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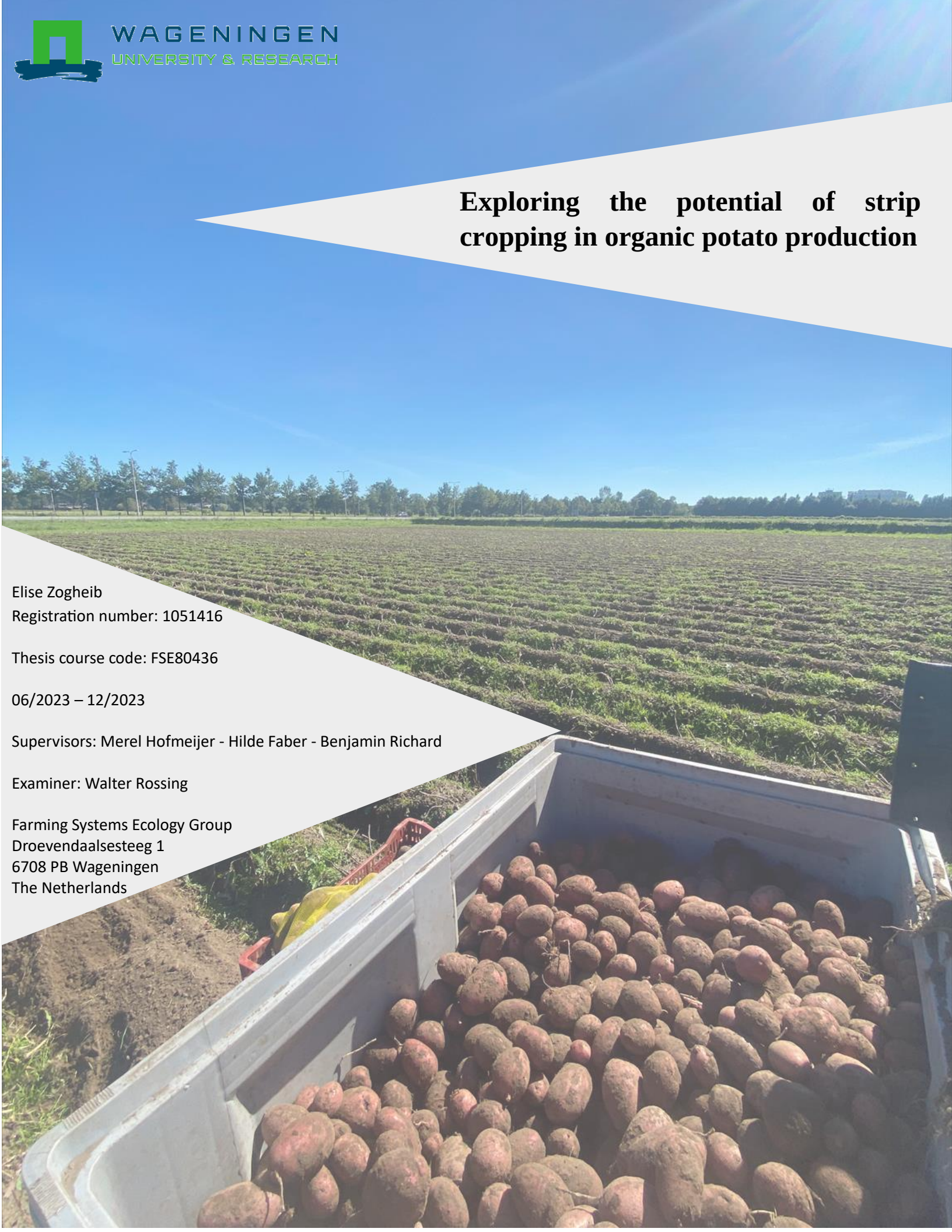
Exploring the potential of strip cropping in organic potato production



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ABSTRACT

Climate change poses serious challenges to global food systems, affecting crop production through altered weather patterns and increased incidence of pests and disease. Agricultural crop diversification, particularly strip cropping, has been proposed as a resilience building strategy against such impacts. This study evaluates the effect of strip cropping on organic potato production in the Netherlands, focusing on yield quality, quantity, and arbuscular mycorrhizal fungi (AMF) colonization. This research was conducted at two organically managed farms in the Netherlands, employing an incomplete block design to compare strip cropping to monoculture in potato production. Yield and quality were measured in terms of marketable yields, starch content and revenues, while AMF colonization was assessed through hyphae, arbuscules and vesicles counts in the roots. Strip cropping did not significantly improve potato yields and quality but showed some positive trends. As for the AMF colonization, it was not significantly different between strip cropping and monoculture This study suggests that strip cropping can be as productive as monoculture in terms of marketable yields and quality, without compromising economic returns. Although no significant differences in AMF colonization were observed, the potential ecological benefits necessitate further investigations. The findings emphasize the need for multi-seasonal studies to better understand the long-term implications of strip-cropping on potato production.

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1.Introduction

Climate change represents one of the defining issues of our time, and we are currently facing a critical moment that requires our attention and action (UN, 2023). The current situation has notable impacts on various areas, including important elements of our food systems. A significant amount of evidence is available today that demonstrates the detrimental impact of climate change on crop production in numerous regions worldwide (Mirzabaev et al., 2023). According to the Intergovernmental Panel on Climate Change (IPCC), climate change exerts an influence on the operations of all elements comprising food systems, including various stakeholders and their interconnected activities that contribute to value creation (IPCC, 2019). In addition, climate fluctuations such as rising temperatures, shifting precipitation patterns and high occurrence of extreme weather are affecting crops, livestock, and fisheries productivity. These fluctuations also affect water availability and quality, inducing heat stress, and modifying the pests and disease environment (Mirzabaev et al., 2023). In the coming years, significant improvements to the global food systems are key for the nourishment of the expanding world population, nutritionally, and sustainably (Devaux et al., 2021). Nonetheless, there is proof that climate change will affect quality, quantity, and food safety as well as the dependability of its distribution (Vermeulen et al., 2012). Evidently, climate change is anticipated to affect all four dimensions of food security, namely the availability of food, access to food, utilization of food, and the stability of the food supply (FAO, 2020). However, current food systems fail to adequately meet the nutritious demands of the global population while adhering to environmentally sustainable practices (Devaux et al., 2021).

An integrated solution to building resilience into agricultural systems to mitigate the consequences of climate change may be agricultural crop diversification (Lakhran et al., 2017). Crop diversification involves introducing new crops or cropping systems into agricultural production on a specific farm, considering the varying benefits gained from value-added crops offering complementary marketing opportunities (Lakhran et al., 2017). The findings from the Crop Diversity Experiment, initiated in 2018, provided robust evidence that diversifying annual crops systems not only enhances yield relative to monocultures but strengthen resilience under environmental stress through mechanisms like complementary nutrient uptake and improve pest and disease suppression (Schöb et al., 2023) Crop diversification offers numerous benefits, including increased income and resilience to fluctuating commodity prices and climate change-induced weather variability. It can also improve food variety and quality, reduce pest pressure, enhances pollinator populations, improves soil quality, creates employment opportunities, and has the potential to increase crop yields (Walia, 2020). Therefore, by cultivating diverse combinations of crops, the agroecosystem will expand its ability to withstand external disturbances linked to the changing climate conditions (Scialabba & Müller-Lindenlauf, 2010). This results in a more resilient system overall. In addition, crop diversification can be classified based on temporal, spatial or genetic diversification (Diltzer et al., 2021) with the management practices of strip cropping corresponding to spatial diversity.

Strip cropping, a form of intercropping, refers to the cultivation of one or more crops within the same field in strips. These strips are wide enough to allow independent management of each crop using existing machinery, while still being narrow enough to facilitate interaction between the different crop components (Hauggaard-Nielson, 2010). Intercropping serves as a method to enhance diversity within an agricultural ecosystem. It exemplifies sustainable agricultural systems that aim to achieve objectives such as efficient resource utilization, increased quantity and quality of crops, and reduced yield losses caused by pests, diseases, and weeds (Mousavi and Eskandari, 2011). Strip cropping allows for more efficient resource utilization on the same land, promotes the potential for carbon sequestration, and reduces economic risks (Campanelli et al., 2023). Intercropping typically demonstrates higher productivity compared to traditional sole cropping methods, primarily because of the complementary utilization of resources across different species in terms of both timing and spatial distribution (Gou et al., 2016). In their study, Hauggard-Nielson and Jensen (2005) also demonstrated that intercrops not only have higher yields than crops grown alone, but also exhibit greater stability.

The potato, ranked as the third most crucial food crop worldwide in terms of consumption, has received strong endorsement from the Food and Agriculture Organization of the United Nations as a crop that enhances food security, particularly in light of the increasing global population and the associated challenges concerning food availability (Devaux et al., 2014). A field experiment conducted in 2015 and 2016 revealed that intercropping potatoes with a higher density of legumes not only boosts overall system but also leads to greater land-use efficiency and economic advantages, primarily due to increased potato yields. (Gitari et al., 2020). In a previous master thesis in the same experimental design as this research, it was observed that potato yields were significantly higher at Droevendaal, with a notable 6% increase ($p=0.018$) in comparison with monoculture (Xu, 2023). However, the underlying mechanisms contributing to these yield enhancements remain unclear. This gap in understanding prompts further investigation into the role of arbuscular mycorrhizal fundi (AMF) colonization as a potential influential factor in crop yield optimization. According to recent study, potato-legume intercropping practice has been found to effectively reduce soil erosion, enhance moisture retention in the soil, and ultimately result in higher crop yield (Nyawade et al., 2018). Research conducted in the Netherlands in a mixed cropping systems with potatoes, showed that the disease incidence of the late blight (Phytophthora infestans) in potatoes planted in strips was significantly lower compared to the monoculture reference. The former, in addition to the inclusion of cultivar mixing within the strips, proved to be more effective in reducing the impact of late blight compared to spatial diversification alone (Ditzler et al., 2021). Reduced disease pressure in potato crops extends the growing period, allowing potatoes more time to mature and develop. This extended growth phases enables the potatoes to accumulate more biomass, leading to larger tubers. During the stages of tuber growth, the starch content increases due to an augmentation in both the quantity and size of starch granules (Grommers & van der Krogt, 2009). Potatoes that undergo a longer maturation process, extending beyond active photosynthesis and into post-senescence, tend to have larger starch granules and, consequently, higher starch content. Therefore, the duration of tuber development, including both the growth and maturation phases, is crucial for enhancing the starch content of potatoes (Christensen & Madsen, 1996).

Arbuscular mycorrhizas (AM) represent an essential functional group within the soil biota, offering significant potential to enhance crop productivity and promote the sustainability of ecosystems in emerging plant production approaches (Lone et al., 2015). These fungi possess the ability to establish a symbiotic relationship with the root systems of 80% of plant families (Gianinazzi et al., 2010). Arbuscular mycorrhizal fungi (AMF) play a crucial role as part of the soil microbial community, contributing to the formation of fertile soils and promoting agricultural sustainability (Guzman et al., 2021). In addition, the symbiotic mycorrhizal fungi offer various advantages to crop production through their ecosystem functions. In fact, AMF exhibits a symbiotic relationship with herbaceous plant species, particularly cereals and vegetables. They facilitate water and nutrient uptake, regulate allelopathic interactions and enhance plant defense mechanisms, thereby promoting root colonization and overall plant growth (Trinchera et al., 2019). On the other hand, a pot experiment indicates a positive influence of AMF on potato yields, as evidenced by the net increase in both above and below-growth of plants at 20 days intervals after seedling emergence. The mycorrhiza infected plants, compared to non-inoculated ones, exhibited higher levels of chlorophyll content, morphological growth parameters, and fresh and dry weight content in both cultivars of plants (Lone et al., 2015),This suggests a beneficial association between AMF inoculations and enhanced overall growth and productivity in potato cultivation.

However, there is a significant knowledge gap regarding the colonization of AMF in open field conditions. Validating the benefits of AMF for crop growth and yield, specifically the mycorrhizal growth response, requires confirmation through field trials conducted across diverse environmental conditions (Ryan & Graham, 2018). Existing research predominantly relies on pot experiments to assess AMF colonization, while studies specifically investigating AMF colonization in open field settings are scarce.
In addition, the interactions between different species of AMF within diverse communities and the soil microbiota are not well comprehended. Limited research has been carried out under field conditions to investigate this aspect (Ryan & Graham, 2018). By highlighting this gap in the literature, there is the need to investigate AMF colonization in real-world agricultural environments and the potential significance of my research in filling this gap.

This research aims to investigate the impact of strip cropping on potato production in the Netherlands, with a focus on quality, yield, and AMF colonization. Implemented on two organically managed farms, the study also seeks to understand the potential correlation between higher yields in strip cropping and increased AMF colonization.

Therefore, this research aims to answer three main questions: 1) How does the overall yield of potatoes in strip cropping systems compare to that of monocropping? 2) What are the differences in potato quality between strip cropping and monocropping systems? And 3) How does strip cropping affect the colonization of AMF in potato plants compared to monocropping? It is hypothesized that a higher overall potato yield will be observed in a strip cropping system than in a monocropping system. In addition a higher portion of the harvested potatoes, the strip cropping will have

higher marketable yields. Finally, that the colonization of AMF in potato plants is higher in a strip cropping system in comparison to a monocropping system.

2.Methodology

2.1. Site description

This research was conducted on two organically managed farms in the Netherlands during the summer of 2023. The first one is the Droevendaal experimental farm in Wageningen (51o59'33.06" N,5o39'43.56"E) and the second one is the Broekemahoeve experimental farm in Lelystad (52o32'23.70"N,5o33'44.92"E) (see Appendix A). In both locations, ongoing experiments related to strip cropping systems are taking place. They differ mainly in terms of their soil types, with Droevendaal having sandy soil and Broekemahoeve having clay soil. The focal crop in this study is potato (Solanum tuberosum L.), featuring two different varieties: Allouette for all treatments, except for the climate treatment variety, Twinner. The full experiment ran for five years from 2018 to 2022, whereby potatoes have been cultivated following a set of rotation across fields during these five years. Finally, it is crucial to highlight that management practices on both sites were diligently synchronized. The aim was to ensure that any observed differences in yields and quality of potatoes could be attributed with greater confidence to the cropping system rather than variations in management. With the primary focus on STRIP_3 (strip cropping) and REF_SPACE (monoculture) treatments, which both underwent identical management practices.

2.2. Experimental design

The experimental design is an incomplete block design that is divided into fields, blocks, treatments, strips, and rows, allowing us to systematically evaluate the variables of interest (see Figure 1). Within the experimental setup, both monocropping systems and strip cropping systems are incorporated. Monocropping refers to the traditional practice of growing a single crop in each area, while strip cropping involves cultivating two or more crops next to each other in distinct 3-meter-wide strips. In Droevendaal, there are six fields, each divided into three blocks (see Appendix A, Figure 1). For fields 1, 2, and 3, each block is allocated for strip cropping of a crop pair, accompanied by a smaller monoculture field as a reference. In contrast, for fields 4, 5, and 6, out of the three blocks in each field, one block is used for the strip cropping of a crop pair, while the remaining two blocks are dedicated to monocropping. These monocrop blocks served as the main reference plots for the individual crops. In Broekemahoeve, there are four fields, each divided into three blocks and follows the same design as Droevendaal (see Appendix A, Figure 2). In addition, the two experimental sites are undergoing several treatments throughout the fields that can be found in Table 1 below.

The focus in this study was on the pair potato/grass-clover. They are sown in strips, where each strip contains four rows of potato, as seen in Figure 1 below. As for the STRIP_DIVERSITY, it is the combination of the cultivation of the six crops sown in strips next to each other. In the first part of the study, all the treatments will be taken into comparison as for the second part of the study which relates to the AMF colonization, the focus will be on specific treatments, namely STRIP_3, REF_TIME, and REF_SPACE.

Table 1:Description of the different treatments on both strip-cropping experimental farms, Droevendaal and Broekemahoeve

Treatments	Description	Droevendaal	Broekemahoeve
STRIP_3	Strips of 3 m wide sown with a sole crop species of a single variety. Varieties selected to suit local markets and environmental conditions. Sole-crop strips are sown in pairs with a second crop.	X	X
STRIP_CLIMATE	Strips of 3 m wide sown with a sole crop species of a single variety. Variety is chosen based on climate adaptation. Choice for early harvestable varieties with water demand early in the year and low demand during dry summer.	X	X
STRIP_DIVERSITY/NATURE	Strips of 3 m wide sown with a sole crop of a single variety, all six crops next to each other in strips. Aim to enhance diversity.	X	X
REF_SPACE Spatial reference	Large-scale monoculture plots of the same crop species sown in STRIP_3, used as a reference for comparing large-scale spatially explicit effects of crop diversification.	X	X
REF_TIME Temporal reference	Small-scale monoculture plots of the same crop species sown in STRIP_3, used as a reference for comparing temporally explicit effects of crop diversification.	X	X

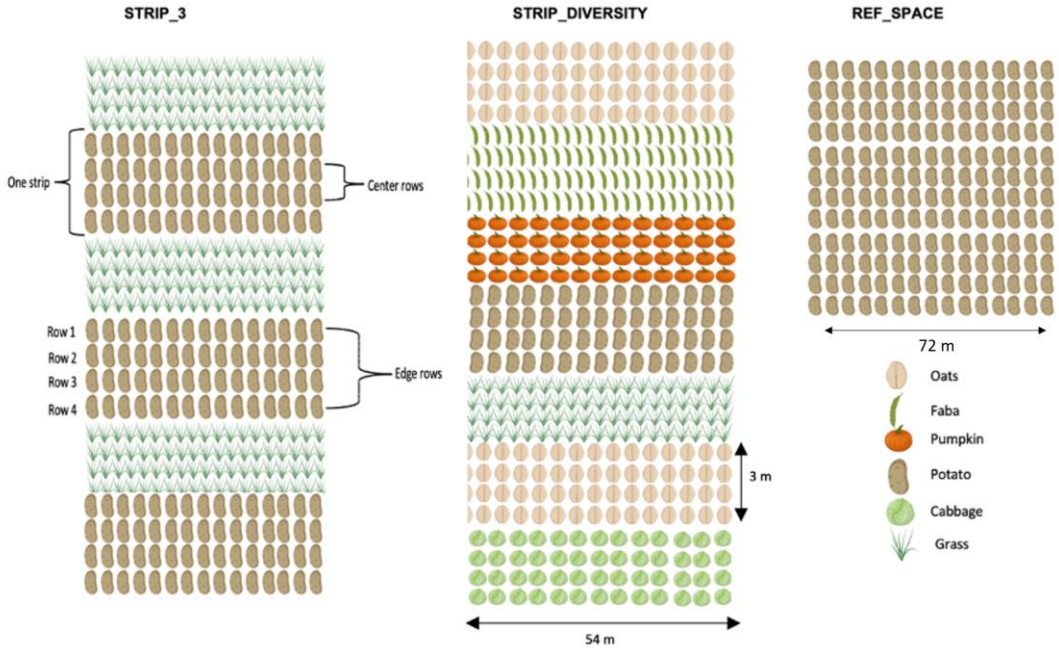

Figure 1:Graphical representation of the different treatments on both strip-cropping experimental farms, Droevendaal and Broekemahoeve

2.3. Data collection

This study focused on the potato/grass crop pair, with data collection conducted during the summer of 2023 from the Droevendaal and Broekemahoeve locations. The objectives of the first and second research question was to assess the yield quantity and quality of potato production by examining specific treatments: STRIP_3, STRIP_DIVERSITY, STRIP_CLIMATE, REF_TIME, and REF_SPACE (Table 2). In the intercropping system, sampling was conducted in both the edge rows and center rows of the strips. Only the inner two potato strips were sampled from the STRIP_3 treatment, with the outer strips being excluded. Each strip consists of four rows of potatoes, and for sampling purposes, the North and South rows were grouped together as the edge rows. The two rows in the center of the strips were considered the center rows.

2.3.1. Yield assessment

The assessment of the yields of potatoes was based on two indicators: the fresh marketable yields and dry marketable yields. At the Droevendaal site, we utilized a one-row harvesting machine for the collection of potatoes (see Appendix B). The yield measurement process began by weighing the crates filled with harvested potatoes to establish the "brute yield", which refers to the harvested kilograms without adjusting for area, for each two-rows, combining the two center rows and two edge rows. During this process, a sample of 5 kilograms of potatoes was collected and enclosed in mesh bags. These bags containing the potatoes samples were then stored in a cooling facility pending subsequent sorting and quality evaluation. For the determination of the fresh yields, brute yields were used and then corrected for the harvested area.

As for the determination of dry yield, a subsample of around 300 grams was chosen at random. The subsamples were initially weighed to record the total weight of the subsamples. They were then dried in an oven at 70 degrees Celsius for a duration of 48 hours. Post-drying, the samples were weighed immediately to obtain the dry weight (see Appendix C). This process enabled the calculation of both fresh weight and dry matter ratio, which I then used to derive the fresh marketable yields and dry marketable yields.

$$Fresh\ Marketable\ Yields = Fresh\ Yields \times Marketable\ Factor$$

Equation 1:The calculation of fresh marketable yields in kg/m² with Marketable Factor calculated in Equation 3.

$$Dry\ Marketable\ Yields = (Fresh\ Yields \times Dry\ matter\ ratio) \times Marketable\ Factor$$

Equation 2: The calculation of dry marketable yields in kg/m² with Marketable Factor calculated in Equation 3.

2.3.2. Quality assessment

To assess the quality of potatoes, they were sorted into various size categories using an optical sorter known as "Smart Grader". The sizes were classified as less than 35mm, 35-50mm, 50-65mm, and greater than 65mm (see Appendix D). The marketable yields consisted of the three categories: 35-50mm, 50-65mm, and greater than 65mm where we could extract the marketable factor. The marketable factor was calculated taking into consideration only the

three categories of potatoes with marketable sizes.

$$Marketable\ Factor = \frac{\text{g\_35\_50mm} + \text{g\_50\_65mm} + \text{g\_m65mm}}{\text{g\_s35mm} + \text{g\_35\_50mm} + \text{g\_50\_65mm} + \text{g\_m65mm}}$$

Equation 3: The calculation of the marketable factor taking into consideration only the three marketable categories.

These marketable categories, in addition to the fresh marketable yields, were merged to serve as a quality indicator, represented by the revenues.

$$Revenues = Fresh\ Marketable\ Yields \times 0.30$$

Equation 4:The calculation of revenues in euros per m² with 0.30 euros the selling price per kilogram.

Moreover, the quality of potatoes is also linked to their starch content, which is determined by the specific gravity method. This method involves calculating the ratio of the potato's density to that of water. To do so, a sample of potatoes was first weighed in air, and then weighed again when submerged in water. The specific gravity was calculated using the weight measurements from these two states (see Appendix C)

$$Specific\ Gravity = \frac{\text{Above\_water\_weight\_kg}}{\text{Above\_water\_weight\_kg} - \text{Below\_water\_weight\_kg}}$$

Equation 5: The calculation of the specific gravity

Once the specific gravity was determined, it was used to estimate the starch content of the potatoes, utilizing a specific gravity formula that correlates the specific gravity to starch content (Sadebo et al., 2021):

$$Starch\ content = 17.546 + 199.07 \times (specific\ gravity - 1.0988)$$

Equation 6: The calculation of the percentage of starch content

2.3.3. AMF colonization assessment

In the second part of the study, a distinct data collection procedure was conducted specifically to evaluate the AMF colonization in strip-cropping compared to monoculture. In each field, 5 samples were collected from the monoculture labeled REF_CENTER, 5 from the edge row of STRIP_3 (STRIP_EDGE), and 5 from the center row of STRIP_3 (STRIP_CENTER), totaling 15 samples per field. This well-designed approach enabled the collection of 60 samples (Table 4). Importantly, it should be noted that this sampling procedure was exclusively conducted in the Droevendaal location. The Broekemahoeve farm was omitted from this part of the research since one of the two fields is new and has no history of strip cropping. The second site has been in strips for four years, but there was only one replication at Broekemahoeve. Moreover, the differences in soil types would have complicated the meaningful comparison with the data obtained from Droevendaal.

For the assessment of AMF colonization in potato plants, a destructive method was employed. The entire plants were collected from the soil during the destructive sampling process, allowing for the extraction of root subsamples for subsequent AMF analyses (see Appendix E). In the laboratory, a distinct protocol developed by Koppert was followed to stain the subsamples and conduct AMF analysis (see Appendix F).

The specific procedure for root rehydration and staining, which aids in AMF detection, is based on the work of Vierhellig et al. (1998) and Vierheilg & Piche (1998), as detailed in the Appendix G. The quantification of AMF root colonization was carried out using the method outlined by Brundrett MC (1998). This approach involves stained roots within a petri dish market with a grid. The roots were examined under a microscope at 10x magnification. 100 observations per sample was done to be able to classify observations into four distinct categories: “No AMF”, which indicates no colonization, “Hyphae”, “Arbuscules” and “Vesicles”.

Table 2: Sample size for quality and yield analysis

	Droevendaal		**Broekemahoeve**	
	Fields 1-2-3	Field 4	Fields J8 -J9	Field J9.3
REF_TIME	6			
REF_SPACE		8		4
STRIP_3	12	4	8	14
STRIP_DIVERSITY	6		8	
STRIP_CLIMATE	12		8	

Table 4:Sample size for AMF analysis

	Droevendaal			
	Field 1	Field 2	Field 3	Field 4
REF_CENTER	5	5	5	5
STRIP_EDGE	5	5	5	5
STRIP_CENTER	5	5	5	5

2.4. Statistical analysis

All Statistical analyses were performed with the statistical program R. Linear mixed-effects models (LMMs) were used to test the effects of different treatments on the fresh marketable yields (kg/m^2), dry marketable yields (kg/m^2), starch content (%) and revenues (€/m^2). As for the proportion of AMF root colonization, a generalized mixed-effect models (GLMM) with a binomial distribution were used, exclusively in Droevendaal.

The data from each location was analyzed using distinct statistical models. This was due to the inability to meet the assumptions necessary to validly apply the diagnostic tests provided by the DHARMa package when attempting to use a combined model for both locations. This specific package is used for diagnostic assessment of hierarchical mixed models in R, it checks if residuals from these models meet the assumptions of homogeneity, independence and normality (Hartig, 2022). The diagnostic tests for overdispersion assumption using the DHARMa package indicated that this assumption was not met for the model. Consequently, further analysis based on this model may not be reliable, and as a result, each location was analyzed separately.

In each location, the analysis was divided into two parts: one for the main comparison between STRIP_3, representing the strip cropping system, and REF_SPACE, representing the monoculture, and another analysis for the comparison between STRIP_3, STRIP_DIVERSITY and STRIP_CLIMATE (Table 2). Therefore, we have a total of four main models, with two in each location.

In the statistical analysis conducted at Droevendaal for Field 4, the LMM assigned "Line" as the random effect when examining the REF_SPACE and STRIP_3 treatments, to account for variation between lines. Similarly, for Fields 1,2 and 3 at Droevendaal, "Field/Block/Line" was used as the random effect in the LMM to account for variation within the same location, which analyzed the treatments REF_TIME, STRIP_3, STRIP_CLIMATE and STRIP_DIVERSITY.

Over at Broekemahoeve, an equivalent analysis was carried out; for Field J9.3," Line" was considered the random effect for investigating the REF_SPACE and STRIP_3 treatments. Additionally, for Fields J8 and J9, "Field" was the random effect used in the LMM, with the treatments being STRIP_3, STRIP_CLIMATE and STRIP_DIVERSITY.

All models were validated by performing the normality of the models' residuals using the Shapiro-Wilk test with a p-value > 0.05. Following the LMMs and GLMMs, the one-way analysis of variance (ANOVA) and the Tukey's Honestly Significant Different (HSD) tests were performed.

3. Results

3.1. Droevendaal

3.1.1. Yield analysis

Fresh Marketable Yields

In field 4, STRIP_3 and REF_SPACE did not demonstrate a significant difference in fresh marketable yields (p-value = 0.9333). The fresh marketable yields were similar for both treatments, with REF_SPACE yielding 3.40 kg/m^2 (±0.14) and STRIP_3 yielding 3.41 kg/m^2 (±0.22).

In fields 1-2-3, the treatments compared were STRIP_3, STRIP_CLIMATE, STRIP_DIVERSITY and REF_TIME. The results indicated significant differences among

the treatments (p-value= 4.769e-16). STRIP_CLIMATE had the lowest mean yield of 1.14 kg/m^2 (±0.30), significantly lower than the others. REF_TIME and STRIP_3 had similar yields, with means of 1.94 kg/m^2 (±0.31) and 2.13 kg/m^2, (±0.30) respectively, and were not significantly different from each other but were from STRIP_CLIMATE. As for STRIP_DVERSITY, it had the highest mean yield of 3.06 kg/m^2 (±0.31) but was not significantly different from STRIP_3 but from REF_TIME and STRIP_CLIMATE.

Dry Marketable Yields

In field 4, STRIP_3 and REF_SPACE did not demonstrate a significant difference in dry marketable yields (p-value = 0.1486). The dry marketable yields were similar for both treatments, with REF_SPACE yielding 0.68 kg/m^2 (±0.03) and STRIP_3 yielding 0.76 kg/m^2 (±0.06) (see Figure 2A).

In fields 1-2-3, all treatments demonstrated a significant difference in dry marketable yields (p-value=3.554e-12). STRIP_CLIMATE had the lowest mean dry yield of 0.20 kg/m^2 (±0.06), which was significantly different from all other treatments. REF_TIME and STRIP_3 had moderate yields of 0.37 kg/m^2 (±0.06) and 0.40 kg/m^2 (±0.06), respectively, and were not significantly different from each other. Yet, both were significantly different from STRIP_CLIMATE and STRIP_DIVERSITY treatments. STRIP_DIVERSITY showed the highest mean dry yields of 0.64 kg/m^2 (±0.06), significantly outperforming all other treatments (see Figure 2B).

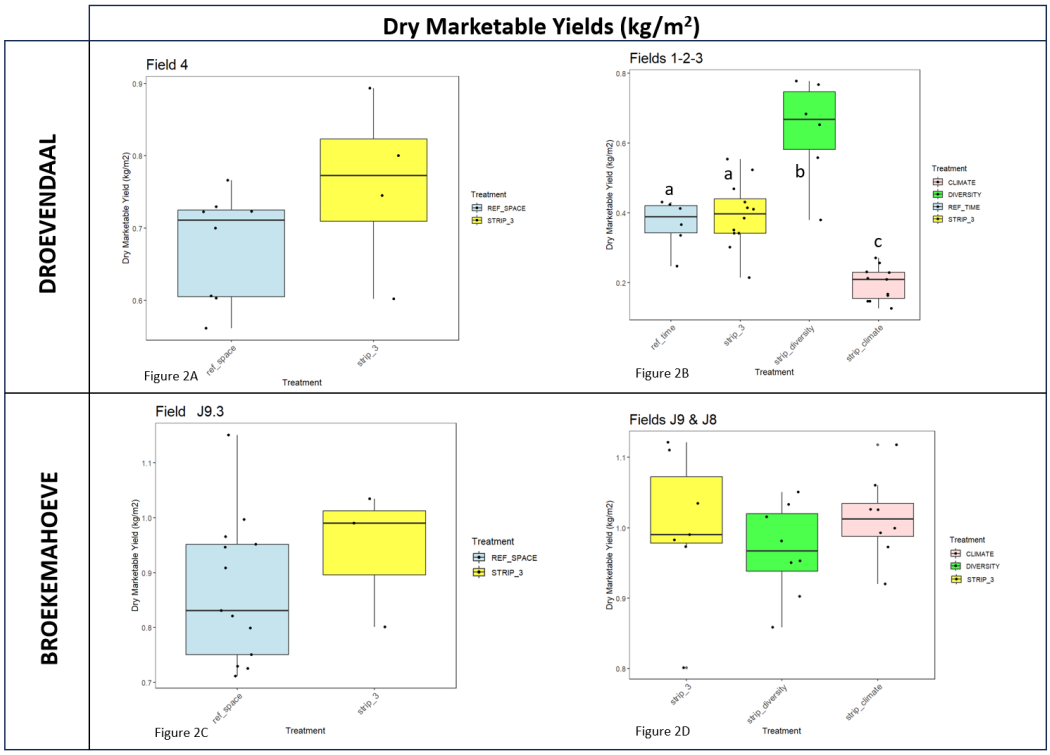

Figure 2:Dry marketable yields (kg/m2) per location: Indication of significant differences by the letters a, b, c

3.1.2. Quality analysis

Starch Content

In field 4, STRIP_3 and REF_SPACE did not demonstrate a significant difference in starch content (p-value =0.3467). The starch content percentages were similar for both treatments, with REF_SPACE scoring 14.0% (±0.002) and STRIP_3 scoring 14.3% (±0.003) (see Figure 3A).

In fields 1-2-3, all treatments demonstrated a significant difference in starch content percentage (p-value=4.897e-09). However, REF_TIME, STRIP_3 and STRIP_DIVERSITY were not significantly different from each with their starch content of 12.7% (±0.005), 12.5% (±0.003) and 13.7% (±0.005) respectively. As for STRIP_CLIMATE, it scored the lowest starch content of 10.9% (±0.002) and was significantly different from all the other treatments (see Figure 3B).

Revenues

In field 4, STRIP_3 and REF_SPACE did not demonstrate a significant difference in revenues (p-value = 0.9333). The estimated revenues were similar for both treatments, with REF_SPACE generating 1.02 €/m^2 (±0.04) and STRIP_3 generating 1.02 €/m^2 (±0.07) (see Figure 4).

In fields 1-2-3, all treatments demonstrated a significant difference in revenues (p-value=4.679e-16). The estimated average revenues were 0.34 €/m^2 (±0.09) for STRIP_CLIMATE, 0.58 €/m^2 (±0.09) for REF_TIME, 0.64 €/m^2 (±0.09) for STRIP_3 and 0.92 €/m^2 (±0.09) for STRIP_DIVERSITY. These results show that STRIP_DVERSITY yields the highest revenue, significantly different from REF_TIME and STRIP_CLIMATE but not from STRIP_3. In contrast, STRIP_CLMATE generated the least revenue, significantly different from other treatments. As for REF_TIME and STRIP_3 are not significantly different from each other but significantly different from STRIP_CLIMATE (see figure 4B).

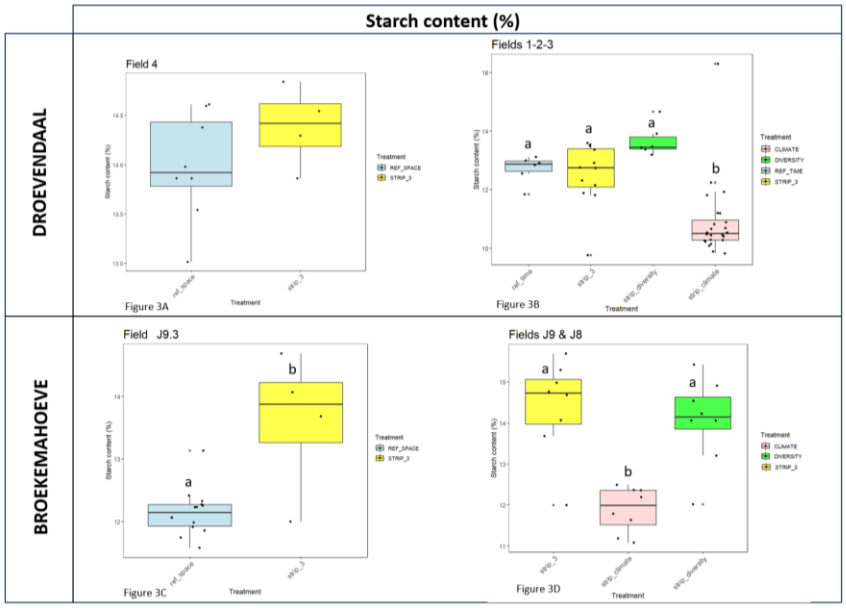

Figure 3:Starch content (%) per location: Indication of significant differences by the letters a, b, c.

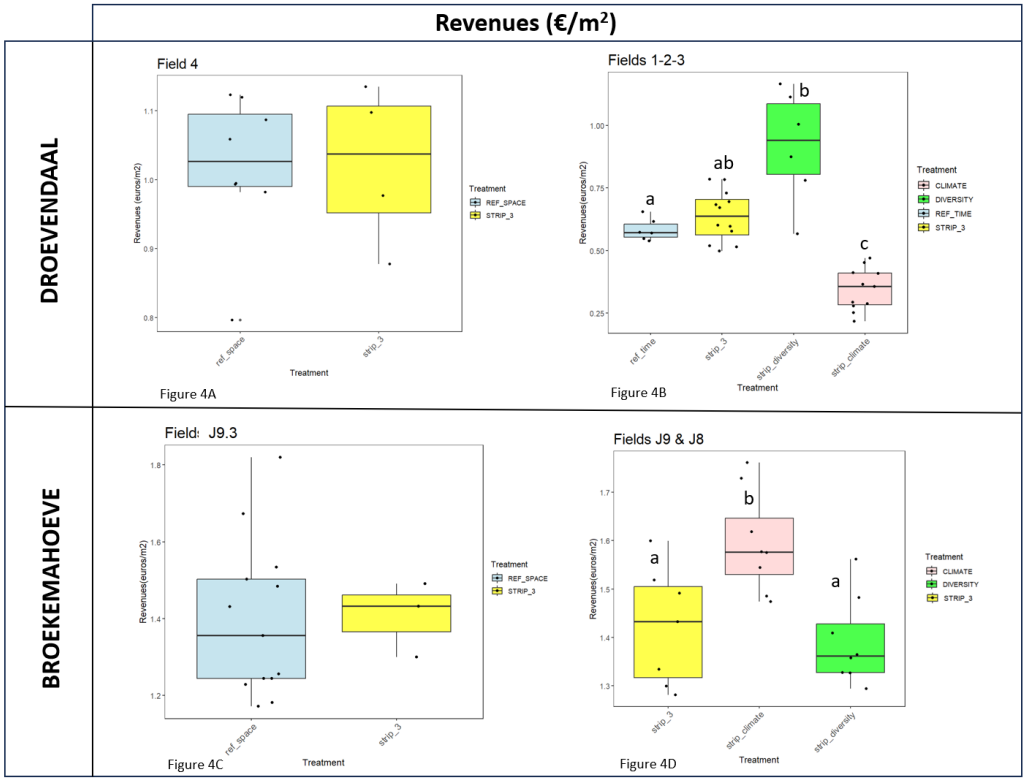

Figure 4::Revenues (€/m2) per location: Indication of significant differences by the letters a, b, c.

3.1.3. AMF colonization analysis

In fields 1-2-3-4, REF_TIME and REF_SPACE were compared to STRIP_3 (edge) and STRIP_3 (center) using an ANOVA test to determine the association between the treatments and the total number of AMF. No significant difference was found in the count data of hyphae, arbuscule and vesicles (p-value=0.5534). Therefore, it indicates that there is not statistically significance in AMF colonization between the strip cropping system and monoculture.

3.2 Broekemahoeve

3.2.1. Yield analysis

Fresh Marketable Yields

In field J9.3, STRIP_3 and REF_SPACE did not demonstrate a significant difference in fresh marketable yields (p-value=0.914). The mean yields were almost similar for both treatments, with REF_SPACE yielding 4.65 kg/m^2 (±0.18) and STRIP_3 yielding 4.69 kg/m^2 (±0.41) .

In fields J8 and J9, all treatments demonstrated a significant difference in fresh marketable yields (p-value=0.0001966). The post hoc test revealed that STRIP_DIVERSITY and STRIP_3 had similar yields, with means of 4.64 kg/m^2 (±0.13) and 4.74 kg/m^2 (±0.14), respectively, and they were not significantly different from each other. However, both were significantly different from STRIP_CLIMATE yielding 5.32 kg/m^2 (±0.13).

Dry Marketable Yields

In field J9.3, STRIP_3 and REF_SPACE did not demonstrate a significant difference in dry marketable yields (p-value=0.3764). REF_SPACE had a mean of 0.87 kg/m^2 (±0.04),

while STRIP_3 had a slightly higher mean yield of 0.942 kg/m^2 (±0.08) (see Figure 2C).

In fields J8 and J9, STRIP_3, STRIP_DIVERSITY and STRIP_CLIMATE did not demonstrate a significant difference in dry marketable yields (p-value=0.4781). STRIP_DIVERSITY had an average yield of 0.97 kg/m^2 (±0.03), STRIP_3 had 1.02 kg/m^2 (±0.03) and STRIP_CLIMATE had the highest at 1.01 kg/m^2 (±0.03) (see Figure 2D).

3.2.2. Quality analysis

Starch Content

In field J9.3, STRIP_3 and REF_SPACE demonstrated a significant difference in starch content (p-value=2.614e-16). The starch content percentages for REF_SPACE was 12.2% (±0.02) and STRIP_3 was 13.5% (±0.004) (see figure 3C).

In fields J8 and J9, all treatments demonstrated a significant difference in starch content percentage (p-value=1.624e-15). However, STRIP_3 and STRIP_DIVERSITY were not significantly different from each with their starch content of 14.8 % (±0.005) and 14.0% (±0.005) respectively. As for STRIP_CLIMATE, it scored the lowest starch content of 12.3% (±0.005) and was significantly different from all the other treatments (see Figure 3D).

Revenues

In field J9.3, STRIP_3 and REF_SPACE did not demonstrate a significant difference in revenues (p-value=0.914). The estimated means from the model indicated that REF_SPACE generated 1.39 €/m^2 (±0.05) and STRIP_3 generated a slightly higher 1.41 €/m2 (±0.11) (see Figure 4C).

In fields J8 and J9, all treatments demonstrated a significant difference on revenues (p-value=0.0001966). The estimated mean revenues were 1.39 €/m^2 (±0.04) for STRIP_DIVERSITY, 1.42 €/m^2 (±0.04) for STRIP_3 and 1.60 €/m^2 (±0.04) for STRIP_CLIMATE. Following a post-hoc test, the highest revenue was associated with STRIP_CLIMATE, which was statistically different from the other two treatments, as indicated by its separate grouping. Both STRIP_DIVERSITY and STRIP_3 were assigned to the same group, indicating no significant difference in revenue generations (see Figure 4D).

4. Discussion

In this study, the aim was to address three research questions surrounding the impact of strip cropping on potato yield, quality, and AMF colonization. This research encompassed two experimental locations, Droevendaal and Broekemahoeve, each providing valuable insights into the effects of strip cropping compared to monocropping. The results from Droevendaal revealed nuanced differences in fresh and dry marketable yields, starch content, and revenues across various strip cropping treatments. Similarly, the Broekemahoeve results explained variation in fresh and dry marketable yields, starch content, and revenues. This research demonstrated the promising role of strip cropping in organic potato production.

4.1. Comprehensive yield analysis

In Droevendaal and Broekemahoeve, it was observed that the marketable yields of REF_SPACE and STRIP_3 (field 4) and REF_TIME and STRIP_3 (field J9.3), respectively, were not significantly different from each other. However, the strip cropping systems performed at least as well as the

monoculture this year and did not underperform. The means of fresh marketable yields of the strip cropping systems at both locations were similar to those of the monoculture systems. The primary goal of strip cropping practices is to ensure that it does not result in lower yields than conventional methods as this could negatively impact profitability. The observations that marketable yields from the two systems were not significantly different from each other are quite promising. It suggests that the strip cropping can match the performance of monoculture systems in terms of yields. This is due to its potential for increased yields per unit of land compared to monocultures, as well as lower risk of crop failure and enhanced resilience to market fluctuations (Glaze-Corcoran et al., 2020). In fact, grass-clover as an adjacent crop to potatoes seems beneficial due to its lower competitive pressure on edge row compared to cereals (Bouwst & Finckh, 2008). A study conducted on strip cropping in Germany provides compelling evidence for the benefits of integrating grass-clover with potato cultivation. Over a three-year period, potatoes with grass-clover consistently demonstrated superior yields. This contrasted with plots adjacent to cereals, which yielded the least (Bouwst & Finckh, 2008). Therefore, the combination of potato and grass clover emerges as a promising pair.

In both experimental locations, the Twinner potato variety, represented by STRIP_CLIMATE, stands out as a unique cultivar chosen for its specific climate adaptation features and early maturity, facilitating an early harvest. However, the performance of STRIP_CLIMATE varied notably between Droevendaal and Broekemahoeve. In Droevendaal this variety yielded the lowest among the treatments, while in Broekemahoeve, it excelled by producing the highest yields. This spatial variation in the performance of the Twinner variety is likely attributable to differences in environmental conditions, including distinct soil types and climate factors.

4.2 Comprehensive quality analysis

A high dry yield indicates that the potato contains a good amount of solid matter such as starch content. This is mainly associated with better taste, texture and nutritional content. The dry marketable yield is derived from the dry matter ratio of the marketable potatoes in this case, thus providing an indication of the dry matter content of potatoes. The dry matter ratio of potatoes refers to the proportion of the potato's weight excluding the water. In general, potatoes are composed of 70-80% water, so the dry matter usually makes up about 20 % of the total weight of the potatoes (Robertson et al., 2018). The dry matter and the starch concentration in potato are an important indicator as it informs the nutrient content including starch, proteins, fibers and minerals. In addition, the quality of the end products and the efficiency of their processing are directly influenced by these properties (Haase, 2003). Therefore, it serves as a key quality indicator for potatoes encompassing aspects such as starch content, firmness, flavor and processing, with the focus of this study being on starch content. A high dry matter content usually indicates a higher starch content. This is due to the significant correlation between dry matter and starch content in tubers, suggesting that an increase in dry matter would likely correspond to higher starch content (Grommers & van der Krogt, 2009). In both locations, STRIP_3 consistently demonstrated a higher starch content compared to REF_SPACE, which served as the primary comparison between strip cropping and monoculture. In contrast, STRIP_3

and STRIP_DIVERSITY consistently achieved the highest scores in terms of starch content. Furthermore, higher starch content in potatoes is translated to higher carbon storage. Starch serves as a storage form of carbon, synthesized during photosynthesis and can therefore be converted back into sucrose during carbon partitioning (Aliche, 2020). This results in an ecological benefit to the system as the potato plants are contributing to soil health through the conversion and storage of carbon which adds organic matter to the soil. This implies more nutrients available in the soil, better soil structure and improved water retention.

Shifting the focus to the economic aspect of crop production, revenues generated from potatoes by the two systems were examined. The profitability is assessed based on the marketable factor, which categorizes the potatoes into three size class that are suitable for the market which are 35-50 mm, 50-65 mm, and over 65 mm. These classifications are crucial in determining the income potential, with the unit price established at 0.30 euros per kilogram (KWIN-AGV, 2022). This approach allows us to evaluate the financial outcomes of the harvest and understand the implications for overall farm profitability when considering strip cropping systems. Looking at the economic outcomes from the two cropping systems studied, it was observed that in both Droevendaal and Broekemahoeve, strip cropping, and monoculture systems produced similar revenue levels per unit area. This parity in financial returns indicates that the choice of cropping system did not affect the economic performance in terms of revenue. The significant quantity of marketable potatoes resulted in increased revenues. In this case, the profitability of the farmers reflects both the quality and the quantity of the potatoes out of the strip cropping system. For farmers, the primary incentive is to ensure that their farm is profitable and generates income. Strip cropping leads to more stable yields and potentially lower input costs, and therefore a more resilient system that supports the farmers' economic objectives. Based on a study conducted in the Netherlands using mathematical modeling to assess the financial performance of strip cropping under high uncertainty, the research suggests that strip cropping demonstrates greater financial resilience compared to traditional monocropping. This is particularly evident in the face of uncertainties such as supply chain shocks and climatic events (Matar,2022). However, a comprehensive economic evaluation is necessary mainly for the farmers to evaluate the practical feasibility of a strip cropping system. A more complete picture of the economic viability of such a design would be interesting to look at, such as input cost added to the revenues.

4.3 Comprehensive AMF colonization analysis

A pot experiment conducted in a greenhouse in the Netherlands, involving the inoculation of AMF on potato plants, demonstrated increased root colonization in strip cropping after six and 10 weeks of planting (Akangbe,2022, Caruso 2022). This high level of AMF colonization is attributed to the high plant diversity in the system (Lee et al., 2023). Research finding indicate that polyculture fields, such as those in strip cropping systems, possess a more abundant and diverse community of AMF compared to monoculture fields. This suggests that implementing strip cropping, characterized by diverse crops, could contribute to the recovery and enhancement of AMF richness. Considering that monoculture farming is associated with decreased AMF diversity over time, the results imply that strip cropping may offer a more favorable

environment for AMF colonization and, consequently, higher levels of AMF diversity in agricultural systems (Gussman et al., 2022). Although this search did not show statistically significant differences in AMF colonization between strip cropping and monocropping treatments, the hypothesis aligns with existing research indicating the positive impact of AMF on potato plant growth and development. A study supports our expectation that higher AMF colonization could contribute to enhanced overall growth, chlorophyll content, and potentially higher yields in potato plants within a strip cropping system (Lone et al., 2015). An ongoing study at Droevendaal farm also confirms that strip cropping systems, particularly at the strip edges, had greater AMF DNA copies per g of soil (qPCR) compared to the monoculture system (Pers. Comm. Laura Riggi). In contrast with previous results, my findings indicated no significant difference in the AMF colonization between the strip cropping and monoculture systems nor between the edge and middle of the strips. The disparity between my study's finding and literature may be explained by the atypical weather conditions experienced during summer in the Netherlands. My data collection took place in July 2023 during wet conditions, which could have significantly influenced AMF colonization in potato roots. Excessive moisture in the soil affects the mycorrhizal associations, disturbing soil's microbial environment and may hinder AMF activity and their symbiotic relationships with plant roots. (da Silva Barros et al., 2019). However, future research in the understanding of the impact of wet conditions on AMF colonization is crucial. In addition, the timing of my data collection in July 2023 may have played a pivotal role in shaping the observed patterns of AMF colonization in potato roots. Dutch potato growth is known to be the most nutrient-demanding during the early stages of development, and potatoes have a greater propensity to associate with AMF for enhanced nutrient acquisition during this period. Collecting data at an early stage may have been a critical phase, capturing the initial stages of colonization and highlighting variations between treatments before the root systems reached full maturity. In addition, we aimed to obtain insights into whether higher yields could be attributed to AMF colonization. A study showed that the notable augmentation in plant width, the increased number of leaves per plant, and enhanced root length observed in AMF-inoculated plants compared to their control counterparts after a 40-day sampling period underscores the positive impact of AMF on the overall growth and development of plants (Lone et al, 2015). However, a more detailed investigation of how AMF colonization performs in open field strip cropping systems could give a clearer idea on how the interactions between these fungi and their host plants works. Therefore, open field studies are important as many external factors may have influenced the results as many interactions in the environment may have been overlooked.

Strip cropping and crop diversification are strategies aimed at enhancing and stabilizing both the yields and quality of crops. The results from both sites in the Netherlands highlighted the promising role of strip cropping within potato production systems. Several studies have indicated that growing crops together in intercropping systems tend to be more productive overall than growing a single type of crop alone (Van Der Werf et al, 2020, Li et al,2023). The increase in productivity that comes with intercropping could assist farmers in minimizing

fluctuations in their crop yields (Raseduzzaman & Jensen, 2017). In addition, strip cropping offers additional ecological benefits such as improving soil health, improving crop protection, increasing biodiversity, and reducing pest and disease pressure that are not immediately quantifiable in yields comparisons (Campenelli et al., 2023). These environmental services result in an increase in the resilience of the cropping system and can be a compelling reason for farmers to consider strip cropping as a viable and sustainable alternative to monoculture. Therefore, while the study provides preliminary insights, there is a significant opportunity for further research to develop a more comprehensive understanding of strip cropping systems in agriculture.

5. Conclusions

This research conducted sheds a compelling light on strip cropping as a sustainable agricultural practice with potential benefits for yield production, agronomic factors such as starch content, and economic aspects. The comparison of strip cropping to monoculture systems in organic potato production yielded insightful results. In terms of overall yield, strip cropping was found to perform comparably to monoculture, suggesting that it can be viable alternative without compromising productivity. As for potato quality, specifically starch content, the findings were not significantly different between the two systems overall, but this supports the fact that strip cropping can match the performance of the conventional monoculture systems in terms of marketable output. Regarding the AMF colonization, the findings did not show significant differences between the treatments, but this leads to further investigation. Continued research across diverse conditions is crucial for a comprehensive understanding of strip cropping's impact, paving the way for more informed and resilient agricultural practices. While the current findings may not conclusively establish the superiority of strip cropping in all aspects, they offer a promising glimpse into its potential benefits for sustainable agriculture.

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7. Appendix

Appendix A: Farms' layout

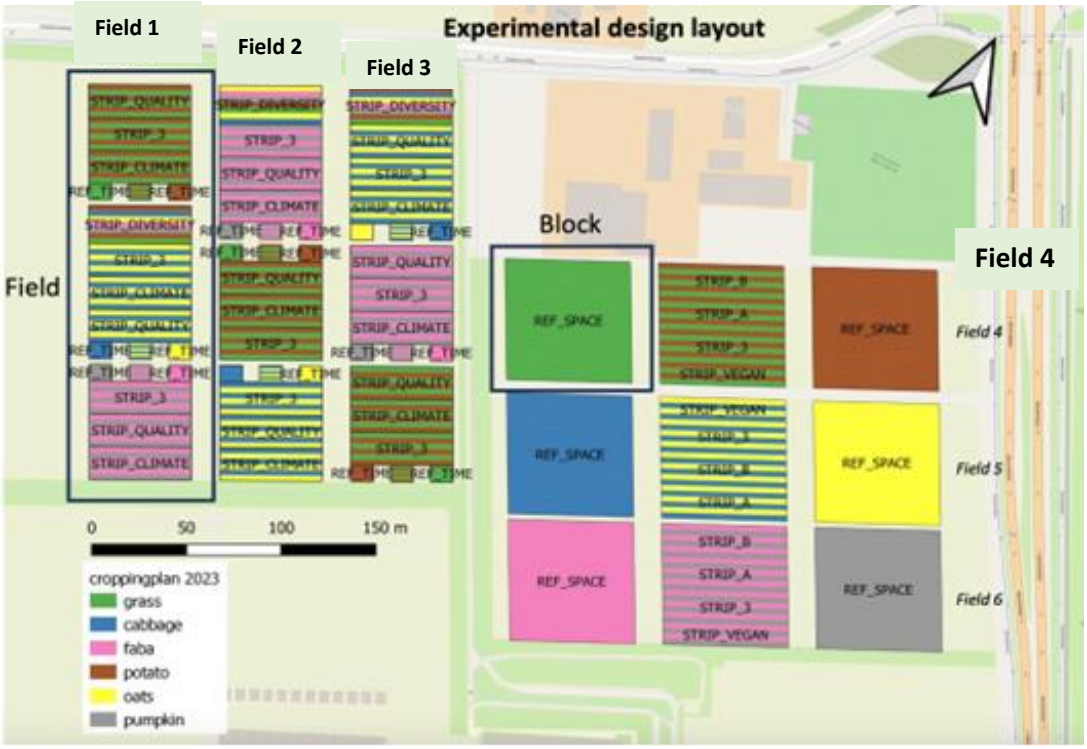

Figure 6: Droevendaal farm layout

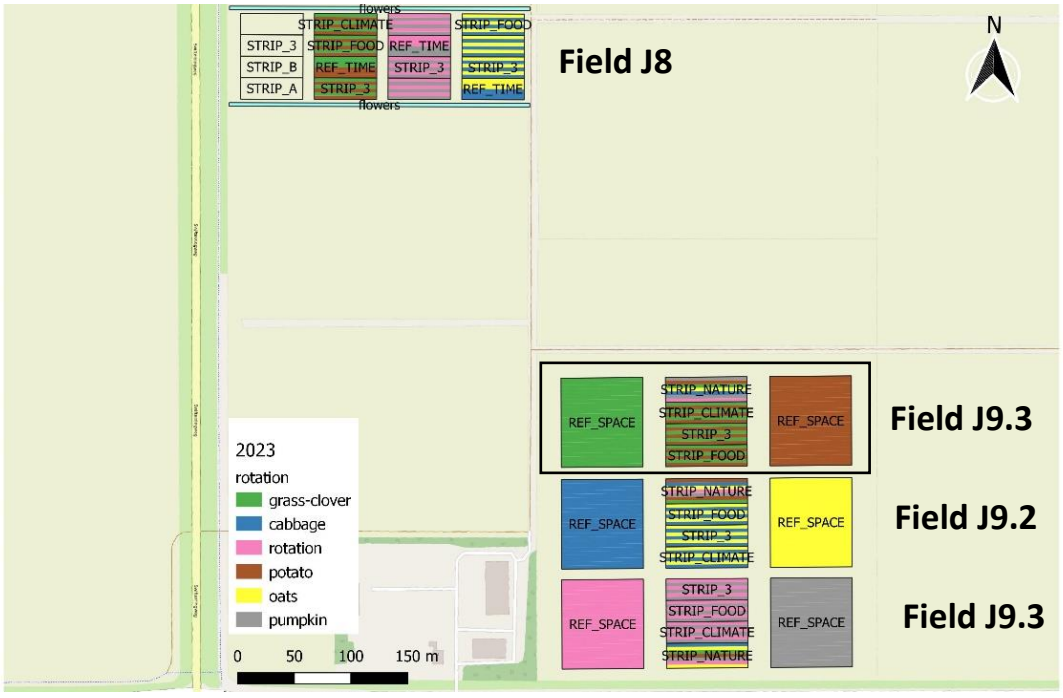

Figure 5:Broekemahoeve farm layout

Appendix B: Protocol Harvesting potato (with machine) in Droevendaal and Broekemahoeve-Lelystad
Last updated: 14/08/2020 by Muhammad Adham | 23/11/2018 by Annet Westhoek

Update: details in title, details in materials, time estimation, addition of field maps and field sheets to illustrate where samples were taken.

Goal: Quantification of potato yield per strip per two rows (Lelystad) or one row (Droevendaal)

Materials needed:

1. 2-rijige aardappel rooimachine (potato harvester two rows) (Lelystad)
or 1-rijige aardappel rooimachine (potato harvest one row) (Droevendaal)
2. Crate/container for 350-400 kg potatoes (Lelystad) or 200 kg (Droevendaal)
3. Cart (wagen) for crate/container
4. Pallet scale (on cart)
5. Labels, pencils, printed tables of crop harvest
6. Raincoat, boots, and gloves
7. 140 mesh bags (for Droevendaal) or 36 bags (for Lelystad) for 5-10 kg potatoes
8. Crate to store mesh bags with samples in
9. 1 m 3 crate for transportation of total yield

*the italic words materials are provided by Unifarm/ other employees.

Time estimation: 2-3 days for Droevendaal fields (with team of one students & four to five Unifarm employees), 1-2 days for Lelystad fields (with team of one students & two to three Lelystad employees)

Methods:

1. Every strip that borders a different treatment is regarded as buffer, so these are harvested, but no need to be sampled (depends on your research question)
2. Weigh empty crate
3. With potato harvester, harvest complete 60 m to crate
4. Weigh crate with potatoes
5. Write down weight brute yield
6. Take sample in mesh bag (minimum 5 kg)
7. Label mesh bag (one label inside bag and one attached to bag)
8. Put mesh bag in crate for samples
9. Empty crate in 1m 3 crate.
10. Store mesh bags in cooling for sorting and quality assessment in Wageningen.

Appendix C: Protocol Quality Assessment Potato
Written by: Marieke Datema

Written on: 19/09/2021

Last updated: 19/09/2021

Goal 1: Determining under water weight potatoes and preparations for dry matter determination

Materials needed:

- 5 kg sample of potatoes collected by the sorting procedure
- underwater weighing machine wet lab at uniform
- water
- sample sheet
- fries cutting machine
- scale
- waste bin
- aluminum trays (+/- 10cm x10cm x 25cm)
- drying oven
- big bucket or bag for left over potatoes

Time estimation: 128 samples 1-1.5 day

Methods:

1. Remove the bucket from the hook of the machine
2. Press on for 1 second
3. Hang the bucket on the hook, it is tared automatically
4. Fill the squared bucket at the bottom for 80% with water
5. Put your sample in the round bucket and keep it still (save the label for later)
6. Press F6 for the dry weight and note down
7. Roll the bucket down in the water with the handle
8. Press F7 to obtain the under water weight and note down
9. Roll the handle again to get the bucket up
10. Put 4-6 potatoes in the fries cutting machine discard the rest of the potatoes, make sure there is a bucket below the machine
11. Put a aluminum tray on the scale and tare it
12. Fill the tray for 75% (+/- 300 gram) with fries
13. Put the label on top and put the sample in the drying oven for at least 48 hours at 70 degrees Celsius
14. Clean the bucket below the fries cutting machine and put it back
15. Repeat step 5 till 14 for the next sample

Goal 2: Determining dry weight

Materials needed:

- aluminum trays from the drying oven
- grinding machine
- scale
- little brown bags
- spoon
- sample sheet

Time estimation: 128 samples 1-1.5 day

1. Retrieve the dried samples from the drying oven
2. Weigh the samples on a scale tare an empty tray first
3. Note the dry weight on the sample sheet

Appendix D: Protocol: Quality (size) assessment of potato yield
Written by: Anke ter Horst

Written on: 13-08-2020

Updated by: 01-09-2020 by Muhammad Adham and Cecilia Revol; 28-04-2022 by Anna de Rooij

Update: Clarification title to match document title, change in sieve size (70mm to 65mm), time estimation, details in method

Goal: This protocol allows you to do quality assessment on potato yield. Tubers are sorted in size.

Materials needed:

1. optical sorter ("Smart Grader") (barn 2, Unifarm)

2. Protocol: Operating the optical sorter (“Smart Grader”)

3. laptop

4. Measurement files (print-out) and pencil

5. Digital scale (accuracy of 0.1kg)

6. Mesh bags (can reuse the bags that the potatoes are stored in)

7. Labels (Can reuse the labels that were used during harvesting of the samples)

8. 2 buckets (one for on the scale and one to put under the Optical sorter)

Time Estimation: 25 minutes per mesh bag of sample

Method:

1. Start up the Optical sorter (incl. Laptop) using the Optical sorter manual (See Pro_091)

2. Extend the transportation belt of the Optical sorter.

3. Place a crate underneath the end of the transportation belt on the scale of the optical sorter so it can measure the weight of the potatoes.

4. Place the digital scale on a table next to the optical sorter and put a bucket on the scale to estimate the weight more accurately. The Optical sorter measures based on volume which can sometimes be inaccurate.

5. Install the right size classification in the setup which can be found on the screen of your laptop. There should be four sizes, namely <35mm, 35mm, 50mm and ≥65mm (or 70mm).

6. Make sure there is a bucket beneath the transportation belt to collect all tubers that fell of the transportation belt.

7. Start running using the button on your laptop screen, afterwards put in the first sample code. Every potato that now goes through the machine is put under this sample code.

8. Put the potatoes one by one on the transportation belt and make sure this is done carefully. When the potatoes move too much the size and volume estimation become less accurate. The accuracy of the run can be found on your laptop screen as well.

9. When all potatoes went through, it can be checked whether the run was accurate.

10. Put the potatoes from the crate into the bucket on the digital scale and note done to total weight.

11. Take a subsample for drying (cube of about 2x2x2 cm) and put it in a mesh bag (one mesh bag per sample). The total weight of subsample needs to be approximately 1 kg.

12. Label the mesh bag for drying.

13. Fill in the code of the next sample and push enter.

14. Put through the next sample and redo steps 9 to 19 .

15. When all samples are finished, data can be exported in either an excel or csv file. A description of how to do this can also be found in the Optical sorter manual.

16. Put the subsample in cool and dry area, also not exposed to direct sunlight.

Appendix E: Protocol: AMF sampling for potato

Written by: Bent Elvers (bent.elvers@web.de | bent.elvers@wur.nl)

Written on: November 4, 2020 | Last update: November 4, 2020

Goal

To determine the potato-root colonization of arbuscular mycorrhizal fungi (AMF) at the strip cropping experiment at Droevendaal experimental farm. AMF are especially important for plant phosphorus acquisition, soil structure and carbon sequestration (glomalin).

Materials needed

- Soil auger (⌀ 7 cm x 20 cm)
- Soil bags & labels
- 2 mm soil sieve
- Wash bottle
- Soil Biology Lab access

- Autoclave
- Sieve-tubes
- 5% ink & 5% vinegar solution
- 10% KOH
- 1% vinegar
- Object slides
- Microscope (100x magnification)

Time estimation

Sample taking with an auger takes less than 5 minutes per sample.

Cleaning of roots takes about 10 minutes per sample as the careful use of a wash bottle is required.

Staining takes about 1.5 h, 20 samples can be stained simultaneously.

Microscopic counting takes about 30-45 minutes per sample.

Method

Sampling

Healthy plants are selected arbitrarily in the middle of the plot (I took three samples per plot). Using a soil auger (⌀ 7 cm x 20 cm) soil cores directly next to these potato plants are excavated and stored in labelled soil bags at 4°C and processed within one week. Sampling in buffer strips is encouraged, as it is a disruptive measurement.

Root Preparation

Soil cores are rinsed in a 2 mm soil sieve, potato roots are then taken up with tweezers and thoroughly cleaned from soil using a wash bottle. Then roots are cut into 2-3 cm pieces and stored in a labelled container. It should contain at least 0.5 g of roots per sample. For staining (SBL access required, contact Tamas Salanki) according to Vierheilig et al. (1998) the root fragments are placed into centrifuge tubes and covered with KOH 10% solution. Then they are autoclaved at 121 °C for 10 minutes. Afterwards the roots are washed again using custom build sieve tubes (tip of centrifuge tube replaced by fine mesh). Then, a mixture containing equal parts 5% ink and 5% acetic acid solution is added to the roots before autoclaving for one minute at 105° C. After rinsing the roots in sieve tubes using tap water, the roots are stored in containers filled with 1% acetic acid solution.

Microscopic identification

For microscopical assessment of the samples (gridline intersection method by McGonigle et al., 1990) randomly chosen roots are placed lengthwise on an object slide representing five horizontal lines. Utilizing a microscope under 100x magnification, stained arbuscular, vesicular, and hyphal structures are assessed for 100 randomly chosen objective projection surfaces (20 per line). (Contrary to the original protocol, counts were generated if a structure was visible within the whole field of view as opposed to only if it crossed an imaginary central vertical line, as overall infection was judged to be rather low.)

Appendix F: Protocol Koppert: Step-by-Step potato root staining protocol

Preparation: Take each root samples and place in a cassette (If roots were stored in ethanol, leave 15-30 min to dry the ethanol). Pre-heat solutions for clarification (10% KOH), acidification (1% HCI) and staining (0,1% (w/v = weight by volume) ink in 2% (w/v) acetic acid in water) up to 70°C.

Clarification: Submerge all the cassettes simultancously in the KOH (10% solution for 60 mins at 70°C. After 30 minutes of incubation, check KO solution color. If it becomes brownish, replace it and incubate for another 30 minutes. Check if roots are completcly white/transparent. Ropcat if not completely clarified yet.

Washing: Wash the roots 4-5 times with tap water until all the KOH is removed.

Acidification: Submerge the roots in the HCI (1%) solution for approximately 30 min at 70°C for the acidification of the roots for a proper binding of the stain to the fungal structures.

Staining: Submerge the roots in the 0,1% (w/v) ink in 2% (w/v) Acetic acid in water solution for 20 min at 70°C. Collect the cassettes from the staining solution.

Washing: Wash the roots in water until no more stain is diffused and the water gets clear.

Storage: Keep in water at 4°C for improve the contrast of staining and short-term storage. In case of long-term storage is needed, keep in water-glycerin and acid lactic (1:1:1, v/v/v) at 4°C. 37