Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Faculty of Landscape Architecture, Horticulture and Crop Production Science

New methods for seed potato production: an investigation into the production and farmer uptake of mini tubers in South Africa

Alba Sáez Fernández

Degree Project • 30 credits
Agroecology - Master´s Programme
Alnarp 2018

New methods for seed potato production: an investigation into the production and farmer uptake of mini tubers in South Africa

Alba Sáez Fernández

Supervisor: Laura Grenville-Briggs Didymus, SLU, Department of Plant Protection Biology

Co-supervisors: Kristina Blennow, SLU, Department of Landscape Architecture, Planning and Management

Examiner: Erik Alexandersson, SLU, Department of Plant Protection Biology

Credits: 30 credits
Project level: A2E
Course title: Master's Thesis in Agricultural Science/Agroecology
Course code: EX0829
Programme: Agroecology – Master's Programme

Place of publication: Alnarp
Year of publication: 2018
Cover picture: Name of photographer
Online publication: http://stud.epsilon.slu.se

Keywords: mini-tubers, Chlorophyll fluorescence, Leaf Area Index, small-scale farming,

SLU, Swedish University of Agricultural Sciences
Faculty of Landscape Architecture, Horticulture and Crop Production Science
Department of Biosystems and Technology

Table of contents

Foreword

I did my bachelor's in microbiology because I believed that microorganisms could be efficiently used to solve environmental problems through bioremediation of contaminated media , and enrolled in the Agroecology programme because I wanted to merge microbiology and environmental sciences, though I had no knowledge whatsoever of agroecosystems. During the past two years, I underwent an introspective phase in which I started to see myself not as an individual that is isolated from the rest, but as a dynamic system that belongs in and affects a bigger one, while being in turn affected by it. Simultaneously, agroecology taught me how much interconnected systems are, how much they affect and rely on each other and how difficult it is to draw a line between systems. I might not have acquired practical skills in farming, yet I have acquired the tools to comprehend, through the application of systems thinking, agricultural systems and how they are affected by socio-economic and environmental externalities. Systemic problems need to be tackled with systemic approaches. It is true, nonetheless, that the reductionist approach allows for a more detailed analysis of a component of the system and that the more components included in the analysis, the lesser we know about each one. I find it very challenging to know how much I should know about a system, how many parts I should integrate and where I should set the boundaries, for the information is unlimited and there will always be elements that will be left out of the analysis even when they interact with the inner ones. Along the journey of this programme, I have learned the importance of running sustainable agri-food businesses in a responsible way for the sake of humanity, all other organisms and the environment, which all should coexist in harmony, as they are irreplaceable pieces of the system in which food production takes place. Agriculture is indispensable for the survival of humankind and is completely dependent on natural resources. Thus, compromising food systems or the environment in which they are embedded impairs inexorably the well-functioning of society. Having realised that, I made up my mind regarding my purpose as agroecologist: to bring about change through the improvement of agricultural systems. Through the completion of the Agroecology programme I shoulder this purpose as my duty, for I firmly believe in the words of Albert Einstein when he said "Those who have the privilege to know, have the duty to act".

The major change that I experienced through undertaking the Agroecology Master Programme was that I developed a sense of responsibility. Learning about systems thinking has influenced the way I see the world. Identifying all the components of a system allows us to see the effects that our actions have on each one of these components. Taking responsibility is about taking into consideration such effects or consequences when making decisions, so that the minimum damage is caused. During the programme and especially during my thesis research journey, I have learned to apply the systems theory and take responsibility for my actions in order to find the most sustainable way of living.

Summary

Potatoes are by far the most produced fresh vegetable crop in South Africa. Remarkably, potato sales represent more than 30% of the turnover in the Fresh Produce Markets (FPM). The potato industry in South Africa has experienced great improvements mainly due to the development of a reliable and healthy seed industry, the introduction of potato production under irrigation and of locally developed potato cultivars that are better-adapted to the South African climate. The Agricultural Research Council (ARC), founded in 1990, played an important role by introducing the potato breeding program. Furthermore, the foundation of the Potato Certification Services in 1995 brought about a sound structure for the production of clean, healthy and viable potato seeds, which are most commonly commercialised as mini-tubers or potato seeds.

The use of suitable potato cultivars and certified seeds is pivotal from an IPM point of view, as it reduces the need for agrochemicals and the incidence of diseases, therefore increasing the profitability of the crop. However, the use of agrochemicals is the main strategy to combat diseases in South African agricultural systems. Inputs-intensive, as opposed to knowledge-intensive agriculture, is environmentally degrading and not always affordable. According to the literature, the reuse of late-generation seeds from one year to the next one as a result of money shortages by poverty-stricken smallholding farmers increases the occurrence of diseases.

Certified mini-tubers, namely pathogen-free potato seeds that have been cultivated in sterilised medium, are currently being produced, although not commercialised, at the potato section of the department of Vegetables and Ornamental Plants of the ARC (ARC-VOP). Mini-tubers allow for the multiplication of seed for several generations before tuber-borne diseases reach dangerous levels that compromise food and economic security. The aim of the social research is to find out, through a survey carried out in a small-scale farming community of the Kwazulu-Natal province (KZN), whether the use of mini-tubers could improve the overall sustainability of smallholding agriculture. To this end, questionnaires and semi-structures interviews are conducted in a total of 30 farms and a descriptive analysis of the data is performed. Flip Steyn, potato breeder and mini-tuber producer at the Council, is already including IPM techniques such as germplasm management and sanitation, as he certifies the mini-tubers and uses developed varieties. The biological research of this work focuses on the improvement of such IPM strategy through the use of plant growth-promoting microorganisms, namely plant growth-promoting rhizobacteria (PGPR) and fungi. Seventeen different combinations of PGPR and fungi are inoculated into the substrate where potatoes are cultivated in order to compare plant performance from three different potato cultivars. Differences in plant growth rate and performance among treatments and varieties are determined by measuring leaf area and chlorophyll fluorescence through non-destructive methods. Post-harvest tests consisting of tuber size and yield measurements are also a part of the methodology, although not carried out within the timeframe of this study, in order to observe differences between treatments and varieties. The ultimate goal of this thesis research is to communicate the findings to the ARC and the Provincial Department of Agriculture of KZN (DARD-KZN) with the purpose of engaging them in future projects regarding the use of mini-tubers to empower small-scale farmers. Results indicate that differences between varieties are statistically significant, which suggests that cultivar plays a role in plant

performance. However, differences between treatments are not as obvious, and drawing conclusions with the available data is difficult. Further research needs to be carried out. The incidence of pests and diseases in small-scale farming, which reported to be the major of the surveyed farmers' challenge, can be overcome by introducing mini-tubers in their system, yet this initiative need to be accompanied with a sound project including trainings on specific topics. The low-cost methodology developed in this study has given satisfactory and reliable results.

List of acronyms

AMF= Arbuscular Mycorrhizal Fungi
ARC= Agricultural Research Council
ARC-VOP= ARC- department of Vegetables and Ornamental plants
CFA= Chlorophyll Fluorescence Analysis
ChlF= Chlorophyll Fluorescence
KZN= Kwazulu-Natal
DARD-KZN= Department of Agriculture and Rural Development of KZN
LSD= Least Significant Difference
NA= Nutrient Agar
PDA= Potato Dextrose Agar
PGPR= Plant Growth-Promoting Rhizobacteria
PSA= Potatoes South Africa

Treatments applied to mini-tubers:
B= *Bacillus subtilis* (Extrasol)
C= control
P= *Pseudomonas fluorescens* (N04)
G= *Trichoderma harzianum* (Gliogrow)
M= AMF mixture consisting of *Glomus mosseae, G. intraradices* and *G. etunicatum* (Mycorrhizae WS)
T= *Trichoderma harzianum* (Excalibur™)
BP= a combination of *Bacillus subtilis (*Extrasol) + *Pseudomonas fluorescens* (N04)
MB= a combination of AMF (Mycorrhizae WS) + *Bacillus subtilis* (Extrasol)
MBP= a combination of AMF (Mycorrhizae WS) + *Bacillus subtilis* (Extrasol) + *Pseudomonas fluorescens* (N04)
MP= a combination of *Bacillus subtilis* (Extrasol) + *Pseudomonas fluorescens* (N04)
TB= a combination of *Trichoderma harzianum* (Excalibur™) + *Bacillus subtilis* (Extrasol)
TBP= a combination of *Trichoderma harzianum* (Excalibur™) + *Bacillus subtilis* (Extrasol) + *Pseudomonas fluorescens* (N04)
TM= a combination of *Trichoderma harzianum* (Excalibur™) + AMF (Mycorrhizae WS)
TMB= a combination of *Trichoderma harzianum* (Excalibur™) + AMF (Mycorrhizae WS) + *Bacillus subtilis* (Extrasol)
TMBP= a combination of all microorganisms except for *Trichoderma harzianum* (Gliogrow)
TMP= a combination of *Trichoderma harzianum* (Excalibur™) + AMF (Mycorrhizae WS) + *Pseudomonas fluorescens* (N04)
TP= a combination of *Trichoderma harzianum* (Excalibur™) + *Pseudomonas fluorescens* (N04)

List of figures and tables

Introduction

The potato industry in South Africa

According to the World Bank collection of development indicators, agricultural land in South Africa comprises 79.83% of land area, 12% of which can be used for crop production with great limitations on availability of water and of high-potential arable land (World Bank, 2014; Theron, 2003). Potato constitutes the biggest vegetable crop within the country and the second largest field crop grown in South Africa (Agricultural Research Council [ARC], 2014) and in 2015, 53.933 ha were planted, out of 12.5 million ha of total arable land (Potatoes South Africa [PSA], 2016). Interestingly, the crop size has increased over the past decade while the number of hectares has more or less remained stable (see Figure 1), which can be attributed to the use of high-yielding varieties, improved quality seed, increased production under irrigation and better management of resources (PSA, 2016; Theron, 2003).

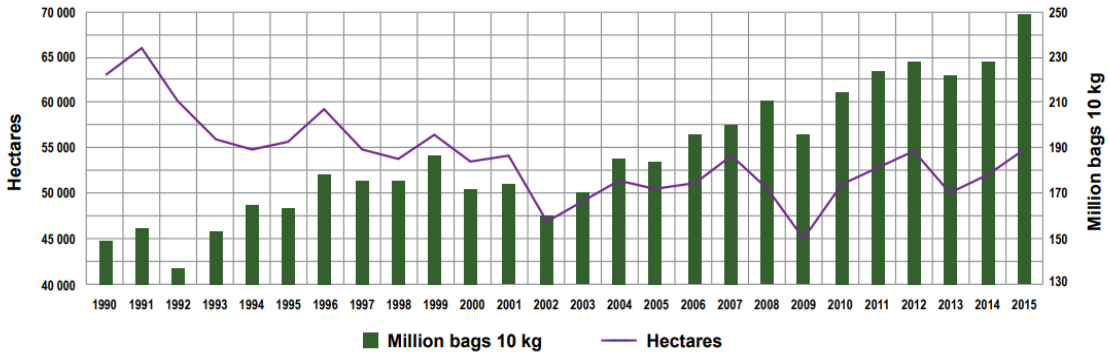


Figure 1: Potato crop size and hectares planted over time.
Source: Potato South Africa, 2015.

The ARC and the breeding programme

The ARC is the principal agricultural research institution in South Africa. Established in 1990, it conducts research with partners in forestry and agriculture, develops human capital and fosters innovation by ensuring that the outcomes of the research are applied in the industry and shared with society. It gives special emphasis to rural and poor communities through communicating knowledge and helping them participate in the country's economy (ARC, 2014; South African Agency for Science and Technology [SAAST], National Research Foundation [NRF] & ARC, n.d.).

Plant breeding involves the artificial selection of varieties, an evolutionary process quickened by human intervention. The mission of the potato breeding program at the ARC is to produce and commercialise varieties that are suitable and well-adapted to local conditions in Africa (ARC, 2014). The programme starts with the acquisition of selected varieties from all over the world in the form of *in vitro* plant material or mini-tubers from approved institutions that can certify that there is no risk of importing tuber-borne diseases. The importation of conventional potato seeds into the country is otherwise not allowed (Theron, 2003). Most of the breeding varieties developed globally are meant to be used in the traditional potato producing

countries, where the climatic conditions are more favourable compared to those in countries of Southern Africa: high temperatures, low humidity and erratic rainfall. Besides being adapted to temperate climates, traditional varieties are resistant to the diseases that occur in such countries, but they are generally susceptible to the ones occurring in warmer climates (ARC, 2014). Potato production in South Africa was seasonal and very limited to specific areas of the Mpumalanga and Free State provinces until the breeding programme introduced locally developed varieties, which led to the potato crop to spread out to other regions in the Free State, Limpopo, the Western Cape and Kwazulu-Natal (*ibid*).

Potato Certification Service (PCS)

Before the Second World War, the potato industry in South Africa relied completely on the importation of potato seeds, which were multiplied locally at the expenses of quality and safety, as the presence of pests and pathogens was not tested. The onset of a healthy seed potato industry was in the early 1970's with the establishment of a potato seed farm in Lydenburg. Testing laboratories in the seed production regions and *in vitro* multiplication and production of mini-tubers arose during the 1980s and were the base of clean potato seed production. The increasing establishment of potato seed businesses hugely improved potato farming and made the country independent of imports and the risks associated with them (Nortjé, 2003). In 1995, seed growers' requests led to the foundation of Potato Certification Service, an article 21 company, namely a non-profit company, that provided certification services. During the same year and after many drastic changes and amendments were procured, a seed programme and certification scheme were introduced (*ibid)*, which set stringent requirements regarding seed production facilities, field inspection and seed testing. Certified seed potatoes in South Africa are labelled from generation 0 (G0) to G8 according to the number of times the propagative material has been multiplied and to the insect and pathogenic infestation levels of the sample, which cease to be 0% after G1 and increase as generations go by. G1-G8 Tubers are sampled and tested for six different virus diseases and bacterial wilt, and an additional bacterial test is carried out when G0 plant material is to be certified. If seed potatoes of a particular generation exceed the permissible levels of infestation, the material is downgraded to the generation that allows such levels. G0 plant material corresponds to mini-tubers that originate from *in vitro* plant material grown in accredited greenhouses or screenhouses, whereas certified seed potatoes ranging from G1 to G8 have undergone subsequent field multiplication and are therefore exposed to pests and diseases. Cultivar authenticity and incidence of diseases are visually examined during field inspections, followed by tuber inspection after harvesting (*ibid*). Laboratory tests must be conducted in laboratories registered with the Department of Agriculture, Forestry and Fisheries (DAFF) and approved by the Independent Certification Council for Seed Potatoes (ICCSP) (PCS, n.d.).

The South African Seed Potato Certification Scheme is applied by seed growers in conjunction with the protocol emanated by the same institution, which describes actions, procedures and processes in more detail than the guidelines provided by the Scheme and is only available to registered seed potato growers. This protocol gives provision for the registration of plantings, conduction of field inspections, sampling, tuber inspection, certification and inspection reports. A plethora of requirements need to be met for a seed grower to be registered with

the PCS. These requirements appear in the Plant Improvement Act 1976 (Act No. 53 of 1976), under which the Scheme is promulgated. Complying with the scheme entails time and money investment, as the grower needs to pay registration fees of plantings per hectare, provide bags and available labourers for the sampling and sacrifice a portion of the harvest to get the certification of seeds. Besides, the seed grower bears the expense of the certification service in itself.

Mini-tuber production

Potato mini-tubers are potato nuclear seeds that have been cultivated *in vivo* and that are originated from vegetative *in vitro* propagating plant material. Thus, certified mini-tubers are disease-free because they are cultivated in artificial soil and therefore have not been in contact with soil-borne pathogens. They will be issued the G0 label provided that no diseases, insect damage or non-pathogenic deviations are found. In contrast, G1-G8 potato seeds, which have been cultivated in the field, are the most demanded type of potato seed in the potato seed market. In 2015, 78% of the overall potato production came from G1-G4 potato seeds (Coleman, 2015).

Flip Steyn, potato breeder and PhD student at the ARC, produces mini-tubers with research purposes and gives away the limited amounts obtained according to demand. Flip's vision with mini-tuber production is to assist small-scale farmers with their businesses and develop smallholder agriculture. Potato seeds represent the highest input cost in agricultural systems, and many farmers obtain their seeds from the harvest of the previous year (Coleman 2015). Vegetative tuber propagation is not a problem in itself, but purchasing late-generation seeds, which contain increasing levels of diseases, and planting a stock of the harvest obtained thereof the following season can have devastating effects for the crop. Purchasing early-generation disease-free plant material allows for the multiplication of seeds without compromising food security and the economic viability of the crop. Production costs are higher for mini-tubers compared to those applicable to tuber seed production due to the costs of the *in vitro* plant material and the stricter cultivation requirements, because the former is developed in greenhouses or screenhouses that need to be maintained pest and disease-free. The facility where mini-tubers are being produced at the ARC is not insect-proof, which downgrades the product to G1, notwithstanding it does not exceed pathogenic or non-pathogenic damage levels. This is an advantage with respects to product pricing, as later-generation-labelled seeds have lower costs, which would make mini-tubers from the ARC more accessible to low-income farmers.

An Integrated Pest Management (IPM) approach

The onset of the South African industrial revolution dates back to the third quarter of the 19th century, when chemical companies started to rise. The first agricultural chemicals company was founded as such during the last quarter of the 20th century by the South African chemical company Sentrachem, after the acquisition of Agricura, an insecticides and herbicides formulator (Majozi & Veldhuizen, 2015). In 1999, South Africa was the fourth largest importer

of pesticides in sub-Saharan Africa (Osibanjo *et al.,* 2002). Notably, the United Nations Environment Programme developed a country ranking according to pesticides imports, agricultural production and Persistent Toxic Substances (PTSs) in which South Africa ranked first (*ibid*). PTS refers to unwanted and banned pesticides and persistent organic pollutants (POPs). The data yielded by the same report and by more recent studies provide evidence that these compounds have deleterious effects in the environment and human health (*ibid;* Quinn *et al,* 2011; Dabrowski, 2016).

Integrated Pest Management (IPM) involves the integration of plant protection methods in a compatible manner in order to keep populations of harmful organisms below a level of economic injury (Bajwa & Kogan, 2002). The regulation (EC) No 1107/2009 and the Directive 2009/128/EC require EU member states to introduce an integrated pest management strategy in their action plans from 2014. However, the use of chemicals is generally the most practiced method to combat and even prevent pests and diseases in South Africa. For this reason, the application of an IPM strategy in agricultural systems is vital for the conservation of natural resources, human health and the socio-economic status of South African farms. Humans have been in constant competition with pests and diseases since the beginning of agriculture. Crop profitability began to increase as farmers gained competences and skills due to a better understanding of their systems. The first methods used to control pests and diseases in primitive agriculture included handpicking and crushing insects, crop rotation and selection of high-yielding plants for seed (Gray, Ratcliffe & Rice, 2011). Today, four types of management strategies have been described for the farmer to adopt, depending on the interaction between the host, the pest and the environment: do nothing strategy, reduction of pest numbers, reduction of host susceptibility to pest damage and a combination of the last two strategies (*ibid*). In order to implement the chosen strategy, multiple methods or IPM tactics should be applied to avoid economic injury and pest outbreaks. These tactics are classified in 4 categories: cultural, physical-mechanical, biological and chemical control methods (University of Nevada Cooperative Extension [UNCE], n.d; Morse, n.d).

The way mini-tubers are produced at the ARC takes into consideration cultural and genetic preventive methods to achieve a reduction of pest incidence. In fact, the ARC published a guide to potato production in 2003 dedicating one entire chapter to the IPM methods available and being used within the potato industry in South Africa (Theron & Mienie, 2003). Such methods involve the use of pathogen-free propagative material, the sterilisation of the substrate to eliminate soil-borne pathogens and the selection of varieties that are tolerant to environmental disturbances and/or certain pests and diseases. Preventive practices usually fall into the cultural and genetic management categories. Nonetheless, some sources such as the UNCE include all preventive methods in a separate category (UNCE, n.d). The IPM strategy that the ARC uses and promotes with the breeding program is, therefore, that of reducing host susceptibility to pest damage.

- *Biological control*

Theron and Mienie provided a comprehensive description of the IPM methods that potato farmers and growers could apply to their agricultural systems in South Africa (Theron & Mienie, 2003). Many of these methods involve soil management, which does not apply to mini-tuber production, as mini-tubers are cultivated in sterilised artificial substrate. Commonly used sterile growth media include vermiculite, sawdust, coir and wood shavings (Ferreira 2013). The trade-off of using sterilised substrate is that the diversity and abundance of life in the soil, which provides a myriad of ecosystem services, is totally absent. There is

overwhelming evidence for the notion that the interactions between soil inhabitants and plant roots positively affect plant growth and performance (Huang *et al* 2014; Beneduzi, Ambrosini & Passaglia 2012; Frew *et al.* 2017). For instance, soil invertebrates modify soil structure and impact nutrient immobilization and availability in the soil, which positively affects water and nutrient uptake by the plant. Earthworms, springtails, millipedes and isopods have the potential to increase nitrogen (N) uptake by plants, whereas invertebrate biomass may store and therefore immobilize significant amounts of carbon (C), N and phosphorous (P), which prevents them from being leached (Mehring *et al.* 2016). Microbial communities play a crucial role in the well-functioning of the soil ecosystem. Plant roots release a wide variety of exudates that attract and select microorganisms in the rhizosphere (Huang X. F *et al* 2014). Plant-microbe interactions provide ecosystem services such as nutrient cycling and carbon sequestration (*ibid*). Moreover, positive effects on the plant and on pest and disease management have also been observed in such interactions (Beneduzi 2012). Examples of beneficial associations between plants and microbes include those with plant-growth-promoting rhizobacteria (PGPR), epiphytes and mycorrhizal fungi (Huang X. F *et al* 2014).

Biological control was defined by Eilenberg as "the use of living organisms to suppress the population density or impact of a specific pest organism, making it less abundant or less damaging than it would otherwise be" (Eilenberg p.1, 2016). Natural enemies of pests are used in biological control strategies to decrease pest population density. Alternatively, beneficial microorganisms may be inoculated in order to directly or indirectly promote plant growth (Eilenberg 2006). Direct growth-promotion mechanisms involve either providing the plant with a compound synthesised by the microbe itself or facilitating nutrient uptake from the soil (Beneduzi 2012). Indirect growth promotion occurs when antagonistic microorganisms lessen the impact of pathogens, resulting in the plant performing better. Production of antagonistic substances such as antibiotics, bacteriocins or siderophores and induction of systemic resistance are mechanisms of indirect plant growth promotion (*ibid*).

Plant-microbe and microbe-microbe interactions at the plant root level have been proven to promote plant growth and health by means of increasing nutrient availability and uptake from the soil, enhancing immunity to abiotic and biotic factors, and supressing diseases through antagonistic interactions (Huang X. F *et al* 2014). They may be a more sustainable alternative to the intensive resource-depleting chemical control practices that largely constitute the agriculture scenario in South Africa. In fact, biological control agents are currently being commercialised in the country. Examples of companies supplying them are Stimuplant CC, ABM™ Africa Division and Rolfes Agri (Pty) Ltd.

Small-scale farming

Small-scale potato production in South Africa within the agricultural framework sits among several threats. Climatic conditions favour the occurrence and persistence of potato diseases and limit the production due to lack of rainfall and sometimes extreme temperatures, above 30˚C, that impair tuber initiation, which is optimal at a soil temperature between 15˚C and 20˚C (Steyn, 2003). However, many small non-commercial farms currently lack irrigation systems in their fields. Breeding programs make sure that different varieties adapted to different abiotic and biotic factors are available on the market, but some farmers do not know which improved cultivars are most suitable for their fields. Large-scale commercial farmers

avoid the prevalence of diseases in the soil by rotating their crops with maize and wheat in a 3-4-year rotation system (*ibid.)*, but many small-scale farmers cannot rotate because of the size of their farms, and soil-borne diseases are almost impossible to treat if the hosts are permanently present in the soil. Moreover, for a small-scale farmer who wants to produce seeds, certification services are hardly affordable, due to the reasons mentioned above (see *Potato Certification Service* section). The use of non-tested seeds potentially harbouring tuber-borne diseases, the stressful environmental conditions in which potatoes are cultivated and the lack of knowledge about IPM and sustainability places poverty-stricken smallholder farms in a situation in which they are up a river without a paddle, that is to say, without the means to improve their systems or progress.

As mentioned in the "mini-tuber production" section, the high costs of seeds force many farmers to multiply the seeds they buy one year and use them several times, which results in the accumulation of tuber-borne diseases and a higher degree of production instability. The use of mini-tubers would allow small-scale farmers to multiply potatoes for more generations without having the risk of losing their crops.

Motivations, aims and research questions

If I were to define agroecology in three words or concepts, I would say it is about sustainability, responsibility and systems thinking. Given the overuse of the word sustainability, I find it imperative to give an accurate definition of the term.

> *"Sustainable development (in the agriculture, forestry and fisheries sectors) conserves land, water, plant and animal genetic resources, is environmentally non-degrading, technically appropriate, economically viable and socially acceptable"* (FAO Council, 1989).

Since the day I read this definition, sustainable development has been my motto and what I strive for in life, therefore it is also my motivation for carrying out this thesis study. In order to develop agricultural systems in a sustainable way, a holistic approach for the analysis of the production site must be taken. In other words, systems thinking must be applied to reach a comprehensive understanding of the system and find solutions for improvement. Systems' thinking involves the use of methods from different disciplines and local knowledge, which takes into account ecological, social and economic concepts and principles (Wezel *et al.* 2009). Within the scope of this study, environmental, economic and social issues associated to mini-tuber production are being tackled to some extent, because agriculture is not only about the farm and the money that can be made out of it, but also about the environment and the community that encompass it. Agroecology was introduced to me as an inter-disciplinary science and I also adopted it as a philosophy. As a result, I cannot see agricultural systems as isolated and independent entities anymore. In line with Gliessman's regard of agricultural systems as agroecosystems, I believe that food production sites should be analysed as wholes and integrated into the environment which they are inexorably connected to instead of putting so much effort in removing externalities.

The benefits of mini-tubers over later-generation potato seeds have already been discussed above. However, they are not being used by small-scale farmers. Moreover, mini-tuber production can still be improved with regards to costs of production. On these grounds, my aims for this research are as follows:

- ♣ To find out if the use of microorganisms as plant-growth promoters in mini-tuber production at the ARC improves plant growth and increases yield.
- ♣ To come up with an efficient but economically reasonable methodology for the analysis of the trials.
- ♣ To find out, through a survey carried out in a small-scale farming community of KZN, whether the use of mini-tubers could improve the overall sustainability of smallholding agriculture.
- ♣ To engage the ARC and the DARD-KZN in a discussion, based on the results, for future projects with smallholder farmers through presenting the findings from this research, which should include the outcomes of the survey, to the two institutions.

The following research questions guide my thesis study:

- ♣ Do the microorganisms applied to the substrate have an impact on plant performance, growth and yield? If so, which treatment is the best?
- ♣ Could any of the challenges that small-scale farming in the surveyed area face be fulfilled by introducing mini-tubers into agricultural systems?
- ♣ What do the aforementioned farmers, the ARC and the DARD-KZN need to know to engage in such discussion?

Biological and social research: setting the boundaries

As mentioned above, the social and environmental sustainability are tackled in this work. To this end, a primary research project consisting of two parts is developed. On the one hand, a biological research involving the use of microorganisms is carried out. On the other hand, a survey of small-scale farmers from the Kwazulu-natal province is conducted and accounts for a social research. For the biological research, I chose to undertake the project that deals with the use of microorganisms as plant growth promoters for the purpose of improving the overall sustainability of the cultivation of mini-tubers G1 at the ARC. Five different microorganisms are tested in combination with each other to see the effects that they have on seven commercialised South African varieties and newly bred lines' plant performance and yield. However, the results presented and discussed in this report refer only to the three breeding lines that the ARC is planning to release, which will nonetheless allow me to compare not only treatments, but also varieties.

Bacterial species include *Bacillus subtilis* and *Pseudomonas fluorescens,* whereas fungal species consist of Arbuscular Mycorrhizal Fungi (AMF) and two strains of *Trichoderma harzianum.* If results indicate that using beneficial microbes has a positive effect on productivity, that is to say, crop yield, further research on the field could allow the ARC to come up with a standardised mini-tuber production method that includes plant growth-promoters and is less costly than the current one. Lower production costs might make mini-tubers more affordable by small-scale farmers. Further research on the effects of such microorganisms on the potato crop regarding tolerance to diseases by the induction of systemic resistance could lead to a lesser use of agrochemicals. The drift from chemical pest management practices toward a more integrated strategy is essential not only for the environment, but also for society, particularly for poverty-stricken small-scale farmers who struggle to pay the high costs of pesticides.

As for the social research, I aim to understand how farming is practiced in a small scale in order to identify key problems and communicate them to the relevant institutions so that future projects involving the use of mini-tubers in small-scale farming can be discussed.

Only two communities from one single province of South Africa are surveyed because of time constraints. The selected are is the coastal province Kwazulu-Natal (KZN), located in the southeast of the country. The reason I chose to survey this area was because the Department of Agriculture and Rural Development of KZN (DARD), which is affiliated with the ARC, enabled me to gain access to local farmers from remote areas.

Literature review

Plant-growth promoting microorganisms colonise naturally the rhizosphere of potato plants with variable abundance and diversity (Senés-Guerrero *et al.* 2014, Kesaulya *et al.* 2015). Kotan *et al.* showed that some PGPR strains are effective biocontrol agents against several plant pathogenic bacteria and fungi in *in vivo* and *in vitro* conditions (Kotan *et al*. 2009). The same study claims that *Pseudomonas* sp., *Pantoea* sp., *Enterobacter* sp., *Bacillus* sp. and *Trichoderma* sp. may be used to control potato dry rot, caused by *Fusarium* sp. (*ibid*). In fact, biological control agents based on *Bacillus subtilis* and *Trichoderma harzianum* strains are registered in USA for potato cultivation (Wharton & Kirk 2014). Such commercial products appear to be effective against some of the major potato diseases in South Africa: late blight, black scurf, dry rot and silver scurf (*ibid*, Theron & Mienie 2003). Moreover, the data yielded by Wharton & Kirk provides evidence that the aforementioned biocontrol agents reduced sprout rot and seed piece decay caused by *Fusarium sambucinum* on seed re-stored under optimal conditions. Another study showed that *Trichoderma harzianum* reduced the incidence of the disease caused by *Verticillium dahliae* and increased total potato yield when inoculated in potato fields infested with the pathogen (Ordentlich, Nachmias & Chet 1990).

Similarly, *Bacillus* and *Pseudomonas* species have been implied in biocontrol due to their ability to produce antibiotics and induce systemic resistance in plants (Beneduzi, Ambrosini & Passaglia 2012, Larkin 2016). Additionally, *Pseudomonas* species produce siderophores, namely low molecular weight iron chelators, with particularly high affinity to the ferric ion. Iron is solubilised and extracted from mineral or organic complexes (*ibid*). This iron can be easily taken up by the plant when such strains are in symbiosis with plant roots. As far as arbuscular mycorrhizal fungi are concerned, *Funneliformis mosseae* (syn. *Glomus mosseae*) appears to naturally colonise potato plants in the Peruvian Andes and has proven to be a good colonizer in greenhouses (Senés-Guerrero *et al.* 2014).

This study employs a methodology based on plant phenotyping techniques, which allow for the analysis of plant traits. The tests carried out in this project are based on the analysis of responses of different genotypes, i.e. the three breeding lines used as subject of the experiments, to different environmental triggers, namely the 17 treatments based on plant growth-promoting microorganisms that are applied to such lines. According to Walter, Liebisch & Hund, phenotyping is more complex than the determination of the arrangement of genes in a genotype, because it encompasses a myriad of processes that occur at different levels or, as they put it, dimensions. Fortunately, the increasing and urgent need for superior varieties to improve crop management has led to the development of non-destructive optical analyses of

plant traits, which are used in this study to analyse the products of gene-environment interactions (Walter, Liebisch & Hund 2015). Breeders and farmers pursue the best genetic variation to maximise breeding efficiency, and plant phenotyping allows for the identification of quantitative traits related to growth, yield and adaptation to stress (Li, Zhang & Huang 2014). The techniques used in this study involve plant imaging, to estimate leaf area, and Chlorophyll *a* fluorescence, to measure photosynthetic efficiency. Yield-related tests are also part of the mini-tubers research at the ARC but will not be carried out within the timeframe of this study. Thus, results derived thereof are not included in this report.

Materials and methods

Trials design

- *The facility:*

The plants were grown at Modaersbond, a screenhouse located in the ARC-VOPI campus, at Roodeplaat, Gauteng province, South Africa. The layer net of the facility allows for the entrance of small insects, but sticky traps are placed in order to monitor them and control that no thrips or aphids, which are potential virus carriers, feed on the plants. The potatoes were planted in pots along 22 tables which carried 38 crates each (see Figure 2), except for three smaller tables, which could only fit 31 crates. Each crate contained 6 pots, which consisted of plastic bags filled with previously sterilised wood shavings and 2, 3 or 4 plants, depending on the variety and the trial intended to be performed on them (see Table 1 below). The three breeding lines that this study focuses on, which make up a total of 9 tables, are shaded on table 1.

Figure 2: table design at Moedersbond.
The left picture shows how the 19 rows (2 crates placed horizontally) are arranged within a table, whereas the right picture displays the arrangement of tables. Source: author.

- *Fertirrigation:*

The setup of the trials at the Moedersbond screenhouse was totally new with regards to the irrigation equipment. As shown in the pictures above, water was provided by means of a drip irrigation system based on on-line pressure compensating button drippers that delivered

water at a flow rate of 12L/hour (Woodpecker PC CNL Dripper, Netafim™). The components were ensembled and set in place from January 28th to February the 1st, with one delivering dripper per crate and each bag receiving an individual emitter (see Figure 3). Further pressure adjustments were needed afterwards and on February 23rd, an automated irrigation controller was installed (X-core, Hunter®), which controlled daily water times, volume applied and to which tables it should be applied. Fertilisation was added to the system on the same. In order to solve pressure problems, four stations consisting of 5 or 6 tables each were set for different irrigation times and equal water delivery. A bigger pump was installed later on.

Figure 3: crate with installed drip irrigation system.
Source: author.

- *Cultivars:*

The plant material was obtained from the In Vitro Genebank facility located at the ARC-VOP campus (South Africa), which is in charge of ensuring the *in vitro* conservation of plant germplasm. One of the services provided is the mass propagation of *in vitro* plants based on the cutting of plant stems and the subsequent growth of the plantlets in a sterile micro-environment. Eight different potato varieties were selected for their traits and planted immediately after receiving such plantlets. Three tables were used for each variety, except for one (table 22, see Table 1), which was planted using only one table.

Table 1: layout of the tables at Moedersbond, indicating variety planted on each table, date of planting, number of crates per table and number of plants per pot.
The shading applies to those varieties that this study is focusing on.

Table	Name of variety	Date of planting	Number of crates	Nbr of plants/bag
1	Mondial	31/01/18	38	3
2	Mondial	31/01/18	38	3, except for the 1st row of bags on right tables (4 plants/bag)
3	Mondial	31/01/18	38	3
4	92-047242(8)VF	31/01/18	38	3
5	92-047242(8)VF	31/01/18	38	3
6	92-047242(8)VF	01/02/18	38	3

7	Darius	01/02/18	38	3
8	Darius	01/02/18	38	3
9	Darius	01/02/18	38	2
10	BP1	02/02/18	38	3
11	Mnandi	02/02/18	31	2
12	Mnandi	02/02/18	31	2
13	Mnandi	02/02/18	31	2
14	BP1	02/02/18	38	3
15	BP1	02/02/18	38	3
16	95-0521-126 (6)	02/02/18	38	3
17	95-0521-126 (6)	05/02/18	38	3
18	95-0521-126 (6)	05/02/18	38	3
19	96-0568-2(13)	05/02/18	38	3
20	96-0568-2(13)	05/02/18	38	3
21	96-0568-2(13)	05/02/18	38	3
22	94-0530-8(22)VF	05/02/18	38	3

- *Maintenance:*

Chemical control was applied to the plants once a week. The list of pesticides can be found on the *Appendix 1*, which includes the application dose and the date to be applied. None of the pesticides used in this study appear in the list of PTSs analysed by Osbanjo *et al.* (2002).

The irrigation system needed maintenance and reparation several times, with regards to the pipes, the pump and the bottom drippers. The pressure problem lasted long enough for some plants to die due to water shortages. For the statistical analyses to give accurate results, ensuring that all the plants are growing under the same conditions is pivotal, hence why the number of plants in each pot must be the same for all the pots belonging in the same variety. The same conditions will apply for different varieties if they are to be compared with each other. In other words, the three varieties to be analysed in this study can be compared with each other because the number of plants per pot is the same for all the tables, whereas Mnandi cannot be compared with any other variety because the number of plants per pot is two (see Table 1 above). For this reason, replacement of dead plants was carried out in several occasions from February the 8th to the 26th. *In vitro* plant material was provided for the replacement of dead plants until there was no more. Alternatively, stems from the best-performing plants were cut and replanted in those pots where plants were missing. Although this was intended to reduce variation within the results, it must be born in mind that plants' root systems were not equally developed in all plants, as replanted stems had barely any roots, replanted *in vitro* plant material's roots were very young and surviving plants had the most developed root systems.

o Treatments:

Five inoculants, most of which are commercial products, containing plant growth-promoting microorganisms were tested in the potato trial in a total of 16 treatments —and one control— consisting of a single or a combination of up to four products. Each table was subject to the whole set of treatments, that is to say, 22 tables were treated with all the possible combinations of microorganisms. Each plot, consisting of two crates, corresponded to one treatment, and the edges of the tables were not treated, leaving room for 17 experiments to take place. All treatments were randomised within each table, whereas different varieties were not mixed and the tables belonging to each variety were placed next to each other (see Table 1 above).

The microorganisms used in this experiment included two commercial products containing *Trichoderma harzianum* (Gliogrow, Molcast Holdings (Pty) LTD; and Excalibur™ Gold, ABM™ Advanced Biological Marketing, Africa Division), a mixture of three arbuscular mycorrhizal fungi that includes *Glomus mossae, G. intraradices* and *G. etunicatum* (Mycorrhizae WS Water Soluble, Biocult), two strains of *Bacillus subtilis* in one formulation (Extrasol, Stimuplant) and a non-commercial isolate of *Pseudomonas fluorescens* named N04. The acronyms used for each treatment are displayed in Table 2.

Table 2: name and description of each treatment used in this study.

Treatment Name	Description
B	*Bacillus subtilis* (Extrasol)
C	**Control**
G	*Trichoderma harzianum* (Gliogrow)
M	AMF mixture consisting of *Glomus mosseae, G. intraradices* and *G. etunicatum* (Mycorrhizae WS)
T	*Trichoderma harzianum* (Excalibur™)
P	*Pseudomonas fluorescens* (N04)
BP	a combination of *B. subtilis (*Extrasol) + *P. fluorescens* (N04)
MB	a combination of AMF (Mycorrhizae WS) + *B. subtilis* (Extrasol)
MBP	a combination of AMF (Mycorrhizae WS) + *B. subtilis* (Extrasol) + *P. fluorescens* (N04)
MP	a combination of *B. subtilis* (Extrasol) + *P. fluorescens* (N04)
TB	a combination of *T. harzianum* (Excalibur™) + *B. subtilis* (Extrasol)
TBP	a combination of *T. harzianum* (Excalibur™) + *B. subtilis* (Extrasol) + *P. fluorescens* (N04)
TM	a combination of *T. harzianum* (Excalibur™) + AMF (Mycorrhizae WS)
TMB	a combination of *T. harzianum* (Excalibur™) + AMF (Mycorrhizae WS) + *B. subtilis* (Extrasol)
TMBP	a combination of all microorganisms except for *T. harzianum* (Gliogrow)
TMP	a combination of *T. harzianum* (Excalibur™) + AMF (Mycorrhizae WS) + *P.fluorescens* (N04)
TP	a combination of *T. harzianum* (Excalibur™) + *P. fluorescens* (N04)

The inoculation of microorganisms was conducted from Friday the 16th of February to Friday of the following week, on the 23rd. 1ml of *B. subtilis, P. fluorescens* and Excalibur equally diluted were inoculated on each plant. The mixture of AMF came as a 100g powder formulation, which was dissolved in 5l distilled water. 0,6ml of the solution were applied to each plant. Gliogrow

came as a liquid solution that was recommended to be applied at a dilution ratio of 1:100. 1ml of the solution was applied to each plant.

Tests

o Leaf Area Index

Leaf Area Index was used as a measurement of plant growth and was used to assess whether or not the microbial treatments had a measurable impact on overall plant growth. Leaf area measurements were taken using the software Easy Leaf Area, developed by Easlon & Bloom in 2014, on pictures taken with a phone. The software performs rapid, automated digital image analyses with little user inputs and gives an estimation of the leaf area of individual images within seconds (Easlon & Bloom 2014). The output is a spreadsheet-ready CSV file with image names, pixel counts, leaf area in cm^2 and percentage of canopy cover values. In order to get pictures of the same size, we developed a low-cost cell phone shuttle made of two poles, one at each end of the table, and wires connecting them, serving as a track over the plants (see device diagram on Figure 4*)*. Plants were filmed with a phone placed in a case which was mounted on this hanging track for smooth horizontal movement generated by pulling a rope attached to the shuttle from one of the sides of the table (see Figure 4 and Figure 5). The wires were as tight as possible to avoid the bouncing of the case while pulling and the position and tilt of the poles was adjusted as to ensure that the crates were properly framed in the videos. Videos were then snipped with the Snipping Tool of Windows®, capturing one single plot, namely two crates wherein the same treatment had been applied, in each screenshot (Figure 5*B*). Images were analysed using the auto-settings provided by the software, except for the minimum leaf size, which was adjusted according to the growth stage of the plant. Figure 5C highlights the green areas of the picture, and the percentage of canopy cover is given thereof. The sampling times are referred to as days after planting (DAP). Videos were taken at 20, 25, 32, 42 and 50 DAP, approximately once a week until the percentage of canopy cover was nearly 100% for most of the plants.

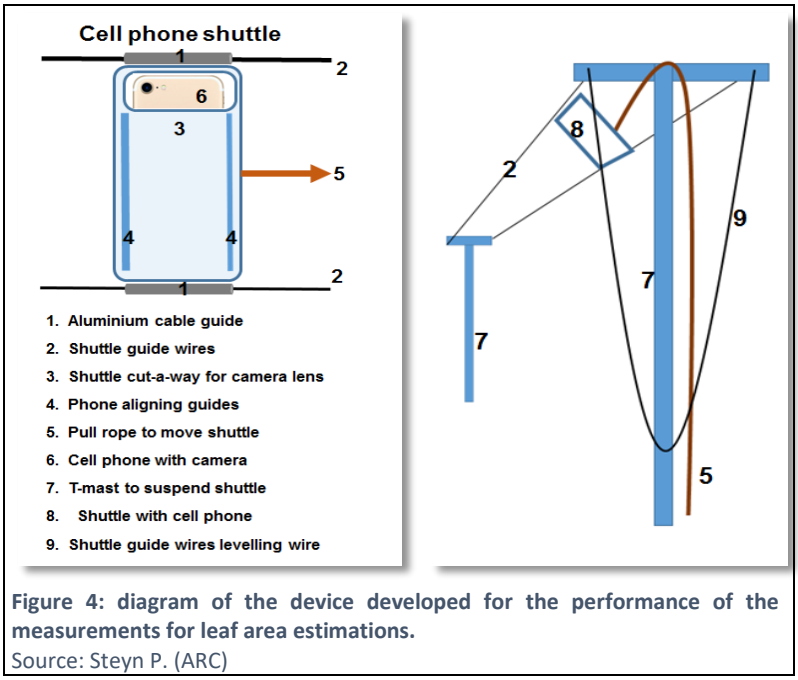


Figure 4: diagram of the device developed for the performance of the measurements for leaf area estimations.
Source: Steyn P. (ARC)

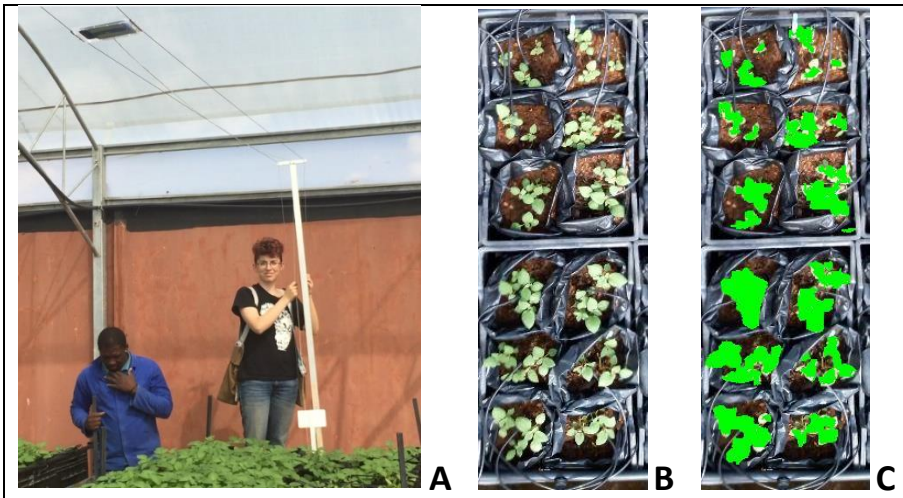


Figure 5: methodology for the measurements of leaf area in three steps.
Videos of the plants are taken with the phone shuttle device (A). The snipping of videos gives pictures of each plot (B). A picture with highlighted green areas is obtained after the analysis with the Easy Leaf Area software (C). Source: author.

o Chlorophyll Fluorescence Analysis (CFA)

Chlorophyll fluorescence (ChlF) technology is widely used for photosynthesis probing purposes (Kalaji *et al*. 2017; Hansatech Instruments Ltd 2006; Murchie & Lawson 2013). It is based on the principle that absorbed solar radiation by antenna pigments from PSI, PSII and Light-harvesting Complexes (LHCs) is converted into energy through three processes that compete with each other, which can be classified as photochemical and non-photochemical processes. Photochemical processes involve the donation of excited electrons from chlorophyll molecules to electron acceptors from the photosystems, which then drives photochemistry. The state of reduction and oxidation (redox) of electron carriers along the thylakoid membrane is the base of the chlorophyll fluorescence analysis procedure (Murchie & Lawson 2013). When key electron carriers such as the bound quinone Q_A receive an electron, they are not able to accept another one until they have passed the one they carry to the next acceptor, and in that state, that reaction centre (RC) is considered to be closed (*ibid*). In non-photochemical processes, on the other hand, absorbed sun light can be dissipated either as heat or fluorescence, depending on whether the energy is re-emitted in the form of infra-red or red/far-red radiation, respectively (Hansatech Instruments Ltd 2006). Heat dissipation or non-photochemical quenching (NPQ) occurs when plants are exposed to light, as it is a photoprotective process that releases excess excitation energy from chlorophyll-containing structures in order to prevent the formation of damaging free radicals (Murchie & Lawson 2013). Importantly, a reduction in one of the processes is associated with an increase in the ones competing with it. Thus, measuring chlorophyll fluorescence not only gives information about energy dissipation within the red/far-red radiation, but also allows the user to infer information about photochemical processes (*ibid;* Cendrero-Mateo *et al.* 2016; Handsatech Instruments Ltd 2006).

In this study, a non-modulated fluorescence system, namely a system that analyses fluorescence induction transients that result from the application of light sources after a period of darkness, is utilised. The fluorescence instrument used was the advanced continuous excitation chlorophyll fluorimeter Handy Pea (Hansatech Instruments, Norfolk, UK). The

fluorimeter illuminates the sample with a 1 sec light pulse of high intensity that induces a polyphasic rise in chlorophyll fluorescence. This process is known as the Kautsky induction phenomenon and the fluorescence induction kinetics curve shows four peaks that occur within the first 300msec and are denoted by the letters O, J, I and P, which the literature refers to as the OJIP transients (Handsatech Instruments Ltd 2006; Bussotti *et al. 2010;* Paul 2016). The analysis of the OJIP transients is known as the JIP test and translates the readings into a plethora of biophysical parameters that can be classified in three groups: specific energy fluxes, flux of ratios or yields and the phenomenological energy fluxes.

Leaves were dark-adapted for 20min following instructions from the manufacturer's operations manual (Hansatech Instruments Ltd, 2006). They were covered with leafclips, which have a small shutter plate that should be closed to exclude light and allow dark-adaptation in the sample area. The fluorimeter performed the measurements using the default protocol and readings were taken on the adaxial surface of the third leaf of each plant, following directions from Robert Laurie, researcher at the Crop Science division of the ARC-VOPI. Cendrero-Mateo *et al.* (2016), in a study where they compared active and passive techniques assessing Chl F at different temporal and spatial scales, put the claim that leaf-to-leaf heterogeneity causes uncertainty at leaf-scale measurements due to stomatal conductance, leaf photosynthesis and leaf chlorophyll content, and suggested that averaging a number of representative leaves to a unique value reduces such uncertainty. In order to reduce leaf-to-leaf variability, the same two plants from each crate were sampled every time, which gave a total of 4 values per treatment and day within a table. Considering that each variety was planted using three tables, the total number of values corresponding to one treatment (i.e. the number of replicates) for a single variety was 12. The maximum sample size was 612 plants, but some crates were not performing well and it was therefore not possible to test all crates. Measurements were taken once a week starting from March the 29th to April the 27th. The reason for taking weekly measurements was to test whether there would be any differences in the results over time. As plants go through different developmental stages, which in turn might affect, or be affected by the population dynamics of the microbial communities living in the rhizosphere, one might expect to see differences within treatments and varieties over time (Senés-Guerrero *et al.* 2014). The crates containing poorly-performing plants were not analysed.

The two basic parameters used for calculations are the O and P transients from Kautsky induction phenomenon mentioned above, namely the minimum level of fluorescence emitted or fluorescence origin, termed F_O, and the maximum level of fluorescence, termed F_M. In order to obtain F_M, leaves need to be fully dark-adapted because all reaction centres are open and all the primary electron acceptors (Q_A) are oxidised after a period of darkness. In other words, only when photochemistry is not being carried out and consequently, no NPQ is present, fluorescence emission can reach its highest levels. F_O corresponds to the signal emitted when Q_A is oxidised at the onset of the illumination. All the readings were computed using a Windows® software package, PEA Plus (Handsatech Instrument Ltd). The parameters derived from the JIP test used for this experiment were as follows: (1) transients F_O and F_M; (2) partial vitality indexes, that is, the density of active reaction centres (RC/ABS), the maximum quantum yield of primary photochemistry (φ_{Po}), the probability to move an electron further than the quinone acceptor Q_A (Ψ_{E0}) which allows for the determination of the ability to convert sunlight into chemical energy and the probability to reduce and an end electron acceptor (δ_{Ro}) with which it is possible to calculate the ability to use chemical energy; (3) vitality indexes calculated from the partial vitality indexes, that is Performance Index (PI_{ABS}) and total Performance Index (PI_{Total}).

o Statistical analysis

Prior to the statistical analysis, a histogram of all the LAI data was created with the software Microsoft Office Excel and values located too far from the trend were regarded as missing values. The analysis was made with a total of 758 values. For each day, 560 Chlorophyll fluorescence measurements were collected. After a detailed examination of the dataset, bad measurements giving negative values or values standing out too far from the trend were regarded as missing values. The final dataset amounted to 2154 samples.

In order to compare the effect of days, varieties and treatments on the different fluorescence parameters used, an analysis of variance by ANOVA, regression or REML was carried out using the software Genstat 18th edition on a single set of data corresponding to 5 sampling times for the leaf area analysis and 4 for the CFA, 3 varieties and 17 treatments. Differences were considered to be statistically significant up to P=0.05. Means of significant effects were determined with the LSD-test (Least Significant Difference).

o Microbial tests

The preliminary plan included the determination of microorganisms isolated from the rhizosphere of the plants in order to confirm that the inoculants had established. One of the aims of the work was to come up with a low-cost and efficient methodology for the analysis of the trial. To that end, a pilot trial to figure out a suitable method for the isolation of rhizosphere microorganisms and endophytes was carried out. However, the identification of the isolates to the species level was not possible due to time constraints, and the confirmation of the presence of the inoculants was therefore missing.

The pilot trial involved the sampling of the roots of two randomly selected plants from the variety Mondial subjected to two different treatments: the combination of Excalibur *T. harzianum,* AMF, *Bacillus subtilis* and *Pseudomonas fluorescens* (TMBP) and *B. subtilis* alone (B). Plants were carefully removed from the soil with properly sterilised tweezers and placed in plastic bags for a rapid transport to the lab. Once they arrived in the lab, the top part was cut off and the roots were plated out in Nutrient Agar (NA) and Potato Dextrose Agar (PDA) following three different methods:

- Direct plating of the roots, without washing the excess of wood shavings attached to them. That should allow microorganisms from the rhizoplane and the ectorhizosphere to grow on the plates. Small pieces of roots were cut and plated in NA and PDA to select for bacteria and fungi, respectively. No antibiotics were added to PDA for a more efficient isolation of fungi.
- Selection of microorganisms from the rhizoplane, namely those living on the root epidermis and mucilage, by washing the roots with distilled water to get rid of microorganisms belonging in the ectorhizosphere, mainly those attached to the wood shavings. Once the roots were washed, they were transferred to an Erlenmeyer flask with 100ml distilled water and shaken for 10min. 100µL from the flask, as well as 4 dilutions, were spread plated onto NA and PDA plates.
- Selection of endophytes, i.e. microorganisms from the endorhizosphere. The roots were surface sterilised with commercial laundry bleach (sodium hypochlorite) that had been diluted to a final concentration of 1%. The plant material was immersed in this solution for 5min and rinsed 3 times with distilled water, after which it was transferred to a mortar and crashed with a pestle and 1ml distilled water to get the endophytes

out of the inner plant tissues. 2 dilutions were made for each plant and 100µL of dilutions 0, -1 and -2 were spread plated onto NA and PDA plates.

All the plates were incubated at 25˚C for 48h under light exposure.

Interviews to small-scale farmers

The ARC partners with the Cedara College of Agriculture, a training institution from the Department of Agriculture of KZN, which allowed for the arrangement of the social research of this study. A meeting with Mr Morgan Naidoo of the Cedara College took place on February the 7th in order to define the objectives of the interviews and schedule the visits. He provided insights into how small-scale farming is carried out in KZN, as well as the point of view of the farmers, which influenced the questions asked in the questionnaires, as some of them were aimed at confirming or rejecting Naidoo's affirmations. Based on the objectives of the study, the Cedara College took the task of selecting potato growers and informing them about my visit. The criterion to select farmers was to have experience in potato cultivation. All the selected farmers had been trained in potato cultivation at least once within the previous 5 years and had some experience in potato farming, most of which were cultivating potatoes at the time of the interview. 30 farmers from two communities, Appelsboch and Swayimane, were interviewed. A total of 7 wards were surveyed. An MSc student from the ARC, Lesiba Klaas Ledwaba, joined the trip as a translator because most of the farmers could only speak Zulu. Furthermore, an extension officer of each community accompanied us to all farms.

The questionnaires were written with the help of Versity Kekana, a researcher from the department of Vegetables and Ornamental Plants of the ARC (ARC-VOP). A bibliographic review of the suggested literature from the courses at SLU was carried out prior to the development of the questionnaires in order to get knowledge about the type of questions that should be asked for the particular purpose of the survey. Meetings with experts from the ARC and SLU were conducted to develop and narrow down such purpose. Finally, the questions were reviewed and modified to comply with the Personal Data Act (1998:204), which ensures and protects people against the violation of their personal integrity. Consequently, data processing also complied with the Act. Questions asking explicitly about race or disability, which were suggested by Mr Kekana, were deleted from the template. The sections that constituted the questionnaires were as follows: interviewer declaration, participant consent, socio-economic characteristics, financial information and personal interests (see questionnaire template on the *Appendix 2)*. The visits to the farms were performed from the 13th to the 16th of March. Each visit was performed in approximately 40min and included a quick visit to the fields and a semi-structured interview combined with questionnaires that would be filled in by the interviewer. All respondents signed the consent form at the end of the visit.

Data from the questionnaires and semi-structured interviews was categorised and coded for further descriptive analyses using Microsoft Office Excel. A data matrix including nominal, ranked, mix-typed and open-ended questions was manually set up. Each case was laid out in a separate line and each question, in a separate column. Most of the questions on the questionnaire were pre-coded. Answers from the semi-structured interview were examined for data relevant to the overall research question, after which they were either added into the data matrix and treated as multiple-choice nominal or open-ended questions or brought up in

the discussion to complement data from the questionnaires. Answers to open-ended questions were categorised in themes. Questions with multiple answers were coded as several variables.

Results

Leaf Area Index

The statistical analysis showed a normal distribution of LAI data after the removal of outliers. There were significant differences between plants belonging in different varieties (>0.001) and a statistically significant interaction between varieties and days (>0.001). The first graph on Figure 6 (see green curve on "all treatments") shows that MC2 experiences a more rapid development of the canopy, which could explain the significant interaction between variety and day. Treatments alone did not show significant differences (P value= 0.560). The interaction between treatments and varieties gave a P value slightly above 0.05 (0.052), which could be regarded as statistically significant. The analysis of the data with Genstat gave the constants for the formula of the leaf area increase over time for each treatment, which was represented by an exponential curve with an average R^2 value of 91.14% and a maximum R^2 value of 97.44% (see Figure 7). The development of potato plants was determined according to this formula for the different varieties and also for all the treatments and is shown in Figure 6.

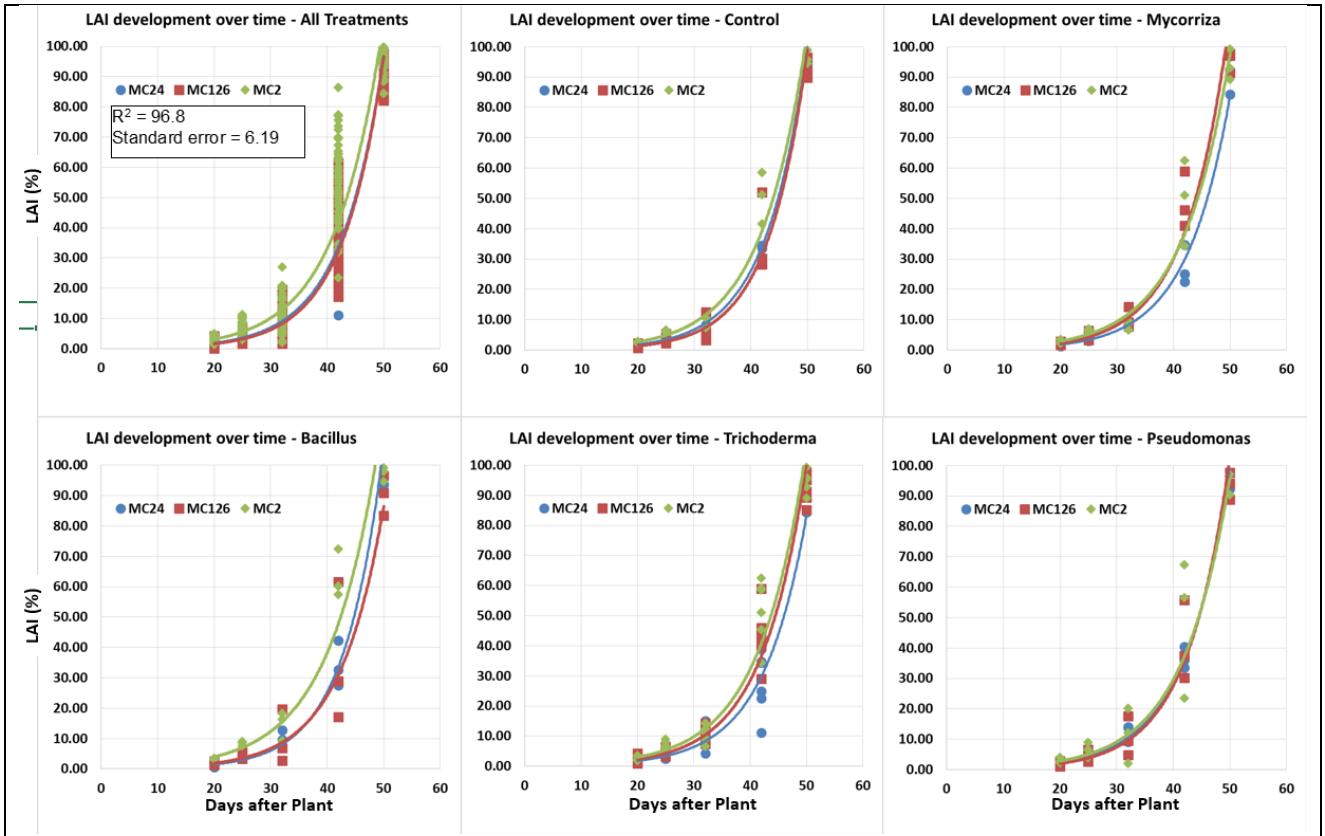


Figure 6: Leaf area development for the three varieties MC24, MC126 and MC2 over time (at 20, 25, 32, 42 and 50 DAP) as determined from RBG values with Easy Leaf Area.

Plotted values were calculated with the formula A + B*(R^X), where X corresponds to days after planting (DAP) and the constants A, B and R are specific for each treatment, the values of which were extracted from the interaction between percentage of canopy cover and treatment determined by the analysis with Genstat. In order to make the interpretation of results simpler, only data from single treatments have been considered.

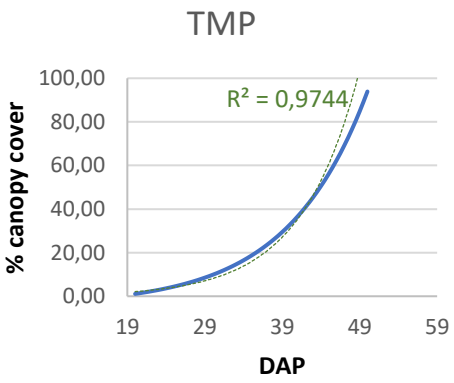
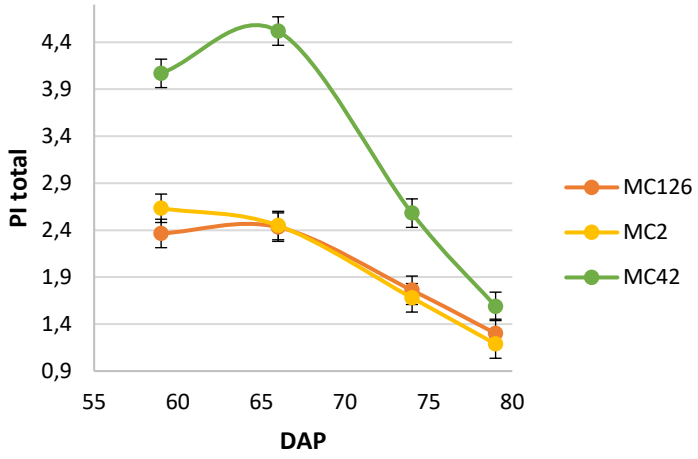


Figure 7: exponential curve representing increase of percentage of canopy cover over time (from 0 to 50 DAP) for the treatment TMP.
Values for each day were calculated with the formula A + B*(R^X), where X corresponds to days after planting (DAP) and the constants A, B and R are specific for each treatment, the values of which were extracted from the interaction between percentage of canopy cover and treatment determined by the analysis with Genstat.

Chlorophyll Fluorescence Analysis

A significant positive relationship (<0.001) was observed between varieties and days for the total performance index parameter (PI_{TOTAL}), which is an indicator of plant vitality. Besides, the variable treatment alone also showed a high degree of significance for the same parameter (<0.001). In order to know which group differs from the rest, i.e. which of all treatments or varieties has a greater effect on plant performance, the Least Significant Difference (LSD) test was performed. Any difference between values larger than the LSD value is considered significant. Results over time show that the best performing cultivar is MC42, with greater significant differences from day 66 after planting, after which the performance index values drop. MC2 displayed decreasing vitality from the first sampling time (59 DAP), although the differences between this variety and MC126 are larger than the LSD (0.1515) only on day 59, therefore suggesting that only MC42 has a statistically significant effect on total plant performance index (Figure 8 *left)*. Figure 8-*right* shows the means of each treatment sorted from highest to lowest. The LSD value, which amounts to 0.2124, allows for the grouping of data to mark significant differences. The letter-coded groups indicate the statistical significance of the values. Treatments with no significant differences get the same letter and make up a group. The group with the highest values gets the letter *a,* and the following groups get *b, c, d, e* and *f,* in this case. 'M' (PI_{TOTAL} prediction=2.483) appears to have the highest value, yet the closest significantly different treatment is 'G' (Figure 8 *right)*. In other words, group *a*, consisting of 'M', 'TMBP', 'TP', 'TM', 'MP', 'P', 'MB', 'TB' and 'TMP', has greater positive effects on plant performance than the rest of the groups.



TreatName	PI_total	
M	2.483	a
TMBP	2.451	ab
TP	2.446	ab
TM	2.410	abc
MP	2.376	abcd
P	2.318	abcd
MB	2.310	abcd
TB	2.307	abcd
TMP	2.282	abcd
G	2.253	bcde
MBP	2.241	bcde
C	2.211	cdef
BP	2.194	def
TBP	2.192	def
TMB	2.191	def
T	2.065	ef
B	2.026	f

Figure 8: interaction between variety and day (left) and between treatments (right) for PI total.
(Left) total performance index over time (<0.001, LSD=0.1515) for the three varieties tested: MC2 (yellow), MC42 (green) and MC126 (orange). Plotted values represent the means ± standard error (SE). (Right) Means of measurements of total performance index for each treatment, sorted from highest to lowest (<0.001, LSD=0.2124). The data correspond to measurements of chlorophyll a fluorescence which were conducted on the third youngest leaves of 560 plants.

The other total vitality index, PI_{ABS}, differs slightly from PI_{TOTAL} in the formula and consequently, also in the plant functions it is indicator of. PI_{ABS} does not provide information about the ability of the plant to use chemical energy, whereas PI_{TOTAL} does. An interaction between day and variety (<0.001) and between variety and treatment (<0.05) was observed in the analysis of PI_{ABS} (see Figure 9*)*. The breeding line MC2 did not lose vitality from the first day, as the analysis of PI_{TOTAL} in Figure 9*A* shows. Instead, values decrease from day 66 after planting, displaying the same type of curve as the other two varieties. MC42 is again the best performing one, both over time and in response to the treatments (Figure 9*A* and *B*). The interaction treatment-variety gave a least significant difference (LSD) of 0.4771, which is not exceeded by the difference between any of the treatments corresponding to MC126 and the same treatment on MC2, which indicates that there are no differences between the two varieties (Figure 9*B)*. 'G' appeared to have the highest PI abs value on MC126. According to the LSD test, 'G' belongs in the same group as 'MBP', 'MB', 'TP', 'BP', 'TB', 'TMP', 'P', 'TBP', 'B', 'TM', 'TMB', 'MP', 'TMBP' and 'M', under which lies the control. As far as MC2 is concerned, 'TMP' (group *a*) reported the highest value, followed by 'TM', 'MBP', 'TBP', 'TB', 'MP', 'B', 'TMBP', 'TMB', 'M', 'MB', 'T' and the control, which has the smallest value within the group. The performance index of MC42 was higher in plants treated with 'TMBP', 'MP', 'BP', 'TM', 'T', 'P', 'TP' and 'TBP'.

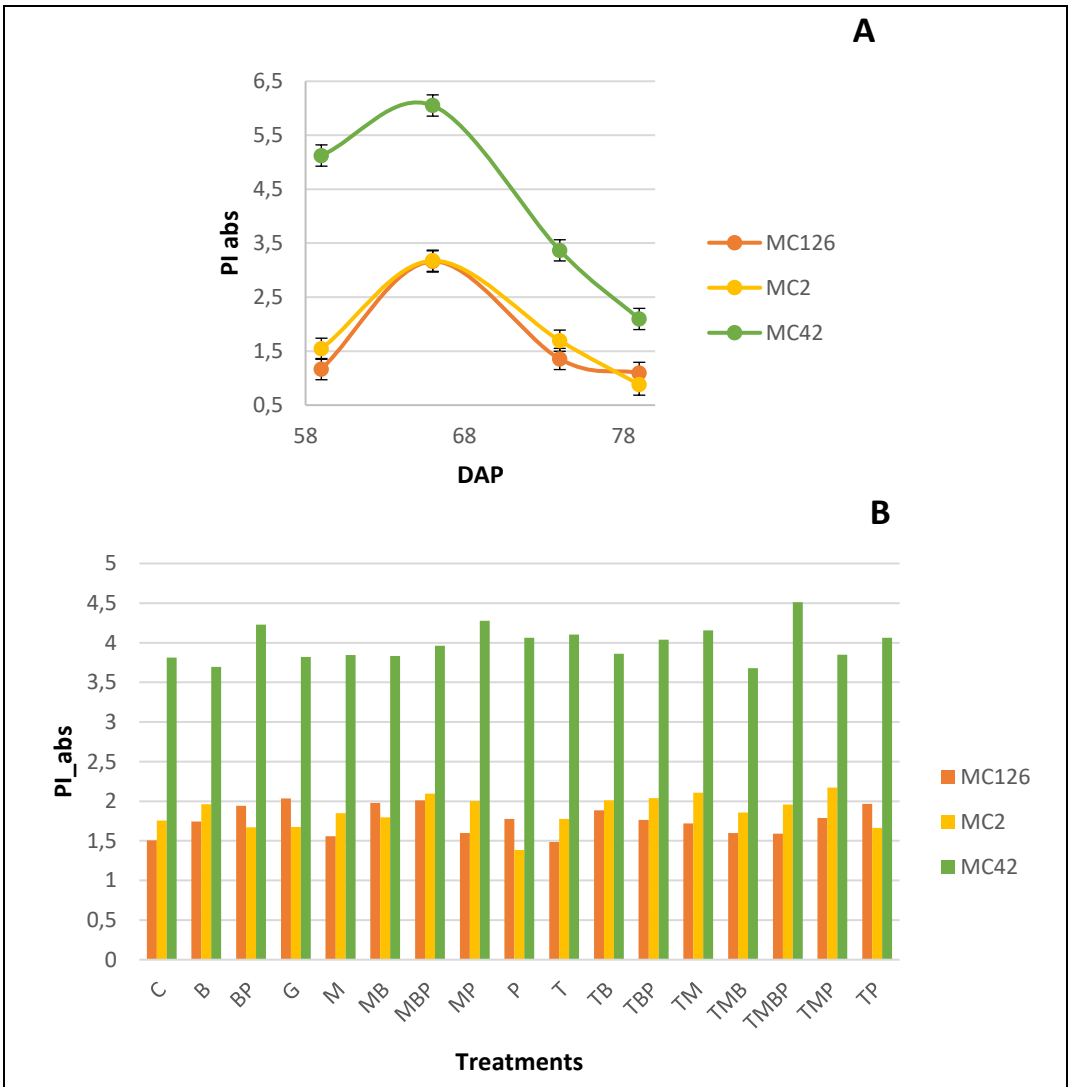


Figure 9: interaction between variety and day (A) and between variety and treatment (B) for PI abs. Performance index over time (A) (<0.001, LSD=0.1965) and per treatment (B) (<0.05, LSD=0.4771), for the three tested varieties: MC2 (yellow), MC42 (green) and MC126 (orange). Plotted values in "A" represent the means ± standard error (SE) of measurements conducted on the third youngest leaves of 560 plants. The negative control on "B" is on the left, under the label "C".

The ability to use chemical energy, expressed as $\frac{\delta o}{1-\delta o}$, is a partial vitality index that is included in the formula of PI total but not in the PI abs one. MC42 did not present a distinctive behaviour for this parameter (<0.001) (Figure 10*B*). On the contrary, this parameter remained stable as well as low over time (Figure 10*A*). Both MC126 and MC2 presented similar behaviours over time, showing a decline at 66 DAP and a rise afterwards. Besides, MC126 experienced another fall after day 74. An interaction variety-treatment was found, with an LSD value of 1.024. All treatment means within the cultivar MC42 were lower than the LSD, which makes the differences between treatments clearly insignificant. Figure 10B shows a peak on 'M' for MC126 that differs strongly from any other treatment, as it is larger than the LSD when compared to all other means. MC2 shows a better ability to use chemical energy when the following treatments, named in descending order, are applied: 'P', 'C', 'TP', 'TM', 'TMB', 'T', 'MB', 'G', 'M' and 'MP' (Figure 10*B*).

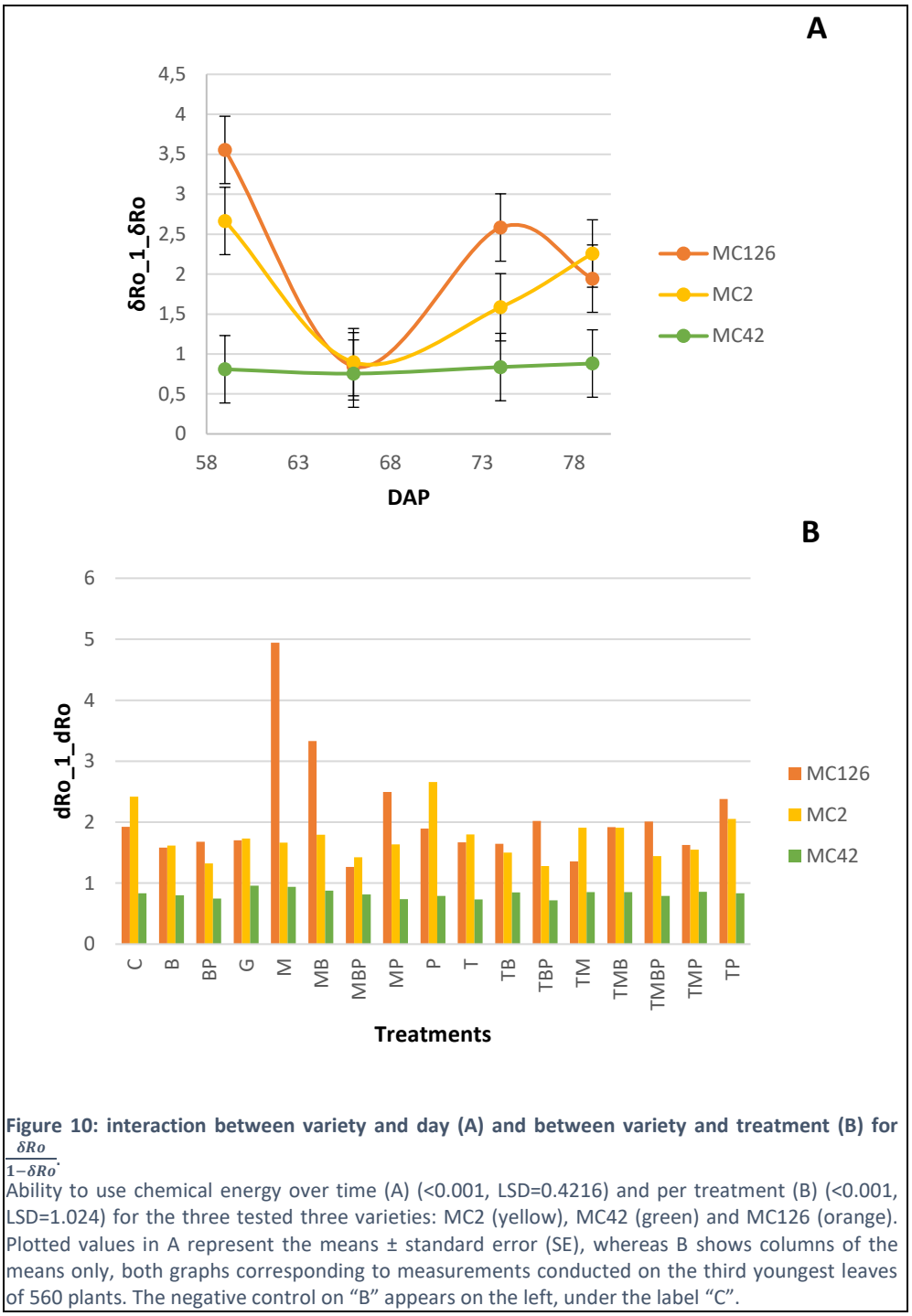
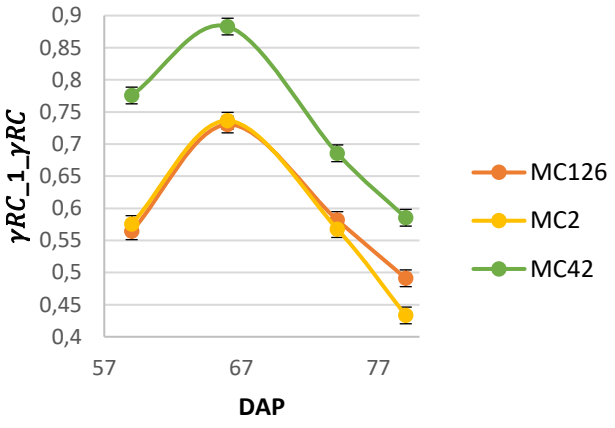


Figure 10: interaction between variety and day (A) and between variety and treatment (B) for $\frac{\delta Ro}{1-\delta Ro}$.

Ability to use chemical energy over time (A) (<0.001, LSD=0.4216) and per treatment (B) (<0.001, LSD=1.024) for the three tested three varieties: MC2 (yellow), MC42 (green) and MC126 (orange). Plotted values in A represent the means ± standard error (SE), whereas B shows columns of the means only, both graphs corresponding to measurements conducted on the third youngest leaves of 560 plants. The negative control on "B" appears on the left, under the label "C".

Measurements of the ability of the plant to absorb sunlight energy, expressed by the formula RC/ABS or $\frac{\gamma RC}{1-\gamma RC}$, indicated a better performance of MC42 over time compared to the other lines (<0.001) (Figure 11 *left)*. All the lines showed a decline in the values from day 66 after planting. The statistical analysis showed no interactions between treatments and days or varieties. Nevertheless, the differences within treatments were significant (<0.001, LSD=0.0182) and 'TM, 'TMBP', 'TB', 'MP', 'MBP', 'TMP', 'BP', 'TP', 'G' and 'MB', which make up group *a*, reported the highest values (Figure 11 *right)*.

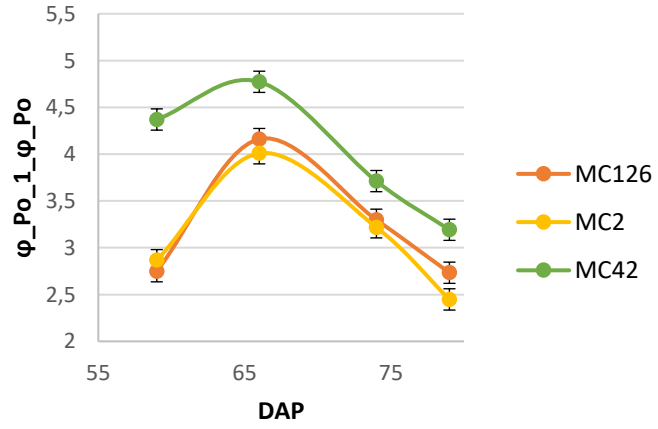


	γ_RC_1_γ_RC	
TM	0.648	a
TMBP	0.646	ab
TB	0.643	abc
MP	0.641	abcd
MBP	0.639	abcd
TMP	0.637	abcd
BP	0.637	abcd
TP	0.637	abcd
G	0.632	abcde
MB	0.630	abcdef
TBP	0.629	bcdef
M	0.627	cdef
B	0.626	cdef
P	0.623	def
TMB	0.622	def
T	0.615	ef
C	0.613	f

Figure 11: interaction between variety and day (left) and between treatments (right) for $\frac{\gamma RC}{1-\gamma RC}$.

Ability to absorb sunlight energy over time (left) for the three tested three varieties: MC2 (yellow), MC42 (green) and MC126 (orange) (0.001, LSD=0.0130). Plotted values represent the means ± standard error (SE). The table on the left shows the means for each treatment (<0.001, LSD=0.0182). Both graphs correspond to measurements conducted on the third youngest leaves of 560 plants.

Measurements of the plant's ability to store the absorbed sunlight, also referred to as the maximum yield of primary photochemistry and represented by the formula $\frac{\varphi Po}{1-\varphi Po}$, were also higher for MC42 than for the other varieties (<0.001), all of them presenting the same type of curves over time (Figure 12 *left)*. Treatments showed significance on their own (<0.05, LSD=0.159), being 'MBP', 'TMP, 'TB', 'TBP', 'TM', 'MP', 'BP' and 'M' the most effective in terms of storage of absorbed sunlight (Figure 12 *right)*.



TreatName	φ_Po_1_φ_Po	
MBP	3.620	a
TMP	3.556	ab
TB	3.543	abc
TBP	3.541	abcd
TM	3.538	abcd
MP	3.526	abcd
BP	3.482	abcde
M	3.465	abcde
TMBP	3.460	bcde
MB	3.435	bcde
TMB	3.410	bcde
B	3.409	bcde
C	3.408	bcde
T	3.400	bcde
TP	3.391	cde
G	3.382	de
P	3.350	e

Figure 12: interaction between variety and day (left) and between treatments (right) for $\frac{\varphi Po}{1-\varphi Po}$.

Ability to store absorbed sunlight energy over time (A) for the three tested three varieties: MC2 (yellow), MC42 (green) and MC126 (orange) (0,001, LSD=0.1134). Plotted values represent the means ± standard error (SE). B shows the means for each treatment (<0.05, LSD=0.159). Both graphs correspond to measurements conducted on the third youngest leaves of 560 plants.

The partial vitality index indicating sunlight conversion ability is expressed by the formula $\frac{\psi o}{1-\psi o}$. MC42 presented the highest levels, compared to the other two varieties, over the entire duration of the experiment (<0.001, LSD=0.04795) (Figure 13*A)* and with respects to all the treatments performed (<0.05, LSD=0.1164) (Figure 13*B)*. Nevertheless, Figure 13*A* shows a decline starting from the first sampling time (59 DAP) until the end of the experiment. The curves corresponding to MC2 and MC126, on the other hand, have a peak on day 66 and start decreasing afterwards. Regarding the interaction variety-treatment, significant differences can be found for the same treatment when comparing MC42 with any of the other two varieties (Figure 13*B)*. However, differences are not as clear when the means of MC126 and MC2 are compared. According to the LSD-test (data not shown), significant differences in sunlight conversion ability between them could be found in only 9 treatments, one of them being the control, out of 17. Differences between treatments within each variety can be found when groups of treatments are considered. MC42 displays the smallest group *a*, consisting of 9 treatments. The same group is made of 12 treatments for variety MC126 and 15 treatments (including the control) for variety MC2, suggesting that nearly all treatments have similar effects on sunlight conversion ability.

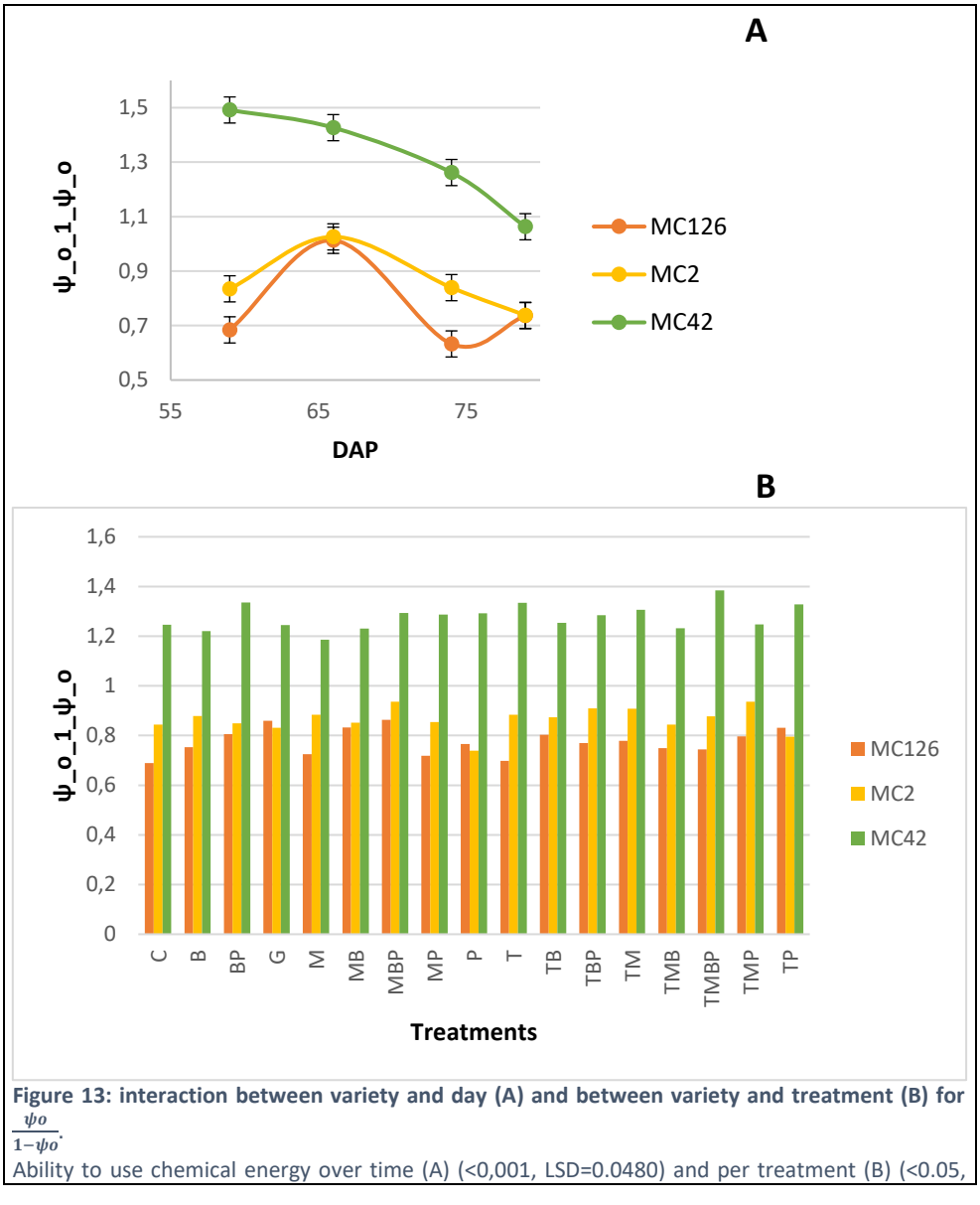


Figure 13: interaction between variety and day (A) and between variety and treatment (B) for $\frac{\psi o}{1-\psi o}$.

Ability to use chemical energy over time (A) (<0,001, LSD=0.0480) and per treatment (B) (<0.05,

LSD=0.1164) for the three tested three varieties: MC2 (yellow), MC42 (green) and MC126 (orange). Plotted values in A represent the means ± standard error (SE), whereas B shows columns of the means only, both graphs corresponding to measurements conducted on the third youngest leaves of 560 plants. The negative control on B is on the left, under the label "C".

Microbial tests

Five dilutions, including the original solution, were plated out to recover microorganisms from the rhizosphere and 4 dilutions, including the original solution, were plated out for the culturing of endophytes. The plates corresponding to dilution 0 displayed scattered colonies the isolation of which was possible, but easier from dilution -1, suggesting that only one dilution of the solution was necessary.

Figure 14: presumable Trichoderma harzianum on a PDA plate isolated from the -1 dilution PDA plate corresponding to the plant treated with 'TMBP'.
Confirmation of the species was missing.

Figure 14 shows a colony isolated from the 'TMBP'-treated root sample plate which has the same appearance as *Trichoderma harzianum,* according to a mycologist of the ARC who performed direct and microscopic observation of the colony. However, this type of colony is not unique, as the colonies of the widely distributed *Penicillium* also display the same characteristics, suggesting that identification of the fungus is required. The confirmation of the microbes could be done at the Biotechnology platform of the ARC and would include DNA extraction and Sanger DNA sequencing of the 16S ribosomal RNA (rRNA), for bacterial isolates, and 18S rRNA for fungal colonies. This study was aimed at identifying the inoculants after they had established within the plant roots. The preliminary protocol included the isolation and subsequent identification of presumptive colonies from samples taken from one of the tables at three different times along the development of the plants. Carrying out these microbial tests at different times allows for a more detailed understanding of microbial population dynamics, for microbial communities vary with plant developmental stages, which has been demonstrated in a study of potato-associated AMF in the Peruvian Andes (Senés-Guerrero *et al.* 2014).

Direct plating of the roots did not show as much biodiversity as other methods, as everything grew around the root piece and different colonies could not be observed nor isolated (see Figure 15).

Figure 15: direct plating of root pieces from plants treated with 'B' (left) or with 'TMBP' (right) on NA plates. Plates were incubated for 48h.

The surface sterilisation and subsequent crashing and diluting of the roots eliminated any microorganisms living on the surface of the roots and allowed the endophytes to be released. Figure 16 shows the growth of endophytes from Mondial plant root samples: one of them corresponding to the treatment with the highest number of inoculants, 'TMBP', and the other one treated only with one microorganism, 'B'. Both PDA (Figure 16A) and NA (Figure 16B) plates show a higher number of colonies for roots colonised only by *B. subtilis* compared to the sample with the highest possible diversity of microbes. *B. subtilis* is known to colonise internal plant tissues and its colonies are typically white or slightly cream on NA, which coincides with the results observed. In contrast, not as many colonies appear on the plates corresponding to plant roots treated with 'TMBP', which also include *B. subtilis,* probably because of competition between the inoculants that resulted in the latter not being able to establish successfully.

Interestingly, although wood shavings had been sterilised, results show a greater microbial diversity than what was expected, as the sample inoculated with only *B. subtilis* appeared to contain more than one type of microorganism (see right plate on Figure 16A and B*).*

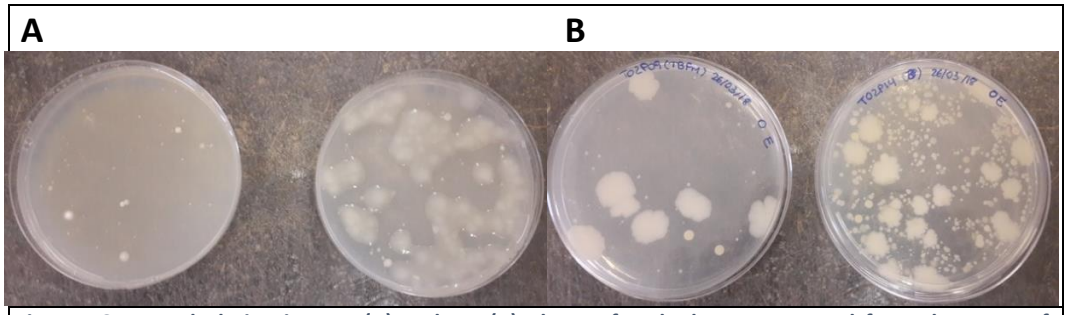


Figure 16: spread plating in PDA (A) and NA (B) plates of endophytes recovered from the roots of Mondial plants treated with 'T', 'B, 'P' and 'M' (left) and with only 'B' (right) after 48 hours of incubation.
Plates were spread plated with 100µL from the original solution, containing the crashed roots diluted in 1ml ('TMBP') or 2ml ('B') distilled water.

In order to determine whether it was necessary to carry out the procedure for the culturing of endophytes or it was possible to recover them by only plating the roots without surface sterilising and crashing, both methods were used on 'B'-treated plant roots and results compared with each other (see Figure 17). A higher diversity and abundance of colonies appears on the endophytes plates, whereas the plate corresponding to microorganisms from the ectorhizosphere shows high abundance of big, white-yellow colonies and very few smaller

colonies of two different types. The biggest colonies from the ectorhizosphere sampling exhibit nearly round edges, whereas the biggest colonies on the endophytes plate seem to have more irregular edges, suggesting that they might correspond to different microorganisms. Further examination of each colony and confirmation of the isolated microorganism should be done in order to determine whether the endophytes used in this study, namely *B. subtilis,* also grow on the ectorhizosphere plate. However, given the content on each plate does not seem to be the same, surface sterilisation and root crashing should be carried out when endophytes are expected to be recovered from a potato root sample.

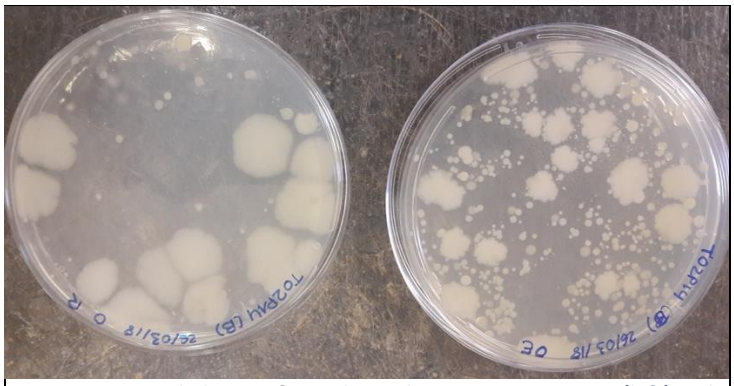


Figure 17: spread plating of ectorhizosphere microorganisms (left) and endophytes (right) from 'B'-treated plant roots in NA plates incubated for 48 hours.
Plates were spread plated with 100µL from the original solution, containing the crashed roots diluted in 2ml distilled water.

Questionnaires and interviews

o General information

Maize and potato are the most cultivated crops within the surveyed area of KZN (Figure 18). 29 out of 30 farmers live on farming (Figure 19 *left*). Some households have other sources of income, such as formal jobs or social grants. Collecting household income data was not possible in many cases, as records of the farm sales were not always kept and farmers having formal jobs were not comfortable disclosing their salaries. From a total of 18 respondents, the majority of them earned between R1000-3000/household/month (Figure 19 *right)*, with an average household members of 8.

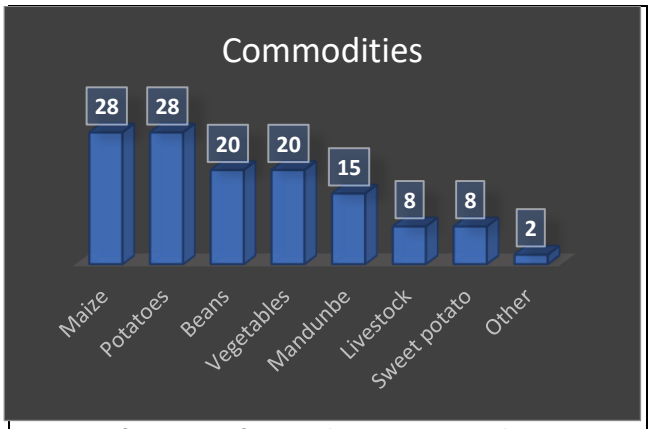


Figure 18: frequency of respondents growing each crop.
The graph shows that maize, as well as potatoes, are cultivated by 28 out of 30 surveyed farmers (n=30).

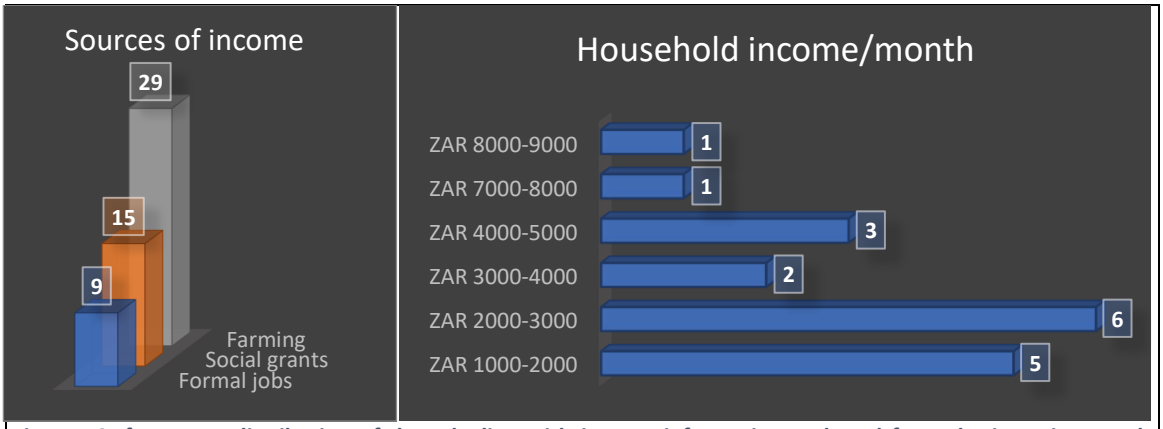


Figure 19: frequency distribution of data dealing with income information gathered from the interviews and questionnaires conducted to 30 small-scale farmers of KZN, South Africa.
The graph on the left indicates the number of respondents living on farming, social grants and/or formal jobs. Each respondent might have more than one source of income. The sample volume for the question "household income/month" (right) was 18 instead of 30, and each row corresponds to the number of respondents having a given range of income.

o Production challenges

Data regarding production challenges was collected mainly through the question "what are the challenges that your farm faces?" which is a multiple-choice question (see Figure 20 *left*), and questions derived from the answers to the main one. However, that question is somewhat subjective and contingent on the knowledge that farmers possess about agricultural practices. Other questions regarding the use of resources, available infrastructure, input expenses and produce sale also gave information about elements hampering the well-functioning of their agricultural systems.

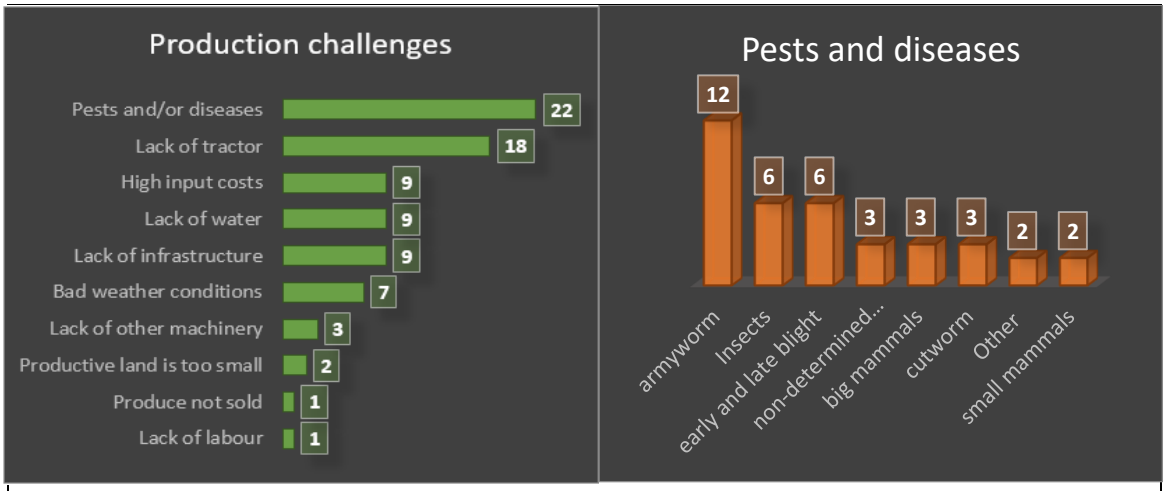


Figure 20: frequency distribution of data dealing with production challenges (left) and pests and diseases (right) gathered from the interviews and questionnaires conducted to 30 small-scale farmers of KZN, South Africa.
Each row or bar corresponds to the number of respondents who face a given challenge/ deal with a given pest or disease.

22 out of 30 respondents reported the presence of pests and diseases to be a major challenge, making it the most frequent one *(*Figure 20 *left).* Among the pests and diseases reported, insects, especially armyworm (40%), and early and late blight (20%) were the most frequent, respectively (Figure 20 *right)*. The follow-up question to those farmers was "How do you deal with these pests and diseases?". None of them showed knowledge of IPM, as the only way they knew to control them was chemical management. In those cases where chemical control did not work any longer, the most common solutions were either to do nothing or to stop growing that crop because the losses were unaffordable. Some farmers did not know about crop rotation systems. The second most frequent challenge was the lack of tractor, scoring 18 out of 30. Tractor availability was of serious concern among farmers of the first community, as most of them could not afford owning one and relied on the few farmers who did and rented it out. The demand for a tractor appeared to be definitely higher than the offer in the first community. One of the respondents, who owned and rented out her tractor, was the chairperson of one of the farmers' organisation and corroborated the importance of the issue among members of the organisation. By the time of the interview she had her tractor broken. 30% of the respondents lacked water and infrastructure on their farms, as well as reported high input costs as a major challenge. 80% of the respondents irrigated at least a portion of their fields, and although KZN is not as dry as other parts of South Africa, the irrigation system used in most of the farms was manual application of rain water with buckets or watering cans (Figure 21*)*.

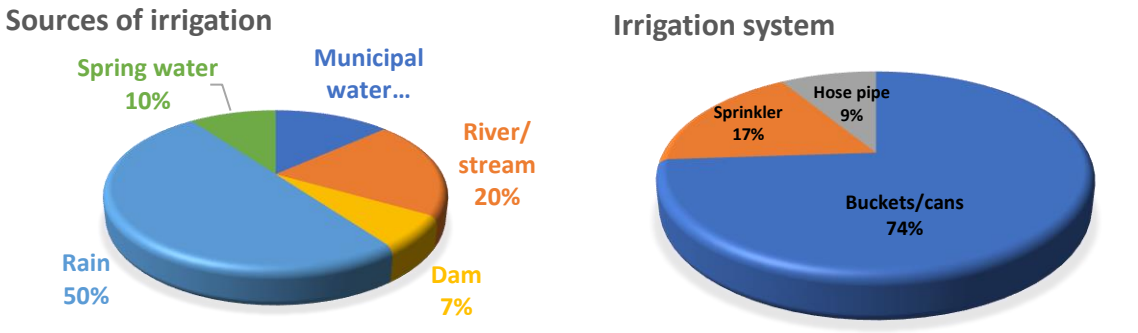


Figure 21: frequency distribution of data dealing with irrigation systems gathered from the interviews and questionnaires conducted to 30 small-scale farmers of KZN, South Africa.
The pie charts show the percentage of respondents who used each source of irrigation (left) or each type of irrigation system (right).

A cold storage room was absent in all the farms, which some farmers pointed out as a weakness of their systems because it accelerated the spoilage of the produce, thus urging them to sell. Additionally, the lack of costumers (see market challenges on

Figure 23*)* as a consequence of transport-related challenges (see transport challenges on Figure 22*)* appeared to be the cause of economic losses for many farmers because they failed to sell the produce before it gets damaged. The main transport challenge observed among the respondents was the higher costs involved, especially for those who had to hire a vehicle (and a driver) or get to the trading location by taxi. Lack of transport, either due to unavailability of vehicles or due to money shortages, is an issue for 23.3% of the respondents (Figure 22 *left)*. Significantly, only 15% used their own vehicles to transport the produce, as opposed to 28% who hired it (Figure 22 *right)*. Moreover, 25 out of 30 farmers sold their produce at the farm gate, which was the only trading point for 16% of them, thus reducing visibility of their business. In other words, 13.3% of the surveyed farmers relied only on buyers' transport to get their produce sold. The market challenges chart reflects that it is not only high costs of transport, as mentioned above, that hamper the farmers' ability to sell the produce on time, but it is also the lack of FPMs or local shops nearby, which was reported especially within respondents from Swayimane. Finally, there is a tendency towards economic insecurity, as 50% of the farmers interviewed negotiate the price of their produce with buyers and often sell it at a lower price than they would be willing to, especially when the harvest has been stored for too long to preserve the characteristics of the produce (see

Figure 23 *right)*.

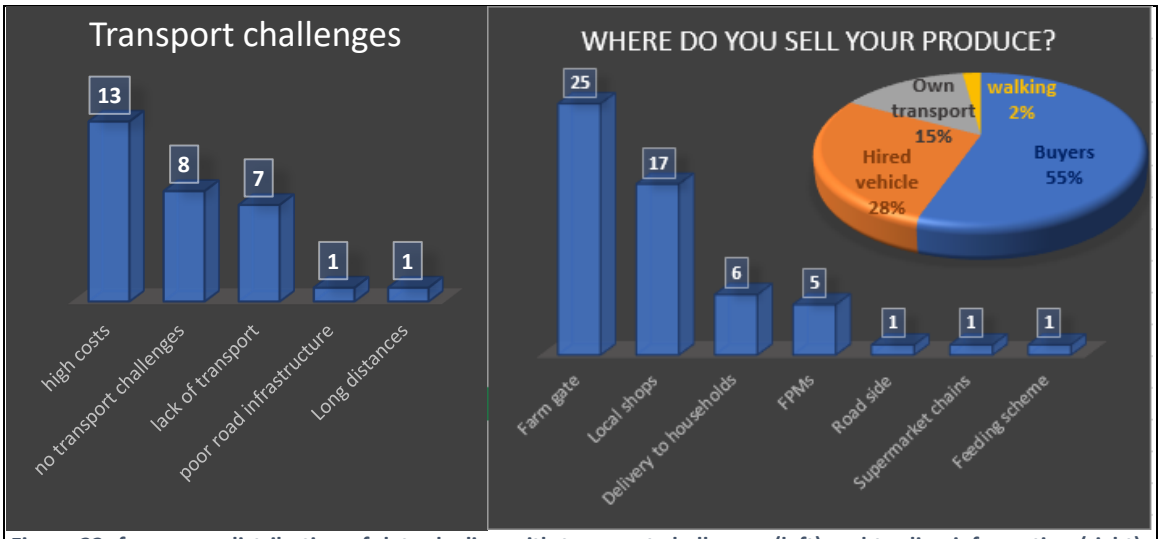


Figure 22: frequency distribution of data dealing with transport challenges (left) and trading information (right) gathered from the interviews and questionnaires conducted to 30 small-scale farmers of KZN, South Africa.
The pie chart shows the answers to the question "how is the produce transported?" and the use of each transportation means in form of percentage. Each respondent might use more than one way of transporting produce.

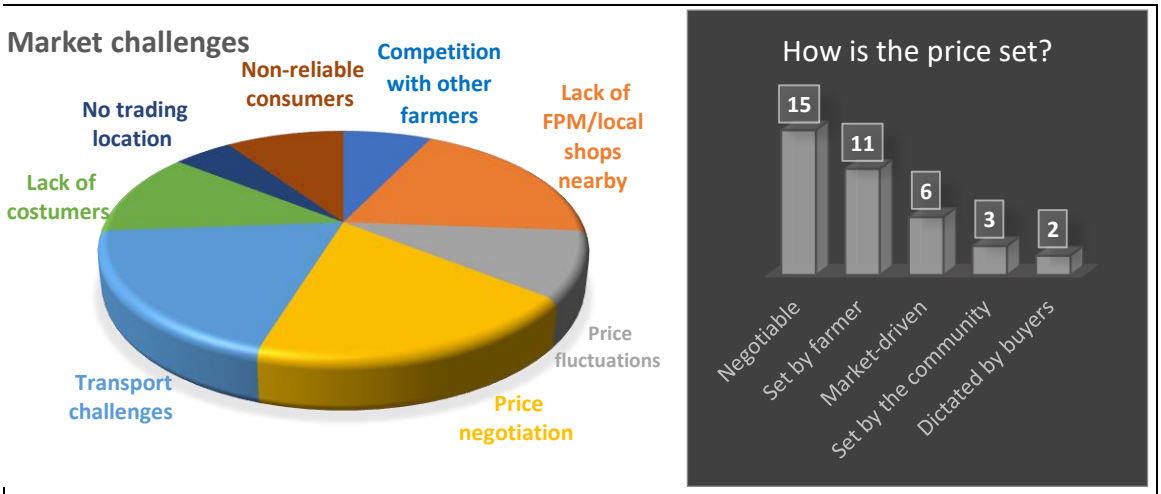


Figure 23: frequency distribution of data dealing with market challenges (left) and price negotiation (right) gathered from the interviews and questionnaires conducted to 30 small-scale farmers of KZN, South Africa.
The pie chart shows the 8 market challenges mentioned by 30 respondents. Each respondent might face more than one challenge. Transport challenges and price negotiation were the two challenges most mentioned. The columns graph shows how many out of 30 farmers uses a given way of setting the price. Each farmer might use more than one pricing strategy.

Regarding farm expenses, all farmers spent money on seeds, inorganic fertilizers and pesticides (Figure 24*)*. Among the respondents who provided information about farm expenses, 64%, 50% and 88% of them spent less than R2000/season on seeds, fertilizers and pesticides, respectively. 37% and 30% of the respondents' expenses on fertilizers and seeds, respectively, ranged from R2001-4000.

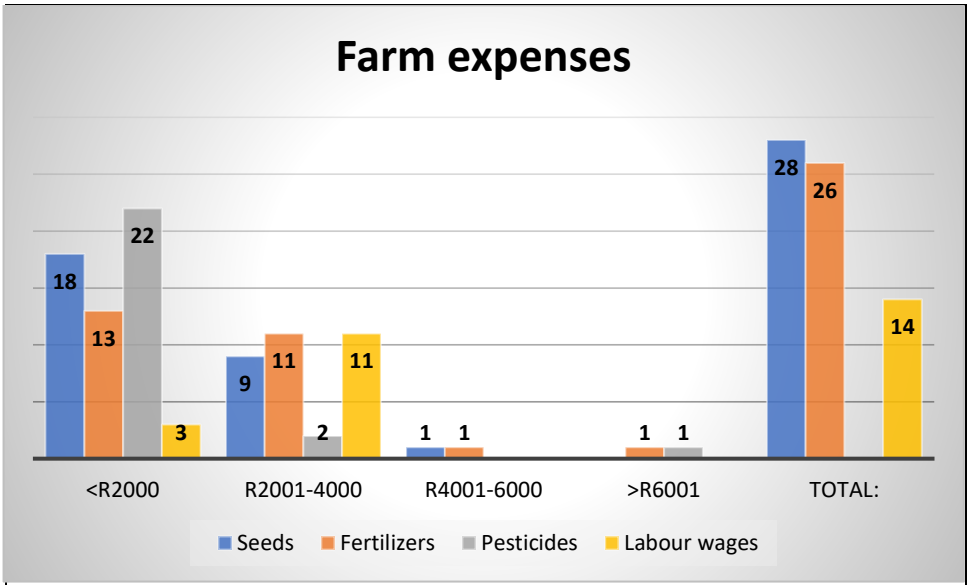


Figure 24: frequency distribution of data dealing with farm expenses per season gathered from the interviews and questionnaires conducted to 30 small-scale farmers of KZN, South Africa. The columns categorised as "TOTAL" correspond to the sample volume for each one of the farm expenses i.e. 14 out of 30 farmers spent money on labour, 26 of them, on fertilizers, and 28 of them, on seeds. The values on top of the other columns correspond to the number of respondents whose expenses on each input fell into a given range of values.

o Knowledge

None of the respondents considered lack of knowledge of agriculture to be a challenge that hinders their ability to properly run their systems. The graph showing the distribution of years of farming experience has its peak on the range 6-10 years and it is right-skewed, having only one value at the range 26-30 years (see Figure 25 *left)*. Frequency of access to information was seasonal for 2/3 of the surveyed population, which coincided with seasonal agricultural trainings (Figure 25 *right)*. Extension services accounted for 83% of the sources of information available, whereas radio/TV and cooperatives together accounted for 17% (see pie chart on Figure 25 *right)*. Specific questions were asked based on the challenges they mentioned in order to understand how they adapted to changes and dealt with hurdles that might weaken their systems. Most of the solutions to their problems were suggested by extension officers from the Cedara College (DARD-KZN). In fact, they appeared to be generally more prone to follow suggestions from the extension officers than relying on their own experience and knowledge to overcome such problems or to try new things to improve the way they farmed. A few farmers acknowledged their lack of entrepreneurial skills due to the fear of failing and losing money. Only one farmer reported coming up with her own ideas to reduce excess harvest.

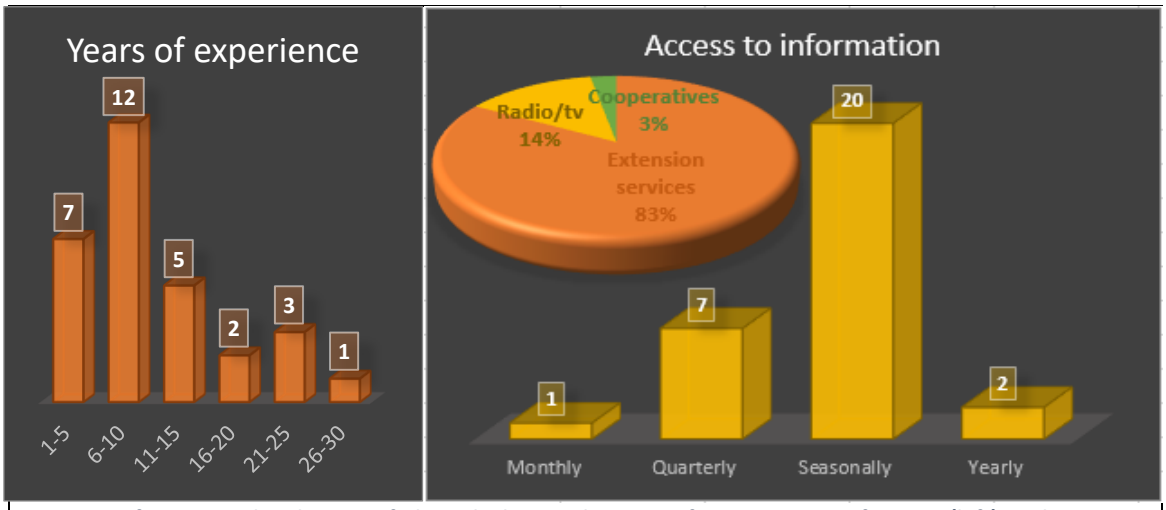


Figure 25: frequency distribution of data dealing with years of experience in farming (left) and access to information (right) gathered from the interviews and questionnaires conducted to 30 small-scale farmers of KZN, South Africa.
The pie chart on the right shows the percentage of respondents choosing each source of information represented on it.

As far as mini-tubers are concerned, most of the respondents affirmed that they knew what mini-tubers were, but only 5 out of 30 really did. The rest, when asked how they got to know them, referred to them as the small-sized tubers of the harvest. Some of them (5/30) argued, out of their own experience or based on the information from the trainings, that small-sized tubers gave lower yield compared to medium or large-sized ones, whereas fewer of them said otherwise and pointed out that the company where they got potato seeds from sells small-sized tubers. The number of respondents who did not know which potato cultivars they were planting accounted for 20% of the total (Figure 26*)*.

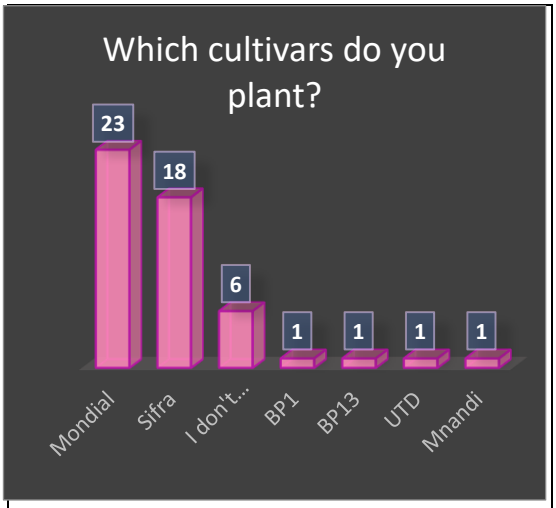


Figure 26: frequency distribution of cultivar used, gathered from the interviews and questionnaires conducted to 30 small-scale farmers of KZN, South Africa.

o Potato seed production

Obtaining potato seeds from the previous year's harvest is a common practice for 37% of the interviewed farmers, though not the only way of obtaining potato seeds (see Figure 27 *left)*. Nearly all farmers obtained potato seeds from private companies. The high inputs costs and the excess harvest (Figure 27 *right)*, especially when market and transport challenges are present, were the main drivers of the reuse of seeds from previous years. The number of bags purchased was contingent on the amount of own seeds produced. The price most paid for a 25Kg bag of potato seeds was R140, with a frequency of 50% (Figure 28*)*. None of the farmers showed to have genuine interest in potato seed production as a business, yet four of them seemed to become interested when we briefly introduced the concept to them. Those who were interested were asked about the certification process of potato seeds, to which they showed no knowledge.

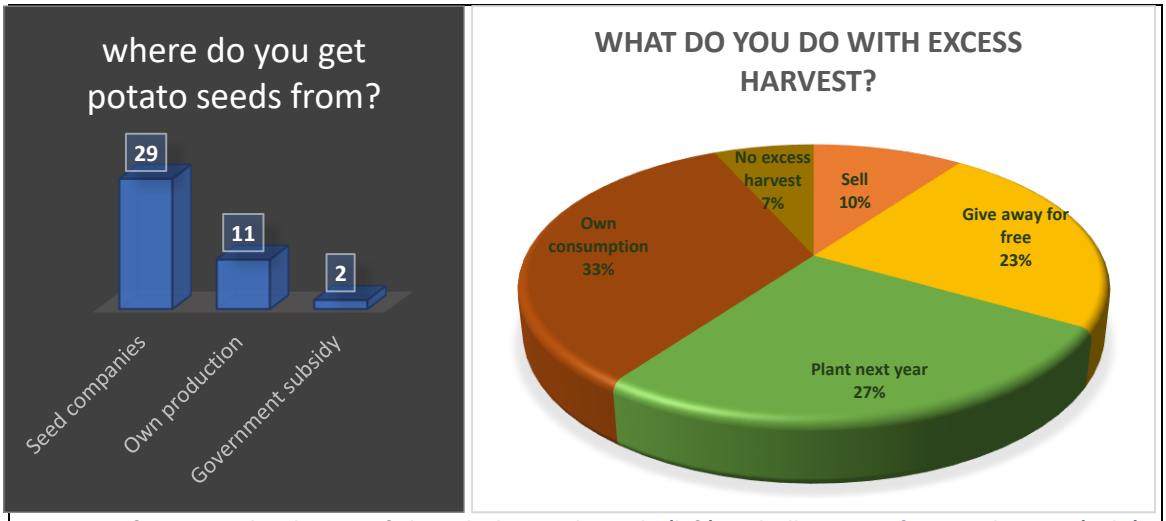


Figure 27: frequency distribution of data dealing with seeds (left) and allocation of excess harvest (right), gathered from the interviews and questionnaires conducted to 30 small-scale farmers of KZN, South Africa.
The columns graph on the left indicates the number of respondents getting seeds from each of the sources represented on it, whereas the pie chart (right) shows what the respondents use excess harvest for, expressed in percentage. Each respondent might choose more than one way to allocate such excess.

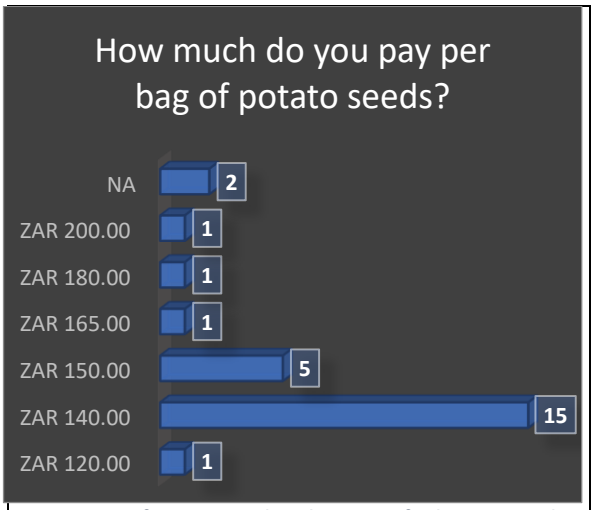


Figure 28: frequency distribution of the price the respondents pay for each bag of potato seeds (n=26). All the data were gathered from the interviews and questionnaires conducted to 30 small-scale farmers in KZN, South Africa, nevertheless four of them did not provide information about the price of one bag of potato seeds.

o Interests

This section deals with the interests of the respondents, their endeavours and purpose in farming. The question dealing with the farmer's vision was an open-ended one, yet 17 farmers gave a very similar answer that could be summarized as "to become a successful farmer" (Figure 29 *left*). Some considered that being successful was the same as being able to make money to sustain their families, whereas others mentioned the well-functioning of a properly equipped farm as an indicator of success. Farmers whose vision had to do with the supply of produce to FPMs, the feeding scheme, local schools or supermarkets were grouped under the subtheme "to reach the targeted market" and accounted for 4 farmers. All farmers mentioned something to look forward to. The old ones, however, did not aim at carrying out changes on the farm, but at preparing their children for taking over. Knowledge transfer, money-making, job creation and providing their children with proper living and education were mentioned by fewer farmers and 2 out of 30 farmers practised agriculture as means to achieve other goals.

Farmers were asked how they allocate the harvest of their crops, which related to their purpose in doing agriculture (Figure 29 *right)*. 29 farmers made a business out of farming and 24 of them allocated part of the harvest for household consumption. There was only one farmer who did not sell the harvest.

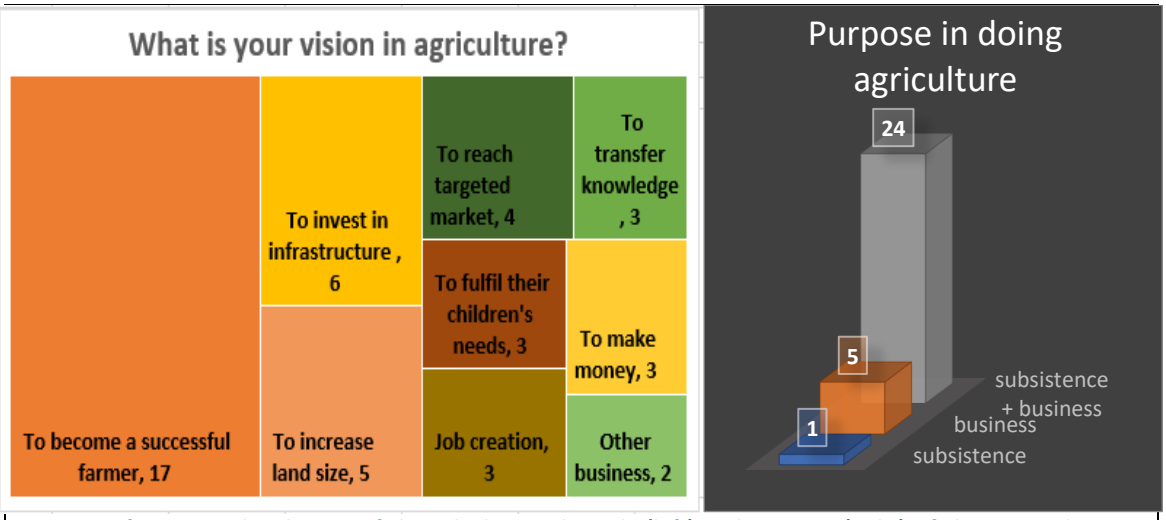


Figure 29: frequency distribution of data dealing with goals (left) and purpose (right) of the respondents in agriculture.
The data were gathered from the interviews and questionnaires conducted to 30 small-scale farmers of KZN, South Africa. Each farmer provided only one answer and all farmers answered both questions (n=30).

Discussion

Discussion of results

As mentioned in the introduction, the selection of high-yielding varieties is an essential part of the integrated pest management strategy. Thus, analysing chlorophyll fluorescence to gain knowledge of plant performance can give valuable insights into the improvement of potato cultivars. The data yielded by the statistical analysis of the CFA shows variety-day interactions in all parameters analysed, which coincides with results obtained from the leaf area index test, thus providing compelling evidence that variety influences chlorophyll fluorescence and canopy cover over time. Furthermore, the data from the CFA test suggests that the variable variety affects plant performance, for the parameter total performance index or PI total takes into consideration all partial vitality indexes, namely the plant's ability to absorb sunlight energy, to store the absorbed sunlight, to convert it into chemical energy and to use such chemical energy, for which the interaction variety-day was significant. The data yielded by the CFA regarding PI total indicates that MC42 is the best-performing variety. MC42 and MC126 experience a decline after day 66, unlike MC2, which displays decreasing values from the first sampling time (59 DAP). A decline in potato plant performance is expected during the tuber bulking stage, as the plant has been accumulating nutrients and energy in the canopy during the vegetative growth stage that needs to be translocated to the tubers. Another contributing factor could be, however, the fact that some plants started showing symptoms of late blight shortly after the readings at 66 DAP were taken. The earlier decline in plant performance observed in MC2 plants compared to MC42 and MC126 can be explained by differences in the length of the growth cycles of each variety. Conversely, all tested cultivars display an increase and subsequent decrease of the other performance index (PI abs), which encompasses all partial vitality indexes except for the ability to use chemical energy. MC42 displays higher values compared to the other varieties for the whole length of the study. A totally different behaviour is observed in all varieties when the parameter indicating the ability to use chemical energy is considered, which bottomed out on day 66 for both MC2 and MC126. The reason for this fall is unknown. MC42 appears to have the poorest ability to use chemical energy,

according to results, but the overall total performance indicates that it is the variety displaying a higher vitality index.

Unfortunately, yield-related post-harvesting tests, which would give information about the correlation between plant performance and yield, could not be carried out within the timeframe of this study and results are therefore not available yet. It seems logical to focus the attention on the parameter PI total for the interpretation of results because it includes all partial vitality indexes in its formula. Nevertheless, it might not be the best indicator of yield, which is ultimately the parameter that breeders need to obtain information about to be able to select the most suitable varieties for a given climate. Assuming the results of the CFA are totally reliable and representative of reality, it will only be possible to determine which ChlF parameter is the best indicator of yield when the results of the post-harvesting tests are available and can be compared with them.

No significant interactions were found between varieties and treatments for the total performance index. In contrast, the performance index PI abs showed a statistically significant variety-treatment interaction. Interestingly, plants treated with only AMF appeared to be the best performing ones in terms of ability to use chemical energy, which is the parameter that is absent in the PI abs formula. This is the only case in which one single treatment is significantly different from all others according to the LSD-test and could be the reason AMF appeared to be the treatment having the highest value for PI total but not for PI abs. Groups of treatments having different degrees of significance have been identified for each parameter, yet it is difficult to find a common denominator due to the large number of treatments belonging in the same group. For instance, although AMF is clearly the treatment having the greatest positive impact on the ability of MC126 to use chemical energy, when PI abs is considered, AMF appears to be as statistically significant as 14 other treatments, which make up the best-performing group (group *a*), and in the case of the PI total, it also belongs in group *a*, together with 8 more treatments. These results suggest that there is a trend towards some treatments positively affecting total performance more than others. Nevertheless, it is not possible to point one of them based on the available data. Furthermore, the slope of the LAI graphs for each treatment was not calculated, which would have been very useful for the determination of the best treatment. A principal component analysis of the different variables of Chl F measurements would shed light on the matter and allow for more developed conclusions to be drawn.

Results suggest that the answer to my research question “Do the microorganisms applied to the substrate have an impact on plant performance, growth and yield?” is “yes”. However, it is not easy to point one or a group of treatments that surpass the rest, for many things need to be taken into consideration. As mentioned in the introduction, the production of mini-tubers at the ARC is a very new initiative and the process still needs improvement. The setting up of the experiment coincided with several modifications regarding the methodology used for the cultivation of potato plants for mini-tuber production, which might have affected the results. For instance, the installation of a high-pressure drip irrigation system caused several inconveniences and variation in the plants’ growth rate due to pressure problems that led to differences in water delivery. A bigger pump was installed some weeks after planting, which fixed the problem, but many plants had already suffered from water deficiency. Many of them even died at different times during the experiment and needed to be replaced, which led to plant roots not being equally developed at the time of the inoculation. This could have affected the rate of successful establishment of the inoculants, causing variation within results.

Moreover, fertilizer was applied through the irrigation system; therefore insufficient water supply affected also the amount of nutrients available for the plant. Some of the old pipes burst when the high pressure was applied and had to be replaced at different times during the experiment.

Results were interpreted based on the assumption that the inoculants had actually established in or on the plant roots. However, there was no evidence of such establishment, since microbial tests did not allow for the identification of the microorganisms down to the species level. Then again, results showing statistical differences between treatments could be the regarded as an evidence of their establishment. It would nevertheless not be unreasonable to entertain the possibility that microorganisms of some crates were washed out from the wood shavings, for example, as the last day of the inoculation and the day after, the plants received a heavy rain. Moreover, the results of the microbial tests provide confirmatory evidence that the inoculants were not the only inhabitants of the plant root systems. Contamination of the substrate through human manipulation of the plants and growth medium surely occurred during the setting up of the experiment, the maintenance, the planting and the replacement of dead plants before and after the inoculation. There is no reason for disregarding the possibility of cross contamination between inoculants from different crates. If microorganisms as plant growth promoters be further investigated, confirmation of establishment by the inoculants needs to be carried out through microbial tests. The literature provides evidence that the composition of microbial communities varies with plant developmental stages (Senés-Guerrero *et al.* 2014). *F. mosseae* is a generalist, as it colonises the roots at early stages and seems to persist along the plant growth, which makes it a promising candidate for AFM use in potato cropping systems (*ibid)*. On these grounds, isolation of microorganisms from the rhizosphere should be conducted multiple times in further research studies.

Discussion of the methodology

The aim “To come up with an efficient but economically reasonable methodology for the analysis of the trials” has been achieved. Results from the regression analysis on the leaf area index data demonstrate that the methodology employed can be successfully used to determine the LAI, for the graph of the development of the canopy cover had a very good fitting. The Leaf Area Index graph is completed when measurements are taken from the day of emergence until the day the plant dies. The graph exhibits an initial upward slope, which is what the study intended to focus on. That slope determines how quickly a variety reaches 100% canopy cover, which can be compared with that of the ideotype, i.e. a behaviour model of a given cultivar, to direct the breeding efforts to a particular end. Crop ideotype was defined for the first time by Donald C. M (1968): "A crop ideotype is a plant model, which is expected to yield a greater quantity or quality of grain, oil or other useful product when developed as a cultivar." Since the purpose of developing new varieties is to improve the efficiency of agricultural systems, breeders focus their attention on developing high-yielding cultivars that are suitable for a particular set of environmental conditions. The ideotype provides a set of characteristics that suit the plant to its environment, which can be used as guidelines for breeders who are looking for specific traits. The steeper the slope, the better the plant is performing. Unfortunately, slopes of the LAI graphs were not calculated in this study, and therefore conclusions regarding which treatment was the best-performing one could not be drawn. The conclusion drawn was, nonetheless, that the use of a handmade cell phone shuttle device as a plant imaging technique and the processing of data through the free open source

software Easy Leaf Area seems to be a low-cost, rapid method for the collection of measurements of leaf area, which is an indicator of plant growth.

The fluorimeter used for the chlorophyll fluorescence analysis might not be economically affordable for everyone. Nevertheless, all the tests carried out in this study, as well as the yield-related tests, are non-destructive testing (NDT) methods, which allow analytical research to be conducted without causing damage. Data collection through non-destructive methods allows the farmer, breeder or researcher to take as many measurements as necessary without affecting the yield or crop productivity in any way. Using non-destructive techniques is very desirable as far as environmental and economical sustainability are concerned, because it produces no waste and no crop losses. Both the LAI and CFA gave consistent results regarding the effects of variety and treatments on plant performance and growth, suggesting that the methodology used is reliable.

The pilot trial revealed that the best method for the recovery of microorganisms from the rhizosphere is the one in which roots are rinsed with distilled water, which is then spread plated. When endophytes are the targeted group of microbes, surface sterilization and crashing of the roots ensures a higher recovery of bacteria than direct plating or the procedure for the sampling of rhizosphere microbes.

Social research

The results from the questionnaires and semi-structured interviews to small-scale farmers confirm the information available on the literature regarding the weaknesses and threats of smallholding agriculture: that the high input costs pose a major challenge for farmers and that one of the ways to reduce costs is through obtaining potato seeds from the previous year's harvest. This, in turn, jeopardises the resilience of their systems, for the use of non-certified late generation potato seeds increases the risk of pests and diseases, which reported to be the major challenge farmers faced. Crop losses due to pests and diseases damaging the harvest leads to low or no income generation, which makes the purchase of inputs and transportation of produce unaffordable, as farmers themselves explained. The lack of inputs in an input-intensive agricultural system can be devastating for the next year's crop, causing an even worse economic situation. Moreover, affordability of transport is taken for granted in Sweden, but for poverty-stricken small-scale farmers, not being able to transport the produce to market entails lower product sales, which also lowers income generation. The main challenge, however, is none of the ones mentioned by the surveyed farmers, but the only one they are not aware of. Their evident lack of knowledge on IPM prevents their agricultural systems from reaching their full potential, as the main strategy used to combat pests and diseases is chemical control. Putting the environmental issues aside, treating pests and diseases only with pesticides represents a high farm expenditure that negatively impacts the economic sustainability of the farm. From an agroecological point of view, knowledge-intensive, as opposed to input or capital-intensive agriculture, integrates the crop into the ecosystem around it, acknowledging the role each of the elements of the farming system play in it and making use of their properties, which are totally overlooked and even removed from the system in input-intensive farming. Evidently, gaining knowledge is also costly, mainly in terms of time. A knowledge-based agricultural system is inevitably time-intensive too, yet one would expect those farmers who live only on farming to be able to afford such time investment.

Most of the interviewed farmers mentioned that agriculture made them happy, that they saw life in it. All surveyed farmers lived on agriculture and 28 of them endeavoured to pursue goals

dealing with the improvement of their systems. I expected, therefore, these farmers to have genuine interest in gaining knowledge of their farms, to seek solutions to their problems and to come up with new practices to strengthen their business. However, frequency of access to information was mostly seasonal and through trainings and/or courses from the extension services. The reason for this apparent lack of interest towards farming education is unknown. Most farmers belonged to a farmers' organisation, and yet only one farmer reported getting knowledge from other farmers of the organisation. All farmers had access to agricultural trainings offered by the Cedara College of Agriculture at least twice per year, yet the knowledge farmers showed of IPM indicates a lack of information received on the topic. Furthermore, the financial problems they face negatively impacts their entrepreneurial skills by creating a fear of failing that keeps them from practicing the trial and error method of problem solving and makes them apply changes only when the extension officers recommend them to do so, without understanding the particularities of their systems to find more suitable solutions.

Farmers' lack of knowledge and initiative might be more complex than it seems. Results provide overwhelming evidence that lack of money and resources hampers farmer's chances to progress. However, the available data makes it difficult to gather evidence that lack of knowledge has the same effect as lack of money, for there are many factors affecting this situation, and only assumptions can be made in this regard:

First, the support that some of them get from the Cedara college is of high worth, and obviously they would not like to risk it by appearing too self-sufficient or confident in the eyes of a stranger sent from the ARC and the Department of Agriculture. This can lead to them giving misinformation to the interviewer about the state of their farms, if they think that the interviewer poses a threat to them. Secondly, cultural and historical factors might also play a role in hindering their ability to find solutions and trust their own judgement regarding the management of their farms. Indigenous South Africans have for many years lived in a society that looked down on them and abused them, which has affected the way they define themselves and has shaped their identity, possibly influencing how capable they think they are of doing things on their own and of coming up with their own solutions to problems. Thirdly, hidden social issues that affect the agri-food sector in South Africa might exist and that would explain why they do not learn from other farmers or obtain information from the media. Questions such as "Do farmers compete with each other? Does that keep them from sharing knowledge?" were not asked in this study and could reveal the real causes for this lack of knowledge.

These assumptions might be real factors that affect potato farming in the surveyed area or might be not. Understanding the nature of the problem of knowledge would make it easier to find a way to tackle it. A comprehensive analysis of social factors affecting a system is what the social part of agroecology deals with and is the key that opens the door towards social sustainability. The future of small-scale farmers of South Africa can be bright, provided that further research is carried out on this matter.

Given that the incidence of pests and diseases is the major challenge, and that using late-generation potato seeds from the previous year's harvest, which accumulate tuber-borne diseases, worsens the situation, one can argue that using certified mini-tubers would clearly reduce the risk of crop losses, as they are pathogen-free and therefore, diseases could only come from the soil or the environment, but not from the tubers. The answer to the research question "Could any of the challenges that small-scale farming in the surveyed area face be

fulfilled by introducing mini-tubers into agricultural systems?" would therefore be "yes". However, mini-tubers alone are not the solution for small-scale farmers, as they are expensive to purchase and they should be introduced into smallholdings after farmers have been taught what they are and how to use them. The use of mini-tubers, together with the provision of appropriate trainings on their use and on IPM strategies that teach farmers how to identify key elements of their systems, can potentially improve food and economic security, as well as the quality of the harvest. Cultural and physical/mechanical control practices, such as crop rotation, sanitation, mechanical removal of pests, use of trap crops to attract the pests that would otherwise attack the crop of interest, use of certified seeds and suitable varieties etc., are IPM strategies that can reduce the incidence of pests and diseases and do not involve the use of pesticides. Semiochemicals, namely chemicals involving pheromones or other compounds that interfere with the interactions between organisms, are also used in IPM programs as they target a specific pest or group of pests. Further research on IPM applied to South African fields might be required to determine which practices can be used and how they should be carried out. The most used cultivars within the surveyed area were Mondial and Sifra, probably because the extension officers recommended them, suggesting that farmers do not make decisions regarding cultivar selection. Moreover, some of them did not know which cultivars they were planting.

There is no single IPM approach that offers a universal solution because different strategies are suitable for different systems. On this grounds, agroecology gives the tools for the management of a specific farm, as it strives for time investment in order to achieve an understanding of the elements of a farming system that allow the farmer to run the farm in an efficient and sustainable way without using a lot of inputs and therefore, without spending a lot of money. Every farm is a different case, and only someone who regularly spends time in it can get the most out of it while preserving its natural resources.

Using mini-tubers without being able to identify a disease by its symptoms or to use the natural resources and tools that the system itself provides will only represent another input to the farm and, consequently, a higher economic investment. Thus, in order for farmers to overcome their challenges, they need to understand the importance of having knowledge of their farms and learn how to integrate their crop into the environment through the application of an integrated pest management strategy that ultimately should reduce the inputs need and consequently, the farm expenses. Moreover, fostering their entrepreneurial skills and encouraging them to try things by themselves might help them be genuinely interested in learning and seeking information. Nevertheless, understanding why they do not seek knowledge in the first place might give a profound insight into how to deal with them. The purpose of this is to ultimately promote their self-sufficiency and independence. To this end, the extension services of the DARD-KZN, need to provide them with the tools for a proper management of their farms by means of trainings on:

- The use of pesticides: environmental impact, how to make an efficient use and alternative methods, such as semiochemicals and biological control.
- The risks of planting potatoes from the previous year's harvest as seeds.
- IPM with special focus on cultural and mechanical/physical control
- Mini-tubers, their benefits and how to use them.

Communication of the findings to the Agricultural Research Council

A lecture on the project was given to researchers and other employees of the ARC on Friday 4th of May. Unfortunately, nobody from the Department of Agriculture of KZN could attend the lecture. After presenting the findings, I engaged the audience in a discussion about the feasibility of introducing mini-tubers in small-scale agricultural systems. Flip Steyn, potato breeder and mini-tuber producer at the ARC, was very positive about the benefits that mini-tubers would bring in smallholder agriculture, as they would be a solution against the high incidence of pests and diseases. Besides, Mr. Steyn argues that, due to their small size and weight, it would be possible to post them, making them accessible in remote areas where certified potato seed availability is scarce. The purchase of mini-tubers by small-scale farmers is a far-fetched option, as mentioned in the discussion, unless the ARC and/or the DARD-KZN find the support to supply them for free. Ideally, farmers would reach a desirable level of self-sufficiency if they produced mini-tubers or potato seeds themselves, as they would reduce the expenses of seeds. To this end, farmers should be provided with mini-tubers and courses in which they are trained on how to use them. Besides, giving them information about how the seed production process and certification of potato seeds is carried out might encourage the interested ones to try to produce seeds of their own. However, this option has some limitations. First, the *in vitro* plant material from which mini-tubers are produced is very expensive to cultivate as well as to transport from the ARC to the farms, as reported by Ms Nokuthula Myeza, manager of the *in vitro* Genebank facility at the ARC. Secondly, the Potato Certification Services (PCS) only certify seeds of farmers who are registered in the Scheme, which has a cost. As mentioned in the introduction, small-scale farmers are in a disadvantageous position with respect to potato seed certification because of the size of their land and the location of their farms. Poverty-stricken small-scale farmers cannot afford giving up the big sampling volume required for tuber analysis. Transport costs would be higher for those who live in remote areas, as they should pay for the sample to be picked up and transported to the analysis lab. In addition, the seed grower pays for the certification of the seeds. The outcomes of the lecture and the following discussion seemed to provide feasible alternatives to the aforementioned limitations. Cutting stems of the young plants and replanting them is a cheap technique for the multiplication of *in vitro* plant material. This technique has been carried out in this study (see Materials and methods) when *in vitro* plant material ran out and dead plants needed to be replaced, thus proving that it is doable. This technique would allow for lesser plant material to be purchased and transported, thus reducing the costs. As far as the certification is concerned, the ARC is planning on starting its own certification system with the purpose of helping small-scale farmers become seed growers and achieve a higher level of independence. However, the development of a sound and economically reasonable certification system seems to be indispensable for smallholding farmers to be able to become seed growers.

Conclusions

This study provides evidence that variety plays an important role in plant performance and growth, making it imperative for farmers to choose wisely the cultivar they use on their systems. Results indicate that microorganisms also play a role, but further research is necessary for the understanding of the factors that affect plant-microbe interactions. As far as the social part is concerned, I put forward the claim that mini-tubers, together with

appropriate trainings, can hugely improve smallholding agriculture. Further research on IPM might still be necessary, as it does not seem to be a common field of study in South Africa. Extension officers need to provide adequate trainings that empower farmers and provide them with knowledge that they can use to find the most efficient and sustainable way to run their systems.

Critical reflections

Doing my Master's thesis in South Africa has been by far the most wonderful experience of everything I have experience during the 2-year programme. I was expecting to have a lot of work, yet I never imagined I could be engaged and dedicated to such a high degree. I acknowledge, nevertheless, a few things that I would have done differently, had I had the time. First, having several variables (CFA parameters, LAI) that give information about the same thing (plant performance and growth) can be very useful yet also bewildering if the researcher does not know how to handle them. I think that a principal component analysis (PCA) would have allowed me to compare different results with each other. Unfortunately, my basic knowledge of statistics and the time constraints made it impossible for me to carry it out. Similarly, the same reasons kept us (Flip and I) from calculating the slope of the LAI graphs, which would have given information about the effect of each treatment on plant growth. As I mentioned in the discussion, I found it imperative to carry out the microbial determination of inoculants. Regarding the social research, I would have liked to do a correlation analysis of some of the questions such as the incidence of pests and diseases in correlation with the use of pesticides or the potato yield in correlation with the amount of pesticides applied to the field.

Acknowledgements

I would like to express a deep gratitude to my supervisor in South Africa, Flip Steyn, for his dedication and support. I would also like to thank the whole ARC family, specifically Lesiba Klaas Ledwaba, for being my guide and translator in KZN; Versity Kekana, for assisting me during the preparation of the questionnaires and the processing of the data; and Elsie, Renné and Erika, for helping me in several occasions. I profoundly thank the farmers who participated in this study. I also want to express my gratitude to my supervisors in Sweden, Laura Grenville-Briggs Didymus, for rooting for me and making possible for me to do my thesis research in South Africa, and Kristina Blennow, for reminding me of my purpose when I felt confused and for putting me back on track. I am very grateful for the help I received from Jan-Eric Englund with statistics.

To my friend Isaac Nape, a scientist who showed me what being passionate about science means and educated me in many different ways, thank you for being there. And to those who were not physically there but nevertheless invested your time in giving me advice and guidance when I needed it, thank you Iris Pla and Albert de Bobes.

To Mohamed Magdi Hussein, whose unconditional support I had along the journey, and whose laptop I used when mine collapsed, a warm thank you.

To my mother, thank you for being always there.

References

Agricultural Research Council (ARC) (2014): *Plant breeding.* Available from: http://www.arc.agric.za/arc-vopi/Pages/Plant-Breeding.aspx [22-03-2018].

ARC (2014): Breeding and commercialization of potatoes. Available from: http://www.arc.agric.za/arc-vopi/Pages/Plant%20Breeding/Potatoes.aspx [21-06-2018]

ARC (n.d.): ARC Vision and Missions. Available from: http://www.arc.agric.za/Pages/About%20Us/ARC-Vision-Mission.aspx. [25-03-2018]

ARC, National Research Fundation (NRF), South African Agency for Science and Technology Advancement (SAAST) (n.d.): Careers in Agricultural Research. Available from: http://www.nrf.ac.za/sites/default/files/documents/Careers%20in%20Agriculture.pdf. [06-03-2018]

Bajwa, W. I. and Kogan, M. (2002): *Compendium of IPM definitions (CID)- What is IPM and how is it defined in the worldwide literature?* Oregon State University, Corvallis, OR 97331, USA, Integrated Plant Protection Center (IPPC).

Beneduzi, A., et al. (2012): Plant growth-promoting rhizobacteria (PGPR): Their potential as antagonists and biocontrol agents. Genetics and Molecular Biology, 35 (4): 1044-1051.

Bussotti, F. et al. (2010): The JIP test: a tool to screen the capacity of plant adaptation to climate change. Scandinavian Journal of Forest Research, 25 (8): 43-50

Coleman, A. (2015): Seed potato production – not for the faint-hearted. Farmer's weekly. Available from: https://www.farmersweekly.co.za/bottomline/seed-potato-production-not-for-the-faint-hearted/. [12-04-2018]

Directive 2009/128/EC of 21 October 2009 on establishing a framework for Community action to achieve the sustainable use of pesticides.

Easlon, H. M. and Bloom, A. J. (2014) Easy Leaf Area: automated digital image analysis for rapid and accurate measurement of leaf area. *Applications in Plant Science*, 2 (7): 1-4.

EE Publishers (2016) Agricultural pesticide-use maps for South Africa. Available from: http://www.ee.co.za/article/agricultural-pesticide-use-maps-south-africa.html. [30-03-2018]

Eilenberg, J. (2006) Concepts and visions of biological control. In: Eilenberg, J. and Hokkanen, H. M. T. (Eds.) *An ecological and societal approach to biological control*. Springer ed. The Netherlands.

FAO (1989): *The state of food and agriculture 1989*. FAO agriculture series, no. 22. Italy, Rome. ISSN 0081-4539

Ferreira, J. (2013): Mini-tubers for the seed potato market. Farmer's weekly, Available from https://www.farmersweekly.co.za/crops/field-crops/mini-tubers-for-the-seed-potato-market/.

Gliessman, S. R. (2015): The Agroecosystem concept. In: Engles, E. W. (Ed.) *Agroecology: the ecology of sustainable food systems*. 3rd ed. Bosa Roca Fla.: CRC Press. 405 pages.

Gray, M. E. et al. (2008): The IPM paradigm: concepts, strategies and tactics. In: Radcliffe, E. B. H., William D. and Cancelado, R. E. (Eds.) *Integrated Pest Management: concepts, tactics, strategies and case studies.* Cambridge University Press ed. p. 551.

Huang, X.-F., et al. (2014) Rhizosphere interactions: root exudates, microbes and microbial communities. *NRC Research Press*, 92: 267-275.

Handsatech Instruments (2018) Handy PEA: Advanced continuous excitation chlorophyll fluorimeter. Available from: http://www.hansatech-instruments.com/products/introduction-to-chlorophyll-fluorescence/continuous-excitation-chlorophyll-fluorescence/handy-pea/. [19-04-2018].

Kesaulya, H., et al. (2015): Isolation and physiological characterization of PGPR from potato plant rhizosphere in medium land of Buru island. *Procedia Food Science*, 3: 190-199.

Kotan, R., et al. (2009): Biological control of the potato dry rot caused by *Fusarium* species using PGPR strains. *Biological Control*, 50 (2): 194-198.

Larkin, R. P. (2016): Impacts of biocontrol products on Rhizoctonia disease of potato and soil microbial communities, and their persistence in soil. *Crop Protection*, 90: 96-105.

Li, L., Zhang, Q., Huang, D. A (2014): A review of imaging techniques for plant phenotyping. *Sensors*, 14: 20078-20111.

Handsatech Instruments LTD (2006): *Operations Manual, setup, installation & Maintenance: Handy PEA, Pocket PEA & PEA Plus Software*.[Pamphlet] Narborough Road, Pentney, King's Lynn, Norfolk PE32 1JL, England [15-04-2018]

Majozi, T. and Veldhuizen, P. (2015): The chemicals industry in South Africa. CEP: 46-50

Mehring, A. S., et al. (2016): Soil invertebrates in Australian rain gardens and their potential roles in storage and processing of nitrogen. *Ecological Engineering*, 97: 138-143.

Morse, J. (n.d) General Standards/Core- Exam review. Available from: https://conference.ifas.ufl.edu/aw11/presentations/Wednesday/Session%20A/am/0800%20Morse.pdf. [01-04-2018]

Murchie, E. H. and Lawson, T. (2013): Chlorophyll fluorescence analysis: a guide to good practice and understanding some new applications. *Journal of Experimental Botany*, 64 (13): 3983-3998.

Nortjé, P. F. (2003): The South African seed potato industry. In: Niederwieser, J. G. (Ed.) *Guide to Potato Production in South Africa*. Pretoria: ARC-Roodeplaat Vegetable and Ornamental Plants Institute, pp. 103-108.

O'neil, R. J. and Obrycki, J. J. (2008): Introduction and augmentation of biological control agents. In: Radcliffe, E. B., et al. (Eds.) *Integrated Pest Management: concepts, tactics, strategies and case studies*. Cambridge University Press ed. p. 551.

Ordentlich, A., et al. (1990): Integrated control of Verticillium dahliae in potato by Trichoderma harzianum and captan. *Crop Protection*, 9 (5): 363-366.

Osibanjo, O., Bouwman, H., Bashir, N. H. H., Okond'Ahoka, J., Choong Kwet Yive, R., Onyoyo, H. A. (2002): *Regionally based assessment of persistent toxic substances*. (UNEP Chemicals). Switzerland. Sub-Saharan Africa regional report.

Paul, K. (2016): *Screening and characterisation of plant physiological traits using photosynthetic and phenotyping tools*. Diss. Szeged, Hungary: Faculty of Science and Informatics, University of Szeged.

Potato Certification Services (PCS) (n.d): *Certification Scheme Protocol*. Available from: http://potatocertification.co.za/certification/ [16-03-2018].

Potato South Africa (PSA) (2017). *Potato Industry Research Strategy: 2014-17*. Available from: http://www.potatoes.co.za/SiteResources/documents/Potato%20Indutry%20Research%20Strategy-2014.pdf [06-03-2018].

PSA (2016): Potato Industry Report 2015-2016. Potatoes South Africa, Potato Certification Scheme, Potato Laboratory Services, Prokon. 1-98

Quinn, L. P., de Vos, B. J., Fernandes-Whaley, M., Roos, C., Bouwman, H., Kylin, H., Pieters, R., Van den Berg, J. (2011) Pesticide use in South Africa: one of the largest importers of pesticides in Africa. In: *Pesticides in the modern world- Pesticides Use and Management*. Margarita Stoytcheva ed. p. 48.

Senés-guerrero, C., Torres-Cortés, G., Pfeiffer, S., Rojas, M., Schüßler, A. (2014): Potato-associated arbuscular mycorrhizal fungal communities in the Peruvian Andes. *Mycorrhiza*, 24: 405-417.

SFS 1998:204. *Personal Data Act*. *In:* Deparment of Justice

Steyn, P. J. (2003): The origin and growth stages of the potato plant. In: Niederwieser, J. G. (Ed.) *Guide to Potato Production in South Africa*. Pretoria: ARC-Roodeplaat Vegetable and Ornamental Plant Institute, pp. 7-12.

Theron, D. J. (2003): The South African potato industry in perspective. In: Niederwieser, J. G. (Ed.) *Guide to Potato Production in South Africa*. Pretoria: ARC-Roodeplaat Vegetable and Ornamental Plant Institute, pp. 1-6.

Theron, D. J. and Mienie, N. J. J. (2003): An integrated approach to pest and disease management. In: Niederwieser, J. G. (Ed.) *Guide to potato production in South Africa.* Pretoria: ARC-Roodeplaat Vegetable and Ornamental Plant Institute, pp. 109-120.

Tiemens-hulscher, M., et al. (2013): Potato Breeding. A practical manual for the potato chain. (Aardappelwereld Magazine) The Hague.

University of Nevada Cooperative Extension (UNCE) (n.d): Integrated Pest Management. Available from: http://www.unce.unr.edu/programs/sites/ipm/. [18-03-2018]

Walter, A., et al. (2015): Plant phenotyping: from beam weighing to image analysis. Plant Methods, 11 (14): 2-11.

Wezel, A., Bellon, S., Doré, T., Francis, C., Vallod, D., David, C. (2009): Agroecology as a science, a movement and a practice. A review. *Agronomy for Sustainable Development*, 29 (4): 503-515.

Wharton, P. S. and Kirk, W. W. (2014): Evaluation of biological seed treatments in combination with management practices for the control of Fusarium dry rot of potato. *Biological Control*, 73: 23-30.

World bank and Trading economics (2014): South Africa- Agricultural Land (% of land area). Available from: https://tradingeconomics.com/south-africa/agricultural-land-percent-of-land-area-wb-data.html. [25-03-2018]

Appendices

Appendix 1: List of pesticides applied on the plants at Moedersbond

Name	Dosage to 40 litres of water	Date of application
Selecron	80 ml	11/01/2018
Odeon	120 ml	11/01/2018
Hunter	20 ml	11/01/2018
Aquarite 5	30 ml	11/01/2018
Aphox	40 g	19/01/2018
Bellies	24 g	19/01/2018
Biomectin	30 ml	19/01/2018
Citrex-oil	80 ml	19/01/2018
Aquarite 5	30 ml	19/01/2018
Movento	12 ml	24/01/2018
Antracol	80 g	24/01/2018
Aquarite 7	30 ml	24/01/2018
Biscaya	32 Ml	06/02/2018
No Blight	80 g	06/02/2018
Aquarite 7	30 ml	06/02/2018
Steward	36 ml	13/02/2018
Deltathrin	40 ml	13/02/2018
Hydrobuff	160 ml	13/02/2018
Selecron	80 ml	02/03/2018
Odeon	120 ml	02/03/2018
Aquarite 5	30 ml	02/03/2018
Aphox	40 g	13/03/2018
Bellies	24 g	13/03/2018
Aquarite 5	30 ml	13/03/2018
Movento	12 ml	26/03/2018
Antracol	80 g	26/03/2018
Aquarite 5	30 ml	26/03/2018
Hunter	20 ml	05/04/2018
No Blight	80 g	05/04/2018
Biscaya	32 ml	05/04/2018
Aquarite 7	30 ml	05/04/2018
Profenafos	80 ml	11/04/2018
Mission	120 ml	11/04/2018
Aquarite 5	30 ml	11/04/2018
Aphox	40 g	18/04/2018
Bellies	24 g	18/04/2018
Aquarite 5	30 ml	18/04/2018
Movento	12 ml	25/04/2018

Antracol	80 g	25/04/2018
Aquarite	30 ml	25/04/2018
Biscaya	32 ml	03/05/2018
No Blight	80 g	03/05/2018
Aquarite 7	30 ml	03/05/2018

Appendix 2: questionnaire template

ARC QUESTIONNAIRE

Enumerator

Tel (work):

Mobile:

E-mail:

Questionnaire:

INFORMATION LEAFLET:

The main aim for this questionnaire is to get some insights in terms of farmer's perspectives and interest in mini tuber production.

The objectives of the research project:

The project aims to enhance and improve the conditions of production and value addition of mini tuber production and thus, contribute to the food and nutritional security of the farmers.

Please note, information provided will be treated with the highest degree of confidentiality.

INTERVIEWER DECLARATION:

I, …Alba Saez……declare that I have asked this questionnaire as it has been laid out. I declare that all responses which have been recorded are the true responses of the respondent and that I have fully checked the questionnaire.

Signature: ……

Date: ……

PARTICIPANT CONSENT:

I, Name ……Surname:……., agree to take part in the aforementioned survey. I understand that my responses will be treated with confidentiality and that the outcomes of this survey will be presented to relevant people within the agricultural sector. I further understand that I will not receive any compensation for taking part in this study. I agree that you may take a photo of myself in front of my house.

ID No……

Signature: ……..

Date: ……

Contact Details: ……

Address: ……

Village: ……..

District: ……..

Local Municipality: ……

Ward……

Province: ……...

SECTION A: SOCIO ECONOMIC CHARACTERISTICS

No.	Variables	Code	Selected Code
A1	Gender	0= Female 1= Male	
A4	Level of education	0=no formal education 1-12=Grade 1-12 13=Technician diploma/degree 14=University degree 15=Other post-matric qualifications	
A5	Number of Household members	Specify number ……	
A6	Marital status	1 = Single 2 = Married 3 = widowed 4 = cohabitation (staying with partner)	

		5 = Other	
A7	Land acquisition	1 = family land 2 = communal land 3 = Government lease	
A8	Household language	(Specify)…… …….	
A9	Sources of income (select more than 1)	Social grants, family support, temporary jobs, informal trade, formal jobs, no income	
A9.1	Household income per month	Specify……	

SECTION E: FARMING STATUS

E1 Is there active production on farm	1 = Yes 2 = No	
E2 Total Land size***(ha)***	Specify	
E3 Land size under production (***list commodities and land size***)	***commodities***	***land size (ha)***
E6 Farming experience (specify in years)	Farm labour	
	Farm manager	
	Farm residence	
	Other specify	
E7 Available functional farm infrastructure (tick): 1)Electricity, 2)water pump, 3)centre pivot, 4)sprinkler irrigation equipment, 5)reservoir, 6)tractor, 7)rotavator/bed maker, mouldboard plough, cultivator, disk plough, 8)storage cold room, computer, printer, 9)tunnels, boom sprayer,10) knapsack sprayer, 11)bakkie, truck, trailer, 12)fence, 13)water tank		
E8 Do you irrigate?		
E8.1 Main water sources for irrigation: Municipal water, river/stream, well, dam, rain, borehole, lake/pond.		
E9 Did you receive potato production training?	If yes, specify year……..	
E10 Major production challenges: Lack of infrastructure, lack of water, pest and diseases damage the crops, lack of customers/market, bad		

weather conditions, costs of inputs, lack of knowledge/proper farming skills, lack of labour												
E13 Which month do you get more harvest (tick more than one month)	Jan	Feb	Mar	April	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
E14 Which potato production enterprise interest you	1=Seed production 2=Conventional potato production											
E15 Where do you get your potato seeds (tick)	Buy from seed companies, specify: ……..											
	Government subsidy											
	NGO's											
	Produce own seeds											
E16.1 How often do you get them?												
E17 which cultivars do you plant?	Specify ……											
E18 Do you know what mini tubers are?	1=yes 2=no											
E19 If so, have you considered using them?	Why/Why not?											
E20 What are the benefits of using mini-tubers over potato seeds or true potato seeds (TPS)?												

SECTION F: FINANCIAL INFORMATION

F1 What do you do with excess harvest:	Sell (go to F1), give away for free, plant next year…
F1.1 Where do you sell your produce (tick) Farm gate, road side, local shops, FPMs…	
F1.3 How do you transport produce to market? Buyers, hired, own…	
F1.6 What are your transport challenges? Small size of transport, high costs, lack of transport…	

F1.7 How is the price for your produce set	1= I set the price 2=negotiable 3=market driven 4=dictated by buyers 5=other specify	
F2 Type of support the farm receives (tick)	Monetary funds	
	Machinery (tractors, irrigation equipment, implements)	
	Training	
	Tools	
	Other specify	
	None	
F3 farm expenses	Water	1=<R2000

		2=R2001-R4000 3=R4001-R6000 4=>R6001 5=not applicable
	Electricity	
	Seeds	
	Fertilizers	
	Pesticides	
	Labour wages	
	Transport	
	Packaging materials	
	Machinery hire	
F4 What are your market challenges?		
F5 Sources of farming information Radio, local agricultural officers, extension services, market agents, information days		
F6 Frequency of information access (tick)		

SECTION P: PERSONAL INTERESTS

P1 How do you rate condition of the farm	1=very poor 2=poor 3=fair 4=good 5=very good	
P2 What is your purpose in doing agriculture? Feeding the family, feeding the family and selling the surplus, selling the whole harvest…		
P3 What is your vision in agriculture? To produce enough food to feed the family, to become a commercial farmer…		
P4 What are you currently doing to achieve your goal?		
P5 Are you satisfied with the way you do agriculture?		