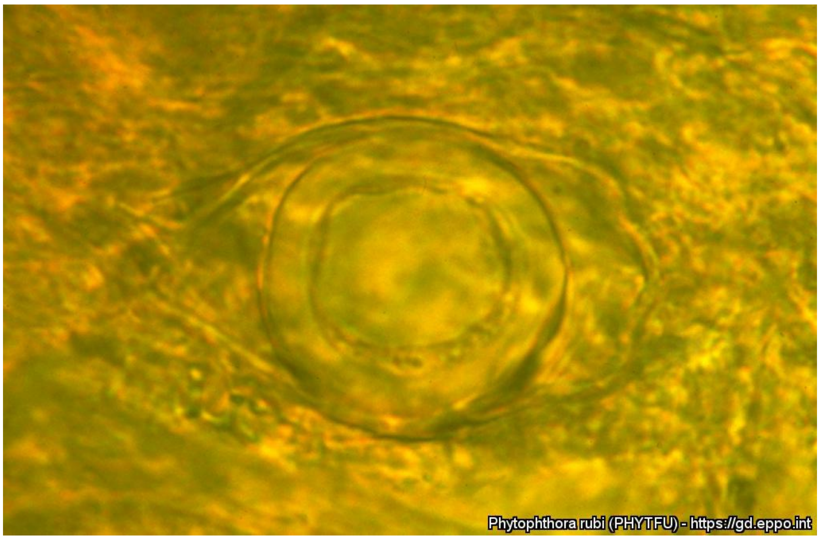
Effect of Phytophthora rubi on yield and fruit quality of Portuguese *Rubus idaeus* ‘Sapphire’



HAMEAU Sklaerenn

Effect of Phytophthora rubi on yield and fruit quality of Portuguese *Rubus idaeus* 'Sapphire'

Which percentage of infected roots by *Phytophthora rubi* is acceptable in 'Sapphire' starting material?

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European Engineer Degree Plant production

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Figure 1: Oospores of Phytophthora rubi (x400) (A. Bolay, 2019)

Preface and Acknowledgements

This study has been done by a French student enrolled in the European Engineer Degree Plant production in AERES UAS during the years 2018-2020.

The sponsor was The Summer Berry Company Portugal. This study aimed to help growers of 'Sapphire' raspberry plants to select out valuable raspberry plants before planting.

- First of all, I would thank Mr CORREIA Carlos for giving me the opportunity to realize my graduation project at his department;

- A big thank you to Mr ALVES Joao, Head grower at T.S.B.C. Portugal for paying attention and helping me with the implementation of my project, for all useful information he accepted to share with me without restriction and for all interesting visits he has invited me;

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Summary

Raspberries are high-valuable soft fruit for growers. But it is even more interesting for them to grow during wintertime when weather as in Portugal is still favorable, as the offer is low on the market and they can distinguish really easily and sell raspberries at high prices.

'Sapphire' is currently considered as the best variety on the market for raspberries, as its shows characteristics that attract the most customers: a really big size, firm, red, juicy and tasty fruit. Both the demand for this fruit and the margin for the grower are really high.

However, as the propagation isn't done into Portugal, growers face the problem of contaminated plants at arrival in Portugal with the oomycete *Phytophthora rubi*. As this is really hard to detect into the propagation field, as it can never express itself as expressing quickly, and symptoms can be confused with *Pratylenchus penetrans*, a nematode. So propagators cannot do anything currently.

Phytophthora gender includes a large range of species well-known all around the world by growers for big damages that they occur to crops when they express.

In that way, the current easiest and relevant solution for growers is to select at planting stage, the ones which show most chances to survive. In that way,a trial has been managed at The Summer Berry Company Portugal, in Herdade dos Almeidans 0, in Longueira-Almograve in the South-West coast of Portugal from November 2019 to December 2019 to determinate the amount of infected roots that a 'Sapphire' raspberry plant can properly live with.

For that, received plants were selected by visual inspection and ranged according to their root infection into several classes: 0% of infection, 30% of infection, 50% of infection, 70% of infection and 100% of infection. *Phytophthora rubi* involvement has been checked by sending samples of the root system to a Spanish laboratory.

Then, they have been transplanted and the crop has been managed as any 'Sapphire' crop at the company until the harvest. A follow-up has been done each week until flowering. Observed criteria were: vegetative and generative stages of development, number of dead plants, number of axillary buds, number of flower abortion and a fruit forecast. Data were analyzed according to the ANOVA One-way by Excel software. Statistically significant differences between infection modalities have been noticed for the number of dead canes and the number of laterals per cane.

0% infected canes got the best behavior *with Phytophthora rubi* with the lowest number of dead canes, one of the highest number of laterals during all the trial (around 13 laterals per cane) and one of the highest development stage (around 53).

Levels of infection which have been considered acceptable in starting material are 0%, 30% and 50%.

These results confirm that *Phytophthora rubi* can kill quickly highly infected canes.

Phytophthora Pocket diagnostic® on-site testing kits cannot be considered as a relevant method to ensure 'Sapphire' raspberry plants are infected with *Phytophthora rubi.*

Chapter 1: Introduction

The Summer Berry Company Portugal (T.S.B.C. Portugal) is a Portuguese company which is producing soft fruits (raspberries, blueberries and blackberries) in polytunnels in the Algarve, on the South West Coast of Portugal. They have 70 hectares among which 35 hectares are raspberries and 35 hectares are blueberries.

Portugal climate allows to grow raspberries in favorable conditions. Most growers are in a conventional-production-way, as The Summer Berry Company Portugal. But indeed T.S.B.C Portugal takes care of their impact on the environment by using integrated pest management as much as possible. In that way, they can sell their fruit into a zero-pesticide-residue policy on the market. They have the Global GAP certification to be allowed selling their products all around the world. They get certifications according to the expectations of their customers, if this certification allows them to sell at higher prices on the market. The British Retail Consortium (B.R.C.) certification, a British food safety certification, allows them to achieve foreign markets while improving their relationship with distributors, strengthening confidence with their clients, increasing their transparency and reducing risky situations at the company. (SARL TREVES ORGANISATION, 2018)

In fact, according to Ms SCHULZE Svenja, Federal Minister for the Environment, Nature Conservation and Nuclear Safety in Germany and who made a speech at the International Conference on Global Action which took place in May 2019, “collaboration at all levels of government and among all stakeholders is essential if climate action is to succeed.” (SCHULZE, 2019) In that way, the demand is pushing growers into producing a zero-pesticide residue and an environmental-friendly product as possible. This is a big challenge nowadays for growers as berries are sensitive to lot of pests and diseases and therefore their appearance is essential for attracting the consumer.

Producing soft fruits as raspberries in polytunnels in the Algarve, on the South West Coast of Portugal is a big opportunity due to favorable conditions to grow, especially during winter. This region benefits of high temperatures during summertime, and winter isn’t so cold, no frost is recorded: the lowest temperature recorded in the weather forecast station in Albufeira do Mar was about 8°C, which allows to grow here all along the year, even if the wind is heavy (around 17km/h). Berries are a high-valued crop, but they are even more during winter due to North European climate, there is not much offer. Besides, T.S.B.C. Portugal is the only berry producer in the surroundings (other growers are mostly located in the North of Portugal). (ALVES, 2019)

1.1 Worldwide *Phytophthora* issues in raspberry

Nowadays, ‘Sapphire’ is one of the best varieties of raspberries in the market: “It is high yielding, has an excellent size and flavor that makes it really valuable for producing”, commented Joao ALVES, Agronomist in T.S.B.C. Portugal in October 2019. (ALVES, 2019)

However, growers as T.S.B.C Portugal, face out some issues with it though, especially with *Phytophthora rubi*, an organism which appears last years in Portugal by young plants propagated in contaminated fields from the United Kingdom.

Phytophthora species are microscopic organisms which produce both zoospores and oospores. Zoospores have the facility to swim into water and are attracted by chemical exudates from the roots of plants. Zoospores cannot swim on long distances, but they can stay on roots and be carried by drainage or run off water. When they are released, roots decompose and they can contaminate the soil but also any surface they have been in contact with, as propagation material, pots, substrate... Oospores are capable of surviving for decades into the soil, even in the absence of a hosting plant, this is why it is complicated nowadays to get rid of it.

Nowadays, this disease is the most destructive disease of raspberries. (The James Hutton Institute, 2019). Already in 1980’s, *Phytophthora rubi* has been a main issue in horticulture by being epidemic in the United Kingdom (Duncan et al., 1987), in Scandinavia but also in Germany (Seemüller et al., 1986) and in Australia during the wet years of 1994–1996 (Wilcox et al., 1993). In the propagated field, *P.rubi* is coming from flooding and sprayers. (ALVES, 2019). On the first year of propagation, T.S.B.C United Kingdom tried to work with raised beds but it didn’t reduce *P.rubi* propagation. In 2018, it has been a rainy year in the U.K and other species as *P.citricola*, a species which appears especially during hot weather conditions, have been noticed within the propagation field. T.S.B.C United Kingdom suppose that they are conveying by contaminated water and set up in drippers. By this way, they contaminate raspberry plants like this. However, unlike Portuguese policy, the U.K is allowed to use methalaxyl and lot of other pesticides as protective and curative solutions against *Phytophthora* species, so the U.K can more easily control it than in Portugal. (ALVES, 2019)

P. rubi is harmful for raspberries as it can kill a plant of ‘Sapphire’ very quickly. Due to their high-value and a lack of suitable land, raspberries are not often rotated with other crops. (ALVES, 2019). This issue isn’t relevant only for Portugal but well present all around the world : “In 90’s we were able to harvest fifty tons per hectare, now we are only able to harvest five tons” gave already evidence Mr Jean-Luc BLANC, a French producer in 2016 about *P.rubi* damages in his raspberry crops. (France TV Infos, 2016). Also, other studies have been done as in Quebec about alternatives to reduce *Phytophthora rubi* apparition from 2014 to 2017 but no studies until now have evaluated with which percentage of contamination does the crop could live with *Phytophthora rubi*.

1.2 *Pratylenchus penetrans*, a nematode which complicates the detection

Besides, *Phytophthora rubi* is not the only cause of root problems which growers have to face. Also, *Pratylenchus penetrans* is another soil organism which makes the detection of *P.rubi* even more complicated as it shows the same symptoms as *Phytophthora rubi*. This is also a nematode where studies are currently done and presents same issues for raspberry growers.

In the United States of America, growers were using fumigation before planting to control it but new regulations make now its fight more complicated, especially because they cannot use anymore methyl bromide for fumigate. In that way, the USDA-ARS Horticultural Crops Research Laboratory, in Corvallis, Oregon (U.S.A.), did a trial in 2010 and 2012 on raspberry plants with different fumigate agents for trying to find out an alternative to methyl bromide. (WALTERS, BOLDA, & ZASADA, 2017) .

WALTERS BOLDA and ZASADA (2017) found that *Pratylenchus penetrans,* is more present in broadcast fumigation into the soil than bed fumigation. However into the roots of raspberries, *Pratylenchus penetrans* is less present with bed fumigation (500 per gram of dry root) than broadcast fumigation (10 per gram of dry root).(Figure 2: Results with Pratylenchus penetrans ￼.

Pratylenchus penetrans is a migratory species which also causes root lesion in a large range of plants. This species is continuously studied and a recent American study has demonstrated that RLNV1 (standing for Root Lesion Nematode Virus 1) is the first virus identified in *Pratylenchus* spp. (VIEIRA & NEMCHINOV, 2019).

Table 1 shows that fumigated beds always get a better cane height (166cm for WA1 ;170 cm and 173 for CA2) and a better yield (1,159 kg/ha for WA1 ; 4,679 kg/ha or 4,231kg/ha for CA1 ; 4,706, 4,381 for CA2) than non-treated one (130cm for WA1; 146 cm for CA2 ; 3,038 kg/ha for CA1 ; 3,865 kg/ha). Tarped beds seem to get higher cane height (166 cm for WA1 ; 258 cm for WA3) than non-tarped ones (137cm for WA1 ; 244 cm for WA3). This difference in plant productivity has been noticed for a year after planting with an increase of 47 to 70% in yield of plants grown into tarped and fumigated beds compared to non-tarped and fumigated beds. (WALTERS, BOLDA, & ZASADA, 2017)This shows that growers growing in full-ground production should use tarped and fumigated beds to manage *Pratylenchus penetrans*.

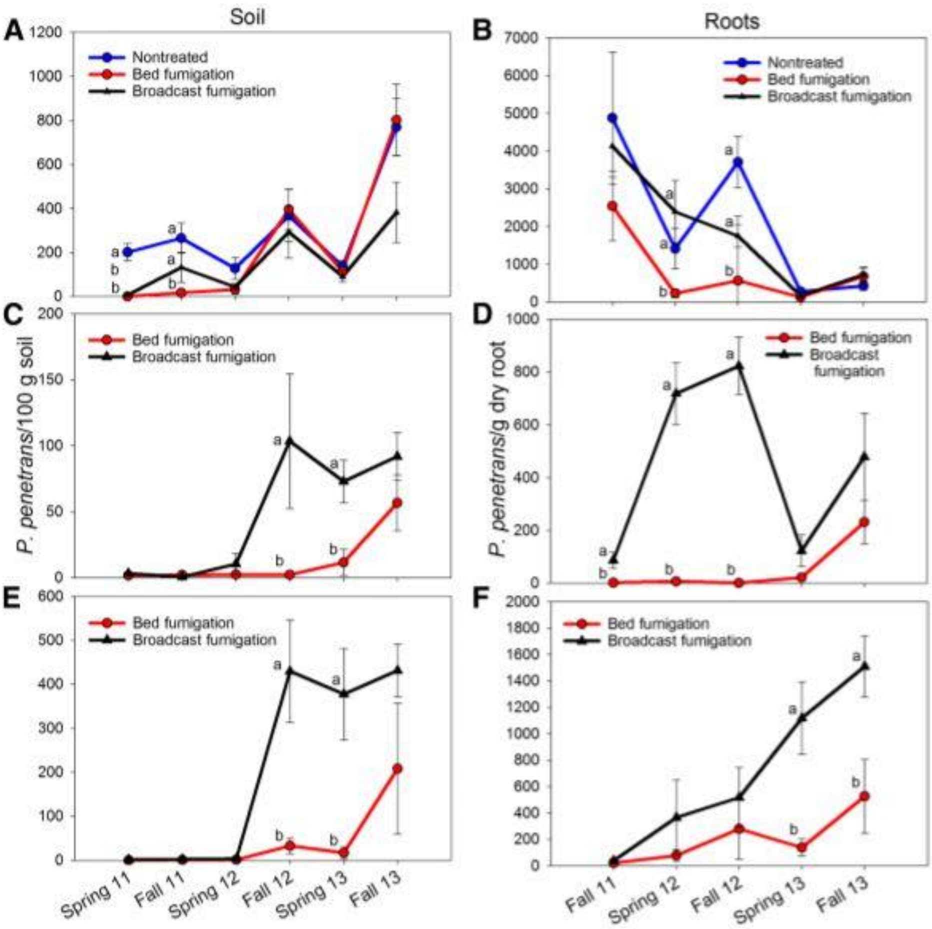

Figure 2: Results with Pratylenchus penetrans (WALTERS, BOLDA, & ZASADA, 2017)

Table 1: Plant performance and yield of raspberry under different alternative fumigation management systems in Washington and California against P. penetrans (WALTERS, BOLDA, & ZASADA, 2017)

TABLE 1
Plant performance and yield of raspberry under different alternative fumigation management systems in Washington and California[v]

Site	Treatment	Cane height (cm)[w]	Yield (kg/ha)[x]
WA1	Bed fumigated, tarped	166 a[y]	1,159 a
	Broadcast fumigated, nontarped	137 b	686 b
	Nontreated	130 b	396 a
	P value	*<0.001*	*<0.001*
WA2	Bed fumigated, tarped	201	1,974
	Broadcast fumigated, nontarped	218	1,924
	P value	*0.1*	*0.7*
WA3	Bed fumigated, tarped	258	3,322
	Broadcast fumigated, nontarped	244	3,389
	P value	*0.4*	*0.4*
CA1	Methyl bromide:chloropicrin	ND[z]	4,679 a
	Chloropicrin:1,3-dichloropropene	ND	4,231 a
	Nontreated	ND	3,038 b
	P value		*0.007*
CA2	Methyl bromide:chloropicrin	170 a	4,381
	Chloropicrin:1,3-dichloropropene	173 a	4,706
	Nontreated	146 b	3,865
	P value	*0.003*	*0.07*

In 2014, another American study was done to evaluate the impact of *Pratylenchus penetrans* in red raspberry cultivation according to the soil type and the soil treatment. Pasteurized treatment was heating the soil to 60°C with steam for 1 h while nematicide one was a combination of the nematicides oxamyl (oxamyl at 367 g/ha and Vydate L at 0.22 ml/liter of soil) and fosthiazate (fosthiazate at 5,044 g/ha and fosthiazate EC900 at 0.13 ml/liter of soil).

Table 2 shows that the propagation of *P. penetrans* is higher in sandy loam soil (around 200 in pasteurized treatment; around 110 with nematicide treatment (in trial 1)), than in silt loam soils (around 160 in pasteurized treatment in trial 2; around 95 with nematicide one). (ZASADA, WEILAND, HAN, WALTERS, & MOORE, 2015). This shows that soil has also an impact on *P. penetrans* development.

Table 2: Pratylenchus penetrans population densities and biomass of raspberry 'Meeker' as affected by soil type and soil treatment in a microplot study (ZASADA, WEILAND, HAN, WALTERS, & MOORE, 2015)

	***P. penetrans* nematodes**			
	Per 100 g of soil		**Per g of dry root**	
Trial, soil type, treatment[y]	**Initial**	**Final**	**Final**	**Total plant biomass (g)**
Trial 1				
Sandy loam				
Pasteurized[z]	0–	0–	0–	192 a
Nematicide	6 c	23 b	113 bcd	110 c
Low	11 c	11 b	36 d	112 c
High	50 ab	39 b	69 cd	91 c
Silt loam				
Pasteurized[z]	0–	0–	0–	179 ab
Nematicide	18 bc	31 b	244 abc	95 c
Low	51 b	46 b	335 ab	124 bc
High	126 a	100 a	448 a	83 c
Trial 2				
Sandy loam				
Pasteurized	0–	0–	0–	212 a
Nematicide	41 b	51 ab	71 c	123 bc
Low	12 b	52 a	162 b	111 bc
High	12 b	40 ab	142 bc	113 bc
Silt loam				
Pasteurized	0–	0–	0–	150 abc
Nematicide	15 b	9 b	187 b	169 ab
Low	18 b	41 ab	225 b	101 c
High	72 a	63 a	538 b	143 bc

1.3 Loss of yield in edible crops due to *Phytophthora* for decades

Phytophthora is a main problem for a lot of valuable crops.

In potato crop, *Phytophthora infestans* can cause a loss from 70 to 80%. This disease is the source of the famine which occurred in 1845-1849 in Ireland. (BLANCARD, 2013)

In chicory, *Phytophthora cryptogea* can cause an average loss of 60% of the expected yield, according to Bayer. In 2013, a study has been done also on *Phytophthora cactorum* and *Phytophthora nicotianae* which are damaging lavender plants in Ontario. During this study, it shows that mulching could increase *Phytophthora* development. (BLANCARD, 2013)

*Phytophthora arecae i*s present since 1960's in the Netherlands on tomato plants and causes quick wilting. (BLANCARD, 2013)

Phytophthora capsici is often present on crops and has caused big losses in yields in Ontario. It affects several crops: it makes collar rot on squashes, zucchini, sweet peppers and chili peppers; it makes fruit and stem injuries on cucumbers, melons and eggplants. It provokes tomato and cucumber drying. (BLANCARD, 2013)

In leeks, especially in North America*, Phytophthora porri* occurs a loss of visual quality on leeks for growers. In fact, tip leaves got turn yellow and color extends (red, orange) for several centimeters down the leaf blade. Symptoms are leaves may be distorted or twisted. (North Carolina State University, 2016). Ravaged tip leaves as in case of rust, could be cut by growers at harvesting stage but this leads to a loss of production, to answer to a visual demand. (LE CORRE, 2019).

In carrots, a study has been done by the I.N.R.A, the Agronomical Research National Institute in France, from 2004 to 2006 to evaluate the evolution and the impacts of *Phytophthora* mega*sperma* (another name of *Phytophthora rubi)* on carrots, the main species involved in 1980's, and which has reappeared since 2002. (BRETON, PRUNIER, & MONTFORT, 2007).

Results are showed in Table 3**.**

Table 3: Presence of P. Megasperma on Daucus carota. (BRETON, PRUNIER, & MONTFORT, 2007)

	2004	**2005**	**2006**
Total of fields surveyed	14	29	60
% fields with *P.sp*	79	76	83
% fields with *P.megasperma*	14	10	7
% fields with the 2 species	0	4	3
% fields with no *Phytophthora* isolated	7	10	7

From 2004 to 2006, the mean percentage of fields with *P. rubi* was 10% whereas the percentage of fields with other *Phytophthora* species reaches 80%. When both species, this is only at 4% of the fields. (BRETON, PRUNIER, & MONTFORT, 2007)

This study showed that *Phytophthora rubi* is not anymore causing the ring rot disease on carrots but new species substitute it as *Phytophthora porri* and *P.brassicae*. This study also reveals that "there is a risk of inefficiency of mefenoxam into the fields. In fact, this is not so surprising because this fungicide, used by growers, is said to be inefficient to protect carrot from ring rot disease in the fields." In that way, there is also as an evidence a lack of knowledge for crop protection against *P.sperma*. (BRETON, PRUNIER, & MONTFORT, 2007)

Phytophthora species are involved in many essential crop damages and *Phytophthora* has already showed that it could endanger human survival.

1.4 Current *Phytophthora* knowledge in *Rubus idaeus*

About raspberries, a study published in 2004 (Table 4), from the Cornell University in the U.S.A, had evaluated the resistance of some raspberry cultivars with *Phytophthora rubi* in hydroponic culture (PATISSON, WILCOX, & WEBER, 2004). This study shows a strong negative correlation (-0,94) between plant disease index and root generation: more the plant disease index is high, more the score of root generation is low.

Also, a high correlation is noticed between the plant disease index and the percentage of petiole lesions: more the plant disease index is high, more plants show disease lesions.

Another high negative correlation (-0,70) has been noticed also between the percentage of petiole lesions and the root regeneration score. This means that more there are petiole lesions on the plant, less roots are regenerated.

Besides, more there are stem lesions on the plant, more the stem is affected.

Table 4: Correlation coefficients (r) between the different criteria used to evaluate 'Latham', 'Encore', 'Titan'and 51 F1 genotypes for susceptibility to P. rubi (PATISSON, WILCOX, & WEBER, 2004)

Evaluation parameter	Plant disease index	Root regeneration score	Stem lesion length	Percent petiole lesions
Plant disease index	1			
Root regeneration score	−0.94	1		
Stem lesion (cm)	0.64	−0.58	1	
Percent petiole lesions	0.77	−0.70	0.78	1

In this same study(Table 3) shows that 'Latham' cultivar seems to be highly resistant to *Phytophthora rubi*, with a root generation index of 2,5 on a 3-point scale compared to 0 for 'Encore' and 'Titan'. (PATISSON, WILCOX, & WEBER, 2004)

Table 5: Reaction of red raspberry cultivars for susceptibility to Phytophthora rubi grown in hydroponic culture (PATISSON, WILCOX, & WEBER, 2004)

Cultivar	Plant disease index (0–5)[z]	Stem lesion (cm)[z]	Petiole lesion incidence (%)[z]	Root regeneration index (0–3)[z]
'Latham'	1.5 a	0 a	15 a	2.5 a
'Encore'	4.4 b	5.0 b	50 b	0 b
'Titan'	4.7 b	9.8 c	87 c	0 b

[z]Values represent the means from three replicate hydroponic basins with six plants of each cultivar per replication. Mean values not followed by a common letter are significantly different (Fisher's LSD, $P = 0.05$).

In Massachusetts, U.S.A, a study revealed that peaks of *Phytophthora* species in the Connecticut River Valley have been noticed and so that rivers can especially serve as a source of inoculum for pathogenic *Phytophthora* species in the northeast of the U.S.A. (BRAZEE, WICK, & HULVEY, 2017).

For raspberries, in the production field, the mortality rate is mostly related to the propagation. In fact, when set up into the soil, no solution exists to avoid the spreading of this organism to the plant. "In the production field the average of mortality can be 10-15% dead plants, but in worst case scenario can reach up to 60%." gave evidence a Portuguese grower. (ALVES, 2019)

Due to 'Sapphire' being a floricane type variety, it means that it will only produce fruit in canes that are one year old. These long canes are propagated for one year in a propagation field in batches of thousands of canes per hectare. *Phytophthora* can infect the canes in these cycles where it can show symptoms in propagation, where then they are selected out, or they can never show symptoms and be present nonetheless and send to growers all around the world. Besides there is no pesticide available for raspberries which is curative for *Phytophthora rubi*, which limits its control. (ALVES, 2019) In fact, according to the James Hutton Institute, "the negligible area of raspberry crop production within the overall agro-industry means that no fungicide would be developed by the agrochemical industry on the basis of its specific potential to control raspberry root rot" (The James Hutton Institute, 2019). Even if Ridomil Gold®, a fungicide came on the market, this is only a short-term solution (efficient only for 14 days) to the main problem. Besides, according to Syngenta, it showed that "strains of fungus resistant to metalaxyl-M (one of the two active components of Ridomil®) and S-isomer may develop" (SYNGENTA, 2017). This is why Ridomil cannot be considered as a sustainable solution to raspberry root rot both for its short-term action. Besides, nowadays companies are looking for environmental-friendly solutions and Ridomil® has negative impacts on the environment according to Syngenta: It has the R50/53 sentence: "R50/53 Very toxic to aquatic organisms, may cause long-term adverse effects in the aquatic environment." due to 64% of mancozeb in its recipe. Also raspberry plants are set up in a sandy soil and Syngenta Canada has reported in 2019 that “the use of this product may result in contamination of groundwater, particularly in areas where soils are permeable (e.g. sandy soil)” (SYNGENTA CANADA, 2019).

A study has also showed that untreated raspberry plants had their yield equal at 28% of the metalaxyl-treated one (BRISTOW & HUMMEL, 1993).

When The Summer Berry Company Portugal receive contaminated plants from its propagator in the United Kingdom, they complain to the propagator, but communication isn’t easy between them. Indeed, it will mean to throw away too many plants and some knowledge is missing based on which percentage of contamination should they throw away these contaminated plants. Because some plants and others aren’t, it is easier for managing raspberry planting to order throwing away all contaminated ones to the workers. However, this leads to a loss of money for the company on both sides : on one side, they can throw away contaminated-looking plants but which would develop well and getting high-valuable fruits ; and on the other side, they will plant out healthy-looking plants but which already caught *Phytophthora rubi* without showing symptoms first. In that way, each contaminated plant makes them losing money because it will die before producing fruit.

In fact, by knowing with which percentage of contamination does the plant can get normal vegetative and generative developments, growers will be able to sort more easily these plants when receiving them, and also suppliers will be able to better sort them before sending them, which in consequence will lead to less complains from their customers. This will allow for growers to get healthier plants and higher yields for finally making higher profits, as this variety is really high-demanded due to its traits of “strong and distinctive flavor and firmness, very large fruit size, large weight and morphology and very uniform” according to its inventors, Mr. SWARTZ Harry Jan and Ms. MCCARTHY Eva, which really attract international customers. (United States FIVE ACES BREEDING LLC (Oakland, MD, US) Brevet n° 20150181783 , 2015).

1.5 Research question

In that way, for conducting this study small samples have been taken and several repetitions have been made in order to get relevant result to be able to answer to the main question “Which percentage of infected roots is acceptable in ‘Sapphire’ starting material? “

To evaluate with which percentage does a ‘Sapphire’ raspberry potted plant can live with *Phytophthora rubi* is the main objective of this trial, in order to help growers and propagators to evaluate at the field when they receive (or for propagators, when they send) the plants to be able to better answer to the demand during wintertime, which is high.

1.6 Research sub-questions

To conduct this study, the following questions have been formulated:

- Is there a significant difference between the percentage of infected roots and the number of laterals?
- Is there a significant difference between the percentage of infected roots and the number of flowers per lateral?
- Is there a significant difference between the percentage of infected roots and the flower abortion?
- Is there a significant difference between the percentage of infected roots and the average fruit size?
- Is there a significant difference between the percentage of infected roots and the dead plants?

During this study, only the percentage of contaminated roots by *Phytophthora rubi* when plants are received in pots has been studied in relation with the development of the plant at vegetative and generative stages. All factors before planting as the propagation itself haven't be explored, as they couldn't be controlled (and so solved) at the grower stage. Because of a lack of samples with 100% infected roots when received, only few plants have been set up but haven't been set up into the trial; they have been set up only to control that they will die as expected.

Data have been analyzed thanks to Excel with F-test to check variances and then thanks to ANOVA One-way variance analysis. Then, when differences were noticed between groups, a Tukey-Kramer Multiple Comparison has been made with Excel.

Chapter 2: Materials and Methods

The research for conducting this study has been quantitative.

Origin and reception of the starting material

In this trial, raspberry plants from 1.8L-pot of variety 'Sapphire' have been used. The starting material has been received on Tuesday, October 15th, 2019.

They were grown in the United Kingdom from mother- plant-cuttings themselves grown by micropropagation. Cuttings were done on small sprouts and then transplanted out into trays at the nursery of Tuesley Farm, Godalming, U.K. Then, they were moved outside to dripping plants and planted out at the Tuesley Farm's nursery. Then, they grow until 10-meter-canes and are stored in a cold room until shipping to Portugal.

Two-long-cane 'Sapphire' raspberry plants were coming from the Tuesley Farm of Hall Hunter, located in Godalming, U.K. They were packed on December, 10th, 2018. Plant passport was 122518. Plant class was number 1.

Grading

On Wednesday, October 30th, 2019, a group of 4 people has graded 180 two-long-cane raspberries at planting stage by estimating the percentage of roots affected by rots. As this is a visual estimation, this had to be conducted by several people to ensure the rating is the most objective as possible. For that, these 4 people have been trained to get the same appreciation on the root system. A color code has been done for each modality tested for the root system to collect data more easily later.

Each plant has been put into the box related to its percentage to facilitate the planting later.

Cultivation in polytunnels

On the same day that they have been graded, in the afternoon, the 180 plants have been transplanted out from 1.8L black pots to 5L black pots into the middle row of 3 "High leg pioneer"-type polytunnels of 175 linear meter each, at a density of 1 cane per meter. Two drippers have been put per pot, closed to each cane of the pot. These drippers were connected to one pipe per row (ref.HSCR PEBD PE 32 DN16-1.4 PN 4 161011 1712 MTS 017- 4 bar).

The planting area was located into the sector number 29.4 of The Summer Berry Company Portugal, at Heldade dos Almeidans in Odemira, Alentejo, Portugal.

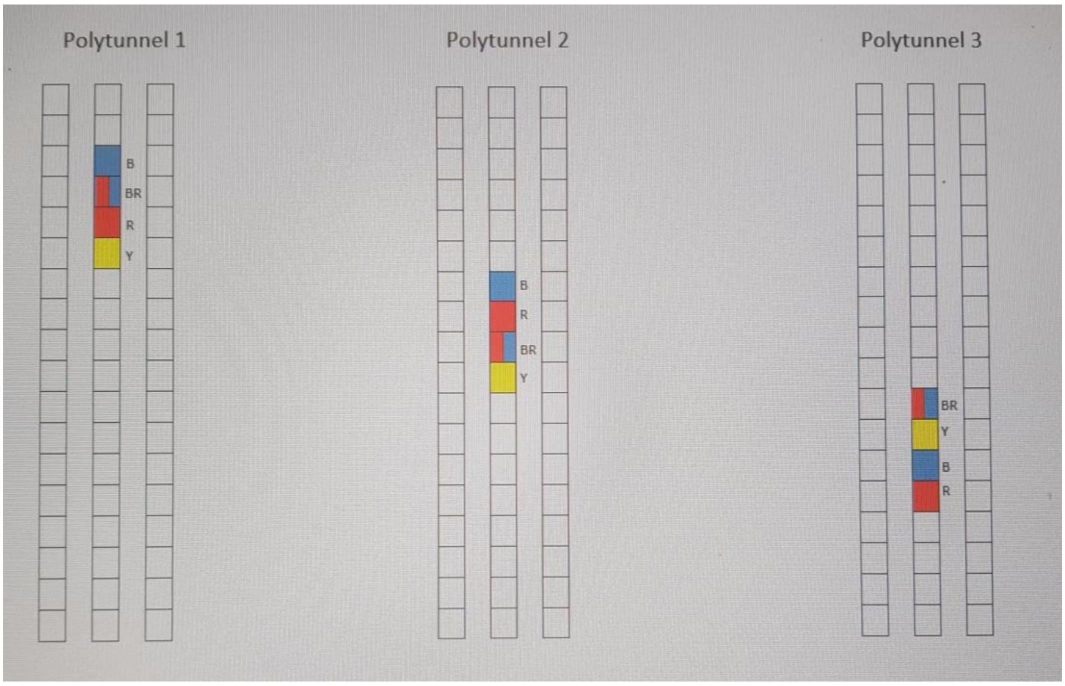

Figure 3: Implementation map of the trial. BR (blue-red): 0% / B (blue): 30% / Y (yellow): 50% / R (red):70% (HAMEAU, 2019)

Each polytunnel was divided into 3 rows of 270m. Each row has been divided into 18 plots longitunidally. The distance between the 3 rows of the polytunnel was about 2m each. Only middle rows have been used for the trial. Each middle row had 60 tested canes. First 30 meters and last 30 meters of each row haven't been used due to a risk of uncontrolled conditions in these places (wind...). Extra canes were planted out on both left and right rows of each polytunnel as a normal use for the company.

Figure 4: One of the three tunnels where canes were planted out in the middle row - © (HAMEAU, 2019)

Transplanting substrate used was coconut fiber "Cocogreen UK Lanaka (Pvt) Ltd" coming from seeduwa in Sri Lanka in 5kg coco peat bales. Its composition was 70% coir peat and 30% coco fiber.

5 samples of the root system have been taken randomly on 5 plants per modality (all polytunnels included) to check Phytophthora presence thanks to 25 Phytophthora on-site tests from the "Pocket diagnostic" brand on October, 30th, 2019. Another check has been done one week later, on November, 7th, 2019 to confirm results.

Signs, made from 24 laminated and printed sheet of papers, with the origin, date of planting, variety, number of canes and the tested modality have been put at the start and at the end of each 15 tested plants. These signs have been hung on thanks to two clips (ref. 2.5x100mm "MacFer" ASN-01P diameter 2~22) per sign.

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Beneficial insects and cure, as a normal conduct of a raspberry crop, have been put before critical stages to prevent and also when the disease/pest has been observed into the field at a critical quantity, according to couples pest/beneficial insect (e.g. aphids/parasitic wasp) and disease/cure (e.g. rust/sulfure). Ones which have been used are: *Bombus terrestris, Phytoseiulus persimilis, Aphidius colemani*, *Pherodis*, *Spodoptera* and *Orius laevigatus.*

Data collection

- **Percentage of Phytophthora infection for each plant**

It has been collected thanks to a visibly general appreciation of the root system of each plant from the people who is rating. As it is quite subjective, a meeting point has been done before rating to ensure everyone was rating according to the same criteria.

The roots have been then sorted by tested modalities according to the appearance of the root system:

-0%: Roots are all white when scratched, strong and clean, and well-spread within the full root system. The root system is homogeneous. Decision: this pot is colored in blue+red.

-30%: Many white and strong roots but few roots are brown. Decision: this pot is colored in blue.

-50%: Half of the roots are white and strong; half is brown and thin. Decision: this pot is colored in yellow.

-70%: Most roots present are thin and dark. Only a few are white and strong. Decision: This pot is colored in red.

-100%: the full plant is contaminated; all roots are dark and thin if there is some. This pot is colored in red and yellow.

Then they have been marked with by a color on the pot, done thanks to 4 permanent markers (to avoid marks to go away when watering). However, laminated signs have been put on rows as well, in case these marks went away.

Figure 5: Example of root infected at 0% (HAMEAU, 2019)

Figure 9:Example of root system infected at 30% (HAMEAU, 2019)

Figure 7: Example of root system infected at 50% (HAMEAU, 2019)

Figure 8: Example of root system infected at 70% (HAMEAU, 2019)

Figure 6: Example of root system infected at 100% (HAMEAU, 2019)

- **Presence *of Phytophthora sp*. into 'Sapphire' raspberry plants**

Before planting, on Tuesday, October 15th 2019, samples were sent to a laboratory. Results of this analysis are shown with more details in Appendix 4: Results of data analysis . Another little analysis has been made by collecting samples after planting of the root system of raspberry plants. Samples have been taken randomly on two dates. Samples have been collected from the three different rows and on 2 dates: on Thursday, October 31st, 2019 and on Friday, November 8th, 2019. Samples have been classified per modality and per collection date.

The presence has been detected by on-site tests from Pocket diagnostic® from Lab028 V2.00, received in a box of 50 on-site tests. They have been stored as required in a dry place and at room temperature before using them. The expiration date of these kits was July, 2020.

- **Generative and vegetative development stages**

This data has been collected by a visual estimation of the vegetative or generative stage done each week thanks to a rating scale , and has been done by 2 people. The rating scale which has been used is available in Appendix 1.

- **Average dead plants**

This data has been collected by a visual counting of the number of dead plants each week for each modality by taking a sample of 10 plants randomly per modality.

- **Average number of axillary buds per class**

This data has been collected by a visibly counting at the vegetative stage on 10 plants of the number of axillary buds per plant for each modality. Then, an average per class has been calculated.

- **Average number of flowers per lateral per class**

This data has been collected by a visibly counting at the flower stage of the number of flowers for each plant per modality on a sample of 10 plants per class taken randomly. Then, an average per class has been calculated.

- **Average number of flower abortion per class**

This data has been collected by a visibly counting at the start and at the end of the flowering stage of the number of flowers per plant on a sample of 10 plants per class taken randomly. Then, an average per class has been calculated at the beginning and at the end of flowering.

The data collected have been analyzed thanks to ANOVA One-way statistical test made with Excel software. Then, when differences were noticed between groups, a Tukey-Kramer Multiple Comparison has been made with Excel.

All lectors must be aware that these data are related to Southern-Western Portugal climate, during the fall and winter seasons. For these reasons, conclusions made of these results cannot be relevant to a global result of the behavior of ‘Sapphire’ to *Phytophthora rubi* for all Portugal and around the world, or applicable to another season.

Besides, because of a lack of time as the study has been conducted during autumn-wintertime, the growth and development of plants are slower than during summertime. In that way, the fruit stage hasn't been evaluated as first fruits only started in February 2020 (the expected week was week 10). However, it has been decided, as the flowering stage already gives good information on the expected yield, that only the flower stage, expected at the beginning of January, would be observed instead of both flower and fruit stages.

Chapter 3: Results

Data which were collected were at vegetative stage: stage of development, mortality, number of axillary buds per cane and number of laterals per cane. Data of generative stage (number of flowers per lateral, flower abortion, fruit forecast) haven't been collected before the deadline finally due to a lack of time and of winter season. In fact, flowers were expecting to bloom only at the beginning of January.

Data were analyzed according to ANOVA One-way analysis, even if the condition of normality wasn't checked. In fact, scientific studies show that under non-normally distributed conditions, ANOVA gives still robust results. (SCHIMDER, ZIEGLER, DANAY, BEYER, & BUHNER, 2010). Besides "The effects of nonnormality on the power of the F test have been investigated by David and Johnson (1951), Srivastava (1959), and Tiku (1971). Their results indicate that, for the conditions studied, mild departures from normality have little effect on the power of the F test." (Harwell, Rubinstein, Hayes, & Olds, Winter 1992).

For the equality of variances, "the effect of variance heterogeneity may be more serious when group sizes are unequal than when they are equal". (LIX, KELSELMAN, & KESELMAN, 1996). As group sizes were equal (10 plants taken out randomly for each modality), the effect of variance heterogeneity is less serious. However, a variance analysis has been conducted as well.

Appendix 4: Results of data analysis shows with more details results of data analysis for below subtitles.

- **Presence *of Phytophthora sp*. into 'Sapphire' raspberry plants**

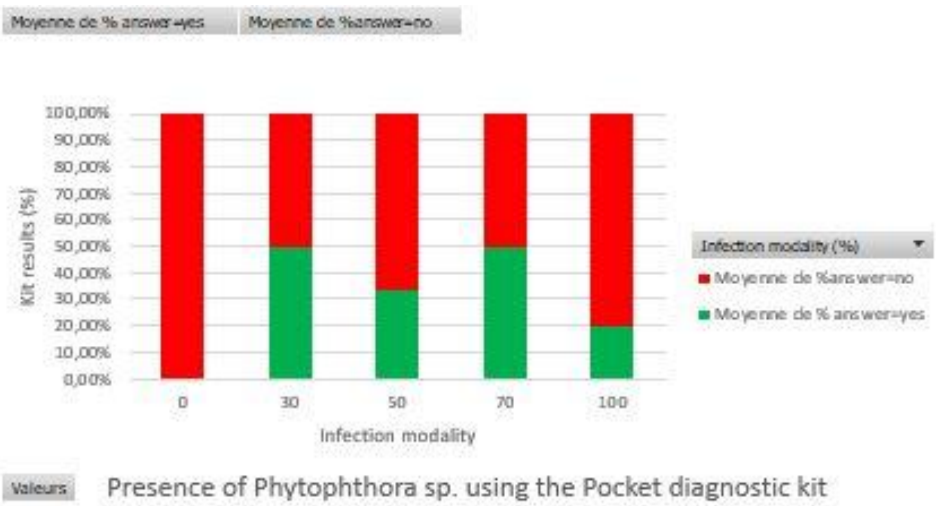

Figure 10: Phytophthora sp. results with Pocket diagnostic on-site kits (HAMEAU, 2019)

Results with the Phytophthora kit shows that all plants considered with 0% of infected roots were also shown with negative results for *Phytophthora sp.* while using the Phytophthora testing kit.

For other modalities, results are more mixed. However, the tendency is that for results which must be normally positive (30,50,70,100), it shows negative results.

However, the result of root analysis done by the company proves the presence of *Phytophthora sp*. into the planted canes.

- **Development stage**

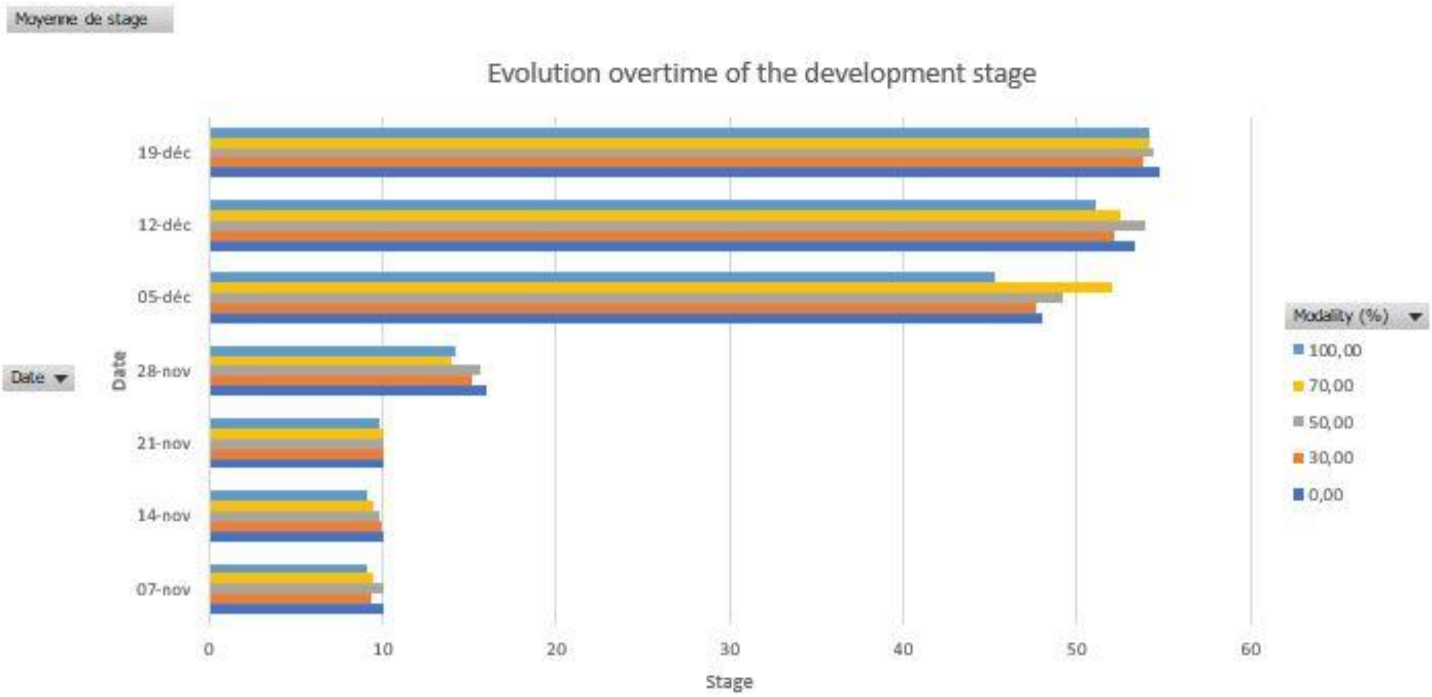

Figure 11: Results on the development stage according to the infection modality (HAMEAU, 2019)

The ANOVA One-way analysis showed that there is no significant difference between the stage and the modality (p=0,99). In fact, as p-value is highly up to 0,05 we can conclude that there is no significant difference.

Figure 14:Root system appearence of 0% infected canes on 19-12-2019-© (HAMEAU, 2019)

Figure 12: Root system appearence of 50% infected canes on 19-12-2019 -© (HAMEAU. 2019)

Figure 13: Root system appearance of 70% infected canes on 19-12-2019 -© (HAMEAU, 2019)

- **Number of dead canes**

The ANOVA One-way analysis showed that there is a significant difference between the number of dead canes and the modality (p=0,07). In fact, as p-value is close to 0,05 ; data suggest that **<u>there is a significant difference between modalities.</u>**

However, after conducting the two-sample t-test analysis, significant differences were noticed **30% and 70%** ; **0% and 100%** ; **0% and 70%** infection modalities at an error threshold of 5%.

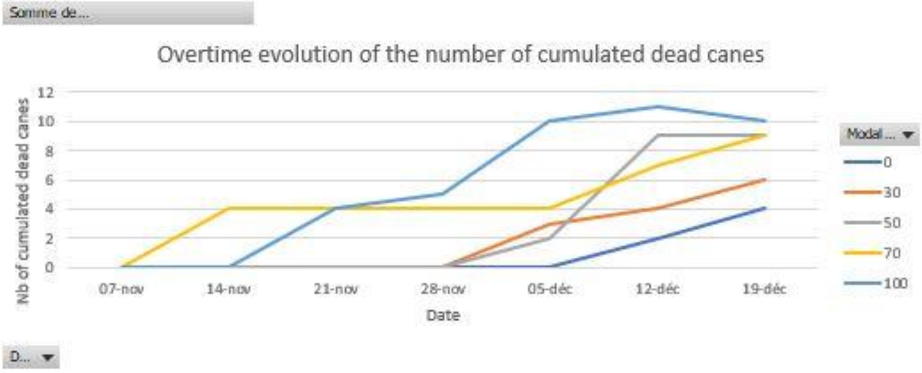

Figure 15: Results on the mortality according to the infection modality (HAMEAU, 2019)

Figure 17: 100% infected canes on 05-12-19© (HAMEAU, 2019)

Figure 16: Some 0% infected canes on 05-12-19 -© (HAMEAU, 2019)

Figure 18: Overview of some 70% infected canes on 05-12-2019 -©

- **Number of axillary buds**

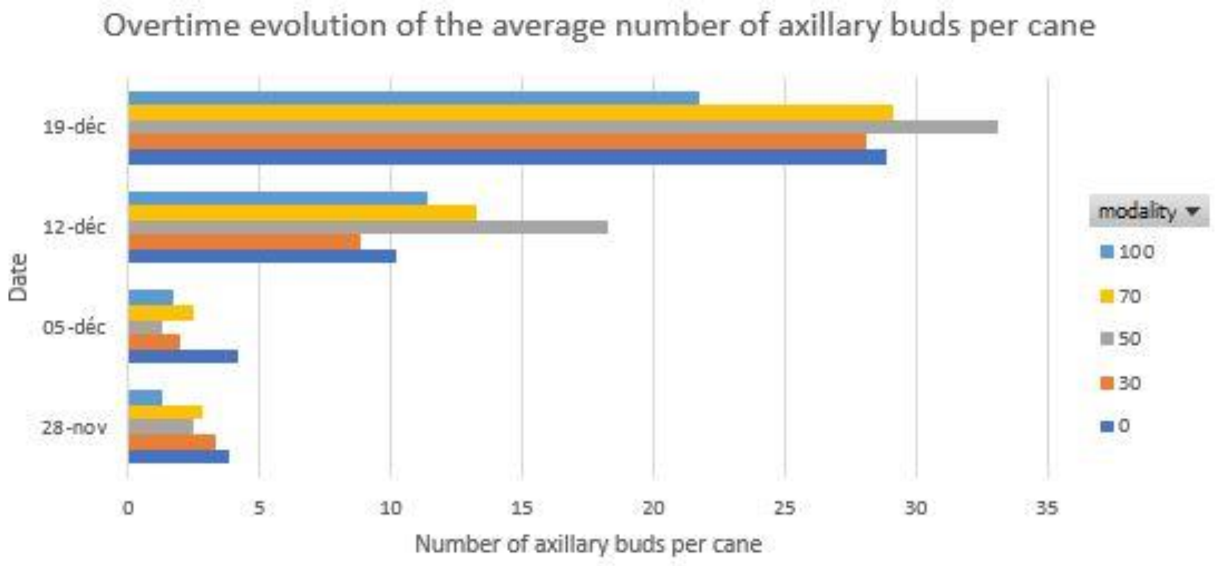

Figure 19: Evolution of the number of axillary buds (HAMEAU, 2019)

The ANOVA One-way analysis showed that there is no significant difference between the stage and the modality (p=0,99). In fact, as p-value is highly up to 0,05 we can conclude that there is no significant difference between modalities.

Figure 20: An example of axillary buds on the plant, here for the 70% infection modality on 05-12-19-© (HAMEAU, 2019)

Figure 21: Some axillary buds for the 30% infected canes on 05-12-19 -© (HAMEAU, 2019)

- **Number of laterals per cane**

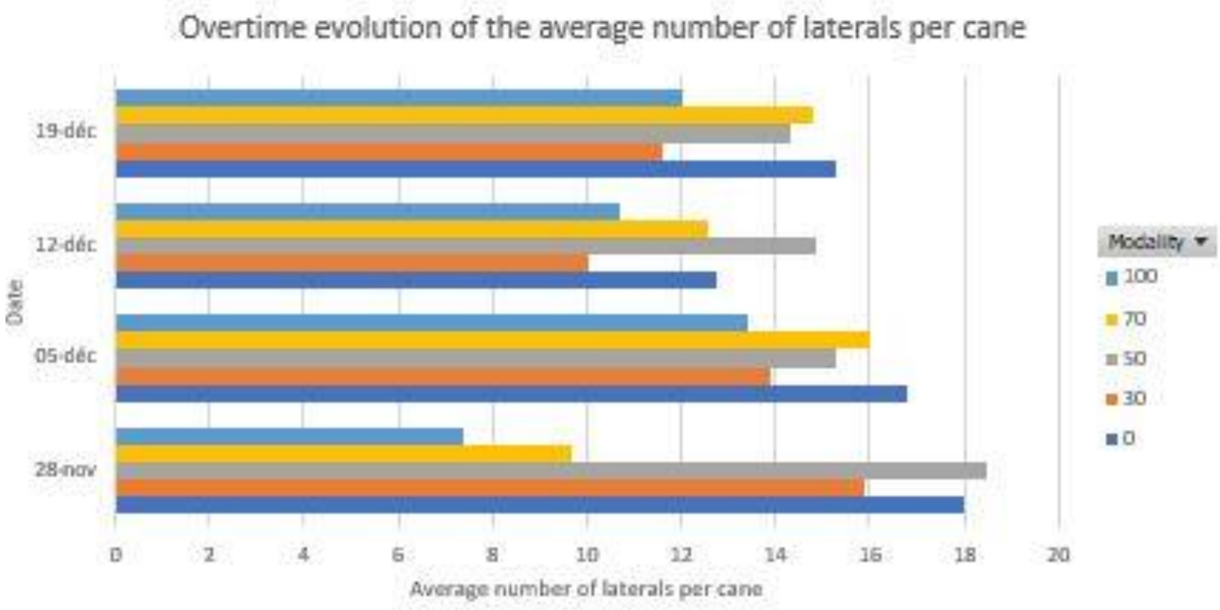

Figure 22: Results on the evolution of the number of laterals per cane according to the modality (HAMEAU, 2019)

The ANOVA One-way analysis showed that there is a significant difference between the number of laterals and the modality (p=0,058). In fact, as p-value is close to 0,05 data suggest that **there is a significant difference**.

However, after conducting the two sample t-test, at a standard error of 5%, data suggest that **no significant difference has been noticed between infection modalities**.

- **Number of flowers per lateral**

Due to winter conditions, plants grow more slowly. The collection of data has been stopped before flowering. So this data hasn't been collected.

- **Flower abortion**

Due to weather conditions, plants haven't given flowers before the end of result collection. Thus, this data hasn't been collected.

- **Yield forecast**

For the same reasons as above, this data hasn't been collected.

Chapter 4: Discussion of results

The objectives of this thesis were to determinate with how much percentage of infected roots by *Phytophthora rubi* does a 'Sapphire' raspberry plant could grow and develop properly. This could allow for 'Sapphire' raspberry growers to order to their propagator only plants which will grow properly once established into their growing field. This will allow for the company to save space and money while the propagator will be able to improve its reflection image by getting less complains from its clients.

What have been noticed during this study are:

- **Presence *of Phytophthora sp*. into 'Sapphire' raspberry plants**

Some root systems were looking bad at planting stage already while the Pocket diagnostic kit hasn't revealed that it was infected by a *Phytophthora* species. On the opposite picture, the Pocket diagnostic didn't reveal that the root system of the collected sample estimated at 100% infected, was infected while it should be. This can reveal that the Pocket diagnostic is insufficient to ensure that the plant is infected by *Phytophthora sp*.

Figure 23: Example of biased results obtained with the Pocket diagnostic on-site tests: here, the 100% infected modality has showed a negative result for Phytophthora sp. while it was well-infected. (HAMEAU, 2019)

However, a study from 2007 demonstrated that Rapid Diagnostic Field Test (as Pocket diagnostic test) are valuable for "useful primary screen for selecting samples for laboratory testing to determine the species identification". (GENERAL TECHNICAL REPORT PSW-GTR-214 , 2007); Besides, an article written by CELETTI Michael, OMAFRA Plant Pathologist in the daily magazine "Daily crops" said that "The only test kit that appeared to work specifically, consistently and provided a clear strong reaction was the Phytophthora Pocket Diagnostic Test Kit. It also performed well in diagnosing late blight caused by *P. infestans* on infected tomato leaf and fruit tissue in the field in both 2009 and 2010". (CELETTI, 2012).

Growers should be more attentive to the way of propagation of their raspberry bushes, to receive healthier plants and then wasting less time by sorting plants and throwing some good ones as well. For that transparency from their propagator on their methods is the key, to ensure good conditions for 'Sapphire' root development. If this is impossible, probably another propagator of 'Sapphire' should be envisaged.

- **Stage of development**

0% and 50% were growing faster compared to others on first weeks of development. However, after few weeks, they stabilized and 30%, 70% and 100% came to the same stage of development as 0% and 50%. Then, 0% overpassed all other modalities.

- **Mortality**

Significant differences have been noticed between modalities.

100% and 70% showed died plants quicker after planting while no one were noticed for other modalities on first weeks. However, one month after plantation, the overtime tendency shows that more the plant is infected by *Phytophthora rubi*, more dead plants there were.

- **Number of axillary buds**

50% showed finally the highest number of axillary buds. The tendency for first weeks was that most plants seem to be infected, most they got a high number of axillary buds early. But then, 50% infected canes overpassed clearly all other modalities.

- **Number of laterals per cane**

No significant differences have been noticed between modalities.

However, 50% and 0% first showed the highest number of laterals per cane and 100% clearly the lowest. Then, finally, 0% and 70% got the highest number of laterals per cane, followed by 50%.

– **The process**

About the process, plants have been followed every 7 days as planned. Data have been collected rigorously each week.

However, looking at the same plants by distinguished them (with a number/sign/color) for collecting data instead of taking randomly some samples all the time could be more representative of the behavior of each modality. Like this, it ensured more to notice how the plant is evolving each week. Besides, only one person was rating plants. To get a more objective point of view, as foreseen in the R.P.P, a group of several people should be better to ensure an objective point of view. For that, several people should do it together next time.

Due to a lack of number of 100% infected plants received, only 10 plants (20 canes) were implemented into the trial. As done for other modalities, getting 30 plants (60 canes) in total should be better for sure more representative in order to get reliable data for this modality.

About methodology, a study made with an implementation of more plots could be more relevant, than getting 13 plots in total. This is relevant especially for 100% modality.

Besides, the same plants should be ranked every week to see better the evolution in term of stage, number of laterals, axillary buds instead of taking 10 plants per plot randomly every week.

Dead plants should be removed from the trial little by little to facilitate their counting.

Botrytis cinerea has been noticed on plants on week 48, but according to the manager of pests and diseases in the company, this wasn't risking affecting the growth and the development of plants.

On week 51, *Amblyseius cucumeris* has been set up into the crop against thrips (*Frankliniella occidentalis*).

Also, these results confirm the fact that *Phytophthora rubi* is a really harmful organism which, when well-established into the root system, can kill quickly a plant as shown by plants infected at 70% and 100%.

Chapter 5: Conclusions and recommendations

The aim of this thesis was to study the behavior of raspberry plants ‘Sapphire’ growing with *Phytophthora rubi* in the South-West Coast of Portugal during autumn-winter. Several infection modalities of the root system were tested: 0%, 30%, 50%, 70% and 100%. Vegetative stage was only studied finally because of the season which did not enable plants to reach the generative stage before the deadline of the thesis report.

Conclusions

- Data suggest that there are significant differences between modalities during this trial statistically on the number of dead canes and the number of laterals per cane.
- 0%-plants seem to get the best behavior with *Phytophthora rubi* with the lowest number of dead canes, one of the highest number of laterals during all the trial (around 13 laterals per cane) and one of the highest development stage (around 53).
- These results confirm that *Phytophthora rubi* can kill quickly highly infected canes.
- *Phytophthora* Pocket diagnostic® on-site testing kits cannot be considered as a relevant method to ensure ‘Sapphire’ raspberry plants are infected with *Phytophthora rubi*.

Recommendations

- Growers should plant canes infected until 50% by *Phytophthora rubi*.
- Due to these controversial results obtained for *Phytophthora* detection, the best current method to consider is to send samples of roots to a laboratory to get relevant results. However, this will ask to spend more money for companies and to wait some time to obtain results than using Pocket diagnostic ® Phytophthora on-site testing kits. This will ask also for companies to be more organized to get enough time to get results for *Phytophthora* from the laboratory before the planting date. So, this must be managed as well with the propagator.
- Growers should be more attentive to the way of propagation of their raspberry bushes, for trying to get healthier plants at reception by minimizing favourable conditions to the development of *P. rubi*. In that way, a real transparency from their propagators should be needed and if weather conditions couldn’t better be controlled from the latter, probably another propagator should be envisaged.
- A study with more randomly distributed data should be done, to get larger groups for each modality and more plots.

- The 100% infection modality should be more repeated as well and should be present this time in each plot as for all other modalities. For that, more plants with 100% infection should be received from the U.K. This should be foreseen with the propagator to do not pay them if possible, on a common agreement between both parties.
- Dead canes should be removed little by little after being considered in the data collection to ensure they won't be counting again on the week after.
- The same living plants for each plot and each modality should be observed week after week, to see better the evolution of each one and avoiding getting weird results as less average of axillary buds one week later as for 30% or less laterals as for 50% (instead of getting the same number if not more). Putting a sticker, using a marker, giving a number to these plants could be used for that.
- More spacing out plants during the implementation of the trial would facilitate to rank plants, especially the number of laterals and the number of axillary buds because bottom canes were difficult to reach. This conducted to complicate the observation, sometimes which was more approximated. It damaged some laterals as well, while manipulating them by counting.
- A group of several people should be ranking plants every week to ensure the objectivity and the agreement on each variable for each plant and each modality while collecting data. Also, they could help to count laterals and axillary buds located in the bottom part of the row where the counter couldn't reach.
- In a recent report of ADHB Agriculture, a trial showed that during winter, potted raspberry canes should be better stored within ambient conditions than in cold stores. (Agriculture and Horticulture Development Board, 2019). So I highly recommend to apply this method before planting these canes to limit *Phytophthora rubi* development.
- This trial should be repeated but this time conducted until the harvest to get the most expected results for the grower: which percentage of infected roots gives the best acceptable yield?

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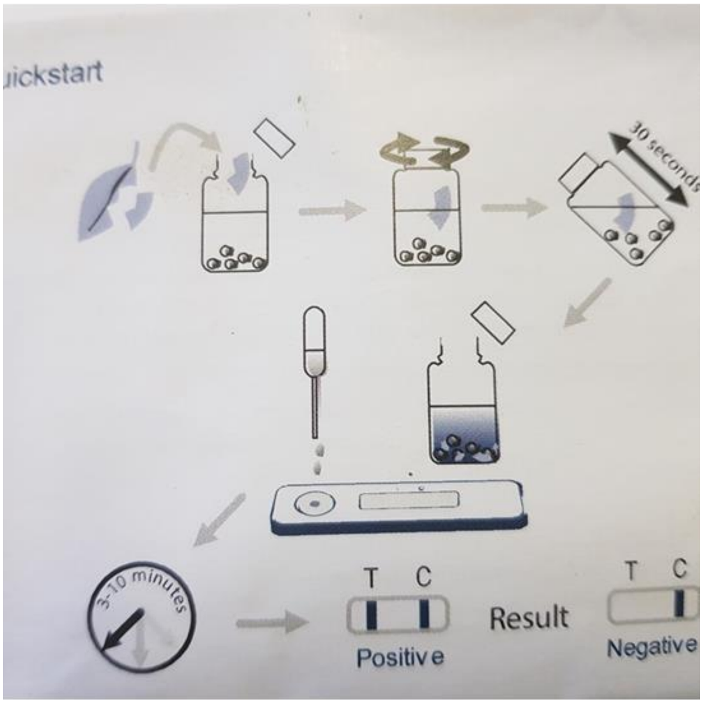
Figures

Tables

Appendices

Appendix 1: Instructions for using the Pocket Diagnostic® on-site Phytophthora test (Brevet n° IFU004 Version 1.00 Serial number: 506, 2015)

Step 1: Select plant material for testing. Refer to the Keycard (25mm square) in the kit for any specific guidance on sampling.



Step 2: Unscrew the extraction bottle lid and add the plant material. Replace the lid tightly.

Step 3: Shake the bottle firmly for 30 seconds (60 seconds if the material is hard or woody), or until the plant material has been throughly broken up. Refer to Keycard in each test in each test kit.

Step 4: Remove the test device from its goil packing and place on a level surface with the viewing window upwards. DO NOT TOUCH THE VIEWING WINDOW. The test can be carried out with the device held horizontally in the hand.

Step 5: Remove the lid from the extraction bottle and draw some of the liquid into the pipette. Gently squeeze 2 or 3 drops of the sample liquid into the sample well of the test device. Take care not to flood the sample as well.

Step 6: After about 30 seconds blue dye will appear in the viewing window liquid flows along the test device. A line (the Control line) will appear next to the letter 'C' on the device. This line confirms the test is working properly.
If the test is positive, a second line, the Test line (next to the letter 'T'), will appear. The lines appear winthin 10 minutes after adding sample to the test device. Note that somes types of Pocket Diagnostic take longer to run than others – refer to the Keycard.

Appendix 2: Phenological Rating scale (BBCH) for long-cane raspberries (The Summer Berry Company Portugal, 2019)

0.Branch development

- 00-Dormancy
- 07-Bud break
- 09-Green tip

1.Foliar development

- 10- First leaves visible
- 11- First leaf separated
- 12- 2 leaves separated
- 19- 9 leaves separated

3. Vegetative development

- 31- 10% of total lateral growth
- 33-30% of total lateral growth
- 36-60% of total lateral growth
- 39-100% of total lateral growth

5. Flower bud development

- 51- First flower bud visible
- 53- Flower buds together
- 55- Flower buds separated
- 57- Flower buds begin to swell
- 59- Flower sepals begin to separate, white petals visible, but flower still largely closed

6. Flowering

- 60-First flower open
- 61- 10% of flowering
- 63- 30% of flowering
- 65- 50% of flowering (full flowering)
- 66- 60% of flowering (first fruits visible)
- 69- End of flowering. Most flowers with dry petals and already show the green fruits growing.

7. Fruit development

- 71- 10% of green fruits visible
- 73- 30% of green fruits visible
- 75 – 50% of green fruits visible
- 79 – All green fruits visible

8. Fruiting

- 80- First fruits growing and gaining colour. (Fruit is white and getting pink)
- 85- First fruits gaining colour (Pale pink to red)
- 89 – First fruits completely red/ripe. Harvest initiates.
    - 891- 10% of the fruit picked.

893- 30% of the fruit picked.
895- 50% of the fruit picked.
897-70% of the fruit picked.
899- All fruit picked. End of harvest.

Appendix 3: Results of analysis of Rubus idaeus ‘Sapphire’ root system for Phytophthora (The Summer Berry Company Portugal, 2019)

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Report

CropHealthMonitor
PlantDoctor
Raspberry Sapphire

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Original

Sample	Research-/ordernumber: 729998/004860067	Date sampling: 15-10-2019	Date report: 07-11-2019	
	Test code: 780	Receiving date: 22-10-2019	Sample was taken by: Third party	Contactperson sampling:

Results

The roots from the Raspberry plant, variety Sapphire, are in a very bad condition, they are brown and rotten. The tissue in the stem base has a grey appearance. Directly beneath the bark the tissue is reddish-brown. The symptoms are suspected from Phytophthora infestation.

The sample was initially assessed microscopically.
Oospores of Phytophthora and/or Pythium are visible, although a little different from normal.
Dark mycelium with septae is present.
Nematodes are present, but it is not clear whether they are plant parasitic.

Furthermore a DNA multiscan is applied for the presence of plant pathogenic fungi and oomycetes.
Phytophthora spp., Pythium spp. and Fusarium spp. are detected in high levels.
Fusarium solani, Cylindrocarpon destructans and Alternaria spp. are detected at low levels.

Phytophthora spp. can cause stem and root rot.
Fusarium species can be harmless or harmful, harmful species can cause stem- and root rot, in case of Fusarium oxysporum wilting disease.
Fusarium solani and Cylindrocarpon destructans can cause root rot.
Pythium spp. can cause root rot, but is often secondary.
Alternaria spp. can cause leaf spot disease and is here secondary.

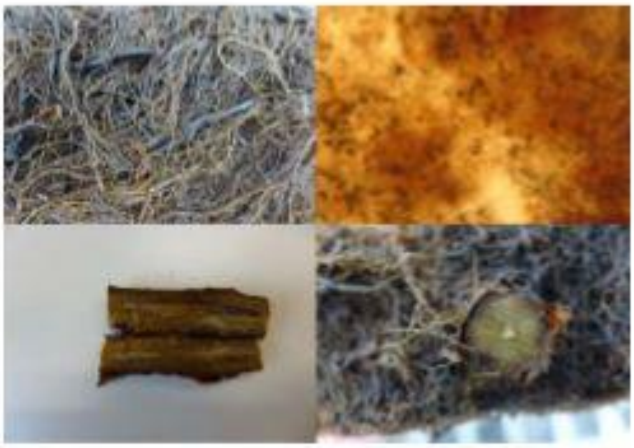
Subsequently the roots and the soil has been analysed for the presence of plant parasitic nematodes, but they were not found.

Based on these results we can conclude that Phytophthora spp. is the main cause of the symptoms in the Raspberry plant, variety Sapphire.
Pythium spp., Fusarium solani and Cylindrocarpon destructans are secondary root rot pathogens.

Phytophthora and Pythium species can spread easily by zoöspores through water.
Both pathogens form oöspores (survival structures), which can survive in soil and infected material for a long time.

It's important to avoid too wet growing conditions. Take care of proper drainage.
Start with clean soil, pots, water and of course clean starting material. Disinfect recirculation water if necessary/possible.

Material upon arrival



Method

The reported results only refer to the processed material.

Appendix 4: Results of data analysis (HAMEAU, 2019)

- **Stage of development**

F-Test

	Modality (%)	*stage*
Mean	48,8261851	33,67268623
Variance	1133,46067	445,2274649
Observations	443	443
Degrees of freedom	442	442
F	2,545801325	
P(F<=f) unilatéral	2,53018E-22	
F critical (unilateral)	1,169579013	

ANOVA One-way

DETAILED REPORT

Groups	*Number of samples*	*Sum*	*Mean*	*Variance*
0	7	202,1333333	28,87619048	478,4406349
30	7	198	28,28571429	466,6680952
50	7	202,9344086	28,9906298	490,9069945
70	7	201,7111111	28,81587302	511,3100353
100	7	192,8227273	27,5461039	459,1554093

VARIANCE ANALYSIS

Variations	*Ssquare sum*	*Freedom's degree*	*Square mean*	*F*	*Probability*	*F critical*
Between groups	10,06464666	4	2,516161664	0,005227886	0,999942153	2,689627574
Within groups	14438,88702	30	481,2962339			
Total	14448,95166	34				

- **Dead canes**

F-test		
	Modality	*cumulated_nb_dead_canes*
Mean	50	0,246666667
Variance	1162,583519	0,18623608
Observations	450	450
Degrees of freedom	449	449
F	6242,525712	
P(F<=f) unilatéral	0	
F critical (unilateral)	1,168143115	

ANOVA One-way

DETAILED REPORT

Groups	*Number of samples*	*Sum*	*Mean*	*Variance*
0	7	6	0,857142857	2,476190476
30	7	13	1,857142857	6,142857143
50	7	20	2,857142857	18,14285714
70	7	32	4,571428571	7,952380952
100	7	40	5,714285714	22,23809524

VARIANCE ANALYSIS

Varaitions sources	*Square sum*	*Freedom degrees*	*Square mean*	*F*	*Probability*	*F critical*
Between groups	109,2571429	4	27,31428571	2,397993311	0,072222621	2,689627574
Within groups	341,7142857	30	11,39047619			
Total	450,9714286	34				

- **Axillary buds**

F-test		
	modality	*nb axillary buds*
Mean	48,27586207	11,76206897
Variance	1114,664121	134,7286601
Observations	290	290
Degrees of freedom	289	289
F	8,273400186	
P(F<=f) unilateral	3,51235E-62	
Critical F-value (unilateral)	1,213891028	

ANOVA One-way

DETAILED REPORT

Groups	*Number of samples*	*Sum*	*Mean*	*Variance*
0	4	47,01818182	11,75454545	137,9767493
30	4	42,3	10,575	145,3387963
50	4	55,12580645	13,78145161	225,3387955
70	4	47,65151515	11,91287879	156,1521786
100	4	36,02272727	9,005681818	93,27103822

ANALYSIS OF VARIANCE

Variations' sources	*Square sum*	*Degrees of freedom*	*Square mean*	*F*	*Probability*	*F critical*
Between groups	49,89307496	4	12,47326874	0,082269081	0,986617893	3,055568276
Within groups	2274,232674	15	151,6155116			
Total	2324,125749	19				

- **Number of laterals per cane**

F-test		
	Modality (%)	*Number of laterals*
Mean	48,27586207	13,29310345
Variance	1114,664121	29,64735712
Observations	290	290
Degrees of freedom	289	289
F	37,59741945	
P(F<=f) unilateral	3,0598E-146	
Critical F-value (unilateral)	1,213891028	

ANOVA One-way

DETAILED REPORT

Groups	*Number of samples*	*Sum*	*Mean*	*Variance*
0	4	62,86666667	15,71666667	5,087777778
30	4	51,47575758	12,86893939	6,589515611
50	4	62,89829545	15,72457386	3,479423075
70	4	53,1	13,275	7,6625
100	4	43,5	10,875	6,5825

ANALYSIS OF VARIANCE

Variations' sources	*Square sum*	*Degrees of freedom*	*Square mean*	*F*	*Probability*	*F critical*
Between groups	68,06975351	4	17,01743838	2,893953215	0,05849791	3,055568276
Within groups	88,20514939	15	5,880343293			
Total	156,2749029	19				

- **Cumulated dead canes: two-sample t-test: "TRUE" answers**

A "true" answer was obtained when the bilateral p-value was up to alpha (=0,05), as the failure threshold (=alpha) of 5% was admitted in the test (this is the mostly used failure threshold while conducting statistical tests). Otherwise, a "false" result was obtained. For a wish to do not make invisible results, only the results with a "TRUE" answer to this test have been submitted below.

Equality test of mathematical expectations: two sample t-test of equal variances

	30	70	
Mean	2,166667	5,333333	
Variance	6,566667	4,666667	
Observations	6	6	
Weighted variance	5,616667		
Hypothetical difference of means	0		
Degree of freedom	10		
T statistic	-2,31432		
P(T<=t) unilatéral	0,021594		
t critical value (unilatéral)	1,812461		
P(T<=t) bilatéral	0,043188		TRUE
t critical (bilatéral)	2,228139		

Equality test of mathematical expectations: two sample t-test of equal variances

	0	100	
Mean	1	6,666667	
Variance	2,8	19,06667	
Observations	6	6	
Weighted variance	10,93333		
Hypothetical difference of means	0		
Degree of freedom	10		
T statistic	-2,96833		
P(T<=t) unilatéral	0,007043		
t critical value (unilatéral)	1,812461		
P(T<=t) bilatéral	0,014086		TRUE
t critical (bilatéral)	2,228139		

Equality test of mathematical expectations: two sample t-test of equal variances

	0	70	
Mean	1	5,333333333	
Variance	2,8	4,666666667	
Observations	6	6	
Weighted variance	3,733333333		
Hypothetical difference of means	0		
Degree of freedom	10		
T statistic	-3,88449298		
P(T<=t) unilatéral	0,001518322		
t critical value (unilatéral)	1,812461123		
P(T<=t) bilatéral	0,003036643		TRUE
t critical (bilatéral)	2,228138852		

– **Number of laterals per cane: two-sample t-test: “FALSE” answers**

As for processing two-sample t-test for dead canes, a “true” answer was obtained when the bilateral p-value was up to alpha (=0,05), as the failure threshold (=alpha) of 5% was admitted in the test (this is the mostly used failure threshold while conducting statistical tests). Otherwise, a “false” result was obtained. For a wish to do not make invisible results, only the results with a “TRUE” answer to this test have been submitted below.

Only “FALSE” answers were obtained. Below is submitted an overview of some “FALSE” results obtained. For not getting a patchy visibility, only few of them have been decided to be submitted.

	0	*30*	
Mean	220,25	178,25	
Variance	11870,25	7074,91667	
Observations	4	4	
Weighted variance	9472,583333		
Hypothetical difference of means	0		
Degree of freedom	6		
T statistic	0,610281312		
P(T<=t) unilatéral	0,282030031		
t critical value (unilatéral)	1,943180281		
P(T<=t) bilatéral	0,564060061		FALSE
t critical (bilatéral)	2,446911851		

Test d'égalité des espérances: deux observations de variances égales

	0	*50*	
Mean	220,25	232,5	
Variance	11870,25	19623	
Observations	4	4	
Weighted variance	15746,625		
Hypothetical difference of means	0		
Degree of freedom	6		
T statistic	-0,138056654		
P(T<=t) unilatéral	0,447356181		
t critical value (unilatéral)	1,943180281		
P(T<=t) bilatéral	0,894712361		FALSE
t critical (bilatéral)	2,446911851		

Test d'égalité des espérances: deux observations de variances égales

	30	*50*		
Mean	178,25	232,5		
Variance	7074,916667	19623		
Observations	4	4		
Weighted variance	13348,95833			
Hypothetical difference of means	0			
Degree of freedom	6			
T statistic	-0,664035124			
P(T<=t) unilatéral	0,265670816			
t critical value (unilatéral)	1,943180281			
P(T<=t) bilatéral	0,531341632		0,05	FALSE
t critical (bilatéral)	2,446911851			

Test d'égalité des espérances: deux observations de variances égales

	70	100	
Mean	197,25	135,5	
Variance	16246,25	3395,6667	
Observations	4	4	
Weighted variance	9820,9583		
Hypothetical difference of means	0		
Degree of freedom	6		
T statistic	0,8812011		
P(T<=t) unilatéral	0,2060567		
t critical value (unilatéral)	1,9431803		
P(T<=t) bilatéral	0,4121135		FALSE
t critical (bilatéral)	2,4469119		