmer

Fescion Farmer is the result of a single man's unexpected collision with the world of farming. In 2017 Fabio Costantini's father got injured and was unable to manage the family's half-hectare garden on his own. As a result, Fabio took some time off work to help his father, and once he touched the soil, he never looked back. Here we are, six years later and Costantini can be found splitting his time between managing the half-hectare garden of U Giancu, a slow food restaurant only a few hundred meters away from his house, and working to mold his own garden into something that is meaningful to the greater community of Rapallo.

Costantini is a smiling, trouble-making, Instagram-posting, terrace-worshipping, Genoese-speaking farmer dedicated to expanding people's understanding and appreciation of where their food comes from. In his terraced land one encounters a diverse array of fruit, vegetables and grains, as well as donkeys, goats, sheep, chickens and bees. He considers himself an agroecological farmer and employs many of the major agroecological principles. He is also eager to continue to move in a more agroecological direction, and is constantly studying and discussing ways to improve his farming practices.

The majority of the products are grown as food for the restaurant and his family. The excess is sold directly to customers in the surrounding landscape, including shops, restaurants, individuals and groups. However, the farm is also considered a means by which to teach others. Costantini engages with schools, local events and curious individuals. His entire operation is based on relationships within the community. Materials, ideas and labor flow freely along the streets of San Massimo, connecting Costantini, the restaurant, the dairy farmer, the baker, the artists and the friends. On top of this, Costantini has tapped into the potential of social media and has built a significant network through his being an influencer. In conclusion, it can be said that though this one-hectare operation appears trivial to an untrained eye, the impacts of Fescion Farmer's work is clearly evident within the community surrounding it.

3.1.2 San Massimo

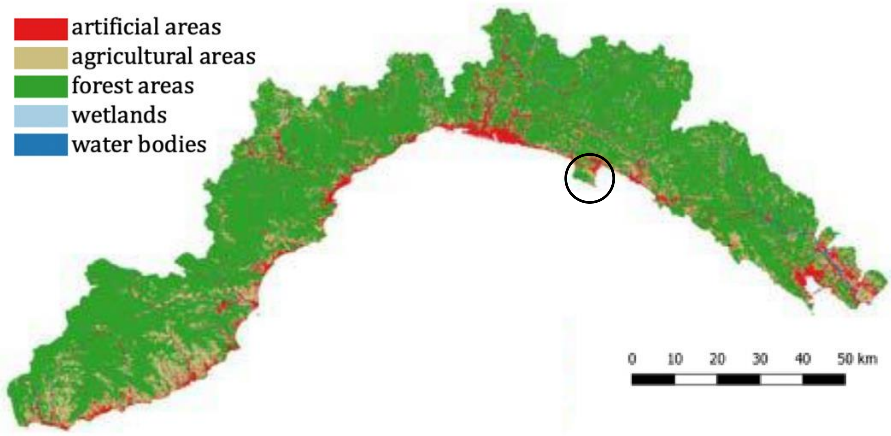
The town of San Massimo, where Fescion Farmer is located, is a microclimate unto its own, and must therefore be considered with special attention. It is located in the middle of a small peninsula on the Ligurian coast and contains a rich mixture of forest, urban and agricultural land (Regione Liguria, 2022). The land is characterized by the steep slopes of the Apenine Mountains descending down into the depths of the Mediterranean Sea. On the other side of the mountains is the Po Valley and Piedmont Region. This unique landscape results in a weather phenomenon that some refer to as the Genoa Low. The Genoa Low occurs as the warm, moist south-easterly flow over the Mediterranean converges with the cooler air from the north, which is funneled through the low passes of the mountains (Gallus, 2017). This convergence intensifies rainfall.

This year was particularly wet and humid (see appendix for weather history). There were two days of late hail, once on April 13th and once on May 12th. These events damaged the plants in the garden, including the tomatoes used in the experiment. The rains were so consistent that we only watered the tomatoes once in the whole season.

The region is also characterized by its terraced landscape. The mountainous region boasts terraces that date back centuries where they used to be studded with olive and chestnut trees. Now many of the terraces are abandoned and have been absorbed by nature. Wild boars pose a particularly irksome problem for farmers and homeowners alike. They rummage through the gardens and tear down the terrace walls, creating rubble that can be carried downhill by the heavy rains.

Figure 1 Liguria is located on Italy's North Coast (Liguria, Italy, n.d.)

Figure 2 San Massimo is Located on the peninsula just East of Genoa (Regione Liguria, 2022)

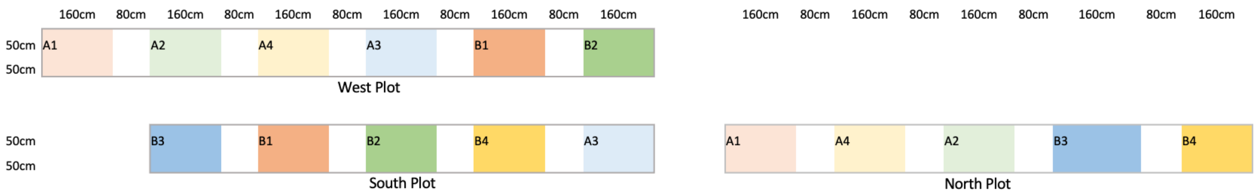


3.2 Materials and Methodology

3.2.1 Research Design: Field Experiment

The bulk of this thesis involves a field experiment that was conducted in San Massimo, Liguria. In this experiment, 160 tomato seedlings were grown in a greenhouse and planted in three outdoor plots on a single terrace (see image below for plot design). Half of these tomatoes were of the hybrid variety, "San Marzano," and the other half were a local variety of "Costoluto Genovese." The tomatoes were grouped by ten and distributed between the plots in a random order. On these tomatoes three different biostimulants were tested against each other and against a control. They received an inoculation of their prescribed stimulants every two weeks. During the course of the trial, data was collected for germination rate, dry root and shoot weights at transplant, height, and yield in number and weight. Foliar NIRS and pH analysis were also taken and a Brix test was conducted to estimate the sugar levels of the tomatoes. Litterbags were also used and analyzed to measure soil activity. For a more detailed explanation of the field experiment, refer to the experimental design in the appendix.

Figure 3 Out planting design: tomatoes were planted in groups of ten referred to in the paper as "blocks" and labeled according to their variety and treatment. There were three plots located on the same terrace where the tomatoes were randomly distributed.



3.2.2 Treatments and Tomato Varieties

Treatment 1: Compost Tea

The creation of the compost tea recipe is more of a story than a formula. Recipes were read (Darwish, 2013; Douds et al., 2016; Douds Jr. et al., 2010; Lowenfels & Lewis, 2010; McCoy, 2016), and conversations were had. It was a learn-as you go process, and creativity and ingenuity were definitely of use. A touch of sentimentalism also played a role, and an exploration of the past.

The base formula was one liter of mature compost submerged in 10 liters of water. The tea was then aerated with a fish pump for about 24 hours. On top of that, adjustments and additions were made based on the perceived situation with the aim to give the plants whatever was needed to help them grow.

The first three inoculations were created with the aim to establish a strong root system equipped with a host of native microorganisms including mycorrhizal fungi. This method was inspired by David Douds, Gopal Selvakumar, and Peter McCoy, though due to time limitations, their methods could not be followed exactly (Douds Jr. et al., 2010; McCoy, 2016; Selvakumar et al., 2016). The process was to collect soil samples from uncultivated or abandoned terraces nearby as well as from the forest. We took particular care to collect any visible mycelium we could find around plant roots.

As we got deeper into the brewing of the compost tea, the more creative we became. The tea became an embodiment of the farm. It told a story that coincided with Costantini's own history. Samples were taken from an abandoned cow pasture where he used to visit. Or from under the ancient terrace walls where he would play. For the third batch of tea, we took a piece of alder root. Costantini reminisced about how as a child he was fascinated by the red hue of the spindly wood. Now, as an adult and a farmer, he is more fascinated by the nodules that house the nitrogen fixing bacteria, *Frankia Alni.* We collected these roots per chance the actinomycete could aid our tomato plants.

In mid-June, the San Marzano tomatoes began showing signs of blossom-end rot, which can be a result of a calcium deficiency. In response, we added pulverized eggshells to the tea. I had also just read about the importance of sugar (typically molasses) to give the fungi a boost (Lowenfels & Lewis, 2010). As we do not grow sugarcane in San Massimo, we decided honey could be a good substitute. We therefore made a base compost tea that was enhanced with eggshells for calcium and honey for sugar.

As my writing has shown, the compost tea was a way for us to experiment and use our imagination. We took ideas from our surroundings and took the liberty to make it our own. As there is no strict recipe for compost tea, and as we were determined to make it from 100% farm material, it felt only right to customize it. We observed the plants, learned from other's and adapted to give the plants whatever we believed they needed most.

Treatment 2: Aquabella "Unleash"

Unleash is a bacteria-based plant biostimulant. It contains no mycorrhizal fungi, but it works in collaboration with the native fungi that is already present in the soil. Due to a lack of communication with the company, no more information about the makeup of this inoculant is available.

Treatment 3: Micosat F®

The third product used in this experiment is considered a microbial consortium (Micosat F®, by CCS – Aosta). A combination of three different products from this company were used for this experiement: Micosat F® MO, TAB Plus and LEN. The first product, Micosat F® MO, is specialized in developing an expansive root system. It was applied three times: once at seeding and then once for each transplant. The objective of Micosat F® MO is to expand the root system of the plant. Forty percent of the inoculant is made up of mycorrhizal fungi. The other sixty percent consists of other microorganism. The symbiotic fungi include: *glomus spp. GB 67; glomus viscosum GC 41; glomus mosseae GP 11.* Along with the symbiotic, this product also contains saprophytic fungi (*trichoderma harzianum TH 01, trichoderma viride TV 03, pochonia clamydosporia PC 50).* These fungi break down organic matter and unlock material otherwise unavailable to plants (McCoy, 2016). The *Trichoderma* species are also known to be mycoparasites and can act as a natural biocontrol for common plant infections such as *Botrytis* and *Fusarium* (Lowenfels & Lewis, 2010; McCoy, 2016). Beyond that they have been credited with enhancing the growth of the host plant (Lowenfels & Lewis, 2010, p. 127; McCoy, 2016, p. 315). The bacteria that is present in this inoculant include: *Bacillus amyloliquefaciens BA 41, Pseudomonas fluorescens PN 53,* *Pseudomonas sop. PT 65, Streptomyces spp. SB 14, Streptomyces spp. SA 51, Streptomyces spp. SL 81*. Actinomycetes (genus *Streptomyces*), are known to improve soil structure and decay cellulose and chitin, thereby increasing available carbon in the soil (Lowenfels & Lewis, 2010, p. 47). Both the *Trichoderma* and *Streptomyces* are claimed to stimulate root elongation and proliferation of new capillitium according to the catalog (*Catalogo MICOSAT 2021*, 2021).

Micosat F® TAB Plus is a foliar treatment that was applied every two weeks following outplant into the field. The same saprophytic fungi that are present in MO (*Trichoderma harzianum TH 01, trichoderma viride TV 03)* are also present in TAB stimulating plant growth and improving the mineral salt absorption efficiency (*Catalogo MICOSAT 2021*, 2021). Though only ten percent of the product is mycorrhizal fungi, there is still a good variety present, including: *Glomus coronatum GU 53, Glomus caledonium GM 24, Glomus mosseae GP 11, Glomus viscosum GC 41,* and *Rhizophagus irregulans RI 31*. Just as with MO, this product contains a range of bacteria as well. There is actinomycetes (*Streptomyces spp. SB 19),* ascomycetes (*Pichia pastors PP 59),* and *Bacillus amyloliquefaciens BA 41*. These bacteria act as biological control, work to improve soil health, and aid in plant protection and development.

In combination with Micosat F® TAB plus, Micosat F® LEN was applied to the leaves as well. This product is recommended to be used in combination with TAB Plus as the synergies created between the microorganisms multiplies their affects. LEN was intended to help plants overcome stress through the improvement of the root system. The logic is, the more developed the root system is, the better a plant can handle damage or stress. The arbuscular mycorrhizal fungi include *Funnelformis coronatum GU 53, Funnelifornis caledonium GM 24, Funneliformis mosseae GP 11, Septoglomus viscosum GC 41* and *Rhizophagus inegulars RI 31*. The saprophytic fungi include *Pochonia chlamydosporia PC 50*. The bacteria component is made up of *Streptomyces averitilis SC 43, Streptomyces spp. SL81,* and *Bacillus firmus BF 90*.

Tomato A: San Marzano

The other variety I will use is a variety of plum tomato called San Marzano F1. This tomato is a hybrid variety originated in Campania. It started to gain popularity in the 1800s as a key ingredient of the classic Neapolitan pizza. It continued to grow in popularity when the first cannery opened in the region, allowing the tomato to be shipped and sold all across the continent (*San Marzano tomatoes)*. In 1996 the peeled, canned tomatoes earned a DOP label for specifically the Pomodoro San Marzano dell'Agro Sarnese-Nocerino 2 and/or KIROS varieties (*Commission Implementing Decision of 8 April 2019 on the Publication in the Official Journal of the European Union of the Application for Approval of an Amendment, Which Is Not Minor, to a Product Specification Referred to in Article 53 of Regulation (EU) No 1151/2012 of the European Parliament and of the Council for the Name 'Pomodoro S. Marzano Dell'Agro Sarnese-Nocerino' (PDO)*, n.d.). We are not working with the PDO varieties, though the history of the crop is still quite relevant and that is why I have included it in this section.

Tomato B: Costoluto Genovese

Pomodoro Costoluto Genovese *(Lycopersicon lycopersicum)* is a variety of tomato from the region where the research is being conducted. Cultivation of this tomato in Liguria presumably dates back to the 19^{th} century. It has a bright red, round fruit with a ribbed surface. It is often praised by nurseries and seed shops for being hardy and resistant to pests and diseases (*Pomodoro Costoluto Genovese (lycopersicon lycopersicum); Pomodoro Costoluto genovese - 1 gr.)*.

3.2.3 Data Collection

Germination Rate

Four weeks after the tomato seeds were sown, the seedlings were transplanted into larger cells of 5 cm x 5 cm. At this time the germination rate was calculated.

Early Root Development

As 30 plants of each trial was seeded, but only 20 were transplanted, we were able to sample the extra plants for root development. Two plants from each trial that appeared the strongest were taken and dried in the sun for 48 hours. Once dried, the total weight of the plant was taken. Then the plant was cut and the root and shoot each weighed separately.

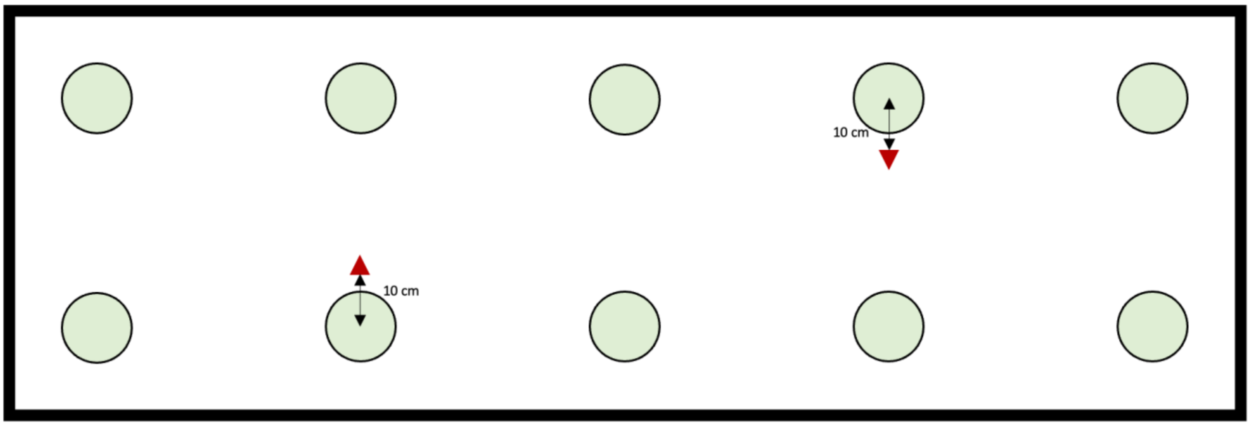
Height and Other Observations

Every one to two weeks after transplant the height was measured in centimeters. A growth curve was created based on the averages to capture plant development. General observations of the plants and the ecosystem were also taken and recorded.

Litterbags

The litterbag-NIRS method developed by Masoero et al. was employed to capture soil activity during the experiment (Masoero et al., 2018, 2023). Fifteen days after transplant 60 litterbags were placed in the beds with the tomatoes. Each litterbag contained one rooibos tea bag, one green tea bag, and one mesh bag filled with dried and shredded hay. The three bags were enclosed in a permeable sachet and marked with a paper tag. Once created, the bags were shipped by Masoero to San Massimo where they were distributed among the beds. Each block had two bags that were buried 12 cm deep and 10 cm from the base of one of the tomato plants.

Figure 4 Diagram of how litterbags were distributed in a single block of ten. The same pattern was followed for all 16 blocks.



After 60 days the bags were collected and dried in the sun for 24 hours. Once dried, the bags were opened and weighed. They were then sent to Dr. Giorgio Masoero for further testing. For more information on procedure and analysis see Masoero et al. (2023).

Foliar Measurements

On July 25th one leaf of at least 15cm in length was taken from each plant and sent to Dr. Giorgio Masoero for testing. The leaves were analyzed for pH and NIR spectroscopy (Giovannetti et al., 2019; Masoero et al., 2023).

Yield and Fruit Quality Measurements

Once the tomato fruits reached full maturity, harvests took place once a week. The tomatoes from each individual plant were collected, counted and categorized into two groups:

sellable and unsellable. The total weight for each group of each plant was then recorded in grams.

All the tomatoes from each block were then grouped together in plastic bags and transported to a room where they could cool to about 20° C. Three to four of the best-looking tomatoes were selected from the bag and blended into a liquid to be tested for sugar content, expressed as a Brix° level. A portable refractometer from GrandBeing® (USA) with a measuring range of 0-90% was used to calculate the Brix° level. The test was repeated three times for each sample to minimize error.

Statistical Analysis

All of the statistical analysis was conducted by Dr. Giorgio Masoero. In general the Linear Discriminant Analysis (LDA) was used to test for statistical significance with a threshold of 25%.

3.3 Boundaries and Limiting Factors

The primary limitation faced by this thesis is lack of time. The seemingly most successful trials for collecting and inoculating local mycorrhiza take over a year to prepare (Douds Jr. et al., 2010; McCoy, 2016). Therefore, shortcuts had to be taken that could possibly limit the success of AMF colonization. The other factor that was constrained by time was data collection. As tomato plants can live through until October or November, data collection, especially on the fruits, could also be extended until the fall. This would give a more complete and accurate picture of the yield and quality measurements. Given the circumstances however, data collection has been limited to early harvest.

The second limiting factor is related to resources and competences. I have little to no background in lab work and field work related to biology, and therefore I had to rely on the help and guidance of others. I also had no direct access to a lab which also limited what kind of data I could collect. Luckily, my professor Paola Migliorini connected me to Dr. Giorgio Masoero, who could analyze the leaves and fruit. His support throughout the thesis was crucial, and the resulting paper wouldn't be nearly as significant without his input. That said, there are certain tests that could have been interesting for this thesis if all resources were attainable. Two factors that I think would have been particularly interesting would be to run a sensory analysis test for a deeper and more developed understanding of the differences in fruit quality, and to test for AMF root colonization. To test for root colonization would be helpful to know if arbuscular mycorrhizal fungi actually played a role at all. By understanding if colonization occurred and to what extent, we could better understand the importance of this particular microorganism.

4. Results

Germination Rate

The San Marzano F1 had a higher germination rate of 94% compared to that of the Costoluto Genovese, which was only 88%. The seeds inoculated with compost tea had the lowest germination rate of 83% and the Control had the highest with 95%. The table below shows the full results.

Table 1 Germination rate of tomato seedlings

Variety	Treatment	Germination Rate
San Marzano	Compost Tea	28/30
San Marzano	Unleash	27/30
San Marzano	Micosat	29/30
San Marzano	Control	29/30
Costoluto Genovese	Compost Tea	22/30
Costoluto Genovese	Unleash	29/30
Costoluto Genovese	Micosat	27/30
Costoluto Genovese	Control	28/30

Unforeseen Circumstances – *Didymella lycopersici*

In mid-June the tomato plants began showing signs of a fungal infection called *Didymella lycopersici*. This fungal infection was fairly common in the early part of the 20th century, though since then infection rates have slowed significantly. The first sign of infection is the appearance of a dark brown lesion girdling the base of the stem just above soil level. Secondary lesions occur higher up the stem and from there the infection spreads to the leaves and fruits as well, making the fruits inedible (Sheard, 1943). Once infected, the chance for survival is slim, but the life of the plant can be prolonged through careful pruning. The fungus thrives in a warm, moist environment, and can spread during rainfall. It was therefore crucial to keep space between the plants and to prune to allow for airflow.

It is impossible to know for certain where the fungus emerged from. It could have come from the seeds or the canes (Sheard, 1943). It could have also come from one of the inoculants, though the chances are low as neighboring farms and others in the Rapallo area also reported cases of infection. It was a particularly wet year (see appendix for weather reports), and the tomatoes experienced a number of heavy storms including one hail storm. It is suspected that these storms could have opened wounds, leaving the plant vulnerable to the fungus (Croxall et al., 1957). Similarly, many signs of infection were observed at the points where the plant was tied to the cane (see image below). This is because often when the stems are tied, they get cut in the process, leaving potential openings for infection.

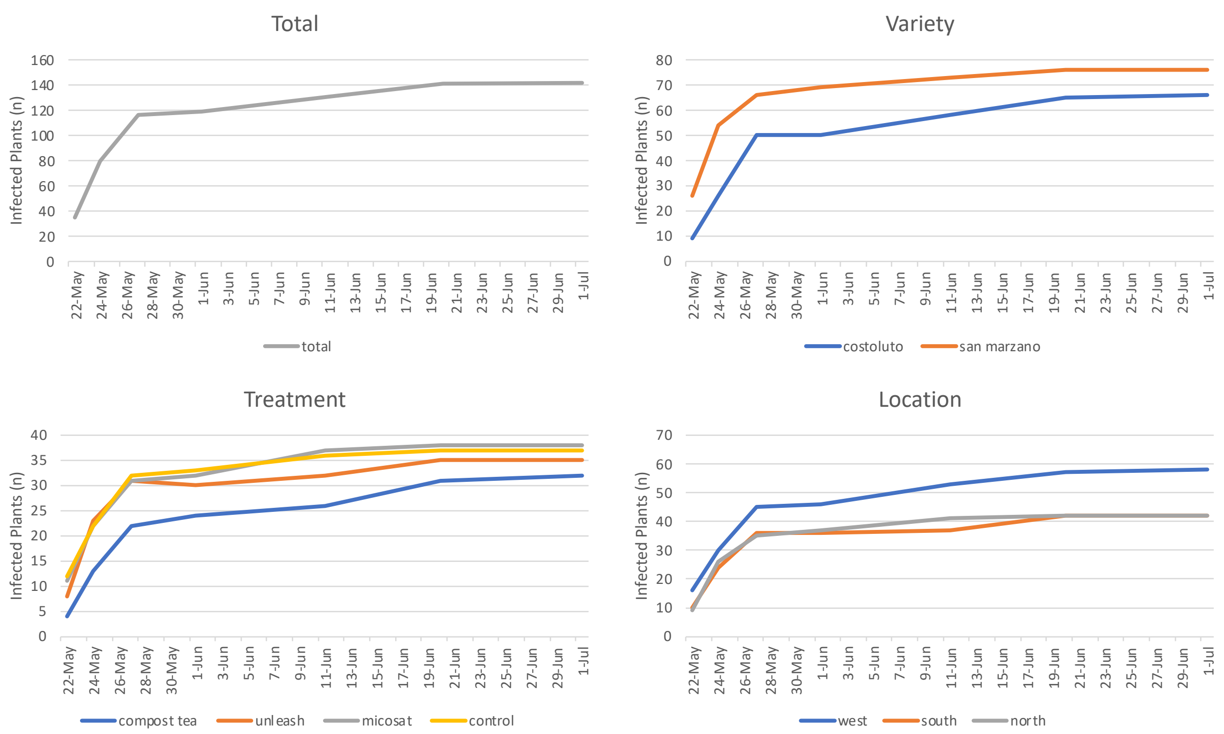
Figure 5 Image of stem lesions caused by *Didymella lycopersici*

The infection spread quite rapidly, starting with two plants suddenly dead on May 22nd, and ending with a total of 29 dead, or 18%, on July 19th. At the last recording of stem infection taken on July 1st, 89% of the plants were either dead or infected. It's assumed that the spread of the disease would have most likely continued if it weren't for the experiment getting cut short for unrelated reasons. However, the spreading of the infection did slow near the end of the trial, as represented in the first set of graphs depicted below.

Table 2 Number of plants infected with *Didymella Lycopersici* identified by presence of at least one stem lesion

variety	22-May	24-May	27-May	1-Jun	11-Jun	20-Jun	1-Jul
costoluto	9	26	50	50	58	65	66
san marzano	26	54	66	69	73	76	76
total	35	80	116	119	131	141	142
treatment	22-May	24-May	27-May	1-Jun	11-Jun	20-Jun	1-Jul
compost tea	4	13	22	24	26	31	32
unleash	8	23	31	30	32	35	35
micosat	11	22	31	32	37	38	38
control	12	22	32	33	36	37	37
total	35	80	116	119	131	141	142
location	22-May	24-May	27-May	1-Jun	11-Jun	20-Jun	1-Jul
west	16	30	45	46	53	57	58
south	10	24	36	36	37	42	42
north	9	26	35	37	41	42	42
total	35	80	116	119	131	141	142

Figure 6 Number of plants infected with *Didymella Lycopersici* identified by presence of at least one stem lesion

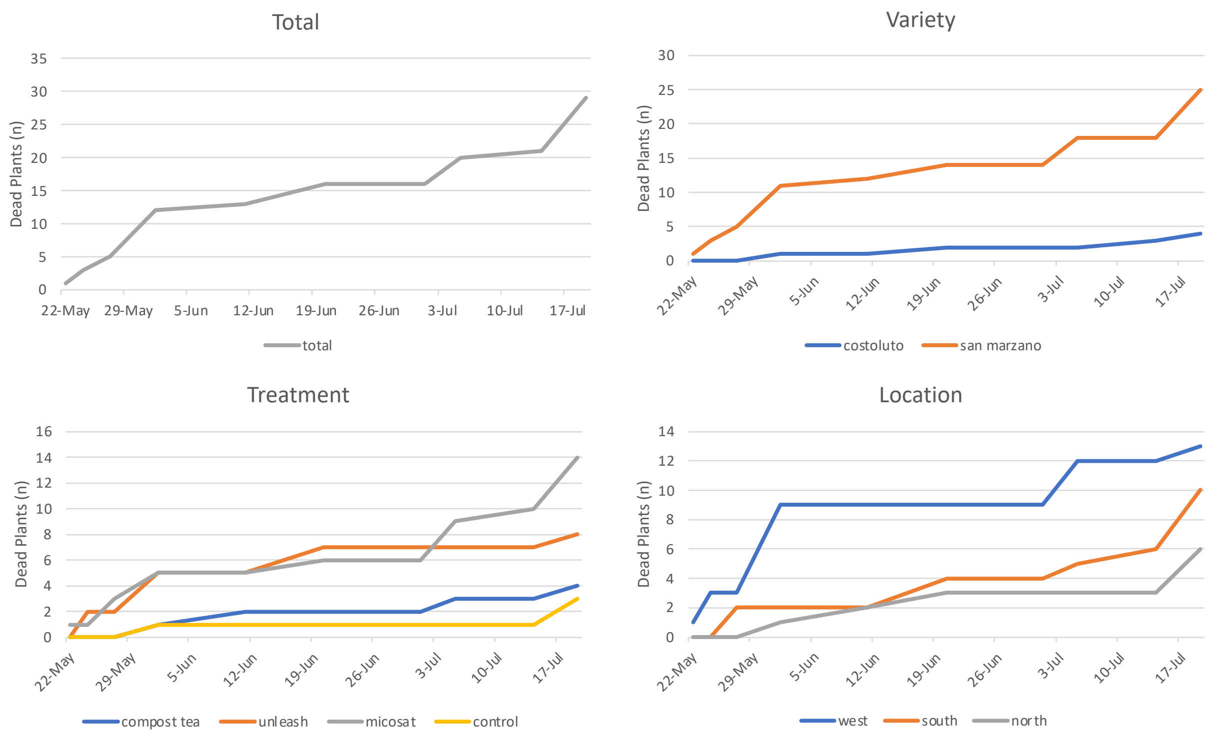


Though unintended, this event did lead to some interesting results that can be considered relevant to this thesis. The graphs represented above in Figure 3 show that the plants treated with compost tea had the lowest rate of infection. The local variety, called here “costoluto,” also remained significantly less infected than the hybrid “san marzano.” The results presented in table 2 show that the local variety was the least affected by the infection, showing only 4 deaths compared to the 25 of the hybrid. There is also a significant difference in the survival rate depending on the inoculation used. The control and the compost tea had the highest rate of survival and the Micosat the lowest. The west plot had the highest number of infections and deaths, which can probably be attributed to it being more shaded and protected from the wind than the others. It is interesting however to note that this plot had two blocks of plants treated with compost tea, which was one of the most resilient treatments to the fungal infection.

Table 3 Number of tomato plants that have died due to *Didymella Lycopersici* infection

variety	**22-May**	**24-May**	**27-May**	**1-Jun**	**11-Jun**	**20-Jun**	**1-Jul**	**5-Jul**	**14-Jul**	**19-Jul**
costoluto	0	0	0	1	1	2	2	2	3	4
san marzano	1	3	5	11	12	14	14	18	18	25
total	**1**	**3**	**5**	**12**	**13**	**16**	**16**	**20**	**21**	**29**
treatment	**22-May**	**24-May**	**27-May**	**1-Jun**	**11-Jun**	**20-Jun**	**1-Jul**	**5-Jul**	**14-Jul**	**19-Jul**
compost tea	0	0	0	1	2	2	2	3	3	4
unleash	0	2	2	5	5	7	7	7	7	8
micosat	1	1	3	5	5	6	6	9	10	14
control	0	0	0	1	1	1	1	1	1	3
total	**1**	**3**	**5**	**12**	**13**	**16**	**16**	**20**	**21**	**29**
lcoation	**22-May**	**24-May**	**27-May**	**1-Jun**	**11-Jun**	**20-Jun**	**1-Jul**	**5-Jul**	**14-Jul**	**19-Jul**
west	1	3	3	9	9	9	9	12	12	13
south	0	0	2	2	2	4	4	5	6	10
north	0	0	0	1	2	3	3	3	3	6
total	**1**	**3**	**5**	**12**	**13**	**16**	**16**	**20**	**21**	**29**

Figure 7 Number of tomato plants that have died due to *Didymella Lycopersici* infection



Height

From the time of transplanting into the field until the second-to-final harvest on July 19th, the height of the plants was recorded every two weeks. This data is somewhat compromised and must be looked upon with scrutiny as the plant height was influenced by the infection. Occasionally, a stem lesion would result in the top half of a plant breaking off, in which case we would resort to the secondary leaves to become the primary plant stalk. Despite this setback however, the average plant height grew at a predictably stable curve (as seen in the graphs in figure 8). From this data it appears that the *San Marzano* grew to be taller in the end, which can likely be attributed to a physical characteristic of the variety. Of the treatments, all the plants averaged similarly, although the Micosat did end up being shorter in the end. There is some variability by location, though it is difficult to understand if the cause is environmental or due to the treatment spread.

Figure 8 Average plant height over time measured in centimeters.

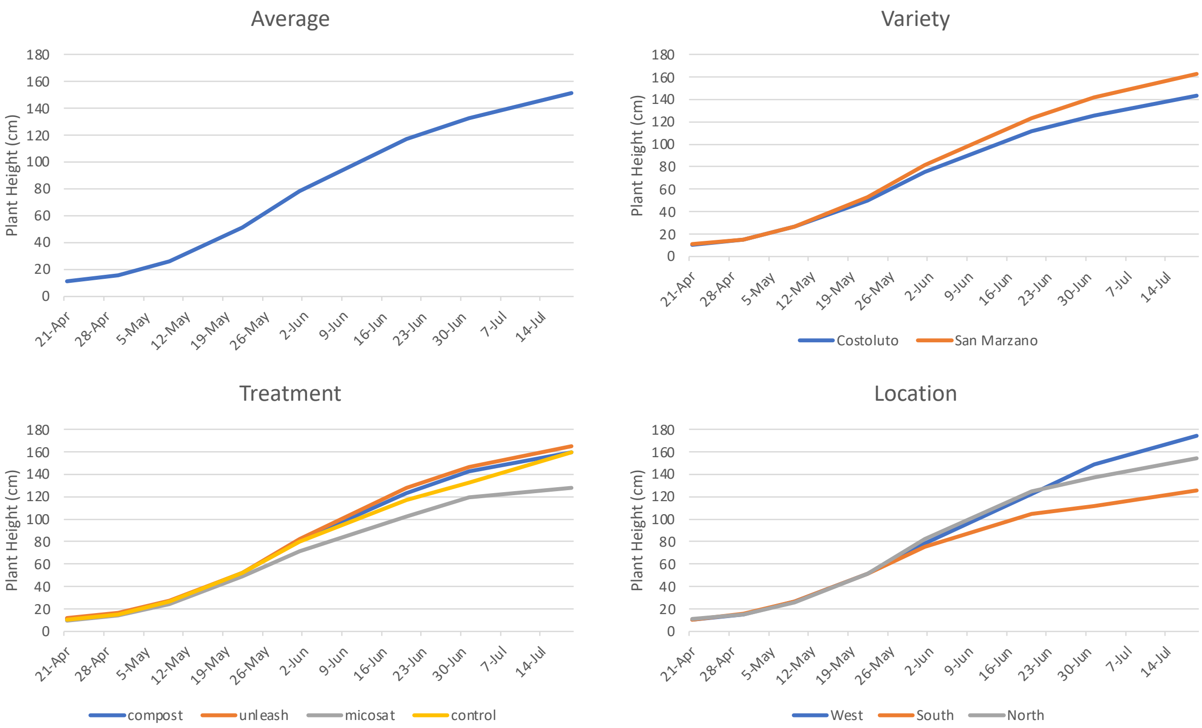


Table 4 Average plant height over time measured in centimeters.

Average Height								
Variety	21-Apr	30-Apr	9-May	22-May	1-Jun	20-Jun	1-Jul	19-Jul
Costoluto	10.376	15.16	26.173	49.405	75.004	111.829	125.76	143.45
San Marzano	11	15.28	26.435	53.125	81.853	123.235	141.517	162.371
Treatment	21-Apr	30-Apr	9-May	22-May	1-Jun	20-Jun	1-Jul	19-Jul
compost	11.183	15.508	26.138	51.97	80.35	123.171	142.436	159.535
unleash	11.443	16.098	27.515	51.828	82.553	127.589	146.274	164.774
micosat	9.64	14.135	24.423	49.216	71.271	102.319	119.169	127.719
control	10.488	15.138	26.625	52.048	79.541	117.052	132.753	159.613
Location	21-Apr	30-Apr	9-May	22-May	1-Jun	20-Jun	1-Jul	19-Jul
West	10.587	15.113	26.517	51.116	78.066	122.491	148.669	174.065
South	10.264	15.382	26.456	51.04	75.052	104.343	111.764	125.919

North	11.234	15.184	25.484	51.67	82.267	124.771	137.474	154.515
Total	21-Apr	30-Apr	9-May	22-May	1-Jun	20-Jun	1-Jul	19-Jul
	10.695	15.226	26.152	51.275	78.462	117.202	132.636	151.5

Yield

On July 5th the first round of tomatoes were harvested. Then they continued to be harvested once a week for a total of four weeks. As the tomato plants had been significantly affected by the infection of *didymella lycopersici*, so was the yield and fruit quality. This factor has been taken into consideration when evaluating the results and explains why it made the most sense to calculate the average productivity per plant as opposed to the total of each collection of plants. It also explains why the tomatoes were categorized into the two groups of sellable and unsellable. In figure 6, an example of what is considered sellable and what is considered unsellable is depicted.

Figure 9 Example of sellable (left) and unsellable (right) tomatoes of the Costoluto Genovese variety

The average total yield showed that the hybrid variety “San Marzano F1” was significantly more productive than the local variety “Costoluto Genovese.” It also shows the north block to be more productive than the other two plots. This could be attributed to the north block receiving significantly more sun and exposure due to wind. The other two blocks were in the shade of an old cherry tree and where therefore more hidden from the sun. The data shows no treatment to be significantly more successful than the others, though the Micosat averaged to be least productive and the Compost Tea appeared to produce the most when recorded in weight. Figure 10 provides a visual of the data. Take special notice of the tomatoes treated with unleash and how in the graphs it appears that these plants produced more tomatoes, but at a lesser weight compared to the rest. This is a point to be considered when we address quality and what the intended characteristics of the product are.

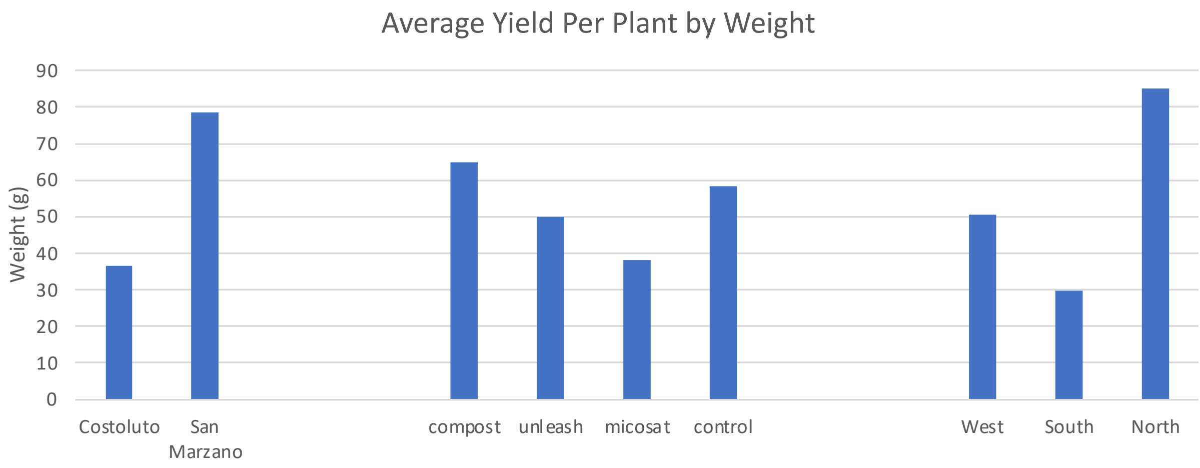
Table 5 Average yield per tomato plant measured in grams. The data is broken down into three tables: total, sellable and unsellable.

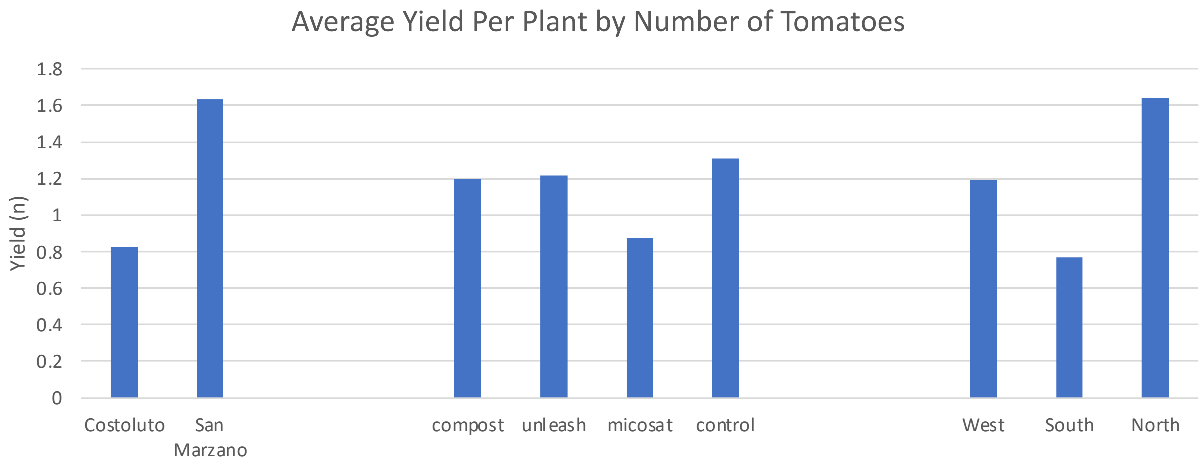
Average Yield Weight				
Variety	5-Jul	14-Jul	19-Jul	25-Jul
Costoluto	47.84	38.86	17.62	42.39
San Marzano	74.44	50.08	36.96	152.31
Treatment	5-Jul	14-Jul	19-Jul	25-Jul
compost	63.23	46.78	41.28	108.48
unleash	58.94	44.42	19.34	77
micosat	38.94	28.9	19.93	64.55
control	72.36	52.67	20.7	87.61
Location	5-Jul	14-Jul	19-Jul	25-Jul
West	67.69	26	20.12	88.15
South	43.18	22.91	18.29	34.09
North	74.19	85.06	38.6	141.95

Average Weight Sellable				
Variety	5-Jul	14-Jul	19-Jul	25-Jul
Costoluto	16.31	23.62	17.37	42.39
San Marzano	40.79	32.66	28.86	152.31
Treatment	5-Jul	14-Jul	19-Jul	25-Jul
compost	40.94	35.24	38.33	108.48
unleash	21.12	28.91	18.31	77
micosat	18.1	10.64	18.89	64.54
control	25.87	33.28	12.43	87.61
Location	5-Jul	14-Jul	19-Jul	25-Jul
West	8.76	13.42	18.86	88.85
South	13.82	12.82	17.27	34.1
North	58.94	57.94	32.64	142.02

Average Weight Unsellable				
Variety	5-Jul	14-Jul	19-Jul	25-Jul
Costoluto	31.54	15.23	0.25	0
San Marzano	33.66	17.41	8.11	0
Treatment	5-Jul	14-Jul	19-Jul	25-Jul
compost	22.78	11.54	2.94	0
unleash	37.81	15.79	1.03	0
micosat	20.87	18.26	1.04	0
control	46.49	19.38	8.27	0
Location	5-Jul	14-Jul	19-Jul	25-Jul
West	58.93	12.58	1.26	0
South	29.35	10.09	1.02	0
North	15.26	27.13	8.59	0

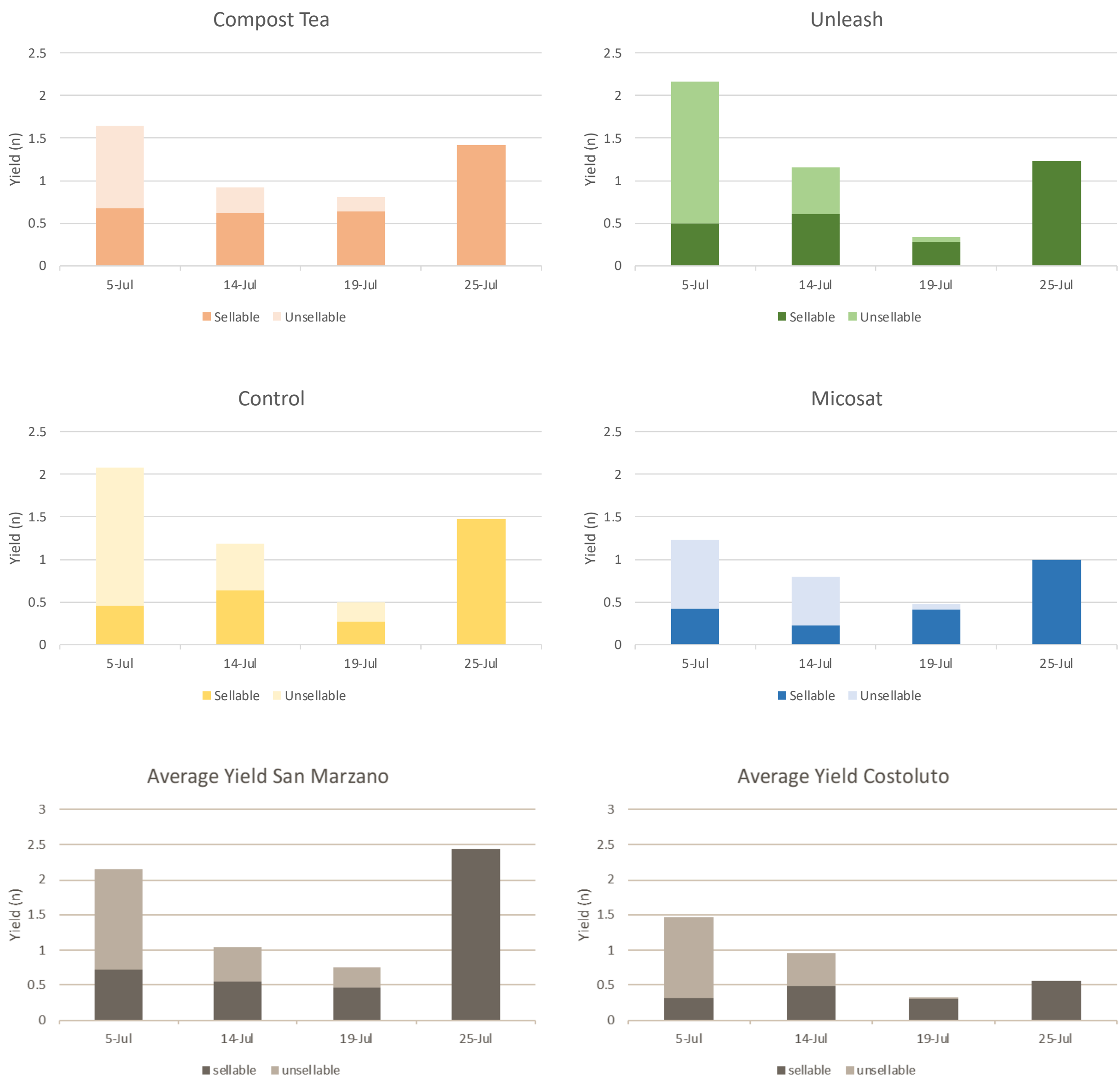
Figure 10 Average yield per tomato plant measured by total weight and number.

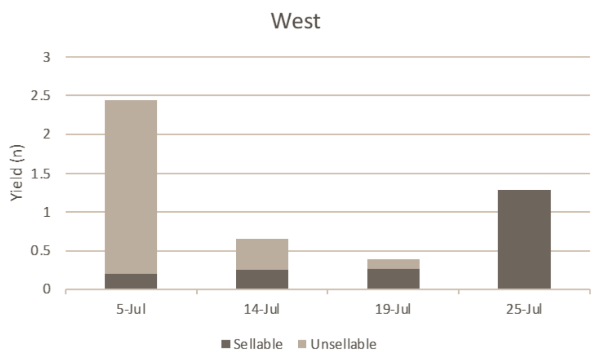


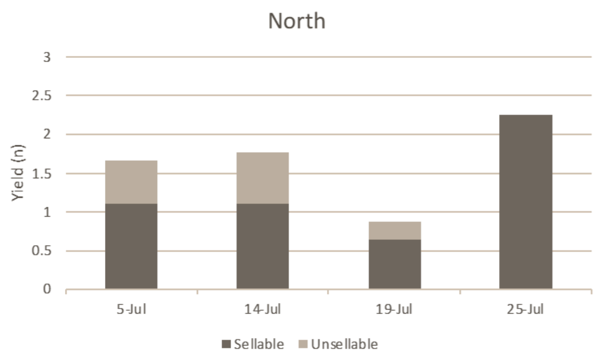


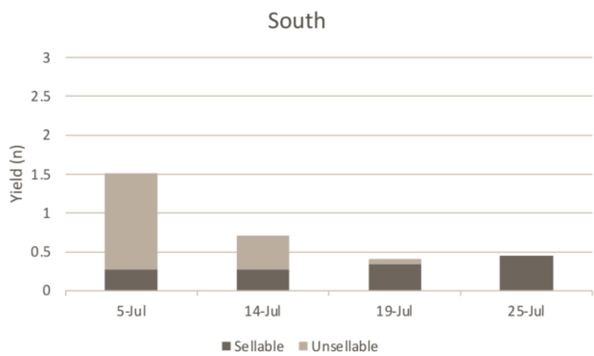
Another interesting outcome taken from this data is the percentage of the yield that is damaged to the point of being unsellable. The data shows this percentage to decrease over time as the number of sellable increased (see table 4). By the last harvest on July 25th, there was not a single unsellable tomato collected. Throughout the month however, it is interesting to see how the control had the highest number of unsellable tomatoes and the compost tea the lowest. This is however congruent with the number of plants infected with the disease as the compost tea was one of the lowest and the control the highest. See the graphs below for a full depiction of the results.

Figure 11 Average sellable and unsellable yield per tomato plant in number over time. The graphs are organized into treatment, variety and location respectively.









Foliar pH and NIR Spectra

The average pH of the leaves tested ranged from 5.56 to 5.80. The maximum foliar pH for control decreased by 2% (calculated as ln(treatment/control)) with a treatment of compost tea and Micosat. The level decreased by 4% for leaves treated with Unleash. A linear discriminant analysis (LDA) classifies the discriminatory capability of the compost tea and Micosat treatments to be minimal. The higher pH of the control has a better classification of 33.33% and the Unleash treatment appears to be the best with a discriminatory capability classification of 60.75%.

With regards to the NIR spectra tests, the Micosat and compost tea treatments are correctly classified. However, the control and the Unleash groups are significantly under classified indicating them to be more dispersed and variable. This can be attributed to the Micosat and compost tea leaves having reinforced the phenotype affinity and homogeneity whereas the Unleash and Control groups are composed of more dispersed heterogenous phenotypes.

Figure 12 ANOVA results of the Foliar pH according to the Treatments (N= 1088)

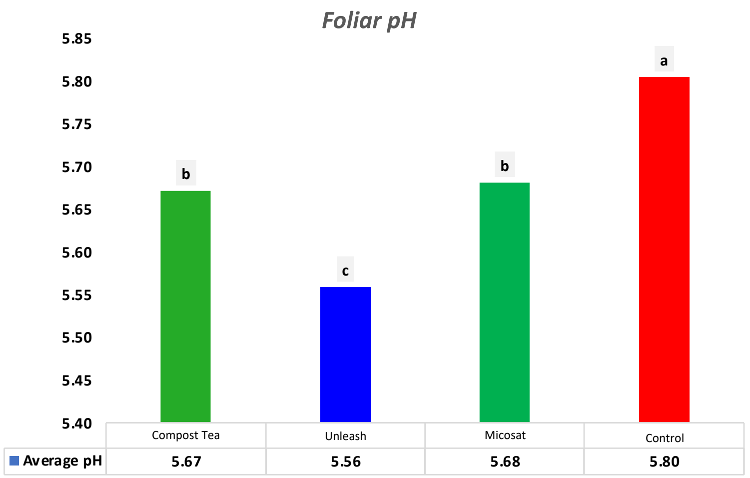


Figure 13 Linear discrimination of the Treatments based on the Foliar pH values

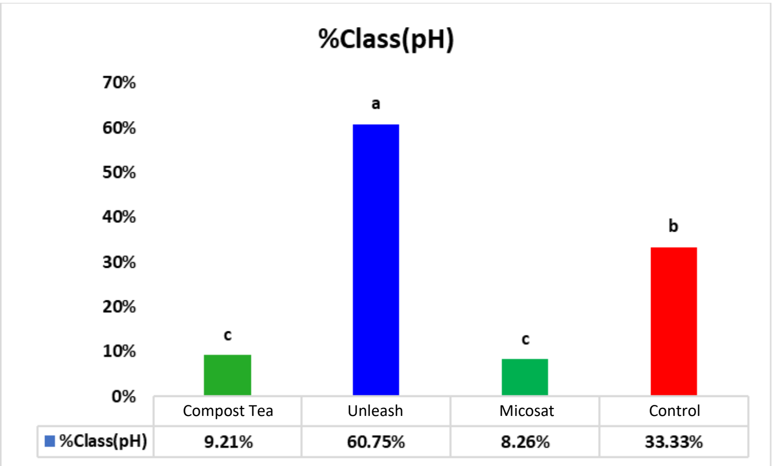
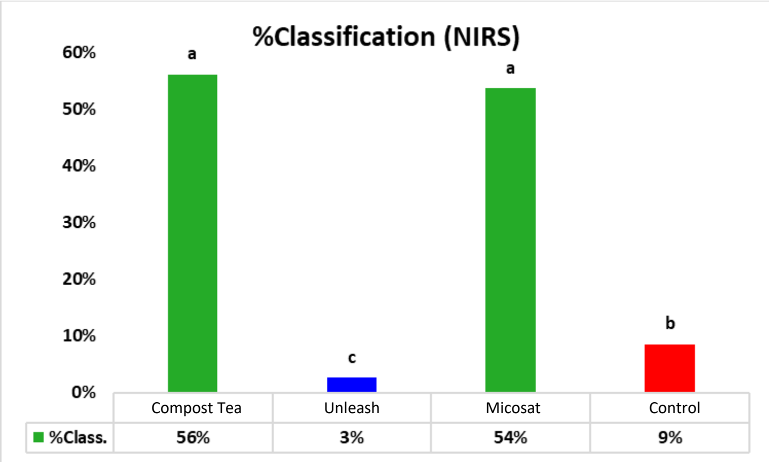


Figure 14 Classification % of the Treatments based on the partial Least Squares Discrimination (PLS-D) of the NIR spectra (N=1088)



Fruit Quality Analysis

A one-time collection of fruit was delivered to Dr. Giorgio Masoero on July 25th containing three tomatoes from each block. On these tomatoes Masoero was able to test the pH, Brix and NIR spectra. Together, this data sheds some light on the quality of the tomato fruits and how variety and treatment can affect said quality. Figure 12 illustrates the interaction between the treatment and variety in regards to sugar level, measured as Brix°. This figure is interesting as it reveals the lack of clear discrimination between either the treatment, variety, or combination of the two. The *Costoluto Genovese* x Control reported the highest Brix° of 7.0, but the second highest comes from *San Marzano* x Unleash. The Compost Tea seems to have done well with both varieties, displaying a more consistent Brix° level across the board. This lacking of a trend implies that neither the treatment nor the variety have an impact on the sugar level of the tomato fruit. However, it would be interesting to see more tests conducted on the fruit to see if one variety has a greater affinity towards a particular treatment.

For the duration of the harvest time, Brix° was also measured on site using a refractometer. From this data it is also difficult to draw any substantial conclusions. Though it is interesting to note that the *Costoluto Genovese* had a significantly higher reading of 4.897 than the *San Marzano,* which was 4.29. The control tomatoes and those treated with Unleash were also significantly higher than those treated with Micosat or compost tea. Finally, the tomatoes from the North plot had a higher reading of 4.817 compared to the other two which were only at 4.573 and 4.253. This difference could be attributed to the North plot being less shaded.

Figure 15 Significant interaction between treatment and variety in the Brix°

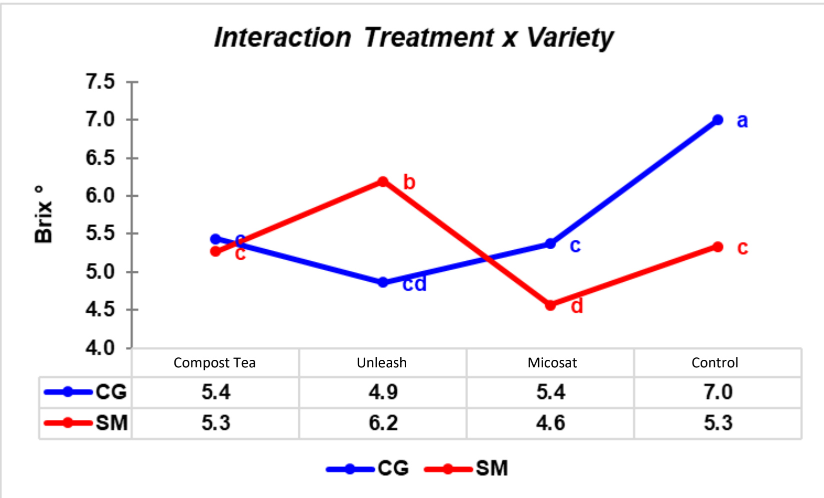


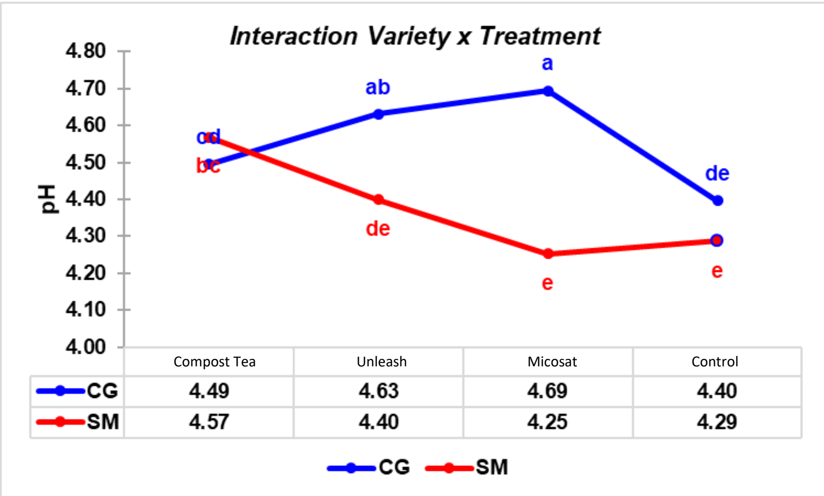
Table 6 Brix° readings taken on-site over the course of four weeks.

Variety	**5-Jul**	**14-Jul**	**19-Jul**	**25-Jul**	**AVG**
Costoluto	4.395	4.834	5.547	4.813	4.89725
San Marzano	4.561	3.904	4.146	4.549	4.29
Treatment	**5-Jul**	**14-Jul**	**19-Jul**	**25-Jul**	**AVG**
Compost Tea	4.083	3.96	4.915	4.583	4.38525
Unleash	4.625	4.58	5	4.625	4.7075
Micosat	4.415	4.223	4.168	4.723	4.38225
Control	4.79	4.793	4.75	4.835	4.792
Location	**5-Jul**	**14-Jul**	**19-Jul**	**25-Jul**	**AVG**

West	4.28	3.9	3.97	4.862	4.253
South	4.33	4.333	4.88	4.75	4.57325
North	4.867	4.965	5	4.434	4.8165

	5-Jul	**14-Jul**	**19-Jul**	**25-Jul**	**AVG**
Average	4.48	4.4	4.617	4.689	4.5465

Figure 16 Significant interaction between treatment and variety in the pH (N = 336)



A similar analysis was made looking at the interaction between the variety and the treatment with regards to the pH value. Here it is clear that the *Costoluto Genovese* variety has a higher pH value in general. When treated with commercial biostimulants the pH seemed to be higher compared to the control or those treated with compost tea. The treatments had a different effect on the San Marzano variety, where the compost tea created a significantly higher pH and the Micosat and control created the lowest pH.

Tables 4 and 5 below summarize the data collected through NIR spectroscopy. This data implies that the variety is the most significant variable in the variation of the NIR spectra (R^2cv = 0.66). We can go on to infer that the position in the plots is only relevant for the *Costoluto Genovese* variety (R^2cv = 0.51), and the weight and Brix° are relevant to the *San Marzano* variety

(R^2cv = 0.65 and R^2cv = 0.68 respectively). The treatment is not relevant when all the data is pooled together (R^2cv = 0.03), however it becomes relevant once considered for each variety separately, where the R^2cv rises to 0.44 and 0.45 for each variety respectively.

Table 5 goes on to consider the average classification of the NIR spectra of the four treatments by their variety. What this table shows is that the classification is equivalent for all the treatments, but significantly higher for the *San Marzano* (79% as opposed to 57% for *Costoluto Genovese*). Thus, it can be inferred that each treatment has a significant fingerprint that is more pronounced in the *San Marzano* tomatoes.

Table 7 Performances of the NIR spectroscopy in fitting the variables of the fruits.

Constituent	N	Mean	SD	SECV	R^2cv	P
Treatment (1-4)	324	2.42	1.10	1.08	0.03	0.0011
Variety	289	1.55	0.50	0.29	0.66	<0.0001
NSW	305	2.14	0.85	0.73	0.25	<0.0002
pH	297	4.46	0.20	0.17	0.26	<0.0003
Weight	299	59.79	19.55	16.54	0.29	<0.0004
Brix	141	54.60	7.33	5.92	0.34	<0.0005
Costoluto Genovese						
Treatment (1-4)	147	2.42	0.98	0.72	0.45	<0.0005
NSW	145	2.05	0.67	0.47	0.51	<0.0005
pH	139	4.56	0.13	0.13	0.00	1.00
Weight	139	61.35	23.16	20.77	0.19	<0.0005
Brix	61	53.52	7.11	7.00	0.03	0.19
San Marzano						
Treatment (1-4)	151	2.47	1.14	0.85	0.44	<0.0005
NSW	168	2.17	0.99	0.99	0.00	0.6066

pH	147	4.36	0.20	0.18	0.15	<0.0005
Weight	153	58.48	16.24	9.64	0.65	<0.0005
Brix	76	54.41	7.68	4.35	0.68	<0.0005

Table 8 Average classification % of the four treatments according to the NIR spectra of the fruits in the two varieties (N = 336).

from \ to	***Costoluto Genovese***	**% Classification**	***San Marzano***	**% Classification**	**P (diff)**
1_Compost Tea	37	59%	48	77%	0.0765
2_Unleash	46	57%	50	78%	0.0285
3_Micosat	51	55%	24	79%	0.0465
4_Control	30	57%	50	82%	0.0158
Total	164	57%	172	79%	<0.0001

All values are significant at P<0.05 *vs*. threshold 25%

Litterbags and Tea Bags

The four methods applied to study the bioactivity of the soil include an NIR spectra test of the green tea bag, the rooibos tea bag and the hay-filled bag. The total weight of the three bags combined was the fourth form of data collection. The NIRS methods produce similar results regardless of the bag filling. The average classification determined by weight is nearly half as efficient as any of the NIRS methods and cannot be classified as significantly different from the threshold of 25%.

When organized by treatment, the litterbags revealed the control group to be the most uniform. The control group showed a 63% classification, where the other treatments ranged from 35-40%.

Finally, it can be concluded that the plant genotype modifies the bioactivity of the soil. The variety of the tomato changed the weight of the rooibos and green tea bags as well as the NIR spectra of all three bags. Except for the hay litterbags placed with the *Costoluto Genovese* variety, all other classifications are significantly higher than the threshold of 50% (P<0.05). The *San Marzano* variety appears to have a more consistent classification across the board as opposed to the *Costoluto Genovese*. However, the green tea bag placed with the *Costoluto Genovese* variety does have the highest classification of 80%.

Figure 17 Average Classification % of the four methods applied to the study of the soil bioactivity (N = 422).

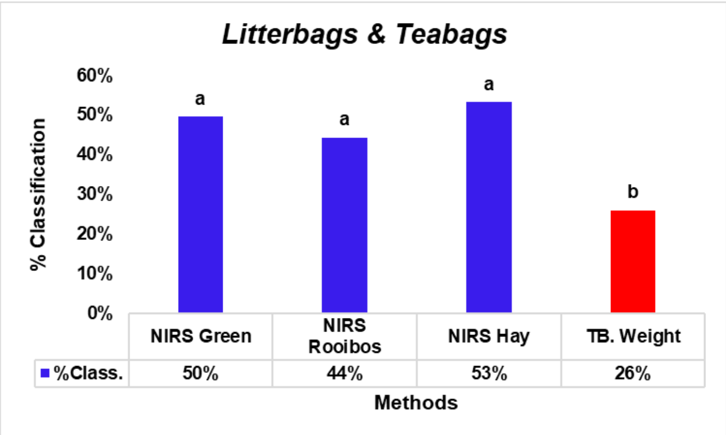


Figure 18 Average Classification % of the Treatments according to Litterbags and Teabags NIRS and Weight (N = 422).

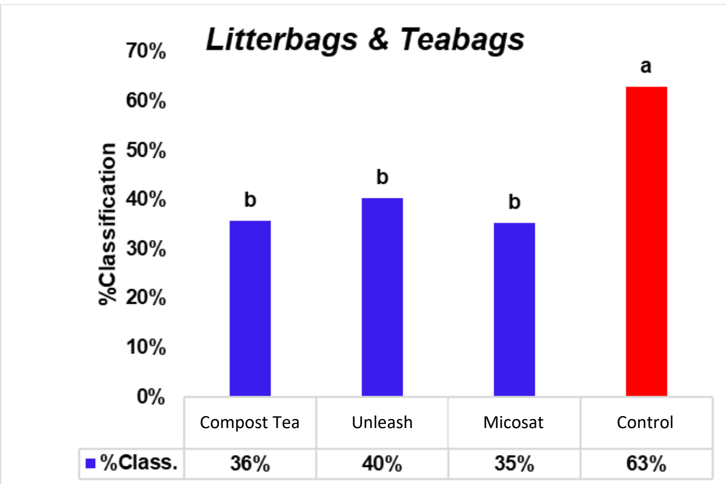
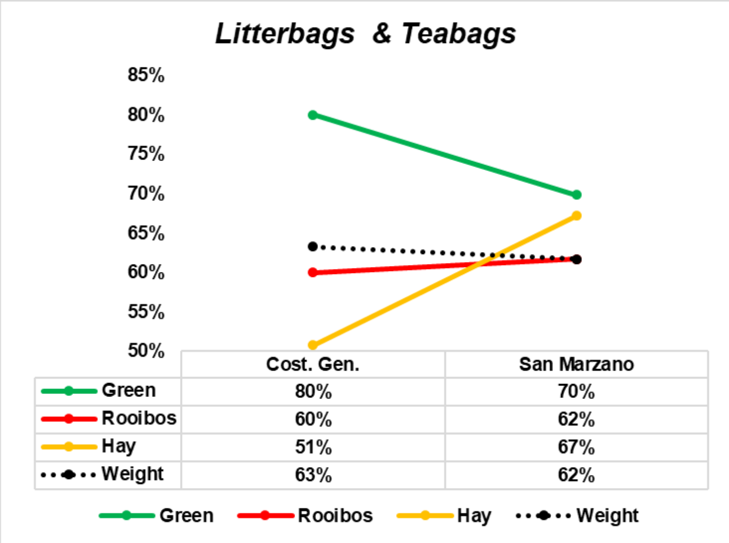


Figure 19 Average Classification % the tomato variety according to the four methods applied to the study of the soil bioactivity.



5. Discussion

When the results are applied to answering the research questions posed at the beginning of this paper, some answers come to light. With regards to the first question on how various microorganisms may influence the growth and quality of tomatoes, there is not much clarity. The germination rate was more influenced by the variety than the treatment, and of the treatments, it was actually the control that performed the best and the compost tea that performed the worst. Studies have shown AMF inoculation to improve plant growth including height and diameter (Arif et al., 2021; Eck et al., 2022; Masoero & Giovanetti, 2015). The results from this experiment did not report any significant evidence to support this point. In this case, the control was of a comparable height to the compost tea and unleash treated plants. Only the plants treated with Micosat averaged shorter than the rest, and this is the inoculum that is certain to contain AMF.

The foliar pH decreased by 2-4% for plants treated as opposed to the control. This is congruent with other studies involving AM (Giovannetti et al., 2019; Masoero & Cugnetto, 2018;

Masoero & Giovanetti, 2015). It is interesting to note the difference in classification of the litterbags examined using the NIRS method as opposed to the weight. Other studies have confirmed the effectiveness of the litterbag-NIRS models for offering a "rational assessment of microbial soil fertility before and during the use of biofertilizers or bioinoculants" (Baldi et al., 2020).

Considering the yield and quality of the tomato plants, there is not any significant differences between the treatments. Contrary to these results, other studies have seen a significant influence of mycorrhizal products on the factors of taste and yield (Baldi et al., 2020; Douds et al., 2016). One important study conducted by professor Paola Migliorini and Luisa Torri actually used a mycorrhizal product from the same company, Micosat, as was used in this study. Their wheat that was grown with Micosat F, and a sensory analysis test proved that people were able to taste the difference between breads baked with the control and the mycorrhizae-boosted wheats (Torri et al., 2013).

However, where the influence of treatment is most apparent is when the plants were exposed to a fungal infection. The plants that were treated with compost tea not only resisted serious infection better than the others, but they also had a higher rate of survival once they were infected. This supports the speculation that local microorganisms are better equipped to help their host plants be resilient in the face of disease or infections (Singh et al., 2016). The fact that the treatments with commercial microorganisms did not resist the disease well, fall in line with the trend of inconsistency presented by other research conducted on mycorrhizal inoculants and biostimulants (Eck et al., 2022; McCoy, 2016).

The second question this paper addresses is focused on the role of plant genotype. One of the varieties used was a local variety called *Costoluto Genovese,* and the other was a hybrid, *San Marzano F1.* The data reflects some a difference in performance between these two varieties to a certain degree. What is of particular interest to this thesis is the interaction between the variety and the treatments. This is most pronounced in the NIRS results. These results show that though the treatments do not appear to have any differentiation in the outcomes when the tomatoes are looked at broadly, the differences do arise when each variety is considered separately. It is also curious to consider how each treatment does have a clear fingerprint, but the fingerprint is more pronounced for the *San Marzano* variety. It is not uncommon for the hybrid variety to take to the microorganisms better than the local (An et al., 2010; Bryla & Koide, 1990). It does however go against the hypothesis set by this research and does call for further research to better understand why this is.

Another chunk of the data concerning the genetic makeup of the plant can be attributed to what we understand of these plant breeds. The hybrid outperformed the local variety in germination and yield. This can be expected as that is the intention of their breeding (An et al., 2010). What did stand out as successful for the local variety was when it was hit with the fungal disease. The Costoluto Genovese proved itself stronger in resisting the infection and surviving with it. Treated with the compost tea that supplied thousands of native microorganisms (Lowenfels & Lewis, 2010), the resilience was even stronger. This supports the theory that the plants that have adapted to a specific region over generations, supported by the life that lives in said region will be more likely to survive when struck with an infection (Singh et al., 2016).

The last point of this research where the plant variety proved to be most significant was the work done to analyze the soil microbiome. The litterbags revealed that the plant genotype can modify the bioactivity of the soil. This supports Jennifer A. Schweitzer (2008) in her findings that "individual plant genotypes could influence the associated belowground soil microbial community (and also vice versa)." The link between plants and the soil food web is ever more intricate than we can imagine and research is just beginning to scratch the surface on this topic.

The final question of how the introductions of external or internal microorganisms influence the soil ecosystem is not within the scope of this research. It is however important to consider when working with this material. As the research presented earlier suggests, it is unclear whether the introduction of foreign microorganisms actually has an impact on the resident community. Some results reveal it not to have an impact (Antunes et al., 2009), others claim that it does (Duchicela et al., 2020), and still others understand that there is not enough information to draw any conclusions just yet (Hart et al., 2018). The litterbags also revealed so significant change based on the treatments, so there is no clear evidence of the impact these biostimulants may have on the agroecosystem of this thesis. In any case, the introduction of foreign microorganisms should be done with caution as it is still unclear what consequences could come from the introduction of exogenous microorganisms. That said, the fact that the compost tea treatment (that of indigenous microorganisms), fared well if not better than the commercial ones, leads one to conclude that if a biostimulant were to be used, the safest and cheapest option would be to make it oneself.

6. Conclusion

Baldi et al. (2020) summarized nicely the reason why inoculation of biostimulants can be so unpredictable and at times difficult: "the greatest problem of inoculating soil for beneficial purposes is the general obstinacy of the soil ecosystem, which normally acts as a buffer against any incoming microorganisms." With this perspective it is quite easy to understand why a biofertilizer might not have the explosive results one might originally expect. This experiment in particular was conducted on a very healthy farm with a diverse and complex ecosystem already established. It is true that the plots were tilled before planting, which would disturb the soil community to some degree (Lowenfels & Lewis, 2010), however, maybe the disturbance was not enough to allow for the consortium of foreign microbes to establish themselves. This could be one explanation for the lack of evidence distinguishing between the different treatments and the control. Maybe the introduced microbes never got the opportunity to show off? Maybe also, the clash between the resident and introduced microorganisms hindered their abilities to support the plant properly. Perhaps more time is needed for the ecosystem to find its balance before the plants begin to experience any benefits.

To come back to the results of this experiment, we can conclude that though interesting, the results are for the most part inconclusive. There are hints that the treatments had an effect on the tomato plants, though more research must be done before anything can be said with certainty. To this point, in most cases the control performed just as well as the other treatments if not better. Therefore, it is important to keep in mind that not every inoculum will work with every plant. Symbiosis is an intricate and complex process and it cannot be forced. Instead, one must continue to test different combinations before finding a successful one, and the successful combination can have significant results (Arif et al., 2021).

As Deguine et al. (2023) states in their study, "optimizing plant–animal–microbial interactions promotes the healthy ecological functioning of agroecosystems, therefore making them less vulnerable." This was certainly the case when the plants were infected with the fungal disease, and the resilience of all the plants was certainly impressive. The fact that the farm on which this study was conducted is already a diverse place practicing agroecological farming techniques could potentially explain why the results were so elusive. Much of the literature cited in this report explain that the introduction of microbes seems to be the most effective in a degraded environment (Hart et al., 2018; McCoy, 2016). It would therefore be very interesting to repeat this experiment on multiple sites and compare across them.

However, going back to what can be taken away from the contents of this paper, it seems as though the most important conclusion is that efforts should be made to harness what is already present in an agroecosystem and promote the development of beneficial relationships between these native species. The use of microbial products may not be necessary on a farm that already has a diverse ecosystem. Instead, what this paper seems to suggest is that these products should be used conservatively and only in cases where they are really needed – to bring a degraded soil back to life for instance. Otherwise, the costs and the risk are too high. If there is an instance when an extra boost of microorganisms could be helpful, perhaps a simple compost tea is the solution. It seemed to be one of the top inoculants in this case study. It will be interesting to see how the world of microbial products progress and I look forward to reading future research on the topic.

Bibliography

An, G.-H., Kobayashi, S., Enoki, H., Sonobe, K., Muraki, M., Karasawa, T., & Ezawa, T. (2010). How does arbuscular mycorrhizal colonization vary with host plant genotype? An example based on maize (Zea mays) germplasms. *Plant and Soil*, *327*(1–2), 441–453. https://doi.org/10.1007/s11104-009-0073-3

Antunes, P. M., Koch, A. M., Dunfield, K. E., Hart, M. M., Downing, A., Rillig, M. C., & Klironomos, J. N. (2009). Influence of commercial inoculation with Glomus intraradices on the structure and functioning of an AM fungal community from an agricultural site. *Plant and Soil*, *317*(1–2), 257–266. https://doi.org/10.1007/s11104-008-9806-y

Arif, A., Husna, Tuheteru, F. D., & Adiansyah, A. (2021). The conservation of endemic and endangered tree species Kalapia celebica Kosterm with Mycorrhizal bio-fertilizer technology in post-gold mining areas. *IOP Conference Series: Earth and Environmental Science*, *800*(1), 012039. https://doi.org/10.1088/1755-1315/800/1/012039

Averill, C., Anthony, M. A., Baldrian, P., Finkbeiner, F., van den Hoogen, J., Kiers, T., Kohout, P., Hirt, E., Smith, G. R., & Crowther, T. W. (2022). Defending Earth’s terrestrial microbiome. *Nature Microbiology*, *7*(11), 1717–1725. https://doi.org/10.1038/s41564-022-01228-3

Baldi, E., Toselli, M., Masoero, G., & Nuti, M. (2020). Organic and Symbiotic Fertilization of Tomato Plants Monitored by Litterbag-NIRS and Foliar-NIRS Rapid Spectroscopic Methods. *Journal of Agronomy Research*, *3*(1), 9–26. https://doi.org/10.14302/issn.2639-3166.jar-20-3363

Bryla, D. R., & Koide, R. T. (n.d.). *Role of mycorrhizal infection in the growth and reproduction of wild vs. Cultivated plants*.

Catalogo MICOSAT 2021. (2021). C.C.S. Aosta s.r.l.

Commission Implementing Decision of 8 April 2019 on the publication in the Official Journal of the European Union of the application for approval of an amendment, which is not minor, to a product specification referred to in Article 53 of Regulation (EU) No 1151/2012 of the European Parliament and of the Council for the name 'Pomodoro S. Marzano dell'Agro Sarnese-Nocerino' (PDO). (n.d.).

Croxall, H. E., Norman, T. M., & Gwynne, D. C. (1957). EFFECT OF 2 :4 :6-TRICHLOROPHENOXYACETIC ACID ON THE SUSCEPTIBILITY OF TOMATO PLANTS TO DIDYMELLA LYCOPERSICI. *Plant Pathology*, *6*(1), 27-27–31. Food Science Source. https://doi.org/10.1111/j.1365-3059.1957.tb00764.x

Darwish, L. (2013). *Earth Repair: A Grassroots Guide to Healing Toxic and Damaged Landscapes*. New Society Publishers.

de Souza, F. A., & Declerck, S. (2003). Mycelium development and architecture, and spore production of *Scutellospora reticulata* in monoxenic culture with Ri T-DNA transformed carrot roots. *Mycologia*, *95*(6), 1004–1012. https://doi.org/10.1080/15572536.2004.11833016

Deguine, J.-P., Aubertot, J.-N., Bellon, S., Côte, F., Lauri, P.-E., Lescourret, F., Ratnadass, A., Scopel, E., Andrieu, N., Bàrberi, P., Becker, N., Bouyer, J., Brévault, T., Cerdan, C., Cortesero, A.-M., Dangles, O., Delatte, H., Dinh, P. T. Y., Dreyer, H., … Lamichhane, J. R. (2023). Agroecological crop protection for sustainable agriculture. In *Advances in Agronomy* (Vol. 178, pp. 1–59). Elsevier. https://doi.org/10.1016/bs.agron.2022.11.002

Douds, D. D., Lee, J., McKeever, L., Ziegler-Ulsh, C., & Ganser, S. (2016). Utilization of inoculum of AM fungi produced on-farm increases the yield of Solanum lycopersicum: A summary of 7 years of field trials on a conventional vegetable farm with high soil phosphorus. *Scientia Horticulturae*, *207*, 89–96. https://doi.org/10.1016/j.scienta.2016.05.013

Douds Jr., D. D., Nagahashi, G., & Hepperly, P. R. (2010). On-farm production of inoculum of indigenous arbuscular mycorrhizal fungi and assessment of diluents of compost for inoculum production☆. *Bioresource Technology*, *101*(7), 2326–2330. https://doi.org/10.1016/j.biortech.2009.11.071

Duchicela, J., Bever, J. D., & Schultz, P. A. (2020). Symbionts as Filters of Plant Colonization of Islands: Tests of Expected Patterns and Environmental Consequences in the Galapagos. *Plants*, *9*(1), 74. https://doi.org/10.3390/plants9010074

Duell, E. B., Hickman, K. R., & Wilson, G. W. T. (2022). Inoculation with native grassland soils improves native plant species germination in highly disturbed soil. *Grassland Research*, *1*(2), 75–83. https://doi.org/10.1002/glr2.12018

Duell, E. B., O'Hare, A., & Wilson, G. W. T. (2023). Inoculation with native soil improves seedling survival and reduces non-native reinvasion in a grassland restoration. *Restoration Ecology*, *31*(1), e13685. https://doi.org/10.1111/rec.13685

Eck, J. L., Kytöviita, M., & Laine, A. (2022). Arbuscular mycorrhizal fungi influence host infection during epidemics in a wild plant pathosystem. *New Phytologist*, *236*(5), 1922–1935. https://doi.org/10.1111/nph.18481

Fracchia, S., Menendez, A., Godeas, A., & Ocampo, J. A. (2001). A method to obtain monosporic cultures of arbuscular mycorrhizal fungi. *Soil Biology and Biochemistry*, *33*(9), 1283–1285. https://doi.org/10.1016/S0038-0717(01)00014-1

Giovannetti, G., Polo, F., Nutricato, S., Masoero, G., & Nuti, M. (2019). Potency of Commercial Symbiotic Bio-Fertilizer Consortium for Mitigating the Olive Quick Decline Syndrome (OQDS). *Journal of Agronomy Research*, *2*(1), 1–21. https://doi.org/10.14302/issn.2639-3166.jar-19-2780

Hart, M. M., Antunes, P. M., Chaudhary, V. B., & Abbott, L. K. (2018). Fungal inoculants in the field: Is the reward greater than the risk? *Functional Ecology*, *32*(1), 126–135. https://doi.org/10.1111/1365-2435.12976

Koziol, L., Bauer, J. T., Duell, E. B., Hickman, K., House, G. L., Schultz, P. A., Tipton, A. G., Wilson, G. W. T., & Bever, J. D. (2022). Manipulating plant microbiomes in the field: Native mycorrhizae advance plant succession and improve native plant restoration. *Journal of Applied Ecology*, *59*(8), 1976–1985. https://doi.org/10.1111/1365-2664.14036

Koziol, L., McKenna, T. P., & Bever, J. D. (2023). Native Microbes Amplify Native Seedling Establishment and Diversity While Inhibiting a Non-Native Grass. *Plants*, *12*(5), 1184. https://doi.org/10.3390/plants12051184

Liguria, Italy. (n.d.). [Map]. Encyclopædia Britannica. Retrieved August 25, 2023, from https://www.britannica.com/place/Liguria#/media/1/340874/283765

Lowenfels, J., & Lewis, W. (2010). *Teaming with Microbes: The Organic Gardener's Guide to the Soil Food Web*. Timber Press, Inc.

Masoero, G., & Cugnetto, A. (2018). The Raw pH in Plants: A Multifaceted Parameter. *Journal of Agronomy Research*, *1*(2), 18–34. https://doi.org/10.14302/issn.2639-3166.jar-18-2397

Masoero, G., & Giovanetti, G. (2015). In vivo Stem pH can Testify the Acidification of the Maize Treated by Mycorrhizal and Microbial Consortium. *Journal of Environmental and Agricultural Sciences*, *3*, 23–30.

Masoero, G., Masoero, G., Delmastro, M., Cugnetto, A., Giovannetti, G., & Nuti, M. (2018). NIRS Footprint of Bio-Fertilizers from Hay Litter-Bags. *Journal of Agronomy Research*, *1*(1), 22–33. https://doi.org/10.14302/issn.2639-3166.jar-18-2084

Masoero, G., Sarasso, G., Delmastro, M., Antonini, M., Solaro, S., & Scapin, I. (2023). *Soluble Biobased Substances in soil or Salicylic Acid on leaves affect the foliar pH and soil biovariability of Grapes-as explained by the NIR Spectroscopy of Litterbags and Teabags*.

McCoy, P. (2016). *Radical mycology: A treatise on seeing & working with fungi*. Chthaeus Press.

Millner, P. D., & Kitt, D. G. (1992). The Beltsville method for soilless production of vesicular-arbuscular mycorrhizal fungi. *Mycorrhiza*, *2*(1), 9–15. https://doi.org/10.1007/BF00206278

Mohammad, A., Khan, A. G., & Kuek, C. (2000). Improved aeroponic culture of inocula of arbuscular mycorrhizal fungi. *Mycorrhiza*, *9*(6), 337–339. https://doi.org/10.1007/s005720050278

Schwartz, M. W., Hoeksema, J. D., Gehring, C. A., Johnson, N. C., Klironomos, J. N., Abbott, L. K., & Pringle, A. (2006). The promise and the potential consequences of the global transport of mycorrhizal fungal inoculum. *Ecology Letters*, *9*(5), 501–515. https://doi.org/10.1111/j.1461-0248.2006.00910.x

Schweitzer, J. A., Bailey, J. K., Fischer, D. G., LeRoy, C. J., Lonsdorf, E. V., Whitham, T. G., & Hart, S. C. (2008). PLANT–SOIL–MICROORGANISM INTERACTIONS: HERITABLE RELATIONSHIP BETWEEN PLANT GENOTYPE AND ASSOCIATED SOIL MICROORGANISMS. *Ecology*, *89*(3), 773–781. https://doi.org/10.1890/07-0337.1

Selvakumar, G., Kim, K., Walitang, D., Chanratana, M., Kang, Y., Chung, B., & Sa, T. (2016). Trap Culture Technique for Propagation of Arbuscular Mycorrhizal Fungi using Different Host Plants. *Korean Journal of Soil Science and Fertilizer*, *49*(5), 608–613. https://doi.org/10.7745/KJSSF.2016.49.5.608

Singh, H. B., Sarma, B. K., & Keswani, C. (Eds.). (2016). *Agriculturally Important Microorganisms*. Springer Singapore. https://doi.org/10.1007/978-981-10-2576-1

Tajini, F., Suriyakup, P., Vailhe, H., Jansa, J., & Drevon, J.-J. (2009). Assess suitability of hydroaeroponic culture to establish tripartite symbiosis between different AMF species, beans, and rhizobia. *BMC Plant Biology*, *9*(1), 73. https://doi.org/10.1186/1471-2229-9-73

Torri, L., Migliorini, P., & Masoero, G. (2013). Sensory test vs. Electronic nose and/or image analysis of whole bread produced with old and modern wheat varieties adjuvanted by means of the mycorrhizal factor. *Food Research International*, *54*(2), 1400–1408. https://doi.org/10.1016/j.foodres.2013.09.045

Vahter, T., Bueno, C. G., Davison, J., Herodes, K., Hiiesalu, I., Kasari-Toussaint, L., Oja, J., Olsson, P. A., Sepp, S., Zobel, M., Vasar, M., & Öpik, M. (2020). Co-introduction of native mycorrhizal fungi and plant seeds accelerates restoration of post-mining landscapes. *Journal of Applied Ecology*, *57*(9), 1741–1751. https://doi.org/10.1111/1365-2664.13663

Wubs, E. R. J., van der Putten, W. H., Mortimer, S. R., Korthals, G. W., Duyts, H., Wagenaar, R., & Bezemer, T. M. (2019). Single introductions of soil biota and plants generate long-term legacies in soil and plant community assembly. *Ecology letters*, *22*(7), 1145–1151. https://doi.org/10.1111/ele.13271

Appendix 1: Experimental Design

Crop A: 120 “"San Marzano" (HYBRID)
Crop B: 120 “Costoluto genovese” (LOCAL)

Inoculum 1: Local AMF + Compost Tea (60)
Inoculum 2: Aquabella “Unleash” (60)
Inoculum 3: MICOSAT (60)
Inoculum 4: Control (60)

A1 30
A2 30
A3 30
A4 30
B1 30
B2 30
B3 30
B4 30

Procedure

<u>Seeding:</u>

The seeds were sourced from a nearby nursery.

In the first week of March, 240 seeds were sown in polystyrene seed trays with cell size of 1cm x 1cm. The soil used was professional seeding soil[1] and a top layer of vermiculite was added.

Inoculum 1:
The compost tea was made by filling a burlap bag with 1 Liter of mature compost and immersing it in 10 Liters fresh water.

Samples of soil and roots from the area surrounding the farm were collected and added to the compost tea in the hope that local species of mycorrhizae and other microorganisms could be introduced to the substrate, enriching its diversity and strengthening its connection to the local ecosystem. Three different locations were selected that had an intact soil system that hadn’t been disturbed in at least 2 years. One location was on a grass terrace where

[1] The soil used was Klasmann Potgrond H, which is made up exclusively of vernalized black peat and blond peat. It is enriched with water-soluble fertilizer and microelements. Nutrients include: Nitrogen (N/l): 210 mg, Phosphorus (P_2O_5/l): 240 mg, Potassium (K_2O/l): 270 mg, Magnesium (Mg/l): 100 mg. Physical and chemical characteristics: pH ($CaCl_2$): 5.5, pH (H_2O): 6, Electrical conductivity: 0.45 dS/m, Dry bulk density: 160 kg/ ^{m3}, Total porosity: 85% v/v.

what could be mycorrhizal mycelium was visible on the root structure of the plants. The other two locations were in the forest and samples were taken from around the roots of young trees growing near older ones of the same species. Sourcing from multiple sites will ensure a diverse mix of species is obtained. The soil was collected from the top 10 cm and the roots were extracted and cut into 2.5-4 cm fragments. The soil, mycelium and roots were bundled in cloth and submerged in the compost solution. Together, the compost and the local soil were submerged in the water and aerated for a total of 24 hours.

The tea was applied using a syringe. Each cell received 2 ml of solution.

Inoculum 2:
1mL of Unleash was mixed with 1L of water. Each seed received 2mL of the solution during seeding by use of a medical syringe.

Inoculum 3:
A Micosat MO – water solution of 4 grams to 1 Liter was created and applied by syringe.

<u>Germination and Seedling Phase:</u>

Once the seedlings reach 3cm in height, the seedlings were transplanted into 5cm x 5cm cells and a second round of inoculation was applied.

Inoculum 1:
A new batch of compost tea was made in the same method as previously described. It was applied to the roots by submerging the trays in the solution.

Inoculum 2:
1mL of Unleash was mixed with 1L unchlorinated water. It was applied to the roots by submerging the trays in the solution.

Inoculum 3:
A second solution of MICOSAT MO was created using the same method as before. It was applied to the roots by submerging the trays in the solution.

<u>Transplant into Field</u>

In April, the seedlings were transplanted into the field. Prior to planting, the first 20cm of soil was mechanically worked and fertilized with mature donkey manure and sheep wool. The soil in the field is clayey and has a pH of 6.5. Chemical pesticide treatments have not been used in the last 6 years.

The plot that has been selected for the seedlings is made up of 3 beds in two rows. Each bed is 1 meter wide. Two seedlings were planted side by side with a spacing of 40 cm between the plants. Each block contained 10 plants and 80 cm was left between each case to minimize contamination. Below is the final plot design.

	160cm	80cm	160cm	80cm	160cm	80cm	160cm	80cm	160cm	80cm	160cm		160cm	80cm	160cm	80cm	160cm	80cm	160cm	80cm	160cm
50cm	A1		A2		A4		A3		B1		B2										
50cm	A1		A2		A4		A3		B1		B2										
50cm			B3		B1		B2		B4		A3		A1		A4		A2		B3		B4
50cm			B3		B1		B2		B4		A3		A1		A4		A2		B3		B4

At this time, a third inoculation of Unleash, MICOSAT MO and compost tea was applied to the plants.

From this time forward, applications of MICOSAT LEN and TAB was applied to the leaves every two weeks. Unleash and compost tea was also applied to their respected plants.

15 days after transplant, 12 litterbags, 12 green tea bags, and 12 red tea bags were evenly dispersed—two for each block. 60 days later the samples were collected, sun dried and cleaned. They were visually examined and weighed to test for soil activity. Later they were also sent away to be tested with NIR spectroscopy.

At the end of May - early June, two leaves were taken from each plant (480 leaves total) and subjected to pH and NIRS tests.

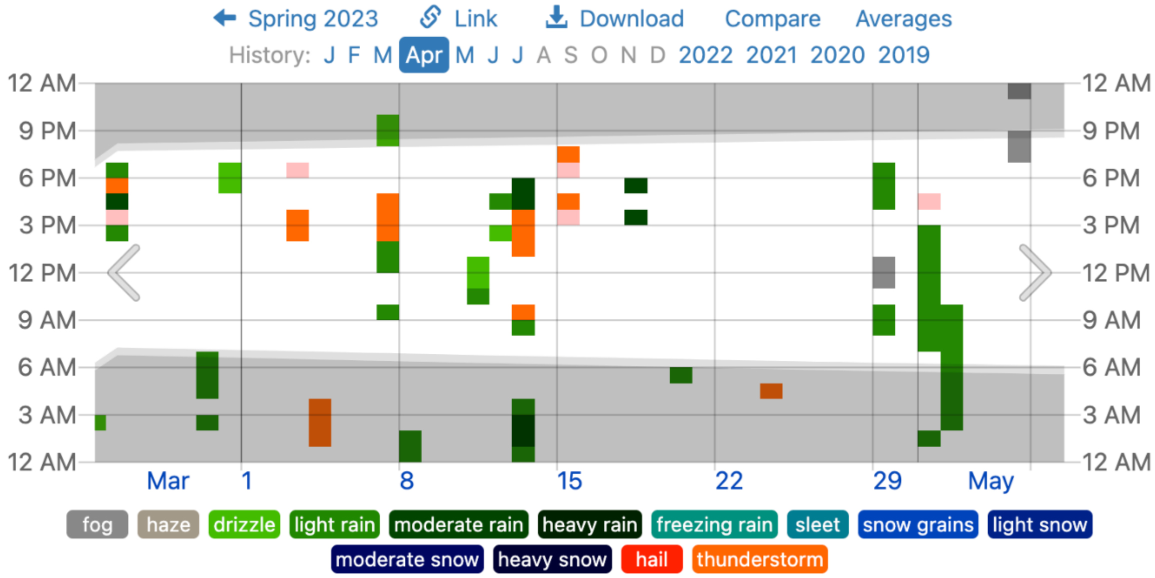
The yield was calculated by weight and number. Quality was measured by conducting a Brix° test.

Appendix 2: Weather History

Day	Observations	Precipitation	Codes
Wed, Mar 1	Light Rain, Light Drizzle		-RA, -DZ
Wed, Mar 8	Light Rain, Light Drizzle, Mist		-RA, -DZ, BR
Thu, Mar 9	Light Rain, Light Drizzle, Mist		-RA, -DZ, BR
Sun, Mar 12	Patches of Fog		BCFG
Mon, Mar 13	Light Drizzle and Rain, Light Rain, Recent Drizzle, Mist		-DZRA, -RA, REDZ, BR
Tue, Mar 14	Rain, Recent Rain, Light Rain, Drizzle, Light Drizzle, Recent Drizzle, Mist		RA, RERA, -RA, DZ, -DZ, REDZ, BR
Sun, Mar 19	Light Rain, Light Drizzle		-RA, -DZ
Thu, Mar 23	Light Rain		-RA
Fri, Mar 24	Light Rain		-RA
Sat, Mar 25	Light Drizzle		-DZ
Sun, Mar 26	Recent Thunderstorm with Rain, Thunderstorm with Light Rain, Recent Rain, Light Rain, Showers in the Vicinity		RETSRA, -TSRA, RERA, -RA, VCSH
Thu, Mar 30	Light Rain		-RA
Fri, Mar 31	Light Drizzle, Mist		-DZ, BR
Mon, Apr 3	Recent Thunderstorm, Thunderstorm, Showers in the Vicinity		RETS, TS, VCSH
Tue, Apr 4	Recent Thunderstorm, Thunderstorm, Thunderstorm with Light Rain		RETS, TS, -TSRA
Fri, Apr 7	Thunderstorm with Light Rain, Thunderstorm with Rain, Rain, Recent Rain, Light Rain, Light Drizzle		-TSRA, TSRA, RA, RERA, -RA, -DZ
Sat, Apr 8	Light Rain		-RA
Tue, Apr 11	Light Rain, Light Drizzle		-RA, -DZ
Wed, Apr 12	Light Rain, Light Drizzle		-RA, -DZ
Thu, Apr 13	Recent Thunderstorm, Thunderstorm, Thunderstorm with Heavy Rain and Small Hail, Thunderstorm with Light Rain, Thunderstorm with Rain, Rain, Recent Rain, Light Rain, Showers in the Vicinity		RETS, TS, +TSRAGS, -TSRA, TSRA, RA, RERA, -RA, VCSH
Sat, Apr 15	Recent Thunderstorm, Thunderstorm, Showers in the Vicinity		RETS, TS, VCSH
Tue, Apr 18	Recent Showers of Rain, Showers of Rain, Showers in the Vicinity		RESHRA, SHRA, VCSH
Thu, Apr 20	Light Rain		-RA
Mon, Apr 24	Recent Thunderstorm, Thunderstorm, Thunderstorm with Light Rain		RETS, TS, -TSRA
Sat, Apr 29	Light Rain, Light Drizzle, Recent Drizzle, Mist		-RA, -DZ, REDZ, BR
Mon, May 1	Light Rain, Showers in the Vicinity		-RA, VCSH
Tue, May 2	Light Rain		-RA
Fri, May 5	Fog, Fog in the Vicinity, Mist, Patches of Fog		FG, VCFG, BR, BCFG
Tue, May 9	Light Drizzle		-DZ
Wed, May 10	Rain, Recent Rain, Light Rain, Light Drizzle		RA, RERA, -RA, -DZ
Fri, May 12	Recent Thunderstorm, Thunderstorm, Thunderstorm in the Vicinity, Thunderstorm with Light Rain, Light Rain, Showers in the Vicinity		RETS, TS, VCTS, -TSRA, -RA, VCSH
Sat, May 13	Recent Thunderstorm, Thunderstorm, Thunderstorm with Light Rain, Showers in the Vicinity		RETS, TS, -TSRA, VCSH
Sun, May 14	Light Rain, Light Drizzle		-RA, -DZ
Mon, May 15	Recent Thunderstorm, Thunderstorm		RETS, TS
Tue, May 16	Light Rain		-RA
Wed, May 17	Light Rain		-RA
Thu, May 18	Recent Rain, Light Rain		RERA, -RA
Fri, May 19	Light Snow, Light Rain, Light Drizzle, Recent Drizzle		-SN, -RA, -DZ, REDZ
Sat, May 20	Light Rain, Light Drizzle		-RA, -DZ
Sun, May 21	Recent Thunderstorm, Thunderstorm		RETS, TS
Mon, May 22	Recent Thunderstorm, Recent Thunderstorm with Rain, Thunderstorm with Light Rain, Rain, Recent Rain, Light Rain, Showers in the Vicinity		RETS, RETSRA, -TSRA, RA, RERA, -RA, VCSH
Sun, May 28	Recent Thunderstorm, Thunderstorm in the Vicinity, Thunderstorm with Light Rain, Light Rain		RETS, VCTS, -TSRA, -RA
Mon, May 29	Recent Thunderstorm with Rain, Thunderstorm, Thunderstorm with Light Rain, Thunderstorm with Rain, Light Drizzle		RETSRA, TS, -TSRA, TSRA, -DZ
Tue, May 30	Recent Thunderstorm, Thunderstorm, Thunderstorm in the Vicinity, Light Rain		RETS, TS, VCTS, -RA
Wed, May 31	Recent Thunderstorm, Thunderstorm in the Vicinity, Thunderstorm with Light Rain, Thunderstorm with Rain, Recent Rain, Light Rain, Showers in the Vicinity		RETS, VCTS, -TSRA, TSRA, RERA, -RA, VCSH

Day	Observations	Precipitation	Codes
Thu, Jun 1	Recent Thunderstorm, Thunderstorm, Light Rain		RETS, TS, -RA
Mon, Jun 5	Rain, Recent Rain, Recent Showers of Rain, Light Rain, Light Drizzle, Showers in the Vicinity		RA, RERA, RESHRA, -RA, -DZ, VCSH
Wed, Jun 7	Light Rain		-RA
Fri, Jun 9	Light Rain		-RA
Sat, Jun 10	Rain, Recent Rain, Light Rain, Showers in the Vicinity		RA, RERA, -RA, VCSH
Sun, Jun 11	Recent Thunderstorm, Thunderstorm, Thunderstorm in the Vicinity, Showers in the Vicinity		RETS, TS, VCTS, VCSH
Mon, Jun 12	Thunderstorm, Thunderstorm in the Vicinity, Recent Showers of Rain, Showers of Rain, Light Rain, Showers in the Vicinity		TS, VCTS, RESHRA, SHRA, -RA, VCSH
Tue, Jun 13	Recent Thunderstorm, Thunderstorm, Thunderstorm in the Vicinity, Rain, Recent Rain, Light Rain, Showers in the Vicinity		RETS, TS, VCTS, RA, RERA, -RA, VCSH
Wed, Jun 14	Recent Thunderstorm with Rain, Thunderstorm with Rain, Rain, Recent Rain, Light Rain, Showers in the Vicinity		RETSRA, TSRA, RA, RERA, -RA, VCSH
Thu, Jun 22	Showers in the Vicinity		VCSH
Thu, Jun 29	Thunderstorm in the Vicinity		VCTS
Fri, Jun 30	Recent Thunderstorm, Thunderstorm with Rain, Rain, Recent Rain, Light Rain, Showers in the Vicinity		RETS, TSRA, RA, RERA, -RA, VCSH
Mon, Jul 3	Light Rain, Showers in the Vicinity		-RA, VCSH
Tue, Jul 4	Light Rain, Showers in the Vicinity		-RA, VCSH
Thu, Jul 6	Thunderstorm, Thunderstorm with Light Rain		TS, -TSRA
Thu, Jul 13	Recent Thunderstorm, Recent Thunderstorm with Rain, Thunderstorm, Thunderstorm with Rain, Light Rain		RETS, RETSRA, TS, TSRA, -RA
Fri, Jul 21	Thunderstorm, Thunderstorm with Light Rain, Recent Rain, Light Rain		TS, -TSRA, RERA, -RA
Mon, Jul 24	Recent Thunderstorm, Recent Thunderstorm with Rain, Thunderstorm, Thunderstorm with Light Rain, Thunderstorm with Rain		RETS, RETSRA, TS, -TSRA, TSRA
Tue, Jul 25	Recent Thunderstorm, Thunderstorm with Light Rain, Recent Rain, Light Rain, Showers in the Vicinity		RETS, -TSRA, RERA, -RA, VCSH

Observed Weather in April 2023 in Rapallo

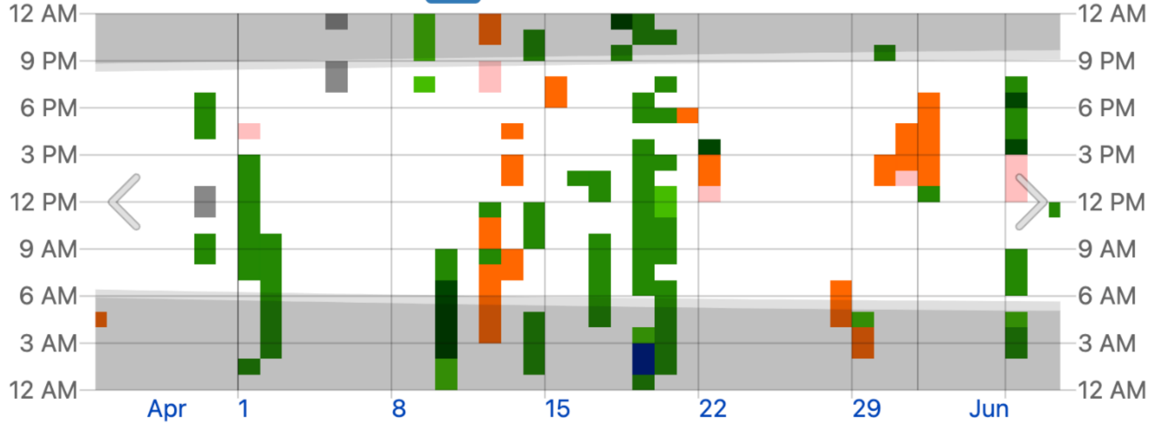


The hourly observed weather, color coded by category (in order of severity). If multiple reports are present, the most severe code is shown.

Observed Weather in May 2023 in Rapallo

Spring 2023 | Link | Download | Compare | Averages

History: J F M A **May** J J A S O N D 2022 2021 2020 2019



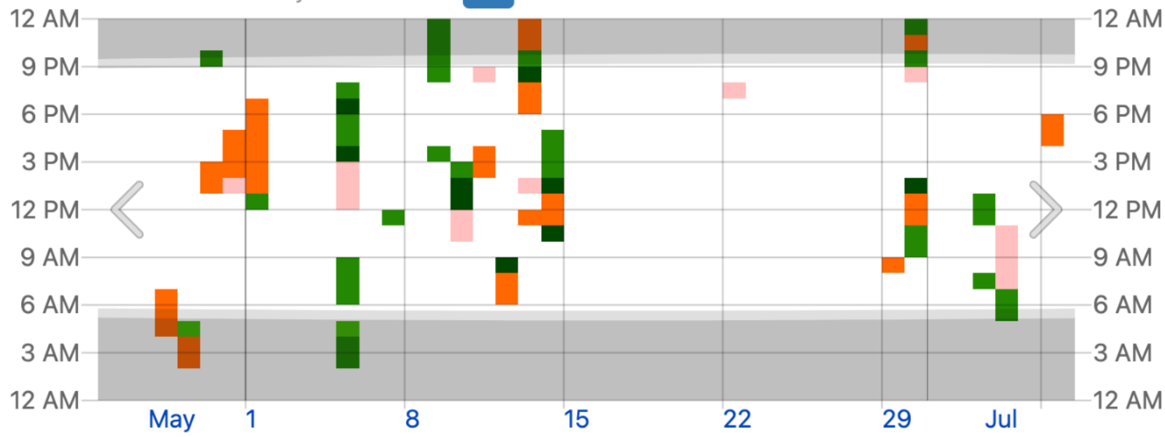
fog | haze | drizzle | light rain | moderate rain | heavy rain | freezing rain | sleet | snow grains | light snow | moderate snow | heavy snow | hail | thunderstorm

The hourly observed weather, color coded by category (in order of severity). If multiple reports are present, the most severe code is shown.

Observed Weather in June 2023 in Rapallo

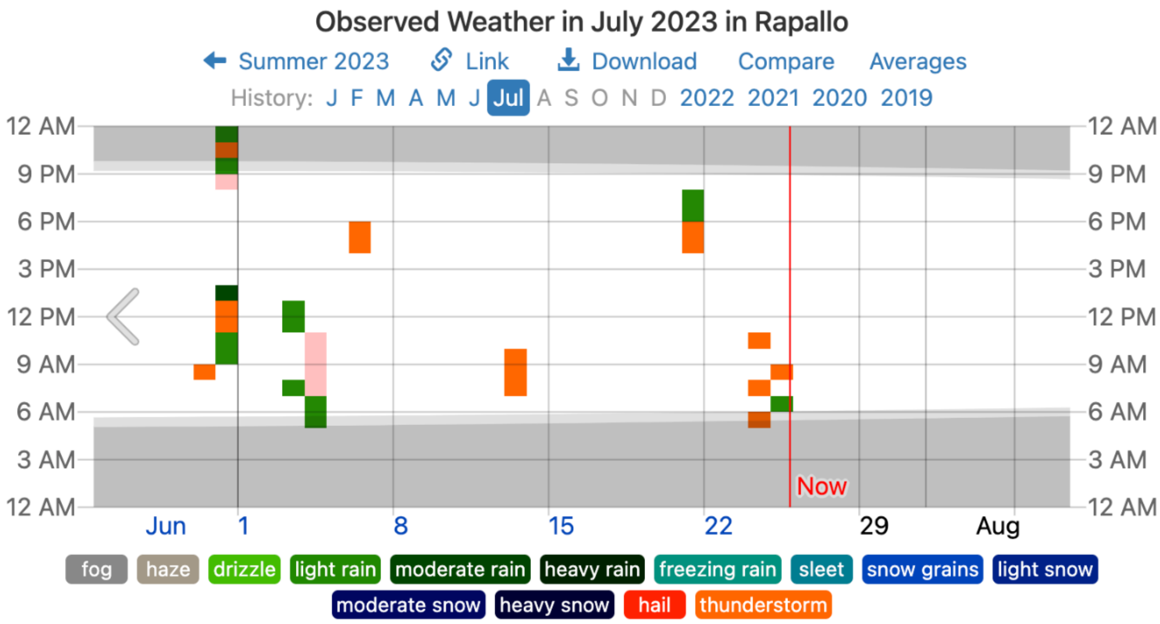
Summer 2023 | Link | Download | Compare | Averages

History: J F M A M **Jun** J A S O N D 2022 2021 2020 2019



fog | haze | drizzle | light rain | moderate rain | heavy rain | freezing rain | sleet | snow grains | light snow | moderate snow | heavy snow | hail | thunderstorm

The hourly observed weather, color coded by category (in order of severity). If multiple reports are present, the most severe code is shown.



The hourly observed weather, color coded by category (in order of severity). If multiple reports are present, the most severe code is shown.

All weather data was taken from Weather Spark. Further information can be found here: https://weatherspark.com/h/m/62182/2023/7/Historical-Weather-in-July-2023-in-Rapallo-Italy#google_vignette