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Metabolomic analysis of low- and partial-resistant cultivars of *Pisum sativum* to *Didymella pinodes*

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List of abbreviations

AM	Arbuscular mycorrhiza
Cv.	Cultivar
GC-MS	Gas chromatography and Mass spectrometry
i	infected
NP	Plants fertilized with nitrogen and phosphorus
m	Mass
M	Plants inoculated with mycorrhiza
Me	Cultivar Messire
MR	Plants inoculated with mycorrhiza and rhizobia
PPA	Prepenetration apparatus
PPFD	Photosynthetic photon flux density
Pr	Cultivar Protecta
QC	Quality control
R	Plants inoculated with rhizobia
RGP	Regression
SL	Splitless
S10	Split10
z	Charge
24 hai	24 hours after infection
48 hai	48 hours after infection
2 wai	2 weeks after infection

1. Introduction

1.1. *Pisum sativum*

Pisum sativum L., also known as the common pea, is an annual plant and belongs to the family of Fabaceae (or Leguminosae). The origin is localized to the Mediterranean basin and Near East, nowadays cultivated in many temperate regions all over the world. While its production has declined in Europe, it increased in China, Canada and the United States. Nevertheless, pea is the most cultivated legume in Europe and globally ranked on the second position (reviewed by KHAN *et al.*, 2013). Leaf tendrils can coil around many supportive objects, therefore *Pisum sativum* can gain up to 2-meters height, besides roots grow in suitable soil conditions up to 1-meter deep.

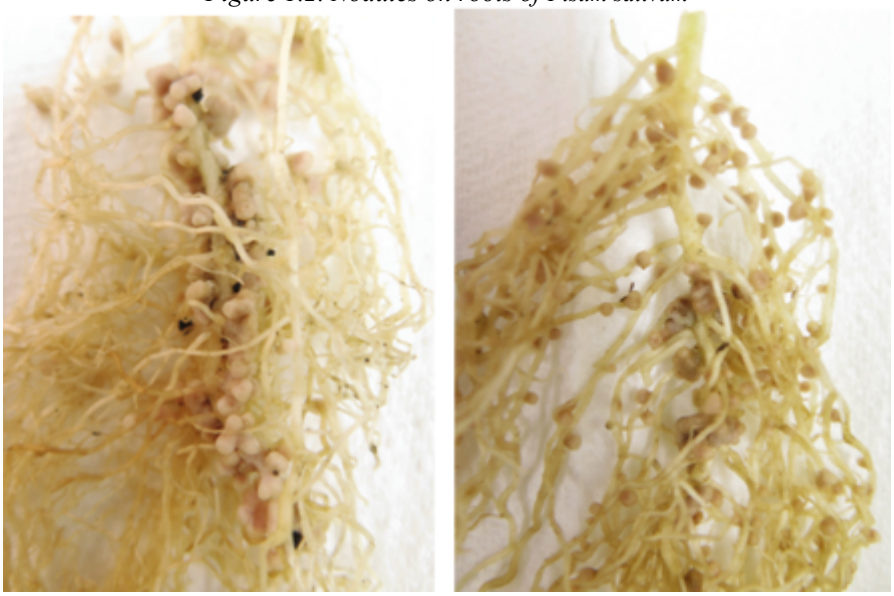
In this study, differences in the two cultivars Messire and Protecta were analyzed. The habitus of cv. Messire exhibits smaller internodes, leading to a shortened stem while cv. Protecta possesses an altered linear growth (Fig. 1.1). Additionally, cv. Messire develops branched shoots, whereas cv. Protecta shows hardly any branches. Regarding infection with *Didymella pinodes* Protecta is known to be the more resistant one, while Messire is more susceptible. In general, *Pisum sativum* is able to form a symbiosis with rhizobia or mycorrhiza which has emphasized fitness benefits to the plant (DENISON *et al.*, 2011; MURRAY, 2010).

Figure 1.1. Cultivar Messire (three pots on the left side) and cultivar Protecta (three pots on the right side).

1.2. Rhizobia and nodules

Nodules are the product of a nitrogen-fixing symbiotic relationship between legumes like *Pisum sativum* and soil bacteria generally termed rhizobia (YOUNG *et al.*, 1989; WIENKOOP *et al.*, 2008). Symbiosis has a beneficial effect for plants, since they can only take up inorganic nitrogen like nitrate or ammonium which is often limited. In order to that, rhizobia which are located in so-called nodules help plants to fixate more nitrogen in return of carbonic compounds. Nitrogen fixation is a very energy-costly process, which is why these bacteria need nutrients from the plant. Sucrose is the main carbohydrate transported from the plant into sink tissues like nodules (WIENKOOP *et al.*, 2008). After fixation of molecular nitrogen, it is transformed into NH_4 or NH_3^-, which is then chemically available for the plant. Plant and bacteria find one another in the soil by chemical signaling. Nodules are formed by meristematic cell development (BREWIN, 1991).

Figure 1.2. Nodules on roots of Pisum sativum



One plant can host different strains of rhizobia (SILVA *et al.*, 1999; MAHERALI *et al.*, 2007; VANDENKOORNHUYSE *et al.* 2007; JANSA *et al.*, 2008). Rhizobia benefits from the symbiosis by getting higher capability of reproduction when inhabiting nodules (Fig. 1.2.) – this way, millions of descendants can be produced in addition compared to soil remaining strains. Some rhizobia can escape from a senescing nodule and can establish in the soil, where they are able to survive for years without any host. In general, rhizobia are attracted by root exudates released from legumes. However, also other bacteria are attracted and competition for nodulation can be high. Furthermore, there is the risk of predation, which gives a hint that

rhizobia stay in suitable soil aggregates over a longer period of time to wait for a less risky nodulation opportunity (DENISON *et al.*, 2011). In agricultural systems the symbiosis between legumes and rhizobia is the main source of biologically fixed nitrogen (LIRA *et al.*, 2005).

1.3. Arbuscular Mycorrhiza

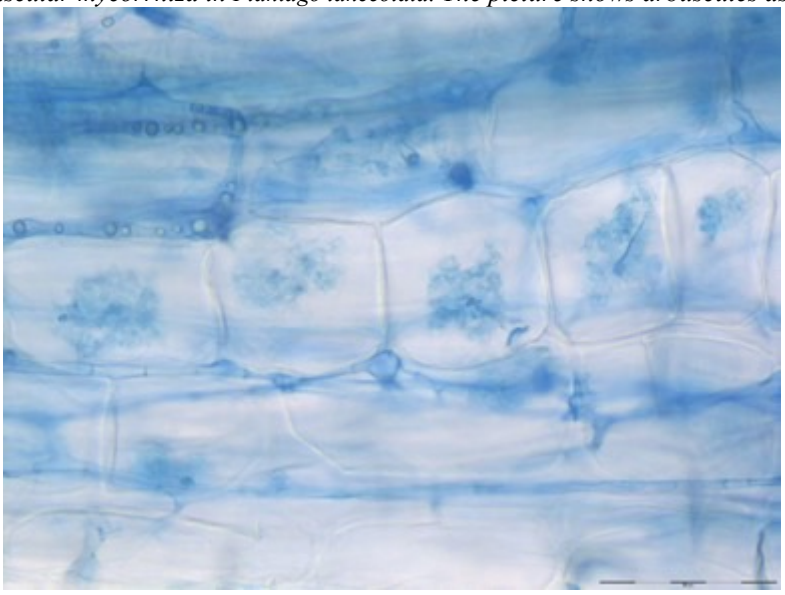
Mycorrhiza are fungi that live in symbiosis with land plants. They colonize roots of the plant by either growing inside the cells and building arbuscules (endomycorrhiza) (Fig. 1.3) or wrapping up the root tips and growing into the extracellular space (ectomycorrhiza) (PARNISKE, 2008). The function of mycorrhiza is simple: the fungus feeds the plant with nitrogen and phosphorus from the ground, whereas the plant exchanges carbon synthesized by photosynthesis (SCHÜSSLER, 2009).

It is estimated that 70-90 % of land plants are living in symbiosis with arbuscular fungi (SCHÜSSLER, 2009). In this case the hyphae grow apoplastic and form membranes inside the cells, which are called arbuscules due to their similarity with little trees (DENISON *et al.*, 2011). Arbuscular mycorrhiza (AM) are fungi of the monophyletic phylum Glomeromycota, which live in symbiosis with plants (PARNISKE, 2008). For many plant species mycorrhiza are essential because of nutrient and water allocation. In most symbiotic plants nitrogen and phosphorus uptake is also improved by mycorrhiza, because the fungus acts additionally as an enlargement of the root system. Furthermore, the hyphae are very small and are able to open up to soil pores. In return, up to 20 % of plant-fixed carbon is allocated to support AM fungi (PARNISKE, 2008).

The colonization of the plant starts, when a fungal spore germinates and hyphae grow towards the plant's roots. The fungus sends signals to the plant which then develops a prepenetration apparatus (PPA) in the outlying cells and guides the fungus into the cell. The fungus enters the cell by building an appressorium which is a special cell helping the fungus to attach to the host surface. Once the fungus colonized the host cells, it immediately starts to develop arbuscules which are used for nutrient transfer. These arbuscules are very short-lived and only functional 4 to 5 days. The nutrient exchange is followed by arbuscule-collapse, where structures are degenerated within a few hours. Some arbuscular fungi also develop vesicles inside the cells which probably serve as carbon storage structure. External hyphae are then developed throughout the soil and take up nutrients like phosphorus, nitrogen and other

micronutrients which then are transferred to the plant. Arbuscular mycorrhiza are completely dependent on the host plant in reproduction and growth, because they would not get any carbon at all without the plant. This is a main difference between rhizobia and mycorrhiza (DENISON *et al.*, 2011).

Figure 1.3. Arbuscular mycorrhiza in Plantago lanceolata. The picture shows arbuscules as well as hyphae.

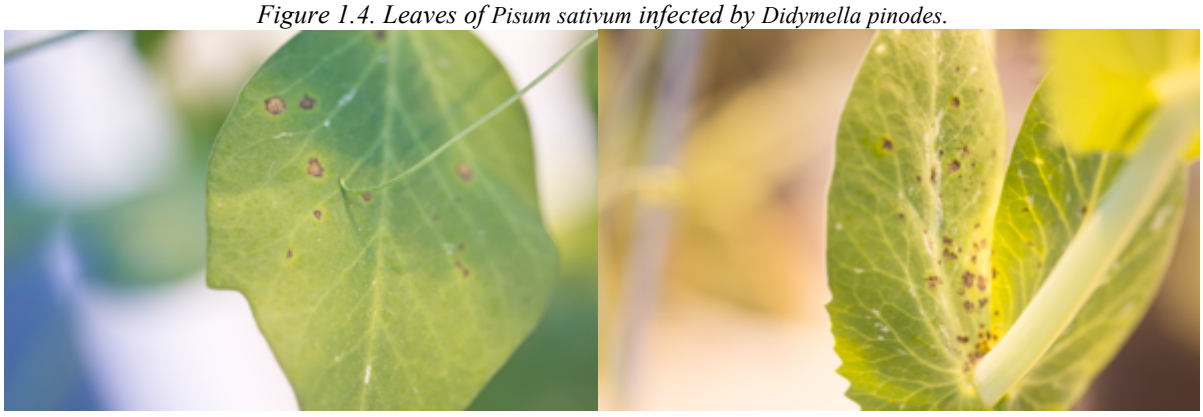


1.4. Ascochyta blight and implications for pea

Ascochyta blight which is developed by several fungi is one of the worst pea diseases worldwide and has huge economic impacts due to possible yield losses of up to 75 % as reported for Australia, France and Canada (ROGER *et al.*, 1999; KAISER *et al.*, 2000; BRETAG and RAMSEY, 2001; MCDONALD and PECK, 2009). Yield losses depend on pathogen frequency, distribution and disease intensity, besides on environmental and seasonal conditions as well as on geographic location (LE MAY *et al.*, 2005; SALAM *et al.*, 2011). In addition to *Didymella pinodes* (Fig.1.4.) also *Aschochyta pisi* and *Phoma medicaginis* cause ascochyta blight (BRETAG *et al.*, 2006; PEEVER *et al.*, 2007). The disease can lead to reduced plant growth, seed number per stem and seed size. The infection happens mainly airborne from infested residues from neighboring fields and is followed by soil-borne inoculum (MCDONALD and PECK, 2009). Ascospores are predominantly released from matured pseudothecia in pea residues under sufficient conditions like rain or high humidity (BRETAG, 1991; SALAM and GALLOWAY, 2005; TIVOLI and BANNIZA, 2007; SALAM *et al.*, 2009a). The dispersion radius of ascospores which are transported by wind

can reach a distance from up to 1.6 km (SCHOENY *et al.*, 2007). The three mentioned fungi cause similar symptoms after an infection. However, *Didymella pinodes* is known as the most frequent and therefore damaging one (MOUSSART *et al.*, 1998; BRETAG *et al.*, 2006; TIVOLI and BANNIZA, 2007). To avoid spreading of ascochyta blight fungicides and agronomic practices like crop rotations, burying of infested residues or intercropping, are executed (XUE, 2003; MCDONALD and PECK, 2009; FERNANDEZ-APARICIO *et al.*, 2010). Buried *D. pinodes* survives much shorter than in pea residues on the soil surface (SHERIDAN, 1973). Intercropping reduces ascochyta blight up to 60 % and is often practiced with *Faba* bean or *Triticale* sp. (FERNANDEZ-APARICIO *et al.*, 2010). The reasons for the decrease of the disease due to intercropping are probably altered microclimate, reduced host biomass and a restriction of spore dispersal due to physical barriers (FERNANDEZ-APARICIO *et al.*, 2010). Another management option is the delay of sowing towards the end of the sowing window. In this case the disease has a shorter period of time for development, which means reduced incidence and severity of ascochyta blight (BRETAG *et al.*, 2000, 2006). Unfortunately, this method is not an option for many areas like Europe and western Canada, where the growing season is very short (reviewed by KHAN *et al.*, 2013). Also, these techniques for controlling ascochyta blight may not be suitable for each farm situation and many fungicides are simply not (cost-) effective (MCMURRAY *et al.*, 2011). Due to all these facts breeding for pathogen resistance would be the most efficient and environmentally friendly method to control the disease (reviewed by KHAN *et al.*, 2013).

Figure 1.4. Leaves of Pisum sativum infected by Didymella pinodes.



1.5. Molecular Biology approaches and the quest for increased plant stress tolerance

1.5.1. General –omics

In general, all "omic"-technologies have the aim to identify all gene products represented in a specific biological sample. These technologies include transcriptomics (identifying transcripts), proteomics (identifying proteins), metabolomics (identifying metabolites) etc., which all are needed to investigate biochemical networks on a large scale approach – the systems biology approach (WECKWERTH *et al.*, 2003). The goals of this approach are to understand the emergent properties of complex biological systems (IDEKER *et al.*, 2001). The main point of interest is the composition of living organisms and their changes in response to physiological stimulation, developmental stages and genetic modification (SALT *et al.*, 2008).

1.5.2. Metabolomics and Mass Spectrometry

Metabolites are small molecules which can be identified and quantified by using several metabolomics approaches. These approaches are used for high throughput detection and analysis of primary and secondary metabolites (ZIVY *et al.*, 2015). Analyzing metabolic networks helps to understand responses of biological systems to changes – genetically and environmentally (MORGENTHAL *et al.*, 2007). Metabolomics gained an increasing interest in the last years in many disciplines like functional genomics, integrative and systems biology, pharmacogenomics and biomarker discovery for drug discovery and therapy monitoring (GOODACRE, 2004). The ultimate goal is to identify and quantify all metabolites in a specific sample which cannot be done by a single analytical technique. Several major approaches have been developed when dealing with metabolomics, of which one is discussed in more detail in the following part: gas chromatography coupled to mass spectrometry (GC-MS). Thereby complex mixtures are analyzed for one or a small number of compounds (FIEHN, 2002). As described by POOLE (2012) gas chromatography is used to separate components of mixtures. This technique enables one to obtain information about molecular composition and amounts of metabolites in samples. After the chromatographic analysis a chromatogram gives information about the heights and the areas of the resolved separated

peaks and their molecular identity. In a GC carrier gas like hydrogen, nitrogen or helium, serve as a mobile phase, where the components of the sample are carried through a column. This column is coated by the so called stationary phase. Mostly this is liquid or gel-like. Before that, the liquid sample needs to be turned into vaporous state. This is done at the beginning of the process, where a heated injector quickly vaporizes the sample and injects it into the GC-device. The different components of the sample react with the stationary phase and stay in the column according to their polarity. At the end of the column a detector measures the moment when the different components leave the column. Retention time and amount of the escaping substances are recorded. The retention time of a component is the total time needed to pass through the whole column. Every component has a different retention time due to typical reactions between the component and the stationary phase. The dead time is the time needed by a component to pass the column without reacting with the stationary phase. For gas chromatographic analysis the substances need to be quite volatile. Less volatile substances like sugars can be turned into more volatile ones by derivatisation. By using derivatisation the resulting product will be less polar and therefore more volatile. The results of the measurements can be evaluated by using software like ChromaTOF® (LECO). This software visualizes chromatograms as well as the specific spectrums – with this information an identification from metabolites in the samples can be performed. The chromatogram shows the separation of different substances according to their retention time. Furthermore, the molecules are fragmented and the spectrum gives information about the fragmentation and leads to an identification. The fragments are listed according to their m/z relation (POOLE, 2012).

1.6. Aims of this work

The aims of this work are to determine metabolic responses of *Pisum sativum* grown symbiotic or non-symbiotic when infected by the fungus *Didymella pinodes*. Two different experiments have been carried out for metabolomic analysis of leaves and nodules of *P. sativum*. In the first experiment metabolites of healthy and infected *P. sativum* grown under four different treatments were analyzed, whereas in the second experiment the metabolites of nodules of healthy and infected plants were analyzed after they were harvested at three different points in time (24 hours after the infection, 48 hours after the infection and 2 weeks after the infection).

In this work the following questions are aimed to be answered:

- Do the various treatments have different impacts on leaves of *Pisum sativum* on metabolite level?
- Does the infection of *Didymella pinodes* have any effects on leaves and nodules of *Pisum sativum* on metabolite level compared to healthy ones?
- Are there significant differences in the nodule weight between cultivars and between three points in time after infection?
- Does the metabolic response of nodules of *Pisum sativum* to the pathogen *Didymella pinodes* change over a time period of two weeks?
- Do leaves and nodules of both healthy and infected cv. Messire and cv. Protecta differ between the cultivars on metabolite level?

In general, it is assumed that different growth conditions of *Pisum sativum* result in different metabolic responses of the plants and furthermore influence the response to the pathogen *Didymella pinodes* on metabolite level.

2. Material and Methods

2.1. Experiment 1: Leaves

Experimental settings

The first experiment was performed in spring 2014. In this experiment two different cultivars of *Pisum sativum* – cv. Messire and cv. Protecta – have been used. These cultivars were subjected to one of the four following treatments: mycorrhiza (M), rhizobia (R), mycorrhiza + rhizobia (MR) or nitrogen and phosphorus fertilization (NP). For inoculation with mycorrhiza a commercial inoculant (Vaminoc) was used containing *Glomus mosseae*. For inoculation with rhizobia a *Rhizobium leguminosarum* bv. *viceae* strain selected for maximum N_2–fixation potential was chosen. Both inoculants have been obtained in powder form from Becker Underwood Ltd. (www.beckerunderwood.com) and were applied as advised by the company.

The plastic pots comprised a volume of 3 l and were disinfected and cleaned with 12 % sodium hypochlorite and deionized water. Each pot was filled with 2 kg growing substrate and moistened with 400 ml sterilized deionized water. The experiment was arranged in a randomized block design with four replicates per pot in a greenhouse in Tulln (Department Für Agrartechnologie, Boku). *Pisum sativum* grew under the following environmental conditions: temperature of 15 ± 5 °C, relative humidity of 60 ± 5 %, 14 h photoperiod / 10 h darkness and 300 µmol m^{-2} s^{-1} light intensity. The plants were watered every other day.

Substrate

The used soil in this experiment originated from arable fields in Tulln, Austria. It was collected from the uppermost 20 cm of the ground, air-dried, sieved through a 2 mm sieve and mixed with expanded clay and silica sand (1:1:1). Afterwards, the soil was autoclaved to get rid of possible contaminations. In general, the soil used in our experiment was very low in plant-available nitrogen and phosphorus. However, according to QIFU *et al.* (1998) nitrogen limitation in early development of legume seedlings can lead to an insurmountable lack in N later in plant growth. Therefore, adding a little starter dose of nitrogen such as 5-10 kg N ha^{-1} can stimulate seedling growth and early nodulation, which leads to enhancement of nitrogen fixation and yield (ATKINS, 1987). This phenomenon is also true for phosphorus, where young seedlings can suffer from P deficiency even in soils with high P availability, due to

limited root systems. This is the reason, why in this experiment each pot received an extra dose of 20 mg N and P per kg soil after planting. The treatments fertilized with nitrogen and phosphorus received additional N and P forms as described by HOFFMANN *et al.* (2009) for beans: 80 mg N kg^{-1} soil and 28 mg P kg^{-1} soil. Treatments containing mycorrhiza or rhizobia received additional N and P as well. Furthermore, 10 ml of a modified nutrient solution which was free of nitrogen and phosphorus was applied on each pot once a week. This solution was prepared according to BROUGHTON and DILWORTH (1971) containing $CaCl_2$ 147 ppm, Fe-citrate 3.35 ppm, $MgSO_47H_20$ 61.6 ppm, K_2SO_4 43.5 ppm, $MnSO_4$ 0.17 ppm, H_3BO_3 0.123 ppm, $ZnSO_4$ 0.144 ppm, $CuSO_4$ 0.05 ppm, $CoSO_4$ 0.028 ppm and $NaMoO_2$ 0.024 ppm with a pH of 6.7.

Plant growth

In this experiment seeds of cultivar Protecta were obtained from Austria, whereas seeds of cultivar Messire were obtained from Spain. Seeds were selected according to their uniformity and healthiness. Before implanting, they were washed with 70 % ethanol for 30 seconds and sterilized with 12 % sodium hypochlorite for five minutes. Afterwards, the seeds were rinsed at least six times with Milli-Q water and pre-germinated in autoclaved perlite. Germination was started on February 3rd, 2014. After three days of pre-germination, five seeds per pot were selected and planted.

Harvesting

Two infections of *Pisum sativum* with *Didymella pinodes* spores were conducted (as described in 2.3). The actual harvesting was done 36 hours after the second infection. The leaves were cut off and put into bags made of aluminum foil which were then immediately frozen in liquid nitrogen to avoid changes in metabolic reactions.

2.2. Experiment 2: Nodules

Experimental settings

In the second experiment again the cultivars Messire and Protecta have been used. However, this time the plants were only inoculated with rhizobia in order to analyze the nodules specifically. Furthermore, a control group was prepared, where plants were fertilized with nitrogen and phosphorus. Overall 88 pots with four seeds each were prepared (Fig. 2.1.). Half

of the pots were infected with spores of *Didymella pinodes*. The other half remained healthy to serve as control group. The substrate – a mixture of soil and perlite – was autoclaved before usage. The pots were filled with substrate up to 3 cm under the edge. After the seeds had been cultivated and germinated, four seeds per pot were inserted. It is very important to treat the seeds gently and not to squeeze them too much in order to make sure they do not get harmed. When inserting the seeds into the substrate it is important that the root points downwards and the seed is located 1 – 2 cm deep under the substrate surface. After seed insertion the pots were gently poured with deionized water and half of the pots were additionally inoculated with rhizobia (Fig. 2.2.). In contrast to the first experiment, this time a liquid inoculant containing a rhizobia strain was used, and 20 ml were pipetted onto each of the 44 pots. The pots were poured with nutrient solution every other day, whereas each treatment was poured with its very own nutrient solution. Rhizobia inoculated plants did not receive any additional nitrogen. The plants grew in a climate chamber for 4 weeks (November 12th, 2014 until December 8th, 2014) under controlled environmental conditions: 14 h photoperiod / 10 h darkness, 600 µmol $m^{-2}s^{-1}$ PPFD, 22°C/16°C day/night temperature, 60-70 % relative humidity. After infection leaves, nodules and roots were harvested at three different points in time: 24 hours after infection (December 9th, 2014), 48 hours after infection (December 10th, 2014) and 2 weeks after infection (December 22nd, 2014).

Figure 2.1. Test design with 88 pots distributed on 4 floors in a climatic chamber. Plastic poles, which served as growth subsidy, were put into the pots before injecting the seedlings. Each pot was labeled with a number.

Figure 2.2. Pot with four seedlings and fertilized with rhizobia. Each pot was labeled with cultivar and treatment.

Plant growth

In total, 250 seeds of cv. Messire and 250 seeds of cv. Protecta were cultivated by putting them in Erlenmeyer flasks and covering them 3 minutes with 69 % ethanol. Afterwards the seeds were washed with water at least 3 times before being put into 75 % chlorine solution for 4 minutes. The flasks were covered with aluminum foil. Then the seeds were washed at least 6 times with water but at any rate often enough until they revealed a neutral smell. Afterwards, the seeds were put into autoclaved wet perlite and covered with transparent plastic. Germination took place from November 10th to 14th, 2014. To avoid lack of material, more seeds than needed were cultivated to compensate not germinated seeds.

Harvesting

As mentioned above, harvesting was performed at three different points in time: 24 hours after infection, 48 hours after infection and 2 weeks after infection (December 9th , 2014; December 19th, 2014; December 22th, 2014). The pots harvested on the three points in time were chosen randomly. After a quick harvest of the leaves, the plants of one pot were marked with different colored rubber bands to distinguish them from one another. The leaves of each plant were put into little bags folded out of aluminum foil. These bags were then put immediately into liquid nitrogen to avoid changes in metabolic reactions. Then the shoots were cut off and remaining substrate was rinsed off the roots. The roots were also collected in plastic bags – one bag for all roots of one plant. Afterwards, the weight of a) roots with nodules b) roots without nodules (nodules were removed carefully) and c) just nodules was

determined. Furthermore, the total number of nodules was counted for every plant. Roots, nodules and leaves were frozen at -80°C until further examination.

2.3. Augmentation and Infection with *Didymella pinodes*

In order to make sure and get enough spores of *Didymella pinodes* for infection of *Pisum sativum* the fungus was augmented on petri dishes. The petri dishes were filled with potato dextrose agar (PDA). Augmentation happened every two weeks on thirty petri dishes. For these thirty petri dishes 39 g PDA dissolved in 1 l of Milli-Q-water were used. The solution was autoclaved and spiked with antibiotics (500 µl ampicillin for 1 l agar and 12 ml chloramphenicol for 1 l agar) before usage. After autoclaving and before adding ampicillin and chloramphenicol it is important that the agar cooled down to about 60 degrees, otherwise the high temperatures will affect the antibiotics' effectiveness. From this point on it is important to work on the sterile bench to not get any undesired infections. After antibiotics were added the fluid was mixed and put on petri dishes, where it further cooled down and at the same time became stiff. When filling petri dishes with agar it is important to make sure not to produce too many bubbles, because they can serve as growth hearth for bacteria. After the agar became stiff an older sample of *Didymella pinodes* growing on agar in a petri dish (Fig. 2.3.) was cut into many small pieces by using a sterile spatula. Each small piece was put on one of the new petri dishes, closed with a lid and sealed with parafilm. *Didymella pinodes* grew under controlled conditions until the moment of infection.

Figure 2.3. Didymella pinodes growing on agar in a petri dish.

To receive spores of the grown fungus for infection of *Pisum sativum* twenty of the best fitting cultivations were chosen and covered with 10 ml of water per petri dish. By using diluting loops the fungus' surface was gently scratched off to get the spores detached. The mixture of water and spores was then filtered through filter paper and filled into Erlenmeyer flasks. By using aerosol cans spores were spread on half of the plants after they were spread with moistening agent. Also the plants, which were not infected, were spread with moistening agent to create a level playing field. To make sure the infection was not transferred to healthy plants, each of the four floors was covered with transparent plastic foil that was fixed on a wooden frame attached to the floors (Figure 2.4.). The plastic foil was removed completely after the first harvest on December 9th 2014.

Figure 2.4. The pots were covered with transparent foil which was fixed on a frame made out of wood and metal. The foil prevented the spores from infecting healthy plants and was intended to intensify the infection.

2.4. Metabolic analysis

Grinding

To extract metabolites from leaves, samples were ground to a fine powder by using mortar and pestle. It is important to chill the utensils and to cool the samples with liquid nitrogen during grinding to avoid changes in metabolic reactions. The ground samples were frozen and stored at -80 °C until metabolite extraction was performed.

Polar Metabolite Extraction

Metabolites of nodules and leaves have been extracted according to the "Standard extraction protocol for metabolites (polar)" (see attachment in 8.8). For leaf-extraction about 7 mg (dryweight) were used of each sample, whereas for the nodule-extraction about 20 mg (freshweight) per sample were used. According to the extraction protocol the samples were solved in 1 ml extraction solvent MCW (methanol/ chloroform/ water-mixture), incubated on ice for 8 minutes and centrifuged in a chilled centrifuge for 4 minutes at 14 000 g. The supernatant which contained the metabolites was transferred into new Eppendorf tubes. Then the extraction procedure was repeated with 500 µl of MCW to extract more metabolites from the samples. Afterwards, 400 µl Milli-Q-water were added for phase separation, which leads to polar and unpolar phases. The samples were again centrifuged for 2 minutes at 14 000 g and the supernatant which is mostly clear and includes the polar phase was put into new Eppendorf tubes. In addition, the polar phase was split into two equal aliquots, each containing about 600 µl, whereas one half of the aliquots was stored at -80°C for further measurements. The other half was used subsequently and dried in the Speed-Vac. Then the samples were derivatised by methoximation, where the samples were resolved in 20 µl methoximation reagent (1 ml Pyridine containing 40 mg Methoxyaminhydrochlord), and silylation, where 80 µl silylation mix (1 ml MSTFA containing 30 µl alkane mixture) were added to the samples. After this step, the samples were again centrifuged and the final supernatant was transferred into GC-microvials and closed with crimp caps.

In total, 48 leaf-samples were extracted and measured, whereas 24 originated form cv. Messire and 24 from cv. Protecta. Half of the samples were infected, whereas the other half remained healthy. For extraction and measurement three biological replicates were used.

When it comes to extraction and measurement of nodules 40 samples were analyzed of which 20 samples originated from cv. Messire and 20 samples from cv. Protecta. Half of the used samples were harvested at the first point in time (24 hours after infection), whereas the other half was harvested at the third point in time (two weeks after infection). All utilized plants originated from rhizobia inoculated treatments, with in total five biological replicates. All remaining nodule-samples of the second point in time (48 hours after the infection) were not extracted according to the assumption that changes in infection intensity would be seen best between first and third point in time. In general, each pot from rhizobia inoculated treatments was sampled. Except for one case, at least three different plants per pot were chosen.

GC-MS

For GC-MS analysis, the instrument PEGASUS® 4D GCxGC TOFMS was used. Furthermore, two different measurement modes – splitless and split10 – were executed.

2.5. Data mining and statistics

Metabolite identification and peak area extraction

After measuring the samples by using GC-MS, data were analyzed by using LECO® ChromaTOF® (Version 4.50.8.0 optimized for Pegasus®), a computer program that analyzes the peak areas of detected metabolites for further processing.

Normalization

Metabolite quantification was done in Microsoft Excel®. Altogether, QC-mixes (Quality Control) with five different quantities (5 µl, 10 µl, 20 µl, 30 µl and 40 µl) were used for normalization of the metabolite data. The QC-mix contained a mixture of in total 48 known substances, therefore metabolites contained in both, the sample and QC-mix, could be quantified absolutely. To check whether the metabolite values were within the range of the QC-mix, the average of all values of one metabolite (measured splitless) was evaluated, which was then divided by the minimum and maximum values of the QC-mix (measured splitless). The average metabolite value could be 25 % higher than the maximum QC-mix value or 25 % lower than the minimum QC-mix value.

The samples of either leaves or nodules were divided into three batches each. For these three batches four QC-mixes were prepared for normalization and quantification of the metabolites in the samples. Usually, the first batch is normalized via the first and second QC-mix, the second batch is normalized via the second and third QC-mix and the third batch is normalized via the third and fourth QC-mix. However, there was an error when analyzing the metabolites of the leaves and one of the QC-mixes was lost, which is why only three QC-mixes were left. Therefore, the first batch was normalized via the first and second QC-mixes and the second and third batches via the second and third QC-mixes. In general, the normalization was done according to the following steps:

1. If the average metabolite value (measured splitless) was within the range of the QC-mixes (5 µl to 40 µl), every metabolite value measured splitless was divided by the average RGP (regression) of the QC-mixes (splitless) of two batches.

2. If the average metabolite value measured splitless was > 25 % than the maximum QC-mix value, the quantification was done by using the metabolite values measured in split10. In this case, the average metabolite value measured split10 had to be within the range of the QC-mixes ± 25 %. This was done by again dividing the average metabolite value (split10) by the maximum value of the QC-mixes (split10). If this value was within the range of the QC-mixes, every metabolite value (split10) was divided by the average RGP of the QC-mixes (split10) of two batches.

3. If the average metabolite value (split10) was not within the range of the QC-mix (split10), every metabolite value (split10) was divided by the average RGP of the QC-mixes (splitless) of two batches. The resulting values were multiplied by the factor of the RGP of the QC-mix (splitless) divided by the RGP of the QC-mix (split10).

4. If the average metabolite value (splitless) was 25 % smaller than the minimum QC value (splitless) every metabolite value (splitless) was divided by the average RGP of the QC-mixes 5 µl to 20 µl splitless (not 5 µl to 40 µl) of two batches.

The average RGP of the QC-mixes which was used for metabolite values of the first batch was determined by the RGPs of first and second batch. The average RGP used for metabolite values of the second and third batch was calculated from the RGPs of second and third batch. Furthermore, the resulting metabolite values were normalized over the fresh weight and dry weight respectively and subsequently were multiplied by 2 (samples were divided into two aliquots) and 100 (only 1 µl of samples was injected into GC-MS).

Outlier-identification, missing-values- and ratios-evaluation

The calculations in this part were performed using RStudio® (Version 0.99.467, © 2009-2015 RStudio). Outliers were defined as values being higher than 1.5 of the 50%-range of the boxplot. Identified outliers as well as zeros – where we did not detect any value – were erased which resulted in missing values. Such missing values were imputed with values calculated by the other given values ("nearest neighbor" → minimum value divided by 2). Thus, entirely

filled data matrices have been set up for evaluation of metabolite ratios. These metabolite ratios were represented in metabolic maps which compared metabolite quantities of treatments, points in time, states of health and cultivars.

Significance-tests

To check the ratios for significance either a T-test or an ANOVA with Tukey-test was executed. T-test was carried out in Excel, whereas ANOVA and Tukey-test were performed in RStudio®.

3. Results

3.1. Results of leaves of *Pisum sativum*

In the following section the results of the metabolite analysis of leaves of *Pisum sativum* are presented. The results are shown in metabolic ratio maps (Figure 3.1a-t and Figure 3.2a-h), additional figures can be found in the section of supplementary data.

3.1.1. Treatment ratios

In this part ratios of two different treatments each are shown in healthy and infected cultivars Messire and Protecta (Figure 3.1a-t). Significant results were evaluated using the T-test in Excel, because the use of ANOVA with Tukey-test in R-Studio only revealed less significant results. Only figures containing significant results or striking trends are represented, whereas all remaining figures were attached in the supplements.

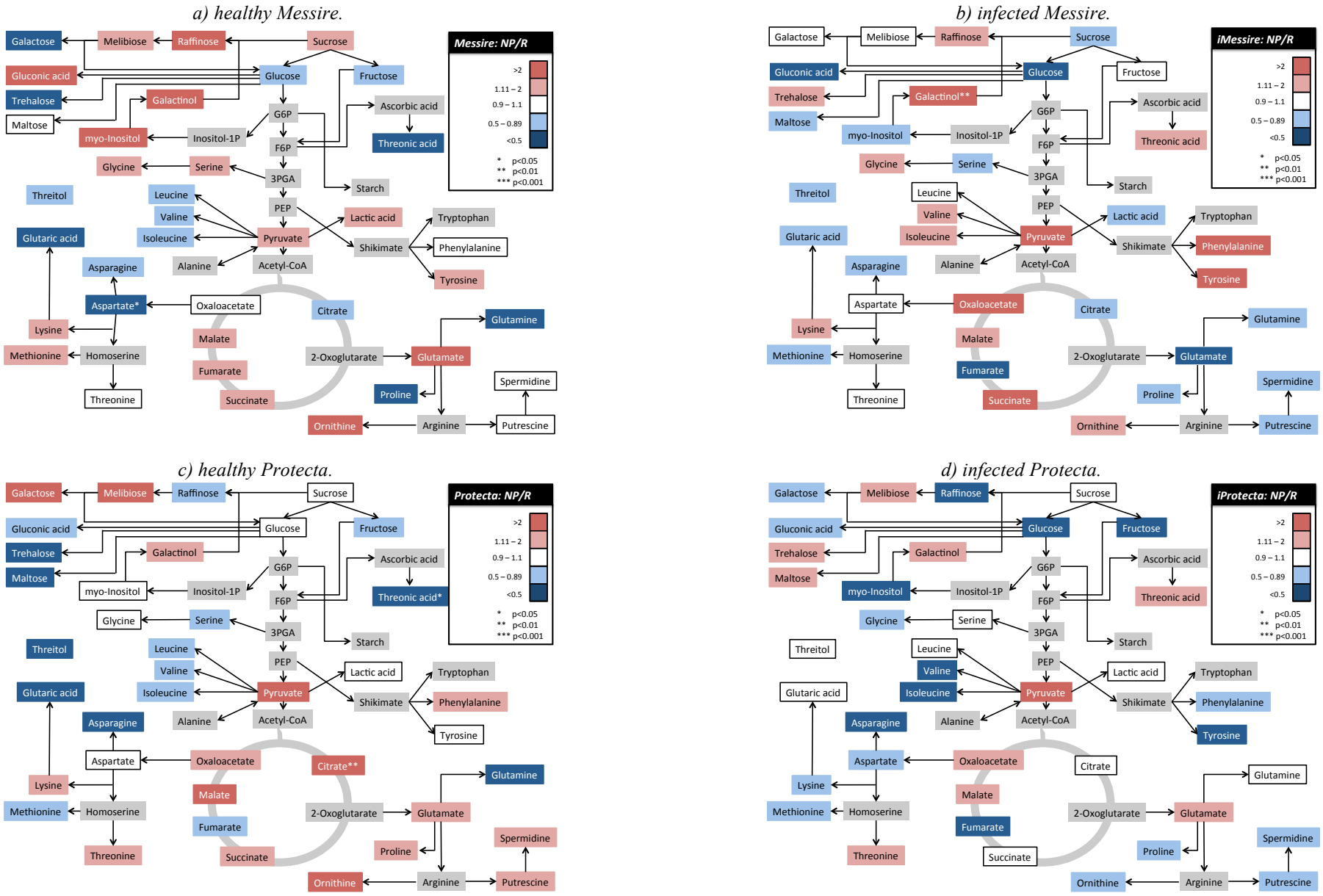


Figure 3.1a,b,c,d. Ratios of treatments NP (fertilized with nitrogen and phosphorus) and R (inoculated with rhizobia) of the different cvs (Messire a,b; Protecta c,d) comparing healthy and infected plants. Metabolites, which show a grey background, were not detected, but were pictured due to cross-linkage. The eventual asterisks on the sides of the metabolites represent significant results: p<0.05 (*), p<0.01 (**) or p<0,001 (***). These ratios reveal, which of the comparing treatments showed higher amounts in metabolites. A dark blue background means the ratio is smaller than 0.5, a light blue background implies a ratio of 0.5 – 0.89, a white background means the ratio is between 0.9 – 1.1, a light red background stands for a ratio of 1.11 – 2 and a dark red background means the ratio is higher than 2. In general, 39 metabolites were identified.

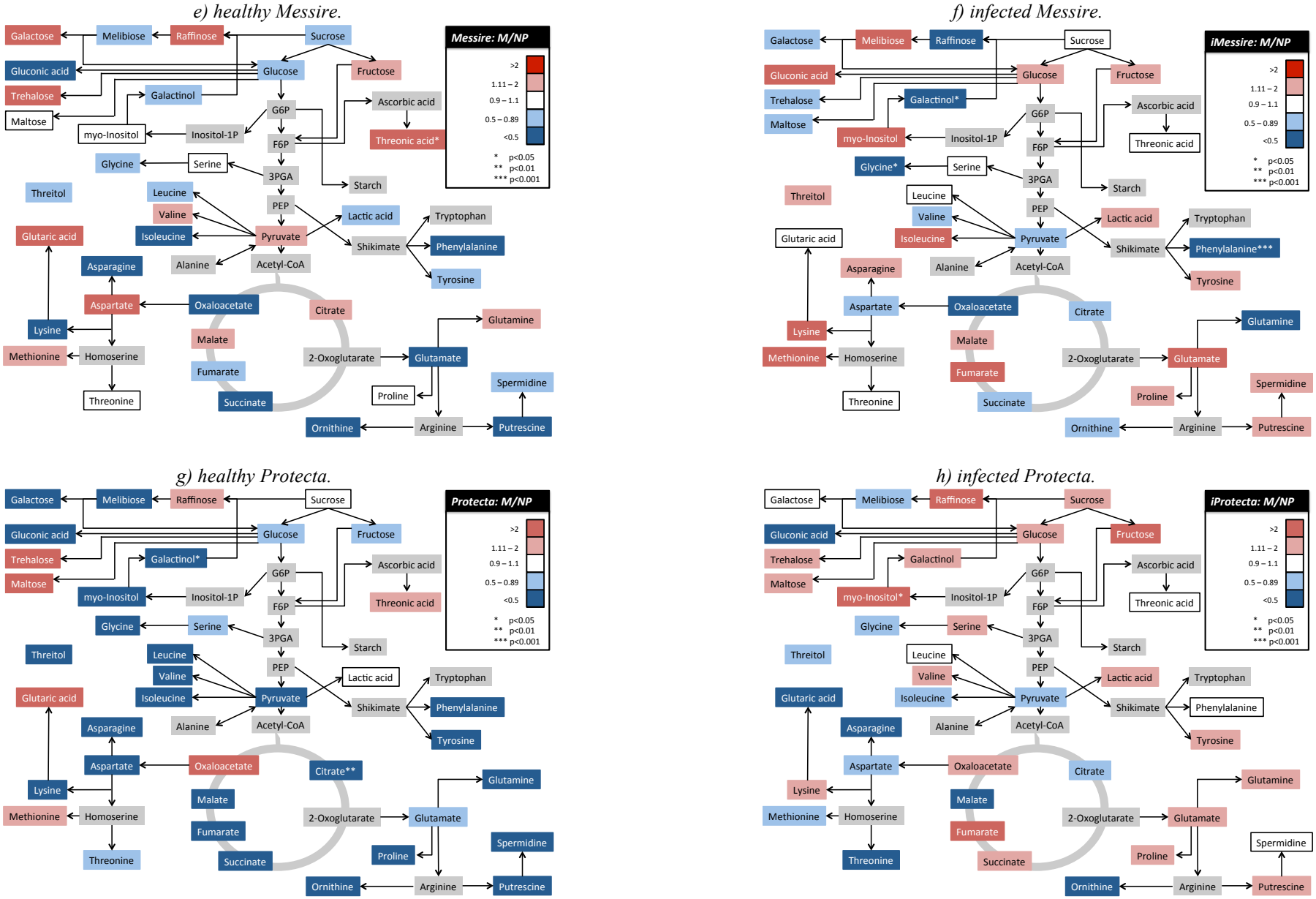
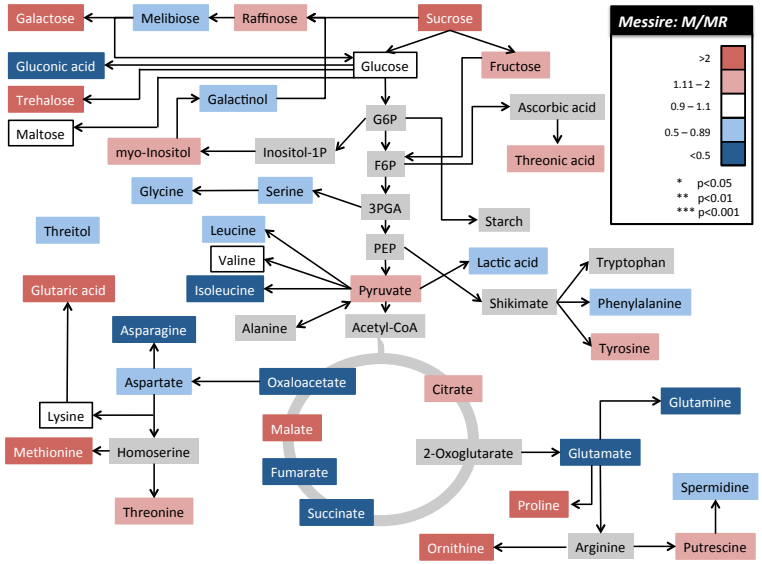
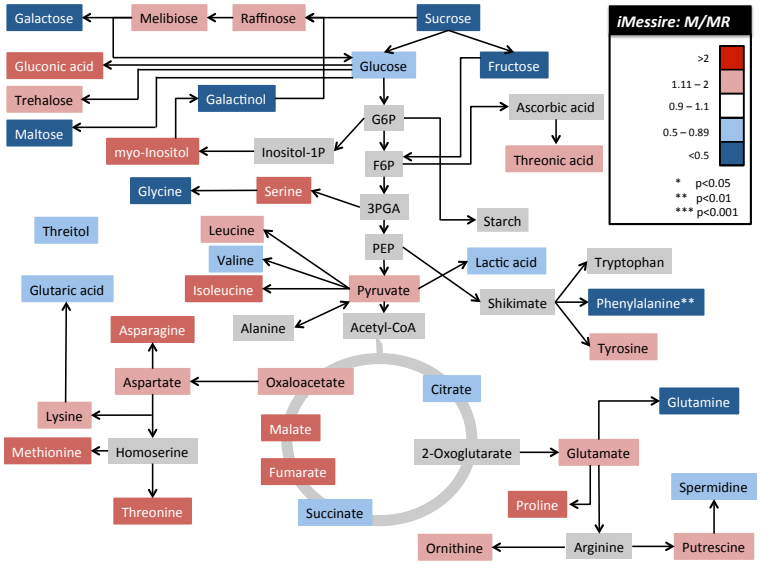


Figure 3.1e,f,g,h. Ratios of treatments M (inoculated with mycorrhiza) and NP (fertilized with nitrogen and phosphorus) of the different cvs (Messire e,f; Protecta g,h) comparing healthy and infected plants. Metabolites, which show a grey background, were not detected, but were pictured due to cross-linkage. The eventual asterisks on the sides of the metabolites represent significant results: p<0.05 (*), p<0.01 (**) or p<0,001 (***). These ratios reveal, which of the comparing treatments showed higher amounts in metabolites. A dark blue background means the ratio is smaller than 0.5, a light blue background implies a ratio of 0.5 – 0.89, a white background means the ratio is between 0.9 – 1.1, a light red background stands for a ratio of 1.11 – 2 and a dark red background means the ratio is higher than 2. In general, 39 metabolites were identified.

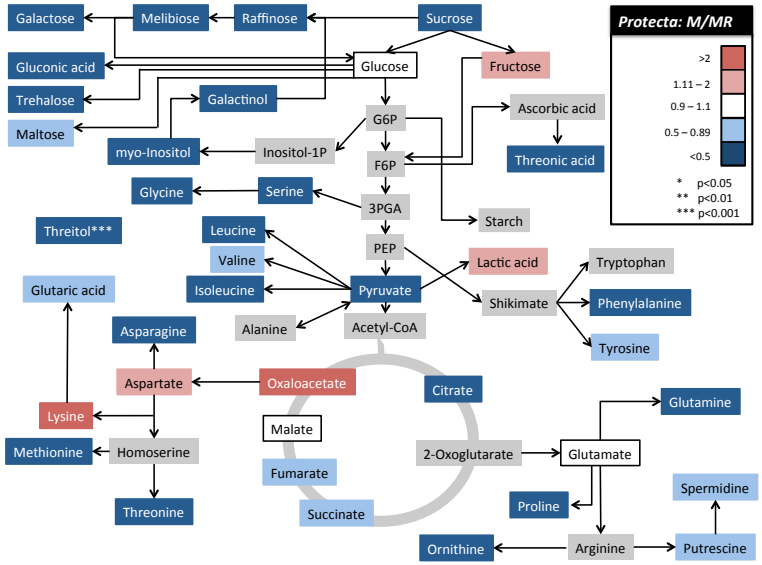
i) healthy Messire.



j) infected Messire.



k) healthy Protecta.



l) infected Protecta.

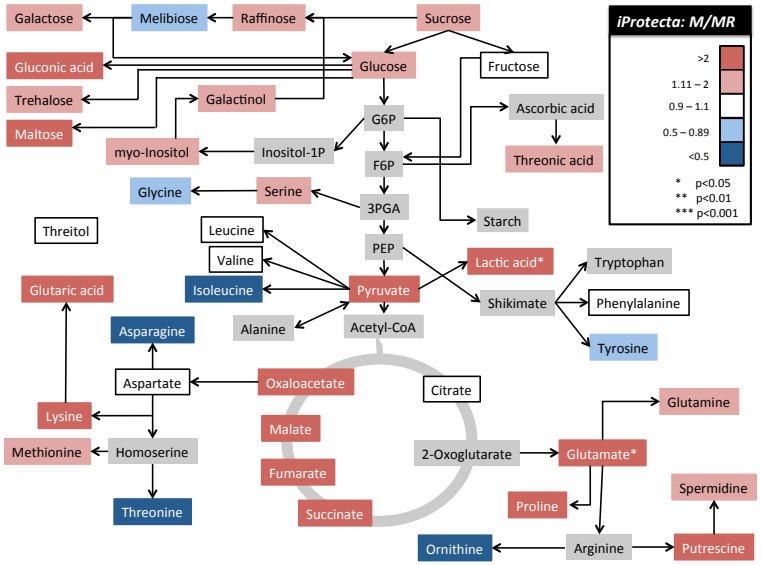
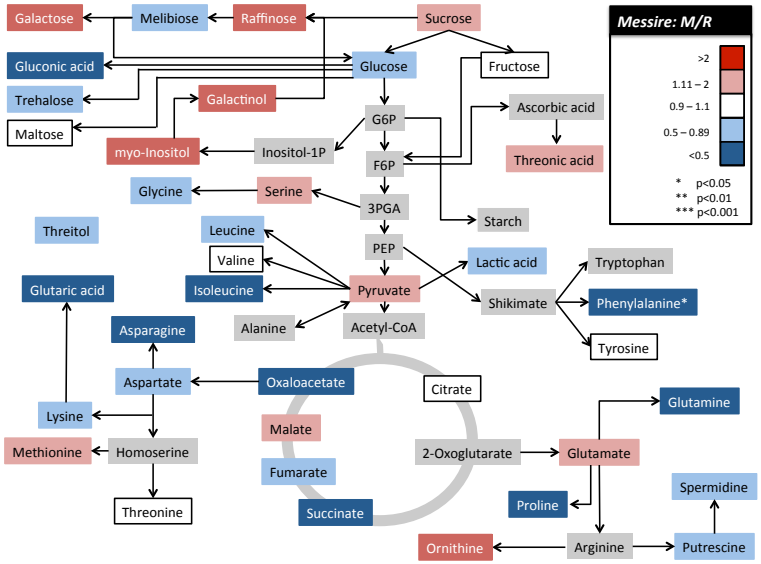
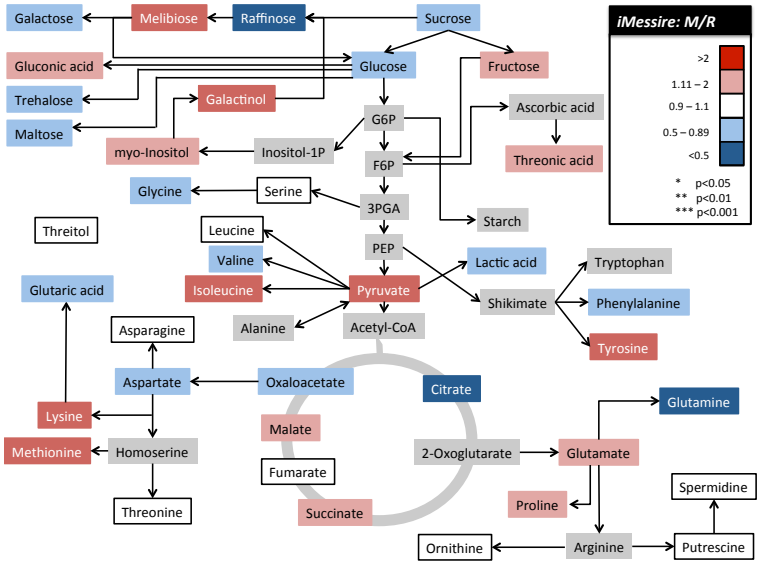


Figure 3.1i,j,k,l. Ratios of treatments M (inoculated with mycorrhiza) and MR (inoculated with mycorrhiza and rhizobia) of the different cvs (Messire i,j; Protecta k,l) comparing healthy and infected plants. Metabolites, which show a grey background, were not detected, but were pictured due to cross-linkage. The eventual asterisks on the sides of the metabolites represent significant results: p<0.05 (*), p<0.01 (**) or p<0,001 (***). These ratios reveal, which of the comparing treatments showed higher amounts in metabolites. A dark blue background means the ratio is smaller than 0.5, a light blue background implies a ratio of 0.5 – 0.89, a white background means the ratio is between 0.9 – 1.1, a light red background stands for a ratio of 1.11 – 2 and a dark red background means the ratio is higher than 2. In general, 39 metabolites were identified.

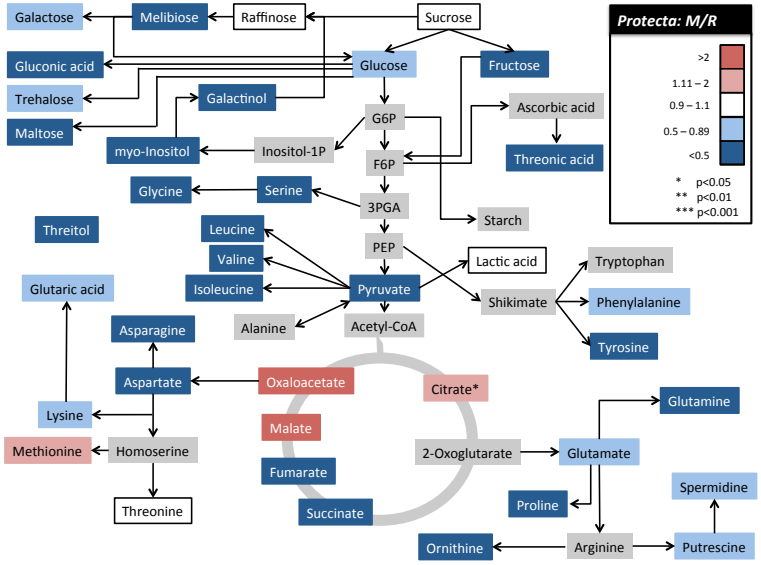
m) healthy Messire.



n) infected Messire.



o) healthy Protecta.



p) infected Protecta.

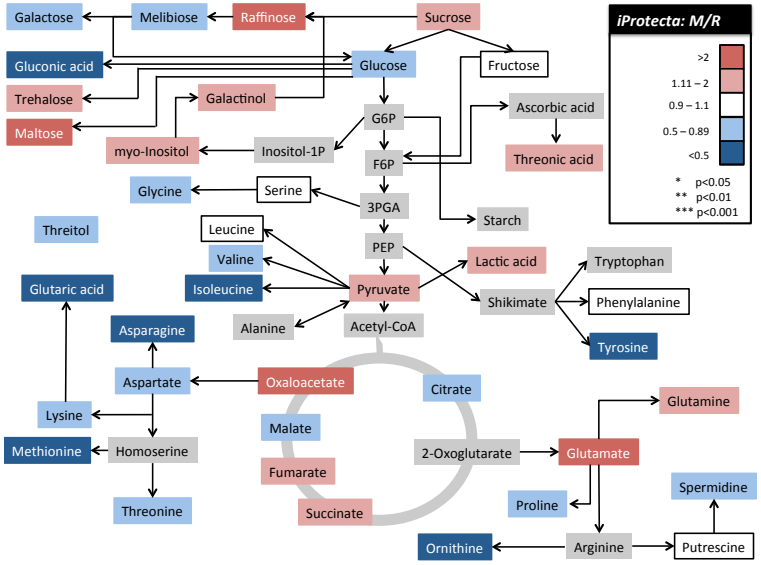


Figure 3.1m,n,o,p. Ratios of treatments M (inoculated with mycorrhiza) and R (inoculated with rhizobia) of the different cvs (Messire m,n; Protecta o,p) comparing healthy and infected plants. Metabolites, which show a grey background, were not detected, but were pictured due to cross-linkage. The eventual asterisks on the sides of the metabolites represent significant results: p<0.05 (*), p<0.01 (**) or p<0,001 (***). These ratios reveal, which of the comparing treatments showed higher amounts in metabolites. A dark blue background means the ratio is smaller than 0.5, a light blue background implies a ratio of 0.5 – 0.89, a white background means the ratio is between 0.9 – 1.1, a light red background stands for a ratio of 1.11 – 2 and a dark red background means the ratio is higher than 2. In general, 39 metabolites were identified.

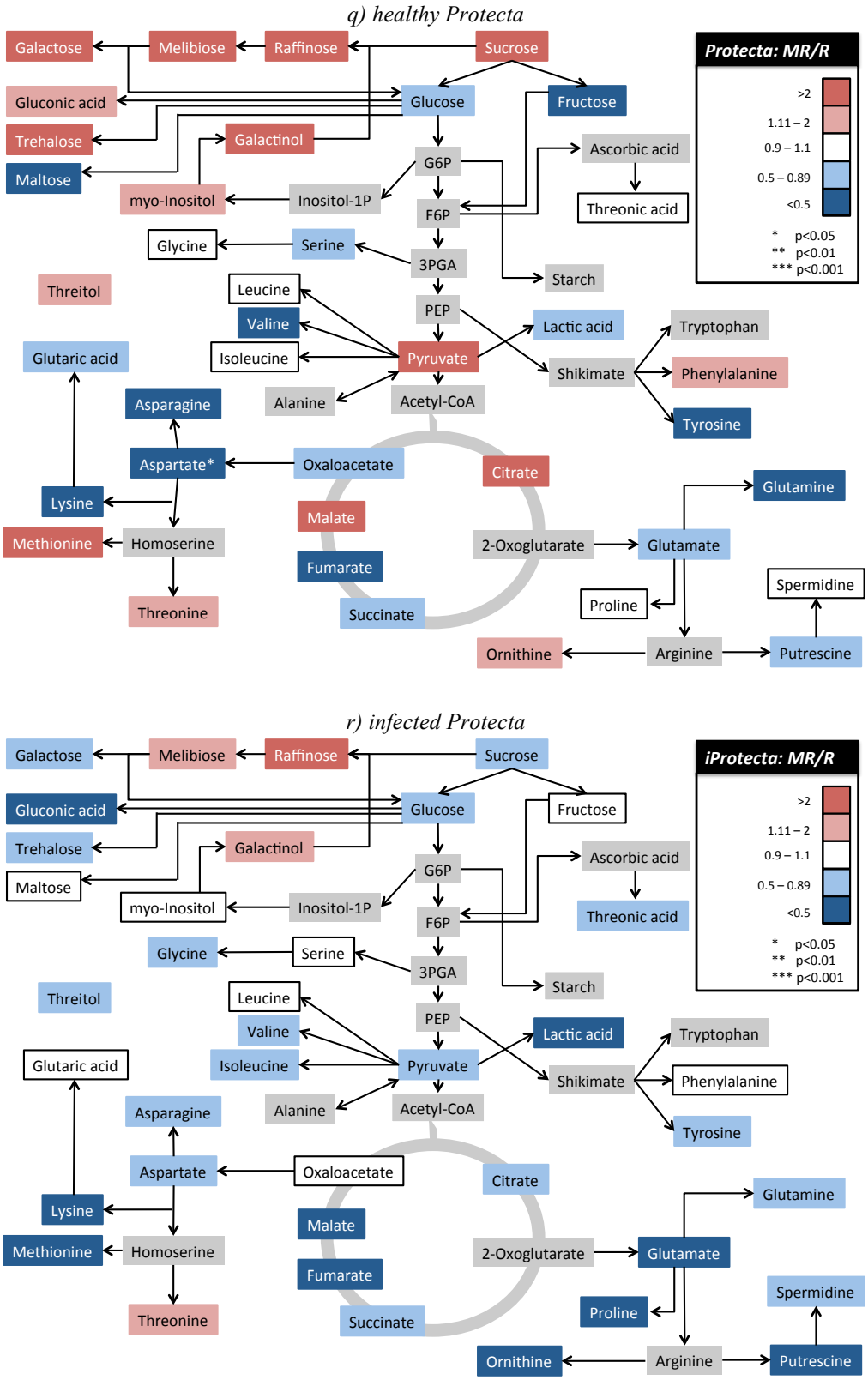


Figure 3.1q,r. Ratios of treatments MR (inoculated with mycorrhiza and rhizobia) and R (inoculated with rhizobia) of cv. Protecta comparing healthy and infected plants. Metabolites, which show a grey background, were not detected, but were pictured due to cross-linkage. The eventual asterisks on the sides of the metabolites represent significant results: p<0.05 (*), p<0.01 (**) or p<0,001 (***). These ratios reveal, which of the comparing treatments showed higher amounts in metabolites. A dark blue background means the ratio is smaller than 0.5, a light blue background implies a ratio of 0.5 – 0.89, a white background means the ratio is between 0.9 – 1.1, a light red background stands for a ratio of 1.11 – 2 and a dark red background means the ratio is higher than 2. In general, 39 metabolites were identified.

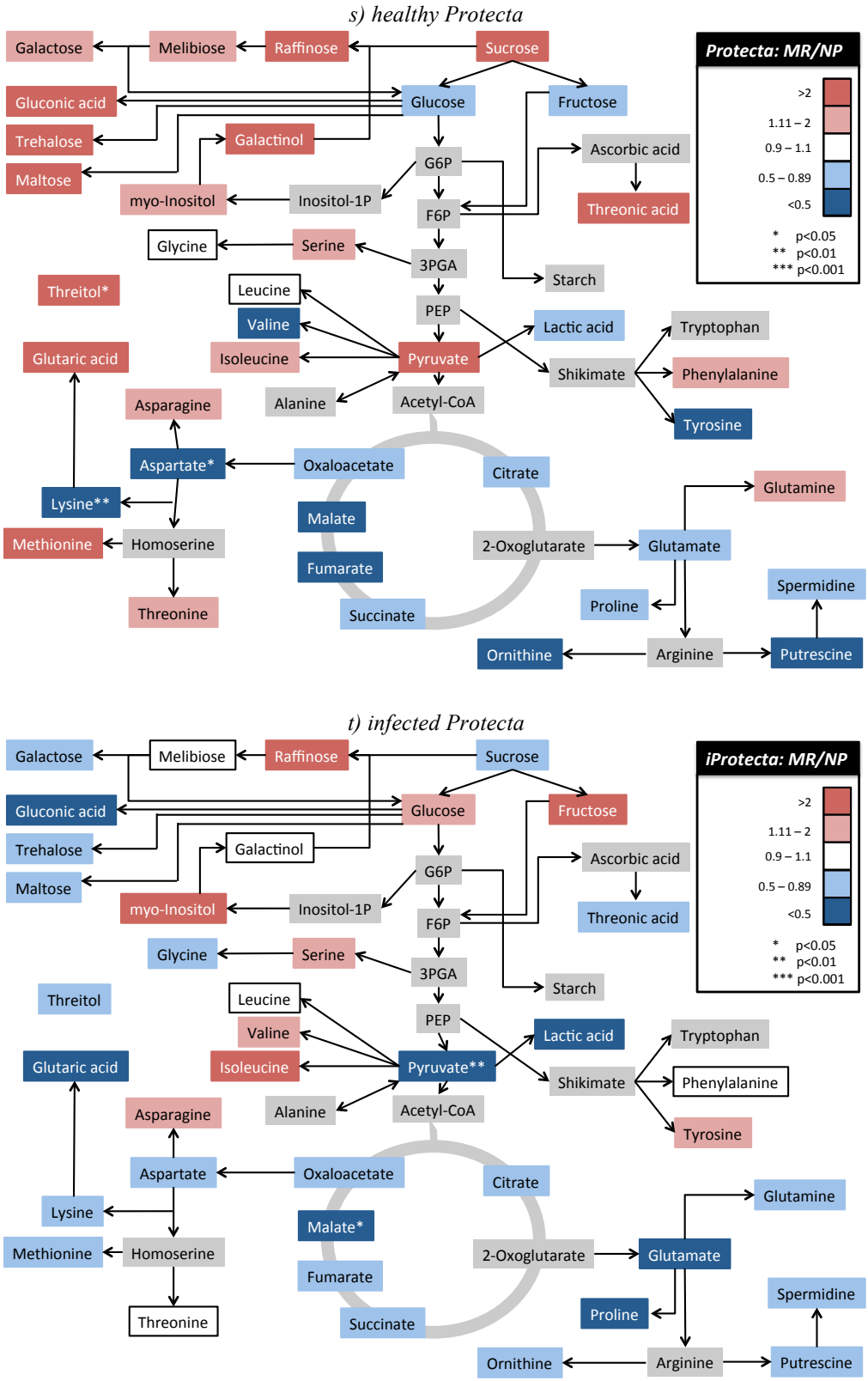


Figure 3.1s,t. Ratios of the treatments MR (inoculated with mycorrhiza and rhizobia) and NP (fertilized with nitrogen and phosphorus) of cv. Protecta comparing healthy and infected plants. Metabolites, which show a grey background, were not detected, but were pictured due to cross-linkage. The eventual asterisks on the sides of the metabolites represent significant results: p<0.05 (*), p<0.01 (**) or p<0,001 (***). These ratios reveal, which of the comparing treatments showed higher amounts in metabolites. A dark blue background means the ratio is smaller than 0.5, a light blue background implies a ratio of 0.5 – 0.89, a white background means the ratio is between 0.9 – 1.1, a light red background stands for a ratio of 1.11 – 2 and a dark red background means the ratio is higher than 2. In general, 39 metabolites were identified.

The treatment ratios of nitrogen and phosphorus fertilized and rhizobia inoculated plants of healthy and infected Messire did not show any obvious trends (Fig. 3.1a and Fig. 3.1b). Anyway, aspartate was significantly higher in rhizobia inoculated plants of healthy cv. Messire ($p<0.05$) (Fig. 3.1a), whereas galactinol was significantly higher in NP treatments in infected plants of cv. Messire ($p<0.01$) (Fig. 3.1b), which stands for slight metabolic differences between the treatments.
The ratios of nitrogen and phosphorus fertilized and rhizobia inoculated treatments of healthy and infected Protecta did not show any striking trends as well (Fig. 3.1c and Fig. 3.1d). Still, citrate was found in significantly higher amounts in NP treatments ($p<0.01$), whereas threonic acid was significantly higher in R treatments in healthy Protecta ($p<0.05$) (Fig. 3.1c). The same treatment ratios of infected Protecta did not show any significant differences between the treatments (Fig. 3.1d).

In healthy Messire leaves threonic acid was found in significantly higher quantities in mycorrhiza inoculated than in fertilized plants ($p<0.05$) (Fig. 3.1e). Infected Messire showed three metabolites, which were significantly higher in fertilized plants (Fig. 3.1f): galactinol ($p<0.05$), glycine ($p<0.05$) and phenylalanine ($p<0.01$).
Consider the treatment ratios of M and NP in healthy and infected Protecta a clear trend becomes visible: in healthy Protecta most of the metabolites were found in nitrogen and phosphorus fertilized plants (Fig. 3.1g), whereas in infected Protecta the biggest part of the metabolites was higher in mycorrhiza inoculated plants (Fig. 3.1h). Galactinol and citrate were significantly higher in NP treatments in healthy Protecta ($p<0.05$, $p<0.01$) (Fig. 3.1g), whereas myo-Inositol was significantly higher in M treatments of infected Protecta ($p<0.05$) (Fig. 3.1h).

The treatment ratios of M and MR in healthy Messire did not show any significant differences between the treatments (Fig. 3.1i). No striking distinction became visible. Regarding the ratios of M and MR in infected Messire, the majority of the metabolites was higher in mycorrhiza inoculated plants (Fig. 3.1j). However, a significant change in metabolite levels was found for phenylalanine; it had higher amounts in MR treatments of infected Messire ($p<0.01$).
Cultivar Protecta showed striking trends in the treatment ratios of M and MR in both healthy and infected plants (Fig. 3.1k and Fig. 3.1l). In healthy Protecta most metabolites occurred in higher quantities in plants inoculated with mycorrhiza and rhizobia, including threitol which was significantly higher ($p<0.001$) (Fig. 3.1k). However, in infected cv. Protecta most

metabolites were higher in mycorrhiza inoculated plants, especially glutamate and lactic acid were both significantly higher ($p<0.05$) (Fig. 3.1l).

The treatment ratios of M and R in healthy Messire showed that most of the metabolites were found in higher amounts in rhizobia inoculated than in mycorrhiza inoculated treatments (Fig. 3.1m). Phenylalanine was significantly higher in M treatments ($p<0.05$). Infected Messire did not show any significant outcomes as well as no striking trend (Fig. 3.1n). Comparing the same treatment ratios in healthy and infected Protecta, the majority of the metabolites was higher in rhizobia inoculated than in mycorrhiza inoculated plants for both healthy and infected plants (Fig. 3.1o and Fig. 3.1p).

The treatment ratios of double inoculated (MR) and only rhizobia inoculated plants of healthy cv. Protecta show that most sugars were found in higher amounts in MR treatments than in R treatments (Fig. 3.1q). Still, aspartate was significantly higher in rhizobia inoculated plants of healthy Protecta ($p<0.05$). However, no further trend was observed for healthy ones. Hence, infected Protecta on the other hand showed an obvious trend: most metabolites were higher in rhizobia inoculated treatments (Fig. 3.1r).

In healthy Protecta most sugars were higher in MR treatments than in NP treatments, including the sugar alcohol threitol, which was significantly higher ($p<0.05$) (Fig. 3.1s). However, aspartate and lysine were significantly higher in those plants of healthy Protecta that were fertilized with nitrogen and phosphorus ($p<0.05$ and $p<0.01$). Furthermore, all detected metabolites which are a part in the citrate cycle were found in higher amounts in fertilized plants. On the other hand, in infected Protecta most of the metabolites occurred in higher values in nitrogen and phosphorus fertilized plants, including pyruvate and malate which were significantly higher ($p<0.01$ and $p<0.05$) (Fig. 3.1t). Again, all metabolites which are players in the citrate cycle were found in higher quantities in nitrogen and phosphorus fertilized plants.

3.1.2. Ratios of infected and healthy cultivars

In this section the ratios of infected and healthy plants of one cultivar and one treatment are represented (Fig. 3.2a-h). The significant results were evaluated by using the T-test in Excel, because ANOVA in R-Studio only revealed less significant results.

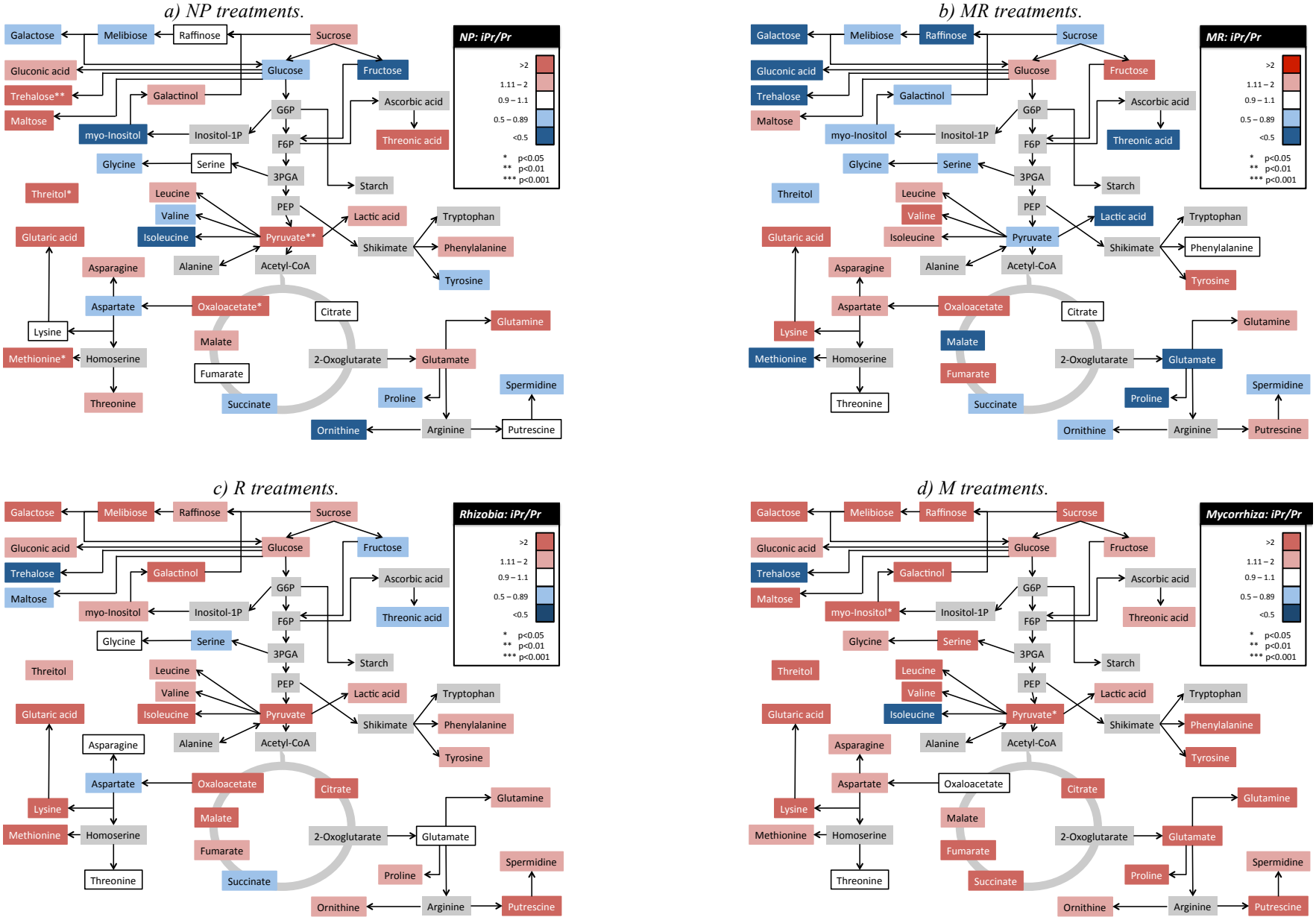


Figure 3.2-abcd. Ratios of healthy and infected plants of cv. Protecta treated with M (mycorrhiza inoculated), R (rhizobia inoculated), MR (inoculated with mycorrhiza and rhizobia) or NP (fertilized with nitrogen and phosphorus). Metabolites, which show a grey background, were not detected, but were pictured due to cross-linkage. The eventual asterisks on the sides of the metabolites represent significant results: p<0.05 (*), p<0.01 (**) or p<0,001 (***). These ratios reveal, which of the states of health showed higher amounts in metabolites. A dark blue background means the ratio is smaller than 0.5, a light blue background implies a ratio of 0.5 – 0.89, a white background means the ratio is between 0.9 – 1.1, a light red background stands for a ratio of 1.11 – 2 and a dark red background means the ratio is higher than 2. In general, 39 metabolites were identified.

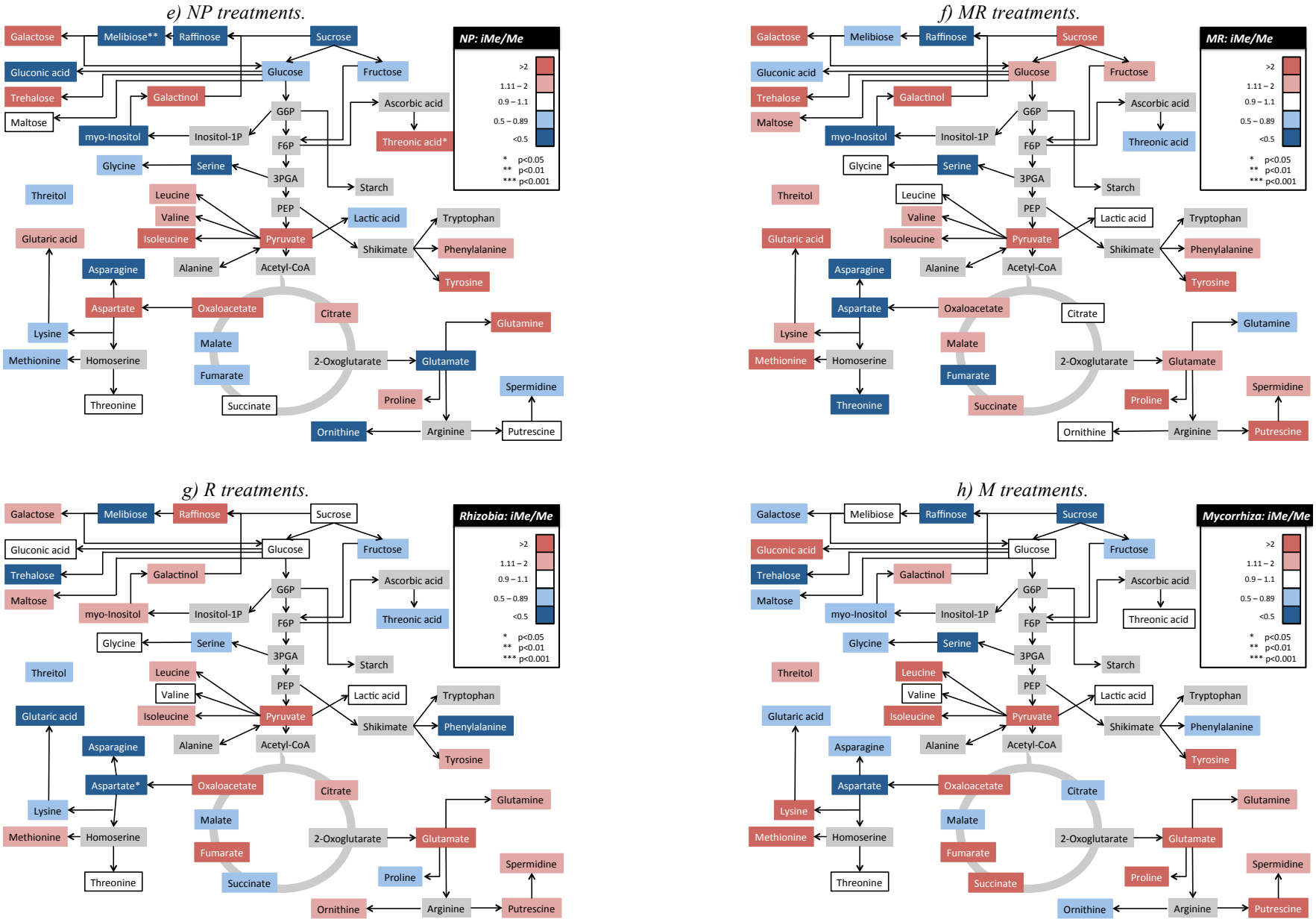


Figure 3.2e,f,g,h. Ratios of healthy and infected plants of cv. Messire treated with M (mycorrhiza inoculated), R (rhizobia inoculated), MR (inoculated with mycorrhiza and rhizobia) or NP (fertilized with nitrogen and phosphorus). Metabolites, which show a grey background, were not detected, but were pictured due to cross-linkage. The eventual asterisks on the sides of the metabolites represent significant results: p<0.05 (*), p<0.01 (**) or p<0,001 (***). These ratios reveal, which of the states of health showed higher amounts in metabolites. A dark blue background means the ratio is smaller than 0.5, a light blue background implies a ratio of 0.5 – 0.89, a white background means the ratio is between 0.9 – 1.1, a light red background stands for a ratio of 1.11 – 2 and a dark red background means the ratio is higher than 2. In general, 39 metabolites were identified.

The ratios of infected and healthy Protecta fertilized with N and P revealed five metabolites which were significantly higher in infected than in healthy plants (Fig. 3.2a): trehalose, threitol, methionine, oxaloacetate and pyruvate ($p<0.05$ to $p<0.01$). Furthermore, those metabolites which occurred in higher amounts in infected Protecta accounted for the bulk.
Regarding the ratios of infected and healthy Protecta of MR treatments the majority of the metabolites was found in higher quantities in healthy than in infected Protecta which includes almost all sugars (Fig. 3.2b). Unfortunately, the results obtained were not significant.
R treatments revealed a clear trend, since almost all metabolites occurred in higher amounts in infected plants of cv. Protecta than in the healthy ones (Fig. 3.2c). However, none of those were significantly higher.
Mycorrhiza inoculated plants of cv. Protecta showed the same trend as rhizobia inoculated plants (Fig. 3.2d). Yet, this trend was even more obvious due to many metabolites which showed a ratio of >2. In addition, myo-Inositol and pyruvate were significantly higher ($p<0.05$) in infected Protecta.
When comparing all four treatments of cultivar Protecta with one another, one can see similarities between M (Fig. 3.2d) and R (Fig. 3.2c) inoculated plants, whereas the treatments NP (Fig. 3.2a) and MR (Fig. 3.2b) differ quite from each other as well as from M and R treatments.

In the ratios of infected and healthy Messire fertilized with nitrogen and phosphorus two significant results were found (Fig. 3.2a): melibiose was significantly higher in healthy plants of cv. Messire, whereas threonic acid was significantly higher in infected plants of cv. Messire. Moreover, a slight trend became visible regarding the sugars, because the majority of them were higher in healthy plants.
Mycorrhiza and rhizobia inoculated plants of healthy and infected Messire showed a more obvious trend, based on the fact that most metabolites were higher in infected plants (Fig. 3.2b). Still, non of the results were statistically significant.
When looking at the ratios of infected and healthy plants of cv. Messire inoculated with rhizobia (Fig. 3.2c) or with mycorrhiza (Fig. 3.2d), no distinct trend was visible in either of the two. Still, aspartate was significantly higher in healthy plants of cv. Messire inoculated with rhizobia than in infected ones (Fig. 3.2c).
After all, plants of cultivar Messire did not show such clear differences between the treatments as plants of Protecta. Although treatments R (Fig. 3.2c), M (Fig. 3.2d) and MR (Fig. 3.2b)

showed higher amounts in the metabolites in infected than in healthy plants of cv. Messire, in total it was just a slight trend in R and M treated plants.

3.2. Results of nodules of *Pisum sativum*

In the next part the results of the nodules of the two cultivars Messire and Protecta either healthy or infected with spores of *Didymella pinodes* are represented. This contains the results of the nodule weight as well as the results of the extracted metabolites.

3.2.1. Nodule weight

In this part of the master thesis the results of the nodule weight are presented. The weight per nodule as well as the total fresh weight of nodules are pictured in boxplots. The boxplots were devised in RStudio ®.

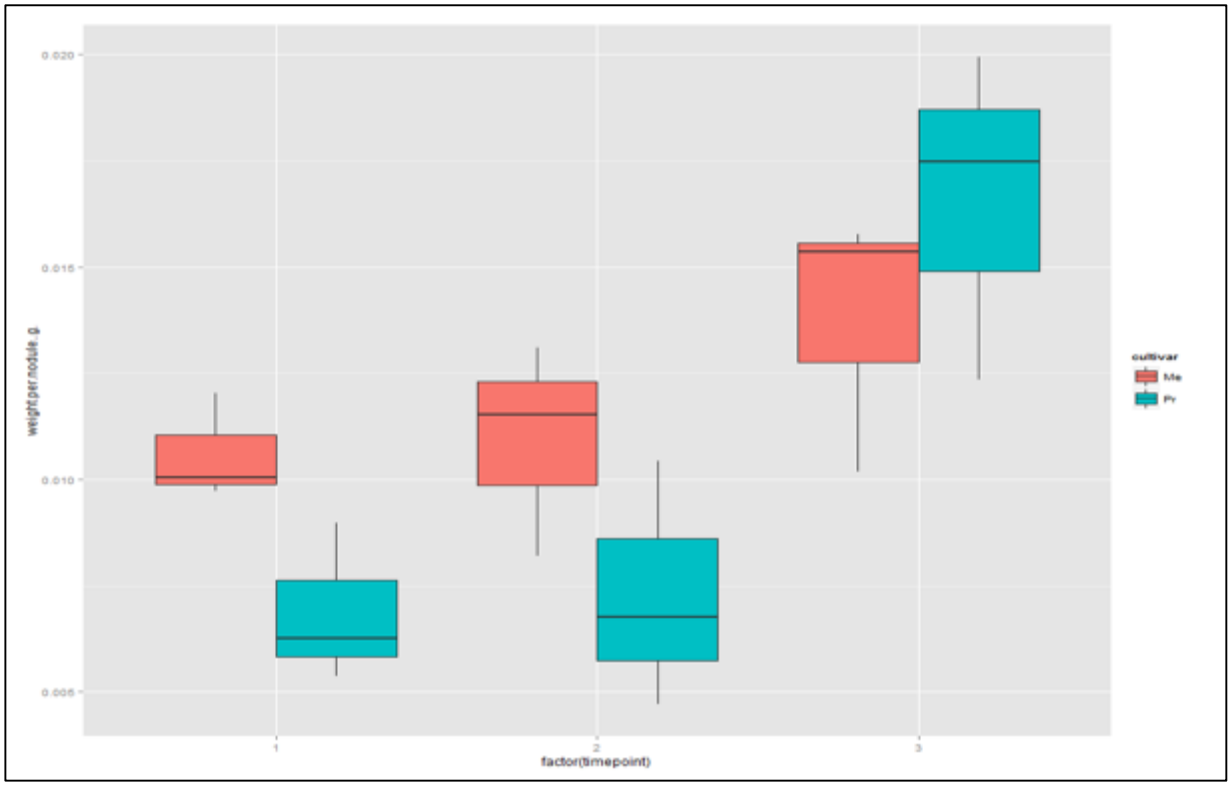


Figure 3.3. Weight per nodule of Messire and Protecta at three different points in time. Red bars depict nodules of cultivar Messire, whereas turquoise bars depict nodules of cultivar Protecta. On the x-axis the three different points in time (1=24 hai, 2= 48 hai, 3= 2 wai) are depicted, whereas on the y-axis the weight (g) per nodule is displayed.

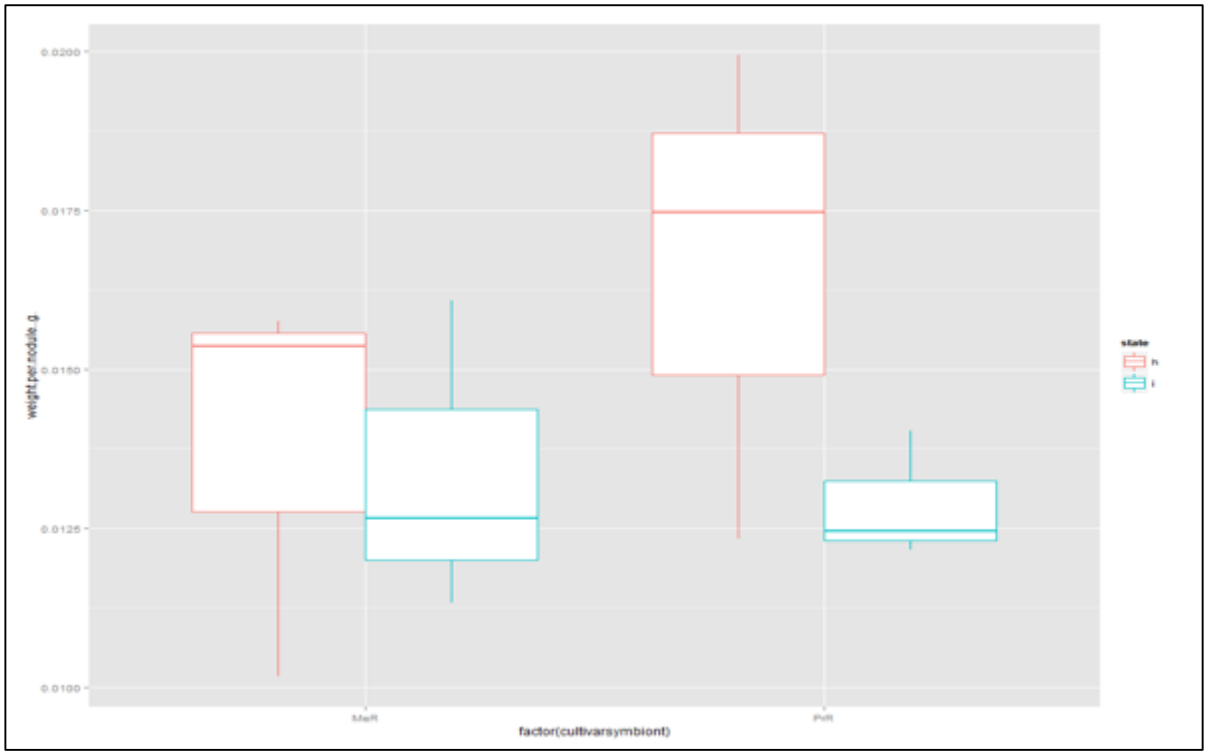


Figure 3.4. Weight per nodule in total in healthy and infected Messire and Protecta. Red-framed bars depict nodules of healthy plants, whereas turquoise-framed bars depict nodules of infected plants. The two left bars respresent cv. Messire, whereas the two right bars represent cv. Protecta. The weight per nodule is shown in g.

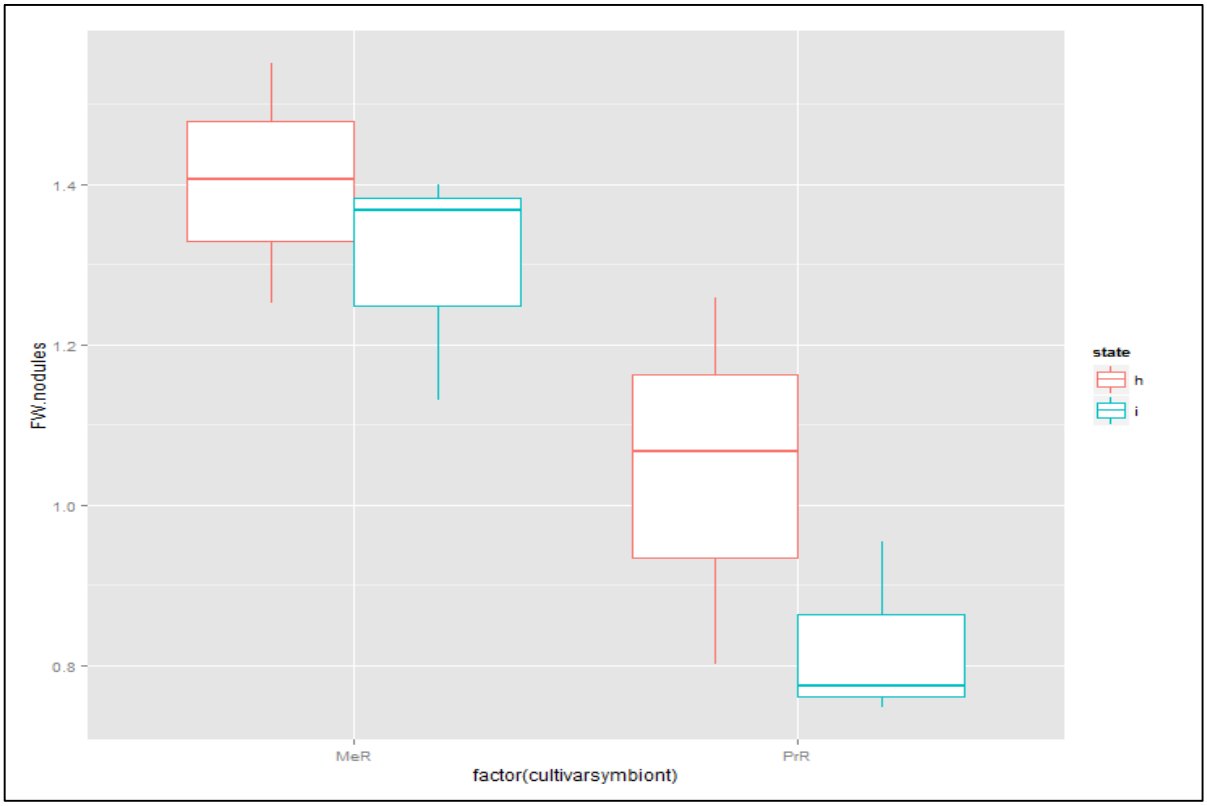


Figure 3.5. Fresh weight of nodules in healthy and infected cultivars Messire and Protecta. The red framed bars represent healthy plants, whereas the turquoise-framed bars depict the infected ones. The two left bars depict results of cv. Messire, whereas the two right bars are those of cv. Protecta.

Figure 3.3. shows the results of the weight per nodule of Messire and Protecta at three different points in time. The weight per nodule increased apparently after two weeks (third point in time) compared to the first two points in time (24 hai, 48 hai) in both Messire and Protecta. At the first two points in time cv. Messire showed a higher weight per nodule than cv. Protecta. However, two weeks after the infection cv. Protecta caught up and developed a higher weight per nodule than cv. Messire.

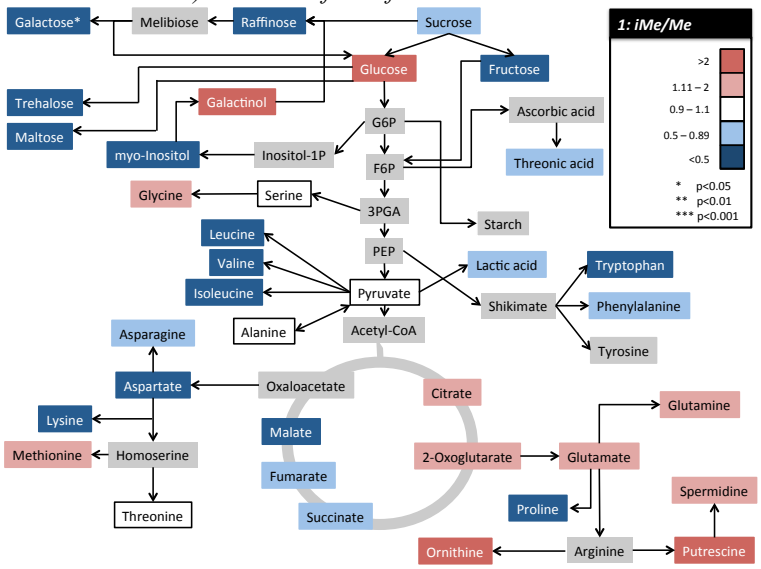
Figure 3.4. depicts the results of the total weight per nodule of healthy and infected Messire and Protecta. Generally, healthy plants of both cultivars showed a higher weight per nodule than infected ones. The highest weight per nodule was found in healthy Protecta. When infected, the weight per nodule of cv. Protecta was less than of cv. Messire. Also the differences in the weight per nodule between healthy and infected state were bigger in cv. Protecta than in cv. Messire.

Figure 3.5. represents the results of the total fresh weight of the nodules in healthy and infected cv. Messire and Protecta. In general, the fresh weight of the nodules was always higher in healthy plants of both cultivars. Still, Messire's nodules achieved the highest fresh weight. Furthermore, the differences between healthy and infected state are less in cv. Messire than in cv. Protecta, just like in Figure 3.4., where the weight per nodule is depicted.

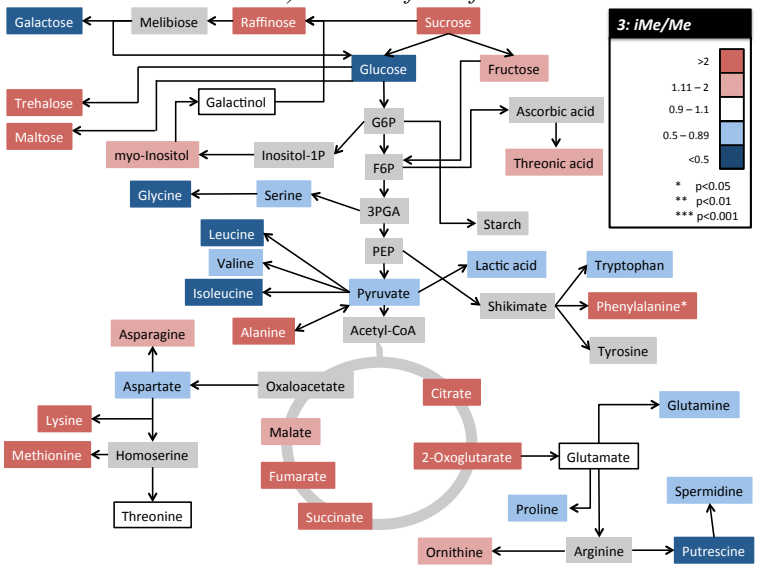
3.2.2. Metabolic results of nodules of *Pisum sativum*

In the following section the results of the metabolites of the nodules are presented. The results are shown in metabolic ratio maps, just like in paragraph 3.1. reviewed above. Healthy and infected plants of one cultivar at one of the two different points in time (24 hai or 2 wai) are shown.

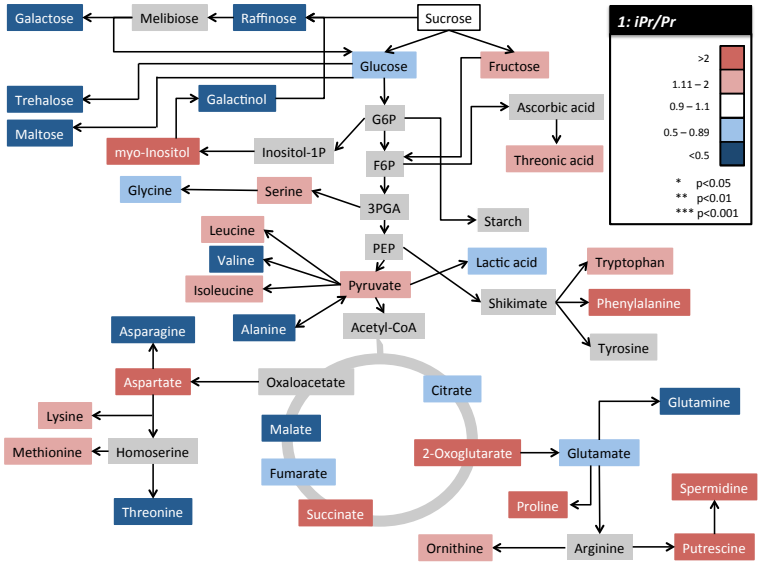
a) 24 hours after infection.



b) 2 weeks after infection.



c) 24 hours after infection.



d) 2 weeks after infection.

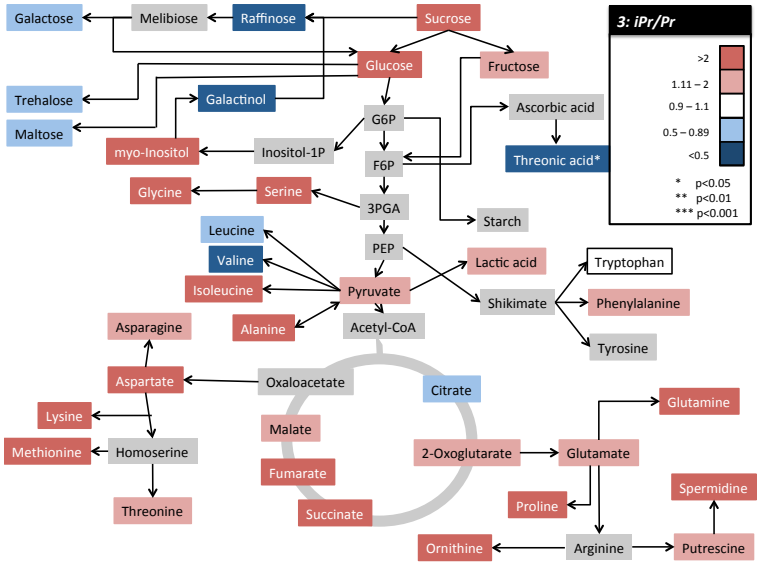


Figure 3.6a,b,c,d. Ratios of healthy and infected plants of the different cvs (Messire a,b; Protecta c,d) at the two different time points (1 = 24 hours after the infection a,c; 3 = 2 weeks after the infection b,d). Metabolites, which show a grey background, were not detected, but were pictured due to cross-linkage. The eventual asterisks on the sides of the metabolites represent significant results: p<0.05 (*), p<0.01 (**) or p<0,001 (***). These ratios reveal, which of the states of health showed higher amounts in metabolites. A dark blue background means the ratio is smaller than 0.5, a light blue background implies a ratio of 0.5 – 0.89, a white background means the ratio is between 0.9 – 1.1, a light red background stands for a ratio of 1.11 – 2 and a dark red background means the ratio is higher than 2. In general, 36 metabolites were identified.

Figure 3.6a reveals the metabolite ratios of infected and healthy Messire 24 hours after the infection. There was a clear trend, since the majority of the metabolites occurred in higher quantity in healthy than in infected Messire 24 hours after the infection. Galactose was significantly higher in healthy Messire ($p<0.05$). Only 11 metabolites, including both polyamines, were found in higher amounts in infected Messire. The metabolite ratios of infected and healthy Messire two weeks after the infection show no clear trend (Fig. 3.6b). Except when comparing them to the ratios of the first point in time, more metabolites were found in higher amounts in infected plants two weeks than 24 hours after the infection. Furthermore, phenylalanine was significantly higher in infected Messire and all metabolites from the citrate cycle as well as most sugars were found in higher amounts in nodules of infected Messire. Also, asparagine was found in higher amounts in infected plants two weeks after the infection.

The metabolite ratios of infected and healthy Protecta 24 hours after the infection show no striking trends, except for the sugars, since most of them were higher in healthy Protecta (Fig. 3.6c). Figure 3.6d shows clearly that the majority of the metabolites was found in higher amounts in infected Protecta two weeks after the infection. Also, comparing the ones harvested first with the ones harvested after two weeks the same trend as in Messire (Fig. 3.6a and Fig. 3.6b) occurred: there were more metabolites found in higher amounts two weeks after the infection than 24 hours after the infection in nodules of infected plants of cv. Protecta (Fig. 3.6c and Fig. 3.6d). However, threonic acid was significantly higher in healthy Protecta two weeks after the infection (Fig. 3.6d).

Except for cultivar Messire two weeks after the infection, the polyamines spermidine and putrescine were always found in higher amounts in nodules of infected plants of both Messire and Protecta than in those of healthy plants. Additionally, nodules of infected plants of both cultivars showed higher amounts in sugars two weeks after the infection than 24 hours after infection.

4. Discussion

4.1. Leaves of *Pisum sativum* after infection with *Didymella pinodes*

In healthy Protecta the ratios between mycorrhiza (M) and the other treatments (NP, MR and R) showed obvious trends, since most metabolites occurred in lower amounts in mycorrhiza treated plants (Fig. 3.1g, Fig. 3.1k, Fig. 3.1o). This may indicate that NP, MR and R treatments provide more nutrients for these plants than M treatment, resulting in higher metabolite levels. The fact that also the double inoculation (MR) showed higher metabolite levels than plants only inoculated with mycorrhiza indicates that paired inoculation positively effects mycorrhiza nutrient provision compared to single mycorrhiza treatment. This may result from enhanced nutrient uptake on the basis of two symbionts. Many reports dealing with co-inoculation of rhizobia and mycorrhiza in legumes argue for a synergistic effect of both symbionts on plant development, yield and nutrient content – especially in levels of N and P (PACOVSKY *et al.*, 1986; AZCON *et al.*, 1991; IANSON and LINDERMAN, 1993; AHMAD, 1995; SAXENA *et al.*, 1997; XAVIER and GERMIDA, 2002).
Furthermore, leaves of healthy plants of cv. Protecta revealed highest amounts of sugars in plants inoculated with rhizobia (R and MR treatments) (Fig. 3.1c, Fig. 3.1k, Fig. 3.1o, Fig. 3.1s). This might be due to higher sugar production of plants living in symbiosis with rhizobia, needed for the exchange of fixed nitrogen. KIERS *et al.* (2011) described that host plants are able to reward cooperating mycorrhiza with more carbohydrates. This may also be true for the symbiosis between cv. Protecta and rhizobia. Hence, an effective plant-microbe cooperation enhances plant sugar production which is then transferred to symbionts for reward.

Infected plant leaves of cv. Protecta treated with M or R revealed increasing amounts in sugars compared to leaves of healthy Protecta (Fig. 3.2c and Fig 3.2d). Not only sugars but also most other metabolites were found in higher amounts in M and R treatments of infected Protecta. Many biochemical studies have revealed that stress conditions like drought and high salinity can lead to accumulation of metabolites in plants, including nitrogen-containing compounds like amino acids and hydroxyl compounds like sugars (MCCUE and HANSON, 1990; BOHNERT *et al.*, 1995). This phenomenon was also described by SCHUBERT *et al.* (1995), where an increase in amino acid concentrations was found in nodules and shoots,

whereas sugars were increasing in shoots of *Medicago sativa* suffering from drought stress. They argued that limited extension growth was the reason for amino acid accumulation in shoots, because the demand for nitrogenous compounds decreased. KAMELI and LÖSEL (1996) described sugar accumulation in leaves of drought stressed durum wheat, due to maintenance of CO_2 assimilation and therefore sugar production, while drought stress caused inhibition of expansion growth and therefore sugar translocation. Inhibited expansion growth might also be the reason for sugar and amino acid accumulation in leaves of infected cv. Protecta, except that growth is inhibited by *Didymella pinodes*. However, expansion growth was not measured in this experiment.

Another suggestion is that *Didymella pinodes* leads directly to sugar accumulation in leaves of Protecta due to the pathogen's nutrient demand. Pathogens need nutrients from their hosts to grow and survive (BROWN *et al*., 2008). Therefore, if *Didymella pinodes* needed carbon compounds like sugar for its own development, it would make sense if it induced Protecta to produce more of it.

However, in our study the suggestion of an interruption of the plant-symbiont connection due to the infection of *Didymella pinodes* makes most sense. In this case sugars etc. are no longer allocated from leaves to roots and are therefore accumulating. YADAV *et al.* (1999) described that amino acid content increased under drought stress due to hydrolysis of proteins in chickpea (*Cicer arietinum*). It might be that a plant-symbiont connection breakdown happened in this study due to hydrolysis of proteins which are responsible for compound transportation from and to leaves of *Pisum sativum* caused by *Didymella pinodes*. If sugars are accumulating in leaves of cv. Protecta and are not translocated to symbionts, the latter may suffer from this carbon shortage. Thus, their reproductive success may decrease. Such decrease in reproductive success of rhizobia has already been shown in soybeans as a result of non-cooperation on the part of rhizobia (KIERS *et al.*, 2003).

With respect to metabolite amounts which can be found in supplemental Table 8.2. a clear trend regarding an increase in most metabolite levels in infected leaves of cv. Protecta could be found. This was true for R, M and NP treatments. In contrast, only MR treated plants showed mostly higher metabolite levels in healthy plants of cv. Protecta compared to infected ones. Hence, *Didymella pinodes* has an impact on plants primary metabolism of cv. Protecta with an interesting antagonistic effect in mycorrhiza treated plants. However, pathogen infection showed the same level in all treatments. According to BROWN *et al.* (2008) pathogenesis is only successful if the pathogen acquires necessary nutrients for growth and

survival from the host. Therefore, the reason for the affection of Protecta's primary metabolism might be due to its nutrient supply for the pathogen. Although environmental stresses like drought stress have been shown to affect primary metabolism of *Pisum sativum* (CHARLTON *et al.*, 2008), impact of *Didymella pinodes* on pea's primary metabolism has not yet been described and is therefore shown in this study for the first time. However, according to CARRILLO *et al.* (2012) and FONDEVILLA *et al.* (2013) *Didymella pinodes* affects pea's secondary metabolism by inducing it to defend against the pathogen, e.g. cell wall reinforcement.

Regarding healthy plants of cv. Messire – similar to cv. Protecta – NP and R treatments induce more nutrients than those treated with mycorrhiza (M, MR) (Fig. 3.1e and Fig. 3.1m). According to XAVIER and GERMIDA (2002), who worked with rhizobia and mycorrhiza in legume lentil, treatments with rhizobia or co-inoculated with rhizobia and compatible mycorrhiza achieved higher productivity than those inoculated only with mycorrhiza or co-inoculated with rhizobia and incompatible mycorrhiza strain. This might also be true for cv. Messire. The fact that in this experiment MR treated plants of cv. Messire did not show higher metabolite amounts than R treated plants, indicates – just like described by XAVIER and GERMIDA (2002) – that mycorrhiza and rhizobia symbioses-efficiency was rather antagonistic in cv. Messire. This is why rhizobia worked better alone. Still, NP treated plants of cv. Messire showed higher metabolite amounts than R treated plants (Fig. 3.1a), which suggests that NP-fertilization provides even more nutrients to cv. Messire than inoculation with rhizobia. According to FAGERIA *et al.* (2014), who worked on nitrogen fertilization and rhizobia inoculation of dry bean, rhizobia inoculation alone does not lead to maximum productivity, but use of N fertilizers is required. If this was also true for cv. Messire, the data from this experiment supported the assumption. The data also reveal a clear difference in establishment of symbiosis interaction depending on the plant cultivars, since microsymbiont strains were the same for Messire and Protecta.

MR treatments revealed obvious metabolic differences between infected and healthy plants of cv. Messire (Figure 3.2f). Infected plants showed twice as many metabolites with higher levels as healthy plants. It is striking, that the major part of sugars and sugar alcohols like (sucrose, glucose, fructose, maltose, trehalose, galactose, galactinol and threitol) and most organic acids which are part in the TCA cycle (pyruvate, oxaloacetate, malate and succinate), accumulated in leaves when cv. Messire was infected. The reasons for sugar accumulation in

leaves of mycorrhiza and rhizobia treated plants of infected cv. Messire might be the same as in infected cv. Protecta: a) expansion growth is inhibited due to the infection followed by a decrease in carbon demand, b) sugar accumulation is directly induced by the pathogen or c) the connection between plant and symbionts is interrupted as a result of *Didymella pinodes* which is why sugars cannot be translocated to the symbionts. However, suggestion c) makes most sense in this study. Organic acid accumulation suggests a disruption of export of these compounds from the leaf as well, since organic acids are common constituents of sap exudates in various plant species (BIALCZYK and LECHOWSKI, 1995; CHARLTON *et al.*, 2008; NAKAMURA *et al.*, 2008).
Mostly, infected leaves of cv. Messire showed more metabolites revealing higher amounts than healthy leaves (Fig. 3.2f, Fig. 3.2g, Fig. 3.2h). This proves the impact of *Didymella pinodes* on leaves of cv. Messire on metabolite level. Therefore, *Didymella pinodes* affects primary metabolism of both cv. Protecta and also cv. Messire which might be due to their nutrient supply for the pathogen.

In total, aspartate (Fig. 3.1a,q,s), citric acid (Fig. 3.1c,g,o), galactinol (Fig. 3.1b,f,g), glutamate (Fig. 3.1l), glycine (Fig. 3.1f), lactate (Fig. 3.1l), lysine (Fig. 3.1s), malate (Fig. 3.1t), myo-Inositol (Fig. 3.1h), phenylalanine (Fig. 3.1f,j,m), pyruvate (Fig. 3.1t), threitol (Fig. 3.1k,s) and threonic acid (Fig. 3.1c,e) revealed significant changes in response to the nutritional treatments of cv. Messire and cv. Protecta. Aspartate is the precursor of five other amino acids (asparagine, lysine, isoleucine, threonine and methionine) and is therefore important in amino acid synthesis. Citric acid, pyruvate and malate are essential parts of the TCA cycle. Galactinol, derived from myo-Inositol and UDP-glucose, is an important intermediate product in the production of raffinose which is a trisaccharide and a crucial compound for transportation and storage of carbohydrates. Glutamate is part of the nitrogen-metabolism and also plays a role in amino acid synthesis. Glycine is essential in photorespiration and amino acid synthesis in plants. Lactate is part of fermentation processes. Phenylalanine is built in the Shikimat pathway and serves further in the synthesis of phenolic compounds like flavonoids, tannins and lignin. Threitol is a sugar alcohol, which meaning in plant biochemistry is not yet further determined and threonic acid is a sugar acid derived from threose (HELDT and PIECHULLA, 2008). The above mentioned significant changes on metabolite level suggest that the treatments have differential impacts on leaf key metabolic pathways discriminating cv. Messire and cv. Protecta in both healthy and infected plants.

Regarding the differences between infected and healthy plants of cv. Messire or cv. Protecta significant changes were found for aspartate (Fig. 3.2g), melibiose (Fig. 3.2e), methionine (Fig. 3.2a), myo-Inositol (Fig. 3.2d), oxaloacetate (Fig. 3.2a), pyruvate (Fig. 3.2a,d), trehalose (Fig. 3.2a), treithol (Fig. 3.2a) and threonic acid (Fig. 3.2e). The roles of aspartate, myo-Inositol, pyruvate, threitol and threonic acid in plants have already been described above. Melibiose, a reducing disaccharide, is part of cell wall degradation. Methionine is part of the amino acid synthesis and is derived subsequently from aspartate. Oxaloacetate plays a major role in the TCA cycle. Trehalose, which occurs only in very little amounts in plants, is an important signaling substance when it comes to regulation of plant metabolism. Initial substrates are glucose-6-phosphate and UDP-glucose (HELDT and PIECHULLA, 2008).
The data suggest an influence of *Didymella pinodes* on leaf metabolism of *Pisum sativum* cultivars. However, responses seem cultivar specific (Figures 3.2a-h), meaning Messire and Protecta are not only differing in their habitus, but also in their reaction to infection of *Didymella pinodes*. These metabolic differences between the two cultivars might be an explanation for the fact that Protecta is more resistant to *Didymella pinodes*. Apart from that, infection was significantly changing the metabolite content of both Messire and Protecta.

4.2. Nodules of *Pisum sativum* after infection with *Didymella pinodes*

4.2.1. Nodule weight, an indicator for cultivar specific responses to infection

The fact that the nodules of cv. Protecta showed a lower weight per nodule than those of cv. Messire 24 hours and 48 hours after the infection indicates that *Didymella pinodes* had a bigger impact on rhizobia symbiosis of cv. Protecta than of cv. Messire. This might be due to different plant developmental stages between the two cultivars including their nodules. This hypothesis is substantiated by the fact that 24 hours after the infection the nodules of cv. Messire were already quite well developed and therefore heavier and bigger in their size compared to the nodules of cv. Protecta. Thus, *Didymella pinodes* might have had bigger impact on nodules of cv. Protecta by inhibiting their growth in an early developmental stage, when symbiont-metabolic exchange might not have been fully established. This also supports the hypothesis of sugar-accumulation in leaves due to impaired plant-symbiont connection. The fact that nodule weight did not quite differ between healthy and infected plants of cv. Messire, may strengthen the hypothesis of different developmental stages between the

cultivars and the idea that nodules of cv. Messire were already quite well developed when infection was taking place and therefore not that much impaired compared to nodule-development of cv. Protecta. However, there is no current literature proving developmental differences between cv. Protecta and cv. Messire, which means it is suggested in this study for the first time. In general, other studies already reported that nodule fresh weight is decreased by abiotic stress. RAMOS *et al.* (2003) argued that water stressed common bean (*Phaseolus vulgaris L.*) leads to reduced nodule fresh weight just like SAITO *et al.* (1984), who observed a sharp decrease in nodule fresh weight under the same conditions in common bean. Additionally, GALLACHER and SPRENT (1978) described retarded nodule growth when soil water potential is decreasing. SPRENT (1971) showed that the rate of nitrogen fixation in nodules and therefore their activity is reduced by drought stress. If *Didymella pinodes* affects on activity of young nodules in cv. Protecta remains to be proven. However, this study is the first one claiming that pea nodule development is impaired by *Didymella pinodes* and indicating a systemic effect.

Very surprisingly however, Protecta's nodule weight increased after two weeks of infection such that it was higher than the nodule weight of infected Messire. Nodules development of cv. Protecta might have been inhibited during the initial days of infection, but was reactivated along two weeks after infection. This data suggest that Protecta plants could re-establish symbiotic interaction by inhibition of the initial negative systemic pathogen effect. However, the reason for this interesting symbiont-protection mechanism remains thus far unknown.

4.2.2. **Effect of *Didymella pinodes* infection on root nodule metabolism – Indication for a cultivar independent down-regulation of the symbiotic interaction**

In general, 24 hours after infection the majority of the metabolites was higher in nodules of healthy than of infected plants of both cultivars, whereas two weeks after the infection it was the other way around. This indicates the induction of different phases of response to *Didymella pinodes* infection within two weeks. Additionally, there were differences between nodules of Messire and those of Protecta. The latter showed more nodule metabolites being accumulated when infected at both points in time (24 hai and 2 wai). This suggests cultivar-specific impacts of *Didymella pinodes* on root nodule metabolism.

Nevertheless, in general nodules of infected Messire and Protecta showed sugar accumulation two weeks after the infection. According to GONZÁLEZ *et al.* (1998), who described water-deficit effects on carbon and nitrogen metabolism of pea nodules, this might be due to a decline in nodule sucrose synthase activity, which then results in reduced potential to metabolise sucrose. Hence, this might be a general nodules stress response also being true for *Pisum sativum* exposed to *Didymella pinodes*.
Moreover, nodules of infected Messire and Protecta showed higher amounts in Asparagine and other amino acids than nodules of healthy plants two weeks after infection. Since inhibition of metabolic exchange between plants and bacteroids, which was already indicated earlier in this study, might also be the reason for amino acids being accumulated in nodules. RAMOS *et al.* (2003) already described transport hindrance of nitrogen-products away from the nodule when water is limited and ASHRAF and IRAM (2005) reported amino acid accumulation in leaves and nodules of water stressed *Phaseolus vulgaris* and *Sesbania aculeata*. Just as described in paragraph 4.1., the accumulation of amino acids in nodules of infected Messire and Protecta in this study might be due to hydrolysis of proteins, which are responsible for transportation of compounds from and to nodules, caused by *Didymella pinodes*. In this case amino acids cannot be transported into the shoot and are therefore accumulating in the nodules.

Except for cv. Messire two weeks after the infection (Fig. 3.6b) Spermidine and Putrescine were always higher in nodules when the plants were infected by *Didymella pinodes*. Putrescine is built by Arginine and Ornithine Decarboxylase, enzymes which form Putrescine out of Arginine or Ornithine, respectively (GALSTON and KAUR-SAWHNEY, 1990; SLOCUM *et al.*, 1991; TIBURCIO *et al.*, 1997). Spermidine is formed by the subsequent addition of an aminopropyl moiety onto Putrescine catalyzed by the enzyme Spermidine Synthase (SLOCUM *et al.*, 1991; TIBURCIO *et al.*, 1997). In general, polyamines have been shown to directly protect plants, e.g. by activating the antioxidant system, and participate in acclimation signaling pathways under stress conditions (reviewed by PÁL *et al.*, 2015). Furthermore, it has been shown that polyamines are able to enhance photosynthetic activity in plants under stress conditions by conjugation to photosynthetic complexes and proteins (IOANNIDIS *et al.*, 2012). Therefore, it is suggested that the accumulation of Putrescine and Spermidine in this study happened due to protection mechanisms of *Pisum sativum* to *Didymella pinodes*.

5. Conclusion and Outlook

This study showed that *Didymella pinodes* infection has a strong impact on primary metabolism of *Pisum sativum* leaves and root nodules. In general, the infection seems to impair the interaction between plant and symbionts which results in metabolite accumulation in nodules as well as in leaves of both cultivars. However, the results of the nodule weight indicate that cv. Protecta might be able to re-establish the symbiotic interaction by inhibition of the initial negative systemic pathogen effect. It also has been shown that the infection of *Didymella pinodes* seems to reinforce over a time period of two weeks, which increases the impact on *Pisum sativum* and symbionts on metabolite level. Importantly, the study indicates that the effects of *Didymella pinodes* on leaf and nodule metabolism as well as the impact of the treatments on leaf metabolism are mostly cultivar-specific. This finding may give first indications to explain higher tolerance for cv. Protecta to the pathogen compared to cv. Messire. However, future studies are needed to fully understand the cultivar-specific mechanisms that lead to improved pathogen protection.

In future, measurements of secondary metabolites are planned of both leaves and nodules of *Pisum sativum* healthy or infected with *Didymella pinodes*. The ultimate goal is to combine data sets of primary and secondary metabolism of nodules and leaves to create a relation between them. At best this would result in highlighting correlations or finding hints for new pathways or compounds which correlate with resistance of *Pisum sativum* to *Didymella pinodes*.

6. Abstract

6.1. Abstract (English)

Pea (*Pisum sativum*) is the most grown legume in Europe and the second-most in the world. However, a large part of the crop is lost throughout all cultivation areas due to ascochyta blight, one of the worst pea diseases worldwide. Ascochyta blight invades the plant mainly via the fungus *Didymella pinodes*. Many management options, like crop rotation, burying of infested residues and the use of fungicides, have failed in successfully and economically defeating the disease in the past. This is why breeding for pathogen resistance would be the most efficient and environmentally friendly method to control ascochyta blight.

In this study two different experiments have been carried out to determine metabolic responses of two cultivars (cv. Messire and cv. Protecta) of *Pisum sativum* grown symbiotic or non-symbiotic when infected by *Didymella pinodes*. In the first experiment metabolites of healthy and infected pea growing under four different treatments were analyzed, whereas in the second experiment the metabolites of nodules of healthy and infected pea inoculated with rhizobia were analyzed after they were harvested at three different time points. This study showed that *Didymella pinodes* infection has a strong impact on primary metabolism of *Pisum sativum* leaves and root nodules. In general, the infection seems to impair the interaction between plant and symbionts, which results in metabolite accumulation in nodules as well as in leaves of both cultivars. However, the results of the nodule weight indicate that cv. Protecta might be able to re-establish the symbiotic interaction by inhibition of the initial negative systemic pathogen effect. It also has been shown that the infection of *Didymella pinodes* seems to reinforce over a time period of two weeks which increases the impact on *Pisum sativum* and symbionts on metabolite level. Importantly, the study indicates that the effects of *Didymella pinodes* on leaf and nodule metabolism as well as the impact of the treatments on leaf metabolism are mostly cultivar-specific. This finding may give first indications to explain higher tolerance for cv. Protecta to the pathogen compared to cv. Messire.

6.2. Zusammenfassung (Deutsch)

Die Felderbse (*Pisum sativum*) ist die am häufigsten angebaute Hülsenfurcht in Europa und die am zweithäufigsten angebaute weltweit. Die Ernteerträge werden jedoch jedes Jahr in allen Anbaugebieten von einer ernsthaften Krankheit gemindert. Diese Krankheit, die in erster Linie durch den Pilz *Didymella pinodes* verursacht wird, wird Brennfleckenkrankheit genannt. Viele Managementoptionen, wie zum Beispiel Fruchtwechsel, Vergraben von befallenen Restbeständen und der Einsatz von Fungiziden, ergaben in der Vergangenheit weder die gewünschten dauerhaften Ergebnisse noch waren sie ökonomisch rentabel. Deshalb wäre es am effizientesten *Pisum sativum* durch gezielte Züchtung eine Resistenz gegen die Krankheit zu erlangen.

In dieser Studie wurden zwei Experimente durchgeführt, um die metabolische Antwort von symbiontisch- und nicht-symbiontisch-wachsenden Felderbsen auf die Infektion von *Didymella pinodes* zu untersuchen. Bei den Erbsen handelte es sich um die Kultivare Protecta und Messire. Im ersten Experiment wurden die Kultivare unter vier verschiedenen Bedingungen angezogen und mit *Didymella pinodes* infiziert. Nach der Ernte von gesunden und befallenen Blättern wurden diese auf Metabolit-Ebene analysiert. Im zweiten Experiment wurden die Knöllchen von gesunden und infizierten *Pisum sativum* zu drei verschiedenen Zeitpunkten geerntet und ebenfalls auf Metabolit-Ebene untersucht. Die Studie zeigt, dass *Didymella pinodes* einen großen Einfluss auf den Primärmetabolismus von Protecta und Messire und deren Wurzelknöllchen ausübt. Die Studie deutet darauf hin, dass die Infektion die Verbindung zwischen Pflanze und Symbiont unterbricht, was in weiterer Folge dazu führt, dass Metabolite in Knöllchen sowie in Blättern beider Kultivare akkumulieren. Die Ergebnisse des Knöllchengewichts lassen jedoch vermuten, dass cv. Protecta in der Lage sein könnte, die symbiontische Interaktion wiederherzustellen, indem es den ursprünglich negativen pathogenen Effekt hemmt. In weiterer Folge kommt es zur Annahme, dass sich die Infektion durch *Didymella pinodes* über eine Zeitspanne von zwei Wochen intensiviert, was den Einfluss auf *Pisum sativum* und Symbionten auf Metabolitlevel verstärkt. Darüber hinaus wird angenommen, dass die Auswirkungen von *Didymella pinodes* auf den Metabolismus von Blättern und Wurzelknöllchen, sowie der Einfluss der Treatments auf den Metabolismus der Blätter zumeist Kultivar-spezifisch sind. Die Studie gibt erste Hinweise darauf, dass cv. Protecta in Bezug auf *Didymella pinodes* widerstandfähiger ist als cv. Messire.

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8. Attachments

8.1. Work design for experiment of leaves

MR - Me	**NP - Pr**	**R - Me**	**M - Pr**	**NP - Me**
1	2	3	4	5
R - Pr	**M - Me**	**NP - Pr**	**NP - Me**	**M - Pr**
10	9	8	7	6
NP - Me	**R - Pr**	**M - Me**	**R - Pr**	**R - Me**
11	12	13	14	15
M - Pr	**MR - Me**	**MR - Pr**	**MR - Me**	**MR - Pr**
20	19	18	17	16
MR - Pr	**NP - Me**	**R - Pr**	**M - Me**	**NP - Pr**
21	22	23	24	25
R - Me	**M - Pr**	**NP - Me**	**NP - Pr**	**M - Me**
30	29	28	27	26
NP - Pr	**R - Me**	**M - Pr**	**R - Me**	**R - Pr**
31	32	33	34	35
M - Me	**MR - Pr**	**MR - Me**	**MR - Pr**	**MR - Me**
40	39	38	37	36
healthy				

MR - Me	**NP - Pr**	**R - Me**	**M - Pr**	**NP - Me**
41	42	43	44	45
R - Pr	**M - Me**	**MR - Pr**	**NP - Me**	**M - Pr**
50	49	48	47	46
MR - Me	**R - Pr**	**M - Me**	**NP - Pr**	**R - Me**
51	52	53	54	55
M - Pr	**NP - Me**	**MR - Pr**	**MR - Me**	**R - Pr**
60	59	58	57	56
MR - Pr	**NP - Me**	**R - Pr**	**M - Me**	**NP - Pr**
61	62	63	64	65
R - Me	**M - Pr**	**NP - Me**	**MR - Pr**	**M - Me**
70	69	68	67	66
NP - Pr	**MR - Me**	**M - Pr**	**R - Me**	**R - Pr**
71	72	73	74	75
M - Me	**MR - Pr**	**R - Me**	**NP - Pr**	**MR - Me**
80	79	78	77	76
infected				

variety	Messire (**Me**)
	Protecta (**Pr**)

treatment (5 replicates)	**M** mycorrhiza	**MR** myc & rhiz	**R** rhizobia	**NP** fertilized

Figure 8.1. Work design of first experiment. 80 pots planted either with the cultivar Messire or Protecta of Pisum sativum were split up on four floors. Four treatments with five replicates were used in this experiment: M (inoculated with mycorrhiza), R (inoculated with rhizobia), MR (inoculated with mycorrhiza and rhizobia) and NP (control group, which was fertilized with nitrogen and phosphorus). Half of the pots were infected with spores of Didymella pinodes, whereas half of them remained healthy.

8.2. Work design for experiment of nodules

Floor 1 (healthy)				
Me - R	Pr - NP	Pr - R	Me - NP	Me - R
1	2	3	4	5
Pr - R	Me - R	Me - NP	Pr - NP	Pr - R
6	7	8	9	10
Me - R	Me - NP	Pr - R	Pr - R	
11	12	13	14	
Pr - NP	Me - R	Me - NP	Pr - NP	
15	16	17	18	
Me - NP	Pr - R	Pr - NP	Me - R	
19	20	21	22	

Floor 3 (infected)				
Me - R	Pr - NP	Pr - R	Me - NP	Me - R
45	46	47	48	49
Pr - R	Me - R	Me - NP	Pr - NP	Pr - R
50	51	52	53	54
Me - R	Me - NP	Pr - R	Pr - R	
55	56	57	58	
Pr - NP	Me - R	Me - NP	Pr - NP	
59	60	61	62	
Me - NP	Pr - R	Pr - NP	Me - R	
63	64	65	66	

Floor 2 (healthy)				
Pr - NP	Me - R	Me - NP	Pr - R	Me - NP
23	24	25	26	27
Me - NP	Pr - NP	Me - R	Me - NP	Pr - NP
28	29	30	31	32
Pr - NP	Pr - R	Pr - NP	Me - R	
33	34	35	36	
Me - R	Pr - NP	Me - R	Pr - R	
37	38	39	40	
Pr - R	Me - NP	Pr - R	Me - NP	
41	42	43	44	

Floor 4 (infected)				
Pr - NP	Me - R	Me - NP	Pr - R	Me - NP
67	68	69	70	71
Me - NP	Pr - NP	Me - R	Me - NP	Pr - NP
72	73	74	75	76
Pr - NP	Pr - R	Pr - NP	Me - R	
77	78	79	80	
Me - R	Pr - NP	Me - R	Pr - R	
81	82	83	84	
Pr - R	Me - NP	Pr - R	Me - NP	
85	86	87	88	

Figure 8.2. Work design of second experiment. 88 pots planted either with the cultivar Messire or Protecta of Pisum sativum were split up on four floors. Two treatments were used in this experiment: R (inoculated with rhizobia) and NP (control group, which was fertilized with nitrogen and phosphorus). Half of the pots were infected with spores of Didymella pinodes, whereas half of them remained healthy. Nodules and leaves were harvested at three different time points: 24 hours, 48 hours and two weeks after the infection.

8.3. Nutrient solutions

Table 8.1. Nutrient solutions, which were used for pouring the plants. The stock solutions A to D were used for all plants, whereas stock solution E was only added to NP treatments.

B&D (Broughton and Dilworth 1971) nutrient solution							
Stock Solution	**Element**	**Final Molarity (µM)**	**Form**	**Mol.Wt.**	**gram/liter**	**Molarity of Stock Solution**	**ml in 25L**
A	Ca	1000	$CaCl_2 \cdot 2H_2O$	147,03	294,1	2	12,5
B	P	500	KH_2PO_4	136,09	136,1	1	12,5
C	Fe	10	Fe-Citrate	335,04	6,7	0,02	12,5
D	Mg	250	$MgSO_4 \cdot 7H_2O$	246,5	123,3	0,5	12,5
	K	1500	K_2SO_4	174,06	87	0,5	
	S	500					
	Mn	1	$MnSO_4 \cdot H_2O$	169,02	0,338	0,002	
	B	2	H_3BO_3	61,84	0,247	0,004	
	Zn	0,5	$ZnSO_4 \cdot 7H_2O$	287,56	0,288	0,001	
	Cu	0,2	$CuSO_4 \cdot 5H_2O$	249,69	0,1	0,004	
	Co	0,1	$CoSO_4 \cdot 7H_2O$	281,12	0,056	0,0002	
	Mo	0,1	$Na_2MoO_4 \cdot 2H_2O$	241,98	0,048	0,0002	
E	N	########	KNO_3	101,1032	318,75	#########	40

8.4. Sum of measured metabolite levels of leaves

Table 8.2. Sum of measured metabolite levels of leaves (µg per g fresh weight). Pr = Protecta; Me = Messire; R = plants inoculated with rhizobia; M = plants inoculated with mycorrhiza; MR = plants inoculated with mycorrhiza and rhizobia; NP = plants fertilized with nitrogen and phosphorus; i = infected.

	PrR	PrNP	PrMR	PrM	MeR	MeNP	MeMR	MeM	iPrR	iPrNP	iPrMR	iPrM	iMeR	iMeNP	iMeMR	iMeM
Asparagine	877560,99	352644,20	358448,52	186502,32	478162,98	380771,18	283484,36	108659,61	556344,94	504297,09	394431,71	124326,72	149515,10	130089,07	54354,30	75080,88
Aspartic acid	34605,29	24152,41	24244,71	10395,95	22404,50	31938,16	22333,95	23367,03	22546,12	22523,73	13121,16	16558,35	8508,63	11227,56	5966,02	6722,72
Citric acid	61728,47	51477,85	133671,33	44444,09	76578,63	73701,85	82245,16	77737,10	195244,46	110723,20	135498,16	137119,52	113809,77	76867,89	90189,82	64168,25
Fructose	21152,79	15807,22	23691,46	9748,27	19501,76	24120,77	18842,69	23681,90	24832,94	18499,82	20005,03	27321,83	22679,48	29562,60	30177,28	23999,24
Fumaric acid	119554,77	35476,44	21374,80	3741,46	10069,22	41522,04	24557,77	27272,03	113870,19	39433,59	26330,63	116489,84	30031,29	9095,65	14153,17	30696,61
Galactinol	20,29	20,44	74,37	17,05	53,52	67,93	170,36	65,00	43,82	51,83	52,81	79,18	13,68	164,95	138,83	60,08
Galactose	3687,87	3455,43	6603,90	1529,84	2816,63	2906,06	2309,76	3969,69	5163,31	5554,87	3069,36	5789,49	3454,59	3901,58	4607,17	2958,46
Gluconic acid	994,90	352,99	1528,84	313,45	574,53	1240,22	502,21	205,09	1663,75	1017,43	501,11	403,95	602,19	420,69	343,93	942,20
Glucose	24166,51	16497,87	28128,07	10451,81	20716,08	23914,10	15052,40	21620,49	25770,02	22597,35	14528,68	25572,89	20686,21	24556,53	23031,34	20374,14
Glutamic acid	1367515,00	818839,18	1202250,18	443346,97	936569,63	796751,54	710657,69	624905,68	1849706,93	1276358,45	952793,51	1840540,23	645389,80	349099,39	788317,26	783143,35
Glutamine	76304,78	40025,39	53135,78	31078,84	36570,05	11208,64	46359,98	1978,77	274599,87	83445,80	51848,79	168692,70	63828,30	33668,66	29937,20	26027,09
Glutaric acid	667,85	414,18	709,23	437,48	6147,72	1044,17	898,69	2293,17	16754,71	15565,73	1614,82	7629,31	2648,78	3098,86	3242,59	3043,65
Glycine	1019,72	665,88	986,34	361,92	657,27	975,32	510,33	408,42	1053,46	1027,31	708,58	707,84	475,55	607,04	533,92	657,69
Isoleucine	726,14	563,46	673,48	451,72	436,10	342,40	1030,25	40,26	1557,90	89,70	922,37	81,42	832,58	1570,64	1207,45	2433,95
Lactic acid	1574,87	1064,39	1512,25	1373,65	1375,54	1689,20	1432,98	1586,70	1372,34	1243,40	1087,14	1734,49	1415,70	839,69	1419,01	1224,43
Leucine	819,62	477,44	789,61	305,79	830,67	693,05	868,58	613,97	1210,14	977,91	1010,02	1399,90	1229,33	844,54	1280,08	2046,72
Lysine	443,52	278,63	273,98	156,92	322,28	423,87	205,40	289,96	746,26	423,82	247,16	499,79	342,62	378,17	408,81	706,59
Malic acid	473343,94	417507,91	548706,33	255134,22	503194,67	761392,88	417009,56	534746,26	418908,35	595845,26	413826,17	405613,94	375097,41	522299,55	385059,64	413036,06
Maltose	1884,13	1194,78	1579,09	467,47	1453,31	2242,40	1463,08	1407,41	2102,78	2205,63	1666,04	2153,25	1932,87	2287,29	2599,64	2119,90
Melibiose	198,10	80,24	120,77	44,46	200,57	237,14	188,83	165,62	100,78	92,03	92,81	73,05	112,85	56,07	105,77	160,53
Methionine	85,03	29,77	640,56	66,74	103,13	125,65	69,68	170,53	233,17	149,18	134,47	195,32	154,53	194,62	243,62	572,25
Ornithine	61,90	116,99	68,48	42,22	43,94	328,63	64,61	110,45	391,86	114,10	60,58	29,81	84,21	138,26	66,92	82,17
Oxaloacetate	168,99	48,32	111,85	124,91	123,20	48,28	109,97	9,30	332,65	246,87	1156,17	193,28	101,96	905,45	163,71	173,50
Phenylalanine	2552,31	1200,92	3065,50	1045,07	2159,62	2231,41	1948,41	1004,81	3910,78	2570,44	2813,88	3659,43	2302,46	2717,65	2918,51	2376,76
Proline	847,18	635,79	936,36	112,68	1611,77	532,40	208,17	528,78	1360,04	846,28	137,31	1167,68	1128,46	900,06	630,27	1325,28
Putrescine	554,66	420,74	447,31	219,89	463,03	494,84	294,78	321,31	982,65	662,98	489,82	768,64	594,54	634,62	642,87	575,09
Pyruvatic acid	62,58	118,35	92,93	24,47	90,53	316,50	136,94	173,48	1059,79	1372,12	837,12	928,45	1179,85	1154,88	1294,16	1275,29
Raffinose	60,98	129,62	946,98	66,79	0,00	318,48	432,71	660,08	393,05	285,44	461,38	519,51	185,91	178,89	13,19	29,15
Serine	24733,67	15646,12	21490,17	8050,28	36663,26	34305,93	24250,86	20988,99	24486,93	16967,88	15416,34	17121,50	14870,67	13091,75	11636,71	10423,65
Spermidine	149,49	103,76	151,27	79,19	132,77	138,07	118,56	73,06	201,05	153,35	121,46	138,63	131,24	89,60	162,28	118,60
Succinic acid	9230,56	4780,56	7793,41	3436,76	11197,21	10604,06	8653,66	8423,44	8164,40	6915,18	5910,61	9914,03	8978,90	10012,38	10038,14	9696,18
Sucrose	689141,32	550240,55	815056,94	287586,80	516792,17	567192,98	349626,92	458135,37	756658,07	723719,80	517026,35	660329,78	399016,20	469507,60	491520,03	353262,99
Threitol	187,81	34,07	217,31	11,64	209,37	162,95	145,80	120,78	239,28	235,80	164,68	248,68	212,49	236,74	248,82	171,84
Threonic acid	8274,21	4655,02	11158,53	4021,87	9961,61	8595,21	7656,10	7913,28	7182,65	5926,69	5027,65	8137,55	8150,70	7703,26	8807,08	7706,53
Threonine	8640,22	6491,86	7449,99	3531,43	6791,82	7995,19	5775,42	6443,83	9716,59	7010,48	6859,26	7728,46	6055,27	6577,86	5595,45	5876,29
Trehalose	2234,05	13,19	3457,55	1522,82	2162,22	9,31	15,09	1543,09	59,42	69,77	42,47	80,26	217,23	337,95	208,15	418,79
Tyrosine	408,96	259,43	371,07	129,29	322,00	415,64	363,76	291,00	924,81	585,77	451,45	751,48	789,69	1388,40	1152,53	1811,69
Valine	3573,74	2981,14	3547,70	1220,74	3136,94	4567,06	2879,56	2876,36	4413,37	3627,65	3273,65	4541,08	3334,27	4680,87	3812,98	5086,85
myo-Inositol	53485,81	36301,11	52448,63	16597,42	27743,32	41481,41	30516,47	40020,16	55515,06	35497,77	43226,08	60479,73	33667,27	22069,42	36690,44	29531,97

8.5. Weight of nodules

Table 8.3. Results of nodules after first time point (9th December 2014, 24 hours after infection). Me = Messire; Pr = Protecta; R = plants inoculated with rhizobia; FW = fresh weight.

Pot	Plant	Treatment	Condition	Nr nodules	FW nodules
1	**blau**	Me- R	healthy	122	0,968
1	**rot**	Me- R	healthy	95	1,174
1	**gelb**	Me- R	healthy	122	0,8615
1	**grün**	Me- R	healthy	57	2,315
16	**blau**	Me- R	healthy	93	0,887
16	**rot**	Me- R	healthy	71	0,741
16	**gelb**	Me- R	healthy	52	0,5416
16	**grün**	Me- R	healthy	28	0,2863
36	**blau**	Me- R	healthy	78	0,712
36	**rot**	Me- R	healthy	68	0,766
36	**gelb**	Me- R	healthy	84	0,757
36	**grün**	Me- R	healthy	60	0,588
13	**blau**	Pr- R	healthy	38	0,307
13	**rot**	Pr- R	healthy	100	0,317
13	**grün**	Pr- R	healthy	43	0,347
34	**blau**	Pr- R	healthy	68	0,632
34	**rot**	Pr- R	healthy	88	0,6122
34	**grün**	Pr- R	healthy	58	0,676
43	**blau**	Pr- R	healthy	116	0,566
43	**rot**	Pr- R	healthy	98	0,6407
43	**grün**	Pr- R	healthy	108	0,7486
43	**gelb**	Pr- R	healthy	61	0,2769
60	**blau**	Me- R	infected	84	0,783
60	**rot**	Me- R	infected	119	0,3609
60	**gelb**	Me- R	infected	70	0,55
60	**grün**	Me- R	infected	65	0,7561
74	**blau**	Me- R	infected	68	0,463
74	**rot**	Me- R	infected	62	0,78
74	**gelb**	Me- R	infected	58	0,232
74	**grün**	Me- R	infected	96	0,5525
83	**blau**	Me- R	infected	53	0,883
83	**rot**	Me- R	infected	49	0,644
83	**gelb**	Me- R	infected	89	0,46
83	**grün**	Me- R	infected	34	0,2249
50	**blau**	Pr- R	infected	79	0,472
50	**rot**	Pr- R	infected	117	0,696
50	**gelb**	Pr- R	infected	91	0,511
64	**blau**	Pr- R	infected	65	0,5477
64	**rot**	Pr- R	infected	67	0,4748
64	**gelb**	Pr- R	infected	53	0,5034
78	**blau**	Pr- R	infected	87	0,4139
78	**rot**	Pr- R	infected	78	0,6526
78	**gelb**	Pr- R	infected	48	0,3597
78	**grün**	Pr- R	infected	48	0,45

Table 8.4. Results of nodules after second time point (10th December 2014, 48 hours after infection). Me = Messire; Pr = Protecta; R = inoculated with rhizobia; FW = fresh weight.

Pot	Plant	Treatment	Condition	Nr nodules	FW nodules
7	**blau**	Me- R	healthy	61	0,8402
7	**rot**	Me- R	healthy	70	0,9507
7	**gelb**	Me- R	healthy	59	0,7322
7	**grün**	Me- R	healthy	71	0,487
30	**blau**	Me- R	healthy	83	0,5538
30	**rot**	Me- R	healthy	106	0,8
30	**gelb**	Me- R	healthy	67	0,6054
30	**grün**	Me- R	healthy	66	0,679
39	**blau**	Me- R	healthy	NV	NV
39	**rot**	Me- R	healthy	56	0,8493
39	**gelb**	Me- R	healthy	83	0,9698
39	**grün**	Me- R	healthy	131	0,656
14	**blau**	Pr- R	healthy	54	0,2488
14	**rot**	Pr- R	healthy	55	0,2053
14	**gelb**	Pr- R	healthy	92	0,6552
14	**grün**	Pr- R	healthy	50	0,223
26	**blau**	Pr- R	healthy	125	1,1904
26	**rot**	Pr- R	healthy	108	0,873
26	**gelb**	Pr- R	healthy	77	0,6982
41	**blau**	Pr- R	healthy	107	0,8238
41	**rot**	Pr- R	healthy	116	0,6244
41	**gelb**	Pr- R	healthy	95	0,3751
51	**blau**	Me- R	infected	95	0,7128
51	**rot**	Me- R	infected	71	0,5636
51	**gelb**	Me- R	infected	76	1,0158
51	**grün**	Me- R	infected	40	0,8943
66	**blau**	Me- R	infected	68	0,5802
66	**rot**	Me- R	infected	79	0,6575
66	**gelb**	Me- R	infected	79	0,5783
66	**grün**	Me- R	infected	97	0,7816
81	**blau**	Me- R	infected	54	0,3699
81	**rot**	Me- R	infected	77	0,8922
81	**gelb**	Me- R	infected	151	0,9766
81	**grün**	Me- R	infected	53	0,44
57	**blau**	Pr- R	infected	78	0,3836
57	**rot**	Pr- R	infected	71	0,4836
57	**gelb**	Pr- R	infected	59	0,2726
58	**blau**	Pr- R	infected	136	0,51
58	**rot**	Pr- R	infected	85	0,4768
58	**gelb**	Pr- R	infected	98	0,3854
58	**grün**	Pr- R	infected	105	0,517
87	**blau**	Pr- R	infected	162	0,541
87	**rot**	Pr- R	infected	57	0,366
87	**gelb**	Pr- R	infected	136	0,688
87	**grün**	Pr- R	infected	85	0,7002

Table 8.5. Results of nodules after third time point (22th December 2014, 2 weeks after infection). Me = Messire; Pr = Protecta; R = plants inoculated with rhizobia; FW = fresh weight.

Pot	Plant	Treatment	Condition	Nr nodules	FW nodules
11	**blau**	Me- R	healthy	121	1,9128
11	**rot**	Me- R	healthy	87	1,0285
11	**gelb**	Me- R	healthy	124	1,5846
11	**grün**	Me- R	healthy	72	1,6789
22	**blau**	Me- R	healthy	109	0,9573
22	**rot**	Me- R	healthy	222	1,3522
22	**gelb**	Me- R	healthy	144	1,5433
22	**grün**	Me- R	healthy	116	1,154
37	**blau**	Me- R	healthy	64	1,1744
37	**rot**	Me- R	healthy	76	1,3225
37	**gelb**	Me- R	healthy	110	1,8663
37	**grün**	Me- R	healthy	107	1,2635
3	**blau**	Pr- R	healthy	53	0,826
3	**rot**	Pr- R	healthy	54	0,3841
3	**gelb**	Pr- R	healthy	111	2,233
3	**grün**	Pr- R	healthy	16	1,3069
10	**blau**	Pr- R	healthy	100	0,528
10	**rot**	Pr- R	healthy	73	0,838
10	**gelb**	Pr- R	healthy	66	0,9416
10	**grün**	Pr- R	healthy	56	0,9
40	**blau**	Pr- R	healthy	31	0,4473
40	**rot**	Pr- R	healthy	112	1,2578
40	**gelb**	Pr- R	healthy	72	1,5784
45	**blau**	Me- R	infected	49	0,659
45	**rot**	Me- R	infected	109	0,5536
45	**gelb**	Me- R	infected	119	1,3578
45	**grün**	Me- R	infected	96	1,3771
49	**blau**	Me- R	infected	73	1,4068
49	**rot**	Me- R	infected	88	0,5207
49	**gelb**	Me- R	infected	151	1,2755
49	**grün**	Me- R	infected	87	0,709
80	**blau**	Me- R	infected	112	1,7246
80	**rot**	Me- R	infected	48	1,4981
80	**gelb**	Me- R	infected	62	0,7692
80	**grün**	Me- R	infected	87	1,6036
54	**blau**	Pr- R	infected	59	0,7562
54	**rot**	Pr- R	infected	112	1,4554
54	**gelb**	Pr- R	infected	102	1,1513
54	**grün**	Pr- R	infected	77	0,4253
70	**blau**	Pr- R	infected	66	0,3233
70	**rot**	Pr- R	infected	66	0,9244
70	**gelb**	Pr- R	infected	106	0,931
70	**grün**	Pr- R	infected	59	0,467
84	**blau**	Pr- R	infected	53	0,4799
84	**rot**	Pr- R	infected	67	1,0139
84	**gelb**	Pr- R	infected	26	0,38
84	**grün**	Pr- R	infected	103	1,7181

8.6. T-test of metabolic results of leaves

Table 8.6. Results of T-test of leaves of Pisum sativum (Asparagine to Maltose). Green colored values show p-values. Pr = Protecta; Me = Messire; R = plants inoculated with rhizobia; M = plants inoculated with mycorrhiza; MR = plants inoculated with mycorrhiza and rhizobia; NP = plants fertilized with nitrogen and phosphorus; i = infected.

T-test	Asparagine	Aspartic acid	Citric acid	Fructose	Fumaric acid	Galactinol	Galactose	Gluconic acid	Glucose	Glutamic acid	Glutamine	Glutaric acid	Glycine	Isoleucine	Lactic acid	Leucine	Lysine	Malic acid	Maltose
iMeM/iMeMR	0,3447	0,4158	0,5476	0,2387	0,4880	0,3632	0,1455	0,4688	0,6783	0,8608	0,4574	0,4490	0,0864	0,5249	0,5464	0,7251	0,4971	0,1278	0,3189
iMeM/iMeNP	0,6747	0,7635	0,6728	0,7441	0,4025	0,0245	0,6760	0,3830	0,7246	0,2704	0,2808	0,9712	0,0486	0,4002	0,5632	0,8978	0,3588	0,8325	0,7775
iMeM/iMeR	0,9545	0,6668	0,1413	0,7705	0,9794	0,1759	0,6562	0,7119	0,3175	0,7971	0,3978	0,5971	0,1363	0,2902	0,5222	0,9946	0,2548	0,5619	0,4051
iMeM/iPrM	0,8712	0,4408	0,7302	0,7800	0,3291	0,8509	0,3993	0,5661	0,6610	0,1330	0,3076	0,4503	0,3544	0,2100	0,1274	0,8166	0,6133	0,4322	0,2929
iMeM/MeM	0,6892	0,4180	0,2761	0,7647	0,4083	0,8070	0,4032	0,4129	0,9534	0,2612	0,8501	0,8455	0,3059	0,2111	0,9467	0,5208	0,2746	0,5010	0,8198
iMeMR/iMeNP	0,3782	0,4081	0,9040	0,1350	0,6721	0,7296	0,2732	0,4849	0,4900	0,2401	0,7538	0,3675	0,5100	0,8596	0,3178	0,7567	0,6092	0,3279	0,4066
iMeMR/iMeR	0,3823	0,2007	0,2945	0,1604	0,5347	0,2020	0,3488	0,3841	0,6703	0,9325	0,9019	0,6728	0,5485	0,6399	0,9903	0,6918	0,2727	0,4759	0,5959
iMeMR/iPrMR	0,3926	0,2827	0,9618	0,2510	0,5607	0,3279	0,1339	0,6221	0,8261	0,2131	0,5073	0,2913	0,5193	0,9624	0,0626	0,8531	0,5052	0,4172	0,2508
iMeMR/MeMR	0,3823	0,2007	0,2945	0,1604	0,5347	0,2020	0,3488	0,3841	0,6703	0,9325	0,9019	0,6728	0,5485	0,6399	0,9903	0,6918	0,2727	0,4759	0,5959
iMeNP/iMeR	0,6686	0,9974	0,2769	0,9805	0,4478	0,0048	0,9516	0,2188	0,2159	0,1864	0,6509	0,4410	0,2296	0,7037	0,3055	0,8755	0,5523	0,7573	0,5903
iMeNP/iPrNP	0,4050	0,3723	0,6216	0,7231	0,4565	0,0397	0,6721	0,2422	0,9495	0,2441	0,0497	0,4039	0,6111	0,4045	0,5355	0,8017	0,4889	0,3288	0,7360
iMeNP/MeNP	0,3372	0,3587	0,8791	0,6415	0,3282	0,1231	0,2543	0,2799	0,4571	0,3393	0,2522	0,4042	0,7882	0,5482	0,2215	0,4130	0,4992	0,7415	0,8740
iMeR/iPrR	0,1481	0,2170	0,5915	0,5717	0,5753	0,0749	0,5217	0,1559	0,3520	0,9527	0,5331	0,4714	0,0245	0,2061	0,8765	0,8301	0,2868	0,5637	0,3039
iMeR/MeR	0,4137	0,0256	0,3671	0,4428	0,4830	0,6048	0,5740	0,9490	0,9951	0,1529	0,7767	0,6049	0,9746	0,8027	0,8762	0,5588	0,6194	0,7979	0,4262
iPrM/iPrMR	0,4548	0,8930	0,9660	0,9119	0,3137	0,7426	0,6372	0,4395	0,7885	0,0400	0,5965	0,4274	0,5995	0,2777	0,0301	0,9720	0,2517	0,2343	0,2093
iPrM/iPrNP	0,4381	0,6579	0,7891	0,2911	0,3620	0,7535	0,9016	0,3934	0,5621	0,2143	0,8828	0,5804	0,5346	0,2733	0,1459	0,9997	0,4038	0,1062	0,2711
iPrM/iPrR	0,1420	0,4466	0,8463	0,9927	0,7195	0,6319	0,7614	0,1097	0,4496	0,1270	0,9140	0,6099	0,1321	0,1074	0,2193	0,9749	0,7707	0,4474	0,2284
iPrM/PrM	0,6291	0,7963	0,4529	0,4142	0,2374	0,3214	0,2891	0,7875	0,4935	0,0695	0,3093	0,3460	0,6206	0,4361	0,3698	0,4425	0,1253	0,8215	0,0546
iPrMR/iPrNP	0,7858	0,5672	0,6456	0,3488	0,8352	0,9762	0,7349	0,2609	0,6782	0,2254	0,3994	0,3977	0,7798	0,2921	0,0984	0,9608	0,3916	0,0104	0,8082
iPrMR/iPrR	0,7240	0,3662	0,7588	0,9213	0,5555	0,4943	0,5316	0,0920	0,1911	0,3400	0,7060	0,4431	0,2619	0,6730	0,0686	0,9983	0,3802	0,1078	0,9677
iPrMR/PrMR	0,7810	0,6523	0,8594	0,4311	0,4668	0,5902	0,2582	0,2967	0,6123	0,4828	0,5538	0,1908	0,4163	0,6068	0,3387	0,7134	0,3753	0,3627	0,6850
iPrNP/iPrR	0,4016	0,7764	0,9494	0,3126	0,6301	0,8151	0,6915	0,4785	0,1850	0,7256	0,9982	0,9792	0,6654	0,1133	0,6589	0,9699	0,6234	0,3306	0,7882
iPrNP/PrNP	0,8925	0,1637	0,9815	0,3276	0,9539	0,5180	0,5181	0,5510	0,5792	0,8420	0,1018	0,3601	0,7653	0,4335	0,8049	0,6100	0,9466	0,7879	0,2330
iPrR/PrR	0,9555	0,3154	0,2709	0,7841	0,7063	0,1276	0,4123	0,4102	0,3645	0,9560	0,8158	0,4187	0,8247	0,2708	0,6880	0,8261	0,3896	0,1006	0,7311
MeM/MeMR	0,5442	0,5510	0,8587	0,8304	0,2348	0,8174	0,2647	0,3645	0,9492	0,5012	0,4390	0,4617	0,3957	0,2683	0,6930	0,6054	0,9413	0,0502	0,9065
MeM/MeNP	0,4844	0,2617	0,4585	0,7961	0,3417	0,7415	0,2013	0,2990	0,6649	0,3227	0,7234	0,5047	0,4492	0,4521	0,4895	0,8546	0,1857	0,7380	0,9312
MeM/MeR	0,4498	0,3988	0,9628	0,9673	0,8429	0,4423	0,3529	0,2547	0,3146	0,4253	0,3102	0,5758	0,8146	0,4450	0,7604	0,6133	0,7370	0,3623	0,9341
MeM/PrM	0,2782	0,7507	0,0878	0,4276	0,5890	0,4293	0,2878	0,6462	0,6864	0,5311	0,7626	0,3444	0,8221	0,4775	0,9243	0,4939	0,8554	0,2370	0,2024
MeMR/MeNP	0,9381	0,1801	0,7159	0,9830	0,3106	0,9301	0,5908	0,4542	0,6301	0,7103	0,4316	0,7186	0,6014	0,5717	0,5689	0,6729	0,2828	0,1859	0,9636
MeMR/MeR	0,6780	0,9950	0,8685	0,7449	0,2702	0,3476	0,6498	0,8654	0,2961	0,4247	0,6950	0,4572	0,8640	0,7208	0,7838	0,9233	0,8231	0,2233	0,8462
MeMR/PrMR	0,8689	0,2903	0,9737	0,5023	0,2286	0,8362	0,1415	0,4121	0,7644	0,9358	0,8691	0,3096	0,1467	0,9694	0,3711	0,8337	0,4405	0,5259	0,4532
MeNP/MeR	0,7146	0,0119	0,4113	0,6553	0,7790	0,2838	0,3455	0,4932	0,7281	0,2801	0,2973	0,4680	0,5717	0,8639	0,4978	0,6732	0,3166	0,6588	0,8487
MeNP/PrNP	0,9305	0,0001	0,3218	0,9151	0,4521	0,4384	0,0093	0,5217	0,9214	0,7881	0,3351	0,0906	0,4688	0,7041	0,3993	0,9652	0,9543	0,8352	0,1477
MeR/PrR	0,7652	0,2025	0,0150	0,1881	0,4306	0,1731	0,7817	0,5328	0,6774	0,3861	0,2740	0,4407	0,1098	0,6040	0,6831	0,9681	0,9461	0,1581	0,7302
PrM/PrMR	0,2590	0,8500	0,1415	0,8905	0,6954	0,1890	0,1360	0,3393	0,9876	0,9871	0,1865	0,7006	0,1398	0,1943	0,8574	0,1674	0,5838	0,9856	0,8179
PrM/PrNP	0,2827	0,0894	0,0048	0,4728	0,3818	0,0147	0,0707	0,3723	0,4893	0,6204	0,3602	0,3758	0,1417	0,4356	0,9926	0,3895	0,1916	0,3589	0,4972
PrM/PrR	0,4045	0,1020	0,0464	0,1694	0,3957	0,1327	0,5975	0,3245	0,4688	0,7927	0,1736	0,4869	0,1229	0,1804	0,9789	0,1534	0,5871	0,4648	0,3132
PrMR/PrNP	0,8535	0,0268	0,5948	0,4132	0,4025	0,3138	0,5876	0,4615	0,5197	0,6309	0,8819	0,2600	0,9959	0,9008	0,8572	0,8538	0,0042	0,3127	0,4293
PrMR/PrR	0,6176	0,0405	0,1227	0,1447	0,4132	0,2507	0,1901	0,6669	0,4971	0,8038	0,3781	0,7226	0,8933	0,9101	0,8291	0,9022	0,2289	0,3247	0,4229
PrNP/PrR	0,5749	0,7009	0,0055	0,6488	0,9050	0,2195	0,0818	0,5731	0,9557	0,8395	0,3552	0,2102	0,7053	0,8385	0,9704	0,8002	0,3161	0,1686	0,1904

Table 8.7. Results of T-test of leaves of Pisum sativum (Melibiose to myo-Inositol). Green colored values show p-value. Pr = Protecta; Me = Messire; R = plants inoculated with rhizobia; M = plants inoculated with mycorrhiza; MR = plants inoculated with mycorrhiza and rhizobia; NP = plants fertilized with nitrogen and phosphorus; i = infected.

T-test	Melibiose	Methionine	Ornithine	Oxaloacetate	Phenylalanine	Proline	Putrescine	Pyruvatic acid	Raffinose	Serine	Spermidine	Succinic acid	Sucrose	Threitol	Threonic acid	Threonine	Trehalose	Tyrosine	Valine	myo-Inositol
iMeM/iMeMR	0,6210	0,2870	0,8167	0,6776	0,0047	0,4556	0,7167	0,5642	0,5891	0,1416	0,3844	0,4385	0,1020	0,2410	0,4044	0,1374	0,6489	0,6220	0,5564	0,3313
iMeM/iMeNP	0,3752	0,2420	0,4139	0,4372	0,0006	0,6841	0,6549	0,6110	0,4813	0,9076	0,4232	0,4214	0,9815	0,4928	0,9992	0,6917	0,5845	0,7451	0,6155	0,3468
iMeM/iMeR	0,3909	0,2886	0,9737	0,6193	0,6184	0,7626	0,9182	0,2165	0,3290	0,9211	0,8348	0,8221	0,8074	0,9637	0,4770	0,9245	0,5433	0,3721	0,6812	0,7945
iMeM/iPrM	0,3734	0,2503	0,3833	0,3249	0,2022	0,8633	0,4768	0,5456	0,2578	0,3371	0,5670	0,8462	0,0305	0,9598	0,5752	0,4640	0,2536	0,2998	0,8030	0,1529
iMeM/MeM	0,9613	0,3041	0,3899	0,1008	0,1062	0,3822	0,1619	0,1386	0,2778	0,2062	0,2661	0,4385	0,3848	0,4087	0,9418	0,8191	0,4583	0,2829	0,8592	0,5796
iMeMR/iMeNP	0,2577	0,5006	0,0603	0,4168	0,5683	0,7467	0,9539	0,2738	0,4419	0,2866	0,1683	0,9919	0,1204	0,1958	0,3776	0,0737	0,4857	0,7917	0,9892	0,8702
iMeMR/iMeR	0,3840	0,9809	0,6118	0,4730	0,1057	0,5666	0,6162	0,4158	0,2572	0,2824	0,6264	0,3108	0,2316	0,4668	0,8109	0,0916	0,3978	0,4979	0,7885	0,5371
iMeMR/iPrMR	0,8414	0,4518	0,8821	0,3370	0,9297	0,3410	0,5990	0,2886	0,1226	0,1850	0,6203	0,0945	0,7961	0,2886	0,8079	0,2004	0,2258	0,3748	0,7947	0,4201
iMeMR/MeMR	0,3840	0,9809	0,6118	0,4730	0,1057	0,5666	0,6162	0,4158	0,2572	0,2824	0,6264	0,3108	0,2316	0,4668	0,8109	0,0916	0,3978	0,4979	0,7885	0,5371
iMeNP/iMeR	0,9506	0,4902	0,1242	0,4836	0,1394	0,8768	0,5374	0,0897	0,7180	0,8580	0,4847	0,3027	0,8285	0,5440	0,4261	0,6662	0,8721	0,3224	0,8354	0,5686
iMeNP/iPrNP	0,3400	0,4700	0,1240	0,5520	0,8487	0,8908	0,8445	0,6202	0,6226	0,6474	0,0066	0,1377	0,3297	0,2249	0,2979	0,6754	0,3605	0,1938	0,4425	0,0971
iMeNP/MeNP	0,0029	0,7057	0,4957	0,3819	0,3101	0,6394	0,8900	0,0693	0,4474	0,3628	0,8158	0,8748	0,3895	0,5396	0,0295	0,9012	0,2575	0,2266	0,5893	0,2077
iMeR/iPrR	0,7812	0,6996	0,5685	0,8331	0,2652	0,6998	0,6834	0,8364	0,3453	0,6689	0,3269	0,9245	0,3434	0,5239	0,9346	0,6547	0,2199	0,7870	0,5507	0,6911
iMeR/MeR	0,1170	0,4420	0,2125	0,2664	0,2507	0,5731	0,1582	0,3752	0,2299	0,7132	0,6206	0,5898	0,9445	0,7268	0,6622	0,5670	0,4624	0,5144	0,8668	0,8313
iPrM/iPrMR	0,5666	0,8232	0,3530	0,3825	0,9741	0,1522	0,1621	0,0550	0,8786	0,8531	0,8200	0,3987	0,4084	0,9070	0,4466	0,3987	0,2341	0,7697	0,9735	0,3293
iPrM/iPrNP	0,4045	0,3800	0,2349	0,5703	0,8970	0,6402	0,4605	0,1234	0,2947	0,7011	0,4887	0,6081	0,6994	0,5166	0,9392	0,2987	0,6307	0,8310	0,6003	0,0330
iPrM/iPrR	0,7025	0,5330	0,1961	0,3699	0,8925	0,8082	0,9794	0,2631	0,3977	0,8415	0,2621	0,6652	0,6773	0,4949	0,7921	0,8705	0,4206	0,5372	0,6226	0,1935
iPrM/PrM	0,3303	0,5866	0,6526	0,9825	0,3028	0,1301	0,0715	0,0350	0,3030	0,3488	0,4454	0,4029	0,2222	0,1595	0,7036	0,9612	0,6410	0,3361	0,3940	0,0357
iPrMR/iPrNP	0,9900	0,4148	0,8566	0,1896	0,8540	0,4314	0,5392	0,0047	0,1555	0,8758	0,6805	0,6405	0,7887	0,3542	0,2701	0,9600	0,3068	0,6409	0,5220	0,2872
iPrMR/iPrR	0,7755	0,4935	0,3944	0,9183	0,9108	0,1682	0,3361	0,5345	0,2497	0,9944	0,3678	0,6256	0,7585	0,3360	0,5611	0,5784	0,5359	0,6178	0,5863	0,9919
iPrMR/PrMR	0,7820	0,4321	0,8387	0,0757	0,8364	0,1603	0,8583	0,8830	0,5600	0,8613	0,7019	0,6429	0,2188	0,4714	0,3187	0,8589	0,1918	0,3566	0,3363	0,7630
iPrNP/iPrR	0,6975	0,6723	0,4475	0,3236	0,7818	0,5302	0,6041	0,0570	0,1141	0,8587	0,3554	0,9459	0,9885	0,8345	0,6435	0,5135	0,5734	0,4978	0,2679	0,1611
iPrNP/PrNP	0,8395	0,0398	0,3275	0,0254	0,6669	0,4776	0,9000	0,0043	0,9379	0,8837	0,4048	0,3690	0,5623	0,0145	0,0792	0,7923	0,0093	0,6951	0,5516	0,3880
iPrR/PrR	0,3894	0,3753	0,3840	0,3115	0,4691	0,5700	0,3932	0,2111	0,1477	0,2721	0,4049	0,7468	0,5316	0,2036	0,2241	0,9708	0,2549	0,5719	0,5937	0,7659
MeM/MeMR	0,6357	0,5001	0,2284	0,3730	0,4698	0,1474	0,6490	0,7511	0,6866	0,7905	0,5810	0,2728	0,1968	0,6592	0,7523	0,6142	0,4231	0,6918	0,9977	0,4715
MeM/MeNP	0,0904	0,7647	0,5355	0,2907	0,0749	0,9905	0,3223	0,8694	0,4930	0,8625	0,5991	0,1136	0,9620	0,4566	0,0475	0,9430	0,4219	0,3814	0,8319	0,9432
MeM/MeR	0,6115	0,6452	0,1702	0,4490	0,0277	0,2916	0,1166	0,4255	0,2675	0,7865	0,6498	0,2116	0,6034	0,0618	0,7900	0,8688	0,8275	0,8153	0,7854	0,2182
MeM/PrM	0,0151	0,5026	0,1197	0,3051	0,9648	0,1195	0,8292	0,2215	0,3048	0,2247	0,9077	0,7492	0,8040	0,0365	0,3647	0,3211	0,4619	0,3802	0,2248	0,0862
MeMR/MeNP	0,3339	0,3637	0,3689	0,7545	0,6391	0,1708	0,2393	0,8641	0,5178	0,9587	0,9208	0,6134	0,1948	0,7994	0,3278	0,7257	0,5820	0,3045	0,8427	0,5023
MeMR/MeR	0,8641	0,1877	0,5471	0,7166	0,6794	0,1870	0,0592	0,5653	0,1276	0,6682	0,8626	0,9621	0,3616	0,2976	0,9473	0,4868	0,4243	0,5629	0,8211	0,7020
MeMR/PrMR	0,4916	0,4184	0,9053	0,6327	0,2581	0,1484	0,5020	0,4245	0,6389	0,6197	0,5266	0,5353	0,0252	0,2536	0,7458	0,4109	0,1896	0,8178	0,3306	0,5824
MeNP/MeR	0,6067	0,6991	0,3406	0,9116	0,8716	0,2928	0,8899	0,4519	0,1288	0,7214	0,9357	0,5403	0,5774	0,3992	0,2547	0,8478	0,4234	0,4649	0,6550	0,3064
MeNP/PrNP	0,1716	0,1923	0,5441	0,6142	0,9459	0,2832	0,8067	0,8628	0,1756	0,5787	0,2092	0,5511	0,8211	0,0945	0,9996	0,9958	0,1106	0,8028	0,7600	0,6160
MeR/PrR	0,1011	0,0508	0,5085	0,8689	0,6914	0,4774	0,6593	0,3333	0,0195	0,5982	0,4036	0,8139	0,7925	0,3178	0,6977	0,4579	0,4696	0,5143	0,4625	0,8564
PrM/PrMR	0,3165	0,4165	0,0792	0,3292	0,1126	0,1316	0,6887	0,2774	0,3616	0,4291	0,3713	0,6438	0,1217	0,0007	0,4447	0,1902	0,2011	0,8875	0,8646	0,2693
PrM/PrNP	0,2934	0,6325	0,1751	0,3645	0,2996	0,1572	0,1122	0,2583	0,7155	0,4922	0,2281	0,2348	0,9492	0,4137	0,6608	0,4732	0,4496	0,1615	0,2921	0,4454
PrM/PrR	0,2244	0,6837	0,1632	0,3436	0,6217	0,2996	0,4523	0,3357	0,8844	0,1231	0,4040	0,4494	0,9520	0,1648	0,3228	0,9122	0,6551	0,1465	0,1091	0,4934
PrMR/PrNP	0,9018	0,3955	0,3231	0,4995	0,2234	0,5175	0,1213	0,4241	0,3562	0,8437	0,3914	0,3404	0,1194	0,0175	0,2650	0,7134	0,1902	0,2388	0,3520	0,6395
PrMR/PrR	0,3921	0,3993	0,8169	0,7420	0,2944	0,9058	0,6173	0,3147	0,3629	0,4726	0,9770	0,6646	0,1411	0,2802	0,9680	0,3912	0,2098	0,2122	0,1452	0,5941
PrNP/PrR	0,3809	0,8393	0,2956	0,6806	0,6855	0,5117	0,4233	0,4002	0,6422	0,2881	0,5128	0,7092	0,9994	0,2523	0,0330	0,6199	0,2022	0,8513	0,7789	0,9419

8.7. T-test of metabolic results of nodules

Table 8.8. Results of T-test of nodules of Pisum sativum. Green colored values show p-value. Pr = Protecta; Me = Messire; i = infected; 1 = 24 hours after the infection; 3 = two weeks after the infection.

T-test	iMe1/iMe3	Me1/Me3	iPr1/iPr3	Pr1/Pr3	Me1/Pr1	Me3/Pr3	iMe1/iPr1	iMe3/iPr3	iMe1/Me1	iMe3/Me3	iPr1/Pr1	iPr3/Pr3
2-Oxoglutaric acid	0,21	0,37	0,93	0,39	0,20	0,63	0,83	0,18	0,72	0,13	0,35	0,54
Alanine	0,05	0,88	0,14	0,29	0,58	0,67	0,08	0,32	0,98	0,07	0,24	0,17
Asparagine	0,29	0,58	0,24	0,43	0,38	0,62	0,76	0,76	0,82	0,81	0,17	0,56
Aspartic acid	0,51	0,17	0,60	0,39	0,54	0,74	0,16	0,26	0,38	0,47	0,23	0,30
Citric acid	0,25	0,56	0,71	0,40	0,16	0,09	0,85	0,19	0,83	0,10	0,35	0,38
Fructose	0,26	0,65	0,59	0,56	0,74	0,56	0,35	0,47	0,37	0,59	0,84	0,80
Fumaric.acid	0,47	0,23	0,08	0,85	0,60	0,44	0,67	0,66	0,64	0,10	0,57	0,19
Galactinol	0,42	0,45	0,28	0,92	0,62	0,55	0,46	0,64	0,51	0,96	0,47	0,47
Galactose	0,15	0,47	0,23	0,66	0,55	0,19	0,29	0,83	0,02	0,17	0,32	0,82
Glucose	0,53	0,16	0,62	0,43	0,45	0,16	0,94	0,24	0,41	0,18	0,81	0,18
Glutamic acid	0,23	0,22	0,87	0,14	0,11	0,27	0,43	0,67	0,86	0,91	0,66	0,59
Glutamine	0,14	0,39	0,06	0,75	0,26	0,78	0,08	0,12	0,70	0,50	0,09	0,17
Glycine	0,25	0,25	0,14	0,34	0,38	0,23	0,89	0,06	0,52	0,16	0,83	0,07
Isoleucine	0,38	0,68	0,93	0,46	0,54	0,18	0,30	0,44	0,09	0,26	0,66	0,40
Lactic acid	0,95	0,58	0,93	0,44	0,76	0,31	0,69	0,73	0,65	0,36	0,67	0,56
Leucine	0,07	0,20	0,38	0,42	0,18	0,45	0,81	0,11	0,23	0,10	0,70	0,75
Lysine	0,75	0,10	0,71	0,45	0,33	0,26	0,62	0,16	0,31	0,37	0,69	0,16
Malic acid	0,21	0,23	0,69	0,16	0,70	0,49	0,20	0,76	0,10	0,61	0,23	0,45
Maltose	0,11	0,49	0,09	0,66	0,78	0,53	0,53	0,16	0,40	0,17	0,29	0,51
Methionine	0,43	0,50	0,60	0,37	0,76	0,68	0,77	0,97	0,61	0,08	0,47	0,17
Ornithine	0,49	0,87	0,53	0,88	0,93	0,85	0,73	0,33	0,51	0,73	0,67	0,30
Phenylalanine	0,07	0,33	0,68	0,77	0,27	0,98	0,45	0,21	0,68	0,03	0,24	0,46
Proline	0,45	0,20	0,15	0,29	0,19	0,08	0,89	0,64	0,21	0,32	0,96	0,49
Putrescine	0,15	0,17	0,24	0,28	0,25	0,21	0,29	0,13	0,16	0,16	1,00	0,55
Pyruvic acid	0,31	0,49	0,39	0,42	0,81	0,62	0,65	0,63	0,92	0,76	0,60	0,48
Raffinose	0,12	0,36	0,52	0,39	0,43	0,39	0,86	0,11	0,17	0,24	0,35	0,20
Serine	0,93	0,76	0,56	0,29	0,63	0,18	0,71	0,26	0,98	0,64	0,88	0,31
Spermidine	0,69	0,44	0,26	0,77	0,44	0,27	0,91	0,20	0,62	0,48	0,12	0,16
Succinic acid	0,28	0,33	0,58	0,32	0,94	0,69	0,36	0,86	0,74	0,08	0,81	0,10
Sucrose	0,22	0,43	0,51	0,39	0,65	0,55	0,52	0,71	0,59	0,17	0,92	0,25
Threonic acid	0,58	0,08	0,32	0,10	0,35	0,03	0,83	0,79	0,25	0,86	0,86	0,03
Threonine	0,57	0,34	0,38	0,71	0,77	0,28	0,14	0,57	0,88	0,19	0,25	0,65
Trehalose	0,13	0,60	0,29	0,25	0,86	0,36	0,50	0,12	0,32	0,30	0,29	0,44
Tryptophan	0,05	0,88	0,18	0,35	0,51	0,16	0,20	0,04	0,35	0,50	0,86	NV
Valine	0,43	0,25	0,84	0,54	0,24	0,55	0,95	0,51	0,17	0,82	0,26	0,41
myo-Inositol	0,90	0,38	0,73	0,74	0,32	0,69	0,48	0,79	0,40	0,83	0,39	0,41

8.8. Ratios of Leaves

Table 8.9. Ratios of leaves (Asparagine to Maltose). Pr = Protecta; Me = Messire; R = plants inoculated with rhizobia; M = plants inoculated with mycorrhiza; MR = plants inoculated with mycorrhiza and rhizobia; NP = plants fertilized with nitrogen and phosphorus; i = infected.

Ratios	Asparagine	Aspartic.acid	Citric.acid	Fructose	Fumaric.acid	Galactinol	Galactose	Gluconic.acid	Glucose	Glutamic.acid	Glutamine	Glutaric.acid	Glycine	Isoleucine	Lactic.acid	Leucine	Lysine	Malic.acid	Maltose
iMeM/iMeMI	19,5660959	1,90121297	0,65058344	0,49703741	3,06374411	0,43277609	0,4467208	3,71048429	0,75245623	1,14659233	0,09145733	0,58339742	0,47320041	2,00083832	0,86260366	1,48914251	1,72523243	3,21549846	0,4695
iMeM/iMeNP	1,71358781	0,78835304	0,70918389	1,40669267	5,63462716	0,36233499	0,73644726	10,7443992	1,33413504	5,15808244	0,14525172	1,02884235	0,41609868	2,56838316	1,22282453	1,10901031	2,45196518	1,13502765	0,8262
iMeM/iMeR	0,93181356	0,79007748	0,36121898	1,36611481	1,03266834	4,39076054	0,70625938	1,56455293	0,60859897	1,21344241	0,07699959	0,71418784	0,53077441	4,22492179	0,86488846	1,00561206	4,3449695	1,46395868	0,6313
iMeM/iPrM	1,2171324	0,49637002	0,64024094	0,80442843	0,25625067	0,84521739	0,50458782	2,33249367	0,71609528	0,42549644	0,02284287	0,28110933	0,47868039	52,563261	0,70592695	1,26510841	1,41377738	1,58852713	0,5668
iMeM/MeM	0,69097402	0,46792751	0,52883698	0,7856335	5,26269609	1,25861915	0,51854446	6,23621267	0,96188298	5,96242088	1,30914454	0,82503337	0,61861186	44,7214786	1,03615579	2,01253475	3,68499918	0,77239613	0,8670
iMeMR/iMeN	0,08757944	0,41465793	1,09007368	2,83015453	1,83913113	0,8372343	1,64856274	2,89568649	1,77304006	4,49861933	1,58819115	1,76353599	0,87932867	1,28365353	1,4175972	0,74473081	1,42123759	0,35298653	1,7597
iMeMR/iMeR	0,04762389	0,41556495	0,55522315	2,74851509	0,3370609	10,145571	1,58098612	0,42165734	0,80881643	1,05830326	0,84191816	1,22418752	1,12166939	2,11157581	1,00264872	0,67529605	2,51848355	0,45528203	1,3446
iMeMR/iPrMI	0,00972868	0,28933587	1,03018782	1,74384088	0,37003187	2,62827047	1,50126653	1,79957134	1,15159142	6,98358589	0,46829698	2,00825444	0,75360048	1,0606993	3,3703251	0,82225306	1,65661405	3,09416368	2,7463
iMeMR/MeM	0,01643775	0,15832493	0,91084227	1,85492418	0,39673173	2,22536532	3,26860229	0,50561895	1,33504474	1,65116748	0,64667918	3,60829636	1,04622135	1,84891534	0,99050383	0,9561535	1,98543728	1,36038925	1,9980
iMeNP/iMeR	0,54377929	1,0021874	0,5093446	0,97115371	0,18327181	12,1179591	0,95900877	0,14561567	0,45617494	0,23525068	0,53011136	0,69416645	1,27559743	1,64497333	0,70728746	0,90676529	1,77203556	1,28980001	0,7641
iMeNP/iPrNP	0,16209967	0,45060499	0,69238153	1,53160715	0,1499892	3,19948004	0,74439938	0,08282314	0,93298431	0,16267498	0,17926109	0,11813842	0,71601911	10,4796982	0,80495988	1,14034695	0,67995197	0,61072782	1,2619
iMeNP/MeNF	0,17307865	2,90037387	1,12229082	0,66555634	0,55971936	2,4407971	3,14037607	0,07070645	0,53573733	0,24617432	15,143759	1,76094313	0,86099733	2,74722504	0,59276979	1,60708732	0,67987054	0,79908923	1,1000
iMeR/iPrR	0,14482922	0,37740195	1,42986583	0,59243088	0,36950301	0,31230114	0,56448141	0,36196433	0,80272382	0,94951187	0,33740599	0,16428162	0,45191556	0,36703248	1,03101845	1,21506838	0,27000165	0,67350391	2,1344
iMeR/MeR	0,20556908	0,37978753	1,48618159	0,56299965	4,0207674	1,46232169	1,52068845	1,04706305	0,99855798	9,25852184	1,43892265	0,43080745	1,01667978	1,31116506	1,02919573	1,48046593	0,64667656	0,8225679	1,3294
iPrM/iPrMR	0,15639405	1,10822386	1,04682955	1,07747827	4,4241171	1,34575155	1,32909863	2,86272211	1,21006542	18,8187849	1,87494808	4,16781074	0,74497318	0,04037588	4,11835076	0,96786328	2,021566	6,26320971	2,2747
iPrM/iPrNP	0,22821841	0,71566734	0,76693912	2,67829987	3,29807224	1,37158034	1,08645287	0,38151654	1,73821432	1,97202811	1,13987359	0,43237913	0,62240822	0,51206641	1,39437189	0,99964281	1,17926525	0,43637465	1,839
iPrM/iPrR	0,11087851	0,60071474	0,80671923	1,00609149	1,48906563	1,62235131	0,79009099	0,24279266	0,6822233	2,70784395	1,13734064	0,41737474	0,50109681	0,02950128	1,26318447	0,96583613	0,8297975	0,62068937	2,3772
iPrM/PrM	1,90260089	1,30279418	5,33126847	1,91273184	31,1342677	8,83438297	2,92469775	1,36699878	1,83883991	4,08737506	36,4690716	16,7315279	1,57199706	0,49741658	1,57159918	3,19653036	3,18497054	1,28508049	4,6061
iPrMR/iPrNP	1,45925254	0,64577867	0,73263037	2,48571126	0,7454758	1,01919284	0,81743585	0,13327055	1,43646309	0,10479041	0,60794941	0,10374251	0,83547735	12,6824836	0,33857531	1,03283473	0,58334244	0,06967269	0,8085
iPrMR/iPrR	0,70896886	0,5420518	0,77063094	0,93374643	0,33657916	1,20553553	0,59445625	0,08481182	0,56379043	0,14389048	0,60659847	0,10014244	0,67263738	0,73066598	0,30672095	0,99790554	0,41047263	0,09910084	1,0450
iPrMR/PrMR	1,44032808	1,48264868	0,90480834	2,00627749	4,7495713	0,70770862	0,46477554	0,09229583	1,49704088	0,21231589	1,71271197	3,0512519	0,71837561	1,69027597	0,43284012	1,27904856	3,69094303	0,20997898	1,5581
iPrNP/iPrR	0,48584384	0,83937705	1,05186867	0,37564557	0,45149576	1,18283359	0,72722068	0,63638829	0,39248515	1,37312644	0,99777787	0,96529808	0,8050935	0,05761222	0,90591647	0,96618124	0,70365637	1,42237723	1,2924
iPrNP/PrNP	1,16021038	0,50315842	1,01182039	0,40564081	0,92727723	1,7795832	0,73385898	1,68415427	0,61251599	1,18159335	3,23636274	91,8570901	0,86091626	0,15144835	1,13457535	1,37108439	1,01440069	1,1412702	6,7486
iPrR/PrR	0,94043062	0,65152231	11,6547857	0,87614462	1,76532455	2,15996851	2,26077479	1,67227584	1,50530082	1,06421199	1,31810222	24,1233623	1,03301838	2,14533145	1,22065649	1,23489973	2,2856237	16,8406965	0,7327
MeM/MeMR	0,46546257	0,64328216	1,12053227	1,17353283	0,23096232	0,76519168	2,81586776	0,30083823	1,04437105	0,31752471	0,04517725	2,55149776	0,80029563	0,08272045	0,82459823	0,7074903	0,92953638	5,663324	1,082
MeM/MeNP	0,4292281	4,8864803	1,50502063	1,19169208	0,59927647	0,70266385	4,46002523	0,12182047	0,74306954	0,21296508	1,68022477	2,19595102	0,57913512	0,15777489	0,69956029	0,8855879	0,45237972	1,17425288	1,0482
MeM/MeR	0,27722034	0,64125655	1,01512758	0,97898341	0,78897187	5,10138779	2,07118303	0,26268917	0,63180383	1,88424857	0,08463271	0,37292727	0,87232018	0,12386822	0,85907883	0,7397509	0,76249404	1,55905159	0,9681
MeM/PrM	3,35138096	1,38198324	6,45434506	1,95849065	1,51598663	5,9326716	2,84597943	0,51129045	1,36896546	0,29168749	0,63633783	5,70084639	1,2164076	0,58463714	1,07072143	2,00938515	1,22193767	2,64292522	3,0115
MeMR/MeNF	0,92215385	7,59616945	1,34313011	1,01547401	2,59469366	0,91828476	1,58389015	0,40493681	0,71149956	0,67070395	37,1918328	0,86065176	0,72365149	1,90732643	0,84836501	1,2517315	0,48667242	0,2073434	0,9688
MeMR/MeR	0,59558031	0,99685112	0,90593338	0,83421903	3,41601987	6,66681036	0,73553988	0,87319081	0,60496108	5,93417929	1,87334793	0,14616014	1,08999744	1,49743169	1,04181503	1,04559864	0,820295	0,27528914	0,8947
MeMR/PrMR	0,852458	2,70951282	1,02336327	1,8861303	4,42992739	0,83584014	0,21347105	0,32849428	1,29132708	0,89798659	1,24027163	1,69822253	0,51745093	0,9696899	1,47279787	1,09992965	3,07965814	0,47759075	2,141
MeNP/MeR	0,64585786	0,13123076	0,67449413	0,82150702	1,31654073	7,26006863	0,46438819	2,15636314	0,85026205	8,84768802	0,05036987	0,16982495	1,50624638	0,78509461	1,22802687	0,83532183	1,68551775	1,32769663	0,9235
MeNP/PrNP	1,08661422	0,0781712	0,62422835	0,93347826	0,24848447	2,33273832	0,17395502	1,97276123	1,06669402	0,78081123	0,03830977	6,16252216	0,71595169	0,57772222	1,54071218	0,97288547	1,01452218	0,87224985	7,7421
MeR/PrR	0,66255991	0,64742988	11,2131517	0,92194573	0,16223091	0,46129428	0,83920237	0,57809718	1,21008578	0,10914074	0,3090754	9,19906317	0,45917809	0,60053942	1,22281829	1,01352391	0,95429805	13,7888617	1,1763
PrM/PrMR	0,11839516	1,26121737	0,17766505	1,13017431	0,67490457	0,10780605	0,21121243	0,19328277	0,98514145	0,97752885	0,08805396	0,7600645	0,34043993	0,13720165	1,13425068	0,38727745	2,34271709	1,023393	0,7695
PrM/PrNP	0,13916811	0,27640133	0,14555722	0,56799794	0,09822705	0,27628883	0,27261046	0,47003167	0,57899769	0,57008111	0,10115542	2,37378731	0,34086664	0,15590878	1,0066307	0,42877574	0,37559138	0,387541	2,6949
PrM/PrR	0,05480579	0,30041511	1,763584	0,46084957	0,08443057	0,39665789	0,61073586	0,29701292	0,55847781	0,7050295	0,04110692	0,60176705	0,32928956	0,12723747	0,98111169	0,37312669	0,59548583	8,13399733	0,3781
PrMR/PrNP	1,17545438	0,2191544	0,81927887	0,50257551	0,14554213	2,56283222	1,29069323	2,43183424	0,58773051	0,58318597	1,14878909	3,12313929	1,0012534	1,13634768	0,8874852	1,10715388	0,16032298	0,37868249	3,5021
PrMR/PrR	0,46290567	0,23819455	9,92645402	0,40776858	0,12510002	3,67936566	2,89157153	1,53667561	0,56690114	0,72123651	0,46683786	0,79173155	0,96724716	0,92737561	0,86498664	0,96346091	0,25418598	7,94806817	0,4914
PrNP/PrR	0,39380998	1,0868801	12,116087	0,81135783	0,85954504	1,43566388	2,2403244	0,63189982	0,96455966	1,23671788	0,40637386	0,25350504	0,96603633	0,816102	0,97464908	0,87021409	1,58546193	20,9887398	0,1403

Table 8.10. Ratios of leaves (Melibiose to myo-Inositol). Pr = Protecta; Me = Messire; R = plants inoculated with rhizobia; M = plants inoculated with mycorrhiza; MR = plants inoculated with mycorrhiza and rhizobia; NP = plants fertilized with nitrogen and phosphorus; i = infected.

Ratios	Melibiose	Methionine	Ornithine	Oxaloacetate	Phenylalanine	Proline	Putrescine	Pyruvatic.acic	Raffinose	Serine	Spermidine	Succinic.acid	Sucrose	Threitol	Threonic.acid	Threonine	Trehalose	Tyrosine	Valine	myo.Inosit
iMeM/iMeMI	1,51773884	3,72554672	1,22785726	1,23360389	0,1976204	2,10285156	1,21878102	1,43425438	1,70475247	8,3342187	0,72803467	0,60102595	0,29757292	0,68867551	1,90893603	2,87915691	1,25933168	1,57194631	0,67318014	2,598557
iMeM/iMeNP	2,86298395	5,97137985	0,59110533	0,2231064	0,21222674	1,47252889	1,26489856	0,78631215	0,20493012	1,0909395	1,31655659	0,60251993	0,97252808	1,68912274	1,00036021	0,89334412	0,59855799	1,30482728	0,67880962	2,259465
iMeM/iMeR	2,72763613	3,67971863	0,9743477	0,72028543	0,57305707	1,27627536	0,96724455	2,94627784	0,3389597	0,92476115	0,90368303	1,28237268	0,73638818	0,97366556	1,51382103	0,97036487	0,68315359	2,97146303	0,76984384	1,242547
iMeM/iPrM	2,91190339	5,43383204	3,94842527	0,34131003	0,20897391	1,13504324	0,74820688	1,37356524	0,07428451	0,60880399	0,85062803	0,85117964	0,22149998	0,9726374	1,35023299	1,74709156	1,85370734	4,97072624	0,80438718	0,488294
iMeM/MeM	0,96889884	3,35355736	0,52384839	4,63651755	0,57397122	2,50644299	2,47939591	7,35105031	0,04643213	0,4966248	1,62329652	2,74760298	0,41666183	1,42278737	0,97386846	0,91192528	0,09642336	6,21704232	0,8923872	0,737926
iMeMR/iMeN	1,88634822	1,60281975	0,48141209	0,18085741	1,07391111	0,70025337	1,03783907	0,54823758	0,12021107	0,13089883	1,80837072	1,00248571	3,26820093	2,45271207	0,52404072	0,31027976	0,47529813	0,83007115	1,00836252	0,869507
iMeMR/iMeR	1,79717094	0,98769896	0,79353499	0,58388713	2,89978696	0,60692603	0,79361635	2,05422266	0,19883221	0,11095955	1,24126372	2,13363944	2,47464785	1,41382341	0,79301821	0,33703091	0,54247313	1,89030821	1,14359262	0,478168
iMeMR/iPrMI	1,13959893	1,71702628	1,10463987	0,63861434	1,03718276	4,58968101	1,52277995	2,74279068	0,04907174	0,08112702	1,33196275	2,94493527	1,17026693	1,51519944	1,32462548	0,29753762	2,78170497	2,5525804	1,16476912	0,344740
iMeMR/MeM	0,56012018	2,20359841	1,03577922	1,39924532	1,66728258	6,26079845	2,79528736	6,49325849	0,03973299	0,05157365	1,48689037	1,27964951	22,8465653	1,71146234	0,60972858	0,41862535	7,82862709	5,24419716	1,32392529	0,433083
iMeNP/iMeR	0,95272491	0,61622585	1,64834869	3,22843913	2,70021135	0,86672347	0,76468151	3,74695704	1,65402578	0,84767409	0,68639892	2,12834899	0,75718963	0,57643269	1,51327593	1,08621621	1,14133234	2,27728457	1,13410861	0,549929
iMeNP/iPrNP	0,60928524	0,64248036	2,01181225	2,33735243	1,05726839	1,17551074	0,87606894	1,18173024	2,15385101	0,691477	0,58741807	2,02895491	0,28607338	0,43145851	1,2998389	0,93828535	3,56300562	4,3970297	2,06900912	4,681208
iMeNP/MeNP	0,23644135	0,75746588	0,42300502	9,71315692	1,21734148	1,6904788	0,91846293	10,4971419	0,49544928	0,40804484	0,88248713	0,94428429	0,41356076	0,6243556	3,35597163	1,06168746	24,0753445	3,33805808	1,46166523	0,315088
iMeR/iPrR	0,7976263	0,66704143	0,69206523	1,15580718	0,33422035	0,76355172	0,78468864	0,83274068	0,59507996	0,72782001	0,64945088	0,90887529	0,37359012	0,73758511	1,05909905	1,46076981	3,66532599	0,74644022	0,75531999	0,727006
iMeR/MeR	0,29212949	1,49498029	1,91944105	3,21636153	0,4660267	0,64424093	1,28410815	4,77764703	8,03338903	0,66487061	1,35614964	0,61926646	0,91968679	0,84295978	0,73379047	0,89162342	0,10073153	1,89349733	1,06290433	1,209916
iPrM/iPrMR	0,5939804	1,17722844	0,34351418	2,30815702	0,98083285	8,50312792	2,48051087	2,8639772	1,1261455	1,11058785	1,13999896	2,0794465	1,57218857	1,07283635	1,87273258	0,49033348	1,88977468	0,80723	0,97477864	1,834607
iPrM/iPrNP	0,59904936	0,70603843	0,30118158	1,52787273	1,07372557	1,52502872	1,48105875	0,67649415	5,9418706	1,23908449	0,90917428	1,43622533	1,25604701	0,74928888	0,96302425	0,47977549	1,15048662	1,15423059	1,74600408	21,66115
iPrM/iPrR	0,74715196	0,45171156	0,17078002	2,43916382	0,91651314	0,85855958	1,01440635	1,78621688	2,71534572	1,10554411	0,68995814	1,36929595	1,24201973	0,7383648	1,18741464	0,81133682	1,35079609	0,44621639	0,72288377	1,84998
iPrM/PrM	3,15728377	1,57783051	1,32582145	1,02005628	2,64087969	51,4038465	3,97957931	31,5266859	5,35705265	2,12679798	1,71435657	2,20668512	2,39348096	15,179148	1,41912771	0,95242118	0,49864644	2,45068517	2,84473353	9,907915
iPrMR/iPrNP	1,00853387	0,59974633	0,87676606	0,66194488	1,094708	0,17934914	0,59707811	0,23620794	5,2762903	1,11570147	0,79752203	0,69067674	0,79891626	0,69841862	0,51423479	0,97846772	0,60879566	1,42986582	1,79118009	11,80697
iPrMR/iPrR	1,25787308	0,38370765	0,49715567	1,05675818	0,93442338	0,10096985	0,40895058	0,62368404	2,41118551	0,9954585	0,605227	0,65849059	0,78999412	0,68823619	0,63405456	1,6546633	0,71479214	0,55277478	0,74158762	1,008384
iPrMR/PrMR	0,76852994	0,13967194	0,87958225	3,28763545	0,91791964	0,14665677	1,15921902	0,88828727	0,46222959	0,88488594	0,80803189	0,70144599	0,5153094	0,75781111	0,36030983	0,92074153	0,01228471	2,59629773	2,39005191	0,758736
iPrNP/iPrR	1,24722938	0,63978325	0,56703344	1,59644437	0,8535823	0,56297928	0,68491972	2,64040256	0,456985	0,89222658	0,75888436	0,95339911	0,9888322	0,98542073	1,23300595	1,69107602	1,17410847	0,38659206	0,41402181	0,085405
iPrNP/PrNP	0,85636519	4,29176408	0,43285399	3,14901059	1,1733696	0,51473417	0,93444656	10,0528748	1,03905205	0,90664542	0,82822704	0,61656213	1,7717625	5,53287232	2,58086272	1,13542921	3,02934922	0,81975225	0,58232982	0,104426
iPrR/PrR	2,0409585	5,73242218	1,96785516	2,50181366	1,75557785	1,60537846	2,02241798	8,29592029	1,91202764	0,62614899	1,34655977	0,79264244	1,79333934	1,78731198	0,58092027	1,04680788	0,23387874	1,99499134	1,23522383	1,343419
MeM/MeMR	0,87740445	2,44802993	2,42778073	0,37228684	0,57405153	5,25267474	1,37406179	1,26689167	1,45879409	0,86549458	0,66685768	0,27991765	16,3166353	0,82840362	1,19516434	1,3216961	102,245325	1,32596756	0,9987147	1,525074
MeM/MeNP	0,69865684	1,34875179	0,47731467	0,46739119	0,45011389	0,993152	0,46856673	1,12283685	2,18668585	0,89635523	0,71573138	0,20707144	0,9652899	0,7412304	3,44726277	1,04005479	149,450188	0,70058864	1,11184072	0,964774
MeM/MeR	0,82240057	1,64037952	3,57012258	0,49966345	0,46528447	0,32804609	0,50094727	1,9148659	58,6446323	1,23805035	0,75496337	0,28902662	1,62541043	0,57686828	1,14063396	0,94876199	0,71367668	0,90500547	0,91694542	2,037300
MeM/PrM	9,4888186	2,55658843	9,99317167	0,07508986	0,96150284	23,2782428	1,20091697	5,89085343	8,57048857	2,60720586	0,89834465	0,68360876	1,27238916	10,3766785	1,96756865	1,82467473	9,58631319	1,95940199	2,5642089	6,556184
MeMR/MeNP	0,79627683	0,55095396	0,19660534	1,25545988	0,78410016	0,18907548	0,34100849	0,88629271	1,49896813	1,03565666	1,07328955	0,73975842	0,05915986	0,89476963	2,88434205	0,78690917	1,46168237	0,52836032	1,11327161	0,6326
MeMR/MeR	0,93731068	0,67008148	1,47052925	1,34214642	0,81052736	0,06245315	0,36457405	1,51146775	40,2007608	1,43045419	1,13212069	1,0325416	0,09961677	0,69636138	0,95437415	0,71783672	0,00698004	0,6825246	0,91812549	1,335869
MeMR/PrMR	1,56362138	0,10883126	0,93805862	1,50047395	0,57101923	0,1075115	0,63150412	0,37521778	0,57087092	1,3919542	0,72383842	1,61428032	0,02639563	0,6709087	0,78276727	0,65441627	0,00436506	1,2637318	2,10273094	0,603965
MeNP/MeR	1,17711661	1,21622046	7,47959957	1,06904763	1,03370386	0,33030804	1,06910549	1,70538213	26,8189564	1,38120503	1,05481385	1,39578214	1,68385729	0,77825773	0,33088106	0,91222309	0,00477535	1,29177868	0,82470934	2,111686
MeNP/PrNP	2,20676569	3,64026182	2,05865399	0,75777089	1,01907855	0,35793146	0,8913148	1,13171626	4,51703819	1,53641069	0,55130042	1,32478827	1,22558552	3,82346992	0,9996228	1,00345594	0,44832539	1,07981195	0,82429663	1,551445
MeR/PrR	5,57260474	2,55773477	0,70952121	0,8990327	1,2590477	1,90268799	1,23585262	1,44597336	0,14163504	0,68543226	0,64485836	1,16333301	0,72848047	1,56388801	0,83845748	1,71501255	8,51016403	0,78645042	0,8777735	0,807224
PrM/PrMR	0,14458369	0,10421004	0,22789568	7,43917663	0,34091887	0,02425969	0,7225526	0,08069464	0,09716869	0,4620766	0,53731851	0,66100024	0,33848757	0,0535608	0,47547797	0,4740239	0,0465567	0,85519325	0,81897707	0,140492
PrM/PrNP	0,16248302	1,92045367	0,09832972	4,7166882	0,47706715	0,01527093	0,34776798	0,21571284	1,15248313	0,52821673	0,43923344	0,40129067	0,92978261	0,27311939	1,75138106	0,57196449	6,98937255	0,38608922	0,35741493	0,228302
PrM/PrR	0,48298039	1,64111504	0,25348085	5,98234968	0,60927053	0,02681342	0,51552023	0,47002444	0,96915532	0,32548241	0,54193503	0,49185181	0,9305956	0,08694086	0,48606847	0,8917418	0,63356011	0,36324447	0,31388643	0,250841
PrMR/PrNP	1,12379916	18,4286815	0,43146811	0,63403363	1,3993568	0,62947745	0,48130472	2,67319919	11,8606431	1,14313673	0,81745451	0,60709609	2,74687373	5,09924044	3,68341162	1,20661531	150,126025	0,4514643	0,43641629	1,625021
PrMR/PrR	3,34049022	15,7481469	1,11226705	0,80416825	1,78714227	1,10526656	0,71347087	5,82472953	9,97394675	0,7043906	1,00859179	0,74410232	2,74927553	1,6232182	1,02227338	1,88121695	13,6083548	0,42475133	0,38326645	1,785445
PrNP/PrR	2,97249753	0,8545455	2,5778662	1,2683369	1,27711693	1,75584774	1,48236832	2,17893584	0,84092799	0,61619103	1,23382008	1,22567469	1,00087438	0,31832549	0,27753439	1,55908593	0,09064621	0,94083038	0,87821298	1,098721

8.9. Ratios of nodules

Table 8.11. Ratios of nodules (2-Oxoglutaric acid to Malic acid). Pr = Protecta; Me = Messire; i = infected; 1 = 24 hours after the infection; 3 = two weeks after the infection.

Ratios	2-Oxoglutaric acid	Alanine	Asparagine	Aspartic acid	Citric acid	Fructose	Fumaric acid	Galactinol	Galactose	Glucose	Glutamic acid	Glutamine	Glycine	Isoleucine	Lactic acid	Leucine	Lysine	Malic ac
iMe1/iMe3	0,274546097	0,360886	0,47868	2,52565998	0,408264	0,227224	0,6039074	6,246981	0,197815	1,87318	0,375691032	3,994216	2,595098	0,543005	0,964746	7,112533	1,406513	0,2333
iMe1/iPr1	1,246563168	2,147655	1,329912	0,32846472	0,838143	0,260246	1,4797871	4,150475	2,458678	0,92277	0,453479518	18,76598	0,888588	0,187317	0,827723	1,199976	0,623821	0,3856
iMe1/iPr3	1,17743626	0,566111	0,561191	0,47065041	1,18835	0,480833	0,4925503	9,898814	0,235101	0,59219	0,528406615	1,083818	0,283908	0,169556	0,796275	0,583225	0,494863	0,2936
iMe1/Me1	1,412227733	0,984465	0,833476	0,48730416	1,226025	0,221806	0,6601039	3,115574	0,063326	2,5257	1,203651786	1,27305	1,482529	0,190034	0,773575	0,267479	0,43189	0,1407
iMe1/Me3	2,380470479	1,122948	0,555122	1,38778102	2,188937	0,36967	3,0956856	5,991393	0,038035	0,32234	0,403347349	2,395475	0,591645	0,238662	0,573713	1,39875	3,111184	0,3378
iMe1/Pr1	3,569863909	0,655811	0,506445	0,73873163	0,449895	0,321827	0,9991492	1,907587	0,100941	0,68081	0,342084838	3,165789	0,740532	0,29881	0,63645	1,730073	0,832699	0,1758
iMe1/Pr3	1,688847167	1,768873	0,805787	1,91633656	0,68546	0,62082	1,1608278	2,155692	0,182756	2,67841	0,944115562	2,771013	1,656859	0,713298	1,308981	0,405574	1,360262	0,5704
iMe3/iPr1	4,540451244	5,951058	2,77829	0,13005104	2,052946	1,145327	2,4503545	0,664397	12,42915	0,49262	1,207054411	4,698287	0,34241	0,344964	0,857969	0,168713	0,443523	1,6525
iMe3/iPr3	4,288665082	1,56867	1,172371	0,1863475	2,910742	2,116117	0,8156057	1,584576	1,188489	0,31614	1,406492487	0,271347	0,109402	0,312256	0,825373	0,082	0,351837	1,2581
iMe3/Me1	5,143863812	2,727911	1,741197	0,19294131	3,003023	0,976154	1,0930548	0,498733	0,320127	1,34835	3,20383422	0,318723	0,571281	0,349968	0,801843	0,037607	0,307064	0,6031
iMe3/Me3	8,670567547	3,11164	1,159694	0,54947263	5,361576	1,626894	5,1260934	0,959086	0,192277	0,17208	1,073614525	0,599736	0,227986	0,439522	0,594678	0,19666	2,211984	1,4475
iMe3/Pr1	13,00278514	1,817223	1,058004	0,29249053	1,101971	1,41634	1,6544742	0,305361	0,510281	0,36345	0,910548319	0,792593	0,285358	0,550289	0,659708	0,243243	0,592031	0,75
iMe3/Pr3	6,151415682	4,901471	1,683352	0,75874685	1,678964	2,73219	1,9221951	0,345077	0,923872	1,42987	2,513010641	0,693757	0,638457	1,313613	1,356814	0,057022	0,967117	2,4443
iPr1/iPr3	0,944546005	0,263595	0,421976	1,43287967	1,417837	1,84761	0,3328521	2,384983	0,095621	0,64175	1,16522708	0,057754	0,319505	0,905183	0,962007	0,48603	0,793278	0,7613
iPr1/Me1	1,132897048	0,458391	0,626715	1,48358144	1,462787	0,852293	0,4460803	0,750655	0,025756	2,73709	2,654258325	0,067838	1,668411	1,014504	0,934582	0,222904	0,69233	0,3649
iPr1/Me3	1,909626837	0,522872	0,417413	4,22505355	2,61165	1,420463	2,0919804	1,443544	0,01547	0,34932	0,889449983	0,12765	0,665827	1,274109	0,693123	1,165648	4,987304	0,8759
iPr1/Pr1	2,863764951	0,305361	0,380811	2,24904409	0,536775	1,236625	0,6751979	0,459607	0,041055	0,7378	0,754355653	0,168698	0,833381	1,595208	0,768918	1,441756	1,334837	0,4558
iPr1/Pr3	1,354802717	0,82363	0,605895	5,83422345	0,817832	2,385511	0,7844559	0,519384	0,074331	2,90258	2,081936504	0,147662	1,864598	3,807969	1,581425	0,337985	2,180533	1,4791
iPr3/Me1	1,199409073	1,738996	1,485192	1,03538453	1,031703	0,461295	1,3401755	0,314742	0,269356	4,26504	2,277889324	1,174598	5,221857	1,120773	0,971492	0,458622	0,872746	0,4793
iPr3/Me3	2,021740421	1,983617	0,989187	2,94864506	1,841996	0,768811	6,2850142	0,605264	0,161782	0,54432	0,763327593	2,21022	2,08393	1,407571	0,720496	2,398304	6,286955	1,1505
iPr3/Pr1	3,03189568	1,158448	0,902448	1,56959732	0,378587	0,669311	2,0285221	0,192709	0,429352	1,14966	0,647389394	2,920961	2,608349	1,762305	0,799284	2,966391	1,682685	0,5988
iPr3/Pr3	1,434342753	3,124603	1,435853	4,07167719	0,576816	1,291133	2,3567701	0,217773	0,77735	4,52291	1,786721696	2,556716	5,835893	4,206852	1,643881	0,695399	2,748762	1,9428
Me1/Me3	1,685613746	1,140668	0,666033	2,84787437	1,785393	1,666637	4,6896949	1,923046	0,600627	0,12762	0,335103021	1,881682	0,399078	1,255893	0,741639	5,229373	7,203648	2,4000
Me1/Pr1	2,527824532	0,666159	0,60763	1,51595594	0,366954	1,450939	1,5136242	0,612275	1,593996	0,26956	0,284205816	2,486775	0,499506	1,572401	0,822739	6,468058	1,928035	1,2491
Me1/Pr3	1,195874523	1,796786	0,966779	3,93252659	0,559091	2,798933	1,7585533	0,691909	2,885958	1,06046	0,784375991	2,176673	1,11759	3,753527	1,69212	1,516281	3,149556	4,0527
Me3/Pr1	1,499646369	0,584008	0,912313	0,53231138	0,205531	0,870579	0,3227554	0,318388	2,653887	2,1121	0,848114754	1,32157	1,251649	1,252018	1,109353	1,236871	0,267647	0,5204
Me3/Pr3	0,709459404	1,575205	1,451549	1,38086379	0,313147	1,67939	0,3749825	0,359798	4,804912	8,30927	2,340701045	1,15677	2,800427	2,988731	2,281595	0,289955	0,437217	1,6886
Pr1/Pr3	0,473084468	2,697232	1,591065	2,5940903	1,523602	1,92905	1,1618163	1,130062	1,810518	3,93412	2,75988719	0,8753	2,23739	2,387131	2,05669	0,234426	1,633557	3,2444

Table 8.12. Ratios of nodules (Maltose to myo-Inositol). Pr = Protecta; Me = Messire; i = infected; 1 = 24 hours after the infection; 3 = two weeks after the infection.

Ratios	Maltose	Methionine	Ornithine	Phenylalanine	Proline	Putrescine	Pyruvic acid	Raffinose	Serine	Spermidine	Succinic acid	Sucrose	Threonic acid	Threonine	Trehalose	Tryptophan	Valine	myo-Inositol
iMe1/iMe3	0,210533	1,87528113	1,963998	0,361989803	2,064669	5,626938	1,9458039	0,078869	1,054233	1,2572152	0,57819884	0,24133	1,52940853	1,209032	0,150292	0,0524929	0,46	0,90901909
iMe1/iPr1	1,540289	1,24998021	1,344934	0,544349709	1,100028	0,299078	0,7787915	0,824271	1,327126	0,9483138	0,67415051	0,356505	0,86971745	1,816023	0,613958	0,0630461	0,96	0,53486334
iMe1/iPr3	0,636062	1,84389716	0,821245	0,754066729	3,132473	1,455657	1,3662516	1,996852	2,107476	0,3513504	0,53350437	0,175458	1,94214075	0,773766	1,124661	12,356371	0,848	0,71982018
iMe1/Me1	0,239497	1,62712277	2,084324	0,765042366	0,261919	5,054256	0,9470358	0,098557	0,976743	1,3401121	0,79715278	0,517073	0,55811177	1,092856	0,202792	0,0241919	0,116	0,47933553
iMe1/Me3	0,63251	4,23196123	2,503287	1,479046694	1,069391	0,405385	1,5106407	0,240681	0,763797	0,7988405	1,91166112	1,73673	1,77937128	0,604559	0,352091	0,0290864	0,397	1,11008939
iMe1/Pr1	0,328806	2,28930899	1,890659	1,80917899	1,147347	0,2991	1,1081113	0,211602	1,493014	2,0754494	0,75905266	0,319229	0,99875759	0,895311	0,172365	0,0768692	0,425	1,26703831
iMe1/Pr3	0,479453	5,52159931	2,158157	1,499621986	6,939924	2,295427	2,3032807	0,647873	4,424822	1,826523	1,5629452	0,895555	0,36043417	1,116481	0,797487	12,356371	0,177	1,53942492
iMe3/iPr1	7,31614	0,66655617	0,684794	1,503770838	0,532787	0,053151	0,4002415	10,45121	1,258855	0,7542971	1,16594926	1,47725	0,56866261	1,502047	4,085095	1,2010402	2,088	0,58839616
iMe3/iPr3	3,021199	0,98326439	0,41815	2,08311594	1,517179	0,258694	0,7021528	25,31875	1,999062	0,2794672	0,92270052	0,727044	1,26986394	0,639988	7,483164	235,39125	1,844	0,79186476
iMe3/Me1	1,137575	0,86766872	1,061266	2,113436235	0,126858	0,898225	0,4867067	1,249634	0,926496	1,0659369	1,37868277	2,142599	0,36492	0,90391	1,349321	0,4608606	0,253	0,52731073
iMe3/Me3	3,004328	2,25670763	1,274587	4,085879441	0,517948	0,072044	0,7763582	3,051672	0,724505	0,6354047	3,30623477	7,196499	1,16343753	0,500036	2,342708	0,5541012	0,863	1,2211948
iMe3/Pr1	1,56178	1,22078176	0,962658	4,9978728	0,555705	0,053155	0,5694876	2,682975	1,416209	1,6508307	1,31278827	1,322791	0,65303519	0,740519	1,146863	1,4643722	0,924	1,39385226
iMe3/Pr3	2,277327	2,94441149	1,098859	4,142718866	3,361276	0,407935	1,1837168	8,214591	4,197197	1,4528324	2,7031275	3,710918	0,23566899	0,92345	5,306241	235,39125	0,385	1,6935012
iPr1/iPr3	0,41295	1,47514108	0,610621	1,385261562	2,84763	4,867143	1,7543228	2,422567	1,588	0,3705001	0,79137279	0,492161	2,23307091	0,426077	1,831821	195,98948	0,883	1,34580205
iPr1/Me1	0,155488	1,30171883	1,549759	1,405424405	0,238102	16,89944	1,2160326	0,119568	0,735983	1,4131526	1,1824552	1,450397	0,64171618	0,601785	0,330303	0,3837179	0,121	0,89618317
iPr1/Me3	0,410644	3,38562258	1,861271	2,717089159	0,972148	1,355446	1,9397243	0,291992	0,575527	0,8423799	2,83565922	4,871551	2,04591879	0,332903	0,573477	0,4613511	0,413	2,07546358
iPr1/Pr1	0,21347	1,83147619	1,405763	3,32356013	1,043016	1,000072	1,4228601	0,256714	1,124997	2,1885683	1,12593946	0,895442	1,14837019	0,493007	0,280743	1,2192532	0,442	2,36890101
iPr1/Pr3	0,311274	4,41734938	1,604656	2,75488709	6,30886	7,675003	2,9575064	0,785994	3,334138	1,9260745	2,31839206	2,512045	0,41442674	0,614794	1,298927	195,98948	0,184	2,87816495
iPr3/Me1	0,376531	0,88243683	2,538006	1,01455526	0,083614	3,472148	0,6931636	0,049356	0,463466	3,8141757	1,49418228	2,946998	0,28736937	1,412386	0,180314	0,0019578	0,137	0,66591009
iPr3/Me3	0,994416	2,29511782	3,048161	1,961426804	0,341389	0,278489	1,1056827	0,12053	0,362423	2,2736293	3,58321547	9,898292	0,91619069	0,781321	0,313064	0,002354	0,468	1,54217597
iPr3/Pr1	0,51694	1,24156002	2,302186	2,399229301	0,366275	0,205474	0,8110595	0,105968	0,708437	5,9070648	1,42276746	1,819409	0,51425603	1,157083	0,153259	0,006221	0,501	1,76021505
iPr3/Pr3	0,753783	2,99452672	2,627908	1,988712575	2,215478	1,576901	1,6858394	0,324447	2,099583	5,1985798	2,92958273	5,104114	0,18558602	1,442918	0,709091	1	0,209	2,13862429
Me1/Me3	2,640992	2,60088623	1,201006	1,933287304	4,082909	0,080207	1,5951252	2,442052	0,781984	0,5960998	2,39811133	3,358771	3,18819883	0,553192	1,736213	1,2023185	3,416	2,31589216
Me1/Pr1	1,372902	1,40696758	0,907085	2,364808891	4,380543	0,059178	1,1700838	2,147008	1,528564	1,5487133	0,95220475	0,617377	1,78952972	0,81924	0,849956	3,177473	3,657	2,64332236
Me1/Pr3	2,001913	3,3934743	1,035423	1,960181621	26,49647	0,454157	2,4320947	6,573595	4,530182	1,3629628	1,96065953	1,73197	0,64581001	1,021617	3,932528	510,76451	1,524	3,21158112
Me3/Pr1	0,519843	0,54095699	0,75527	1,223206135	1,072898	0,737818	0,7335373	0,879182	1,954725	2,5980774	0,39706445	0,18381	0,56129803	1,480931	0,489546	2,6427881	1,07	1,14138404
Me3/Pr3	0,758015	1,30473769	0,862129	1,013911185	6,489606	5,662345	1,5247045	2,691833	5,793189	2,2864677	0,81758486	0,515656	0,20256265	1,846767	2,265003	424,81632	0,446	1,38675763
Pr1/Pr3	1,458161	2,41190653	1,141484	0,828896419	6,048672	7,674451	2,0785645	3,061747	2,963685	0,8800614	2,05907347	2,80537	0,36088253	1,247031	4,626743	160,74551	0,417	1,21497898

8.10. Further results of metabolite ratios of leaves

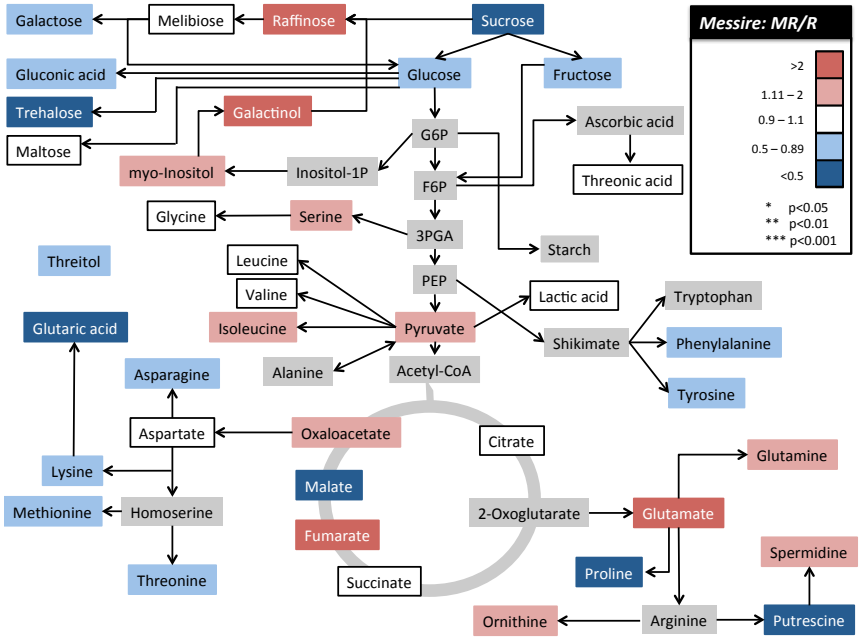

Figure 8.3. Treatment ratios of MR (mycorrhiza and rhizobia inoculated) and R (rhizobia inoculated) in healthy Messire (Me). Metabolites, which show a grey background, were not detected, but were pictured due to cross-linkage. The eventual asterisks on the sides of the metabolites represent significant results: p<0.05 (*), p<0.01 (**) or p<0,001 (***). These ratios reveal, which of the comparing treatments showed higher amounts in metabolites. A dark blue background means the ratio is smaller than 0.5, a light blue background implies a ratio of 0.5 – 0.89, a white background means the ratio is between 0.9 – 1.1, a light red background stands for a ratio of 1.11 – 2 and a dark red background means the ratio is higher than 2.

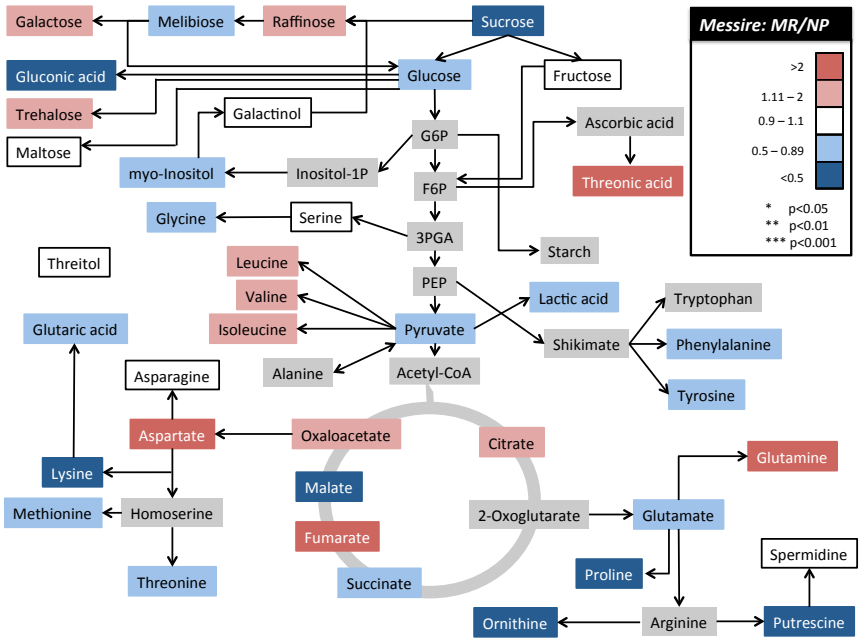

Figure 8.4. Treatment ratios of MR (mycorrhiza and rhizobia inoculated) and NP (nitrogen and phosphorus fertilized) in healthy Messire. Metabolites, which show a grey background, were not detected, but were pictured due to cross-linkage. The eventual asterisks on the sides of the metabolites represent significant results: p<0.05 (*), p<0.01 (**) or p<0,001 (***). These ratios reveal, which of the comparing treatments showed higher amounts in metabolites. A dark blue background means the ratio is smaller than 0.5, a light blue background implies a ratio of 0.5 – 0.89, a white background means the ratio is between 0.9 – 1.1, a light red background stands for a ratio of 1.11 – 2 and a dark red background means the ratio is higher than 2.

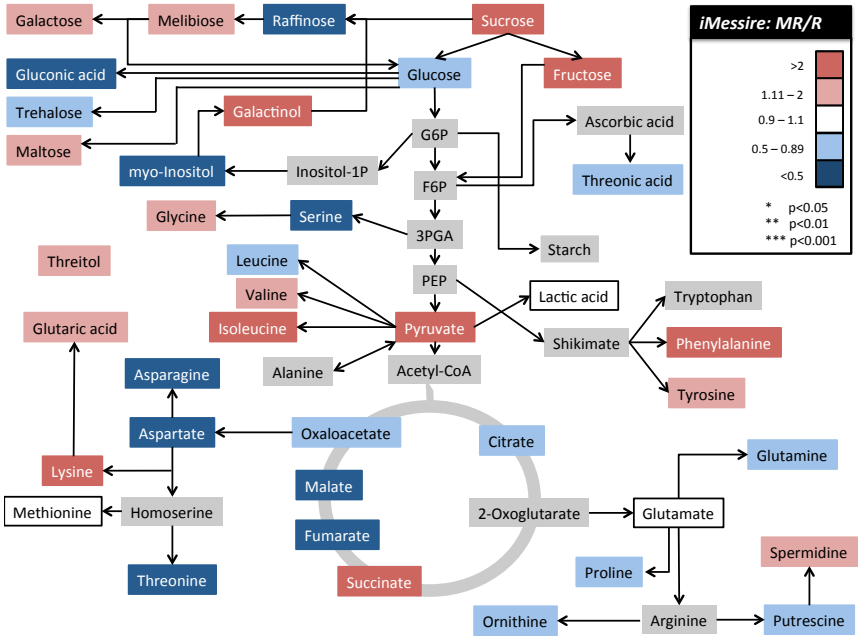

Figure 8.5. Treatment ratios of MR(mycorrhiza and rhizobia inoculated) and R (rhizobia inoculated) in infected Messire. Metabolites, which show a grey background, were not detected, but were pictured due to cross-linkage. The eventual asterisks on the sides of the metabolites represent significant results: p<0.05 (*), p<0.01 (**) or p<0,001 (***). These ratios reveal, which of the comparing treatments showed higher amounts in metabolites. A dark blue background means the ratio is smaller than 0.5, a light blue background implies a ratio of 0.5 – 0.89, a white background means the ratio is between 0.9 – 1.1, a light red background stands for a ratio of 1.11 – 2 and a dark red background means the ratio is higher than 2.

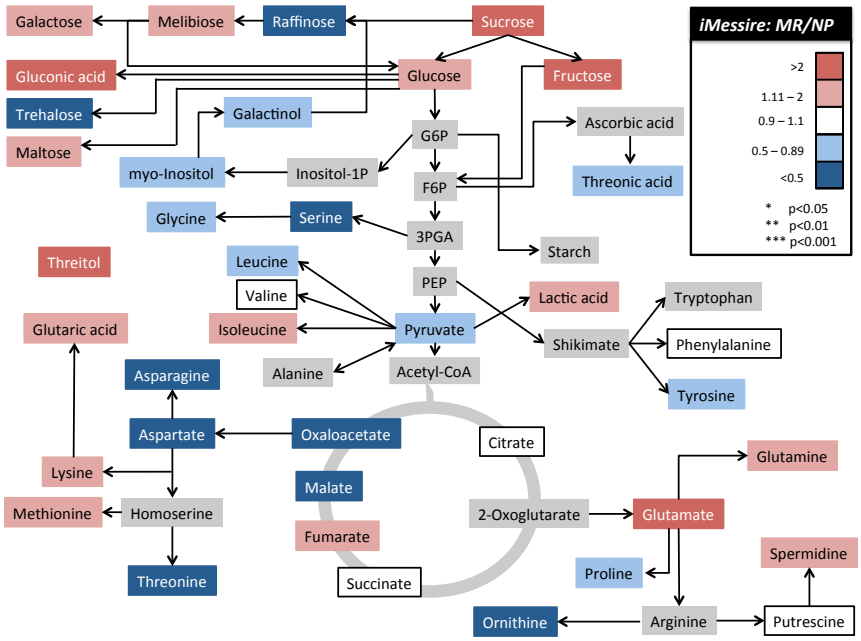

Figure 8.6. Treatment ratios of MR (mycorrhiza and rhizobia inoculated) and NP (nitrogen and phosphorus fertilized) in infected Messire. Metabolites, which show a grey background, were not detected, but were pictured due to cross-linkage. The eventual asterisks on the sides of the metabolites represent significant results: p<0.05 (*), p<0.01 (**) or p<0,001 (***). These ratios reveal, which of the comparing treatments showed higher amounts in metabolites. A dark blue background means the ratio is smaller than 0.5, a light blue background implies a ratio of 0.5 – 0.89, a white background means the ratio is between 0.9 – 1.1, a light red background stands for a ratio of 1.11 – 2 and a dark red background means the ratio is higher than 2.

8.11. Standard extraction protocol for metabolites (polar)

1. **Preparations:**

- Extraction solvent:
  methanol/chloroform/water-mixture → 2.5:1:0.5 [v/v/v]
  prechill at -20°C
- Label 3 series of Eppis:
  - Sample + extraction mix → 1.5 ml Safe lock
  - 1. Supernatant (separate Pellet from liquid) → 2 ml Eppis
  - 2. Supernatant after phase separation→ only polar phase, 1st aliquot
  - 3. Supernatant, polar phase, 2nd aliquot

2. **Extraction of metabolites:**

- Add **1 ml extraction solvent (MCW)** (treat blank in the same way as samples)
- Agitate for 10sec., incubate for **8 min. on ice,** shake once a while
- **Centrifuge** samples 4 min. at 14000 g (prechill centrifuge!)
- Put supernatant in new Eppi (contains metabolites)
- Repeat extraction procedure with **500 µl** of **MCW**
  → pellet contains cell debrits and proteins and starch (freeze at -80°C for protein or starch extraction)
- Add **400 µl H_2O** (Milli Q) for phase separation → polar and unpolar
- Agitate samples shortly and centrifuge (14000 *g*, 2 min)
- Transfer the upper, polar phase (mostly clear) to new Eppendorf tubes
- Split polar phase into 2 equal aliquots (each ca. 600 µl)
- Add internal standard to polar aliquots (e.g. 20 µl of 0.1 g/l C13-Sorbitol solution)
- Dry samples in Speed-Vac
- Derivatization or store at -80°C until measurements

3. **Derivatization:**

Acclimate samples at room temperature when using frozen samples to avoid condensation.

- **Methoximation**

- Prepare mixture:
  40 mg Methoxyaminhydrochlorid (CH_3ONH_2*HCl) in 1 ml Pyridine
- Resolve sample in **20 µl** methoximation reagent

- **Incubate 90 min. at 30°C** using a thermo shaker

- **Silylation:**
- Spike MSTFA with retention index marker (Alkanes C10-C40)
  → add 30 µl alkane mixture to 1 mL MSTFA
- Add 80 µl silylation mix
- Incubate for 30 min at 37°C
- Centrifuge: 2 min at 14000 g
- Transfer supernatant in GC- microvials and close them with crimp caps, **prepare dilutions if necessary using pyridine**

4. GC-MS measurement:

- Full Scan for profiling

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Sprachen	Deutsch - Muttersprache. Englisch - C2*. Französisch – B1*. Latein - A2*.
Programme	Microsoft Office (Word™, Excel™, PowerPoint™). IOS X. LECO® ChromaTOF. RStudio®.
Freizeit	Laufen & Fitness, Reisen, Kulinarik, Gesellschaftsspiele, Film, Zeichnen & Fotografie.

* Gemeinsamer Europäischer Referenzrahmen für Sprachen