Raspberry cane propagation

Phenotype, leaf sap composition and disease susceptibility of organic and conventional raspberry canes

Annemieke Rutten
December 2022

Internship– MSc plant sciences

Vlamings BV
Jelle Gerstel
Tim van den Hurk

WAGENINGEN UNIVERSITY & RESEARCH

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MSc internship Plant Production Systems

Institution:	*Wageningen University*
Student name:	*Annemieke Rutten*
Registration number:	*1024551*
Study program:	*MSc Plant Sciences - Specialization Natural resource management*
Chair group:	*Plant Production Systems*
Course code:	*PPS - 70224*
Supervisor:	*Gerrie van de Ven*
Date:	*December 2022*
Internship company:	*Vlamings BV.*
Supervisors:	*Jelle Gerstel*
	Tim van den Hurk

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Contents

Abstract

Raspberries (*Rubus idaeus)* are grown on biennial canes on a perennial root. Originally raspberry plants grow in soils, but due to susceptibility to soilborne diseases and quality problems, they are increasingly cultivated in substrate. The main component of substrate is peat. However, this is being replaced by more sustainable products. Compost is one of the substitutes for peat and consists of partially decomposed biological (waste) materials. Compost contains nutrients that stimulate plant growth, and it has potential to suppress soilborne diseases through the microbiome. Plant fertilization also influences disease susceptibility. For example, plant material with high nitrogen is more susceptible to biotrophic diseases while material with low nitrogen is more susceptible to hemi-biotrophic and necrotrophic diseases.

Agricultural supplier Vlamings BV commissioned this study. It investigated the differences between conventional and organic raspberry canes (variety: Tulameen and Glen Ample) at a commercial propagator of raspberry canes. The phenotype, leaf sap composition and disease susceptibility to the biotrophic disease Rust (Phragmidium rubi-idaei) and the necrotrophic cane disease Botrytis (Botrytis cinerea) were examined. It was also examined whether the phenotype of raspberry plants can be controlled at a late stage of cultivation by changing the fertilization scheme.

The phenotypic traits analyzed were cane length, cane thickness, leaf size and number of buds on the final cane of 180cm. To determine disease susceptibility, 15 pots of organic raspberries were swapped with 15 pots of conventional raspberries. As a result, each field contained both conventional and organic raspberry plants that could be compared without differences in environment and spraying schedules affecting the results. Rust infection intensity was determined using a score table, Botrytis infection was determined by determining what percentage of the cane was infected. The effect of the cultivation change on phenotype was determined by determining the cane length, length of bud growth, cane thickness, number of buds at the final cane and root score of the switched organic and conventional raspberry canes.

This study showed that the organic plants are smaller and thinner than the conventional plants and have smaller leaves. The organic plants did have more buds at the final length of 180cm and higher root scores than the conventional plants. In addition, Rust and Botrytis infections were less severe on the organic raspberry plants than on the conventional plants. A cultivation change at a late stage of growth had no effect on cane length, bud growth and cane thickness of the raspberry plants, but it reduced rooting and the number of buds at the final length.

The differences in phenotype, leaf sap and disease susceptibility were probably caused by one or more management differences between the organic and conventional plants. These management differences are the amount of nitrogen fertilization (organic: 112 kg N/ha, conventional: 300 kg N/ha), the type of nitrogen fertilization (organic: amino acids, conventional: NO_3^- and NH_4^+), the type of substrate (organic: with compost, conventional: without compost) and the application of root stimulants (organic: weekly, conventional: 3x total). To find out which factors influence to differences in phenotype, leaf sap and disease susceptibility, research must be done in which only one management factor differs for each treatment. Also, examining different years and weather conditions is important to see if the differences between organic and conventional canes are consistent.

1. Introduction

1.1 Background

Globally, between 800 and 900 tons of raspberries are produced annually (FAO, n.d.). Raspberries are grown on biennial canes on perennial roots. Originally, raspberries are grown in the soil, but due to susceptibility to root diseases and quality problems this is increasingly being replaced by substrate cultivation (Linnemannstöns, 2019). Substrate cultivation can take place in several ways. Summer raspberries are grown by growing a cane with flower buds in the first year. After a cold period, the buds break in the second year and raspberries are produced in the summer. Autumn raspberries are grown in one year. In autumn, the upper buds of the cane already produce fruit. The lower part of the cane can then be kept for a two-year production of one stem. After a cold period, the lower buds break in spring (Carew et al., 2000; Linnemannstöns, 2019).

Peat is the most common substrate for plant cultivation (Pascual et al., 2018), but due to the non-renewable nature of peat, alternatives are increasingly being used. Compost is an organic alternative to peat made by the partial decomposition of biological (waste) materials (Pascual et al., 2018). Compost can stimulate plant growth because it contains various nutrients (Gondek et al., 2020) and it can have a suppressive effect on infection by soilborne diseases (Rasool et al., 2021; Milinkovic, 2019; Escuadra & Amemiya, 2008; Darby et al., 2006; Termorshuizen et al., 2006; Kavroulakis et al., 2005). The effect that compost has on soilborne diseases depends on the type of compost, the host plant, and the disease. Termorshuizen et al. (2006) studied 18 compost types and 7 soil diseases and found that in half of the cases the disease was suppressed. Only very rarely (3%) the disease was stimulated by the compost.

Next to the substrate, fertilizer also affects plant growth and disease susceptibility (De & De, 2019). Nitrogen is the most important plant fertilizer, making up to 1.5-2% of plant dry matter. The effect of nitrogen on disease susceptibility has often been studied (Elad, 2021; Rav David, 2021; Barnea et al., 2022; Hoffland et al., 2000; Hoffland et al., 1999), but found to be quite variable. Among others, pathogen type and chemical form of nitrogen influence the effect of fertilization on disease susceptibility (Dordas, 2008). The difference between pathogens can be explained by their food source. Biotrophic pathogens, which get their nutrition from living cells, infect plants with high nitrogen concentration more easily. High nitrogen concentration promotes growth, but relatively less is invested in physical protective structures such as lignin or the cuticle (Sun et al., 2020). This facilitates infection of biotrophic diseases. In contrast, hemi-biotrophic or necrotrophic pathogens infect plants with low nitrogen concentration more easily. This is because these pathogens live on dead or dying plant material (Dordas, 2008) and nitrogen deficiency accelerates aging (Mu & Chen, 2021), which facilitates the infection.

The chemical form in which nitrogen fertilization is applied can also influence the intensity of disease infections (Dordas, 2008; Sun et al., 2020). The form of nitrogen affects secondary metabolites in the plant's biochemical defense system (Sun et al., 2020). These studies mostly consider nitrogen in the forms of NO_3^- and NH_4^+. However, besides nitrate and ammonium, there are other forms of nitrogen fertilization that can influence plant defenses. Plants can also take up organic nitrogen, in the form of amino acids or proteins (Tegeder & Rentsch, 2010; Näsholm et al., 2009). Amino acids can be applied in plant cultivation as fertilizers or bio stimulants (Jeber & Khaeim, 2019; Radkowski, 2018; Baglieri et al., 2014). Amino acids from plant protein hydrolysate can have positive effects on plant nutrient uptake, stress tolerance, microbiome, growth, and product quality (Choi et al., 2022; Paul et al., 2019; Colla et al., 2017; Colla et al., 2015; Baglieri et al., 2014). A recent study by Barrada et al. (2022) investigated the effect of Soy protein hydrolysate on tomato growth, yield and tolerance to Botrytis and Pseudomonas. This study found that the addition of this biostimulant increased growth and yield. No effect was found against Botrytis, but the biostimulant increased tolerance against Pseudomonas infection.

Two common fungal diseases of raspberries are Rust (Phragmidium rubi-idaei) and Botrytis (Botrytis cinerea) (Graham et al., 2019; Dolan et al., 2018). Rust is a biotrophic pathogen (Evans & Bruzzese, 2003) and can show itself early in the season by distinct orange spots on the leaves (Dolan et al., 2018). Toward autumn, Rust produces black teliospores, with which it overwinters. One way to reduce Rust infection is to improve air circulation in the crop. Cane botrytis is a necrotrophic pathogen that lives on dead and dying plant material of all plants (Dolan et al., 2018). Dry air, splashes and insects can spread the fungus. The fungus first infects aging leaves and then spreads through the buds to the stem. An infection of Botrytis can cause serious crop reductions because infected buds do not sprout properly. Fungicides and good ventilation can control and reduce Botrytis.

1.2 Research objective

This research was commissioned by Vlamings BV, an agricultural supplier in Noord-Brabant. Among other things, they supply fertilizers and crop protection products to their customers and advise on applications. One of their clients is Genson, a grower of strawberries, raspberries, and leeks. For several years, Genson has been growing some of its raspberry plants organically using advice and fertilizers from Vlamings. These organic raspberry plants seemed to differ from conventional plants in phenotype (including color and size). Besides this, in 2022, the buds on the two-year-old conventional canes did not break as evenly as on the organic stems. This was possibly due to Botrytis infection of the conventional buds. The purpose of the current study is to identify the differences between organic and conventional raspberry plants. The focus is on phenotype, disease susceptibility and leaf sap composition. Phenotype and disease susceptibility were chosen because of observations from previous years. Leaf sap composition is studied because Vlamings' advisors use this method for fertilization recommendations. Finally, it is investigated whether the phenotype of the plant can be controlled by temporarily applying a different fertilization scheme.

1.3 Research questions

The differences in phenotype, leaf sap composition and disease susceptibility between organic and conventional raspberry canes and the effect of a cultivation change on cane phenotype are examined using three research questions:

1. What are the differences in cane length, cane thickness, number of buds, rooting and leaf sap composition between organic and conventional raspberry plants?
2. What is the effect of cultivation method during propagation (organic or conventional) on Rust and Botrytis infection?
3. Can changing the cultivation method at a late stage of propagation influence the phenotype of the raspberry cane?

2. Methods

2.1 General

This research was conducted from August through December 2022 at a commercial raspberry propagator located in Noord-Brabant. The raspberry varieties used in this study were summer raspberries: 'Glen Ample' and 'Tulameen'. The measurements were conducted on raspberry stems in Westerbeek and Sint-Oedenrode. In Westerbeek is the 'organic field' with the organic raspberries of the varieties Glen Ample and Tulameen. In Sint-Oedenrode, the conventional raspberries are grown. The conventional canes of Glen Ample and Tulameen are in the 'Donte' and 'Rijtvenweg' fields, respectively. Figure 1 shows where the three fields are located and figures 2, 3 and 4 show the arrangement of the surroundings.

Figure 1: Location of the organic and conventional plants. The organic raspberries are on the organic field. The conventional Glen Ample is on Donte and the conventional Tulameen is on Rijtvenweg. Map taken from Google Maps (n.d.).

Figure 2: Area map of conventional raspberries (variety: Glen Ample) on Donte in the fall of 2022. The red square shows the location of the raspberries in this study.

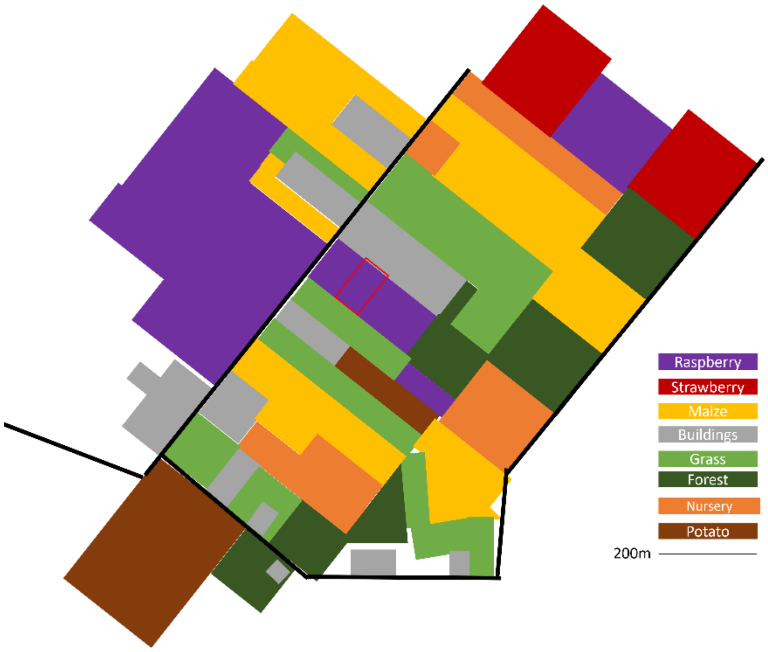


Figure 3: Area map of conventional raspberries (variety: Tulameen) on Rijtvenweg in the fall of 2022. The red square shows the location of the raspberries in this study.

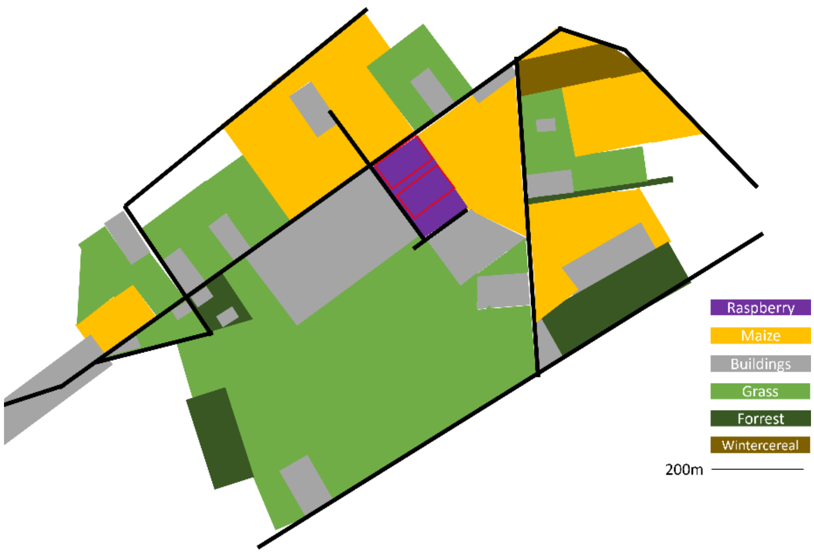


Figure 4: Area map of organic raspberries (varieties: Tulameen and Glen Ample) in the organic field in the fall of 2022. The upper red square shows the location of the variety Tulameen in this study, the lower square that of the variety Glen Ample.

The raspberry plants were put outside on the field in May. The organic Glen Ample were put outside first, on May 3. The conventional Glen Ample were put on the field on May 16, the organic Tulameen on May 23, and the conventional Tulameen on May 27. The raspberry plants are grown in 1.8-liter pots with two canes per pot. The substrate consists of peat moss (conventional 50%; organic 45%), wood fiber (conventional 35%; organic 30%) and perlite (15%). For the organic raspberries, the substrate also contains 10% compost.

The conventional raspberries are fertilized daily with a standard fertigation scheme in which the main and trace elements are supplied in inorganic form by artificial fertilizers. Organic raspberry canes receive fertilization once a week, usually on Tuesdays or Wednesdays, with SKAL certified fertilizer (SKAL, n.d.). The nitrogen in the organic fertilization scheme is given in the form of amino acids from a soy protein hydrolysate (≈112 kg N/ha) (commercial name: FERTICELL) (Agroplasma, 2019). Conventional raspberries receive more nitrogen than the organic raspberries, in the form of nitrate and ammonium (≈300 kg N/ha). The other elements are given in the same chemical form to the organic and the conventional raspberry plants. In addition to regular fertilization, root stimulants have been applied to the raspberries during the season. The conventional raspberries received root stimulants in early June, late August, and early September. The organic raspberries were given root stimulants weekly, with an additional dose in early June.

No sprays against diseases or pests occurred at the organic field. Crop protection products were used on the conventional field. Against Rust and Botrytis, both preventive and curative fungicides were applied. Rust preventatives were sprayed on June 21st (Signum) and on July 6th (Signum) and 27th (Flint). A curative fungicide for Rust was applied on September 19th (Topaz). Treatments for Botrytis were mostly preventive. On September 13th (Captosan), 19th (Switch) and 30th (Teldor), October 21st (Captosan) and once on November 4th (Teldor). The treatment on September 19th was with a product (Switch) that also has a mild curative effect.

In addition to fertilization and crop protection, management was conducted in the form of tipping and defoliating the plants. The conventional Tulameen and Glen Ample were tipped in late August (at 210cm) and early September (at 200cm), respectively. During the same period, the lower 30cm of the conventional plants was defoliated. In the organic field, this management was conducted later. The organic Tulameen was tipped at the end of October (at 200cm), and the first 30cm of the organic plants was defoliated at the end of September. All canes were tipped one to two weeks before packing at the final length of 180cm.

2.2 Phenotype and leaf sap composition (research question 1)

A raspberry cane is during the cultivation characterized by one long cane with compound leaves with 3 or 5 leaflets attached. The phenotype of the plant can be described in, among others, cane length, cane thickness, number of leaves and buds, leaf size, leaf color, cane color, root length and root surface. During the period between Aug. 12th and Nov. 25th, the phenotypic traits cane length, leaf size, rooting, thickness of the final cane and number of buds on the final cane were measured and the composition of the leaf sap was determined. The characteristics leaf and cane color and root length and area were not determined because the necessary equipment was not available for this purpose. In appendix 1, table A1, all measurements and dates are given.

2.2.1 Measurements

Cane length and leaf size

Cane length of the organic and conventional raspberries was measured on August 12th and 25th. The cane length measurement was performed in 15 replicates. The leaf size of raspberries was measured on August 12th. Leaf size was measured in 30 repetitions, 15 repetitions at 50cm height and 15 at 100cm in the crop. The repetitions were conducted in 5 rows spread over the approximately 25 rows of the field.

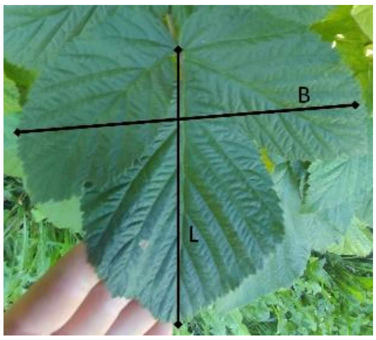


Figure 5: Length and width measurement of the composite raspberry leaf.

Cane length was measured from the potting soil to the tip of the plant. For leaf size, the length of the leaf vein and the width of a mature leaf were measured. Leaf vein length was measured from the tip of the leaf to its attachment to the petiole. Leaf width was measured at the widest point of the leaf perpendicular to the leaf vein (Figure 5). A model by Fallova et al. (2008) was used to calculate the leaf area from the length and width of the leaf. The formula used is LA=0.03 + 0.71 L x W. Here LA is the leaf area, L is the length of the leaf vein and W is the width of the leaf.

Cane thickness and number of buds

After the raspberry canes were tipped at their final length of 180cm, cane thickness and number of buds were measured. These measurements were taken on November 3rd (organic Tulameen) and November 16th (organic Glen Ample and conventional Tulameen and Glen Ample). Measurements were taken with fifteen replicates, in 3 rows of the field.

Cane diameter was measured at 3 heights: 10-20cm, 90-100cm and 160-170cm. These three measurement points were then used to calculate the average cane thickness. The number of buds was measured by counting the number of buds on the cane from the bottom to the top.

Roots

The roots of the raspberries were assessed from photos for which the pots were removed from the roots. From August 26th through November 3rd, photos were taken once or twice a week of 6 root blocks of the organic and conventional Tulameen. On August 30th, photos were also taken of 6 root blocks of the organic and conventional Glen Ample, but due to difficulties in removing the pots of the organic Glen Ample, it was decided not to continue this. At each measurement time, two photos were taken of 3 root blocks side by side. The root blocks in the photograph were scored on a scale of 1-5 as described in Table 1 and shown in Figure 6.

Table 1: description scores to indicate rooting.

Score	Description
1	Poor rooting, little to no white roots visible.
2	Moderate rooting, <30% visible white roots.
3	Good rooting, 30-60% visible white roots.
4	Very good rooting, 60-90% visible white roots.
5	Exceptionally good rooting, >90% visible white roots, potting soil not visible between roots.

Figure 6: Right to left: root score 1 to 5

Leaf sap analysis

Leaf sap composition was analyzed with plant sap measurements performed by NovaCropControl. The exact methods of the analyses are known to NovaCropControl. Sampling was performed as described in the NovaCropControl manual (NovaCropControl, 2019). In week 34, three samples were taken from the organic raspberries, on August 22nd, 24th and 26th. This was done because the organic raspberries receive fertilization only once a week, unlike the conventional raspberries which are fertilized daily. Conventional raspberries were sampled this week on Aug. 24th. After the first week, it was decided to sample Tulameen variety raspberries at the end of each week. Because the nitrogen concentration in the leaves was highest a few days after fertilization. Leaf sap samples were taken on Sept. 2nd, 15th and 29th. Glen Ample was sampled only during the first week. Table 2 shows a summary of the sampling moments. At each sampling moment, 2 samples were taken from all treatments, one from old leaves and one from young leaves.

Table 2: Sampling dates for the leaf sap analyses. A green box means that the treatment was sampled on this date.

Date	Tulameen Organic	Tulameen Conventional	Glen Ample Organic	Glen Ample Conventional
Mon, 22-8				
Wed, 24-8				
Fri, 26-8				
Fri, 2-9				
Thu, 15-9				
Thu, 29-9				

NovaCropControl's leaf sap analysis was used to determine twenty-two major and trace elements. In this study, the elements calcium, magnesium, chloride, sulfur, phosphate, and nitrogen were considered. These elements were chosen at the request of Vlamings. For nitrogen, 3 parameters were considered: total nitrogen, nitrogen from nitrate and the percentage of inorganic nitrogen of the total nitrogen. The last parameter was calculated by totaling the N from ammonium and the N from nitrate and dividing this by N total.

2.2.2 Statistical analysis

Statistical analyses were performed in R with the interface Rcommander. One-way ANOVA tests were used to compare the differences between the averages of organic and conventional raspberries of the two varieties. Side-by-side comparisons were used to see which differences were significant.

Cane length, Cane thickness, leaf area and number of buds at the final length were compared between the four variety-cultivation combinations. Cane length was measured at two dates individually and the comparison between the variety-cultivation combination was done separately for both dates. The average root score over the 14 measurement points for Tulameen and the 1 measurement point for Glen Ample were compared between the 4 variety-cultivation combinations.

The statistical analysis of the leaf sap composition was conducted over the measurements from August 24th and September 2nd, 15th and 29th of the variety Tulameen. On these dates samples were taken from both organic and conventional plants. One-way ANOVA tests were used to test the differences between cultivation methods and leaf age for the elements: calcium, magnesium, chloride, sulfur, phosphate and total nitrogen, nitrogen from nitrate and percentage of inorganic nitrogen.

2.3 Fungal infection experiment (research question 2)

2.3.1 Experimental design

To determine the effect of the cultivation method during propagation on fungal infection, an experiment was set up in which a number of organic plants were swapped with an equal number of conventional plants. By putting organic and conventional plants in the same field with the same fertilization and spraying management (organic or conventional), the effect of the cultivation method during the beginning of the propagation on the infection pressure of Rust and Botrytis can be compared. This set-up minimizes the variation in infection pressure caused by spraying and environmental factors.

On August 26th, 2022, fifteen pots, each containing two raspberry canes, were transferred from the organic field to the conventional field (Rijtvenweg) and vice versa. The fifteen swapped pots were then placed side by side in the second pole section (from the path) of a row in the center of the field (Figure 7). Red and white ribbon was used to mark the start and end of the swapped planting area.

The Tulameen variety was chosen for this experiment because at the time of the swap, the organic Glen Ample canes had high infection pressure from Rust. In the conventional field, the infection of Rust was still much lower, and it was not desirable to bring the infected organic plants to the conventional field. The Tulameen variety is less susceptible to Rust and had low infection pressure at the time of the switch.

Figure 7: Organic raspberry plants (variety: Tulameen) among conventional plants on the Rijtvenweg. The photos were taken on August 26th, 2022, midway through the plant change.

2.3.2 Rust

To determine the infection level of Rust on the leaves of raspberries, photos were taken on August 25th of the lower and upper surfaces of the top leaves of the varieties Glen Ample and Tulameen in the organic field. At the time of the pictures, the infection of Tulameen was still very low (score 0-1 visible). In Glen Ample, the infection pressure of Rust was already higher (scores 1-5 visible). Using these leaves, a scoring table was created from 0 to 5 (Table 3, Figure 8).

The rust scores were determined for the four different cultivation methods of the raspberry canes in the experiment: organic, conventional, organic plants in the conventional field (organic-conventional) and conventional plants in the organic field (conventional-organic). Starting Sept. 2nd, one week after the switch experiment was initiated, Rust scores were measured weekly for 8 weeks. Measurements were taken on leaves in 3 rows across the field, at 3 heights in the crop (≈50cm, ≈100cm, and ≈150cm), and on both sides of the row.

Table 3: description of Rust scores

Score	Description
0	No Rust spores
1	A few spots of orange Rust traces
2	Groups of orange Rust spores scattered over <50% of the leaf blade
3	Groups of orange Rust spores, scattered over >50% of the leaf
4	Groups of orange and black Rust spores >50% of leaf, <10% necrosis
5	Groups of orange and black Rust spores >50% of leaves, >10% necrosis

Figure 8: from left to right rust score 0 to 5

The number of repetitions varied between 30 and 60 per cultivation method per date (Table 4). During the first two measurements, 15 leaves per height were measured, 45 repetitions in total. By September 15th, the lower leaves of the organic canes had been removed. Therefore, from September 15th onwards the measurements on the organic field were taken at only 2 heights (~100cm and ~150cm). At the conventional field, 3 heights were still measured. During the measurements from September 15th onward, 10 (conventional) or 15 (organic) leaves per height per side were measured, a total of 60 repetitions per cultivation method. On some dates there was insufficient time to complete the measurement completely and a lower number of repetitions was measured.

Table 4: number of repetitions of rust score measurements.

Date	organic	Conventional -organic	conventional	Organic-conventional
2-9	42	45	45	48
8-9	45	42	45	45
15-9	60	33	54	42
22-9	40	55	31	61
29-9	60	58	60	60
5-10	60	47	59	51
13-10	61	60	X	X
15-10	X	X	55	60

2.3.3 Botrytis

Botrytis measurements were conducted at the end of the canes' growing season, when Botrytis can become a problem. Measurements on the four treatments: organic, conventional, organic-conventional, and conventional-organic were taken on Oct. 20th, Oct. 27th, Nov. 3rd and Nov. 10th. For each treatment, 15 replicates were measured, in 3 rows spread across the field.

For the measurements, the canes were divided into four compartments starting from the ground: 0-50cm, 50-100cm, 100-150cm and 150-200cm. Four Botrytis characteristics were looked at in each compartment: fungal growth on the petioles (1), buds (2) and cane (3) and dark brown spots on the canes (4) (Figure 9). On Each cane 16 points were inspected for presence of Botrytis. The maximum number of Botrytis characteristic is 16. Then the infection percentage of each cane was calculated using the following formula:

Figure 9: Botrytis characteristics on the raspberry cane. Right: fungal growth on the petiole and dark brown spots on the stem. Left: fungal growth on the petiole and bud.

$$infection\ percentage = \frac{Found\ number\ of\ Botrytis\ characteristics}{Maximal\ number\ of\ Botrytis\ characteristics} \cdot 100\%$$

2.3.4 Statistical analysis

Statistical analyses were performed in R, using the interface Rcommander. ANOVA tests were used to see if the average rust score and Botrytis infection rates were similar for the different cultivation methods.

For the average rust score, three comparisons were made: one for all dates combined, one for the organic field per date, and one for the Rijtvenweg per date. For the comparison with all dates combined, thirty random measurement points were used for each date and cultivation mode. This was done to ensure that each date has the same weight in the total average despite the different numbers of replicates. In Excel, each measurement was assigned a random number between 0 and 1. The lowest thirty were selected for statistical analysis. In the ANOVA tests for the organic field and the Rijtvenweg, comparisons were made between organic and conventional-organic (organic field) and between conventional and organic-conventional (Rijtvenweg). These comparisons use all replicates as listed in Table 4.

The average infection rate of Botrytis was compared between the different cultivation methods. A side-by-side comparison was performed to see if the infection of Botrytis differs between treatments that are in the same field (conventional vs. organic-conventional and organic vs. conventional-organic).

2.4 Change in cultivation method (research question 3)

2.4.1 Experimental design and measurements

The final research question focuses on the effect of a change in cultivation method at a late stage of propagation on the phenotype of the plant. To answer this question, the same experiment was used as described in 2.3. Various plant phenotype characteristics were examined to see if the switched plants developed the same as those still in the original field. The four treatments in this exchange experiment are: organic, organic to conventional (organic-conventional), conventional and conventional switched organic (conventional-organic). The measured plant parameters are thickness of the final cane, number of buds on the final cane, cane length (organic), bud growth (conventional) and root score.

Measurements of cane thickness, number of buds, cane length and root score were made as described in section 2.2. The conventional plants were tipped just before the implementation of the exchange experiment (25-8). Therefore, not cane length, but the length of the growth from the buds was measured. Then the total length of all bud growth per cane was calculated. On September 13th and 22nd, the length of the growth of the buds was measured. Cane length of organic plants was measured on September 2nd, 13th and 22nd. An overview of all measurement times of the different parameters can be found in Appendix 1 table A1.

2.4.2 Statistical analysis

Statistical analysis of the above-mentioned data was performed in R with the interface Rcommander. The differences between the averages of the 4 treatments were statistically analyzed using one-way ANOVA tests. The cane lengths and number of buds on the final length were compared among the 4 treatments (organic, organic-conventional, conventional and conventional-organic) of the variety Tulameen. The cane lengths of the organic and the organic-conventional plants were measured at 3 dates. The differences in cane length for the two treatments and the 3 measurement dates were analyzed using a one-way ANOVA with side-by-side comparison. A similar comparison was made for the total length of bud growth of conventional and conventional-organic.

3. Results

3.1 Phenotype and leaf sap (research question 1)

3.1.1 Observations

Figure 10 shows the organic and conventional raspberry canes of the variety Tulameen on Aug. 25th and Oct. 27th. The leaf color of the organic plants was visibly lighter than that of the conventional plants during the growing season. During autumn, the organic plants released their leaves earlier than the conventional plants. Also, the organic stems lost their leaves gradually from bottom to top while the conventional plants lost their leaves first halfway up the plant.

Figure 10: Organic (left) and conventional (right) raspberry canes on Aug. 25th (top) and Oct. 27th (bottom). Variety: Tulameen

3.1.2 Cane length

Organic raspberry canes averaged lengths of 150 and 176cm (Glen Ample) and 139 and 189cm (Tulameen) on August 12th and 25th. The conventional plants have lengths of 171 and 213cm (Glen Ample) and 173 and 226cm (Tulameen) on these dates (Figure 11). For both varieties, on both August 12th and August 25th, conventional canes are significantly longer than organic canes.

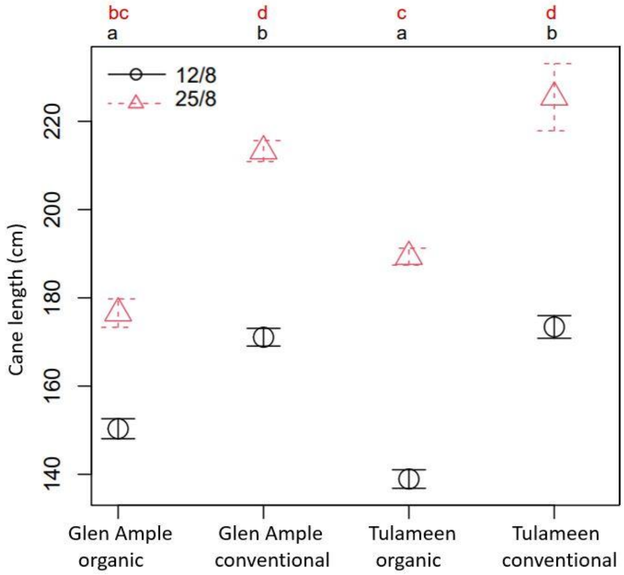


Figure 11: Cane length of organic and conventional Glen Ample and Tulameen. Different letters indicate significant differences (Anova). Error bars show standard error. α=0.05, n=15

3.1.3 Leaf size

The organic and conventional Glen Ample have average leaf sizes of 270 and 386cm2, respectively. Organic and conventional Tulameen leaves have areas of 217 and 324cm2 (Figure 12). For both Glen Ample and Tulameen, the leaves of organic plants are significantly smaller than the leaves of conventional plants.

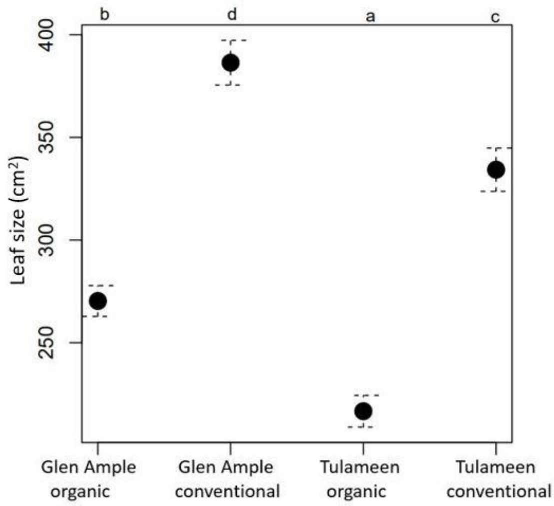


Figure 12: Leaf area of organic and conventional Glen Ample and Tulameen (Fallovo et al., 2008). Different letters indicate significant differences (Anova). Error bars show standard error. α=0.05, n=15

3.1.4 Cane thickness

The average cane thicknesses of the final cane of organic and conventional Glen Ample are 0.64 and 0.76cm, respectively. For Tulameen, the organic and conventional average cane thicknesses are 0.71 and 0.88cm (Figure 13). For both varieties, the organic stems are thinner than the conventional canes.

3.1.5. Number of buds

Organic Glen Ample averaged 37 buds on the final cane, while the conventional Glen Ample averaged 31 buds. For Tulameen, it was found that the organic canes averaged 30 buds and the conventional canes had 27 buds (Figure 14). The organic raspberry canes had more buds at the final length than the conventional canes in both varieties studied.

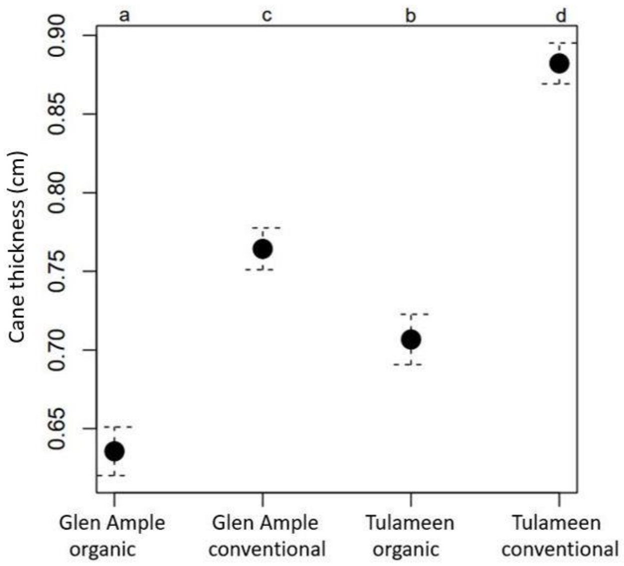


Figure 13: Cane thickness of organic and conventional Glen Ample and Tulameen. Different letters indicate significant differences (Anova). Error bars show standard error. α=0.05, n=15

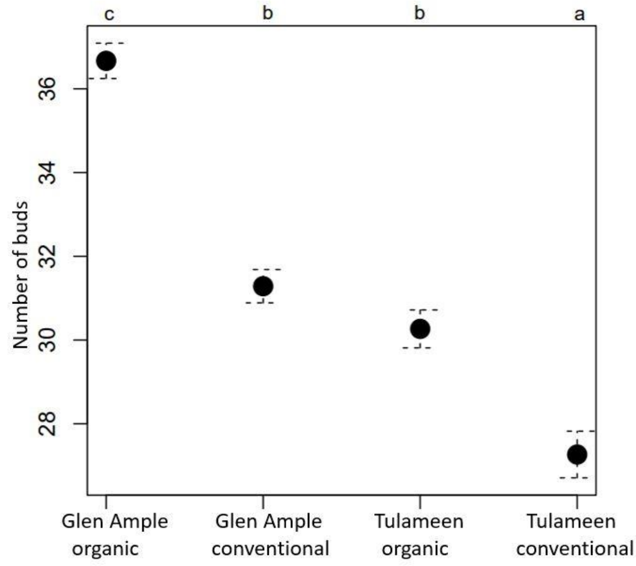


Figure 14: Number of buds at the final cane length (180cm) of organic and conventional Glen Ample and Tulameen. Different letters indicate significant differences (Anova). Error bars show standard error. α=0.05, n=15

3.1.6 Root score

Averaged over all measurement moments, the root scores of the organic plants are higher than those of the conventional plants for both Glen Ample and Tulameen (Figure 15). The root scores of Tulameen were higher than conventional plants in organic cultivation throughout the entire measurement period (Figure 16).

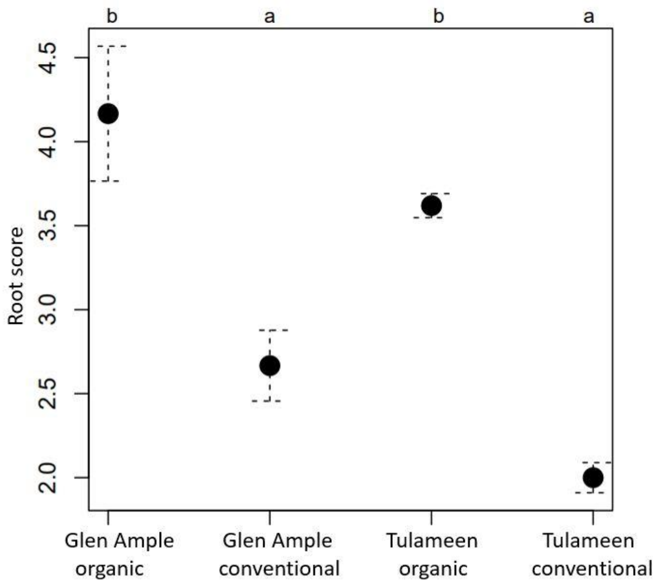


Figure 15: Root score of organic and conventional Glen Ample and Tulameen. Different letters indicate significant differences (Anova). Error bars show standard error. α=0.05, n=6 (Glen Ample), n=84 (Tulameen)

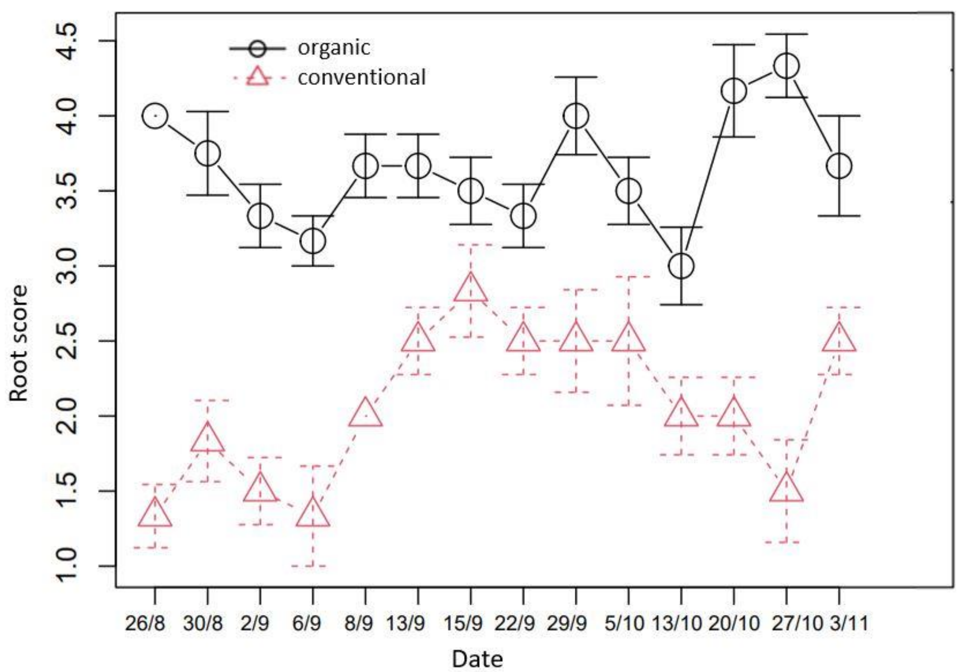


Figure 16: Root score of organic and conventional Tulameen over time. The error bars show the standard error. n=6

3.1.6 Leaf sap

The calcium contents differ significantly between the old and young leaves of conventional plants (Figure 17). In organic leaves, this difference is not significant because the variation is greater than for conventional. No difference in calcium content was found between the organic and conventional leaves. The percentage of inorganic nitrogen is higher in the conventional old leaf than in the organic old leaf (Figure 18). Nitrogen content from nitrate (Figure 19) is also higher in the conventional old leaf than in the organic old leaves. The amount of chlorine in the old leaf is higher in the organic than in the conventional plants (Figure 20). Total nitrogen, phosphate, sulfur, and magnesium did not differ significantly between the organic and conventional leaves for both the young and old leaves (Appendix 2, Figures A1, A2, A3 and A4).

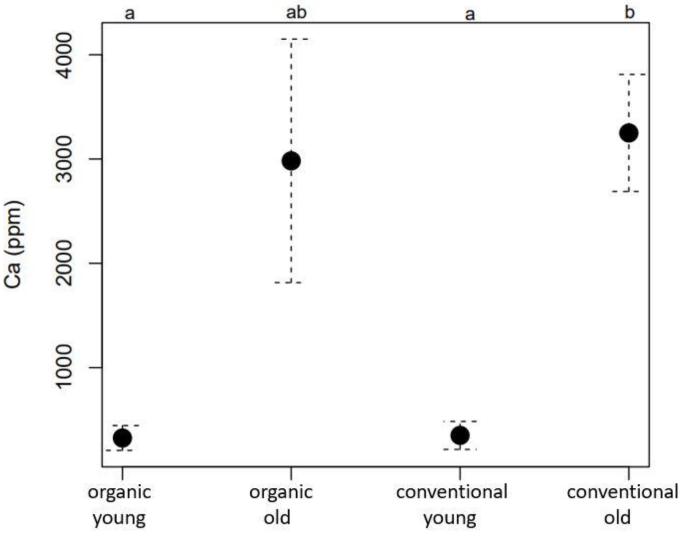


Figure 17: Calcium (Ca) in the leaf sap of young and old leaves of organic and conventional Tulameen. Different letters indicate significant differences (Anova). The error bars show the standard error. α=0.05, n=4

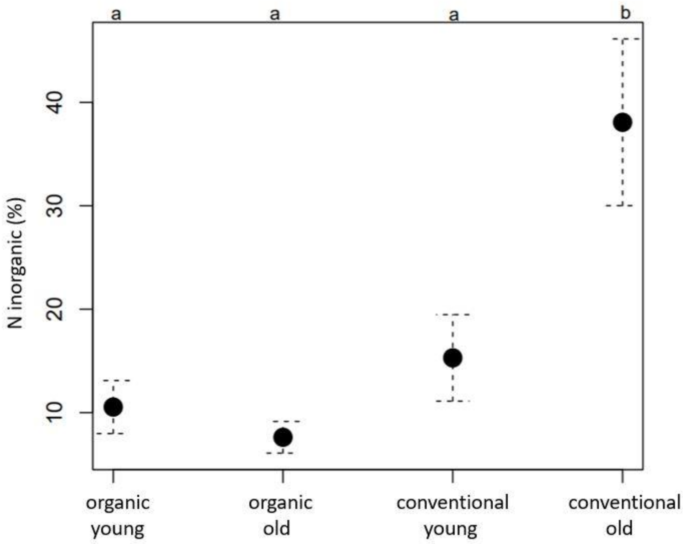


Figure 18: Percent of inorganic nitrogen (N) of total nitrogen in leaf sap of young and old leaves of organic and conventional Tulameen. Different letters indicate significant differences (Anova). The error bars show the standard error. α=0.05, n=4

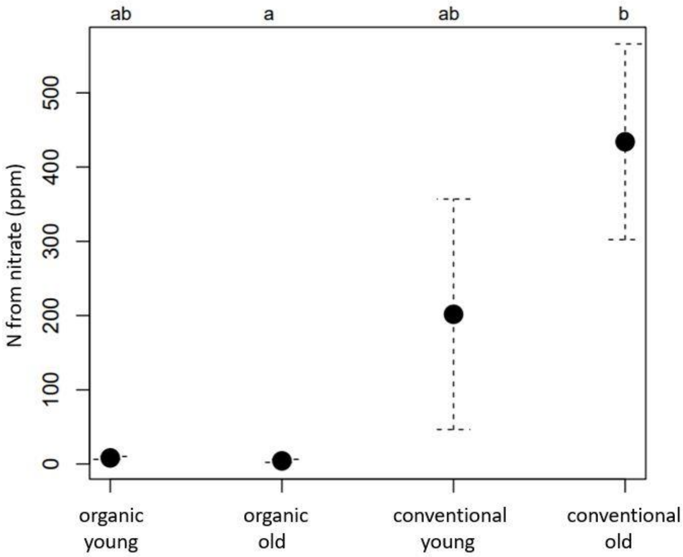


Figure 19: Nitrogen (N) from nitrate in the leaf sap of the young and old leaves of organic and conventional Tulameen. Different letters indicate significant differences (Anova). The error bars show the standard error. α=0.05, n=4

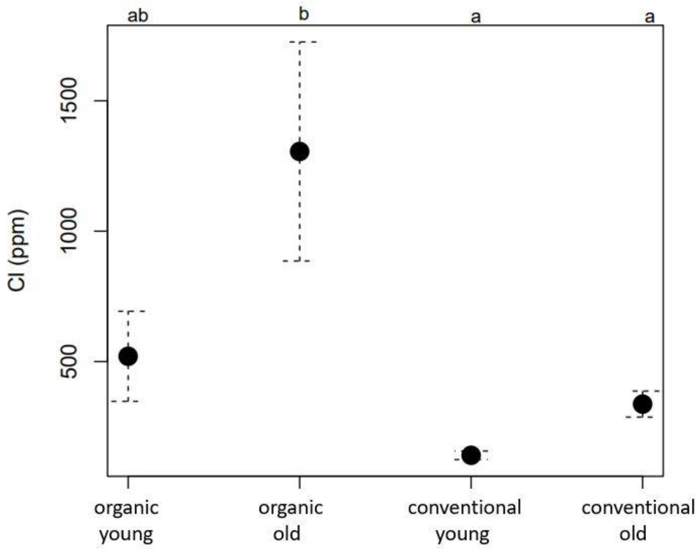


Figure 20: Chloride (Cl) in the leaf sap of young and old leaves of organic and conventional Tulameen. Different letters indicate significant differences (Anova). The error bars show the standard error. α=0.05, n=4

3.2 Fungal infection experiment (research question 2)

3.2.1 Rust

Figure 21 shows the average rust score over 8 weeks for the different cultivation methods. This figure shows that the organic raspberry canes in both the organic field (organic) and in the Rijtvenweg (organic to conventional) have a lower average rust score than the conventional plants in the same field.

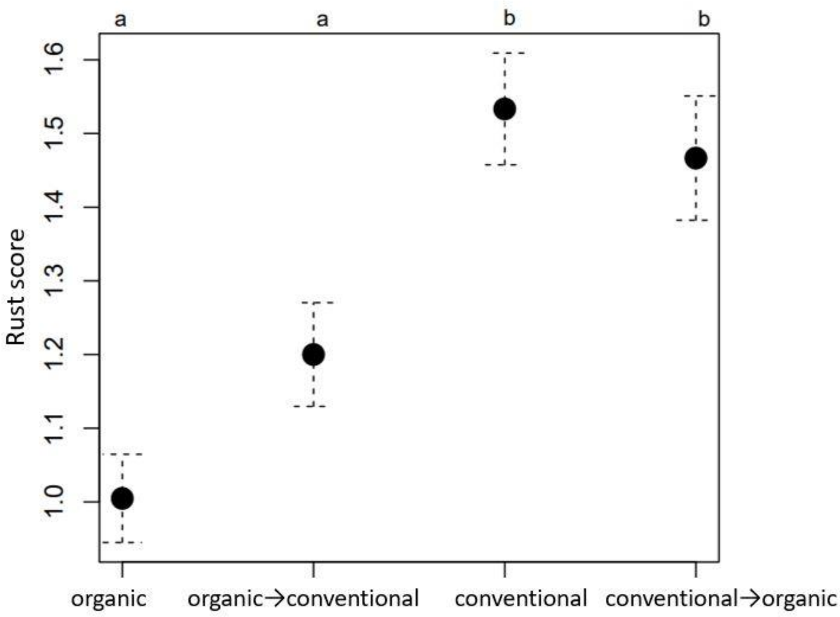


Figure 21: Average rust infection of raspberry plants (variety: Tulameen) in the organic field (organic & conventional-organic) and the Rijtvenweg (conventional & organic-conventional). Different letters indicate significant differences (Anova). Error bars show standard error. α=0.05, n=210

Figures 22 and 23 show the rust scores at the different dates for the organic field and Rijtvenweg, respectively. Here it can be seen that on the organic field, the rust score of the conventional plants was higher than the score of the organic plants. On the Rijtvenweg, the differences are much smaller than on the organic field. On Sept. 8th (Rijtvenweg), Sept. 22nd (organic field), Sept. 29th (organic field) and Oct. 13th (organic field), the conventional plants have significantly higher rust scores than the organic plants in the same field.

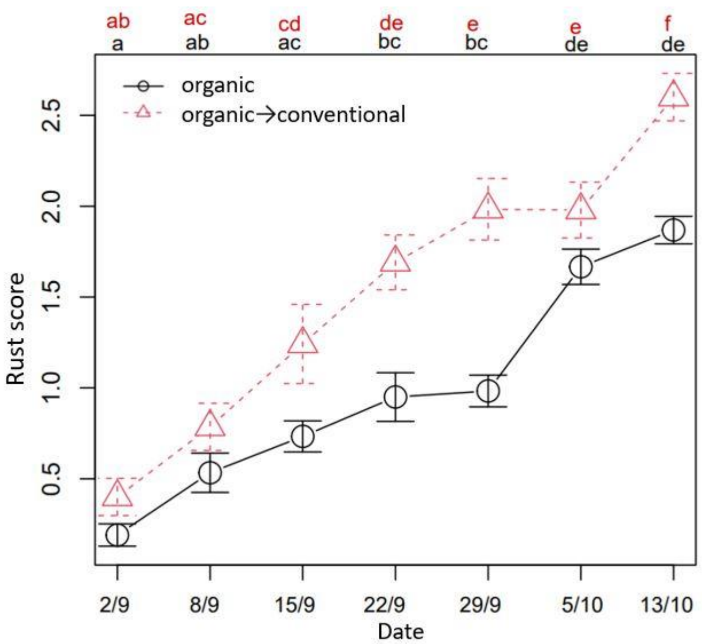


Figure 22: Average rust infection of raspberry leaves (variety: Tulameen) in the organic field (organic & conventional-organic). Different letters indicate significant differences (Anova). Error bars show standard error. α=0.05, n=30-60

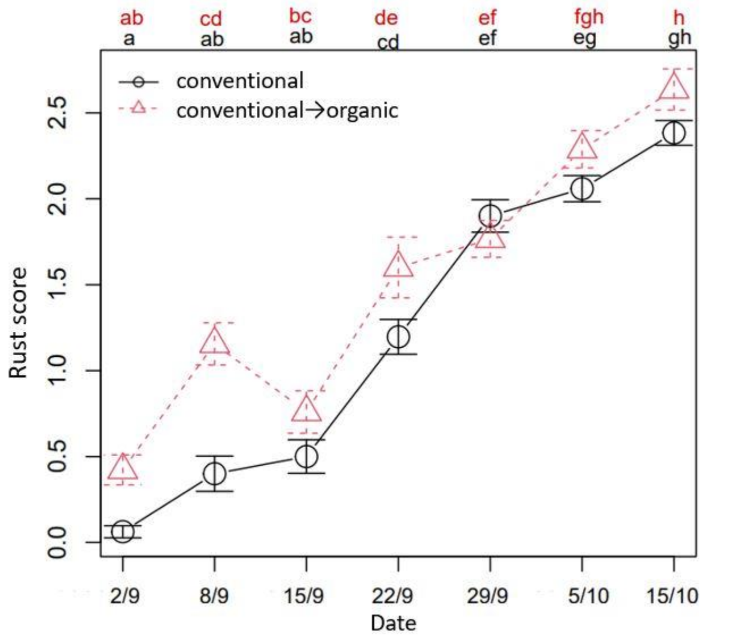


Figure 23: Average rust infection of raspberry leaves (variety: Tulameen) on the Rijtvenweg (conventional & organic-conventional). Different letters indicate significant differences (Anova). Error bars show standard error. α=0.05, n=30-60

3.2.2 Botrytis

On both the organic field and the Rijtvenweg, there was a very low infection pressure (<5%, Figure 24). On the biofield, only one Botrytis trait was found during the observations: a leaf stalk on a conventional stem. More Botrytis was found at the Rijtvenweg, but also only on the conventional plants. The organic plants in the conventional field did not have any Botrytis. On the Rijtvenweg, the infection rate of the conventional plants was significantly higher than that of the organic plants.

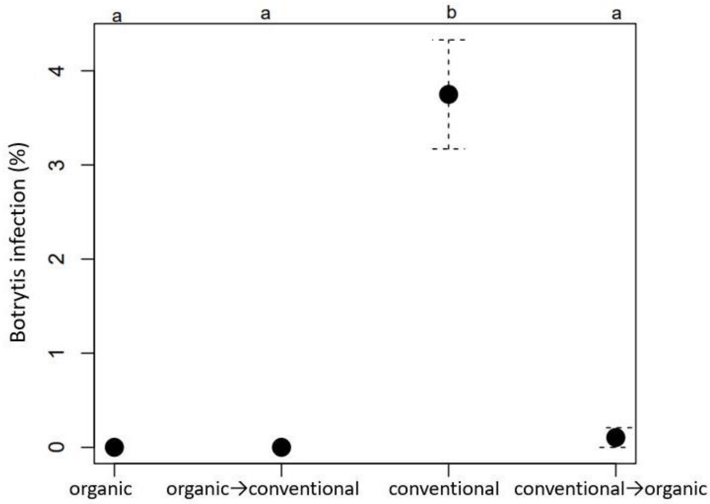


Figure 24: Botrytis infection percentage of raspberry plants (variety: Tulameen) in the organic field (organic & conventional-organic) and the Rijtvenweg (conventional & organic-conventional). Different letters indicate significant differences (Anova). Error bars show standard error. α=0.05, n=60

3.3 Change in cultivation method (research question 3)

3.3.1 Cane thickness

Figure 25 shows the cane thicknesses of both switched treatments, the organic canes and the conventional canes. The thickness of the organic canes switched to the conventional field is not significantly different from the canes that remained in the organic field. The same is true for conventional plants, again there is no significant difference between the plants that were switched and the ones that remained on the Rijtvenweg. There is, however, a significant difference in thickness between the organic and conventional plants (Figure 25).

3.3.2 Number of buds

The number of buds on the final cane length differs significantly between the switched plants and the plants left on their original field (Figure 26). The organic plants that went to the conventional field (organic-conventional) have significantly fewer buds on the cane of 180cm than the organic plants, but the same number of buds as the conventional plants. The conventional plants that went to the organic field (conventional-organic) have fewer buds than the conventional plants that remained at the Rijtvenweg.

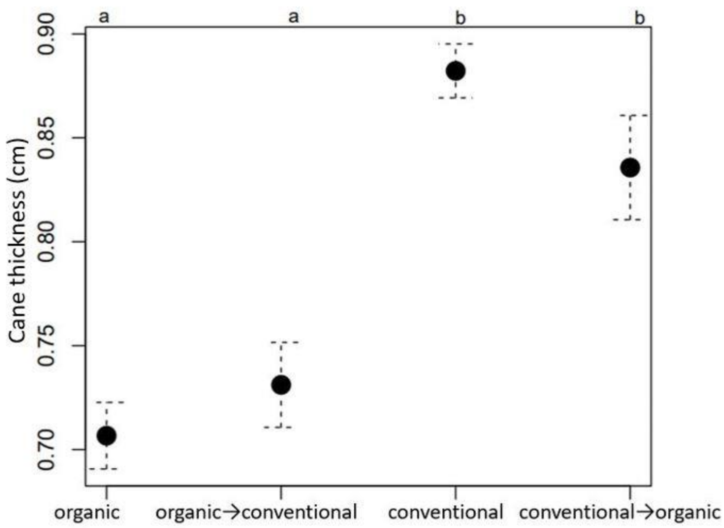


Figure 25: Cane thickness of organic, conventional and switched raspberry canes (organic-conventional and conventional-organic) (variety: Tulameen). Different letters indicate significant differences (Anova), The error bars show the standard error. α=0.05, n=15

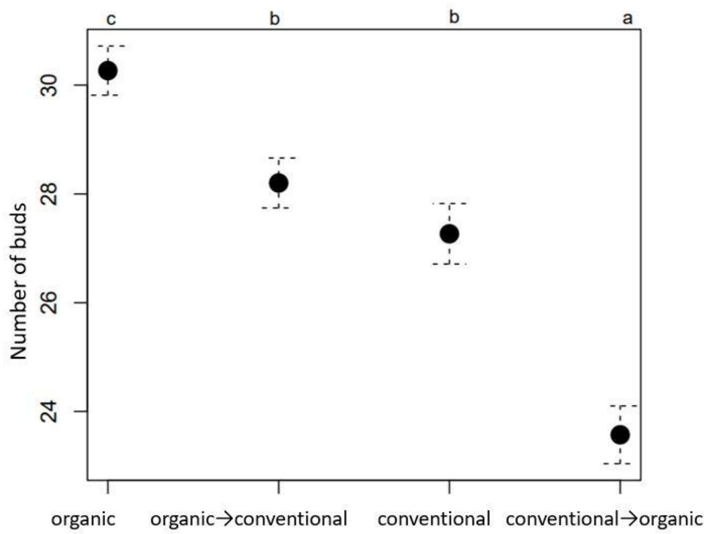


Figure 26: Number of buds at final cane length (180cm) of organic, conventional and switched raspberry canes (organic-conventional and conventional-organic) (variety: Tulameen). Different letters indicate significant differences (Anova), The error bars show the standard error. α=0.05, n=15

3.3.3 Cane length - organic

Figure 27 shows the cane length of organic canes and organic canes that were switched to the Rijtvenweg. At each measurement moment, the differences between the lengths of organic and organic-conventional are not significantly different.

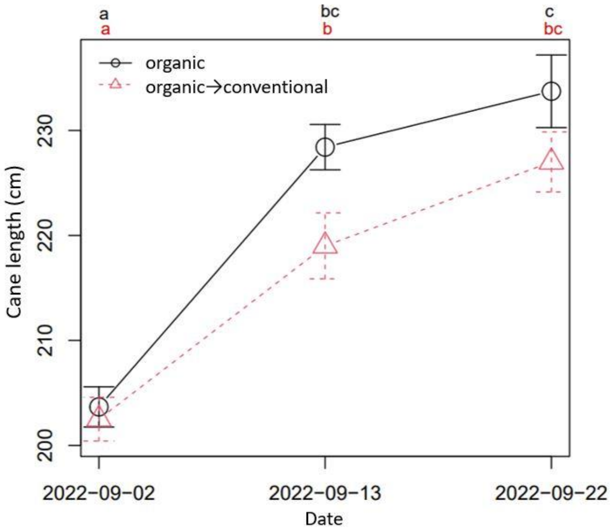


Figure 27: Cane length of organic, and organic-conventional raspberries over time. Different letters indicate significant differences (Anova), The error bars show the standard error. α=0.05, n=15

3.3.4 Total length of bud growth - conventional

The total length of bud growth was the same at both dates for conventional plants and conventional plants switched to the organic field (Figure 28).

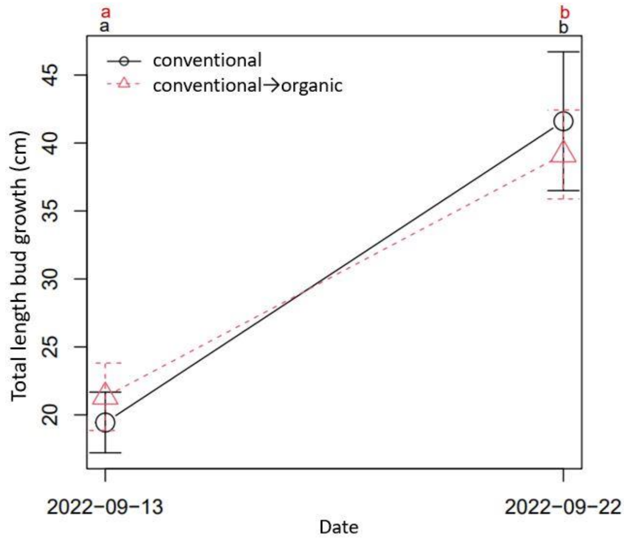


Figure 28: Total length of bud growth of conventional raspberries and conventional raspberries switched to the organic field (conventional-organic) over time. Different letters indicate significant differences (Anova), The error bars show the standard error. α=0.05, n=15

3.3.5 Root score

The differences between the average root scores of all measurements combined were significant for all 4 treatments (Figure 29). The roots of organic-conventional canes were scored lower than those of the organic plants after the switch. Figure 30 shows that in the first two weeks, the root score of organic-conventional is still more or less the same as that of the organic plants. After that, the root score drops and becomes more or less the same as that of the conventional plants. In the conventional plants that were switched to the organic field, the root score decreased compared to the conventional plants that were not switched. Conventional-organic has the lowest average root score of all 4 treatments (Figure 29).

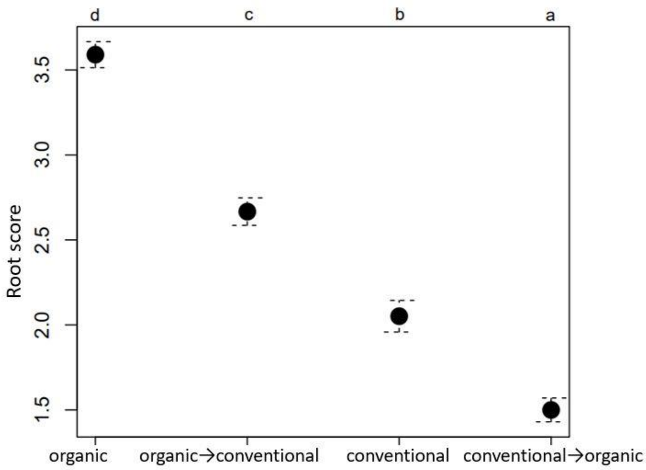
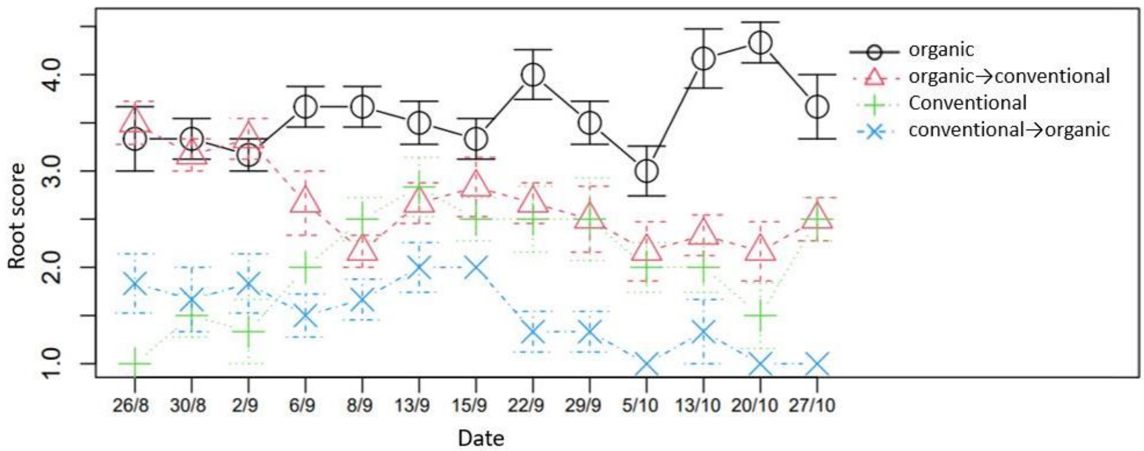


Figure 29: Average root score of organic, conventional and switched raspberry canes (organic- conventional and conventional-organic) (variety: Tulameen). Different letters indicate significant differences (Anova), The error bars show the standard error. α=0.05, n=78

Figure 30: Average root score of organic, conventional and switched raspberry canes (organic-conventional and conventional-organic) (variety: Tulameen) over time. The error bars show the standard error. α=0.05, n=6



4. Discussion

4.1 Phenotype and leaf sap (research question 1)

The explanation of the differences in phenotype and leaf sap between the conventional and organic raspberry canes can be sought in the management differences between the crops: the amount of nitrogen fertilization (≈112 kg N/ha vs. ≈300 kg N/ha), the form of nitrogen fertilization (inorganic vs. amino acids), the compost in the organic substrate and the more frequent application of root stimulants in the organic crop.

Organic raspberries show several symptoms that can be assigned to nitrogen deficiency. Nitrogen deficiency can show itself in plants by reduced growth, leaf discoloration to light green or yellow and faster leaf aging (Mu & Chen, 2021; Uchida, 2000). Also, under nitrogen deficiency, the root/shoot ratio of the plants change (Maradanov et al., 1998). Root growth is stimulated and shoot growth is reduced. These symptoms are visible in the organic raspberries that appeared lighter in color, were smaller, had increased rooting and dropped their leaves earlier than conventional raspberry plants. Although the phenotype of the organic raspberry canes looks like nitrogen deficiency, this is not confirmed with the leaf sap analysis. Total leaf nitrogen did not differ significantly between the conventional and organic leaves. This could be caused by the small number of repetitions and the large variation between repetitions.

Studies on the effect of protein hydrolysates or amino acids on plant growth show a positive effect on growth parameters of tomato (Paul et al., 2019), basil (Aghaye Noroozlo et al., 2019), timothy (Radkowski, 2018), wheat (Jeber & Khaeim, 2019) and lettuce (Choi et al., 2022). Root biomass is also stimulated by protein hydrolysates and amino acids (Choi et al., 2022; Aghaye Noroozlo et al., 2019; Colla et al., 2017; Colla et al., 2015). Besides this, compost in the substrate also has a positive effect on aboveground and belowground plant growth (Milinkovic et al., 2019; Lascano et al., 2009). This study found no positive effect on above-ground growth but did find a positive effect on underground growth. However, it is not clear what the effect of amino acids or compost is because the difference in nitrogen fertilization between conventional and organic plants is very large.

The difference in the number of buds on the bottom 180cm of the cane can be explained by the growth rate of the plant. This is because plant development, including the time between the appearance of two leaves, is determined by degree-days (Hodges, 1990). This is a combination of time and temperature. If we assume that the organic and conventional plants had about the same number of degree-days, we expect the same number of leaves, and buds, to have developed on the canes. However, due to higher growth rate, the internodes of the conventional plants are larger, resulting in fewer buds on the lower 180cm.

The difference in nitrate and percentage of inorganic nitrogen can be explained by the different forms of nitrogen fertilization. Conventional plants are fertilized with nitrate and ammonium and therefore contain more of this inorganic nitrogen. Organic plants receive no inorganic nitrogen through fertilization but only organic nitrogen forms. The difference in chloride in the leaf sap can also be explained by fertilization. The organic fertilization scheme contains more chloride than the conventional scheme (T. van den Hurk, personal communication, 23-11-2022). The leaf sap measurements were made in only 4 replicates and have large standard deviation. This makes differences in leaf sap composition difficult to detect. The large standard deviation can be explained by different weather conditions during the sampling.

4.2 Fungal infection experiment (research question 2)

Nitrogen fertilization affects plant disease susceptibility (Barnea et al., 2022; Elad et al., 2021; Sun et al., 2020; Rav David et al., 2019; Mur et al., 2017; Dordas, 2007; Hoffland et al., 2000). Pathogens that live on living plant material, such as Rust, infect plants with high nitrogen more readily. Pathogens that live on dying or dead material, such as Botrytis, infect plants with low nitrogen better (Dolan et al., 2018; Dordas, 2007; Evans & Bruzzese, 2003). However, these studies were all conducted with inorganic nitrogen fertilization, such as conventional plants. No distinctions are made between organic and inorganic nitrogen in the plant. The leaf sap analyses showed that the organic and conventional plants contained the same amount of total nitrogen, but the conventional plants contained more inorganic nitrogen and nitrate.

Rust and Botrytis resistance of organic and conventional plants can also be influenced by the microbiome. Beneficial organisms in the microbiome can enhance the systemic resistance of the plant leading to improved defense against diseases and pests (Pieterse et al., 2014). According to Blundle et al. (2020), soils and microbiomes that are biologically maintained also have a positive effect on plant defense against pests. The compost and protein hydrolysate in the biological management also have a positive effect on the substrate microbiome and systemic defense of the plant (Neher et al., 2022; Colla et al., 2015; Escuadra & Amemiya, 2008; Aldahmani et al., 2005; Kavroulaki et al., 2005).

A final explanation for the differences in Rust and Botrytis infection between organic and conventional plants can be sought in the microclimate in and around the plant, caused by the phenotype of the plant. According to Dolan et al. (2018), Rust and Botrytis infection can be reduced by better air circulation through the crop. This can be done, for example, by reducing plant density. In this study, it was shown that the phenotype of organic and conventional plants differs. Among other things, conventional plants have thicker stems and larger leaves. It is possible that due to the larger leaves of conventional plants, air circulation in the crop is lower, which promotes Rust and Botrytis infection.

Botrytis infection was very low this year in both the conventional field and the organic field. This may have been due to weather conditions. Botrytis thrives well in a warm and moist autumn. October was very dry this year (KNMI, n.d.), so there were few problems with Botrytis.

4.3 Change in cultivation method (research question 3)

When organic or conventional raspberry canes are switched cultivation methods at a late stage of propagation, there is no significant effect on cane length, bud sprouting and cane thickness. However, there is an effect on the number of buds on final cane length and root score of the plants. Both these parameters are lower in the switched plants than the plants that remained. The fact that the aboveground plant parameters no longer changed significantly at crop change may be due to the late timing of the change. The change experiment was conducted in autumn, when the growth rate of the canes goes down. The decline of the roots could mean that the roots are tuned to a specific fertilization pattern and cannot easily switch to a different fertilization scheme.

5. Conclusions and recommendations

5.1 Phenotype and leaf sap (research question 1)

Organic raspberry plants of the varieties Glen Ample and Tulameen varieties are smaller, thinner and have smaller leaves than conventional plants. Besides this, the organic raspberry canes have more buds at the final length of 180cm and higher root scores than the conventional canes. The leaf sap differs between the old leaves of the conventional and organic raspberry canes. The old leaves of conventional raspberries contain more nitrate, a higher percentage of inorganic nitrogen and less chloride than the organic old leaves. The leaf sap of the young leaves did not differ between organic and conventional plants.

To better understand the background of the differences between organic and conventional plants, studies should be conducted in which only one factor is changed each time. By growing raspberry plants on substrate with and without compost, with both organic and conventional fertilizer, the effect of compost and fertilizer can be evaluated independently of each other. This can also assess whether there is an interaction effect between the compost and the organic fertilizer. In these tests the nitrogen gift should be similar for all treatments. Furthermore, it would be interesting to test the effect of the amount of fertilization and fertilization frequency on raspberries in both cultivation methods. When organic raspberries are fertilized with the same amount and just as often as conventional raspberries, is there an increase in aboveground biomass? Does a decrease in nitrogen fertilization and fertilization frequency of conventional plants make the raspberry canes smaller and thinner? Finally, it is important to test what effect these differences in phenotype in the second year have on bud sprouting and raspberry harvest, quality and storability.

5.2 Fungal infection experiment (research question 2)

The organically propagated raspberry plants suffered less from Rust and Botrytis than the conventional raspberry plants. This indicates that the organic plants are more resistant to Rust and Botrytis than the conventional plants.

To gain more insight into the effect of cultivation method on the susceptibility of raspberries to Rust and Botrytis, follow-up research is needed. It is important to know whether the result of lower Botrytis infection in the organic plants is also valid under situations with higher Botrytis pressure. It is also good to know whether the results for Rust are similar in other years with different weather conditions. More insight into the mechanisms behind the increased resistance of organic plants to Rust and Botrytis could be obtained through research with sterilized substrate. This will give clues as to whether the microbiome in the substrate influences the disease susceptibility of the plants. Studies of higher or lower nitrogen inputs could provide more insight into the effect of plant nitrogen content on disease infection.

5.3 Change in cultivation method (research question 3)

A cultivation change at a late stage of propagation cannot be used to improve cane length, cane thickness, number of buds or rooting. Additional research could show whether a cultivation change at an earlier moment could provide additional cane length and thickness growth. In that case in a less vigorous year, fertilization could be switched halfway through cultivation to still achieve the target length of 180 cm.

6. Acknowledgements

This research would not have been possible without the cooperation of Vlamings, and specifically my supervisors Jelle Gerstel and Tim van den Hurk. Thank you very much for your guidance, your feedback about the experimental setup and especially for explaining everything I could see and measure on the raspberry cane. I would also like to thank Gerrie van de Ven, my supervisor from the WUR, for her good advice and feedback on this report.
Besides this, I am thankful for the opportunities I received from the company Genson. I would like to thank Thijs van Doorn and Tom van den Berg for making the raspberries available and answering all my questions about their cultivation practices.
Finally, I would like to thank Serena Horsmans for helping me move the raspberry plants between the organic field and the Rijtvenweg.

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8. Appendix

Appendix 1: Measurements and dates

Table A1: summary of all measurements and measurement times for the 4 cultivation modes of Tulameen and the 2 cultivation modes of Glen Ample.

	Tulameen				***Glen Ample***	
	Organic	**organic→ conventional**	**conventional**	**Conventional →organic**	**organic**	**conventional**
Cane length	12-8 25-8 2-9 13-9 22-9	2-9 13-9 22-9	12-8 25-8		12-8 25-8	12-8 25-8
Length bud growth			13-9 22-9	13-9 22-9		
Cane thickness	3-11	16-11	16-11	3-11	16-11	16-11
Number of buds	3-11	16-11	16-11	3-11	16-11	16-11
Root score	26-8 30-8 2-9 6-9 8-9 13-9 15-9 22-9 29-9 5-10 13-10 20-10 27-10 3-11	30-8 2-9 6-9 8-9 13-9 15-9 22-9 29-9 5-10 13-10 20-10 27-10 3-11	26-8 30-8 2-9 6-9 8-9 13-9 15-9 22-9 29-9 5-10 13-10 20-10 27-10 3-11	30-8 2-9 6-9 8-9 13-9 15-9 22-9 29-9 5-10 13-10 20-10 27-10 3-11	30-8	30-8
Leaf sap composition	22-8 24-8 26-8 2-9 15-9 29-9	2-9 15-9 29-9	24-8 2-9 15-9 29-9	2-9 15-9 29-9	22-8 24-8 26-8	24-8
Rust score	2-9 8-9 15-9 22-9 29-9 5-10 13-10	2-9 8-9 15-9 22-9 29-9 5-10 15-10	2-9 8-9 15-9 22-9 29-9 5-10 15-10	2-9 8-9 15-9 22-9 29-9 5-10 13-10		
Botrytis	20-10 27-10 3-11 10-11	20-10 27-10 3-11 10-11	20-10 27-10 3-11 10-11	20-10 27-10 3-11 10-11		

Appendix 2: Additional graphs leaf sap analysis

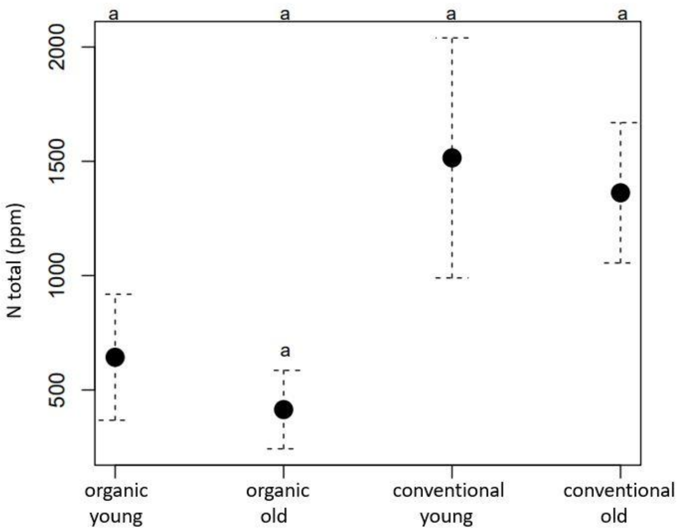

Figure A1: Nitrogen (N) total in the leaf sap of young and old leaves of organic and conventional Tulameen. Different letters indicate significant differences (Anova). The error bars show the standard error. α=0.05, n=4

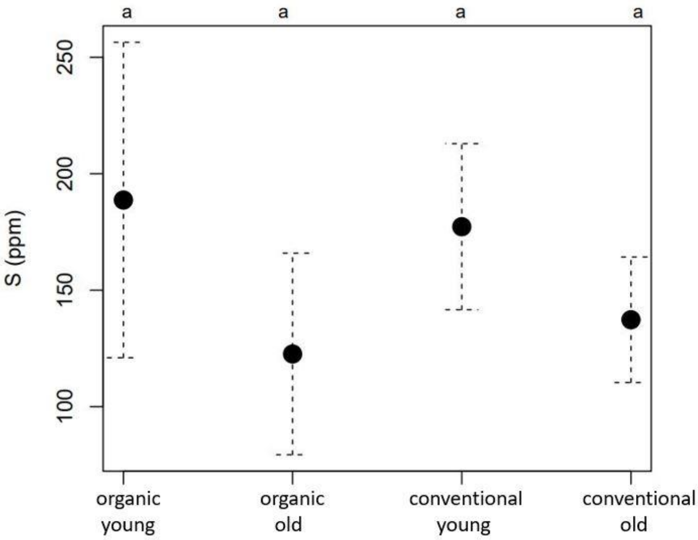

Figure A2: Sulfur (S) in the leaf sap of young and old leaves of organic and conventional Tulameen. Different letters indicate significant differences (Anova). The error bars show the standard error. α=0.05, n=4

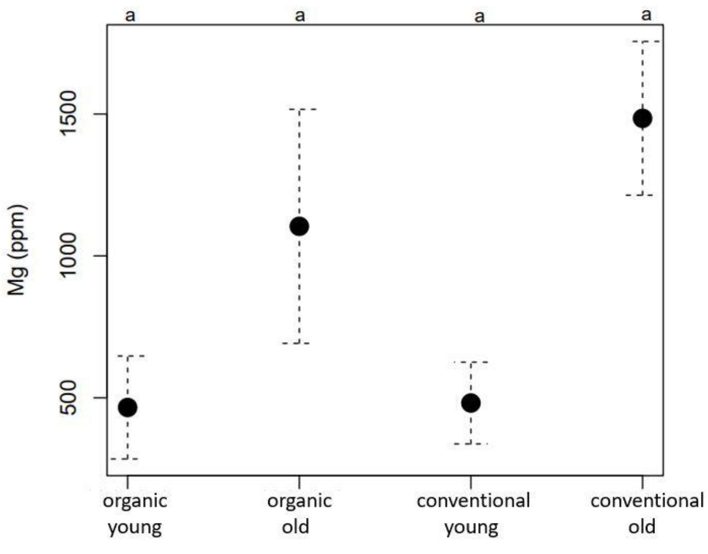

Figure A3: Magnesium (Mg) in the leaf sap of young and old leaves of organic and conventional Tulameen. Different letters indicate significant differences (Anova). The error bars show the standard error. α=0.05, n=4

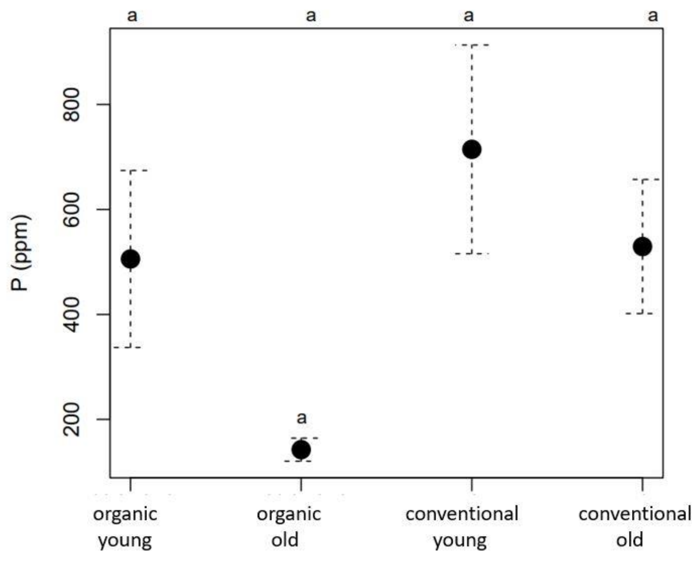

Figure A4: Phosphate (P) in the leaf sap of young and old leaves of organic and conventional Tulameen. Different letters indicate significant differences (Anova). The error bars show the standard error. α=0.05, n=4