The effect of grass mulch and strip cropping on *Phytophthora* infections and Colorado Potato Beetle infestation in potato



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## Abstract

The increasing number of challenges in organic potato cultivation call for innovations. Two of the most challenging problems in the Netherlands are *Phytophthora infestans* and Colorado Potato Beetles (CPB). Phytophthora infestans is the causal agent of late blight which can destroy large fields of potato within a short period, decreasing the yield tremendously. CPB larvae can defoliate whole potato plants, affecting the yield. Conventional farmers counteract *Phytophthora* and CPB with agrochemicals. However, sustainable agriculture is becoming more popular among consumers, and stricter governmental laws pressure the farmers to reduce the use of pesticides. In this research, the goal was to investigate the effect of strip cropping and mulching on CPB occurrence and Phytophthora infections and to see whether these treatments can reduce the damage caused by the CPB and Phytophthora. Strip cropping increases spatial diversity and this reduces the dispersal of pests and diseases, as non-host plants form an obstacle. The experiment included 5 main treatments 4 different strips and one monoculture reference field. The first strip is a reference strip. The second strip had increased genetic diversity by the use of multiple cultivars. In the third strip, mulch was added, mulching has the characteristic to attract predators of the CPB such as ground beetles that feed on the larvae. And in the last strip both mulching and increased genetic diversity was used. This research proved that increasing spatial diversity and increasing genetic diversity both have a reducing effect on both the Phytophthora and the CPB occurrence. Mulching also decreased the CPB occurrence and *Phytophthora* infections, however, the yield was also reduced. Research into the causes of this negative side effect is recommended.

**Keywords:** Strip cropping, mulching, Organic potato, *Phytophthora infestans*, Colorado Potato Beetles

**Abbreviations:** CPB: Colorado Potato Beetle, PI: *Phytophthora infestans,* DEF: Droevendaal Experimental Farm, BH: Broekemahoeve, SOM: Soil Organic Matter, WUR: Wageningen University and Research.

### Introduction

Potatoes are the third most consumed crop in the world (CIP 2021). More than a billion people rely on the potato as their food source. Therefore it is essential to secure potato production to provide enough food for the increasing population (Bond, 2014). Besides, increasing popularity for sustainable food production of consumers and stricter laws on the use of pesticides pressure the farmers to grow more sustainable food (Vermeir et al., 2006; Jess et al., 2014). The crop is suffering from several pests and diseases. The two most dangerous threats to potatoes in the Netherlands are Phytophthora infestans and the Colorado Potato Beetle (Figure 1) (Kromann et al., 2014). Phytophthora, the causal agent of late blight, is an oomycete,

which disperses its zoospores with wind and water (Hirst et al., 1960). It can destroy potato fields very rapidly due to its asexual reproduction (P. Skelsey et al., 2009). Next to that, Phytophthora also produces sexual oospores, which can survive several winters in the soil. This causes much genetic variation within *Phytophthora* making it hard to control the dispersal (Turkensteen et al., 2000). The Potato Beetle, Colorado Leptinotarsa decemlineata (CPB) is a beetle that feeds on potato leaves. The larval stages of the CPB can defoliate the field considerably. The 4<sup>th</sup> instar stage of the CPB is the most devastating as it can eat up to 77% of the leaf area of a plant (Khelifi et al., 2007). Climate change ensures that CPB is getting an increasingly larger habitat as CPB are ectothermic and need warmth to survive. Warmer winters in the Netherlands make it possible for the pupae to survive more often (Popova et al., 2013). To secure a sufficient yield, conventional farms Phytophthora and CPB control bv agrochemicals, which makes the farm highly dependent on inputs. The reliance on pesticides thereby also has the risks of the development of resistant pests and the possible side effects on nature. Besides that, organic farming is an upcoming trend in Europe, and organic farms are not allowed to pesticides, these therefore the use development of alternative pest control methods is necessary (Göldel et al., 2020).

The challenge is to invent new techniques or reinvent old ones to counteract Phytophthora and CPB in an environmentally friendly and sustainable way, to reduce pesticide use in potato farming. Using a decision support system can already help to reduce the agrochemical use during the transition phase to organic (Skelsey et al., 2009). Additionally, breeding for resistant crops is a strategy, however, it takes much time to develop a new potato cultivar that is resistant to multiple pests or pathogens (Fry, 2008). At this moment there is no resistant cultivar against CPB and in the Netherlands, there are 17 marketable partly resistant cultivars against Phytophthora such as Carolus and Alouette, which were used in this research (Alyokhin, 2009; Lammerts van Bueren, 2019). The durability of resistance is uncertain as pests and diseases evolve all the time, but using different varieties and management strategies can increase the durability of a resistant cultivar (Khelifi et al., 2007).

A harder to implement but not less effective technique is to increase spatial and/or genetic biodiversity in the field. In this case, we use strip cropping. Strip cropping is an upcoming agroecological practice, whereby crops are intercropped which implies that multiple crops are grown in strips next to each other, to increase biodiversity and ecosystem services (Gliessman, 2014). In this research, the effect of strip cropping is investigated in the multiyear and multi-site experiment of Wageningen University and Research (WUR) on 3 different locations in the Netherlands. The main findings in the previous years were that strip cropping decreases the infection rate of *Phytophthora* as zoospores are more likely to end up on nonhost or resistant plants, thereby being unable to reproduce (Bouws et al., 2008; Ditzler et al., 2021; Horst ter, 2020). The effect of a strip cropping system with grass-clover as a neighbouring strip on the infestation rate of CPB is less investigated. The research of Patt et al. (1997) found a reduced amount of CPBs after the introduction of strip cropping. However, they intercropped the potatoes with flowers, which attract predators, they did not investigate the effect of grass (Patt et al., 1997). Therefore, in this research strips of potato are alternated with strips of grassclover. The grass-clover acts as the non-host barrier which reduces the Phytophthora infection and the grass-clover possibly attracts natural enemies of CPB (Bouws et al., 2008; Middleton et al., 2021).



Figure 1: The two most important threats to potato cultivation; the Colorado Potato Beetle and Phytophthora infestans (Source: Own picture)

Next to strip cropping, mulching is another technique that reduces CPB. Mulching is the addition of a ground cover to the crop, in this case, mowed grass-clover was added to some strips. It is already widely known that mulching has many benefits for crops, it generates a better water holding capacity, reduces soil temperature within the crop, and increases weed suppression (Dvořák et al., 2015; Dvořák et al., 2012; Stephan Junge et al., 2017). Besides those benefits, it was also discovered that mulch can help to increase the natural enemy prevalence in the crop, which predate on the CPB (Göldel et al., 2020). Multiple studies found that in particular ground beetles and centipedes, which are predators of CPB, were more abundant in mulched plots (Brust,

1994; Szendrei et al., 2009). Several experiments found a reduction in the presence of eggs and larval stages of CPB (Brust, 1994; S Junge et al., 2017; Stephan Junge et al., 2017). Furthermore, Thomas Andersen (2021) found that growing different cultivars in a strip and mulching had a reducing effect on the CPB occurrence, however, they only compared the fields to reference strips and not to a reference field. Therefore, they could not conclude if there was a beneficial effect of strip cropping on the CPB population compared to a monocrop. The effect of mulch on the infection of Phytophthora is less investigated. Nyankanga et al. (2008) did investigate the effect of mulch on Phytophthora infection rates and found no significant effect of mulching, only a slight non-significant increase in the Phytophthora infection rate in some fields (2008). This increased infection rate can be due to higher humidity in the crop, which favours the dispersal of zoospores. This may imply that mulching can have a negative effect on the Phytophthora infection. Therefore, it is possible that there can be a trade-off between the effect of mulch on Phytophthora which should be increased and on the CPB infestation which should be lower.

The implementation of both strip cropping and mulching is less investigated. Therefore, this research focused on the effects of mulching and strip cropping on the *Phytophthora infestans* infections and the infestation of CPB. As it is important to first identify the possible negative side-effects of mulching on *Phytophthora* infections before promoting mulching as a means to manage CPB.

### **Research questions**

The knowledge gaps presented above lead to the following main research question: What are the effects of mulching and strip cropping on the *Phytophthora infestans* infections and the CPB infestation? This question is divided into the following sub-questions:

 What is the effect of applying both mulching and strip cropping on the *Phytophthora infestans* infections and Colorado Potato Beetle infestation in potatoes?

- 2) What is the effect of applying both strip cropping and mulching on potato fresh yield (kg m<sup>-2</sup>) and yield quality, in terms of marketable yield, dry matter, and NPK content?
- 3) Is there an interaction between Colorado Potato Beetle occurrence and *Phytophthora infestans* infection?

### Hypotheses

The following hypotheses are connected to the previously mentioned research questions:

- 1) It is expected that strip cropping has a reducing effect on both Phytophthora infections and CPB abundance because the grass is a non-host which forms a barrier for both the CPB and the zoospores (Nyankanga et al., 2008). Mulching reduces the CPB abundance because it attracts predators of the CPB larvae (Göldel et al., 2020; Khelifi et al., 2007). On the other hand, will increase mulching Phytophthora due dispersal to the increased humidity benefiting the dispersal of zoospores (Nyankanga et al., 2008).
- 2) Applying both strip cropping and mulching will have a positive effect on the yield, as strip cropping decreases the CPB infestation and Phytophthora infections, thereby increasing the yield. The mulching will cause a tradeoff on the yield, as mulching decreases the abundance of CPB on the yield, but enhances Phytophthora infestans infections. Additionally, the mulch enhances soil humidity, counteracting dry conditions, and increasing the yield and yield quality. In total there will be a positive effect on yield.
- Colorado Potato Beetle probably prefers healthy plants, not affected by *Phytophthora*, so more CPB are expected on healthy plants compared to infected plants.

### Materials and Methods

### **Experimental Design**

#### Location

The experimental fields investigated in this study are located on the Droevendaal Experimental Farm (DEF) in Wageningen (51°59'31.9"N 5°39'47.3"E). DEF is located on sandy soil, with a minimal slope of only a few centimetres over about 1 km (PDOK, 2021; AHN, 2021). The experimental fields are part of a long-term strip cropping experiment with a randomized block design in which crops rotate each year. In total, 6 different crop species were grown in the experiment (cabbage, potato, pumpkin, oat/barley, and 2 years of grass-clover). This research focused on potatoes grown in strips with grass-clover. All crops had a monoculture reference plot, three treatments of strip cropping in crop pairs, a rotation strip, and a pixel plot (Appendix A). Also, data from the Broekemahoeve (BH) in Lelystad was used for the Phytophthora and yield data and to create a robust data set the data on CPB from the last two years collected on DEF and the yield data from both DEF and BH of the past four years were also taken into account for the fresh yield.

#### Treatments

All treatments with their characteristics are shown in Table 1 below.

Carolus en Alouette to

calculate the Land Equivalent Ratio Carolus (S)

### Potato varieties

The three different potato varieties that were used at DEF are Agria, Alouette, and Carolus. Agria was used as the main potato variety and was grown in the Strip, Strip-add, Strip\_3, Strip\_6, and the reference fields (Ref\_space & Ref\_time). Alouette and Carolus were grown together with Agria in the Strip\_var and Rotation. Agria was grown on the outer rows and Carolus and Alouette on the inner rows. Carolus and Alouette are partly *Phytophthora* resistant, Agria on the other hand is susceptible to *Phytophthora* (Agrico, 2021). At the BH Agria was replaced with Ditta and only Alouette was used as a resistant variety.

### Data collection

#### Phytophthora incidence

The data collection of *Phytophthora* was based on the protocol of Anke ter Horst (ter Horst, 2020). The *Phytophthora* infection severity was estimated per 9 m<sup>2</sup> rather than counting the lesions per plant. Per strip 5 sampling areas of 3mx3m were randomly selected with the use of R studio (Figure 2). Per sampling area of 9m<sup>2</sup>, the percentage of plant surface that suffered from necrosis due to a *Phytophthora* infection was estimated on a scale from 0% to 100% infection. 0% indicates no *Phytophthora* present and 100% indicates completely infested plants. However, this point was not reached as the crop was burned when it

susceptible (S) or partially resistant (R) to Phytophthora Name Treatment Width (m) Cultivars Field Length (m) Fertilization Reference field 63 Ref\_space Agria (S) 6 72 Animal manure Reference strip 1,2,3 Strip Agria (S) 54 3 Animal manure Strip\_add Strip with grass mulch Agria (S) 1,2,3 54 3 Grass mulch at the beginning of the season (incorporated) and at the end of the season on top of the plants Strip var Strip with 3 cultivars Agria (S) 1.2.3 54 3 Animal manure Carolus (R) Alouette (R) Rotation 54 Grass mulch at the beginning of the season A potato strip part of a 6 Agria(S) 1,2,3 3 year rotation, the strip Carolus (R) ncorporated) and at the end of the season on top of the plants received both mulching Alouette (R) and multiple cultivars were used Ref\_time Small reference field of 1,2,3 12 9 Animal manure Agria (S) 108m<sup>2</sup> next to the strips fo account for field effects Strip\_3 72 Same as Strip but located Agria (S) 6 3 Animal manure next to ref space to make it possible to compared different fields (connected design) Strip 6 6-meter strip to get a 6 72 3 Animal manure Agria (S) better view of the spatial diversification Ref strip Reference strips for Alouette (R) 6 72 3 Animal manure

Table 1: Treatments that were sampled in this research. The S and R behind the cultivars indicate whether the cultivar is suscentible (S) or partially resistant (R) to Phytophthora

exceeded the Dutch legislation (wetten.overheid.nl, 2021). The buffer strips and the first and last 10 meters of a strip were excluded from sampling to prevent external influences from affecting the data. (Appendix B). The field was sampled 2 times, once a week from the first lesions that were spotted till the burning of the field in the second and third week of July.



Sampling direction

Figure 2: Overview sampling points of CPB and Phytophthora, in grey the transects. Note the turned compass rose

#### Colorado Potato Beetle occurrence

The data collection on the Colorado Potato Beetle (CPB) is based on the protocol of Thomas Andersen but slightly adapted as fewer blocks were sampled in this study and the data collection per transect was intensified.

The potatoes were sown from West to East. On the Southern edge, the transects were determined perpendicular to the strip direction the distance between transects was 4 or 5 meters depending on the sampling round (Figure 2). In total 8 transects per strip were sampled. Different plants were selected per sampling round by changing the distance between the transect from 5 to 4 meters. The 10 meters on the sides were excluded to prevent any border effects. The transects were sampled twice. The first sampling round was in the second week of July and the second sampling round was in the third week of July just before burning the crop. Every 4- or 5meter interval, 8 plants were sampled, 2 plants per row. Quantification of the CPB infestation was done by visual inspection. To limit the influence of time of the day for any given date of sampling, the blocks were sampled in random order. The CPB were grouped per life stage in 3 categories larval stage 1 and 2 (L1L2) combined, larval stage 3 and 4 (L3L4) combined, and the adult beetles. Total adult abundance was recorded on a field sheet, L1L2 and L3L4 instar abundance were quantified in units of 5 (in the format: 0, 5, 10, etc.) to make the counting less time-consuming. For example, if 34 larvae were present exactly the abundance was estimated at 35. A record of the abundance for each category per plant was noted also the total infestation was calculated by adding up all adults and larvae. The percentage of infested plants was derived by scoring whether there was at least 1 adult or larvae present (1) or none at all (0). The scores were added and divided by the number of plants scored resulting in an infestation percentage. (Appendix C1 and 2).

#### Potato yield

The potato yield was determined based on fresh yield (kg m<sup>-2</sup>), and quality (% marketable yield 35-65mm).

The fresh yield data was collected by harvesting all strips mechanically by using a 1-row potato harvester with a crate on a scale at the end of the assembly line which determined the weight (Appendix D).

To check whether there was a border affecting the yield of the outer two rows were compared to the inner 2 rows of a strip.

To determine the quality of the potatoes, one sample per row was taken during the harvesting by holding the same bag under the potato harvester every 30 seconds during the harvesting. The sample had to be at least 5 kg to ensure the representativeness of the data. These samples were sorted into different size classes: <35mm, 35-50mm, 50-65mm, and >65mm and were counted and weighed. The size classes 35-50mm and 50-65mm were considered to be the marketable yield (kg m<sup>-2</sup>). The <35 and >65 were excluded from the marketable yield as they are too small or too big. The underwater weight was determined with the use of the HP-Ultra weighing system at Unifarm. The weighing machine had a bucket that was filled with ~5 kg of potatoes. First, the weight above water was determined, after that the bucket was put underwater and the weight was measured again. After determination of the underwater weight, the dry matter weight was calculated by filling a small tray with ~300gr of potato fries. The tray was weighted to get the exact weight and after that, the tray was put in the oven for 48 hours at 70 degrees Celsius. After 48 hours the tray was weighed again and the dry matter percentage was calculated by the formula to dry weight/fresh weight \* 100%. Additionally, the NPK content was determined by grinding the dried potato fries and bringing samples of ~25 grams to the lab where the NPK analysis was executed according to the methods of Houba et al. (1992) (Appendix D2, F).

#### Soil humidity

Soil humidity was measured 3 times in July, once a week to determine a reliable average. To determine the soil humidity, the ML3 ThetaProbe soil moisture sensor was used. By putting the sensor in the middle of each strip into the soil and noting down the moisture percentage that was indicated by the ThetaProbe.

### Nitrogen availability

Nitrogen availability was measured after the growing season. The nitrogen availability was measured by taking multiple soil samples, drying the soil in the oven for 2 days at 70 degrees Celsius, and bringing it to the lab for analysis where the available NO<sub>3</sub> and NH<sub>4</sub> content was determined according to the methods of Houba et al. (1998) (Appendix E)

### Data analysis

All statistical analysis was performed by using R software (version 1.4.1717) for Windows (R Development Core Team, 2021). Multiple datasets were created. For *Phytophthora infestans* infections, the data of 2021 was used from both DEF and BH. At DEF all treatments were sampled twice at BH only the Strip, Strip\_add, Strip\_var, and the Reference field were sampled once. The data of 2019 was excluded as the dry summer caused low infections. For CPB infestation all the data of 2019-2021 was used of the Strip, Strip add, and Strip var. Besides, for 2021 all treatments were sampled, but only the 3 aforementioned strips and the 2 reference fields were taken into account for the main report. All CPB data was collected at DEF. All the available yield, marketable yield, dry matter content, and NPK content data of 2018-2021 collected on both DEF and BH were taken into account. The yield data was averaged over four years. Nitrogen availability in the soil and soil humidity (%) was only collected at DEF in 2021. All datasets were first analysed for normality using the Shapiro-Wilk Normality test and Q-Q plots. The homogeneity and equality of variance were checked with a Bartlett test and residual plot, respectively. Based on the tests the data was used or transformed for further analysis. Most data turned out to be not normally distributed and the variances were also not equally distributed nor homogenous. Therefore, some transformations were tried, however, the logarithmic and square-root transformation did not change the outcomes for the assumptions. This led to the use of Generalized Models. Multiple Linear Mixed error distributions were fit to the data. For the Phytophthora infection, the Gaussian distribution was found to be most appropriate. The function glmmTMB was used to fit a generalized linear mixed model. The General linear hypothesis function (glht) from the Multcomp package was used in combination with the Least-square means (lsm) function of the emmeans package for pairwise comparisons between the treatments. The same approach was used for the CPB data. The negative binomial distribution was found to best fit the errors. The yield data was analysed with a Linear Mixed-Effect Model with field and year taken as random effects. The same glht with the lsm function was used to obtain the differences between the treatments. A correlation plot was constructed to get an insight into the interactions between all the variables of interest in this research. The packages corrplot, Hmisc, and PerformanceAnalytics were combined to make the correlation plot. In all the analyses a significance level of 0.05 was used.

# Results and Discussion

### Mulching and Phytophthora

As a result of the wet summer in 2021, the *Phytophthora* quickly infected almost all Agria plants within a week from 16 July on when the first infections were found. At the end of the week, infections were also found in the partially resistant cultivars Carolus and



Figure 3: Phytophthora infection percentages (% 9m-2) at Droevendaal (DEF) and the Broekemahoeve (BH) in 2021 per treatment. Different letter above the treatment results indicate significant differences (p<0.05)

Alouette. At the BH there was only one sampling point just before burning on 22 July, a couple of days later than et DEF, the infection in the resistant cultivars was as high as the non-resistant cultivars, so all resistances were broken in the end (Figure 3c).

Strip cropping had a significant decreasing effect on the *Phytophthora* infections at all sites and sampling points. Additionally, increasing the number of cultivars within a strip decreased the *Phytophthora* infections even more compared to the reference field and monoculture strip at DEF (Figure 3a, 3b). The addition of mulch in the Strip\_add and Rotation treatment caused significantly lower infection percentages compared to the reference field and the Strip. For DEF there is an additive effect as strip cropping (Strip) already lowers the infection significantly by ~70% compared to the reference field (Ref\_space) and additional genetic diversity is significantly lowering the infections even more with ~8% (Figure 3ab). The effect of mulching was not significantly different from the effect of increasing genetic diversity in the Strip\_var and the rotation treatments with both mulch and multiple cultivars.

It was expected that the *Phytophthora* would have higher infections in the Strip\_add treatment as mulch increases the humidity however no significant differences in soil humidity were found between treatments. Also, no increase in *Phytophthora* infections were observed. At the BH the mulching increased the *Phytophthora* severity slightly but not significantly in both 2019 and 2021 (App. G Table 1).

At DEF the low infections were partly caused by the mulching management as the second mulching was added on top of the potato plant, which caused some difficulty scoring the plants for *Phytophthora* as the plants were barely visible anymore.

Earlier research by Nyankanga et al. (2008) showed little effect of mulching on foliar *Phytophthora* infections. Döring et al. (2006) found a slight decreasing effect in *Phytophthora* infections. Both studies did not show any significant reduction or increase in *Phytophthora* infections. Taking these studies together, it can be concluded that mulching has no to a slightly decreasing effect on *Phytophthora* infections.

The absent effect of increased humidity in the soil by mulching is probably caused by the already wet summer which nullified the effect of the mulch. To be sure about this further research should measure the soil and/or air humidity daily. The absence of the mulching effect on humidity was also found by Döring et al. (2006). The difference between the effect of the treatments on the BH and DEF on the *Phytophthora* infections is probably caused by the different sampling dates, as Phytophthora can disperse quite fast causing way more infections in just a couple of days. Besides, the field at the BH is located next to another trial field where they inoculate the Phytophthora, which also could have caused higher infections at the BH.

#### Mulching and Colorado Potato Beetles

In 2021 the Colorado Potato Beetles were the most abundant in the reference fields (Ref time) located next to the strips, even more than in the big reference field. As all treatments located on field 6 including the big reference field (Ref\_space), the Strip\_6, Strip 3, and the Ref strip treatments showed a significantly lower abundance of CPB hinting at a field effect (App. H Figure 1&2). The statistical analysis did indeed indicate a significant field effect for field 6 (p<0.05). A field effect is a term for a field with a strong location effect caused by the characteristics of the field such as proceeding crop, slope, or available nitrogen. To account for the fieldeffect the Ref time is taken as a reference for the CPB as the CPB are location specific and highly dependent on the start population of CPB that survived the winter in the field.

If the Ref\_time is compared to the strip treatments, increasing the spatial diversification had the most effect on the CPB as all the strip treatments lowered the CPB abundance. Among the strip treatments (Strip, Strip\_add & Strip\_var), there were no significant differences (Figure 4). For 2021 there were not enough sampling points to



Figure 4:CPB infestation in the fields on Droevendaal a-c indicate significant differences (p<0.05)

prove differences for the 3 strip treatments, however, the combined data is promising a reducing effect of mulching on CPB occurrences.

Taking into account the results of the past three years, the strip treatment had the most CPB. The addition of mulch decreased the number of CPB significantly by 7% compared to the strip. Growing multiple varieties in the same strip lowered the number of larvae even more, by 17% (Figure 5). For the adults, there were no significant differences.

Besides the different treatments, an interesting side effect was found as there was a significant difference in CPB presence on different cultivars. Alouette had significantly



*Figure 5: CPB per treatment per year over 3 years, letters indicate significant differences (p<0.05). Error bars deviation within a year* 

lower CPB abundance than the Agria. Carolus also showed a lower abundance, however, this was not significant.

Reverting to the research questions stated in the introduction mulching had indeed a reducing effect on the CPB abundance across the three years (Figure 5D), however, looking at the separate years (Figure 5A-C) did not always result in significant reductions. 2020 contained the most data as 8 sampling rounds were executed in that year. The not-so-mobile larvae show different abundances for different treatments. This can be due to the order in which the potato plants came up as the early potatoes would be infested earlier or by the preference of the adults that position their eggs on specific treatments. The latter explanation could not be proven as there were no significant differences in abundance for the adults. For the mobile adults, it was expected that they would avoid the mulch strips as the appearance of the strip would be divergent compared to the strips without mulch however, this was not proven, possibly due to the fact that the mulch was incorporated at the start of the season and the late addition of the second mulch, reducing the mulching effect. The other research question was whether applying mulch would have an additive effect increasing spatial diversification in on preventing CPB infestation. This was proven by the combined data as the mulching decreased total CPB abundance significantly the compared to the strip without mulching (Figure 4d).

As stated before, the CPB abundance on field 6 was quite low, this was caused by a field effect. Field 6 has not been used to grow potatoes in the last couple of years. Fields 1, 2, and 3 have a 6-year rotation, however, there were potatoes grown on those fields but in other blocks, these blocks are close to each other making it possible for the CPB to move towards the block with potatoes. The CPB hibernates within the soil as pupae, during spring these CPB will search for potato plants near the spot where they woke up. A crop rotation reduces the number of CPB as the absence of a host plant reduces the number of surviving CPB. It is important to take this into account when considering strip cropping as rotating different crops within strips over the same field. As CPB can move some meters within a field, therefore strip cropping might not solve the CPB problem in the long term. To counteract this effect considering multiple fields with different crops might be a solution as the field will get some time to get rid of the soil-borne pests and diseases. This year already showed that not having potatoes as a preceding crop near the field has a significant reducing effect on CPB presence. Of all the fields, field 1 had the highest abundance, this could be caused by the fact that this field was located in the corner of the block and thereby encountered more influences from the outside. Fields 2 and 3 were less infested, an unproven explanation could be the presence of wagtails, during the sampling rounds they were present around these fields. Wagtails have a foraging distance of about 100 meters from their nests which were located near the Droevendaal farm (Badyaev et al. 1998). Field 3 borders the farm, so this field was easily reachable for the birds, field 2 was a little further away, but still, partly within the range, field 1 was located outside the range of the wagtails located on the Droevendaal farm. Wagtails have an average feeding rate of 8.7 items per minute, so if they are foraging they can eat quite some CPB (Badyaev et al. 1998).



*Figure 6: CPB infection per cultivar, the letters indicate significant differences (p<0.05)* 

The wagtails are a natural enemy of the CPB, so they could have predated on the CPB. It would be interesting to take a deeper look into this relationship in further research.

In 2021 the weather did not allow us to do as many sampling rounds as hoped for, as the *Phytophthora* would have spread quickly if the sampling rounds were continued during the week with lots of rain. As a consequence of this wet week, the *Phytophthora* did spread quickly causing the early burning of the field. It would be recommended to do more sampling rounds in further research to increase the reliability and robustness of the data. Furthermore, the management in terms of mulching could be improved by not incorporating the mulch as this increases the visual differences between the treatments. Possibly this could help demonstrate more different abundances of CPB between the treatments.

# Mulching and strip cropping effect on

### potato fresh yield

For the yield, the data from 2018-2021 were taken into account of both DEF and the BH. There was a significant year effect for the yield, so all years were first shown separately to identify trends within the years. In 2021 the yield for the Strip\_var and Strip treatment were significantly higher with up to 1800 kg/ha more (Figure 7d). Strip add yielded significantly less with a yield reduction of about 5000 kg/ha. In 2020 Strip add also yielded significantly less again with about 5000 kg/ha, the other treatments were more or less the same (Figure 7c) In 2018 and 2019 there were no significant differences found (Figure 7a,b). Taken all years together resulted in yields that deviate around 22 tons ha<sup>-1</sup> (Figure 8). There was only one significant difference found, between the strip and the Strip add treatment, whereby the strip with mulch resulted in a lower yield. There were no significant differences found between the reference field and the other treatments.

To check whether there was a border effect present on the yield the difference between the edge and middle rows was determined, it turned out that there were no significant differences.

In the introduction, it is hypothesized that mulching would increase the yield, as there

would be less damage by the CPB and the mulching itself benefits the water holding capacity of the soil. The hypothesis formulated in the introduction was not supported as the yield turned out to be lower for the treatment with mulching. Devaux et al. (1987), concluded otherwise in their research as mulching increased yield significantly, however, this was proven under tropical conditions. Mulching would have a larger effect under dry circumstances than during wet summers in the Netherlands. This year was contrary to the last three years quite wet, therefore this year the yield was very low for the mulch treatment. Furthermore, in 2021 as stated before the mulch was dropped on top of the potato plant, which was damaging for the plants, as the plants were completely covered in the mulch the plants could take up less sunlight the last two weeks compared to the other treatments resulting in a lower yield. The lower yield for the mulch treatment could also be caused by the different fertilization management as the Strip add and rotation treatment did not receive any animal manure because the mulch itself would serve as a fertilizer. The available nitrogen was checked during this research, however, no significant differences in available nitrogen were found. The effect of different fertilization managements can still influence other nutrients such as phosphorus and



*Figure 7: Yield per treatment per year, letters indicate significant differences (p<0.05)* 

potassium which could have been the limiting factor. It is suggested to check this also in further research. Previous research proved an increase in yield after the addition of mulch, therefore whether the mulching results in a higher or lower yield depends on many factors as which cultivar was used, the way of the addition of the mulch, and the weather (Mahmood et al., 2002). The absence of significant difference in 2018 and 2019 can be due to the absence of different growing periods caused by the dry summers, which resulted in low *Phytophthora* infections, thereby making it unnecessary to burn some fields earlier.



*Figure 8: Mean potato yield per treatment. Letters indicate significant differences (p<0.05) and the error bars the standard deviation.* 

# Mulching and strip cropping effect on yield quality

To study the effect of mulching and strip cropping, the marketable yield, NPK content, and dry matter content were used as indicators. The fresh marketable yield mirrors the total fresh yield as the marketable yield is derived from the total fresh yield (Figure 9). The percentage marketable yield of the fresh yield varies between 85 and 95% with no significant differences between the treatments (App. I Figure 2). As it mirrors the fresh yield also the marketable yield is lower for the Strip\_add treatment and the Ref\_strip is differing from the Strip, Strip 3, and Strip 6.



Figure 9: Marketable yield (kg ha<sup>-1</sup>) per treatment 2018-2021, letters indicate significant differences (p<0.05)

The mean nitrogen content was 1.3%, most treatments deviated around this mean. However, some significant differences were obtained. The potatoes from Ref\_strip contained 0.1% less nitrogen, this was significantly lower than all the other treatments (Figure 10a). Another interesting observation is that the treatments with Carolus and Alouette tended to have a higher nitrogen content although not significant. The amount of nitrogen taken up by the tubers can possibly be a cultivar characteristic. Lastly, it was hypothesized that the Rotation and the

Strip\_add treatment would have affected the nitrogen content because those treatments received different fertilizer management. The Rotation and the Strip\_add only received grass-clover mulch, the other treatments received also animal manure. Though no significant differences in N content were observed. Moreover, there were also no differences in the soil available nitrogen between the treatments. More research into the nitrogen release and uptake during the growing season would be interesting to verify whether the nitrogen in the mulch is released with the same speed as the manure.

Continuing to the phosphorus content of the potatoes the mean phosphorus content was 0.39%. But the Strip\_add, Strip\_var, and Rotation treatment contained 0.02% less phosphorus which was significantly lower (Figure 10b). This lower uptake of phosphorus on the Strip\_var and Rotation strips is possibly again a characteristic of the cultivars Alouette and Carolus. The phosphorus content tended to be higher in the potatoes grown on the reference fields (Ref\_space and Ref\_time) and Strip and Strip\_6, however, this was not significant.

For potassium content, the potatoes grown on Ref\_time and Strip had a significantly higher potassium content of about 0.18% (Figure 10c). There seems to be no simple explanation for this as both treatments received the same fertilization as most other treatments. Although a high number of Colorado Potato Beetles was noticed in the Ref\_time and to some extend also in the Strip treatment, so it can be questioned whether that could have something to do with it. A far-fetched explanation could be found in the underinvestigated topic of above and below-ground



Figure 10: Nitrogen, Phosphorus and Potassium content in the potatoes in 2021. Letters indicate significant differences

interactions. It is known that damage of plants by pests can induce certain chemical mechanisms within a plant. So an interesting thesis topic could be whether it is possible that the feeding of Colorado Potato Beetles induces the uptake of potassium.

Additionally, there was also a significant difference between the Strip and Strip\_add treatment (p<0.05), this could possibly be caused by the difference in fertilization management.

No significant differences were observed between the treatments in dry matter content. In the introduction, it was hypothesized that mulching would have a beneficial effect on the tuber quality, however, none of the indicators scored significantly better after the addition of mulching. Additionally, it could also be that the potatoes did not yet get to the point where the different treatments would have had an effect, due to the early burning of the field.

#### Interaction between variables

After analysing all separate variables the interactions between the parameters were calculated to check whether the parameters influenced each other. Some significant interactions between indicators were found. The marketable yield is strongly correlated with the fresh yield (r=0.99), as the marketable yield is a percentage of the fresh yield (Figure 11). Furthermore, there was a small positive correlation between dry matter and (marketable) yield, indicating that more yield also increased the dry matter content of the yield. The NPK content was negatively correlated with the dry matter content implying more dry matter reduced the amount of NPK present in the potato and vice versa, this phenomenon can be caused by the content of the dry matter. If the potato stores more sugars the dry matter content will go up, but the NPK content will become lower. Consequently, Phytophthora infection caused an increase in phosphorus content, but on the other hand decreased the dry matter content thereby also reducing the quality of the potato, as high-quality potatoes preferably have a high dry matter content (Neele et al., 1989). At the start of this research, it was hypothesised that Colorado Potato Beetles would have a preference for healthy plants not affected by Phytophthora, however, no correlation was found. A possible explanation for that is the order in which the data was collected, as there was one sample point on which both the CPB and the Phytophthora were present in the field. The other sampling round of the CBP was before the Phytophthora hit, thereby the Phytophthora did not make any difference in the preference of the CPB. Surprisingly no negative correlation was found between the occurrence of either CPB or Phytophthora infections on yield. For the CPB, this could be due to not reaching the damage threshold or

due to the field effect. A highly infested field could be cancelled out by a less infested field. The *Phytophthora* on the other hand did not reduce the yield either, as all the treatments had to be burned at the same time. No yield differences could be obtained due to the lack of differences in the growing time. Also, there is no reference field where no *Phytophthora* was present, making it impossible to know what yield would have been obtained in the absence of *Phytophthora*. It would have been interesting to compare the yield with and without the presence of *Phytophthora* to get an insight into the yield loss the *Phytophthora* causes.



Figure 11: Correlation plot of all the parameters of interest

The quality of the yield was contrary to the yield influenced by the *Phytophthora* infection as differences in dry matter and phosphorus content were observed. The number of infected tubers was however not counted, this could be done in further research giving a better insight into the quality reduction by *Phytophthora*.

### Conclusions

This research showed that both strip cropping and mulching reduced the occurrence of Colorado Potato Beetles and the severity of *Phytophthora*. All treatments performed better than the reference for *Phytophthora*. So, it can be concluded that strip cropping decreased the Phytophthora infection. Applying mulch or using multiple cultivars helped reduce the infections even more. Appling both mulching and multiple cultivars at the same time did not yield an additive effect, so using either one of them should be sufficient to decrease the infections from *Phytophthora*.

The Colorado Potato Beetles were strongly reduced by the implementation of strip cropping compared to the small reference fields. A decrease in Colorado Potato Beetle infestation by the use of mulching has been found in 3 consecutive years. Additionally, a strong field effect was observed caused by the absence of potatoes in the proceeding years. This emphasizes that thinking about crop rotation is important and that not only shifting the strips over years but also changing fields is crucial to prevent soil-borne pests and pathogens from surviving and multiplying. Another side effect that was discovered was that Colorado Potato Beetles tend to have a cultivar preference as they were more present on Agria than on Alouette. For potato breeders, it would be interesting to investigate which characteristics the Colorado Potato Beetles like or dislike in a plant and to breed for these characteristics.

A drawback of the mulching treatment found in this research was a decrease in yield, however, this decrease does not have to be found every year as the effect of mulching on yield is weather dependant. The mulch can be beneficial in dry periods but be a disadvantage during wet summers, when the field is already suffering from too much water. Also, improving the mulch application method and timing can decrease or nullify the yield reduction. Mulching had a significant reducing effect on marketable yield compared to the Strip treatment, the same reduction was observed for phosphorus uptake caused by the different fertilization management.

There turned out to be no correlation between the number of Colorado Potato Beetles or larvae on a plant and the severity of the *Phytophthora* infection.

In conclusion, strip cropping in combination with mulching and/or increasing genetic diversity can be a promising set of practices in the potato cultivation of organic potatoes.

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# Appendix A



### Appendix B Protocol\_Phytophthora estimation

Written by: Marieke Datema Written on: 01/05/2021 Last updated: 01/05/2021

Goal: Estimate Phytophthora infection level Materials needed:

- Measuring tape
- Rain pants and boots
- Measurement files

### Time estimation: Unknown

- 1) The first 10m and buffer strips are excluded from sampling due to side effects (Figure)
- 5 transect locations are determined transversal to the strip direction by random selection using RStudio. The transects are 3x3m, so a total area of 9m<sup>2</sup> per transect is sampled.
- 3) Phytophthora infection is estimated visually. The leaf area of all plants present in the  $9m^2$  transect will be sampled together and get a score from 0-100%, whereby 0% indicates the absence of Phytophthora on the leaf area and 100% indicates that the plants are completely infected by Phytophthora. The sample unit therefore will be the leaf area in the full transects and all transects will be scored.



# Appendix C1

**Protocol Colorado Potato Beetle Counting** 

Written by: Marieke Datema Written on: 01/05/2021 Last updated: 01/05/2021

Goal: Count CPB per life stage Materials needed:

- Measuring tape
- Rain pants and boots
- Measurement files
- Labels
- Field sticks

### Time estimation: Unknown

- 1) The first 10m and buffer strips are excluded from sampling due to side effects (Figure)
- 2) 8 transect locations will be labeled every 5 meters in one strip. Per transect 2 plants will be checked in every row, the plant closest to the transect label and the plant on the right side next to it will be measured. So in total 8 plants per transect and 64 plants per strip.
- 3) CPB will be grouped by their life stage as follows:
  - a. eggs
  - b. larval instar L1 and L2
  - c. larval instar L3 and L4
  - d. pupae
  - e. adult potato beetle
- 4) Only the larvae stages and adult beetles were counted. The larval stages are differentiated from each other by their colour. Larval instar L1 and L2 are red and larval instar L3 and L4 are orange and pink (see Figure below).





### Appendix D Protocol Fresh yield collection potato

Used from: Anke ter Horst Updated by: Marieke Datema Last updated: 15/05/2021

Goal: Fresh yield collection

Machinal harvest Fresh yield (kg m-2 ) will be collected using protocol 14: Protocol potato harvest by one or two rows.

### Materials needed:

- 1. 1-rijige aardappel rooimachine (single row potato harvester)
- 2. Crate/container for 200 kg
- 3. Cart (wagen) for crate/container
- 4. Pallet scale (on a cart) (accuracy of 1kg)
- 5. Labels
- 6. Mesh bags for 5-10 kg tubers
- 7. Crate to store mesh bags with samples in
- 8. 1 m<sup>3</sup> crate for transportation of total yield

- 1. Weigh empty crate.
- 2. With potato harvester, harvest complete one row of 60 m to crate.
- 3. During harvest collect a sample by holding the mesh bag multiple times under the end of the harvester during harvest.
- 4. Weigh crate with tubers, including the mesh bag with the sample.
- 5. Write down the weight of the brute yield.
- 6. Label mesh bag ( one label inside the bag and one attached to the bag).
- 7. Put mesh bag in the crate for samples
- 8. Empty crate in 1m<sup>3</sup> crate.
- 9. Store mesh bags in cooling for sorting and quality assessment in Wageningen.

# Appendix D2

### **Protocol Quality Assessment Potato**

Written by: Marieke Datema Written on: 19/09/2021 Last updated: 19/09/2021

Goal 1: Sorting potato Materials needed:

- samples collected in the field
- 3 sieves (35cm, 50 cm, and 65 cm)
- grey crate
- bucket
- balance
- sampling sheet

### Time estimation 128 samples 3 days

- 1. Put the 3 sieves on top of each other on a grey crate, the biggest size (65cm) on top and the smallest size at the bottom.
- 2. Put the bucket on top of the scale and tare the scale
- 3. Put the potato sample on top of the sieves
- 4. Shake the upper sieve (65cm) and try if the potatoes fit through the sieve
- 5. Count the potatoes that do not fit through the sieve and put them in a bucket
- 6. Weigh the potatoes with the bucket on the scale and note the number of potatoes and their weight on the sample sheet
- 7. Put the potatoes in a bag for further analysis
- 8. Shake the middle sieve (50 cm) and try if the potatoes fit through the sieve
- 9. Count the potatoes that do not fit through the sieve and put them in a bucket
- 10. Weigh the potatoes with the bucket on the scale and note the number of potatoes and their weight on the sample sheet
- 11. Put the potatoes in a bag for further analysis until it reaches 5kg, discard the rest
- 12. Shake the bottom sieve (35 cm) and try if the potatoes fit through the sieve
- 13. Count the potatoes that do not fit through the sieve and put them in a bucket
- 14. Weigh the potatoes with the bucket on the scale and note the number of potatoes and their weight on the sample sheet
- 15. Put the potatoes in a bag for further analysis if the 5kg is not yet reached, otherwise discard them
- 16. Collect the smallest potatoes from the bottom of the grey crate, count and weigh them, and note the results down
- 17. Put the potatoes in a bag for further analysis if the 5kg is not yet reached, otherwise discard them
- 18. Put the sieves back in order and repeat from point 3 with the next sample

# Goal 2: Determining underwater weight potatoes and preparations for dry matter determination

### Materials needed:

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

### Time estimation: 128 samples 1-1.5 day

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

# Goal 3: Determining dry weight and preparations for NPK analysis Materials needed:

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

### Time estimation: 128 samples 1-1.5 day

- 1. Retrieve the dried samples from the drying oven
- 2. Weigh the samples on a scale tare an empty tray first
- 3. Note the dry weight on the sample sheet
- 4. Grind the dried fries with a grinding machine (Samples of row 1-4 and 2-3 can be put together for the STRIP, STRIP-ADD, REF\_SPACE, REF\_TIME, STRIP\_3, and STRIP\_6)
- 5. Put 5 grams of ground potato in a brown bag
- 6. Label the bag
- 7. Repeat step 2 till 6 for all the samples
- 8. Bring all the brown bags with samples to Hennie Halm for NPK analysis

# Appendix E

Protocol for collecting soil samples and processing for mineral N analyses Written by: Tshering Choden (tshering.choden@wur.nl) Written on April 12, 2019 Last updated: October 31, 2019 Goal: Beginning of the growing season, in-season, and after harvest soil sampling and

analysis to monitor the availability of soil mineral nitrogen for crop growth and development

### Material needed:

In the field

- ✓ Soil augers (Figure 2a)
- ✓ Knives for removing soil from the auger (Figure 2a)
- ✓ Special soil bags as per the number of samples you need to take (Figure 2a).
- ✓ Field map
- ✓ Gloves
- ✓ Gumboots
- ✓ Marker pen to label the bags
- ✓ Plastic tray

### In the laboratory

- ✓ Wheel trolley to carry soil samples
- ✓ Aluminum foil tray for drying the soil
- ✓ Metal aluminum tray to put the aluminum foil tray in it for oven
- ✓ Small papers for labeling
- ✓ Special pencil to write labels

### **Time estimation**

Half-day to obtain 27 composite samples (consisting of 12 subsamples in each composite sample), with four persons. Approximately two hours to put the soil in the tray, labeling and to put in the oven, with two persons.

### Method (Figure 1 a and Figure 1b):

- 1. Use a soil auger to obtain the soil samples.
- 2. Label the bag for the soil sample with field number and strip number.
- 3. Put soil auger into the soil till 25 cm depth and take a soil sample in a zigzag way with strip length intervals of 8.4 meters leaving out 10 meters from two sides.
- 4. Take 12 random subsamples from each strip and mix them to make one composite sample.
- 5. From each treatment (variety mix, mono strip, and additive) take subsamples from two strips.
- 6. Leave the third strip located next to different treatment as a buffer zone to avoid border effect.
- 7. For treatment: rotation, take subsamples from 1 strip and for mono, reference takes one subsample from the middle strip.
- 8. Turn and pull the soil auger slowly and carefully to get out the soil.
- 9. Using a special knife (Figure 1a), cut the excess soil and remove the soil into the bag.

10. Close the bag properly and put it in the tray away from the sun to transport to Radix agros



Figure 1a: Tools and methods to collect a soil sample

- 11. In case the samples are needed to take throughout the day, put the samples in the freezer (5°C) to avoid any microbial processes taking place in the sample.
- 12. Transport the soil samples to radix agros and put the soil in the aluminum foil tray.
- 13. Put a label in it and put the samples on the stove and dry them at 70°C for 48 hours.
- 14. After 48 hours when samples are dried, for chemical analysis (soil mineral N), the soil should be sieved using a 2mm sieve.
- 15. The soil particles that are difficult to sieve need to be mashed with smashing wood (Figure 1b).
- 16. Scoop the sieved soil and put 4 grams into the tube and put a label on it.
- 17. Bring to Hennie's laboratory for chemical analysis at Lumen.

# Appendix F

### Chemical analysis plant/soil samples

Samples were digested with a mixture of  $H_2SO_4$ –Se and salicylic acid (Novozamski et al., 1983). The actual digestion is started by  $H_2O_2$  and in this step, most of the organic matter is oxidized. After decomposition of the excess  $H_2O_2$  and evaporation of water, the digestion is completed by concentrated  $H_2SO_4$  at elevated temperature (330°C) under the influence of Se as a catalyst. In these digests total, N and P were measured spectrophotometrically with a segmented-flow system (Skalar San++ System).

In the same digests, K was measured with Varian AA240FS fast sequential atomic absorption spectrometer. (Terneuzen, the Netherlands).

### Remark:

Salicylic acid is added to prevent loss of nitrate-N. This is done by coupling the nitrate to salicylic acid, a reaction that proceeds easily in the acid medium. In this way, 3-nitrosalicylic acid and/or 4-nitrosalicylic acid are formed. These compounds are reduced to their corresponding amino forms by the plant organic matter.

### **Reference**

Soil and plant analysis, a series of syllabi 1997

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# Appendix G (*Phytophthora*)

| Year | Location | Ref_space | Strip | Strip_add | Strip_var | Rotation | Ref_time | Strip_6 | Strip_3 | LER | Total |
|------|----------|-----------|-------|-----------|-----------|----------|----------|---------|---------|-----|-------|
| 2021 | DEF      | 68%       | 23%   | 9%        | 10%       | 7%       | 47%      | 52%     | 28%     | 1%  | 279   |
| 2021 | вн       | 83%       | 69%   | 71%       | 65%       | NA       | NA       | NA      | NA      | NA  | 72%   |
| 2019 | DEF      | 0%        | 0%    | 0%        | 0%        | 0%       | 0%       | 0%      | 0%      | NA  | 0%    |
| 2019 | вн       | 2%        | 0%    | 1%        | 0%        | NA       | 1%       | 4%      | 3%      | NA  | 2%    |





Figure 1: CPB infestation (%) per treatment at DEF in 2021



Figure 2: CPB infestation (%) per field at DEF in 2021

# Appendix I



Figure 1: Mean potato yield at both DEF and the BH n 2018-2021 per treatment



Figure 2: Percentage of marketable yield with respect to the fresh yield per treatment