Establishing a method to investigate the impact of root-galling grape phylloxera (*Daktulosphaira vitifoliae* (Fitch)) on *Vitis vinifera* L.

Master Thesis submitted by

Jo Pfisterer

University of Natural Resources and Life Sciences, Vienna

Department of Crop Sciences,

Division of Viticulture and Pomology

Univ. Prof. Dr. Astrid Forneck

Supervisor: Dr. Michaela Griesser

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List of abbreviations

%	Percentage
°C	Degree Celsius
°dH	German degree of hardness
‰	Per mille
Aver.	Average
BBCH-Code	A scale used to identify the phenological development stages of a plant named after the **B**iologische Bundesanstalt (Federal biological research centre), **B**undessortenamt (German National Office for Plant Varieties) and **ch**emische Industrie (Chemical industry)
BS	Bundle sheet
CC	Companion cells
Ch	Chamber
CF	Chlorophyll fluorescence
CIRAS	CIRAS-2 Portable Photosynthesis System
cm	Centimetres
cm²	Square centimetre
CPU	Central Processing Unit
e.g.	Exempli gratia
Fruct.	Fructifying
g/l	Grams per Litre
h	hour
HandyPEA	Handy PEA Chlorophyll Fluorimeter
Inoc.	Inoculation
LED	Light-emitting diode
m	Metres
M	mesophyll cells
m²	Square metres
ml	Millilitres
Non-fruct.	Non-fructifying
PAM	PAM-2500 Chlorophyll Fluorometer
PAR	Photosynthetic active radiation
PD	plasmodesmata
PP	Phloem parenchyma
rel.	Relative
s	Seconds

SE	Sieve elements
ST	phloem sieve tube
Temp.	Temperature
UniSpec	UniSpec-SC Spectral Analysis System
VE	Demineralised water

I. Introduction

Since grafting of European cultivars of *Vitis vinifera* L. onto rootstocks of American *Vitis* species, the pest grape phylloxera- *Daktulosphaira vitifoliae* (Fitch) is commonly seen as non-problematic anymore, especially by producers. But grafting and the resilience of resistant rootstocks as primary management tool to handle with phylloxera may be challenged in the future. Host-plant interactions with diverse grape phylloxera clonal lineages and potential climate effects on both, grapevine and distribution of phylloxera (BENHEIM, et al., 2012) may change conditions in the future and can set this pest into a new context. New strains can evolve of which strong infestations, also of resistant rootstock types, are possible and already were revealed in the past (GRANETT, et al., 1985). Such strains have been reported in various countries and also in Europe (SONG & GRANETT, 1990; FORNECK, et al., 2001; YVON & PEROS, 2003) . Thus ongoing research following new aspects and approaches is needed.

Also its presence all over the world (EPPO European and Mediterranean Plant Protection Organisation, 2014) might be one reason why research never stopped. Most research focusses on detection, pest management, development of the pest, consequential damages of the plant and genetic variation of phylloxera (POWELL, et al., 2013; BENHEIM, et al., 2012), while research about a possible quality lowering effect of grapes by phylloxera infestation is investigated rarer and often not current (STEFFAN & RILLING, 1981; STRAPAZZON, et al., 1986; RYAN, et al., 2000). Some research on leaf-galling phylloxera found that it is not affecting the wine quality of grafted European cultivars (STRAPAZZON, et al., 1986) while research on root-galling phylloxera found an effect on source sink relations of grapevine (STEFFAN & RILLING, 1981). This issue did find attention by recent studies which provide evidence that metabolism of leaves and roots, infested by phylloxera significantly altered, with the consequence of changes in several metabolic pathways (GRIESSER, et al., 2015; GRIESSER, et al., 2013; LAWO, et al., 2013; LAWO, et al., 2011). Thus a quality lowering effect on grapes and consequently wine is possible. Verification and quantification of this loss in quality of grapes can be one future approach, to set phylloxera as a pest in a new and nowadays barely perceived and investigated context and is the background hypothesis and one reason for this work.

This work shall deliver a new method which enables investigation on this research question, independent from the season of the year and with respect to strict quarantine conditions needed for this pest (POWELL, et al., 2013; EPPO- European and Mediterranean Plant Protection Organisation, 2004). A new approach to clear the impact of root galling phylloxera

on grapevine shall be designed and proved by the first experimental set up as well as measurements.

This research question opens several approaches to gain data on which answers can be given. The investigation of distribution patterns of assimilates, namely source-sink relations within grapevine is one approach which is already widely explored (HALE & WEAVER, 1962; KOBLET & PERRET, 1972; COOMBE, 1988; LEBON, et al., 2008; KELLER, 2010). Another approach is to research photosynthesis and thus the source of assimilates, namely carbon, whose distribution afterwards can be explained by source sink relations within the plant. Of course also measurements of berry sap, containing total soluble solids (° Brix), titratable acidity (g/L), pH value, tartaric acid (g/L) and malic acid (g/L) and other important parameters of must analysis are needed to find answers.

Performing trials regardless of the seasons requires the creation of an artificial environment. This results naturally in different conditions than those on the field, but brings advantages also. The ability to gain data during the whole year and repeat the same trial even several times a year, in order to adapt conditions of plants and measurements and to confirm gained insights, makes results more significant.

I.1 Problem

Phylloxera as a quality-lowering aspect in viticulture receives not enough attention and research. Until today neither a method exists to investigate the impact of phylloxera on grape quality, nor one combining a comparable development of plants under an isolated and controlled environment with that under field conditions, to relate effects clear to phylloxera.

I.2 Reason for this work

The reason why this work and thus the design and implementation of a new method was done are following “Background Hypotheses”

i. Root galling of phylloxera on common used rootstocks causes an additional sink (Evidence: Appearance of more carbohydrates in phylloxerated roots)
ii. Infestation by phylloxera influences the partitioning of carbohydrates with negative impact on fruits and/or green tissue
iii. Infestation by phylloxera causes an increase in photosynthetic activity needed to compensate the carbohydrate losses

I.3 Aims

Following aims should be reached by reference to the method developed in this work.

Establishment and production of samples to prove:

1. Effects of carbohydrate translocation (with an impact on fruits, roots, leaves and other organs)
2. Causal effects of infestation by phylloxera on the activity of enzymes which are relevant for carbohydrate translocation (e.g. invertases etc.)
3. Causal effects of infestation by phylloxera on photosynthetic activity of leaves (e.g, chlorophyll fluorescense)
4. Causal effects on vegetative and generative development of vine (e.g. leaf mass, root mass, etc.)

I.4 Objectives

These are the reasons for shifting the content actual belonging to material and methods into results:

Main issues:

1. Designing an experimental set up to measure sink source translocation of fructifying vines under controlled conditions
2. Development of an experimental set up including interactions of root-galling and sucking phylloxera

Subsections:

1. Establishment of a setting and verification of environmental conditions (light, temperature, water, nutrients) for trial plants
2. Establishment and verification of an infestation by phylloxera
3. Verification and control of treatments (infested and non-infested)
4. Development and verification of plant- and tissue sampling

II. Literature Review

II.1 Partitioning of assimilates in grapevine

The beginning of this chapter will give a brief introduction to *Vitis vinifera* L. Therefore the botanic classification is important which can be seen below:

Domain	*Eukarya*
Kingdom	*Plantae*
Division	*Angiospermae*
Class	*Dicotyledoneae*
Order	*Rhamnales*
Family	*Vitaceae*

In terms of the genus *Vitis,* the species which is in the focus of this trial, is the Eurasian species *Vitis vinifera* L. This species is the most important in terms of grape varieties cultivated today. *Vitis vinifera* L. is, like other species within the genus *Vitis,* a perennial vine with tendril bearing shoots. The flowers of *Vitis vinfera* L. are mainly hermaphroditic. All species within this genus can be grafted onto each other.
The members of this genus can be roughly divided into two groups: the American and the European group. Because of their different agronomical traits, breeders had hoped to combine the positive traits through crossing them. By now the grafting of phylloxera-susceptible European wine grape cultivars to rootstocks, which are, in most cases, hybrids of tolerant American *Vitis* species, is the most important gain, whereas a long history in crossing between both groups brought no satisfying result (KELLER, 2010). Two species out of the American group are of special interest in this trial: *Vitis berlandieri* and *Vitis riparia*. The rootstock *Kober 5 BB* arose due to a crossing of both and is the rootstock onto which the cultivar *Zweigelt* is grafted, building the trial plants of this work. A rootstock is the lower part of European cultivars in today's viticulture. Grape cultivars with desirable fruit properties are grafted on the respective rootstock and are called scion. The rootstock also has desirable properties, but in this case more in terms of soil adaption properties and vigour-influencing traits, on the grafted variety.

The anatomy of the grapevine structure is very similar to that of many other woody perennial plants. The root system features three main functions. Anchoring the plant in the soil, gathering and sending water and nutrients to support plant growth and acting as a storage pool for carbohydrates. The trunk also has different functions. It serves structural, is also an important storage pool for carbohydrates and links the canopy with the root system. Thus it is

important for the transport of assimilates, water and nutrients (CREASY & CREASY, 2009). Finally the branches, which form, together with the trunk and the reproductive organs, the canopy, are supporting the shoots, fruits and leaves (CREASY & CREASY, 2009; KELLER, 2010).

II.1.1 Source sink relations in grapevine

In general, nutrients and assimilates can be transported within the plant via the xylem or the phloem, both building the vascular tissue.

The phloem occupies a central position in the investigation of source-sink relationships within a plant. Next to the transport and allocation of photo-assimilates and nutrients, it also offers other important functions. It can be seen as a "neural network", conducting electric signals, which result from environmental stimuli and thus regulate physiological functions (FROMM & LAUTNER, 2007). The transport of phytohormones is another important feature of the phloem. Phytohormones act as chemical signals and can be seen as a remote control for the regulation and the control of physiological processes (KELLER, 2010). Nevertheless the allocation and partitioning of solutes, especially sucrose, is clearly linked to the phloem, too.

There are several differences between the phloem and the xylem. The phloem consists, in contrast to the xylem, of living cells. The phloem's structure of a transport channel results of a sieve tube, consisting of sieve tube cells, which are connected through sieve plates. Those sieve plates are the end walls of the sieve tube cells and include pores for the transport of solutes, for instance sugar. Companion cells are connected to the sieve tube cells via numerous plasmodesmata and provide metabolic functions to the sieve tube cells (MARSCHNER & MARSCHNER, 2012).
In contrast to the xylem, which is driven by transpiration, the phloem relies on pressure driven mass flow (LALONDE, et al., 2004). Another difference is, that flow of solutes within the phloem underlies the control of the whole plant metabolism and changes with the source sink development; meaning that long distance transport is bidirectional depending on the nutritional requirements of various plant organs or tissues (MARSCHNER & MARSCHNER, 2012). Thus the direction of the flow within the phloem is dynamic and often in opposite to the direction of transpiration (KELLER, 2010). Despite these differences there is an exchange between the two. A xylem to phloem transfer predominantly occurs in the vascular bundle of leaf traces, whereas a phloem to xylem transfer is of importance at the roots, redirecting solutes, transported by the phloem, to the shoots (LALONDE, et al., 2004).
The sap constellation within phloem depends on species, cultivar, and rootstock and varies according to changes of physiological environment, the part of the plant, and vegetation

period (KELLER, 2010). In grapevine, sucrose builds the major compound of the phloem sap in terms of concentration and translocation (KOBLET, 1969).
The pressure flow theory by *MUENCH* (1930) delivers the principle of sap movement within the phloem from sites with high pressure (source) to sites with lower pressure (sink). This theory has been further developed by *PATRICK* who showed that a pressure works on the sieve tubes of the phloem, which is detached from turgor pressure of source and sink cells to ensure a steady phloem transport (PATRICK, 1997). The transport via phloem also depends on solutes, especially sucrose, which is mainly responsible for the osmotic pressure of the phloem sap. Thus it can be seen as the fuel for the phloem system (Hellmann, et al., 2000). That means that the transport rate within the phloem depends on the concentration of solutes and a pressure gradient from source to sink, generated by osmosis at the source phloem (PATRICK, 1997; LALONDE, et al., 2003; KELLER, 2010) .

The sink source relationship is an essential aspect in grapevine's physiology. Allocation of assimilates and mineral nutrients via phloem is directed from source- to sink organs. A source organ may be defined as an organ which produces carbohydrates over its own needs and therefore is exporting carbon skeletons. Organs which require the import of carbon can be defined as sinks. The strength of a sink means its ability to mobilize photo-assimilates as well as its capacity to import and store further compounds coming from a source organ or tissue. The activity of a sink organ correlates with the rate of respiration (BLANKE, 2009). An example for a typical source organ is a grapevine leaf reaching half of its mature size, or the basal leaf of a shoot with five to six leaves (KOBLET, 1969). Typical sink organs are flowers, petals, fruits or roots that may change during their development from sink to source (BLANKE, 2009). The Translocation of the solutes occurs in the sieve elements of the phloem tissue and follows a gradient of concentration, usually being higher concentrated in the source than in the sink (KELLER, 2010).
The shift of carbohydrates in *Vitis vinifera* L. can be separated into three physiological mechanisms: phloem loading, phloem unloading and storage of monosaccharides in sink tissues. The phloem itself can be divided into three parts: collection phloem, transport phloem and release phloem (LALONDE, et al., 2003).

II.1.2 Phloem loading

Sucrose is transported into the vein of a leaf through the phloem loading mechanism. There are three principle pathways of sucrose on its way into the collection phloem of source leaves (VAN BEL, 1993).

The first way is the symplastic phloem loading. Symplasmic phloem loading species transport sucrose from mesophyll (M) cells to phloem sieve tube (ST) through the connection

of those via plasmodesmata (PD). This route is called symplastic, because the cytoplasms of all interveining cells are joined into a single cytoplasm. The concentration of sucrose in M cells is higher than the concentration in the phloem sieve tube. Thus sucrose can move along a concentration gradient to enter the phloem ST (BRAUN, et al., 2014). This mechanism is also called “passive” loading not requiring energy input for the entry of sucrose into phloem ST due to downwards diffusion along a concentration gradient (RENNIE & TURGEON, 2009; SLEWINSKI & BRAUN, 2010). Although there is no need for sucrose transporters to move sucrose across the plasma membrane in order to enter the collection phloem, this need remains for sucrose entering the transport phloem. Through sucrose leaking out along the transport phloem, it must be retrieved via sucrose transporters to maintain a high chemiosmotic gradient along the transport path (BRAUN, et al., 2014).

The second way to load sucrose into the phloem is the apoplastic phloem loading mechanism. The apoplast is bounded by a plasma membrane continuum and includes the cell walls, intercellular spaces and the xylem vessels. Apoplasmic phloem loading species do not transport sucrose symplasmically from M cells all the way to the phloem. Due to a paucity of plasmodesmata, connecting the companion cells (CC) - sieve element (SE) complex to surrounding cells, sucrose has to take another route (BRAUN & SLEWINSKI, 2009). Instead, sucrose moves through PD into the bundle sheet (BS) cells and from there further via PD into the phloem parenchyma (PP) (MA, et al., 2008). The export of sucrose could either follow into the BS-CC cell wall space, or, across the PP plasma membrane delivered by SWEET transporters into the phloem apoplasm, prior an import of sucrose into the CC – SE complex (CHEN, et al., 2012). Sucrose transporters, located on the plasma membrane of the CC – SE complex, subsequently import sucrose (BRAUN, et al., 2014). Then sucrose gets imported into the CC cytoplasm and moves via PD into the sieve tube for its long distance transport from source to sink (BAKER, et al., 2013). The import of sucrose into the CC-SE complex requires energy due to the need of moving sucrose from its lower concentration in the apoplast to a higher concentration in ST symplasm (GIAQUINTA, 1983). In contrast to symplasmic phloem loading species, apoplasmic phloem loading species are able to attain a high concentration of sucrose within the phloem sieve tubes (GEIGER, et al., 1973). Getting symplastically loaded, sucrose would diffuse back via plasmodesmata, which connect adjoining cells, towards the mesophyll cells to reach equilibrium (RENNIE & TURGEON, 2009).

Finally, the last mechanism for loading sucrose into the phloem, is the so called polymer trapping (BRAUN, et al., 2014). Some plants build large polymers of the synthesised sucrose, e.g. raffinose or stachyose (RENNIE & TURGEON, 2009). The synthesis of these large molecules is located in specialised companion cells, which are called intermediate

cells. The route of sucrose starts at mesophyll cells and goes further via PD into the cytoplasm of the intermediate cells. The polymers are thought to be too large to diffuse back the same way sucrose took to the intermediate cells. Like sucrose, raffinose and stachyose can further move through PD, connecting the CC and SE to get finally long-distance transported in the sap within the sieve tube (BRAUN, et al., 2014).

All three types of mechanisms might be used by plants, even within a single vein (VAN BEL, 1993). However, the phloem loading mechanism of polymer trapping seems to play a minor role in terms of sugar transport within grapevine (KELLER, 2010). Both other modes operating in tandem in grapevine leaves are thought to be possible (LALONDE, et al., 2003).

II.1.3 Phloem unloading

The second step of carbohydrate transport shifts the non-reduced sugars to the sites of usage or storage: the sinks. The first step of phloem unloading is the exit of assimilates and nutrients from the SE. Afterwards, a complex series of short distance transport events takes place (OPARKA, 1990). The unloading of sucrose from the SE – CC complex occurs either symplasmically, through interconnecting PD, or apoplasmically, into the surrounding cell wall matrix, or through the simultaneous combination of both (BRAUN, et al., 2014). The unloading path is influenced by the function and the development of the sink (PATRICK, 1997). Unloading of the phloem is strongly regulated by the sink strength (MARSCHNER & MARSCHNER, 2012).

The unloading of the phloem happens in reverse order to the phloem loading (LALONDE, et al., 2004). Symplastic phloem unloading is the predominant route of photo assimilates. This process is a passive one because of the concentration gradient between the SE and the surrounding sink cells and because no membranes have to be crossed due to the open PD. Thus sucrose taking the symplastic pathway is driven by passive diffusion and bulk flow. The process is characterised by a large transport capacity and low hydraulic resistance. The import of solutes can be controlled by sink-cells by opening and closing their PD, which is called “facilitated diffusion” (KELLER, 2010). Further sinks can be differentiated into utilization sinks and storage sinks. Utilization sinks use photosynthates for their growth. Examples are root tips or shoot apices. These sinks show an evidence for a symplasmic phloem unloading (MARSCHNER & MARSCHNER, 2012; OPARKA, 1990).

In contrast to the symplasmic pathway, the apoplasmic, as well as the post phloem transport, is one of energy dependence. Active transport pumps, using ATPases, require energy to pump solutes across the phloem and across pericarb cell membranes (GIRIBALDI, 2007). Sucrose and hexose transporter are integrated in the ATPases (HAYES, et al., 2007) and are activated by sugars and absidic acids. There are different paths unloaded sucrose can

take. One option might be the uptake by adjacent recipient sink cells across the plasma membrane through sucrose transporters (SUTs) (WEBER, et al., 1997). Another way is the uptake through H^+/hexose symporters (HXTs), following hydrolysis by cell wall invertase (CWIN) (RUAN, et al., 2010). It also may be further transported, symplasmically or apoplasmically, to distant cells. This transport is called “post-phloem transport” and depends on the type of the sink, the developmental stage and the species (WERNER, et al., 2011). A special feature of apoplastic unloading from the phloem occurs at veraison. Although the symplastic pathway normally is the dominant route of solutes, during berry ripening the apoplastic pathway comes to be predominate at that growth stage (ZHANG, et al., 2006) .

II.1.4 Patterns of Assimilate partitioning

The allocation and partitioning of assimilates underlies the complex interactions between source and sink organs, which are further influenced by plant environment and development of the plant. This chapter shall provide an understanding of the patterns by which fixed carbon from photosynthesis is distributed within the plant.

A leaf’s export of fixed carbon depends on its carbon balance. The carbon balance is determined by the rate of photosynthesis and the metabolic activity. Allocation means the regulation of distribution of fixed carbon within a source leaf to the different metabolic pathways. It is also important for a carbon importing sink organ. There are three ways existing which describe the allocation of fixed carbon. The first one is the utilization for own needs, such as consume by respiration, or synthesis of components which are of importance for cell metabolism or growth, e.g. amino acids. Storage is the second way fixed carbon can be allocated. Therefore carbon is converted into starch at day and stored within the chloroplast to get remobilised at night or during stress. The third way is the transportation of fixed carbon. Therefore it gets converted into sucrose and either temporarily stored in the vacuole or exported to sink organs. The coordination of allocation of fixed carbon to either starch or sucrose is important because only sucrose can be exported immediately. Sinks partly coordinate this allocation by their demand of sucrose. A high demand of sucrose by sinks detracts sucrose from sources and favours further production instead that of starch (KELLER, 2010).

Distribution of exported assimilates is called partitioning and is driven by the source-sink turgor gradient within the phloem. Transport of assimilates is a dynamic process and depends on developmental stage of a vine and changing environmental conditions. For instance KOBLET showed, that leaves which recently became sources, export their assimilates at first to the growing shoot tip and the unfolding leaves. Once new leaves are grown on the shoot, the older ones are more distant from the shoot tip and begin with basipetal export (KOBLET, 1969). Normally sources supply nearby sinks. But this does not

happen in every case. Certain sources seem to supply certain sinks, whereas the way of supply is flexible (WARDLAW, 1990). Further, sinks compete during their development on the vine on different resources, such as light, space or nutrients. These are reasons for a hierarchy of sinks with relative priorities between them. This hierarchy is dynamic and sensitive to environmental influences. For instance, inflorescences are weak competitors, whereas after fruit set, berries and seeds dominate the shoot and outcompete the roots (HALE & WEAVER, 1962; WARDLAW, 1990). The import rate of a sink depends on its strength and is related to all sink strengths on a vine and the amount of available assimilates of the different source organs. Sink strength can be defined as a product of sink size, which is the total weight of a sink, and sink activity, which is the rate of assimilate import per unit sink weight (KELLER, 2010). Confusingly low sink strength cannot be related to a low sink priority. Seeds with their small size and weight are one example, having normally top priority in the sink hierarchy. In contrast to them the storage pools, like perennial organs, normally have the lowest sink priority (MINCHIN & LACOINTE, 2005).

The relative priority of a sink depends on its ability to attract sucrose. Thus it is higher if the sink is able to reduce sucrose concentration within the phloem which is in other words the rate of phloem unloading, by maintaining the favourable pressure gradient to the source at the same time (WARDLAW, 1990; PATRICK, 1997). Most of the imported sucrose is normally converted to hexose sugars like glucose and fructose, to use the sugar for own metabolic processes, for instance the reassembly of starch. For this conversion the invertase and/or the sucrose synthase are used (HAWKER, et al., 1991).

Changes in activity of sink size influence the assimilate transport pattern. The pattern of assimilate partitioning between a source and sink underlies several important traits. To name the most important: Proximity, Connection, Interference, Communication, Competition and Development.

Proximity thereby means, that the closer a source and a sink are, the more likely this source will supply the nearby sink (WARDLAW, 1990). Leaves which are near to the shoot tip, for example, export their assimilates to it, whereas basal leaves prefer the supply of clusters and perennial organs (HALE & WEAVER, 1962; KOBLET, 1969).

Another trait is the connection between a source and a sink. A source leaf favours a sink which is directly connected through it by a vascular bundle (KELLER, 2010). So an inflorescence or a cluster is mainly supplied by source leaves which are located at the same shoot side (KOBLET, 1969; YANG & HORI, 1980). Interference also has an impact on the pattern of assimilate partitioning. Wounding and pruning can change normal paths of translocation so that a direct connection between source and sink is interrupted. Alternative

connections can be provided by vascular interconnections (KELLER, 2010). Hedging, for instance, stimulates a cross transfer of assimilates via vascular interconnections on both sides of the shoot. Also it induces young leaves to change from acropetal to basipetal export (QUINLAN & WEAVER, 1970; KOBLET & PERRET, 1972).

Communication also is an important trait of the pattern. Grapevine strives for a balanced growth concerning vegetative and reproductive growth. In terms of vegetative growth also the growth of above and below soil organs needs to be balanced. This balancing requires interactions between sinks and sources which are given through a pressure gradient within the phloem, nutrients and hormones (KELLER, 2010). A low pressure within phloem at sink side induces the import of assimilates.

Further competition between sinks has a strong impact on the partitioning pattern. All sinks of a plant compete with each other. The respective priority of a sink related to the priority of all other sinks in terms of assimilate allocation, determines the competition. A competitive sink is one of a fast growth because consume of assimilates lowers the pressure at sink size within the phloem. Thus a competitive sink is seen as one with great sink strength. The more sinks compete about assimilates, the less assimilates per sink are available. A high number of buds remained after winter pruning, lowers the vigour of each shoot due to the higher number of shoots and grapes (KELLER, et al., 2008).

The last important factor which influences the pattern of assimilate distribution is development. During the growth of a grapevine, there is a change in the importance of a sink. Shoot tips, for instance, have high priority after bud burst, which changes to clusters after bloom. Berries become dominant in sink hierarchy after fruit set (HALE & WEAVER, 1962). After veraison there is again a shift of the grapevine's priority in direction to wooden organs and roots, to fill up reserves and increase cold resistance (CANDOLFI-VASCONCELOS, et al., 1994).

There are several other factors influencing the pattern of assimilate partitioning, e.g. environmental conditions, such as water- or nutrient deficiencies. The consequence of such deficiencies is an increase in partitioning assimilates to the root system but a decrease of total plant growth (KELLER, 2010).

Summarising the chapter showed that sources and sinks build an inseparable system showing one part affecting the other.

II.1.5 Key Parameters to investigate source-sink relationships of grapevine in a trial

This sub-chapter shall deliver an overview about important key parameters which are to investigate if data about source-sink relationships of grapevine shall be won. There are different approaches to gain information about sink source relations in grapevine.

One is to investigate sugar physiology of grapevines. In general, grapes can rely on sugars as energy source. Carbohydrates, mainly in form of sugars, are transported through source sink fluctuation and serve as regulators for source sink interactions (ROITSCH, 1999), as well as plant development and the gene expression (GIBSON, 2005). The annual cycle of sugar physiology in grapevine can be divided into two phases:

Phase I: Starch mobilisation out of woody tissue to support annual organs during their early growth

Phase II: Net leaf photosynthesis which supports both: further growth of annual organs and replenishment of the reserves (LEBON, et al., 2008).

Therefore starch metabolism and photosynthesis are key issues for sugar availability. Former research identified starch as main component of sugar reserves (EIFERT, et al., 1960; MULLINS, et al., 1992; ZAPATA, et al., 2001). During the dormancy, 90 percent of the starch is located in the ray parenchyma of the root system (ZAPATA, et al., 2004; BATES, et al., 2002). There it represents up to one third of the root system's dry weight (ZAPATA, et al., 2001). This makes starch during dormancy highly suitable for sampling, especially when comparing the amount of starch at dormancy to the amount of starch at later growth stages of grapevine, for instance at flowering. In early spring, when soil temperature exceeds ten to twelve °C, dormancy is broken and the metabolism gets reactivated. At that time, starch is the only source of carbohydrates (LEBON, et al., 2008) and gets progressively mobilised to support the growth of annual vegetative and reproductive organs (BATES, et al., 2002)

Photosynthesis is another key parameter for the investigation of source sink relationships in grapevine. Research has shown that there is an increase of leaf photosynthesis from bud break to flowering, followed by a decrease until leaf senescence (STOEV, 1952). The maximum of photosynthesis occurs before and during bloom, depending on the cultivar (LEBON, et al., 2008). Depending on the cultivar, maximum performance of photosynthesis gets maintained over a certain period to decrease slowly (SCHULTZ, et al., 1996). Measuring photosynthetic performance, it has to be considered, that it depends on environmental conditions and fluctuation over the day. Mainly changes in temperature have influence on the rate of photosynthesis due to changes of stomatal opening (SCHULTZ,

2003), being highest in the morning under natural conditions (MEDRANO, et al., 2003). PETRIE investigated in his studies the stomatal conductance, the leaf chlorophyll concentration and photosynthesis rates of the fourth leaf of potted Pinot noir grapevines (PETRIE, et al., 2000).

The disaccharide sucrose with its cleavage products glucose and fructose takes over a central role within the partitioning of carbon (ROITSCH & GONZÁLEZ, 2004). Therefore those sugars are of special interest for investigations on source sink relationships. One possible key parameter to investigate is the invertase activity. While starch being the only source of carbohydrates in early spring, from anthesis on, sucrose becomes the most important sugar in terms of mobilisation. Via phloem it is shifted to reproductive organs, typically being sinks in angiosperms, which are in case of grapevine the berries. There sucrose is split into glucose and fructose (LEBON, et al., 2008). The hydrolytic cleavage of disaccharide sucrose, into the hexose monomers glucose and fructose, is mediated by invertase. Three kinds of invertases exist in plants: vacuolar invertases, cell wall bound invertases and neutral invertases. To use sucrose as carbon- and energy source, the α1-β2-glycosidic bound has to be cloven. Cell wall bound invertase catalyses this irreversible hydrolyse at the site of phloem unloading. Metabolisation of the cleavage product glucose and fructose controls the sink's strength to attract sucrose (ROITSCH & GONZÁLEZ, 2004). Both hexoses, glucose as well as fructose, are the sugars being mainly accumulated within berries. They account for 90 percent or more of carbohydrates of grape juice and represent 30 percent of fresh weight of mature berries (HOFACHER, et al., 1976). Accumulation of hexoses begins at the same day with berry softening and once it occurs, both hexose increase linearly (COOMBE, 1988). Earlier research found a connection between hexose accumulation and invertase activity (DÜRING & ALLEWELDT, 1984). Both, soluble and cell wall bound invertase, have been localised in the tissue of grapevine leaves and berries (RUFFNER, et al., 1990). PÉREZ gives examples of how different sources of carbon and sucrose concentration affect fruit growth, hexose accumulation and invertase activity (PÉREZ, et al., 2000). Further RUFFNER gives information about hexose measurements with a DNSA- reagent and invertase measurements (RUFFNER, et al., 1995). Vacuolar invertase was identified as the predominant source of sucrolytic activity in berries (RUFFNER, et al., 1990; DAVIES & ROBINSON, 1996). Transcripts of hexose transporters and a cell wall associated invertase have been investigated by HAYES, in young sink and old source leaves as well as in berries (HAYES, et al., 2007). Furthermore a very detailed overview about carbohydrate dynamics and their measurement is given by HOLZAPFEL et al. 2010 (HOLZAPFEL, et al., 2010)

Also agronomic indicators are of importance when investigating the source sink relationship of grapevine. PALLAS, for instance, counted the length and number of unfolded leaves on each shoot every week. In addition they took total fresh weight and dry weight of ripened clusters. Also the cluster to leaf ratio, which relates the fresh cluster weight in grams to the total leaf area in m² of each plant, was measured in this trial, as well as total plant leaf area. Individual leaf area has been estimated through a quadratic relationship between the length of each leaf lamina and the corresponding leaf area, as described by SCHULTZ (PALLAS, et al., 2008). The leaf area to fruit weight ratio, which is the source to sink ratio, was also investigated in study by PETRIE et al. 2000 which is mentioned above. Further agronomic parameters to research on can be the yield per vine, berry weight, berry number per cluster, cluster compactness and berry skin thickness (PASTORE, et al., 2013).

A much related study in terms of the research question from STEFFAN and RILLING worked with radiocarbon dating, thus with radioactive labelled ^{14}C isotopes. Cuttings of *Vitis rupestris* 187 G. with two buds were infested by phylloxera on leaves and on roots as soon as three to four leaves expanded. After a successful infestation, plants were placed in a gas-proofed plastic tent and exposed to a $^{14}CO_2$ atmosphere for one hour. Then trial plants stood in an isotope extractor fan for 23 hours. In the following, plants were placed for 24 hours with twelve hours under artificial light. After 24 hours passed, they were flooded with liquid nitrogen to kill them and the parasites. Leaf laminas, shoots, wooden part of the cuttings and roots were investigated separately, whereby measured radioactivity was related to dry weight. As a result, leaf lamina of leaf- and root-infested cuttings had the highest amount of radioactivity (STEFFAN & RILLING, 1981). More information about this working technique can be found in a study of YANG et al. 1980 (YANG, et al., 1980).

Composition of phloem- and xylem sap would also be valuable information. Especially when sampled to different growth stages. A lot of studies have investigated these compositions and have optimised techniques for sampling (PEUKE, 2000; KELLER, et al., 1995; PATE, 1976; KING & ZEEVAART, 1974). Further the direct detection of phloem and xylem sap flow velocities, allows non-invasive and precise measurements (HELFTER, et al., 2007) which can also contribute useful information to verify or reject the background hypothesis of this work.

To make reliable statements about the environmental conditions, basic parameters like soil water content, temperature and relative humidity should be measured, in best case automatically, in periodic intervals. Further analysis of grape quality parameters like total soluble solids (° Brix), titratable acidity (g/L), pH value, tartaric acid (g/L) and malic acid (g/L) are needed and useful to link with other measurements. Growth measurements with a possible approach to investigate are also covered in a study of KELLER and KOBLET and

might be useful for the development of additional measurements in this experimental set up (KELLER & KOBLET, 1995).

II.2 Stress

Like other plants, grapevine needs three essential resources for its growth: carbon, water and nutrients (BLOOM, et al., 1985). Environmental stress is a result of suboptimal growth conditions. Stress originates from two categories. Biotic stresses result from the interactions between living organisms, for instance pests or diseases, whereas abiotic stress is a consequence of interactions between an organism and its physical environment, e.g. through overcast sky, water and nutrient deficiency (REHMANN, et al., 2005). Further, stress is limiting either the availability of one or more resources, or is limiting the plant's availability to use this resource (KELLER, 2010). In most stressful environments, more than one stress occurs, e.g. salinity often appears with drought. The ability of a plant's metabolism to deal with certain environmental conditions and thus keep up its functionality displays if these conditions are stress. Effects on the plant depend on the duration and severity of the stress, as well as the time when the stress appears. Acclimatisation processes in grapevine can be triggered by stress (KELLER, 2010). The optimum resource allocation hypothesis implies, that the response of plants on paucity of a resource is the investment in biomass of organs or in processes which lower that paucity. Most times this occurs on the cost of plant parts which have a higher need on the scarce resource (POORTER & NAGEL, 2000; BLOOM, et al., 1985). An example for this hypothesis might be the response of plants on the limitation of carbon, for instance through overcast sky. If so, plants might increase their investment in shoot-, especially leaf growth. In contrast to this nutrient deficiency results in an increased investment in root growth or a decline of shoot growth (KELLER & KOBLET, 1995).

Competing sinks also play an import role during phases of stress. Their size, number and their developmental stage determine where the resources are invested to secure the survival of the plant (GEIGER & SERVAITES, 1991). Many reactions of plants on stress include morphogenetic responses, rather meaning redirection than a stagnation of growth. The consequences are an inhibition of cell extension, local stimulation of cell division and changes in differentiation (POTTERS, et al., 2009).

II.2.1 Low light intensity- an example for abiotic stress

Like an absence or a surplus of water can be an abiotic stress for grapevine, also low light intensities, that curtail photosynthesis, mean stress for vines. Light in general is not only a source of information, but also the source of energy for the plant. Grapevine leaves are able to adapt to low light intensities, like it is done by shaded leaves. Their adaption comprises a larger surface but a lower thickness. Further, they have less stomata per unit leaf area but more chlorophyll per reaction centre and per unit nitrogen. Contents of rubisco and carotenoids are lower and their respiration rate too, even up to 50%, compared with that of leaves exposed to sunlight (EVANS, 1989). These traits enhance light absorption and energy

transfer of shaded leaves (SCHULTZ, et al., 1996; ORTOIDZE & DÜRING, 2001). Leaves produce carbon for the formation of biomass. The border, when light reaches an intensity that a leaf's CO_2 assimilation is zero, is called light compensation point. This threshold level depends on cultivar, species and developmental conditions, but is reached in a typical grapevine leaf at 10-30 µmol m^{-2} s^{-1} (KELLER & KOBLET, 1994).

Under an overcast sky leaves within a canopy are not able to reach light saturation. Generally light saturation is reached at approximately 700-1200 µmol m^{-2} s^{-1}, which is the unit of photosynthetic active radiation (PAR). To get a better idea of this value, one must imagine, that full sunlight reaches up to 2000 µmol m^{-2} s^{-1}, while overcast sky, depending on the clouds, reaches 100-1000 µmol m^{-2} s^{-1} (SMART, 1985). If photosynthesis runs below light saturation, light is limited. Within a canopy, interior leaves receive much less light than leaves on the outside of the canopy. Often the light level is below 10 µmol m^{-2} s^{-1} (MULLINS, et al., 1992). The shaded side of a canopy receives only three to six percent (40-100 µmol m^{-2} s^{-1}) of light intercepted by the side which is exposed to sunlight, while less than 10% of PAR permeate from an upper leaf to the leaf underneath (SMART, 1985). This is the reason for the whole canopy photosynthesis almost never reaching light saturation (PONI, et al., 2003). In case these low light intensities persist for a longer period of time, the leaves consume more water and nutrients than they contribute to whole plant metabolism due to carbon supply. As a consequence, vines can initiate the processes of senescence and abcission (REICH, et al., 2009; POORTER, et al., 2006; TAYLOR & WHITELAW, 2001). Senescence means the ordered degradation of cell constituents. This process ends with death and is followed by abscission of the affected organ due to cell wall's breakdown in respective abscission zones. It can be seen as an adaptive strategy of a plant to sacrifice an organ or other parts to survive a stress period (KELLER, 2010). Phytohormones again act as a remote control. While abscisic acid stimulates senescence, it is accelerated by ethylene and stopped by auxin (ROBERTS, et al., 2002). The earliest and most dramatic consequence during senescence is a breakdown of chloroplast, the photosynthetic machinery of a plant, containing the majority of leaf protein. Cell constituents like carbon, nitrogen and other stored nutrients within leaf protein get rapidly remobilized and can be used to sustain other important sink's metabolism and growth. Strong sink strength, for instance a high crop load, may increase recycling of nutrients (KELLER, 2010). Afterwards abscission occurs, as a consequence of canopy shading or stress. Thereby grapevine repels the surplus of leaves which are not needed for photosynthetic performance or cannot longer be provided due to stress (HIKOSAKA, 2005). Recycling of nutrients involves a loss of approximately 50% of leaf's nutrients (HIKOSAKA, 2005; BERTAMINI & NEDUNCHEZHIAN, 2001). The loss is compensated by remobilizing buffer reserves from permanent storage organs (HUNTER, et al., 1994; GEIGER & SERVAITES, 1991). Grapevine has a quite high shade tolerance.

Adaption to low light conditions by altering leaf and shoot growth at the cost of shoot hydraulic resistance is a possible response to such an environment. Production of new leaves rather than maintain older source leaves is a reaction of grapevine. As a consequence fruit production and root growth are lowered (KELLER, 2010). Studies of KELLER, KOBLET and CANDOLFI-VASCONCELOS investigated the effects of low light conditions on grapevine. Low light intensity (30 $m^{-2}s^{-1}$) lowered inflorescence initiation and bud fertility, further bud break was earlier. Further lateral shoot growth was promoted under low light conditions (KELLER & KOBLET, 1995). Another effect was the triggering of stem necrosis (KOBLET, et al., 1996), a physiological disorder of grapevine, as well as inflorescence necrosis (KELLER & KOBLET, 1994), another expression of this physiological disorder. These studies underline the extensive influence of light on grapevine and stress conditions resulting from low light intensities.

Measuring chlorophyll fluorescence (CF) is one approach to investigate the stress effect of low light conditions. The principle on which chlorophyll fluorescence is based on- is how light energy is used, once it gets absorbed by chlorophyll molecules of a leaf. There are three possibilities for the usage of the light energy. If this energy is used to drive photosynthesis it is called photochemistry. Another option is the dissipation as heat of excess energy. The third opportunity would be that the light energy can be re-emitted as light which is called chlorophyll fluorescence (MAXWELL & JOHNSON, 2000). These three options compete with each other, which means that if one process increases in terms of efficiency, the yield of the other processes will decrease (MAXWELL & JOHNSON, 2000). Thus, by measuring the chlorophyll fluorescence, information about photochemistry and heat dissipation can be won (MAXWELL & JOHNSON, 2000). Only one or two percent of the total light absorbed are re-emitted. There is a difference of the light spectrum of fluorescence and absorbed light. Fluorescence emission usually consists of longer wavelengths than the wavelengths of absorption. The yield of chlorophyll fluorescence can be determined by exposing a leaf to a source of light with a defined wavelength and measure the amount of light re-emitted at longer wavelengths (MAXWELL & JOHNSON, 2000). The most frequently used parameter is the maximum quantum yield of photosystem two (PSII), which is also called the Fv/Fm ratio (KITAJIMA & BUTLER, 1975). Its values are always below one and are almost constant with approximately 0.8 over several plant species (BJÖRKMAN & DEMMIG, 1987). For stressed plants this value is distinctly lowered, which makes it suited for the measurement of stress levels and makes important when analysing CF data (ROHÁČEK, 2002; PFÜNDEL, 1998).

II.2.2 *Daktulosphaira vitifoliae* (Fitch)- an example for biotic stress

Grapevine with its fruits, as widely spread cultivated plant, attracts not only humans but also a great number of other organisms. In most regions these organisms are permanent

companions over the vegetation period. Pathogens like powdery (*Erysiphe necator*) or downy mildew (*Plasmopara viticola)*, bunch rot (*Botrytis cinerea)* or bacteria are next to several pests, only naming a few examples. Cultivated vines have a serious disadvantage compared to these organisms due to vegetative propagation of nowadays common cultivated grape varieties. The evolution of grapevine and of other organisms that liked their fruit, roots or other organs was strongly linked, but ended with vegetative propagation for grapevine. It deprived grapevines of the opportunity to adapt through sexual reproduction while other organisms, partial incredibly fast, are still in a permanent adaption and optimisation to secure their survival by attacking host plants more successful or by developing resistances against pesticides. That puts these organisms at an immense advantage over cultivated grapevine, whose only opportunity to evolve is somatic mutation, thus evolution is only very slow and plants remain often unaltered for several hundred years (KELLER, 2010).

As an example for biotic stress, the root feeding and aphid-like phylloxera- *Daktulosphaira vitifoliae* (Fitch) and their interactions with grapevine will be described here briefly. This insect devastated a big part of European viticulture in the 19th century and still is monitored and researched in many parts of the world. Susceptible *Vitis vinifera* species are grafted on to rootstocks of American *Vitis* species, which co-evolved with phylloxera and tolerate it as a result (BATTEY & SIMMONDS, 2005; POWELL, et al., 2013). Root-feeding stages of phylloxera are the stages of the most economical damage, although it also infests leaves of *Vitis* species. Phylloxera is cecidogenic, meaning gall-forming. *Vitis vinifera* is particularly susceptible to radiole (root-galling) grape phylloxera. Depending on the age of roots, nodosities are induced at young roots; or if they are old and lignified, then tuberosities are induced (POWELL, et al., 2013). Tuberosities disrupt water and nutrient uptake with the effect of a loss of leaf surface area, yield reduction or even vine death after a few years (FURKALIEV, 1999). Nodosities serve as nutrient reservoirs, among others there is a massive starch accumulation within nodosities, depending on gall development (GRIESSER, et al., 2015; KELLOW, et al., 2004). This nutrient mobilisation seems to be highest at post-harvest season (OMER, et al., 2002). When phylloxera infests the root of a susceptible *Vitis* species, the root first acts as a sink for nutrients, namely sucrose. Sucrose afterwards is converted to starch, which accumulates in the cortex. Enhanced levels of free amino acids, especially glutamine have also been reported (KELLOW, et al., 2004).

These effects of an infestation of grapevine by phylloxera can be seen as a stress because the insect does use resources which no longer can be used by the plant. The impact of this biotic stress, of course depends on various factors, for instance susceptibility of the plant, number of root galls, nutrient availability, age of the plants and much more. Thus healthy plants growing in a diverse ecosystem and having a higher tolerance against stress because

of a balanced vigour between reproductive and vegetative growth, are important for wine grower, being less susceptible to stress.

III. Material and Methods

III.1 Plant material and experimental conditions

One year old potted grapevines of the cultivar Zweigelt (*Vitis vinifera* L.), grafted onto the rootstock Kober 5BB (*Vitis berlandieri* × *Vitis riparia*), were trial plants in this work. Planting pods had a volume of three litres and were filled with a standard substrate consisting out of 80% standard potting soil and 20% sand. Plants were placed from a cooling chamber, where they over wintered into a glass house cabin with a minimum of twelve hours light per day and a temperature of 21°C. The number of trial plants decreased from 101, in the beginning of the experiment, to 70 plants, distributed equally over both growth chambers until the end of the trial. To start the experiment within the chambers, 85 plants were selected

Pouring and fertilization occurred to sufficient levels all over the vegetation period. Except for the 35 days plants were located in the coldhouse, where controlled conditions in terms of duration of light and temperature were. Trial plants were poured and fed weekly from the bleeding sap stage on with the fertilizer FERTY® MEGA 3 (Planta Düngemittel GmbH) and VE Water. Constellation of this fertilizer can be found in ANNEX. According to the producer this product is especially suitable for young and potted plants. To simplify the handling a stock solution was made. For this solution 240 grams were solved in 800 ml VE-Water. Therefore the Water was warmed up to 40°C and the lab-bottle which the solution was in, was stirred for 30 minutes with a magnetic stirrer. Thus the stock solution contained 30 percent of the fertilizer. A dosage of 5 ml per 3 litres of VE-Water of this stock solution was added with every watering. The producer recommends a dose of 0.5-1.5 ‰, with respect to growth stage and plant species and 0.4-0.5‰ for young plants (PLANTA DÜNGEMITTEL GmbH, 2014). With the chosen dosage of 5ml of 30% stock solution per 3000 ml of VE-Water, we gave with each watering 0.05%, or 1.5ml fertilizer. With the total amount of 11.65 litres per plant, which was given within the growth chambers until the 20th of August, a total amount of 5.825 ml of fertilizer was dosed to every plant. This corresponds to the recommendation of the producer of 0.5‰ of fertilizer for young plants. The weekly dosage of water slightly fluctuated thus also the dosage of fertilizer. With a mean dosage of 810 ml per week per plant, the weekly dose of fertilizer was 0.405 grams of fertilizer per plant per week.

Vines stayed in the cabin until BBCH 13; then shoots were removed and a single shoot was trained vertically. Following plants were relocated into a coldhouse, after an immerse treatment with a solution of 0.05% KARATE® ZEON (Syngenta), to exclude a possible infection by phylloxera. In the coldhouse, all plants were placed on one table and linked to an automatic trickle irrigation system reaching saturated water field capacity. A fertigation with the same fertilizer was linked to the irrigation system. Lateral shoots were removed weekly

from BBCH 18 on. At BBCH 60, the actual trial plants were selected for uniformity and relocated into two growth chambers (HAUSER), with the same size of 6.681 m². There, 14 hours of light and a constant temperature of 22°C were programmed via a system, which is linked to each chamber. Before, chambers were adapted and disinfected with *Dan Klorix* (COLGATE PALMOLIVE Ges.m.b.H.), a detergent based on sodium hypochlorite. Within each chamber, each plant was topped to 14 leaves per plant and poured and fed two to three times a week. Two crop loads (one cluster per plant/no cluster per plant) were distributed over the two chambers and placed without randomisation equally within the chambers. The number of plants was reduced at BBCH 81 due to scarce space in both chambers, so that in each chamber were 18 replicates of the fructifying vines and 17 of the vines without a grape. The plants were cultivated for 14 weeks within the growth chambers. High quarantine conditions were maintained for both chambers over the whole trial period, because plants of one chamber (213) were inoculated with young and fresh eggs of phylloxera, collected out of a clone breeding of the strain AT1. In total, four inoculations with phylloxera were made, being all different either in terms of the method or in terms of the inoculation material.

Adjustments of the environmental conditions, such as water, nutrients, light and temperature, were done and optimised to ensure equal growth conditions between the two chambers and plants. Further measurements of chlorophyll fluorescence were made in different growth stages of trial plants. For the measurements, different instruments with different techniques and principles, were used. Chlorophyll fluorescence was measured in a similar position on each leaf, located between the terminal and the upper lateral lobe. Each third leaf of plants without a cluster and the leaf opposite to the cluster of fructifying plants as well as the eighth leaf of each vine were measured in case of using the instruments PAM-2500 Chlorophyll Fluorometer and Handy PEA Chlorophyll Fluorimeter. During the further procedure with CIRAS-2 Portable Photosynthesis System only the eighth leaf of 24 plants was measured due to the time consuming and sophisticated measurement procedure. Chamber 195 represents the growth chamber without any phylloxera contact and is called treatment control. Within chamber 213 there were four attempts to inoculate the trial plants with grape phylloxera. The initiation of abiotic stress on the grapevines in terms of a low light intensity was given in both chambers, whereas a biotic stress in terms of infestation by phylloxera was only tried to give in chamber 213. Water and nutrients were given in sufficient quantities over the whole vegetation period.

III.1.1 Location of Plants

All plants used in this trial were located in a cooling chamber, with a constant temperature of 4°C for over-wintering until March 28th. From then until April 9th, the vines were placed in a glasshouse-cabin, with a minimum of 12 hours light per day, ensured by light modules which

switched on, if natural light intensity was too low (see Figure 19). On the 8th of April all plants were relocated into the coldhouse of the Division of Viticulture and Pomology of the Department Crop science at the UFT-Tulln, until relocation into the growth chambers on the 14th of May. Vines stayed within the growth chambers for several weeks. Measurements and observations were made over a period of 14 weeks.

Table 1: Location of the trial crops including important growth conditions

Location of vines	Number of Vines	Duration in days	Temperature	Light per day	Water per vine	Fertilizer
Cooling chamber	101	Not recorded	4°C	-	Not recorded	-
Glasshouse cabin	101	12	21°C	Min. 12 h	600ml	0.36ml
Coldhouse	95	35		Natural day length	Automatic irrigation system	fertigation
Growth chamber (from July 10th)	70	102	26°C	14 h	11.65l*	5.825 ml*

*Over the whole period of vines within growth chambers

III.1.2 Time table

For a structured and informal overview about the trial procedure, the following Table 2 will give needed information. The time table contains all information about cultivation of the vines, measurements, development of the trial plants and their growth conditions. It can also be seen in ANNEX, Figure 18. In Table 2, a brief temporal overview of the trial process is given.

Table 2: Trial design with short description about what was done and important stages of the trial and the trial plants

Winterkill and breaking dormancy	99 vines were taken from a cooling chamber with 4 °C into a Glasshouse cabin at march 28th. There, a minimum of twelve hours of light per day and a temperature of 21°C was ensured. Once per week, plants were watered with 200ml/vine, including fertilizer.
Bud break	After bud-swelling, which was about April 3rd, young shoots were removed except one shoot per vine. Shoots with inflorescence were preferred.
Relocation into coldhouse	After twelve days, the young vines, which were at about BBCH 15, were relocated into coldhouse. Before placement there was a treatment with KARATE® ZEON (Syngenta) through immerse the

	soil and root part of each vine and to ensure no infection with phylloxera. Then they were linked to an automatic irrigation system with integrated fertigation and placed on saucers. Shoots were consecutively removed, except the chosen main shoot.
Cultivation in the coldhouse	On April 24th, lateral shoots were removed for the first time. From that time this was done consecutively, approximately once a week. Plants were fixed on a bamboo bar, which was stuck into the soil with a "Tapener Gun". Also the trial plants were put on one table at that time.
Installation of growth chambers	Both chambers were modified. Light modules were relocated to the top of each chamber and shelves dismantled as far as possible. The "control" chamber number 195 was also disinfected with *Dan Klorix*. There was a waiting period of one day before putting plants in the chambers. Two chambers, the "phylloxera"-chamber number 213 and the "control" chamber 195, gave space and an artificial environment for the trial plants.
Relocation into growth chambers	On May 14th, plants were at about BBCH 60, which marks the beginning of bloom and were put into the chambers. Light was adjusted on a length of 14 hours as well as temperature on 22°C. The amount of liquid fertilizer was attuned on 5ml Ferty per 3 litres of VE Water. The amount of given water was 300ml/week/vine. Within the chambers, watering was done exclusively with VE-Water because of high lime content of the tap water in Tulln an der Donau with 23°dH (ÖSTERREICHISCHE VEREINIGUNG FÜR DAS GAS- UND WASSERFACH, 2013). Also the soil of all potted vines was covered with coconut-fibre blankets.
1st Inoculation	On May 23rd, the first inoculation was done. Samples with fresh, living eggs were prepared within 24 hours. Also shoots were cut on a level of 14 leaves per plant. Finally, the measuring leaves were marked with zip ties, so that there was a loose fixation around the petiole of the 3rd and the 8th leaf of variant 1 and 3. At variant 2 and 4 the leaf opposite of the grape was marked and also the 8th leaf.
Optimisation of cultivation in growth chambers	On May 26th, a new growing system was installed. Therefore each vine was carefully wound around a polypropylene-string, which was fixed below on the trunk and above to a framework of wires

	installed before, on a height of approximately 2 metres. Bigger saucers were installed four days later. From that day on, watering was done from below. The amount of water and fertilizer was the same than before.
2nd Inoculation	One week later, 28th of May. Other technique but same inoc. Material- AT1 Strain. Some light measurements were done to check equal conditions between the chambers.
Beginning of measurements	First measurement with the PAM 2500 on June 5th. BBCH of trial plants at approximately 71. Before the measurement there were several tests to familiarize with the instrument. From now on, there were measurements at important growth stages of the potted vines. Find detailed information in Measurements. Of course there was an ongoing support of the vines with VE-water, which included fertilizer. The amount of water was one litre per plant per week at that time, all given from below.
3rd Inoculation	The 3rd inoculation was on June 24th. The technique differed this time significantly from the inoculations before. Vines were at that time at BBCH 75.
Ongoing measurements and adjustments between the two chambers	At BBCH 79, on June 26th and July 1st and 2nd, there were measurements with the instruments PAM-2500 (light and dark measurements), HandyPea and a Porometer. Trial plants were in BBCH 79. The Porometer measurements for direct information about stomatal conductance were not furtherly done. Values were very low compared to field conditions because of the significantly lower light intensity within the growth chambers and measuring took too much time. Eight lamps in chamber 213 were taken out on July 2nd, so that both chambers had an equal number of lamps. On July 7th there was a measurement with the instruments UniSpec and CIRAS. For the CIRAS-measurement 24 plants were taken out (twelve of each chamber). On July 10th, six plants of chamber 195 and eight plants of chamber 213 were sorted out. Samples were taken from whole shoot, all leaves and in case berries. The plants of chamber 213 were also visually controlled on an infestation with phylloxera. There was no visible infestation of the plants. Plants were rearranged after the sorting and the temperature was raised to 26°C in both chambers. Further

	measurements were made with the instruments HandyPea, PAM-2500 (light and dark measurements and PAR measurements), UniSpec and CIRAS.
4th Inoculation and Hatching control of eggs	The visual control of July 10th showed no infestation. Because of this failure, a significant change in inoculation was decided. On July 25th, leaf gulls of a wild living *Phylloxera* Population were caught. After a careful transport and storing in the infection chamber, the collected whole shoots were prepared to inoculation samples. Therefore fresh and vital eggs were put on a filter paper, which was rolled and put in a 50 ml centrifuge tube on July 29th. Each inoculation sample contained 300 to 400 eggs. The inoculation was done one day later. One week later a hatching control of each inoculation sample was made.
Final measurements and last optimisations on the trial design	The last measurements with HandyPea and UniSpec were made on August 5th and 6th. Vines were at that time at BBCH 89, which is the end of veraison. Plant pots were marked with coloured tape to ensure the same alignment at each measurement. On August 11th and 12th, the last light and dark measurement with PAM-2500 was made. On August 20th, my supervision of the trial ended and it was maintained by employees of the division of viticulture and pomology.
Further maintenance and measurements of the trial	Instructions to simulate the end of the vegetation period were planned. After sampling the ripe berries, light and temperature, were planned to get reduced to initiate vegetation ending. Also the sampling of the measuring leaves should be done. In an adequate state, the potted vines should get relocated into a cooling chamber to simulate dormancy. For the vines of chamber 213 strict quarantine conditions were considered.

III.2 Measurements

The instruments and the date of each measurement are listed in Table 3. Measurements were tried to take at the same time of the day and with the smallest possible time period between the measurements of each chamber. Depending on the chosen instrument, each one was calibrated before. Further the first measurements were taken on test plans, which were treated same as the trial plants but are not included in the trial and the results

Table 3: Measurement dates and instruments and respective growth stag of the trial plants expressed by reference to the BBCH scale

Date	Instrument and Measurement	BBCH trial plants
03.06.	Light measurements- **SPAD**	**73**
04.06.	**PAM** (Light measurements- test)	**73**
05.06.	**1st** measurement **PAM (light)**	**73**
26.06. & 27.06.	**2nd** measurement **PAM (light)**	**75-77**
01.07.	**1st** measurement **Porometer-** no further measurements	**75-79**
	1st measurement **HandyPea**	**75-79**
02.07.	**2nd** measurement **PAM (dark)**	**75-79**
07.07.	**1st** measurement **UniSpec**	**79-81**
	1st measurement **CIRAS (24 plants)**	**79-81**
14.07.	**2nd** measurement **HandyPea**	**81-83**
21.07. & 22.07.	**3rd** measurement **PAM (Dark & Light)**	**83-85**
22.07.	**2nd** measurement **UniSpec**	**83-85**
23.07.	**2nd** measurement **CIRAS (24 plants) +** PAR with **PAM**	**83-85**
05.08.	**3rd** measurement **HandyPea**	**89**
06.08.	**3rd** measurement **UniSpec**	**89**
11.08. & 12.08.	**4th** measurement **PAM (Dark & Light)**	**89**
21.08.	**3rd** measurement **CIRAS (24 plants)**	**89**

III.2.1 Preparation, Calibration and post processing of measurements

The PAM-2500 Chlorophyll Fluorometer (PAM) is produced by the company *Heinz Walz GmbH* in Germany. It is a portable system and a non-destructive method to measure the photosynthetic performance of plants (HEINZ WALZ GmbH, 2015). For internal light sources LEDs (light emitting diodes) are used, including saturation pulses and actinic light. Blue and red actinic light sources are also part of the instrument (HEINZ WALZ GmbH, 2008). Chlorophyll fluorescence is collected by a flexible optical fibre, which modulates the

measuring light and the actinic light to the sample; in this case the measuring leave (HEINZ WALZ GmbH, 2015). To ensure a comparability of all measurements, there is a distance clip (Distance Clip 60° 2010-A) for convenient positioning of the fibre optics relative to the sample (HEINZ WALZ GmbH, 2008) with the same distance of leave and fibre optics at each measurement. The whole system was linked to a CPU, in this case a portable computer (Samsung Electronics GmbH) and the software PamWin-3. With this software, different measuring modes and programs can be adjusted. Data was exported after each measurement into Excel 2010 (Microsoft Corporation), sorted and different chlorophyll fluorescence parameters were calculated. The data and parameters express the photosynthetic performance. Therefore different parameters are given by the PAM-2500. The description of those parameters is taken from the Handbook of Operation for the PAM-2500, provided by the manufacturer. Measuring the PAR of each trial plant on the 23rd of July was done with an adaption of the measuring head. Therefore fibre optics was detached from the leaf distance clip, not to disturb the measured radiation through shading the sample.

In general the relative fluorescence yields can be expressed in five different levels. Two of those levels are measured on dark-acclimated samples, and the others are measured with light-exposed samples (HEINZ WALZ GmbH, 2008). These parameters are shown in ANNEX, Table 23.

In case of the PAM-2500, a “zero-offset” before the measurement of dark-acclimated samples was done. Therefore the leaf clip was kept in the dark. In all measurements the leaf clip of the type “Distance Clip 60° 2010-A” and leaf clip holder “2030-B” with fibreoptics “2010-F”, was used. The distance between the fibre optics and the measured leaf was the same in all measurements. Temperature was measured also by the instrument, but was not changed within the chambers, except the enhancement done at BBCH 83.

Measurements of light- and dark acclimated samples were made separately, meaning that light measurement of both chambers was done first, and dark measurement of both chambers was done, if possible the next day. Chamber 195 was measured first at all measuring dates, to avoid a spreading of phylloxera. In correspondence with the company Walz GmbH, the settings of the measuring program were adjusted. Those settings were kept for all measurements and can be seen beneath:

Table 4: Settings of the PAM adjusted in the PamWin 3 software used for all measurements; chosen in correspondance with the company Walz GmbH

Parameter	Meaning	Intensity	Duration [s]
AL	Actinic light	10	5
ML	Measuring light	10	-
SP	Saturation Pulse	7	0,5
PS I Light FR	Alternative light source far-red (preferably excites PS I in plants)	10	5
Gain	Electronic signal amplification	2	-

For the dark measurements there was an acclimatisation time of one hour before measuring. The measured spot on the leaves was chosen similarly over all measurements. It was a spot between the main vein and the next vein in clockwise direction. Table 23 (ANNEX) gives a brief introduction into measurement values of PAM.

Figure 1 visualises the measurement principle for the relative fluorescence yields due to saturation pulse analysis.

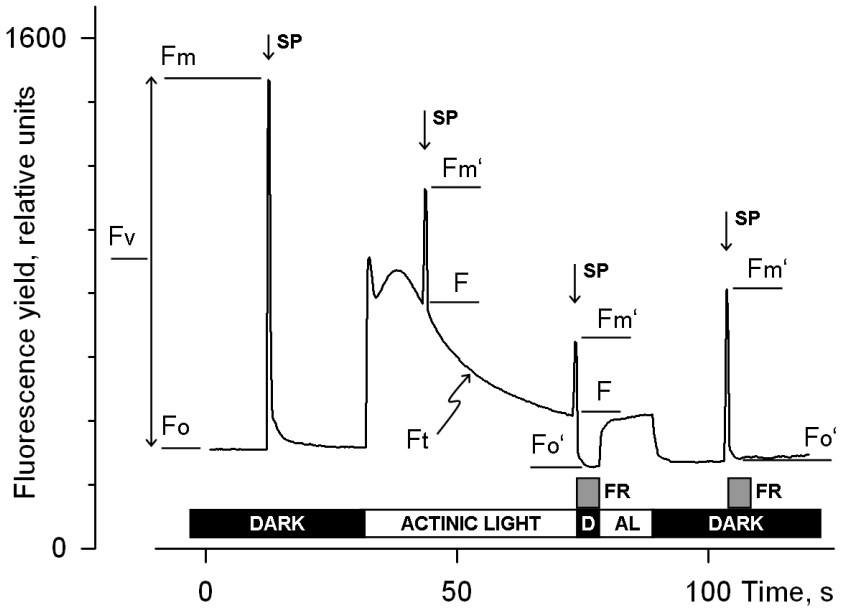

Figure 1: Example for Measurements of Saturation Pulse Analysis. Al=Actinic Light; D=dark; SP=Saturation Pulse; FR=far-red illumination taken from (HEINZ WALZ GmbH, 2008)

The five relative fluorescence yields are needed to calculate the fluorescence ratio parameters. By these fluorescence ratio parameters, photochemical use and non-

photochemical losses of absorbed light energy can be quantified. Thus fluorescence ratio expressions use as data input the relative fluorescence yield measurements Fo, Fm, Fo', Fm' and F (HEINZ WALZ GmbH, 2008).

The fluorescence quotients, which normally are calculated automatically by the software PamWin-3, were calculated manually in this trial with Microsoft Excel 2010. The measurements with dark-acclimated and light-exposed samples were done at different times with respect to a dark acclimation of the samples. Therefore, lights were turned off for at least one hour and the measurement of Fo and Fm was done with a minimum of light provided by a small LED-headlamp. Because of the temporal separation of the two measurements, the calculation of the parameters only could be done separately in Excel. Parameters, their calculation and meaning are shown in Table 24, ANNEX.

In case of the Handy PEA Chlorophyll Fluorimeter (Hansatech Instruments Ltd, Norfolk, England) (HandyPEA) measurements, leaf clips were placed on the measuring leaves 30 minutes before the measurement. Again chamber 195 was measured before chamber 213 to avoid a spreading of phylloxera. The leaf clips were placed on a similar position on the leaf at each measurement, also compared to measurements with other instruments. The chlorophyll fluorometer is able to measure fast fluorescence induction kinetics. The detection of Fmax is ensured through saturation due to a high intensity focused LED array. Three ultra-bright red LEDs submit a peak wavelength of 650 nm, which is readily absorbed by chloroplasts. The maximum intensity on the surface of the sample exceeds thereby 3000 µmol m^{-2} s^{-1}. Further, the LEDs are focused through lenses onto the leaf surface with the purpose to illuminate the area of the leaf, which is exposed by the leaf-clip, evenly. This area has a diameter of 4mm (HANSATECH INSTRUMENTS Ltd., 2015).

Different columns containing different chemicals are placed at the backside of CIRAS-2 Portable Photosynthesis System (PP Systems Inc.) (CIRAS). As measurements were performed with CIRAS, the freshness of the chemicals contained in the absorber columns was always checked and if necessary the chemical were replaced. LED tuning was done with the leaf cuvette closed before each measurement. A selection of 24 plants was measured. Those plants were measured outside the growth chambers due to scarce space. Therefore, plants have been carefully taken out and put back after the measurement in the same position they stood before. To ensure this, every plant pot was marked with coloured tape. Measurements with CIRAS were done at BBCH 79-81, 83-85 and 89. The chosen plants, which were twelve plants of each chamber, were selected with respect to an equal distribution within the chamber, as well as a uniform appearance compared to other trial plants. Selected trial plants and their location can be seen in Table 5.

Table 5: Location of selected trial plants for CIRAS measurement

Chamber	Variant	Plants
195	1	7, 12, 18, 27, 36, 40
195	2	4, 8, 22, 30, 37, 41
213	3	44, 49, 56, 62, 79, 84
213	4	46, 51, 66, 73, 77, 80

The sequence of measured plants at each measurement date was held equal and began with chamber 195. Measured parameters are described below:

EVAP: Leave transpiration rate (stomatal and cuticular transpiration)

GS: Stomatal conductance (stomatal diffusion resistance)

TL: Leaf temperature

CL: Sub-stomatal concentration of CO_2 (content of CO_2 in mesophyll)

PAR: Photosynthetic active radiation (light which is utilisable for the leave to do photosynthesis)

Pn: Assimilation rate (CO_2 - already bound)

Statistical analysis was performed with different software. Data from HandyPea and CIRAS measurements were analysed by using IBM® SPSS® Statistics 21 (IBM Corporation, Armonk, NY). PAM data was statistically analysed using the SAS software, Version 9.4 of the SAS System (SAS Institute Inc., Cary, NC, USA) for checking Gaussian distribution and afterwards using a paired, two tailed, type three t-Test ($p<0.05$) in Excel 2010.

III.3 Inoculation

There were four inoculations in total. The first three inoculations occurred with fresh, vital eggs from an own bred clone offspring of the phylloxera strain AT1. The method differed slightly between the first three inoculations. At the fourth inoculation, eggs collected from the field were used. Further, eggs have been collected with a scalpel or a small painting brush and were placed on filtration paper, either being cut into half or taken as a whole. The filter papers were carefully moistened with two drops of VE water before placing the eggs on them. The collection occurred under a stereo microscope of the type Olympus SZX 10. After the preparation of the inoculation material, all instruments were strictly cleaned with a solution containing 70% of ethanol. The inoculation samples persisted of a rolled filter paper with phylloxera eggs within a two ml Eppendorf tube, at the inoculation one, two and three;

and a filter paper within a 15ml centrifugation tube at inoculation four. They were kept less than 24 hours before inoculating the plants within chamber 213.

III.4 Growth chambers

Both growth chambers were built by the company *HAUSER* and have the same size, which is 262 cm in width, 255 cm in depth and 236 cm in height. Thus 6.681 m² could be used for the cultivation of the vines.

The growth chambers were modified on May 12th in 2014. Therefore lights were rearranged within the two chambers. Equal radiation intensity was given through putting the light modules out of shelves and relocate them on top of each chamber. Plants were relocated from coldhouse into growth chambers on 14th of May.

Environmental conditions were influenced by rearranging several parts within the growth chambers. For instance the light modules of the infested chamber (no. 213) were reduced, taking out single lamps, on July 1st. Therefor an equal number of lamps per chamber was established on July 1st. From that day on, 40 lamps per chamber, within eight light modules, spent light. Before that day, chamber 213 had eight lamps more due to bigger light modules.

Another important growth-factor for the test plants is the day length. It was controlled by an automatic control system (*GIRA Control 9*) in both chambers. Day length was attuned to 16 hours of light on the 14th of May in both chambers. On May 15th we decided to attune the artificial day length to 14 hours of light. Due to measurement methods with the PAM-2500 the time of switch on and off the light was changed at some dates.

IV. Results

IV.1 Evaluation of experimental conditions

IV.1.1 Growth chambers

Figure 15 in ANNEX shows the arrangement of the trial plants within the growth chambers from the 14th of May when they were located into the chambers the first time.

With a total number of 42 plants within chamber 195 (Control), or 43 plants within chamber 213 (Phylloxera), each plant had approximately 0.159 m² space and 0,155m² space. The space might be less because of two narrow corridors between plants located at the side of the chamber, as well as the plants in the middle of the chamber and a small free space between the entrance and the plants in the middle.

The quantity of plants per chamber changed on July 10th. From that day on there were 35 plants per chamber. That raised space per vine from 0.159 m² and 0,155m² to 0.191m² per vine. Again the actual space per vine might be lower because of free space needed for cultivation and measurements.

Samples were taken of the plants which were sorted out. Whole shoots, leaves and berries of the throw-outs were collected and stored separately at -80°C. The sorted out plants of chamber 213 were visually examined on an infestation through phylloxera. An infestation was not detected. Reasons for the reduction of plants per chamber were the narrow conditions in space. Measurements were difficult to take. Also the low light intensity was additionally lowered through the dense placements of the vines. Even 35 plants per chamber can be seen as a limit because of the little space within both chambers.

With this reduction, also a new arrangement was done. The new allocation of the plants is shown in ANNEX, Figure 16**Fehler! Verweisquelle konnte nicht gefunden werden.**. Again, no randomisation was done but an equal distribution of treatments within the two chambers.

Plants were not placed in a randomised order to hold conditions for each group, namely fructifying and non-fructifying vines, of measured plants as similar as possible. Figure 16 shows that nearly the same number of plants was placed at the two sides of each chamber as well as in the middle of each chamber. A difference between chamber 195 and 213 is the double row at both sides in the phylloxerated chamber with the effect that more plants had to be placed in the middle of chamber 195. This difference was given through permanently

installed shelves with a different width. Figure 15 and Figure 16, both in ANNEX, show four different variants so two per treatment “control” and “phylloxera”, which are listed as followed:

- Variant 1- no grape, no infection (chamber 195)
- Variant 2- fructifying (one grape), no infection (chamber 195)
- Variant 3- no grape, inoculation (phylloxera) (chamber 213)
- Variant 4- fructifying (one grape), inoculation (phylloxera). (chamber 213)

Growth conditions also were tried to hold as similar as possible. Each plant received the same amount of water and thus fertilizer, metered with a graduated plastic beaker, which was attached to a bamboo stick. That enabled watering without touching or moving potted plants. Following Table 6 gives a brief overview about factors influencing the growth and development of trial plants.

Table 6: Indicators for equal environmental conditions within both growth chambers

Growth conditions	Chamber 195	Chamber 213
PAR free space [µmol/m²s]		
70 cm height (~cluster zone)	51	55
150 cm	131	145
180 cm (height of biggest plants)	257	214
PAR at plants [µmol/m²s]		
Lower measurement leaves	17.257	22.357
Upper measurement leaves	30.886	39.100
Temperature [°C] (aver. 14.05.-13.7.)	21.516	21.680
Temperature [°C] (aver.14.07.-20.08.)	25.158	25.630
Water per Plant [l] 14.05.-20.08.14	11.65	11.65
Fertilizer per Plant [ml] 14.05.-20.08.14	5.825	5.825

Radiation was first detected by a light measurement with the SPAD LP-80 on July 2nd. The data won through that measurement was taken as an indicator for equal and comparable light conditions. A later measurement at BBCH 81-83 is presented below, in the sub-chapter “Light”.

IV.1.2 Water

Plants were well watered over their whole vegetation period. The field capacity was held at saturation point. Watering was done from above in the beginning, and later from below, or mixed between above and below due to promote the infestation by larvae of phylloxera.

Watering was done with a plastic beaker which was linked to a bamboo-stick. The purpose of this self-made instrument was to reach every plant without touching or replace it, in order to keep conditions the same. All in all, every plant was watered with 11.65 litres during the 14th of May until the 20th of August in the growth chamber. The distribution of the given water was held constantly with exceptions in terms of inoculation dates, plant development and appearance or organisational matters. The absolute amount was about 830 ml added per week per plant. Each plant pot had a soil volume of 0,003 m³. The surface of each pot was 314.16 cm². Pouring of each pot with approximately 0.83 litres per week corresponds to a precipitation of 26.42 mm per plant per week and a precipitation of 370.2 mm per plant over the whole period of plants being located within the growth chambers.

Although water conditions of each plant were the same, not all plants showed the same amount of consume. Trial plants 1, 4, 9, 11 and 15 in chamber 195, as well as the trial plants 40, 55, 57 and 60 in chamber 213, consumed less water than other plants. Nevertheless no differences to other plants could be detected visually.

Water deficiency was not seen on any plant over the whole trial, including the before, with plants being located in the glasshouse and coldhouse.

IV.1.3 Temperature

Within the growth chambers temperature was held constantly. The computer based controlling system of the chambers allowed a programming of constantly 22°C until July 14th. From then on, the temperature was programmed on 26°C, again day and night. It was measured via data-loggers (*onset*, HOBO® data logger) in intervals of 30 minutes and can be seen in Figure 2.

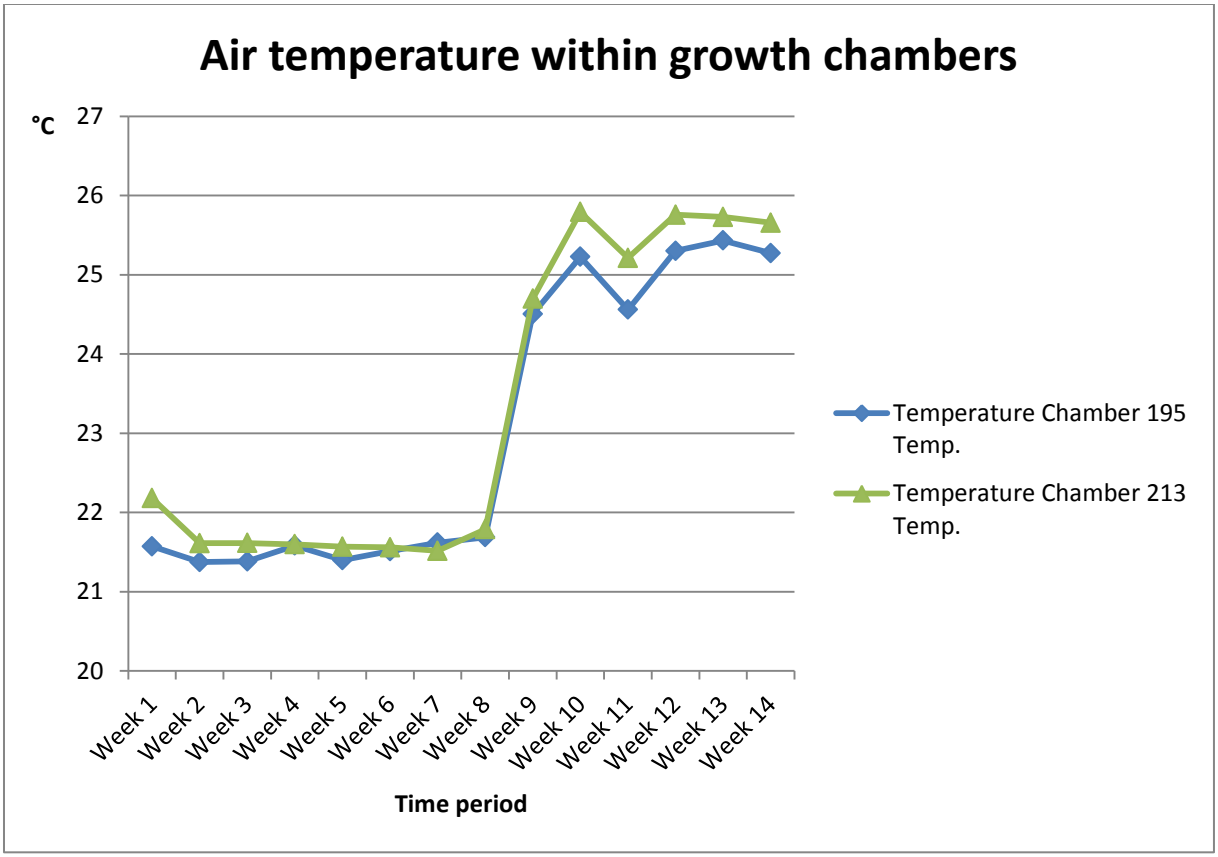

Figure 2: Temperature of growth chambers 195 and 213 over a period of 14 weeks

The figure shows slight differences between the chambers, depending on each week. Chamber 195's temperature was lying slightly below or nearly at the same level of chamber 213's temperature, except for week 7. With increasing temperature, the difference between the chambers increases too. This is shown by week 10 to 14. This data results from always the same location within the chamber. Table 25 in ANNEX shows differences between the two growth chambers of up to 0.652 °C. Differences between the two chambers are lower from week two to week nine.

For more detailed information, also the temperature measured with the PAM is presented below in Figure 3.

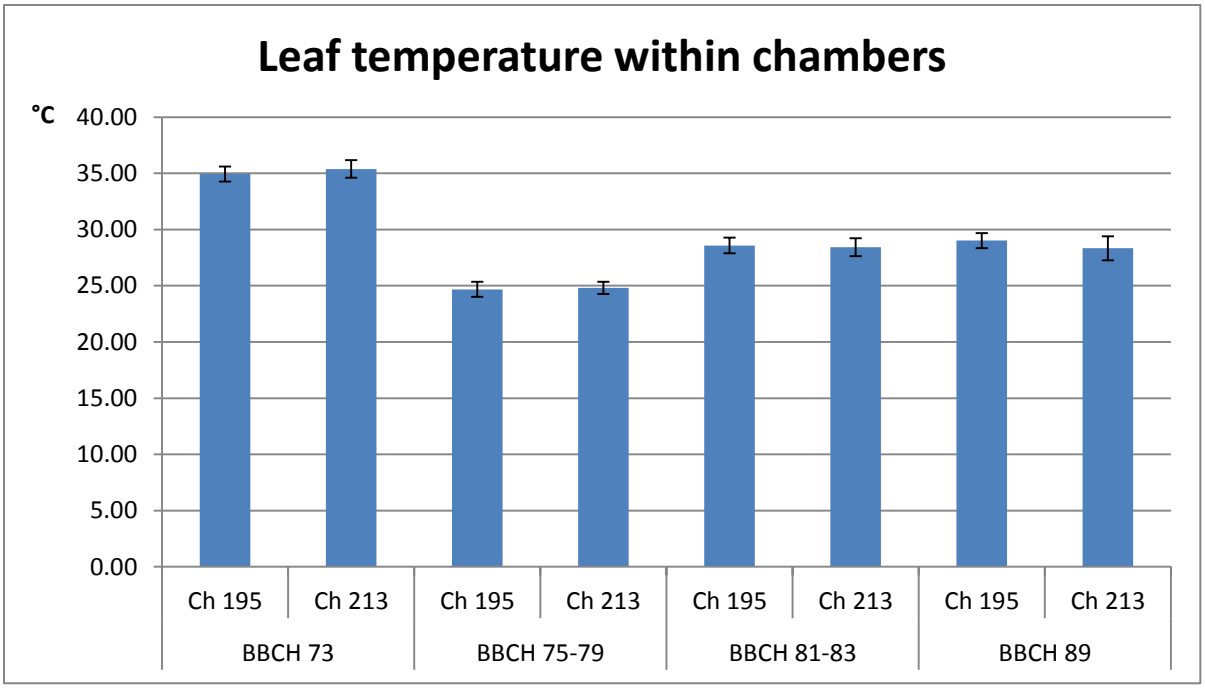

Figure 3: Mean values of leaf temperature in °C, standard deviation as double error bar, measured with PAM at four different dates in Chamber 195 (Control) and Chamber 213 (Phylloxera)

At the first measurement, leaf temperature within both chambers is very high, although the settings of the automatic temperature control were programmed on 22°C. Over the whole investigated period, the temperature is comparable between the two chambers. At measurement one and two the temperature is higher in chamber 213, whereas at measurements three and four, chamber 195 has higher temperature than chamber 213. Standard deviation reaches its highest value at measurement four in chamber 213. Over other measurements standard deviation is more similar between both chambers. The following Table 7 gives a more detailed overview about the temperature on different heights due to showing values of the lower and the upper measurement leaf.

Table 7: Leaf temperature in °C measured with PAM at four dates in both chambers (ch 195, ch213) at different measurement leaves, all meaning respective leaves of fructifying and non-fructifying plants; * symbolizing leaves of fructifying vines; Diff. represents difference between chambers

Temp. [°C]												
	BBCH 73			BBCH 75-79			BBCH 81-83			BBCH 89		
Leaf	**Ch 195**	**Ch 213**	**Diff.**	**Ch 195**	**Ch 213**	**Diff.**	**Ch 195**	**Ch 213**	**Diff.**	**Ch 195**	**Ch 213**	**Diff.**
3rd all	34.87	35.27	**0.39**	24.68	24.79	**0.12**	28.48	28.34	**0.14**	28.82	28.27	**0.55**
8th all	34.73	35.11	**0.38**	24.77	24.85	**0.08**	28.69	28.54	**0.15**	29.22	28.40	**0.82**
3rd*	34.86	35.36	**0.50**	24.54	24.94	**0.39**	28.63	28.38	**0.26**	28.88	28.37	**0.51**
3rd	34.88	35.12	**0.24**	24.68	24.59	**0.08**	28.32	28.29	**0.02**	28.76	28.16	**0.59**
8th*	34.73	35.09	**0.36**	24.73	24.88	**0.15**	28.73	28.57	**0.16**	29.48	28.26	**1.22**
8th	34.74	35.15	**0.41**	24.83	24.81	**0.02**	28.65	28.51	**0.14**	28.94	28.55	**0.39**
All leaves	34.94	35.38	**0.44**	24.68	24.82	**0.14**	28.58	28.44	**0.15**	29.02	28.34	**0.68**

Table 7 shows a correlation between leaf height and temperature. The higher the measured leaf, the higher the temperature. Between BBCH 75-79 and BBCH 81, temperature was elevated to 26°C. Leaf temperature is roughly 2.3 to 3 °C higher than the measured air temperature. The differences between all measured leaves are lower from BBCH 75 to 83 and the highest at BBCH 89. Within chamber 213 there is a decrease in different temperatures between leaf and air temperature over the trial period. While difference in week 3 is 13.77°C, it decrease to a difference of 3.25°C (week 6), 3.23°C (week 11) down to 2.61°C between air and leaf temperature in week 13. In chamber 195 there is a decrease from week 3, with a difference of 13.49°C, to 3.17°C in week 6, between leaf and air temperature. Then an increase to 3.92°C increases difference, but decreases afterwards from week 11 to 13 on a difference of 3.39°C between air and leaf temperature. These differences include leaf temperature of both measurement leaves and of both variants between each chamber.

IV.1.4 Light

Although environment within the growth chambers differed significantly to field conditions- mainly because of the low light intensity and the constant temperature- conditions for the trial plants were tried to hold as authentic as possible. The intention was to cultivate the vines without any unforeseen influences on their growth, except the infestation by phylloxera. Then, changes in photosynthetic performance could clearly be assigned to the infestation by the pest. This would imply a growth of the trial plants without any deficiency symptoms. Nevertheless, there were a few irregularities observed.

For instance, after a while, plants lost their older leaves. Before this loss occurred, there was a de-colouring with an effect of light-yellow leaves before falling off. Also tendril formation seemed to be higher than normal, but was not measured. At BBCH 83-85, a more detailed measurement of photosynthetic active radiation (PAR) was done with PAM on 23rd of July.

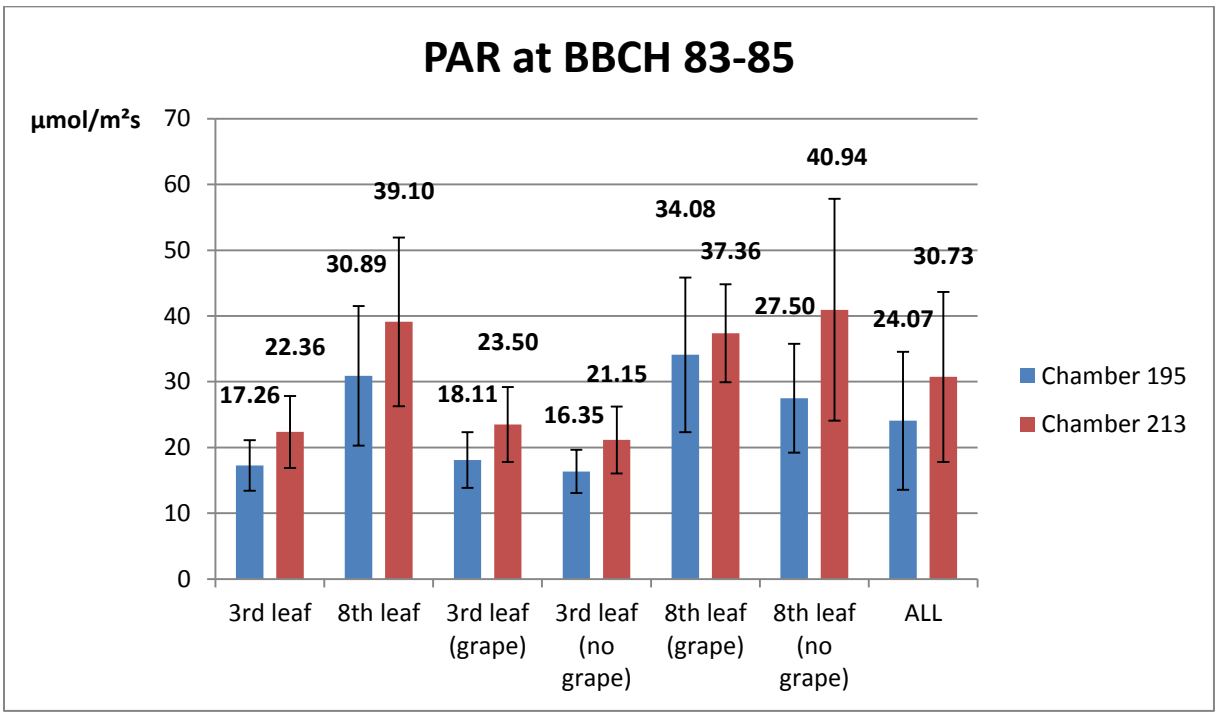

Figure 4: Measurement of the photosynthetic active radiation (PAR) with PAM-2500 in both chambers at BBCH 83-85; standard deviation as double error bar.

Figure 4 shows not only differences between chambers, but also differences within chambers between variants. Each measurement leave was measured twice. The planting pots were marked to ensure the same direction of each plant and thus similar or same radiation intensity at each following measurement. Measurements were taken with PAM-2500. The sensor-head was modulated by removing the fibreoptics “2010-F” because of a possible shading of the sensor head and thus a distortion of PAR values. Like data of temperature, also in case of PAR there is a correlation between the position of a measurement leaf and its PAR. The higher the measurement leaf, the more PAR reaches the leaves. Generally PAR of

the eighth leaves is higher than that of the third leaves. PAR-values of all groups are higher in chamber 213 (Phylloxera) than in chamber 195 (Control). Standard deviation varies and is lowest between the third leaves, more in detail of non-fructifying plants. The highest standard deviation can be observed between measurements of the eight leaf of non-fructifying vines in chamber 213. Measurements of the eight leaves' PAR in both chambers show standard deviations twice as high than those of third leaves' measurement.

Data has been checked via SAS to ensure Gaussian distribution and afterwards in Excel 2010 with a t-test ($p<0.05$, independent samples) and most checked comparisons are significantly different, see Table 8.

Table 8: Significant differences of PAR at BBCH 83-85, t-test (p<0.05, independent samples), red marked data is significant different

Between chambers				
Tested comparison	**p-value**		**Difference [µmol m^{-2} s^{-1}]**	
Leaf 3 - Leaf 3	0.000030		5.10	
Leaf 3 no grape- Leaf 3 no grape	0.002968		4.794	
Leaf 3 grape- Leaf 3 grape	0.003013		5.389	
Leaf 8 - Leaf 8	0.004843		4.794	
Leaf 8 no grape- Leaf 8 no grape	0.007148		13.441	
Leaf 8 grape- Leaf 8 grape	0.326455		3.278	
Fructifying plants	0.002545		4.333	
Non-fructifying plants	0.000502		9.118	
All plants	0.001067		6.657	
Within chambers				
Tested comparison	**p-value ch195**	**p-value ch213**	**Ch195 Diff**	**Ch213 Diff**
leaf 3-leaf 8 no grape	0.000042	0.000185	11.147	19.794
Leaf 3 - leaf 8 grape	0.000022	0.000001	15.972	13.861
Leaf 3 - Leaf 8	0.000000	0.000000	13.629	16.743
Grape- no grape	0.001423	0.043164	4.171	0.614
Leaf 3 grape- Leaf 3 no grape	0.178292	0.206741	1.758	1.143
Leaf 8 grape, leaf 8 no grape	0.064049	0.430010	6.583	3.580

At the time of the measurement, both chambers had the same number of light modules. Only the comparison of leaf eight of fructifying plants between chambers, and the comparison of leaf three and leaf eight between fructifying and non-fructifying plants within chamber 195, as well as chamber 213 show no significant difference. The slightest difference between chambers is between the eight leaf of fructifying plants. Over all plants there is a difference of 6.7 µmol m^{-2} s^{-1} PAR. Most unequal radiation is between measurement leaf eight of non-

fructifying plants. Fructifying plants have less difference in PAR than non-fructifying between the chambers. Within the chambers, differences between leaf 3 to leaf 8 (non-fructifying), leaf 3 to leaf 8 in general, and leaf 3 to leaf 3 are higher within chamber 213 (phylloxera), while leaf 3 to leaf 8 (fructifying), fruct. to non-fruct. and leaf 8 to leaf 8 are higher within chamber 195 (control).

IV.2 Chlorophyll fluorescence

In this part different measurements at different growth stages are presented. Measurements include three different instruments: PAM-2500 Chlorophyll Fluorometer (PAM), Handy PEA Chlorophyll Fluorimeter (HandyPEA) and CIRAS-2 Portable Photosynthesis System (CIRAS), all measuring chlorophyll fluorescence. In case of CIRAS, the measurement system is closed, because gas exchange is also measured. Both other instruments are open and measure only chlorophyll fluorescence (CF). Depending on the software the Parameters might have different names, but are calculated with the same equation. The values Fm (maximum fluorescence of dark adapted sample), Fo (minimum fluorescence of dark adapted sample), Fv (variable fluorescence; Fv= Fm-Fo) and Fv/Fm, the ratio for the maximum quantum efficiency of photosystem two (PSII), are covered by all three instruments. The instruments CIRAS and PAM have even more parameters and values in common. Here, the values ΦPSII (CIRAS) and Y(II) (PAM) are presented, which both declare the effective photochemical quantum efficiency (CIRAS)/yield (PAM) of PSII. Because of the great quantity of data, only three CF parameters are presented. All other gained data can be seen in the ANNEX. To compare the measurements with respect to the growth stage of trial plants, measurements were bundled dependent on a comparable BBCH stage of the vines. Development of vines was similar between both chambers, but is always a floating passage from one into the next growth stage. Following measurement dates with the instruments were bundled and are reviewed in Table 9.

:

Table 9: Review of bundled measurements to compare instruments PAM, CIRAS and HandyPEA, including date and BBCH-stage of trial plants.

Comparison between instruments	Instruments	Measurement date	BBCH of trial plants (Both chambers)
Group 1	HandyPEA	01.07.14	75-79
	CIRAS	07.07.14	79-81
	PAM	26. and 27.6.14, 02.07.14	75-79
Group 2	HandyPEA	14.07.14	81-83
	CIRAS	23.07.14	83-85
	PAM	21. and 22.07.14	83-85
Group 3	HandyPEA	05.08.14	89
	CIRAS	21.08.14	89
	PAM	11.08.14	89

To understand the following chapter, it is also of importance, that with HandyPEA and PAM at each plant leaf three (non-fructifying vines), or the leaf opposite of the grape and leaf eight, were measured. While with CIRAS only the eighth leaf was measured, due to a time consuming and sophisticated measurement procedure. In the presented figures there are bars which present mean values taken of all plants- in case of HandyPEA and PAM this includes both measurement leaves, whereas with CIRAS only one leaf is included. Further, the number of measured plants differs between the instruments. HandyPEA and PAM have measured all plants while with CIRAS, as described in “Material and Methods”, a compendium of twelve plants of each chamber, equally distributed within each chamber and including six non-fructifying and six fructifying trial plants, was measured.

IV.2.1 Fv/Fm ratio- Potential maximum photochemical quantum yield of PS II

This presented parameter arises from the equation $\frac{\mathrm{Fv}}{\mathrm{Fm}} = \frac{(Fm-Fo)}{Fm}$, and can be named as potential maximum PSII quantum yield, or quantum efficiency of open PSII centres. Thus it consists of data of dark adapted samples where Fo is the fluorescence signal after switching on the measuring light, and Fm is the maximum fluorescence resulting of an applied saturation flash.

As with other parameters resulted of the measurements, the statistical analysis was done with SPSS software in case of HandyPEA and CIRAS, and with Excel (2010) in case of PAM. All data was checked on significant differences between various combinations with a T-test, after checking Gaussian distribution of all gained data, which was given. The

significance level was $p<0.05$. Examined combinations were the comparison between both chambers, as well as within each chamber. For instance, fructifying plants were compared with non-fructifying plants within chamber 195 and 213, and between the two chambers. The most data resulted from PAM measurements. Therefore, it was checked more detailed than data of the other two instruments, meaning more comparisons between and within the chambers were checked. All results of statistical analysis are presented in the ANNEX. The object being checked statistically is shown in the following table.

Table 10: Statistically checked comparisons between and within chambers of each instrument

Instrument	**Statistically checked comparisons**	
	Between chambers	**Within chambers**
CIRAS	All plants	No grape- grape
HandyPEA	All plants	Leaf 3 – Leaf 8, no grape
		Leaf 3 – Leaf 8, grape
		Grape- no grape, both leaves
PAM	All plants	Leaf 3 – Leaf 8, no grape
	Leaf 3, no grape	Leaf 3 – Leaf 8, grape
	Leaf 3, grape	Leaf 3 – Leaf 8
	Leaf 8, no grape	Grape - no grape
	Leaf 8, grape	Leaf 3 grape – Leaf 3 no grape
	Both leaves, grape	Leaf 8 grape – Leaf 8 no grape
	Both leaves, no grape	
	Leaf 3	
	Leaf 8	

The following graph shows the mean values of Fv/Fm. It was measured with the instruments HandyPea (July 1st, BBCH 75-79), CIRAS (July 7th, BBCH 79-81) and PAM (26th and 27th June and 2nd July 2014, BBCH 75-79).

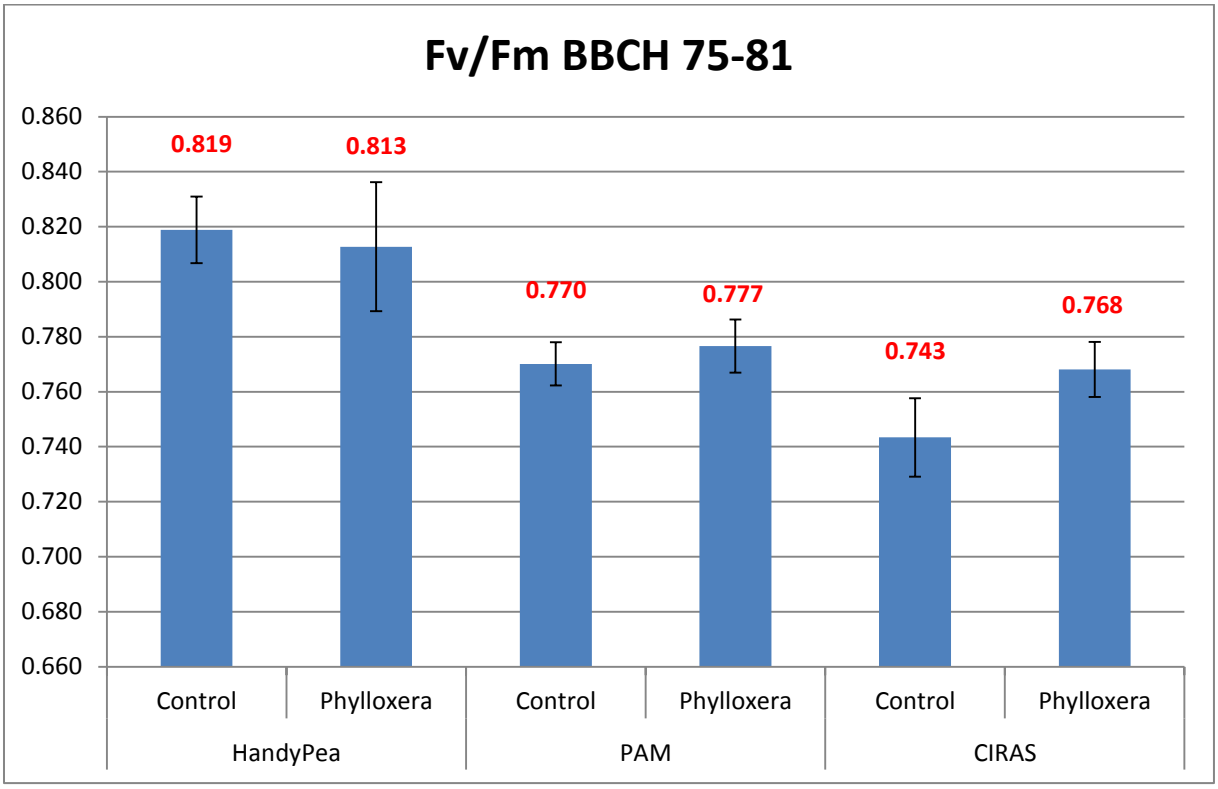

Figure 5: Fv/Fm measured at BBCH 75-81, with instruments CIRAS, HandyPea and PAM; Mean values above each bar; standard deviation as double error bar; Red values representing significant differences between the two chambers proved with paired t-test ($p<0.05$)

The HandyPea measurement shows a higher Fv/Fm of chamber 195 (control). Both other instruments measured higher Fv/Fm values for chamber 213 (phylloxera). Each comparison among chambers showed significant differences. Values of CIRAS and PAM are lower than those measured with HandyPea. The presented data are means of all plants, including both measurement leaves, in case of HandyPEA and PAM, as well as fructifying plants and plants without grapes. More in detail, there unfolded other statistically checked significant differences at that measurement with PAM. The comparisons between the two chambers of the lower measurement leaf of fructifying vines showed significant differences. Other comparisons between plants of the two chambers with a significant difference were leaf eight of fructifying plants, both leaves of fructifying plants, the comparison of leaf three as well as the comparison of leaf eight, both including fructifying and non-fructifying plants

Within the chamber, there were only significant differences in chamber 213. Here, a statistically checked difference was found comparing leaf 3 with leaf 8, fructifying with non-fructifying plants and the lower measurement leaf of fructifying plants compared with that of

non-fructifying plants as well as same comparisons with leaf eight. Control chamber 195 showed no significant differences between plants.

Table 11 gives an overview about the mean values of the Fv/Fm ratio. With each instrument there were different leaves measured within both chambers. The HandyPEA and CIRAS values have a tendency to be slightly higher in chamber control. Values gained through measuring with PAM show this tendency vice versa, being slightly higher in chamber phylloxera.

Table 11: Fv/Fm ratio measured at BBCH 75-81, with instruments CIRAS, HandyPea and PAM; Mean values and (±) standard deviations of measured leaves; Red-dyed values representing significant differences between the two chambers proved with paired t-test (p<0.05)

	HandyPea		PAM		CIRAS	
	Fv/Fm		Fv/Fm		Fv/Fm	
Leaf	Control	Phylloxera	Control	Phylloxera	Control	Phylloxera
3rd	**0.817**±0.008	**0.809**±0.026	**0.769**±0.009	**0.775**±0.011	---------	---------
8th	**0.821**±0.02	**0.817**±0.020	**0.771**±0.007	**0.778**±0.009	---------	---------
3rd (grape)	**0.816**±0.009	**0.813**±0.027	**0.769**±0.007	**0.780**±0.007	---------	---------
3rd (no grape)	**0.818**±0.007	**0.803**±0.025	**0.769**±0.011	**0.769**±0.012	---------	---------
8th (grape)	**0.819**±0.018	**0.819**±0.021	**0.773**±0.006	**0.781**±0.008	**0.743**±0.012	**0.770**±0.009
8th (no grape)	**0.823**±0.008	**0.814**±0.019	**0.769**±0.008	**0.773**±0.008	**0.637**±0.281	**0.767**±0.012
All leaves	**0.819**±0.012	**0.813**±0.023	**0.770**±0.008	**0.777**±0.010	**0.743**±0.014	**0.768**±0.010

Figure 6 again shows Fv/Fm mean values of the same instruments than presented in figure 5. The measurement with PAM was done at July 21st and 22nd, with HandyPEA at July 14th and with CIRAS at 23rd of July. In case of the measurements with HandyPEA and CIRAS, trial plants were at BBCH 79-81. PAM measurement was done when vines were already further developed, at BBCH 83-85.

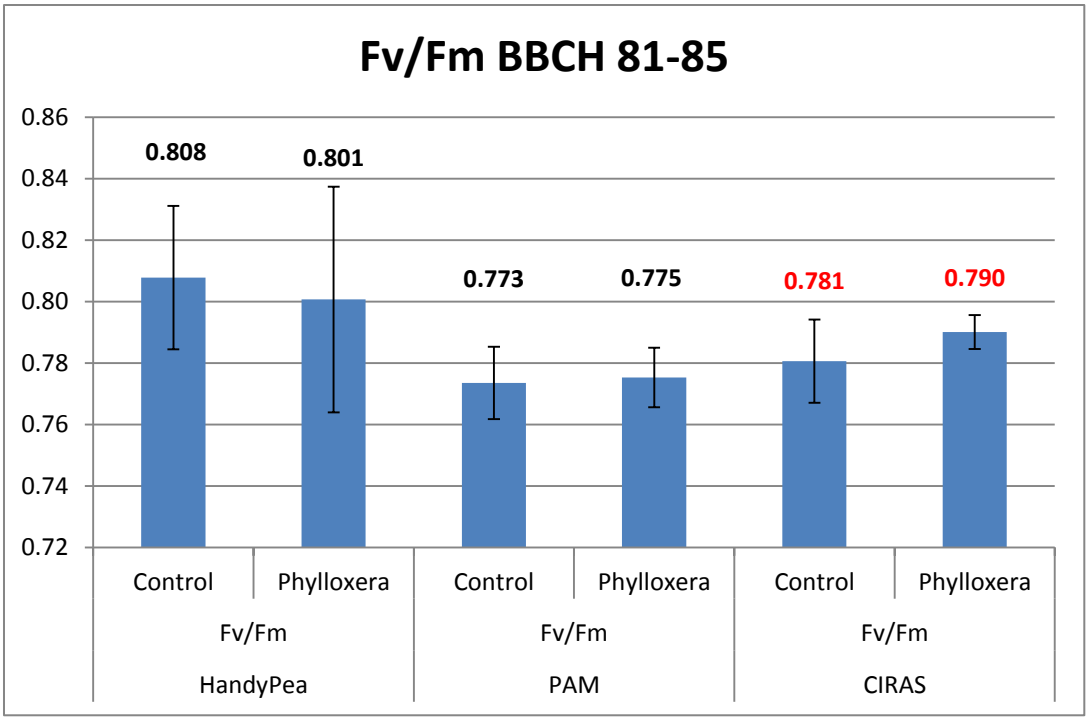

Figure 6: Fv/Fm measured at BBCH 81-85, with instruments CIRAS, HandyPea and PAM; Mean values above each bar; standard deviation as double error bar; Red-dyed values representing significant differences between the two chambers checked with paired t-test ($p<0.05$)

Same as in Figure 5, the mean values of Fv/Fm measured with HandyPEA are higher than those measured with other instruments. In contrast to Fv/Fm measured with PAM and CIRAS, again chamber 195 (Control) exhibits higher values at the HandyPEA measurement. Compared to the first presented measurement, most values sunk, except the Fv/Fm mean value of chamber 195 at the CIRAS measurement. At this measurement date there is only a significant difference between the chambers at the CIRAS measurement, data of both other instruments was also statistically proofed but showed no significant difference, comparing all plants between the two chambers. The check of PAM data provided other statistically differences, but only at comparisons within each chamber and not between the two chambers. Within chamber 195, following comparisons showed statistically significant differences: Leaf 3 – leaf 8 non-fructifying plants, Leaf 3 – Leaf 8 fructifying plants, Leaf 3 – Leaf 8 in general, and fructifying plants compared with non-fructifying vines. In chamber 213, only the comparison between fructifying and non-fructifying trial plants exhibits a significant difference. Also, data gained through the measurement with HandyPEA shows significant difference within the chamber. Comparisons of the third and eighth leaves of vines without a grape and fructifying plants with non-fructifying plants, show significant differences. Table 12 reveals that standard deviation of the HandyPEA data is slightly higher than of both other instruments.

Table 12: Fv/Fm ratio measured at BBCH 81-83, with instruments CIRAS, HandyPea and PAM; Mean values and standard deviations of measured leaves; Red-dyed values representing significant differences between the two chambers checked with paired t-test (p<0.05, independent samples)

	HandyPea		PAM		CIRAS	
	Fv/Fm		Fv/Fm		Fv/Fm	
Leaf	Control	Phylloxera	Control	Phylloxera	Control	Phylloxera
3rd	**0.801**±0.026	**0.797**±0.031	**0.769**±0.014	**0.774**±0.011	---------	---------
8th	**0.814**±0.02	**0.806**±0.040	**0.778**±0.007	**0.776**±0.008	---------	---------
3rd (grape)	**0.810**±0.025	**0.800**±0.024	**0.774**±0.008	**0.777**±0.009	---------	---------
3rd (no grape)	**0.790**±0.025	**0.788**±0.043	**0.765**±0.018	**0.772**±0.013	---------	---------
8th (grape)	**0.820**±0.007	**0.806**±0.041	**0.779**±0.005	**0.779**±0.005	**0.781**±0.011	**0.792**±0.006
8th (no grape)	**0.808**±0.024	**0.809**±0.036	**0.776**±0.008	**0.773**±0.010	**0.780**±0.017	**0.788**±0.005
ALL	**0.808**±0.023	**0.801**±0.037	**0.773**±0.012	**0.775**±0.010	**0.781**±0.014	**0.790**±0.006

The last measurement with all three instruments was done at August 5th (HandyPEA), 11th (PAM) and 21st (CIRAS). The trial plants were all at BBCH 89.

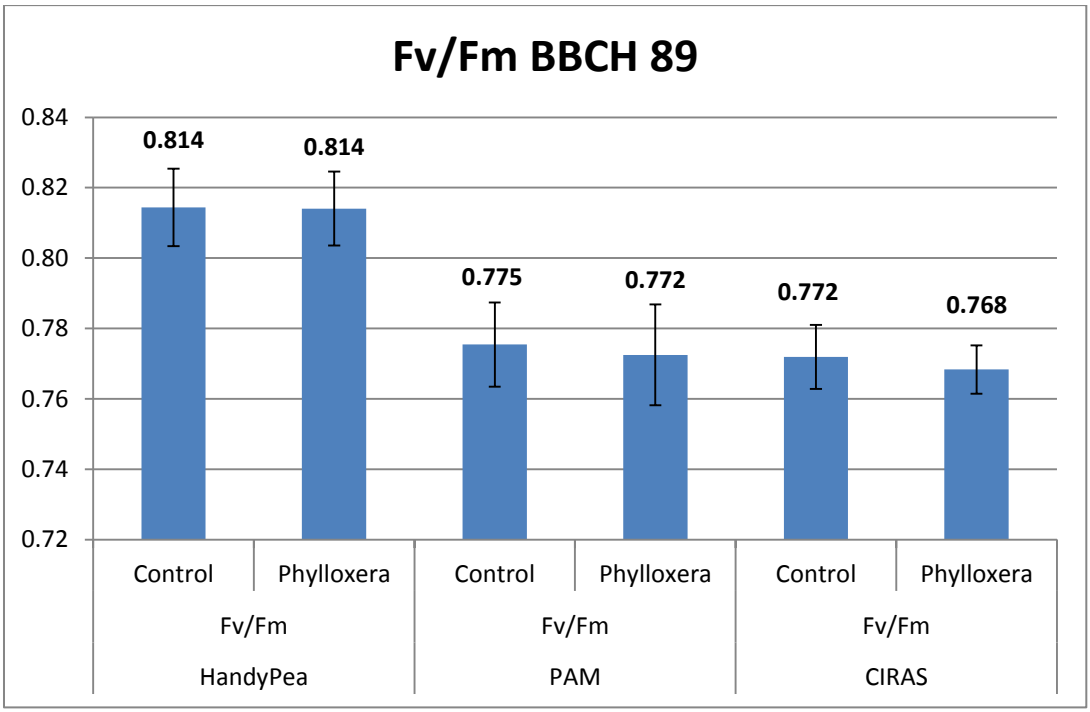

Figure 7: Fv/Fm measured at BBCH 81-85, with instruments CIRAS, HandyPea and PAM; Mean values above each bar; standard deviation as double error bar; no significant differences (p<0.05)

No significant differences were detected when comparing all plants of each chamber with those of the other chamber. In contrast to figure 5 and figure 6, mean values of Fv/Fm from plants in chamber 213, measured with CIRAS and PAM, are not higher any more than those of chamber 195. The data of HandyPEA also shows a difference with chamber 213 slightly being lower than chamber 195 at the measurements before, now being equal between both

chambers. A more detailed statistical check demonstrates that in case of the HandyPEA measurement, there is a significant difference between fructifying and non-fructifying plants within chamber 213. Further, non-fructifying plants, including data of both measurement leaves and the eighth leaf, show a statistically examined significant difference between the two chambers in case of PAM data. More significant differences can be found within chamber 195. There is a significant difference between Leaf 3 and Leaf 8 of fructifying vines, between fructifying and non-fructifying in general, as well as between measurement Leaf 3 of non-fructifying plants, compared with the lower measurement leaf opposite of the grape of fructifying plants.

Table 13: Fv/Fm ratio measured at BBCH 89, with instruments CIRAS, HandyPea and PAM; Mean values and (±) standard deviations of measured leaves; Red-dyed values representing significant differences between the two chambers checked with t-test (p<0.05, independent samples

	HandyPea		PAM		CIRAS	
	Fv/Fm		Fv/Fm		Fv/Fm	
Leaf	**Control**	**Phylloxera**	**Control**	**Phylloxera**	**Control**	**Phylloxera**
3rd	**0.814±**0.014	**0.815±**0.010	**0.773±**0.015	**0.771±**0.017	---------	---------
8th	**0.815±**0.01	**0.813±**0.011	**0.777±**0.008	**0.774±**0.012	---------	---------
3rd (grape)	**0.810±**0.016	**0.819±**0.004	**0.767±**0.015	**0.772±**0.019	---------	---------
3rd (no grape)	**0.818±**0.010	**0.812±**0.012	**0.780±**0.012	**0.771±**0.014	---------	---------
8th (grape)	**0.815±**0.007	**0.816±**0.009	**0.776±**0.007	**0.777±**0.006	**0.774±**0.011	**0.763±**0.006
8th (no grape)	**0.816±**0.009	**0.810±**0.013	**0.779±**0.008	**0.770±**0.015	**0.770±**0.007	**0.772±**0.005
ALL	**0.814±**0.011	**0.814±**0.010	**0.775±**0.012	**0.772±**0.014	**0.772±**0.009	**0.768±**0.007

In Table 13 standard deviations are demonstrated. They are in a comparable range in all instruments. Also the mean values between both chambers are similar in all instruments.

IV.2.2 Effective photochemical quantum efficiency/yield of PSII

In contrast to the sub-chapter before, only the instruments CIRAS and PAM are compared here. The compared measurement value is called Y(II) in the PAM software, and ΦPSII LA in the CIRAS software. It is a value to assess the PSII photochemistry in a light adapted leaf, and results of following equation: $\Phi PS2, Y\ (II) = \frac{Fm\prime - F}{Fm\prime}$, where Fm' is imposed when superimposed with saturating light pulses, and F is recorded under continuous light exposure with a fixed intensity, also called actinic light. The statistical analysis again consists of a t-test ($p<0.05$) after proving the Gaussian distribution of data. Examined comparisons can be seen in Table 10. Data results of same measurement dates than before (see Table 3). With CIRAS, only the eighth leaf was measured, which is the reason for presenting also only the eighth leaf measured with PAM, although also the third, respectively the leaf opposite of the grape, was measured. That needs to be considered when looking at the figures of this sub-chapter, especially at the bars "ALL" plants, which include in case of PAM the values of the lower measured leaf.

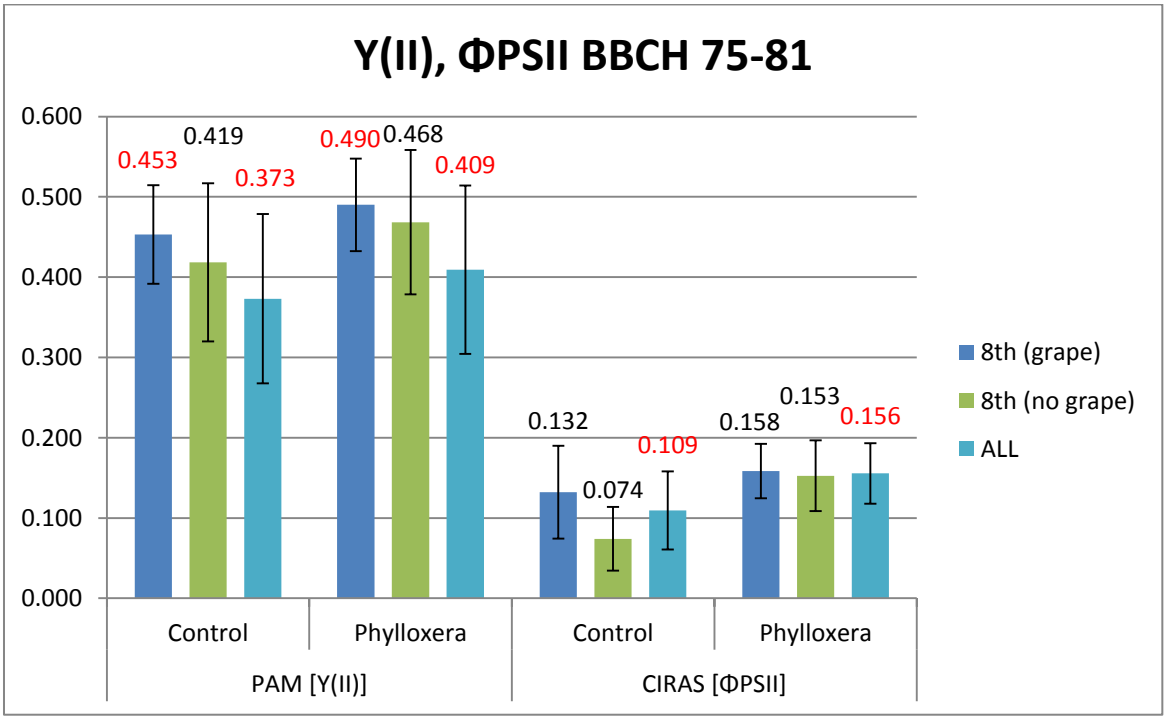

Figure 8: Y(II) (PAM) and ΦPSII (CIRAS) mean values at BBCH 75-81 of trial plants; Mean values presented above bars, bar "ALL" includes lower measured leaf in case of PAM; red-dyed mean values represent a significant difference (paired t-test, p<0.05, independent samples) between the chambers NOT between the instruments; standard deviation as double error bar

This comparison of mean values includes a comparing of all plants between chamber 195 (Control) and chamber 213 (Phylloxera), as well as the eighth leaf of fructifying plants and the eighth leaf of non-fructifying plants between the two chambers. Statistical analysis has been applied to investigate differences between the chambers and not between the measurement instruments. In all cases, the eighth leaf of fructifying plants has the highest

value. Significant differences between the chambers can be found with both instruments. In the CIRAS measurement, only the comparison of all plants is significantly different, while in the measurement with PAM also the eighth leaf of fructifying plants differs significantly between chamber 195 and chamber 213. Mean values of CIRAS are roughly half as high as PAM mean values. In Table 14 also other significant differences between chambers, namely the comparison of the lower measurement leaf of fructifying plants and the third measurement leaf of non-fructifying plants as well as the same comparison with leaf eight, both concerning PAM measurement, are shown.

Table 14: Mean values and standard deviations of the Y(II) (PAM) and ΦPSII (CIRAS) measurement at BBCH 75-81 of trial plants; red marked values represent a significant difference between chambers not between instruments; in case of PAM also the lower measurment leaf is considered in "ALL"

	PAM [Y(II)]		CIRAS [ΦPSII]	
Compared Leaves	**Control**	**Phylloxera**	**Control**	**Phylloxera**
3rd	**0.305**±0.084	**0.338**±0.080	----------	----------
8th	**0.439**±0.079	**0.481**±0.073	----------	----------
3rd (grape)	**0.320**±0.089	**0.354**±0.078	----------	----------
3rd (no grape)	**0.282**±0.073	**0.315**±0.079	----------	----------
8th (grape)	**0.453**±0.061	**0.490**±0.058	**0.132**±0.058	**0.158**±0.034
8th (no grape)	**0.419**±0.099	**0.468**±0.090	**0.074**±0.040	**0.153**±0.044
ALL	**0.373**±0.105	**0.409**±0.105	**0.109**±0.049	**0.156**±0.038

Standard deviations are higher in PAM measurement. Mean values of Y(II) of fructifying plants are higher than those of vines without a grape. More in detail, there are further significant differences within chamber 195 and chamber 213. In both chambers, the comparison of leaf three and leaf eight of non-fructifying plants, same with fructifying plants and leaf three and eight in general are statistically significant different.

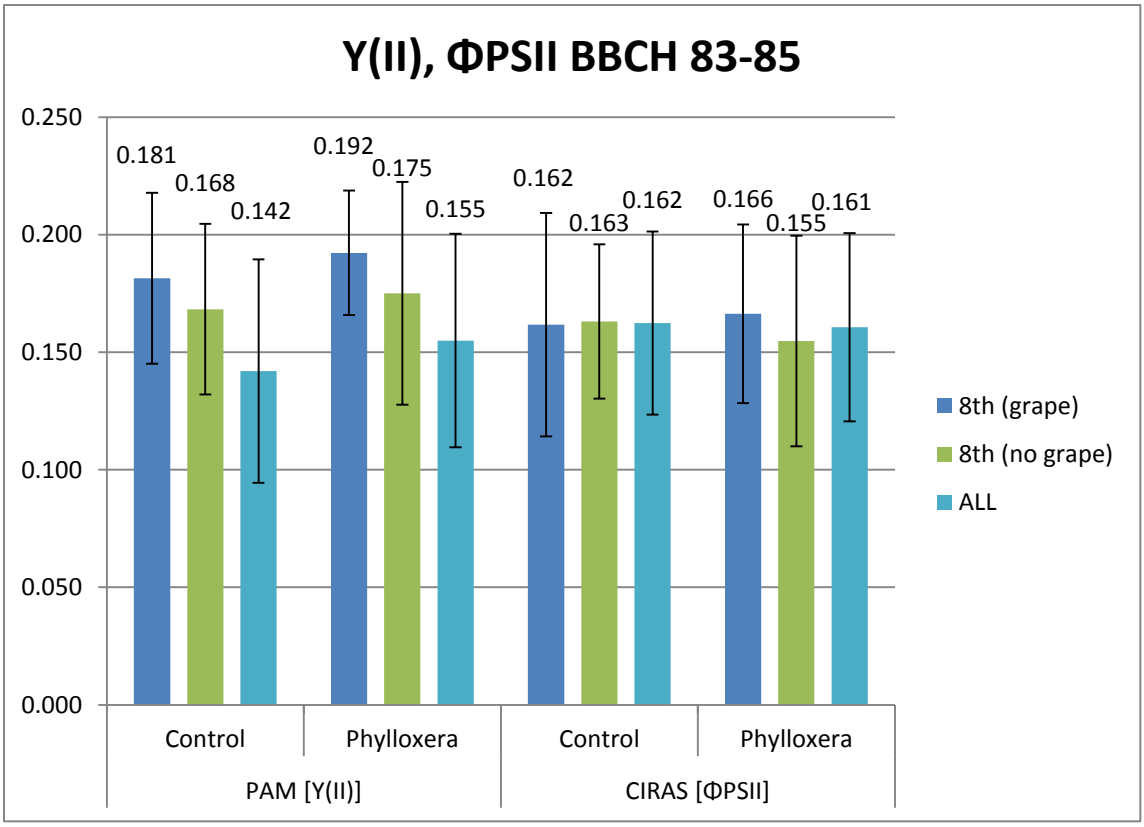

Figure 9: Y(II) (PAM) and ΦPSII (CIRAS) mean values at BBCH 83-85 of trial plants; Mean values presented above bars; BAR "ALL" includes lower measured leaf in case of PAM, no statistical difference (t-test, $p<0.05$, independent samples) detected; standard deviation as double error bar

With further development of the trial plants, the mean values sunk in general. Again, there is a tendency of higher mean values of fructifying plants, except the ΦPSII mean value of control chamber which is slightly lower than that of vines without a fruit. In contrast to the measurement at BBCH 75-81, now the ΦPSII mean value of all plants is higher than Y(II). But if adjusted to take the mean value only from eighth leaf of vines with and without grape, it is still higher with 0.175. At this measurement, the Y(II) values have more than halved compared to the earlier measurement date.

As shown in Table 15, standard deviation is now comparable between PAM and CIRAS, or even lower at the PAM measured mean values. The only significant difference between the two chambers can be found between the third leaf of all non-fructifying vines, concerning the PAM data. More significant differences ca be observed within the chambers, so between variants and measurement leaves. In chamber 213 (Phylloxera), there are significant differences between third leaf and eight leaf of non-fructifying plants, same with fructifying plants and the comparison of the third with the eighth measurement leaf in general. Same differences can be found in chamber 195 (Control), and, additional there are significant differences between vines with and vines without grape as well as between the comparison of the leaf opposite to the grape with the third leaf on non-fructifying vines.

Table 15: Mean values and standard deviations of the Y(II) (PAM) and ΦPSII (CIRAS) measurement at BBCH 83-85 of trial plants; Red-dyed values represent a significant difference between chambers, not between instruments; in case of PAM also the lower measurement

	PAM [Y(II)]		CIRAS [ΦPSII]	
Compared Leaves	**Control**	**Phylloxera**	**Control**	**Phylloxera**
3rd	**0.110±**0.032	**0.126±**0.031	----------	----------
8th	**0.175±**0.036	**0.184±**0.039	----------	----------
3rd (grape)	**0.126±**0.033	**0.132±**0.025	----------	----------
3rd (no grape)	**0.092±**0.021	**0.120±**0.036	----------	----------
8th (grape)	**0.181±**0.036	**0.192±**0.027	**0.162±**0.048	**0.166±**0.038
8th (no grape)	**0.168±**0.036	**0.175±**0.047	**0.163±**0.033	**0.155±**0.045
ALL	**0.142±**0.048	**0.155±**0.045	**0.162±**0.039	**0.161±**0.040

Mean values of Y(II) are comparable with the second measurement with a slight tendency to be lower at the third measurement. Again, like in both measurements before, there is a significant difference of the eight leaves of non-fructifying vines between the chambers, in contrast to fructifying vines, which showed no significant difference of the eight leaves over all three measurements. Also the third measurement leaves of fructifying vines show a significant difference between both chambers, like observed in both measurements before. Comparing this measurement with the second, mean values are slightly lower, but there is less difference to the decrease from first to second measurement. This decrease can also be seen comparing the eight and the third leaves, respectively over all measurements Table 16. Y(II) of chamber 213 is higher than respective values of chamber 195.

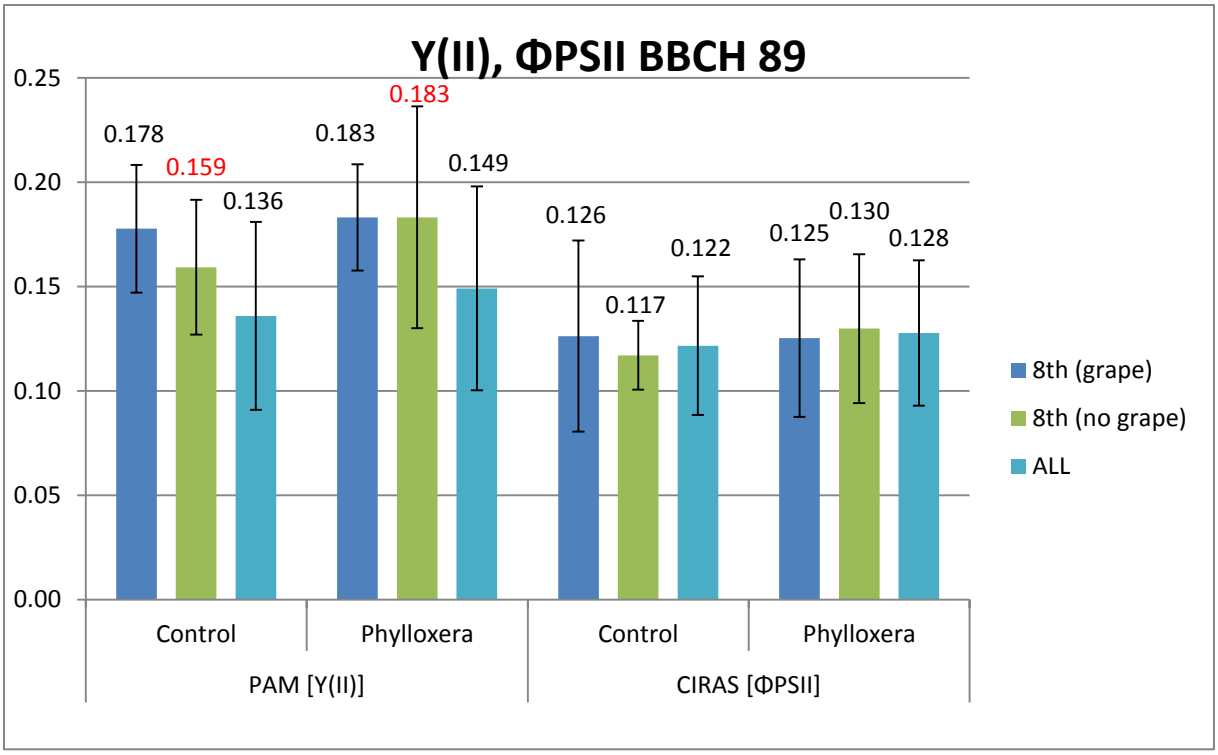

Figure 10: Y(II) (PAM) and ΦPSII (CIRAS) mean values at BBCH 75-81 of trial plants; Mean values presented above bars, bar "ALL" includes lower measured leaf in case of PAM; red mean values represent a significant difference (t-test, p<0.05, independent samples) between the chambers NOT between the instruments; standard deviation as double error bar

Mean values of ΦPSII, measured with CIRAS, sunk if compared with the measurement at BBCH 83-85. Like the Y(II) values in all three measurements, ΦPSII of chamber 213 are higher than those of chamber 195, like it is in the first measurement with CIRAS. ΦPSII between fructifying and non-fructifying trial plants is almost equal. Within chamber 213, fructifying vines have lower ΦPSII than non-fruct., vice versa to both measurements before. In chamber 195, fructifying vines have higher ΦPSII, like in the first measurement.

As presented in Table 16, all comparisons between the two chambers are significantly different regarding the PAM measurement. In the CIRAS measurement there are no significant differences. Within chamber control, there are significant differences between leaf three and leaf eight of non-fructifying plants, as well as of fructifying plants and of the lower measurement leaf and the eighth leaf over all plants. Comparisons within chamber phylloxera only show significant differences between leaf three and leaf eight of non-fructifying plants, and those of fructifying plants.

Table 16: Mean values and standard deviations of the Y(II) (PAM) and ΦPSII (CIRAS) measurement at BBCH 89 of trial plants; Red-dyed values represent a significant difference between chambers not between instruments;

	PAM [Y(II)]		CIRAS [ΦPSII]	
Compared Leaves/Plants	Control	Phylloxera	Control	Phylloxera
3rd	**0.103**±0.029	**0.115**±0.028	----------	----------
8th	**0.169**±0.032	**0.183**±0.041	----------	----------
3rd (grape)	**0.111**±0.033	**0.121**±0.027	----------	----------
3rd (no grape)	**0.094**±0.022	**0.108**±0.029	----------	----------
8th (grape)	**0.178**±0.031	**0.183**±0.025	**0.126**±0.046	**0.125**±0.038
8th (no grape)	**0.159**±0.032	**0.183**±0.053	**0.117**±0.017	**0.130**±0.036
ALL	**0.136**±0.045	**0.149**±0.049	**0.122**±0.033	**0.128**±0.035

Standard deviations are comparable between Y(II) of control chamber and ΦPSII of both chambers, while those of chamber phylloxera are distinctly lower. The mean value of ΦPSII of all leaves does not show such a distinctive higher level than that of the chamber control.

IV.2.3 qP- Coefficient of photochemical fluorescence quenching

The last presented chlorophyll fluorescence parameter qP represents photochemical quenching, and is calculated by the equation: $qP = \frac{Fm\prime - F}{Fm' - Fo\prime}$. Fm' represents the measurement for maximum fluorescence in the light and Fo' the value for minimum fluorescence level. qP expresses the proportion of open PSII reaction centres. Like Fv/Fm presented before, it provides information about underlying processes which have altered the efficiency of PSII photochemistry. This part presents results of the same measurements with CIRAS and PAM, than done in the part before. Statistical analysis and checked comparisons are the same than before (Table 10). Values of all measurement leaves again comprise the third leaves (of fructifying and non-fructifying trial plants) in PAM measurements, whereas only the eight leaf of each plant was measured with CIRAS.

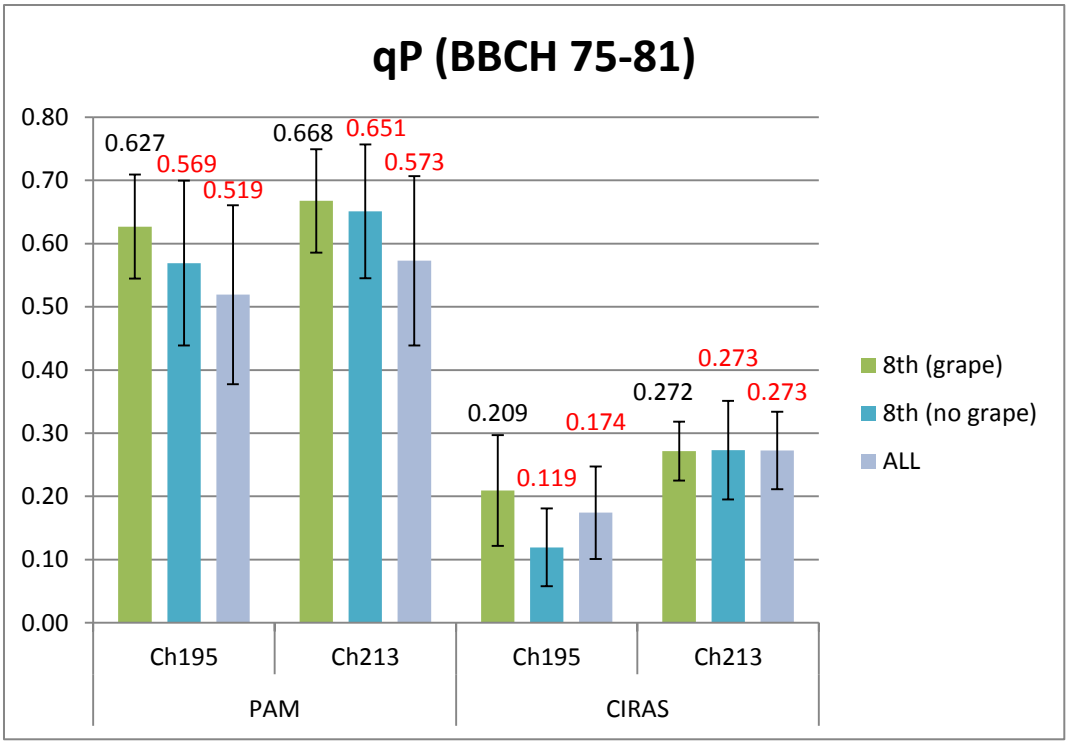

Figure 11: Mean values of qP measured at BBCH 75-81 with PAM and CIRAS; red values represent statistical differences between chambers (NOT instruments); standard deviation as double error bar

As showed in Figure 11, mean values of qP are distinct higher in PAM measurement than in CIRAS measurement. With both instruments there are higher qP values in chamber 213 than in chamber 195, comprising the eighth measurement leaf of fructifying compared to non-fructifying vines as well as all measurement leaves. In the qP values of PAM the fructifying, means of fructifying plants are higher than of others.

As presented by the following table, there were no significant differences, neither between the chambers, nor between variants. The third leaves, only measured with PAM, show distinct lower qP compared to qP of eight leaves measured.

Table 17: Mean values and (±) standard deviation of qP measurement at BBCH 75-81 with CIRAS und PAM, Red-dyed values represent statistical differences between chambers (NOT instruments)

	qP (PAM)		qP (CIRAS)	
Measured leaf	**Control**	**Phylloxera**	**Control**	**Phylloxera**
3rd	**0.433** ± 0.119	**0.485** ± 0.110	----------	----------
8th	**0.603** ± 0.107	**0.661** ± 0.092	----------	----------
3rd (grape)	**0.453** ± 0.123	**0.510** ± 0.110	----------	----------
3rd (no grape)	**0.400** ± 0.107	**0.451** ± 0.104	----------	----------
8th (grape)	**0.627** ± 0.082	**0.668** ± 0.082	**0.209** ± 0.088	**0.272** ± 0.047
8th (no grape)	**0.569** ± 0.130	**0.651** ± 0.106	**0.119** ± 0.062	**0.273** ± 0.078
ALL	**0.519** ± 0.141	**0.573** ± 0.134	**0.174** ± 0.073	**0.273** ± 0.061

In this case, also the comparison of non-fructifying plants between the chambers, measured with CIRAS, was checked statistically. There is a significant difference (p= 0.006543707) between these two variants. PAM data show several significant differences. Comparison of leaf 3 and leaf 8, comprising both variants, as well as of leaf 8 of non-fructifying vines, revealed significant differences, between the two chambers. The comparison of both measurement leaves of variants 1 and 3 also is significantly different. Standard deviations of PAM measurement are slightly lower in chamber 213 than in chamber 195, while in CIRAS measurement they are lower in general compared to PAM data. Within the chambers there are significant differences between the two measurement leaves of all variants, measurement leaves in general, within both chambers, as well as between third leaves of variants 3 and 4.

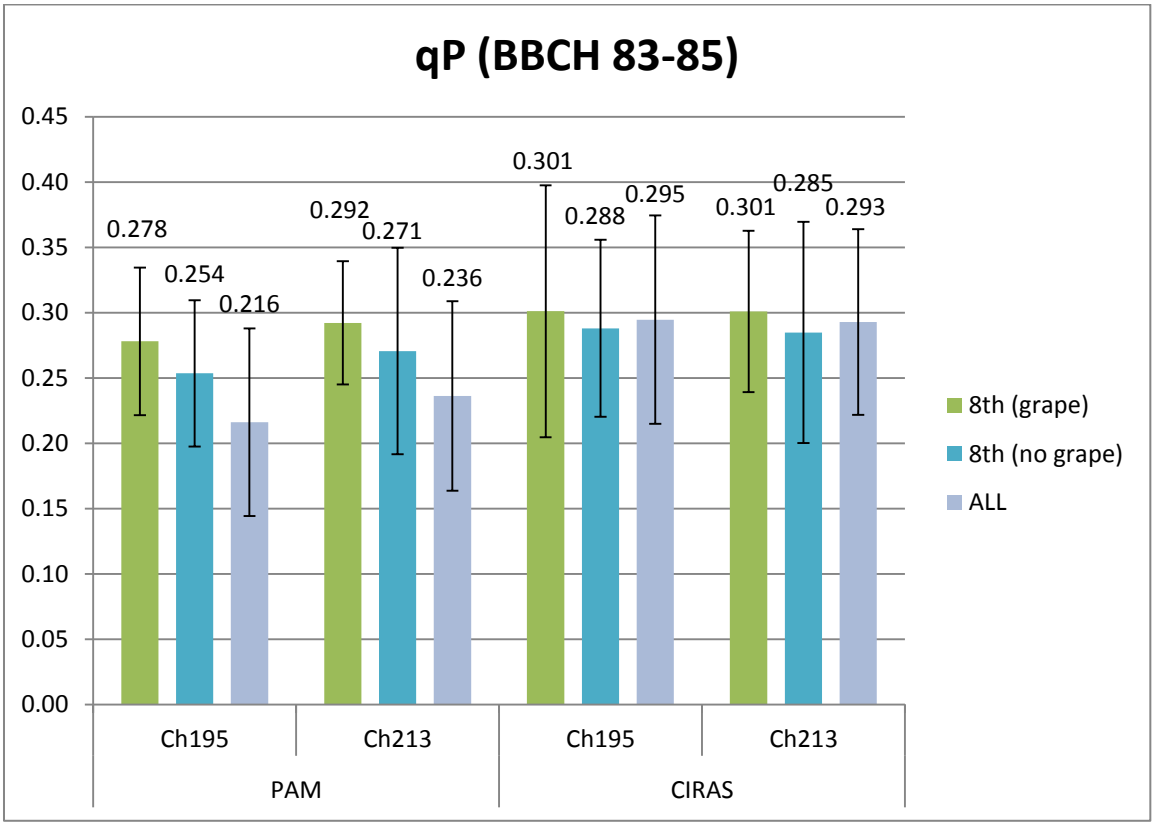

Figure 12: Mean values of qP measured at BBCH 83-85 with PAM and CIRAS, standard deviation as double error bar.

In general, qP values of this measurement are in a similar range comparing both instruments. In contrast to the first measurement, qP measured by CIRAS is higher than qP measured by PAM. The pattern of qP means in PAM measurement is the same, than at BBCH 75-81, meaning qP within chamber 213 is higher than in chamber 195 and variants 2 and 4 have higher qP, than variants 1 and 3. Data of CIRAS measurement is in a very similar range. Variant 3 and 4 even show same qP coefficient.

Table 18: Mean values and (±) standard deviation of qP measurement at BBCH 81-83 with CIRAS und PAM, red values represent statistical differences between chambers (NOT instruments)

	qP (PAM)		qP (CIRAS)	
Measured leaf	**Control**	**Phylloxera**	**Control**	**Phylloxera**
3rd	**0.167** ± 0.047	**0.191** ± 0.047	----------	----------
8th	**0.266** ± 0.057	**0.282** ± 0.065	----------	----------
3rd (grape)	**0.190** ± 0.047	**0.197** ± 0.038	----------	----------
3rd (no grape)	**0.143** ± 0.033	**0.184** ± 0.056	----------	----------
8th (grape)	**0.278** ± 0.057	**0.292** ± 0.047	**0.301** ± 0.096	**0.301** ± 0.062
8th (no grape)	**0.254** ± 0.056	**0.271** ± 0.079	**0.288** ± 0.068	**0.285** ± 0.085
ALL	**0.216** ± 0.072	**0.236** ± 0.073	**0.295** ± 0.080	**0.293** ± 0.071

The only detected significant difference between chambers was found comparing the third leaves of plants in chamber control and plants in chamber phylloxera. Within the chamber (only PAM data) there are again significant differences between leaf 3 and leaf 8 of all four variants. Further comparing third leaves and eight leaves, within both chambers there are significant differences. In chamber 195 there is also a significant difference between third leaves of variant 1 and 2 and fructifying and non-fructifying vines, comprising both leaves.

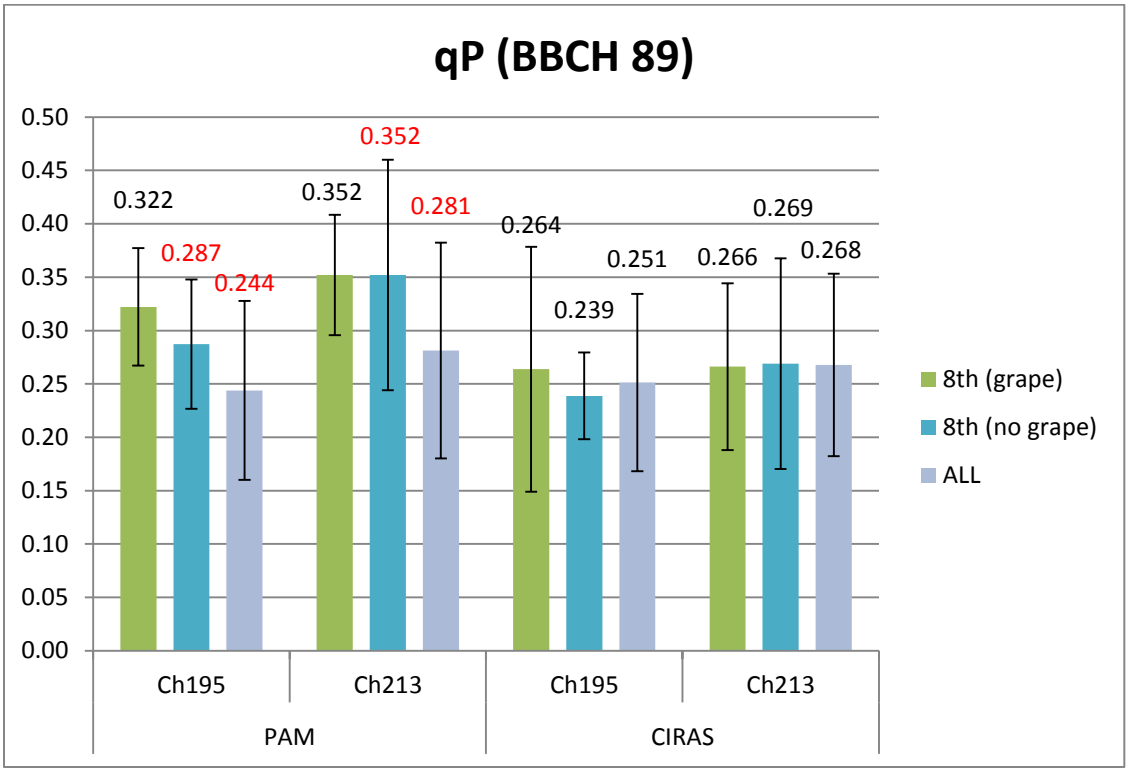

Figure 13: Mean values of qP measured at BBCH 89 with PAM and CIRAS; red values represent statistical differences between chambers (NOT instruments), standard deviation as double error bar.

Means of qP for chamber 195, when measured with PAM, the same pattern than in both measurements before, can be seen in Figure 13. Values of chamber 213 are higher than qP in chamber 195, for CIRAS and PAM measurment. Also the variants 2 and 4 have higher qP

than variants 1 and 3. PAM data show two significant differences- over all leaves (including the third leaves) and variant 1 to 3, both between chambers. The level of qP is in a similar range than at the second measurement. While qP-means measured by CIRAS are lower compared to BBCH 81-83, those of PAM are higher. Variant 3 and 4 have the same qP-level at this developmental stage, measured by PAM. Both instruments measured higher levels of qP in chamber phylloxera than in chamber control. In chamber 195, when measured by CIRAS, also variant 2 has greater qP-means than variant 1. This effect cannot be found within chamber 213. qP-values of CIRAS at full ripening stage of trial plants is on its lowest level, compared to earlier growth stages. Levels of qP measured by PAM are higher at BBCH 89 than at BBCH 83-85 but do net reach the level of qP-values at BBCH 75-81.

Table 19: Mean values and (±) standard deviation of qP measurement at BBCH 81-83 with CIRAS und PAM, Red-dyed values represent statistical differences between chambers (NOT instruments)

	qP (PAM)		qP (CIRAS)	
Measured leaf	**Control**	**Phylloxera**	**Control**	**Phylloxera**
3rd	**0.183** ± 0.054	**0.210** ± 0.058	----------	----------
8th	**0.305** ± 0.060	**0.352** ± 0.084	----------	----------
3rd (grape)	**0.200** ± 0.059	**0.222** ± 0.059	----------	----------
3rd (no grape)	**0.164** ± 0.042	**0.198** ± 0.056	----------	----------
8th (grape)	**0.322** ± 0.055	**0.352** ± 0.056	**0.264** ± 0.115	**0.266** ± 0.078
8th (no grape)	**0.287** ± 0.061	**0.352** ± 0.108	**0.239** ± 0.041	**0.269** ± 0.099
ALL	**0.244** ± 0.084	**0.281** ± 0.101	**0.251** ± 0.083	**0.268** ± 0.085

CIRAS measurement showed no statistically significant differences in contrast to data measured by PAM (Table 19). Here, third leaves and eighth leaves in general, as well as third leaves of variant 2 and 4 differ significantly. Standard deviations of PAM data are higher in chamber 213 than in chamber control. Regarding standard deviations of all leaves, CIRAS data has lower values than PAM data. Third leaves have again lower level of qP than eighth leaves.

IV.3 Inoculation with Phylloxera

There were four inoculations at different dates. They differed not only through material but also in inoculation-techniques. Table 20 gives an overview about the dates, used *Phylloxera*-material and the amount of eggs used in each inoculation. Because of the risk of damaging the larvae of the louses, only after the fourth inoculation a detailed hedging-control was made and can be seen in Table 21. The visual check of a successful infestation of the first three inoculations was done when sorting out trial plants on July 10th. This demonstrated that all inoculations until then were unsuccessful. That was the reason for a change in inoculation material and amount of eggs per inoculation sample.

Table 20: Inoculation dates, material and amount of eggs per plant

Date 2014	Inoculation and Material	Number of eggs per plant
23.05.	1st Inoculation AT 1 Strain	50-60
28.05.	2nd Inoculation AT 1 Strain	50-60
24.06.	3rd Inoculation AT 1 Strain	50-60
30.07.	4th Inoculation Leaf galls (field sample)	300-400

All inoculations were exclusively done in chamber 213. On 23rd -, 28th May and June 24th, inoculation material was the same, whereas on the 30th of July both, inoculation material and technique, differed due to unsuccessful inoculations of the three trials before. The method of the inoculation differed, more or less, at all dates.

Inoculation was done through bringing fresh and vital eggs near young roots at all four dates. Infected root parts were cut off a host plant and collected in a petri dish. From there, vital eggs were carefully collected with a painting brush and put on a filtration paper. According to the chosen method, the filtration paper was coiled in an *Eppendorf-tube*, a centrifugation tube, or was placed directly, without any tube. At all dates, the inoculation samples were tried to place directly or near to young and vital roots. The number of eggs differed: first three inoculations included a number of 50 to 60 eggs per plant, whereas at the fourth inoculation there was a significant increase of 300 to 400 eggs per plant. The origin of the eggs differed also. For the inoculation one, two and three, eggs were taken from *AT 1,* a clonal genetic strain. Eggs of the fourth inoculation were collected in the field.

Host plants of those eggs were potted vines of the rootstocks *5C* and *Fercal* on different, artificially made soil types. Those plants remained from another scientific trial with *Phylloxera.* The collection of eggs of all inoculation-samples was done with a Stereo Microscope of the type Olympus SZX 10.

Figure 14 provides an overview of the technique of all inoculations. Here can be seen, that there were differences between all inoculations.

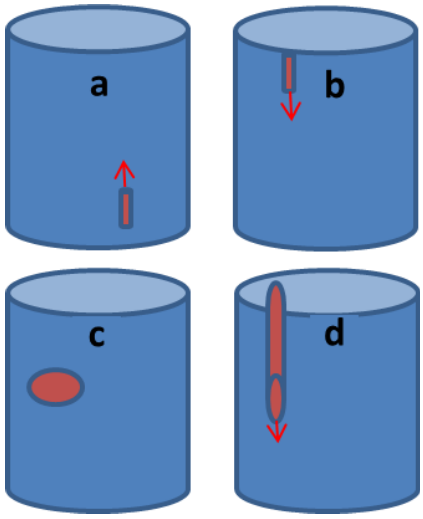

Figure 14: Draft of all four inoculation techniques, alphabetical order for the dates of inoculation, blue pillar symbolising plant pot, brownish tube/ellipse symbolising Eppendorf- (a,b) /centrifuge (d) tube or a filter paper (c) without tube and red arrows mark the opening of the tubes.

First and second inoculation

Infested root parts were visually located, cut off host plants and put into a petri dish on a filter paper. Each filtration paper was wetted with approximately 4 drops of distilled water. Eggs were gathered with a brush to bring them on a quartered filter paper, with a diameter as a whole paper of 90 millimetres. The capture of eggs from infested root material of the host plant can be seen in Figure 26 in ANNEX. Quartered filter papers with 50 to 60 eggs were put into a 2 ml *Eppendorf*-tube, which is shown in Figure 27 in ANNEX. *Eppendorf*-tubes were covered with aluminium foil and held in a closed box until inoculation. At both dates, inoculation samples were made within two days, so that no inoculation sample was older than 24 hours. The slightly different technique of inoculation between these two dates is shown in Figure 14, draft a and b.

Third Inoculation

The third inoculation differed slightly from those before. Instead of a quartered filter paper, it was taken completely this time. Further, it was not rolled within an Eppendorf tube, but given directly near to young roots. This is shown schematically in Figure 14- draft c.

Inoculation with leaf gull-eggs from the field

This inoculation differed significantly from those before, concerning not only the technique, but also the number and origin of eggs. Eggs were collected in the field, directly enclosed to *Götzhof*- an experimental farm of the *HBLA Klosterneuburg,* an Austrian federal college for

viticulture and fruit-growing, which is located on geographical longitude 16°20' and geographical latitude 48°18' and between 190 and 310 m above sea level (HBLA UND BUNDESAMT FÜR WEIN- UND OBSTBAU, 2011). Whole shoots of wild growing vitis-rootstocks, full of infested leaves with leave gulls of phylloxera, were cut and carefully transported to Tulln. Shoots were stored in plastic-bags containing water, placed in a box within chamber 213 (see Figure 28, ANNEX).

The number of eggs per plant increased up to 300 to 400. Eggs were carefully placed on a filter paper, with the same diameter than those used in inoculations before. This procedure did not differ from the other inoculations. Just the source of the vital eggs differed, because they were directly taken from leaf gulls. The gulls were carefully opened with a scalpel, then eggs were taken with the blunt side of the scalpel or a small paintbrush, and put on the filtration paper. Although the amount of eggs per inoculation sample was up to four times higher compared to earlier inoculations, preparation was faster. An average number of 103 eggs per leaf gull was observed, counting eggs of 33 leaf gulls. The filter papers were carefully marked with a pencil (see Figure 28) to avoid damage of eggs through the rolling of the filtration papers. The lines also ensured a consistent distribution of eggs over all inoculation samples. Coiled filter papers with 300 to 400 eggs were placed this time into 50 ml centrifuge-tubes. To visualize this infestation technique it is schematically shown in Figure 14- draft d.

Control and verification of treatments

The first control of treatment phylloxera and treatment control was done visually at 16th of July. Therefore, eight plants from chamber 213 (Phylloxera) and six from chamber 195 (Control) have been randomly chosen and roots were carefully set free through rinsing them with water. Samples of those plants were taken. For those, shoots, leaves and in case of variant 2 and variant 4 grapes, were separately collected into plastic bags and stored at -80°C. The roots of plants were thrown away after autoclaving them. This control revealed that none of the three inoculations until that time has led to an infection of the trial plants.

A control of the hatching of eggs was made one week after the fourth inoculation, to ensure a successful hedging. It is shown in Table 21. In order to proof hedging, the filtration paper within each centrifuge tube of each plant was checked under a stereo microscope. Remaining eggs and hedged larvae were counted, and in case of a high number of larvae or vital eggs, who remained on filtration paper, tubes were carefully placed back to give larvae a possibility to establish on the respective trial plant.

Table 21: Control of egg-hatching seven days after inoculation. light-red fields represent tubes which were carefully put back into pods, dark red fields represent plants which have definitely less than 300 individuals per plant

Pant	Preparation: Eggs/Tube (29. and 30.7.2014)	Hatching control (06.08.2104)		Hatch [%]	No. of individuals/plant
		Eggs/Filter-paper	Larvae/Filter-paper		
43	333	15	11	92.19	307
44	318	13	3	94.97	302
57	412	7	5	97.09	400
46	384	4	9	96.61	371
64	348	6	16	93.68	326
69	331	9	104	65.86	218
47	370	0	1	99.73	369
48	376	14	41	85.37	321
49	370	3	11	96.22	356
50	306	29	18	84.64	259
51	318	5	14	94.03	299
52	369	6	26	91.33	337
55	344	6	1	97.97	337
73	376	2	3	98.67	371
71	321	2	5	97.82	314
68	329	2	15	94.83	312
63	352	4	2	98.30	346
66	347	28	9	89.34	310
80	315	1	25	91.75	289
81	350	5	17	93.71	328
65	382	1	37	90.05	344
67	324	2	0	99.38	322
56	342	10	43	84.50	289
74	305	11	5	94.75	289
75	378	0	17	95.50	361
78	417	6	22	93.29	389
79	405	3	25	93.09	377
59	315	1	50	83.81	264
77	315	5	22	91.43	288
61	365	2	17	94.79	346
62	302	2	14	94.70	286
82	327	3	24	91.74	300
83	391	15	68	78.77	308
84	386	8	77	77.98	301
85	335	2	34	89.25	299
Ø	350.23	6.63	22.60	91.63	321

Table 21 shows five plants with guaranteed less than 300 individuals per plant, because of dead individuals on the filter paper. The average percentage of hatching is 92. For those samples, which contained a high number of living but not yet hatched or migrated individuals from the filter paper, tubes with the filtration paper were carefully put back into the plant pods. Nevertheless this hedging control shows that larvae hedged in each plant pot.

V. Discussion

The present study aimed to design and establish a method, cultivating potted grapevines within two growth chambers, under same conditions and in a controlled environment. The method presented here shall deliver a suitable approach for future trials, investigating the effect of root-galling phylloxera on grafted grapevine, with special focus on sink source relations, photosynthetic performance and carbon partitioning within the plant.

V.1 Setting and verification of environmental conditions (Light, temperature, water, nutrients) for trial plants

The number of trial plants used within this trial decreased during the experiments. When plants were placed into the growth chambers for the first time, space conditions were very scarce. Not only for plants, but also for the work needed to cultivate the vines, for instance ongoing removal of lateral shoots or measurements, space was limited. The number of plants in future trials therefore should be 35, like it was after the reduction on July 10th, or even lower. Plants stood very narrow what may have increased the effect of low light intensity through shading of the lower leaves.

The number of trial plants is also linked to sampling strategy. Taking whole plant samples, separated into the respective organs, to different growth stages would need a higher number of plants as it would be needed with non-destructive methods. KELLER et al. for instance analysed the xylem sap, taken before by a non-destructive method (KELLER, et al., 1995). Since analysis of phloem sap would be more suitable, but requires either non-destructive (PATE, 1976) or destructive measurement techniques (KING & ZEEVAART, 1974), number of trial plants should consider this point. While measurements of photosynthesis are non-destructive, the same plant can be monitored over the whole vegetation period. SCHOEDL et al. describes a sampling strategy which also could be used for future trials of this experimental set up. In this field study chlorophyll fluorescence and leaf temperature were measured, within a randomised block design, on four developmental stages (BBCH 63, BBCH 77, BBCH 87 and BBCH 89). At harvest, sampling included fruit clusters of four plants per block, to analyse grape quality parameters like total soluble solids (° Brix), titratable acidity (g/L), pH value, tartaric acid (g/L) and malic acid (g/L) with a WineScan FT 120 (Foss, Hilleroed, Denmark) (SCHOEDL, et al., 2011). These parameters need to be analysed in future trial-sets of this experimental set up, to clear the impact of root-galling phylloxera on fruit and thus wine quality. Because of the relative success of resistant rootstocks, this might seem to be redundant. But the success and resilience of this primary management tool could be challenged in the future by host-plant interactions with different grape phylloxera clonal lineages, as well as by potential impacts of climate change on both, grapevine and grape

phylloxera distribution (BENHEIM, et al., 2012). Also for new rootstocks-varieties, which might not have a sufficient tolerance level against phylloxera, this effect would be of special interest.

The size of plant pots should also be overthought. Used plant pots with their size of three litre volume were practical when relocating the plants but in terms of space for the root system of the trial plant and water holding capacity, too small. A comparable trial set up of KELLER and KOBLET, also working within an artificial environment ("Phytotron") used 18-liter pots (KELLER & KOBLET, 1995). Bigger sized plant pots would also have the advantage of a higher water holding capacity, if watered from above, thus watering could maybe be reduced to once a week, or even less, with the advantage of less disturbance of controlled environment within each chamber. Maybe this would also have an effect on more constant and equal temperature. Another important factor which is influenced by the size of used plant pots is the infestation and establishment of phylloxera. Bigger plant pots would contribute more space to the root system. Soil exploration is triggered due to more space. A more widely spread root system normally has more young root tips. Combined with partial root zone irrigation, a certain watering technique, ROMERO et al. investigated this effect (ROMERO, et al., 2014). Probably watering from below, like it was down after a few weeks within the growth chambers, has a similar effect on root exploration because roots need to explore deeper soil layers, containing more water. Thus it would be positive for root system and phylloxera establishment. Other studies which worked with phylloxera and potted grapevines used either pots with a higher volume (OMER, et al., 1995) or pots having a slightly lower volume (HERBERT, et al., 2008; BLANCHFIELD, et al., 2006). Thus pot size may not be the most important factor in terms of phylloxera. Although with inoculation techniques tried out in this work, plant pots with a medium size of ten litres would be a good compromise between soil volume per plant and space proportions within chambers.

Observed senescence and abscission of the lower leaves are very likely a result of low light conditions. Studies of HIKOSAKA and of BERTAMIN AND NEDUNCHEZHIAN investigating the effect of nutrient losses due to insufficient recycling, should be taken into account when starting the next experiment with the same plants within the growth chambers (HIKOSAKA, 2005; BERTAMINI & NEDUNCHEZHIAN, 2001). A possible effect of a lowered buffer storage could falsify measurements, e.g. starch content of roots, especially when using same trial plants for more trial sets to gain data over more vegetation periods. Leaf senescence began at the lowest leaf of some trial plants. It occurred to a similar date within both chambers, so it can be seen as an indicator of similar envirnomental conditions between both chambers. Nevertheless it has to be considered if leaf number per plant is adequate. With one grape per plant (variant 2 and 4), a reduction of leaves would be possible without affecting the leaf to

fruit ratio too much. The question is if less leaves per plant under low light conditions might be limitting phyotosynthsis additionally to low PAR. Reduction of only two leaves per plant can lower total leaf area up to 14%, assuming that all leaves might have the same size. On the other hand the senescence and abscission of leaves can be seen as a reaction of trial plants to the artificial environment. If these leaves are not needed it would be better to adjust leave number per plant to a lower level in the beginning of trial, so that no losses due to unsufficient nutrient recycling (HIKOSAKA, 2005; BERTAMINI & NEDUNCHEZHIAN, 2001) affect source sink and carbon partitioning investigation. Also to reduce the canopy effect, which might amplify low light conditions, maybe plants should be topped to a level of ten to twelve leaves per plant for future trial sets.

V.2 Comparability between growth chambers

Adjustment of temperature is a very important factor within this experimental set up. Not only the plants have their optimum temperature range but also phylloxera. Further temperature between both chambers should be comparable to have equal growth conditions. Phylloxera's optimum temperature range reaches from 21°C to 36°C (GRANETT, et al., 1985), while too low temperatures are stemming for the establishment of phylloxera (FORNECK & HUBER, 2009). First setting of temperature within the growth chamber was 22°C. Assuming that air temperature within the chambers is not equal to soil temperature within the pots, this setting might be too low. However, with raising the temperature up to 26°C, it is very likely that soil temperature was appropriate. Additional the effect of different temperatures on growth and photosynthesis (HENDRIKSON, et al., 2004) is also an important reason for keeping conditions between the chambers equal. There is a difference in the measured air temperatures between the chambers of up to 0.6°C, this may be too unequal. These differences are verified by measurements of leaf temperatures, which also vary up to 0.68°C, with the same distribution than air temperature, being higher at the start of the trial and especially at the last growth stage. Because there is no obvious pattern apparent from differences between measurement leaves, it might be a good strategy to measure both leaves in further trials, or even one more. The reason for higher temperature of the upper measurement leaves might be because of proximity to light modules which emit warmth. While differences between leaf and air temperature declined with increasing time within chamber 213, they fluctuate in chamber 195. Thus chamber 213 seems to have more stable conditions for trial plants than chamber 195, concerning this growth parameter.

Light regimes within the two growth chamber also should be in a comparable range to verify equal growth conditions for all variants. The first light measurement on July 2nd, soon after the placement of trial plants into growth chambers gave first impression weather light modules emit comparable PAR in both chambers. At that time there were differences in the

number of lamps, with chamber 213 having two light modules with respectively two lamps more. The values between the growth chambers are similar at cluster zone height but differ in a height of 150 and 180 cm. Further data were needed to verify or reject same light conditions between the two chambers. Therefore another measurement on July 23rd was made with PAM. This time the PAR reaching both measurement leaves was of interest. Statistical analyses showed significant differences between the chambers as well as within the chambers. The only pairing tested without a significant difference between the chambers was leaf eight of fructifying plants (variants 2 and 4). In this context no significant difference means growth conditions are more equal, thus is positive and of importance for further trials. So leaf 8 values of fructifying plants show that these leaves are the ones being most suited for measurements because they show highest comparability due to least difference, with this plant distribution, within the chambers. Interpreting all other values allows the conclusion that light regimes are not comparable between the chambers with this placement of plants and this kind of lamps. This seems surprising with a difference of only 6.7 PAR, comparing all plants between the two chambers. Such low difference would probably make no difference under field conditions with PAR of 2000 µmol/m²s (SMART, 1985) but relating it to the low light regime of this trial shows that within chamber 213, 15% more PAR reaches measuring leaves, what is in line with results showing over all comparisons higher values of PAR in chamber 213.

Another unexpected phenomenon is the high number of significant differences within the chambers between fructifying and non-fructifying vines. This can be interpreted as an indicator that an equal distribution of these variants within the chambers is not suited for this trial design. Significant differences between the third and the eight leaves can be explained by the canopy effect, which means that only 10% of PAR from a leaf below reach the leaf underneath (SMART, 1974) and were expected. Further they are found between fructifying and non-fructifying variants, meaning even within one chamber conditions between both variants are not comparable for this growth parameter. The canopy effect seemed to be stronger within chamber 213 with higher differences although number of leaves per vine was held equal. A possible reason for the more present effect might be the paired placement of the vines at the sides of the chamber. Summarizing the comparability of PAR intensity between both chambers, it might be better to have exactly the same distribution of plants within both chambers. That was not possible because of installed shelves with different depth at the sides of the chambers. Further a randomised arrangement of variants within the chamber, or even between chambers, might erase different conditions between the respective variants and measurement leaves.

Randomising all variants over both chambers would be the best for erasing a possible “chamber-effect”, but would complicate trial design because each trial plant of variant three and four would need a reliable barrier to quarantine phylloxera. Further repeated controls of variants one and three would be needed to verify no infestation by phylloxera. Summarizing, the distribution of plants, number of plants as well as light modules in terms of quantity, position and intensity, have to be adjusted and verified by PAR measurements before the start of the next trial, to lower the difference between the chambers to a not significant level. Then conditions might be better comparable and results are more significant. To avoid an effect of chambers, it would be the best to place plants randomised and distributed over both chambers. Then effective and practical quarantine provisions are needed and have to ensure that there is no spread of phylloxera from variants 2 and 4 to variants 1 and 3.

V.3 Chlorophyll fluorescence

Chlorophyll fluorescence (CF) describes radiation emitted by the electron acceptors of chlorophyll molecules, especially from photosystem II (PSII). This work includes several chlorophyll fluorescence measurements. CF can be detected by different types of fluorimeters either based on the pulse amplitude modulation technique, like the PAM-2500 Chlorophyll Fluorometer (Heinz Walz GmbH, Effeltrich, Germany) and the CIRAS-2 Portable Photosynthesis System (CIRAS) (PP Systems Inc., Amesbury, USA), or on the continuous excitation technique like the Handy Plant Efficiency Analyser (PEA) Chlorophyll Fluorometer (HandyPEA) (Hansatech Instruments, Norfolk, England), all used in this work. Parameters of these measurements have either been calculated manually or were calculated by the respective software of each instrument. Further instruments can measure samples in an isolated atmosphere, as it is done with CIRAS, or measure in an open system, as it is done with PAM and HandyPEA. A huge amount of data resulted from measurements, which were done at different developmental stages of trial plants, namely berries pea-sized (BBCH 75), majority of berries touching (BBCH 79), beginning of berry ripening (BBCH 81), berries developing colour (BBCH 83), softening of berries (BBCH 85) and berries ripe for harvest (BBCH 89). Since these stages pass fluently from one to the next, not all trial plants were exactly in the same developmental status, but were within the same growth stage. Further not all measurements with the different instruments could be done within the same BBCH-stage. Therefore measurements at related stages were grouped into three stages: BBCH 75-81, BBCH 81-85 and BBCH 89, concerning comparisons of all instruments, or BBCH 75-81, BBCH 83-85 and BBCH 89, concerning the comparison of CIRAS and PAM. The maximum time period between measurements with instruments was 16 days at BBCH 89, which were the last measurements. Measurements at BBCH 75-81 were performed within a time period of eleven days, while nine days passed between measurements at BBCH 81 to 85. Because

of the high amount of data, only three CF parameters are presented in results. The ratio of Fv/Fm was measured with all instruments, ΦPSII (CIRAS), which is the same than Y(II) (PAM) and qP, were measured with CIRAS and PAM.

Fv/Fm, also called maximum yield of primary photochemistry (KITAJIMA & BUTLER, 1975) or quantum efficiency of open PSII reaction centres (MAXWELL & JOHNSON, 2000), is determined in dark adapted leaves and is almost constant for many different plant species, reaching values of approximately 0.8 under non-stressful conditions. A decrease in Fv/Fm results from an increase in thermal dissipation at the expense of photochemical activity, thus being an indicator for photo inhibition. Further it provides information which processes altered the efficiency of photochemistry (GALLÉ & FLEXAS, 2010). While in the first measurement all comparisons between chamber 195 (control) and chamber 213 (phylloxera) differ significantly for all instruments, there is a decline in significant difference, ending with no significant difference between the chambers in the last measurement. Measurements of Fv/Fm with HandyPEA resulted in the highest value for the ratio in all three measurements. In contrast, Fv/Fm measured with CIRAS, shows lowest values in the first and third measurement. From BBCH 75-79 to BBCH 81-85 values measured with HandyPEA and PAM sunk, whereas they reached the highest level over the whole measurement period with CIRAS. As low levels of Fv/Fm are an indicator of photoinhibition (BUFFONI, et al., 1998), which means the inhibition of photosynthesis trough light intensity far above light saturation, the instruments CIRAS and PAM seem to be more sensitive in measuring this effect than HandyPEA, under the low light regime, adjusted in this experimental set up. This effect can be explained by the adaption to low light intensity as done by shaded leaves. Studies, performed under field conditions, revealed advantages of this adaption, namely enhanced light absorption and energy transfer under low light conditions, and disadvantages when same leaves are exposed to higher light intensities, in terms of lower efficiency (ORTOIDZE & DÜRING, 2001; DURING, 1998; SCHULTZ, et al., 1996) Nevertheless, this effect cannot be seen by reference to measurements with HandyPEA. Since low quantum efficiency of open PSII reaction centres is also an indicator for stress (PFÜNDEL, 1998), trial plants seemed to have more stress with later development, referring to measurements performed by CIRAS and PAM. Although lower values can also correlate with first signs of a beginning shut down of metabolism, introducing senescence. Lower Fv/Fm values with beginning senescence are investigated in rice (*Oryza sativa*) (KUMAGAI, et al., 2009). Date of sampling in terms of developmental stage of trial plants is said to have no impact on the level of Fv/Fm ratios (FLEXAS, et al., 1998). An influence of measuring technique itself, namely a decrease of values due to oversaturation by laser pulses, is reported to be, at least partly, possible (FLEXAS, et al., 2000). The same study shows Fv/Fm values of severely stressed plants, due to drought and low light acclimatisation of greenhouse grown plants, of 0.6 on their

lowest level (FLEXAS, et al., 2000). Lower values can only be achieved if stresses are combined, such as high light with extremely high or low temperature (SCHULTZ, 1995). Thus conditions for plants in this trial design seemed to be more moderate. All values are above 0.6, so that growth conditions might be near the optimum or plants have adapted very well, compared to other studies (PALLIOTTI, et al., 2009). Since temperature values differ between chambers but are in a good range for the growth of trial plants, it is likely that lower Fv/Fm values are a consequence of the low light regime but this cannot be identified clearly as reason. To identify the reason for lowered Fv/fm values, representing a variance to optimal growth conditions, measurements under a higher light regime, e.g. under field conditions, should be taken in future trial sets. Then these data could be linked to growth chamber conditions, and the lower light regime could be identified or discarded as cause for lower Fv/Fm values. Furthermore this parameter was, beside ΦPSII, investigated by another study on its suitability for pre-symptomatic detection of downy mildew (*Plasmopara viticola*) (CSÉFALVAY, et al., 2009) as well as taken into consideration, when investigating the effects of low temperature on photosynthesis and growth of grapevine (HENDRIKSON, et al., 2004). These examples undermine importance and variety in usage of Fv/Fm, but also show that it is a non-specific stress indicator, not clearly detecting reasons for stress.

Another important and presented CF parameter measures the efficiency of PSII photochemistry. In literature it is usually called ΦPSII, as it is also named when measured with CIRAS, while PAM-software denotes it as Y(II). It is seen as the most useful parameter of photochemical quenching (GENTY, et al., 1989). The proportion of the light absorbed by chlorophyll associated with PSII, which is used by photochemistry, is expressed by this parameter (MAXWELL & JOHNSON, 2000). Due to measuring ΦPSII and qP, the PSII photochemistry of a leaf under light-adapted conditions can be assessed (GALLÉ & FLEXAS, 2010). Measurements were taken to BBCH 75-81, BBCH 81-83 and BBCH 89, with CIRAS and PAM. The longest time period, with eleven days between the measurements was at BBCH 75-81. Days between the other two measurements were ten from PAM measurement at BBCH 89 to CIRAS measurement at the same growth stage of trial plants and one day between the measurements at BBCH 83-85, which makes them probably most suited to compare, because trial plants were under very similar conditions with respect to their growth stage. While in the first measurement all instruments detected significant differences between both growth chambers, concerning the comparison of all measuring leaves between chambers, there is a decline with ongoing development of potted vines. It is a similar effect already observed in Fv/Fm measurments. Comparing the means of Y(II), measured by PAM, there can be seen the same pattern over all three measurements. The eight measurement leaves of variants 2 and 4 (fructifying vines) are higher than those of variants 1 and 3 (non-fructifying vines), with a significant difference in the first measurement.

Y(II) of chamber 213 (phylloxera) is generally higher than in chamber 185 (control). This tendency can also be observed in CIRAS measurements, which show more constant values concerning the range of ΦPSII over all measurements. This range is higher due to high Y(II)-values in the first measurement. Having a more detailed look on data (see ANNEX), distinct higher values of Fm' (light adapted sampling value= maximum fluorescence levels during a treatment; induced by saturation light pulses which temporarily close all PS II reaction centres) can be seen for both chambers. While Fm' of chamber 195 and 213 is 1.646 and 1.631 (means over all measurement leaves) in the second measurement, it is 2.374 (ch195) and 2.166 (ch213) in the first measurement. Relating that to the equation which calculates Y(II) ($Y\ (II) = \frac{Fm\prime - F}{Fm\prime}$) and knowing the comparable range of F-values between both measurements, it clearly shows that this increase in light adapted maximum fluorescence levels is responsible for distinct higher values of Y(II) at first measurement compared to both other measurements. As indicator for overall photosynthesis, it seems, that plants may have not adapted at that time combined with later measurement, and therefore showed a different photosynthetic performance. Although other studies revealed a high and fast ability of grapevine to adapt to other light regimes compared to other plants (CARVALHO, et al., 2001). As a result of this significant increase of Y(II), a change in heat dissipation, for instance non-photochemical quenching, which occurs when internal or external factors change (MAXWELL & JOHNSON, 2000), is observed. Data of non-photochemical quenching is not presented in results but values can be seen in ANNEX. At first measurement (BBCH 75-81) NPQ values are about half as high as in measurements at BBCH 81-83. NPQ-mean is 0.321 (chamber 195) and 0.423 (chamber 213) including values of both measurement leaves, in the first measurement, while being 0.794 (ch195) and 0.791 (ch213) in the second measurement. As NPQ is linearly related to thermal dissipation, changes in its level are related to dark adapted state and an increase results either of processes which protect the leave against light-induced damage or damage itself (GALLÉ & FLEXAS, 2010; MAXWELL & JOHNSON, 2000). However, values shown in results, of this indicator of photosynthesis, are similar to those of different cultivars under drought stress (GUAN, et al., 2004); although watering of trial plants was done evenly in equal amounts over the whole time period of this trial. Thus low light intensity might be the reason for such low ΦPSII levels. Plants without drought stress show values of ΦPSII in a range of 0.4 (MAROCO, et al., 2002). The second measurement is the only one without any significant difference between the chambers and with mean values of ΦPSII/Y(II) within a comparable range. No significant difference means in this trial that conditions seem to be comparable. An example of the importance of prompt measurements between two instruments is given by this timing. This approach secures that plants are within the same growth stage and share, even within a controlled environment,

same conditions, this gives a result the highest comparability and might be the best approach for future trials in this, or a modified experimental design.

Whilst ΦPSII is the proportion of absorbed energy being used by photochemistry, qP relates to the proportion of opened PSII reaction centres. Dates and time periods between are same than ΦPSII values. Measurements of qP show a similar pattern than it is detected in ΦPSII measurements. The only measurement without any significant differences was also the second measurement. That again undermines the importance of keeping time periods between measurements with different instruments as short as possible. As this CF parameter also relates to achieved efficiency of PSII photochemistry, like ΦPSII, it is not surprising that values measured with PAM, show a similar higher level in the first measurement, while they are in a comparable range to qP measured with CIRAS at the second and third date. It remains unclear if this heterogeneity of measured means results from an influence on photosynthesis, what is quite unlikely due to controlled environment within both chambers, or from the instrument. Although in all measurements same settings of the PAM were adjusted. High qP values are characteristic for optimal utilisation of photochemical energy in the carbon metabolism, which includes in this case photorespiration. Low qP values are an indicator for over saturation with light above the requirements of carbon assimilation. Thus this CF parameter is a valuable indicator of light stress. CIRAS measured very similar qP-levels between variant 2 and 4 in chamber 213, while values differed more in chamber 195. PAM data also show this effect, with exception of the second measurement. Another study also cultivated potted grapevines under low light conditions (150µmol m^{-2} s^{-1}) revealed values of qP in a range around of 0.4 and 0.6 (300µmol m^{-2} s^{-1}) (CARVALHO, et al., 2001). If these values are set into relation of qP-mean values in this study 0.25 – 0.35 (around 30 µmol m^{-2} s^{-1}), it shows that they are distinctly lower. This effect can be explained by a study of LEE et al. which reveals that photosynthetic capacity is not influenced until a loss of 40% of PSII reaction centres (LEE, et al., 1999), what seems to be present in this trial. Over all three detected CF parameter, PAM data show the highest variation. Although in this study there is a focus on PAM, the fact that CIRAS also measures gas exchange has a high advantage because due to combination of CF and gas exchange, the relationship between CO_2 fixation, light use efficiency and photoinhibition can be investigated (GALLÉ & FLEXAS, 2010). It was attempted to link PAM data with a porometer, but measurement of stomatal conductance took very long, compared to field conditions, what can be seen as another indicator for an extreme environment for trial plants, due to low radiation intensity.

The three CF parameters presented in this work are only a few resulted from gained data but were chosen because they are seen as the most useful quenching parameters, already researched in several studies (GALLÉ & FLEXAS, 2010; MAXWELL & JOHNSON, 2000;

GENTY, et al., 1989). The longer the plants were placed within the growth chambers, the less significant differences are detected. Maybe measurements at BBCH 89 were performed too late, with plants being in that stage for approximately four weeks when measured with CIRAS. CF-analysis might be a very useful tool within this experimental set up, especially by giving information about the ability of plants to tolerate environmental stresses and with which impact these stresses have influenced or even damaged the photosynthetic apparatus (MAXWELL & JOHNSON, 2000). Therefore it is important in further trials with this experimental set up, to erase other stresses. Then, a clear link to the effects of root-galling phylloxera can be made. Low light conditions should be overthought, differentiating the study distinctly from field conditions. On the other hand, effects probably would be clearer, because the high ability of adaption to stresses of grapevine is then limited. Further it is shown by statistical analysis that there are less significant differences at later measurements. This may result from two influencing factors. One is the adaption of plants to artificial environment, which also needs to be considered when determine the start of future trials. Another one might be the reduction of trial plants before the second measurement, which already showed less significant differences for all CF parameter presented. It seems that plant number should not be too high under such extreme low light conditions, because of the risk of increase the impact of low radiation through shading. Considering the beginning of future trials, also the reusability of trial plants has to be considered. Since overcast conditions during the bloom do not only reduce fruit set and number of berries per cluster for the same season, but also lower bud fruitfulness for the next growing season (KELLER & KOBLET, 1995). This effect might become stronger when using same trial plants for more than one trial within one year, enabled through the artificial environment of this experimental set up. Thus it can be a better option to begin with each trial-set after bloom of trial plants in the glasshouse. If light intensity would be raised due to light sources with higher PAR, trial plants easily could bloom within the chambers and the start of each trial-set could be in an earlier growth stage of trial plants.

Summarizing all results there might be an effect resulting of the spatial separation of trial plants, or at least it cannot be securely excluded.

A very sophisticated but promising approach is delivered by phenomics, meaning the acquisition of high-dimensional phenotypic data on an organism- wide scale. This approach includes large scale phenotyping in contrast to former approaches of measuring a limited set of phenotypes. As understanding phenotype characteristics is a key goal of biology, phenomics declares the aim of study these complex webs of interactions and asks the question why not measure all variables instead of focussing only on those of what are thought to be important nowadays (HOULE, et al., 2010). Applying this high-throughput of phenotyping has already won attention through quantifying various traits in large plant

populations non-destructively. Such systems are already used to collect data in artificial environments, like in growth chambers or within greenhouses, or are field based. By linking data of different types and numbers of sensors, among others fluorescence, this approach can detect traits within and beyond the visible spectrum (GOGGIN, et al., 2015). Gaining such holistic data and results, this current approach could be the chance for an incredible output of research issues of one and the same experimental design. In this experimental design, not only the original research topics could be investigated, but also various other traits and mechanisms of plants and insects adapting to a certain environment, like low light conditions and interacting with it and among each other, would deliver a massive progress, not only for future studies with this set up. Of course that would not only need high financial input but also techniques to handle high-throughput phenotyping, the use of very high-dimensional data and dynamic models which link phenomena across levels, but these are said to be either in reach or already available (HOULE, et al., 2010).

A fundamental aspect for further development and optimisation of this method is to securely exclude an effect resulting from one or both growth chambers. Measurements of both, environmental factors, like PAR and temperature, as well as of CF, cannot clear for sure if there is such an effect. Therefore environmental conditions have to be measured and adjusted before the beginning of each trial-set and further monitored, preferably automatically, including the most important growth factors like light, water status (plants and soil), temperature and nutritional status (plants and soil). Verification of equal conditions for trial plants, between variants and growth chambers of the highest possible comparability should be the first requirement for the start of each trail-set. Resulting from these equal growth conditions, a high and valuable amount of reproducible data can be won, which will be the base for finding answers to the hypothesis and clear the impact of phylloxera on fruit and wine quality in the future.

VI. Summary

“Establishing a method to investigate the impact of root-galling grape phylloxera (*Daktulosphaira vitifoliae* (Fitch)) on *Vitis vinifera* L.”

This study aims to design and establish a method, cultivating potted grapevines in a controlled environment. The method presented here, shall deliver a suitable approach for future trials, investigating the effect of root-galling phylloxera (*Daktulosphaira vitifoliae* (Fitch)) on grafted grapevine. Since grafting of European cultivars of *Vitis vinifera* L. onto rootstocks of American *Vitis* species, the pest grape phylloxera lost attention, but is present all over the world (EPPO European and Mediterranean Plant Protection Organisation, 2014). Future challenges like host-plant interactions of new strains, also detected in Europe (SONG & GRANETT, 1990; FORNECK, et al., 2001; YVON & PEROS, 2003), with different infestation potential, also on cultivated resistant rootstocks (GRANETT, et al., 1985), changes in these interactions and in climate, what may affect both grapevine and distribution of phylloxera (BENHEIM, et al., 2012), can bring up multiple challenges in the future.

An experimental set up and first data shall deliver results for the verification of the background hypothesis: phylloxera is able to cause an additional sink on roots, also of common used rootstocks. As a consequence carbohydrate partitioning might be negatively influenced and fruit quality decreases. Therefore the objectives of this work are to design an experimental set up, to investigate the sink source translocation, as well as the interactions of root-galling and sucking phylloxera. That includes the setting and verification of environmental conditions, the establishment and verification of an infestation by phylloxera, the verification and control of treatments and the development and suggestions of plant and sampling strategies.

The first objective was to cultivate one year old potted grapevines of the cultivar Zweigelt, (*Vitis vinifera* L., Blaufränkisch × St. Laurent), grafted onto rootstock Kober 5BB *(Vitis berlandieri* × *Vitis riparia)*, within two growth chambers. Each chamber contained one treatment (“control” or “phylloxera”) with two variants (non-fructifying vines or fructifying vines with one grape per plant). Trial plants were cultivated successfully within the growth chambers under a low light regime (24-31 µmol m^{-2} s^{-1}), directly after finishing bloom until veraison. Artificial environment was adjusted to 26°C and 14 hours of light, within both chambers. A difference in temperature and PAR between and within both chambers was detected depending on the position of measurement leaves, which were a lower (3^{rd} or opposite to grape) and a higher (8^{th}) leaf, on each trial plant.

The second objective was to inoculate the treatment phylloxera with eggs of grape phylloxera at four different dates, differing either in inoculation-technique, material or both. Infestation by

phylloxera was checked visually in both chambers via 14 randomly chosen trial plants and was not detected after the third inoculation. A control after the fourth inoculation verified hatching of larvae for each plant of the treatment phylloxera.

The first approach to gain information about environmental conditions and the source sink translocation, were measurements of chlorophyll fluorescence (CF), performed to different growth stages of trail plants. Three different types of fluorimeters, either based on the pulse amplitude modulation technique, or on the continuous excitation technique, were used in this study. Various CF parameters were calculated and three are presented in this work. The ratio of Fv/Fm, ΦPSII or Y(II) and qP. Values calculated for ΦPSII or Y(II) are similar to those of drought-stressed plants (GUAN, et al., 2004), although this stress did not occur visually. qP-values are mainly around 0.3, which corresponds with data of other studies, cultivating plants also under a low light regime (CARVALHO, et al., 2001). Calculated Fv/Fm values are near the optimum value of 0.8, which is an indicator for non-stressful conditions (PFÜNDEL, 1998), but decreased at later development stages. Nevertheless they are higher than values of heavy stressed plants in other studies (FLEXAS, et al., 2000; SCHULTZ, 1995). Significant differences between the chambers were rarer with ongoing development of plants within chambers and with closer time periods between measurements. Less differences and thus more comparable conditions could also be detected when reducing the number of trial plants.

Moreover, several adaptions, optimisations and suggestions, which can be implemented in future trials, are named. Most important findings are the requirement of an adaption time of plants, a maximum number of 70 plants, a randomised placement of all variants over both growth chambers, a higher pot volume, as well as a decrease of the number of leaves per plants. Measurements with instruments should be taken on at least two leaves per plant and in closely connected time periods. Under low light conditions, even slightly different PAR values between the chambers, result in significant differences. Thus they have to be verified especially. An effect by each growth chamber, thus unequal growth conditions, could not be excluded. Therefore environmental conditions have to be measured and adjusted before the beginning of each trial-set and further monitored, preferably automatically. The most important growth factors like light, water status (plants and soil), temperature and nutritional status (plants and soil) should be measured continually. Verification of equal conditions, with the highest possible comparability, for trial plants and between growth chambers, should mark the start of each trial-set. Further measurements on sugar physiology, pattern of assimilate transport and distribution, agronomic indicators and grape quality parameters, result in a high and valuable amount of reproducible data. Then answers to the hypothesis can be given and can clear the impact of phylloxera on fruit and wine quality in the future.

Abstract (English – German)

"Establishing a method to investigate the impact of root-galling grape phylloxera (*Daktulosphaira vitifoliae* (Fitch)) on *Vitis vinifera* L."

Aim of this study was to develop a method to investigate the impact of root-gulling phylloxera (*Daktulosphaira vitifoliae* (Fitch)) (PY) on grapevine (*Vitis vinifera* L., Zweigelt), grafted onto rootstock Kober 5BB, within two growth chambers (GC). The underlying background hypothesis says that PY is able to cause an additional sink on roots, also of common used rootstocks. As a consequence carbohydrate partitioning might be negatively influenced and fruit quality decreases. The first objective was to cultivate one year old potted grapevines within the GC, each containing one treatment ("control" or "PY") with two variants (non-fructifying and fructifying vines). Trial plants were cultivated successfully within the GC under a low light regime. Measurements of chlorophyll fluorescence (CF) were taken with different types of fluorimeters. Various CF parameters were calculated and three (Fv/Fm, ΦPSII or Y(II) and qP) are presented. Significant differences between the GC were rarer with ongoing development of plants and with closer time periods between measurements. Less differences and thus more comparable conditions were detected when reducing trial plants. The second objective was to inoculate roots of the treatment PY with eggs of grape PY at four different dates, differing either in inoculation-technique, material, or both. Infestation by PY was checked visually in both GC and was not detected after the third inoculation. A control after the fourth inoculation verified hatching of larvae for each plant of the treatment PY. Further several adaptions, optimisations and suggestions, which can be implemented in future trials, are found. An effect by each GC, thus unequal growth conditions, cannot be excluded. Measurements on sugar physiology, pattern of assimilate transport and distribution, agronomic indicators and grape quality parameters, result in a high and valuable amount of reproducible data. Then an impact of PY on fruit and wine quality can be quantified.

“Etablierung einer Methode zur Untersuchung der Auswirkung wurzelgallen-bildender Reblaus (*Daktulosphaira vitifoliae* (Fitch)) auf *Vitis vinifera* L.“

Ziel dieser Arbeit war die Entwicklung einer Methode zur Beantwortung der grundliegenden Hypothese: Die Reblaus (PY) (*Daktulosphaira vitifoliae* (Fitch)) stellt, auch bei gängig verwendeten Unterlagen, ein zusätzliches Sink-Organ an der Wurzel dar**,** welches sich negativ auf die Rebe und die Fruchtqualität auswirkt.

Hierfür wurden einjährige Topfreben (*Vitis vinifera* L., Zweigelt), gepfropft auf die Unterlage Kober 5BB, in zwei Wuchskammern unter Niedriglichtbedingungen kultiviert. Jede Wuchskammer enthielt dabei ein Versuchsglied (Kontrolle und PY) mit je zwei Varianten (nicht-fruktifizierende und fruktifizierende Reben). Messungen der Chlorophyllfluoreszenz (CF) wurden mit unterschiedlichen Fluorimetern durchgeführt. Neben zahlreichen anderen errechneten CF-Parametern werden drei (Fv/Fm, ΦPSII or Y(II) and qP) in dieser Arbeit vorgestellt. Signifikante Unterschiede dieser Parameter zwischen den Versuchsgliedern nahmen mit zunehmender Entwicklung der Versuchspflanzen und kürzeren Messintervallen ab. Auch durch die Reduzierung der Versuchspflanzen ergaben sich weniger signifikante Unterschiede und somit vergleichbarere Bedingungen. Außerdem wurden die Wurzeln des Versuchsglieds PY mit Eiern der Reblaus zu unterschiedlichen Zeitpunkten inokuliert. Die Inokulationen unterschieden sich entweder in der angewandten Technik, dem verwendeten Material, oder in beidem. Nach der dritten Inokulation konnte visuell kein Befall festgestellt werden. Eine Schlupfkontrolle nach der vierten Inokulation verifizierte den Schlupf an jeder Pflanze des Versuchsgliedes PY. Des Weiteren wurden zahlreiche Optimierungen vorgenommen und umfangreichere Messungen vorgeschlagen, welche zum Beispiel die Zuckerphysiologie, die Assimilatverteilung, physiologische Parameter und die Zusammensetzung der Beereninhaltsstoffe beinhalten, vorgestellt, um möglichst viele aussagekräftige und reproduzierbare Daten zu gewinnen, anhand derer der Einfluss der Reblaus auf Frucht- und Weinqualität erhoben werden kann.

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ANNEX

Table 22: Content of macro- and micronutrients according to Planta Düngemittel GmbH (2014)

Macronutrients		Micronutrients	
18 % N	Total nitrogen	0,02 % B	Boron (B)
- 10 % NO_3-N	Nitrate	0,04 % Cu	Copper (Cu)*
- 8 % NH_4-N	Ammonium	0,10 % Fe	Iron (Fe)**
12 % P_2O_5	Water-soluble phosphate	0,05 % Mn	Manganese (Mn)*
18 % K_2O	Water-soluble potassium oxide	0,01 % Mo	Molybdenum (Mo)
2 % MgO	Water-soluble magnesium oxide	0,01 % Zn	Zinc (Zn)*
* = chelated as EDTA ** = chelated as EDTA and EDDHA			

Table 23: Definitions of generated data with PAM, provided by the Handbook of Operation of PAM 2500, Heinz Walz GmbH, page 79 ff.

Dark-acclimated samples	
Fo	Minimum fluorescence level excited by very low intensity of measuring light to keep PS II reaction centers open
Fm	Maximum fluorescence level elicited by a strong light pulse which closes all PS II reaction centers.
Light-exposed samples	
Fo'	Minimum fluorescence level during a treatment. Measuring routine for Fo' was active, which means that Fo' is determined during a dark interval following the saturation pulse. In this dark interval, far-red light is applied to selectively drive PS I reaction centers to quickly remove intersystem electrons and open PS II reaction centres. Alternatively Fo' can be estimated by following equation: $$Fo' = \frac{1}{\frac{1}{Fo} - \frac{1}{Fm} + \frac{1}{Fm'}}$$ (OXBOROUGH and BAKER 1997)
Fm'	Maximum fluorescence levels during a treatment, induced by saturation light pulses which temporarily close all PS II reaction centres. Value is decreased with respect to Fm by non-photochemical quenching.
F	Corresponds to the momentary fluorescence yield (Ft) of an illuminated sample shortly before application of a Saturation Pulse

Table 24: Fluorescence Ratio Parameters inherited from the Handbook of Operation for the PAM-2500 provided by Heinz Walz GmbH(2008, 81 ff.); All Sources are inherited.

Source	Equation
Maximum photochemical quantum yield of PS II (KITAJIMA and BUTLER 1975)	$\frac{Fv}{Fm} = \frac{Fm - Fo}{Fm}$
Effective photochemical quantum yield of PS II (GENTY et al., 1989)	$Y\ (II) = \frac{Fm' - F}{Fm'}$
Coefficient of photochemical fluorescence quenching (SCHREIBER et al., 1986) (VAN KOOTEN and SNEL 1990) as formulated by (VAN KOOTEN and SNEL 1990)	$q_P = \frac{Fm' - F}{Fm' - Fo'}$
Coefficient of photochemical fluorescence quenching assuming interconnected PS II antennae (KRAMER et al., 2004)	$q_L = q_P * \frac{Fo'}{F}$
Coefficient of non-photochemical fluorescence quenching (SCHREIBER et al., 1986) as formulated by (VAN KOOTEN and SNEL 1990)	$q_N = 1 - \frac{Fm' - Fo'}{Fm - Fo}$
Stern-Volmer type non-photochemical fluorescence quenching(BILGER and BJÖRKMAN 1990).	$NPQ = \frac{Fm}{Fm'} - 1$
Quantum yield of non-regulated heat dissipation and fluorescence emission: this quenching type does not require the presence of a transthylakoid ΔpH and zeaxanthin (GENTY et al., 1996)*	$Y\ (NO) = \frac{F}{Fm}$
Quantum yield of light induced (ΔpH- and zeaxanthin-dependent) non-photochemical fluorescence quenching (GENTY et al., 1996)*	$Y\ (NPQ) = \frac{F}{Fm'} - \frac{F}{Fm}$

* (KRAMER et al., 2004) have derived more complex equations for Y(NO) and Y(NPQ). (KLUGHAMMER and SCHREIBER 2008)have demonstrated that the equations by Kramer et al. (2004) can be transformed into the simple equations of (GENTY et al., 1996) which are used by PamWin-3 software.

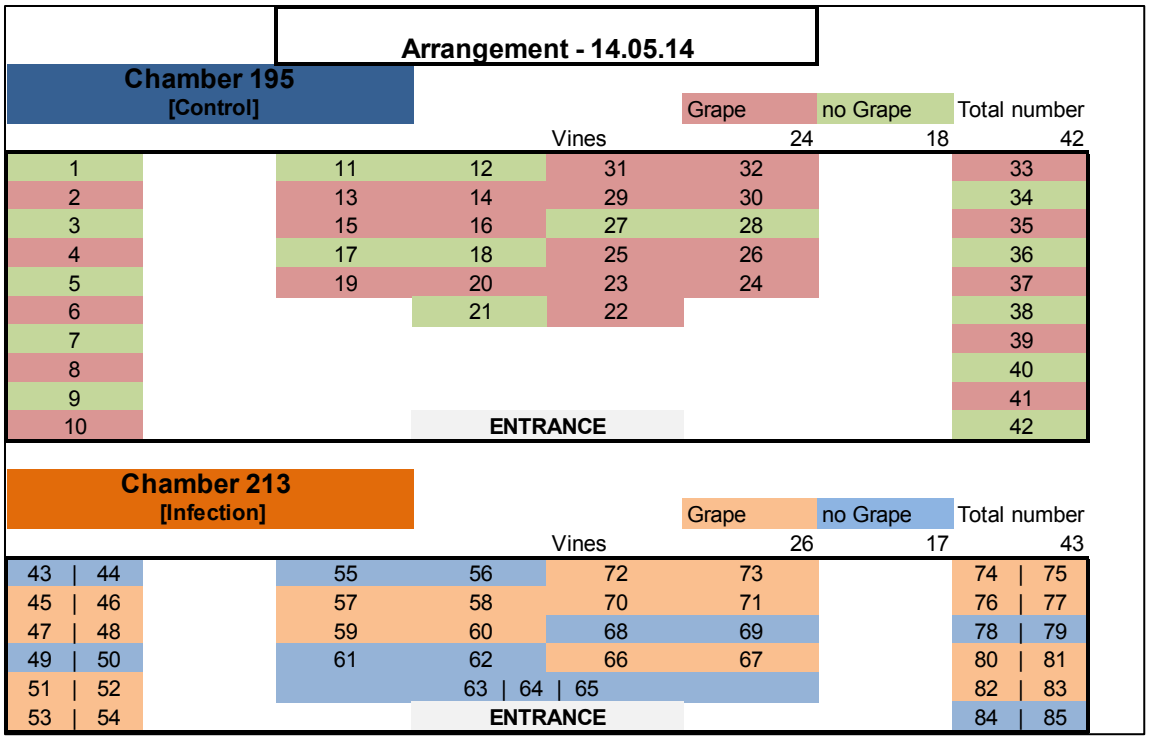


Figure 15: Draft of the arrangement of May 14th of plants in both growth chambers; both chambers have the same size; Each number represents a trial plant; Colouration of squares give information about treatment

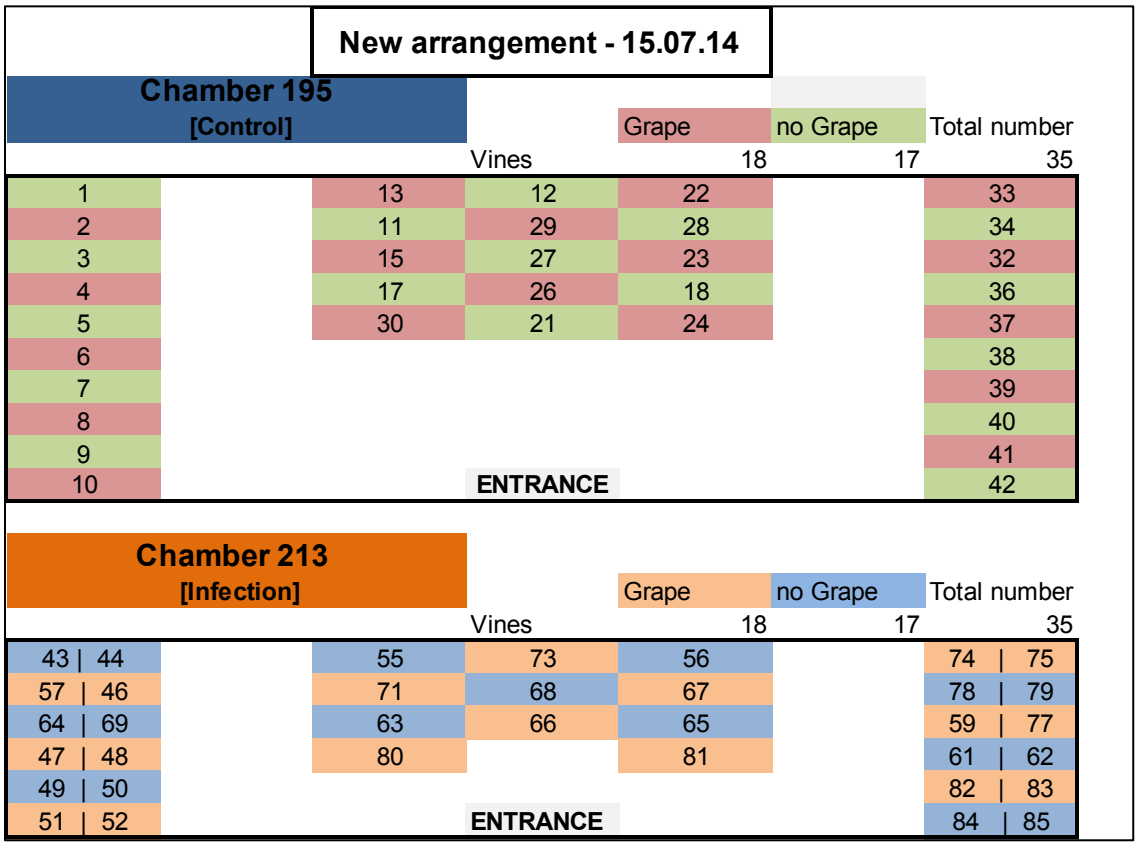
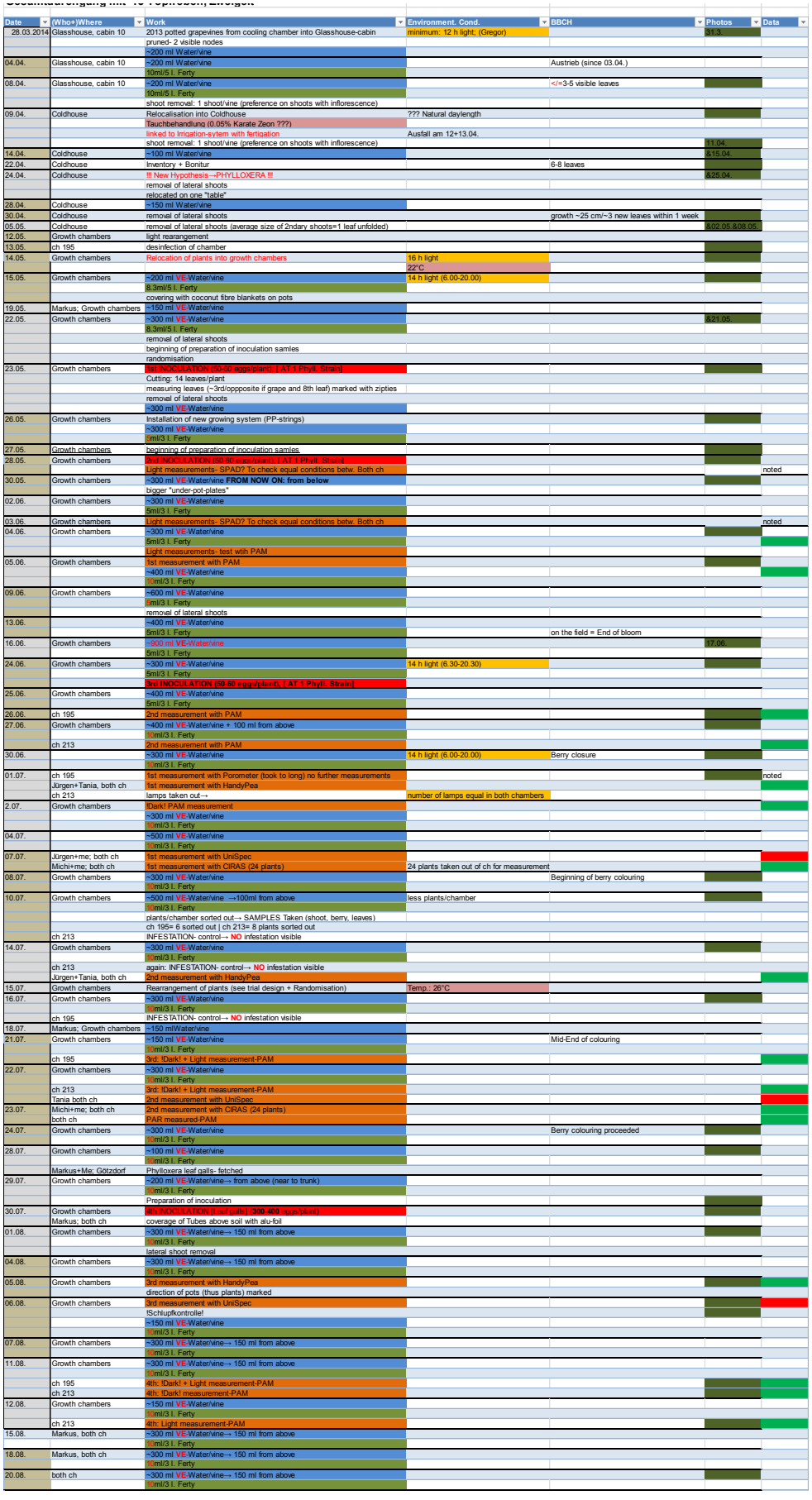


Figure 16: Draft of the new arrangement and randomisation of plants in both growth chambers on July 15th; both chambers have the same size; Each number represents a trial plant; Colouration of squares give information about treatment

Table 25: Air temperature in °C in growth chambers

	[°C]		
Time	Chamber 195	Chamber 213	Difference
Week 1	21.573	22.181	0.608
Week 2	21.374	21.612	0.238
Week 3	21.382	21.614	0.232
Week 4	21.583	21.599	0.016
Week 5	21.398	21.568	0.170
Week 6	21.511	21.560	0.049
Week 7	21.618	21.515	0.103
Week 8	21.687	21.788	0.101
Week 9	24.503	24.696	0.193
Week 10	25.225	25.792	0.567
Week 11	24.560	25.212	0.652
Week 12	25.302	25.758	0.456
Week 13	25.432	25.730	0.298
Week 14	25.273	25.656	0.383

Figure 17: Left side: SPAD LP-80; first measurement of light conditions; Right side: Automatic light control system (GIRA Control 9) of growth chambers



Date	(Who+)Where	Work	Environment, Cond.	BBCH	Photos	Data
28.03.2014	Glasshouse, cabin 10	2013 potted grapevines from cooling chamber into Glasshouse-cabin	minimum: 12 h light; (Gregor)		31.3.	
		pruned- 2 visible nodes				
		~200 ml Water/vine				
04.04.	Glasshouse, cabin 10	~200 ml Water/vine		Austrieb (since 03.04.)		
		10ml/5 l. Ferty				
08.04.	Glasshouse, cabin 10	~200 ml Water/vine		</=3-5 visible leaves		
		10ml/5 l. Ferty				
		shoot removal: 1 shoot/vine (preference on shoots with inflorescence)				
09.04.	Coldhouse	Relocalisation into Coldhouse	??? Natural daylength			
		Tauchbehandlung (0.05% Karate Zeon ???)				
		linked to Irrigation-sytem with fertigation	Ausfall am 12+13.04.			
		shoot removal: 1 shoot/vine (preference on shoots with inflorescence)			†1.04.	
14.04.	Coldhouse	~100 ml Water/vine			&15.04.	
22.04.	Coldhouse	Inventory + Bonitur		6-8 leaves		
24.04.	Coldhouse	!!! New Hypothesis→PHYLLOXERA !!!			&25.04.	
		removal of lateral shoots				
		relocated on one "table"				
28.04.	Coldhouse	~150 ml Water/vine				
30.04.	Coldhouse	removal of lateral shoots		growth ~25 cm/~3 new leaves within 1 week		
05.05.	Coldhouse	removal of lateral shoots (average size of 2ndary shoots=1 leaf unfolded)			&02.05.&08.05.	
12.05.	Growth chambers	light rearangement				
13.05.	ch 195	desinfection of chamber				
14.05.	Growth chambers	Relocation of plants into growth chambers	16 h light			
			22°C			
15.05.	Growth chambers	~200 ml VE-Water/vine	14 h light (6.00-20.00)			
		8.3ml/5 l. Ferty				
		covering with coconut fibre blankets on pots				
19.05.	Markus; Growth chambers	~150 ml VE-Water/vine				
22.05.	Growth chambers	~300 ml VE-Water/vine			&21.05.	
		8.3ml/5 l. Ferty				
		removal of lateral shoots				
		beginning of preparation of inoculation samles				
		randomisation				
23.05.	Growth chambers	1st INOCULATION (50-60 eggs/plant); [AT 1 Phyll. Strain]				
		Cutting: 14 leaves/plant				
		measuring leaves (~3rd/oppposite if grape and 8th leaf) marked with zipties				
		removal of lateral shoots				
		~300 ml VE-Water/vine				
26.05.	Growth chambers	Installation of new growing system (PP-strings)				
		~300 ml VE-Water/vine				
		8ml/3 l. Ferty				
27.05.	Growth chambers	beginning of preparation of inoculation samles				
28.05.	Growth chambers	2nd INOCULATION (50-60 eggs/plant); [AT 1 Phyll. Strain]				
		Light measurements- SPAD? To check equal conditions betw. Both ch				noted
30.05.	Growth chambers	~300 ml VE-Water/vine **FROM NOW ON: from below**				
		bigger "under-pot-plates"				
02.06.	Growth chambers	~300 ml VE-Water/vine				
		5ml/3 l. Ferty				
03.06.	Growth chambers	Light measurements- SPAD? To check equal conditions betw. Both ch				noted
04.06.	Growth chambers	~300 ml VE-Water/vine				
		5ml/3 l. Ferty				
		Light measurements- test with PAM				
05.06.	Growth chambers	1st measurement with PAM				
		~400 ml VE-Water/vine				
		10ml/3 l. Ferty				
09.06.	Growth chambers	~600 ml VE-Water/vine				
		10ml/3 l. Ferty				
		removal of lateral shoots				
13.06.		~400 ml VE-Water/vine				
		5ml/3 l. Ferty		on the field = End of bloom		
16.06.	Growth chambers	~400 ml VE-Water/vine			†7.06.	
		5ml/3 l. Ferty				
24.06.	Growth chambers	~300 ml VE-Water/vine	14 h light (6.30-20.30)			
		5ml/3 l. Ferty				
		3rd INOCULATION (50-60 eggs/plant); [AT 1 Phyll. Strain]				
25.06.	Growth chambers	~400 ml VE-Water/vine				
		5ml/3 l. Ferty				
26.06.	ch 195	2nd measurement with PAM				
27.06.	Growth chambers	~400 ml VE-Water/vine + 100 ml from above				
		10ml/3 l. Ferty				
	ch 213	2nd measurement with PAM				
30.06.		~300 ml VE-Water/vine	14 h light (6.00-20.00)	Berry closure		
		10ml/3 l. Ferty				
01.07.	ch 195	1st measurement with Porometer (took to long) no further measurements				noted
	Jürgen+Tania, both ch	1st measurement with HandyPea				
	ch 213	lamps taken out→	humber of lamps equal in both chambers			
2.07.	Growth chambers	!Dark! PAM measurement				
		~300 ml VE-Water/vine				
		10ml/3 l. Ferty				
04.07.		~500 ml VE-Water/vine				
		10ml/3 l. Ferty				
07.07.	Jürgen+me; both ch	1st measurement with UniSpec				
	Michi+me; both ch	1st measurement with CIRAS (24 plants)	24 plants taken out of ch for measurement			
08.07.	Growth chambers	~300 ml VE-Water/vine		Beginning of berry colouring		
		10ml/3 l. Ferty				
10.07.	Growth chambers	~500 ml VE-Water/vine →100ml from above	less plants/chamber			
		10ml/3 l. Ferty				
		plants/chamber sorted out→ SAMPLES Taken (shoot, berry, leaves)				
		ch 195= 6 sorted out | ch 213= 8 plants sorted out				
	ch 213	INFESTATION- control→ NO infestation visible				
14.07.	Growth chambers	~300 ml VE-Water/vine				
		10ml/3 l. Ferty				
	ch 213	again: INFESTATION- control→ NO infestation visible				
	Jürgen+Tania, both ch	2nd measurement with HandyPea				
15.07.	Growth chambers	Rearrangement of plants (see trial design + Randomisation)	Temp.: 26°C			
16.07.	Growth chambers	~300 ml VE-Water/vine				
		10ml/3 l. Ferty				
	ch 195	INFESTATION- control→ NO infestation visible				
18.07.	Markus; Growth chambers	~150 mlWater/vine				
21.07.	Growth chambers	~150 ml VE-Water/vine		Mid-End of colouring		
		10ml/3 l. Ferty				
	ch 195	3rd: !Dark! + Light measurement-PAM				
22.07.	Growth chambers	~300 ml VE-Water/vine				
		10ml/3 l. Ferty				
	ch 213	3rd: !Dark! + Light measurement-PAM				
	Tania both ch	2nd measurement with UniSpec				
23.07.	Michi+me; both ch	2nd measurement with CIRAS (24 plants)				
	both ch	PAR measured-PAM				
24.07.	Growth chambers	~300 ml VE-Water/vine		Berry colouring proceeded		
		10ml/3 l. Ferty				
28.07.	Growth chambers	~100 ml VE-Water/vine				
		10ml/3 l. Ferty				
	Markus+Me; Götzdorf	Phylloxera leaf galls- fetched				
29.07.	Growth chambers	~200 ml VE-Water/vine→ from above (near to trunk)				
		10ml/3 l. Ferty				
		Preparation of inoculation				
30.07.	Growth chambers	4th INOCULATION [Leaf galls1 (300-400 eggs/plant)				
	Markus; both ch	coverage of Tubes above soil with alu-foil				
01.08.	Growth chambers	~300 ml VE-Water/vine→ 150 ml from above				
		10ml/3 l. Ferty				
		lateral shoot removal				
04.08.	Growth chambers	~300 ml VE-Water/vine→ 150 ml from above				
		10ml/3 l. Ferty				
05.08.	Growth chambers	3rd measurement with HandyPea				
		direction of pots (thus plants) marked				
06.08.	Growth chambers	3rd measurement with UniSpec				
		!Schlupfkontrolle!				
		~150 ml VE-Water/vine				
		10ml/3 l. Ferty				
07.08.	Growth chambers	~300 ml VE-Water/vine→ 150 ml from above				
		10ml/3 l. Ferty				
11.08.	Growth chambers	~300 ml VE-Water/vine→ 150 ml from above				
		10ml/3 l. Ferty				
	ch 195	4th: !Dark! + Light measurement-PAM				
	ch 213	4th: !Dark! measurement-PAM				
12.08.	Growth chambers	~150 ml VE-Water/vine				
		10ml/3 l. Ferty				
	ch 213	4th: Light measurement-PAM				
15.08.	Markus, both ch	~300 ml VE-Water/vine→ 150 ml from above				
		10ml/3 l. Ferty				
18.08.	Markus, both ch	~300 ml VE-Water/vine→ 150 ml from above				
		10ml/3 l. Ferty				
20.08.	both ch	~300 ml VE-Water/vine→ 150 ml from above				
		10ml/3 l. Ferty				

Figure 18: Calendar with detailed information about trial design, environmental- and growth conditions

Figure 19: Left side: Trial-plant within the Glasshouse-cabin at the 31st of March; Right side: Trial plants within the glasshouse cabin on the 8th of April

Figure 20: Left side: Trial plants at the 11th of April, three days after relocation into cold-house; Right side: Trial plants after 25 days in the coldhouse on the 5th of May, short before the bloom

Figure 21: Left side: Growth chamber directly after relocation of trial plants into growth chambers on April 14th; Right side: Growth chamber on July 16th, when berry colouring nearly proceeded

Figure 22: Both pitures taken on May 11th; Left side: Rearrangement of light modules to the top of the chamber; Right side: Equal distribution of light modules

Figure 23: Handy Pea chlorophyll fluorometer at the measurement on June 5th; left side: sensor unit and leafclips; right side: control unit.

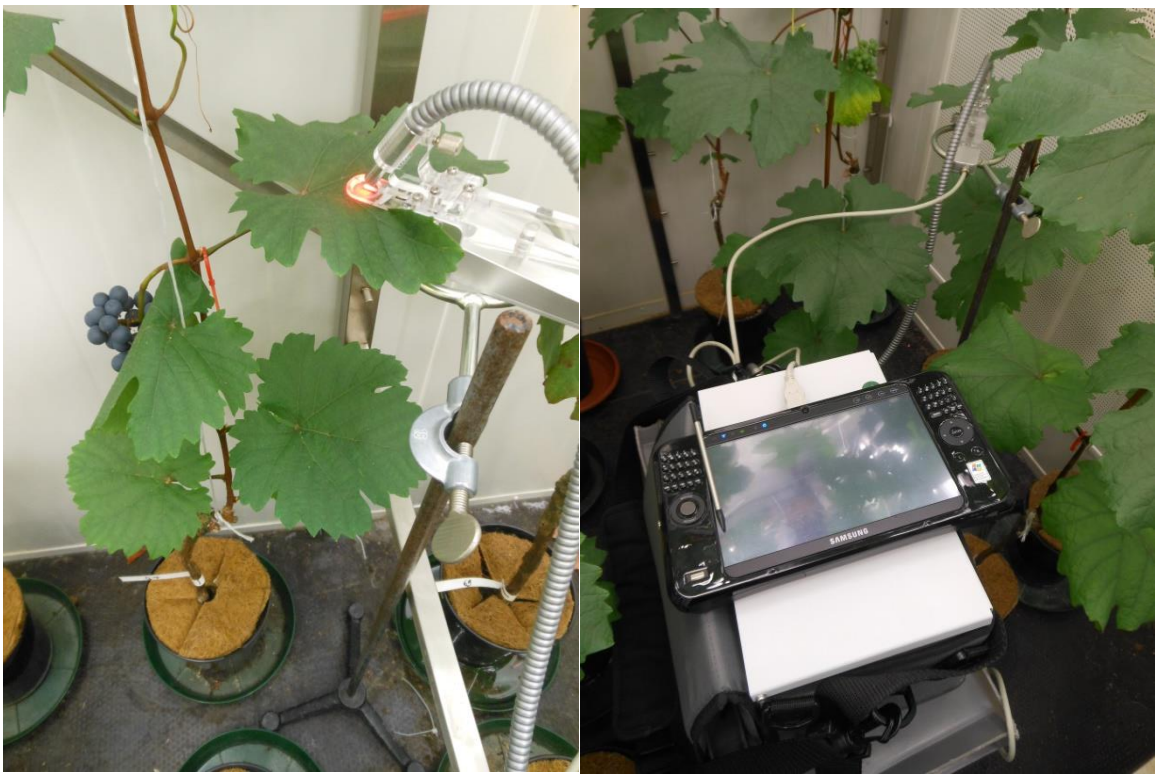


Figure 24: Left side: Fibre optics and leaf distance clip of the PAM-2500 at the fourth measurement; Right side: PAM-2500 and Samsung portable CPU at the 2nd measurement (June 27th)

Figure 25: Both sides: Senescence of basal leaves, very likely due to low light conditions (June 24th)

Figure 26:Left side: Collection of infested root parts of host plants in petri dish, 23.05.14; Right side: Used Stereo Microscope with petri dish containing infested root material for the catching of eggs

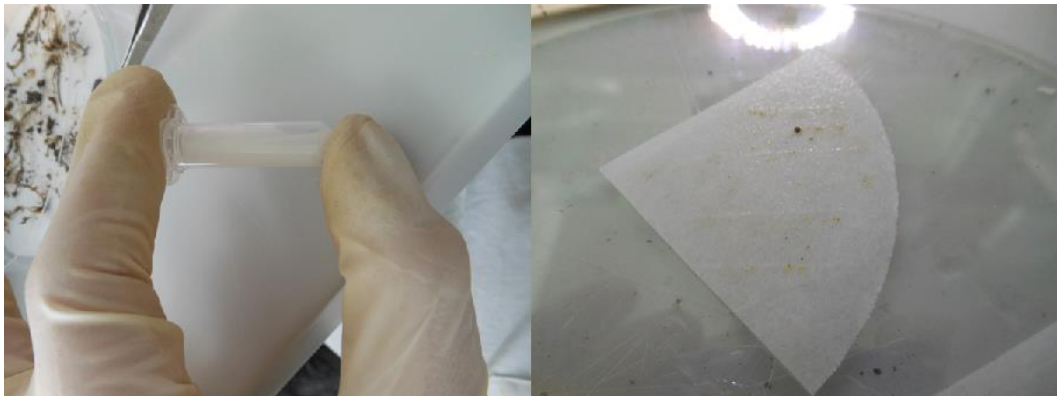


Figure 27: Left side: Quartered filter-paper with eggs from inoculation date 24th of May; Right side: 2 ml Eppendorf-tube with rolled filter paper in it

Figure 28:Left side: Marked filter papers to avoid damage of eggs while rolling the paper; Right side: Shoots with inoculation material for the 4th inoculation

Table 26: Mean values and standard deviations of 2nd PAM measurement, both chambers

Mean ch 195	Light			Dark			Calculated Parameters						
2nd measurement	Fm'	Fo'-Calc	F	F/Fo	Fm=Fm'	Y(II)-measur	Y(II)	Y(NPQ)	Y(NO)	NPQ	qN	qP	qL
3rd	2.349	0.681	1.636	0.741	3.210	0.305	0.306	0.183	0.511	0.379	0.323	0.433	0.192
StaDev	0.242	0.034	0.305	0.040	0.167	0.084	0.084	0.060	0.100	0.147	0.100	0.119	0.078
8th	2.398	0.650	1.343	0.689	3.013	0.439	0.439	0.114	0.447	0.264	0.245	0.603	0.305
StaDev	0.189	0.027	0.211	0.028	0.132	0.079	0.079	0.046	0.072	0.121	0.088	0.107	0.094
3rd (grape)	2.348	0.683	1.599	0.743	3.222	0.320	0.321	0.182	0.497	0.384	0.327	0.453	0.205
StaDev	0.242	0.035	0.306	0.037	0.166	0.089	0.089	0.055	0.094	0.138	0.090	0.123	0.083
3rd (no grape	2.349	0.679	1.693	0.737	3.190	0.282	0.283	0.184	0.533	0.373	0.315	0.400	0.170
StaDev	0.250	0.035	0.305	0.046	0.171	0.073	0.073	0.069	0.107	0.164	0.117	0.107	0.068
8th (grape)	2.329	0.642	1.270	0.686	3.020	0.453	0.453	0.125	0.421	0.305	0.274	0.627	0.325
StaDev	0.180	0.023	0.146	0.028	0.158	0.061	0.061	0.049	0.051	0.128	0.089	0.082	0.076
8th (no grape	2.500	0.662	1.451	0.693	3.001	0.419	0.418	0.098	0.484	0.205	0.203	0.569	0.276
StaDev	0.156	0.029	0.248	0.029	0.082	0.099	0.099	0.039	0.082	0.083	0.070	0.130	0.112
ALL	2.374	0.665	1.488	0.715	3.110	0.373	0.373	0.148	0.478	0.321	0.283	0.519	0.249
StaDev	0.217	0.034	0.299	0.043	0.179	0.105	0.105	0.064	0.092	0.146	0.101	0.141	0.103
Mean ch 213	Light			Dark			Calculated Parameters						
2nd measurement	Fm'	Fo'-Calc	F	F/Fo	Fm=Fm'	Y(II)-measur	Y(II)	Y(NPQ)	Y(NO)	NPQ	qN	qP	qL
3rd	2.100	0.638	1.391	0.713	3.178	0.338	0.338	0.224	0.439	0.523	0.405	0.485	0.231
StaDev	0.187	0.037	0.214	0.040	0.209	0.080	0.080	0.049	0.070	0.147	0.070	0.110	0.073
8th	2.232	0.604	1.147	0.648	2.919	0.481	0.481	0.117	0.402	0.324	0.268	0.661	0.357
StaDev	0.231	0.051	0.121	0.063	0.314	0.073	0.073	0.089	0.093	0.229	0.165	0.092	0.090
3rd (grape)	2.044	0.625	1.323	0.704	3.198	0.354	0.354	0.232	0.414	0.572	0.430	0.510	0.250
StaDev	0.172	0.038	0.221	0.045	0.231	0.078	0.078	0.046	0.062	0.135	0.059	0.110	0.075
3rd (no grape	2.177	0.657	1.485	0.725	3.150	0.315	0.315	0.211	0.474	0.455	0.371	0.451	0.205
StaDev	0.183	0.029	0.166	0.029	0.176	0.079	0.079	0.052	0.067	0.138	0.071	0.104	0.064
8th (grape)	2.253	0.595	1.143	0.633	2.894	0.490	0.490	0.102	0.408	0.290	0.246	0.668	0.356
StaDev	0.198	0.062	0.116	0.075	0.362	0.058	0.058	0.086	0.112	0.181	0.180	0.082	0.085
8th (no grape	2.203	0.617	1.153	0.668	2.954	0.468	0.468	0.138	0.393	0.370	0.298	0.651	0.358
StaDev	0.273	0.028	0.131	0.035	0.238	0.090	0.090	0.090	0.059	0.282	0.139	0.106	0.099
ALL	2.166	0.621	1.269	0.680	3.048	0.409	0.409	0.170	0.420	0.423	0.337	0.573	0.294
StaDev	0.219	0.048	0.212	0.062	0.296	0.105	0.105	0.089	0.084	0.216	0.143	0.134	0.103

Table 27: Mean values and standard deviations of 3rd PAM measurement, both chambers

Mean ch 195	Light				Dark			Calculated Parameters						
3rd measurement	Fm'	Fo'-Calc	Fo'-measure	F	F/Fo	Fm=Fm'	Y(II)-measured	Y(II)	Y(NPQ)	Y(NO)	NPQ	qN	qP	qL
3rd	1.716	0.589	0.744	1.529	0.695	3.016	0.110	0.110	0.382	0.509	0.772	0.512	0.167	0.065
StaDev	0.179	0.046	0.051	0.186	0.067	0.239	0.032	0.032	0.061	0.062	0.200	0.076	0.047	0.020
8th	1.573	0.532	0.668	1.299	0.628	2.826	0.175	0.175	0.364	0.460	0.817	0.525	0.266	0.112
StaDev	0.169	0.027	0.041	0.166	0.030	0.143	0.036	0.036	0.055	0.059	0.220	0.074	0.057	0.032
3rd (grape)	1.724	0.583	0.737	1.509	0.683	3.016	0.126	0.126	0.373	0.501	0.756	0.508	0.190	0.074
StaDev	0.157	0.057	0.045	0.172	0.076	0.293	0.033	0.033	0.052	0.042	0.162	0.060	0.047	0.020
3rd (no grape)	1.707	0.596	0.751	1.551	0.707	3.015	0.092	0.092	0.391	0.517	0.790	0.517	0.143	0.056
StaDev	0.205	0.032	0.058	0.203	0.056	0.174	0.021	0.021	0.069	0.078	0.237	0.092	0.033	0.017
8th (grape)	1.521	0.524	0.663	1.247	0.625	2.831	0.181	0.181	0.377	0.441	0.878	0.546	0.278	0.119
StaDev	0.145	0.028	0.039	0.153	0.034	0.167	0.036	0.036	0.049	0.053	0.223	0.065	0.057	0.031
8th (no grape)	1.632	0.542	0.674	1.358	0.632	2.821	0.168	0.168	0.350	0.482	0.748	0.501	0.254	0.104
StaDev	0.179	0.024	0.043	0.165	0.025	0.114	0.036	0.036	0.059	0.060	0.200	0.078	0.056	0.032
ALL	1.646	0.561	0.707	1.416	0.662	2.922	0.142	0.142	0.373	0.485	0.794	0.518	0.216	0.088
StaDev	0.187	0.048	0.060	0.210	0.062	0.218	0.048	0.047	0.058	0.065	0.210	0.075	0.072	0.035
Mean ch 213	Light				Dark			Calculated Parameters						
3rd measurement	Fm'	Fo'-Calc	Fo'-measure	F	F/Fo	Fm=Fm'	Y(II)-measured	Y(II)	Y(NPQ)	Y(NO)	NPQ	qN	qP	qL
3rd	1.731	0.581	0.733	1.515	0.679	3.015	0.126	0.126	0.370	0.504	0.757	0.505	0.191	0.074
StaDev	0.173	0.037	0.048	0.179	0.041	0.173	0.031	0.031	0.054	0.064	0.192	0.073	0.047	0.022
8th	1.530	0.523	0.663	1.252	0.618	2.764	0.184	0.184	0.362	0.454	0.825	0.529	0.282	0.121
StaDev	0.159	0.033	0.055	0.170	0.036	0.165	0.039	0.039	0.040	0.066	0.209	0.069	0.065	0.039
3rd (grape)	1.769	0.577	0.741	1.537	0.666	2.987	0.132	0.132	0.353	0.515	0.702	0.485	0.197	0.075
StaDev	0.179	0.039	0.052	0.176	0.039	0.161	0.025	0.025	0.053	0.056	0.174	0.069	0.038	0.017
3rd (no grape)	1.691	0.586	0.724	1.491	0.694	3.044	0.120	0.120	0.388	0.492	0.816	0.527	0.184	0.074
StaDev	0.162	0.035	0.043	0.184	0.040	0.186	0.036	0.036	0.050	0.072	0.199	0.072	0.056	0.027
8th (grape)	1.553	0.524	0.659	1.257	0.618	2.795	0.192	0.192	0.357	0.451	0.815	0.526	0.292	0.124
StaDev	0.152	0.031	0.070	0.154	0.033	0.149	0.027	0.026	0.037	0.056	0.193	0.062	0.047	0.030
8th (no grape)	1.505	0.521	0.666	1.247	0.619	2.732	0.175	0.175	0.367	0.458	0.835	0.532	0.271	0.118
StaDev	0.168	0.036	0.035	0.190	0.040	0.178	0.047	0.047	0.044	0.078	0.231	0.077	0.079	0.047
ALL	1.631	0.552	0.698	1.383	0.649	2.889	0.155	0.155	0.366	0.479	0.791	0.517	0.236	0.098
StaDev	0.194	0.046	0.062	0.218	0.049	0.210	0.045	0.045	0.047	0.070	0.202	0.071	0.073	0.039

Table 28: Mean values and standard deviations of 4th PAM measurement, both chambers

Mean ch 195	Light				Dark			Calculated Parameters						
4th measurement	Fm'	Fo'-Calc	Fo'-measure	F	F/Fo	Fm=Fm'	Y(II)-measured	Y(II)	Y(NPQ)	Y(NO)	NPQ	qN	qP	qL
3rd	1.766	0.760	0.744	1.588	1.036	4.578		0.103	0.549	0.348	1.621	0.714	0.183	0.090
StaDev	0.191	0.057	0.043	0.213	0.067	0.239		0.029	0.038	0.051	0.299	0.050	0.054	0.031
8th	1.517	0.676	0.679	1.262	0.951	4.274		0.169	0.535	0.296	1.828	0.746	0.305	0.166
StaDev	0.110	0.038	0.027	0.114	0.062	0.259		0.032	0.030	0.028	0.222	0.030	0.060	0.040
3rd (grape)	1.717	0.759	0.741	1.530	1.045	4.500		0.111	0.548	0.341	1.651	0.721	0.200	0.101
StaDev	0.194	0.066	0.049	0.220	0.069	0.232		0.033	0.041	0.053	0.308	0.050	0.059	0.034
3rd (no grape	1.818	0.761	0.748	1.649	1.026	4.660		0.094	0.551	0.355	1.589	0.708	0.164	0.077
StaDev	0.177	0.047	0.037	0.194	0.065	0.222		0.022	0.037	0.049	0.294	0.050	0.042	0.024
8th (grape)	1.501	0.671	0.678	1.235	0.945	4.216		0.178	0.529	0.293	1.825	0.745	0.322	0.177
StaDev	0.135	0.043	0.032	0.127	0.061	0.232		0.031	0.035	0.030	0.255	0.035	0.055	0.037
8th (no grape	1.533	0.681	0.681	1.290	0.957	4.335		0.159	0.542	0.298	1.831	0.747	0.287	0.154
StaDev	0.074	0.033	0.022	0.094	0.064	0.279		0.032	0.023	0.026	0.189	0.024	0.061	0.041
ALL	1.641	0.718	0.712	1.425	0.993	4.426		0.136	0.542	0.322	1.724	0.730	0.244	0.128
StaDev	0.199	0.064	0.049	0.236	0.077	0.291		0.045	0.035	0.048	0.281	0.044	0.084	0.052
Mean ch 213	Light				Dark			Calculated Parameters						
4th measurement	Fm'	Fo'-Calc	Fo'-measure	F	F/Fo	Fm=Fm'	Y(II)-measured	Y(II)	Y(NPQ)	Y(NO)	NPQ	qN	qP	qL
3rd	2.831	0.866	0.758	0.753	0.970	4.249	0.734	0.734	0.088	0.178	0.528	0.397	1.065	1.244
StaDev	0.554	0.085	0.142	0.136	0.105	0.378	0.009	0.009	0.034	0.031	0.193	0.155	0.043	0.160
8th	2.455	0.829	0.674	0.695	0.970	4.293	0.717	0.717	0.120	0.163	0.750	0.508	1.084	1.297
StaDev	0.119	0.047	0.035	0.037	0.068	0.331	0.013	0.013	0.012	0.016	0.129	0.049	0.028	0.103
3rd (grape)	2.898	0.869	0.768	0.762	0.971	4.267	0.736	0.736	0.084	0.179	0.521	0.381	1.066	1.249
StaDev	0.756	0.085	0.193	0.184	0.092	0.373	0.008	0.008	0.044	0.041	0.238	0.206	0.050	0.190
3rd (no grape	2.761	0.863	0.747	0.743	0.970	4.230	0.731	0.731	0.093	0.177	0.535	0.413	1.065	1.239
StaDev	0.188	0.087	0.058	0.056	0.120	0.395	0.009	0.009	0.018	0.017	0.138	0.074	0.036	0.127
8th (grape)	2.451	0.828	0.666	0.690	0.974	4.369	0.718	0.718	0.123	0.158	0.786	0.521	1.086	1.307
StaDev	0.121	0.037	0.037	0.039	0.054	0.246	0.009	0.009	0.011	0.012	0.113	0.041	0.026	0.097
8th (no grape	2.460	0.830	0.683	0.700	0.967	4.213	0.715	0.715	0.117	0.168	0.712	0.494	1.081	1.286
StaDev	0.121	0.056	0.032	0.035	0.082	0.394	0.016	0.016	0.014	0.019	0.138	0.054	0.031	0.111
ALL	2.643	0.848	0.716	0.724	0.970	4.271	0.725	0.725	0.104	0.170	0.639	0.452	1.075	1.271
StaDev	0.441	0.070	0.111	0.103	0.088	0.354	0.014	0.014	0.030	0.026	0.198	0.127	0.037	0.136

no data- unknown reason

ANNEX

Table 29: Results of statistical analysis as p-values of the 2nd PAM measurement with red values being significantly different (<0.05)

	Ch 195 vs Ch 213	Temp.	ETR	Fm'	Fo'-Calc	Fo'-measured	F	F/Fo	Fm=Fm'	Fv	Fv/Fm	Y(II)-measured	Y(II)	Y(NPQ)	Y(NO)	NPQ	qN	qP	qL
Control vs Phylloxera	leaf 3 no grape	0.0000000	0.0068252	0.0298214	0.7070315	0.0280579	0.5136762	0.4706520	0.2781553	0.4641958	0.5187794	0.2840468	0.2170675	0.2819667	0.1258281	0.1111641	0.1469799	0.0766080	0.0766080
	Leaf 3. grape	0.0000000	0.0038769	0.0000066	0.0000009	0.0006805	0.0015679	0.6777821	0.1659733	0.7442443	0.0000060	0.1685239	0.0011112	0.0006338	0.0000125	0.0000256	0.0933706	0.0511959	0.0511959
	Leaf 8, no grape	0.0000000	0.0001785	0.0004592	0.0000504	0.0001855	0.0267012	0.4309589	0.1285021	0.6663072	0.1238478	0.1280610	0.0961092	0.0008805	0.0274935	0.0159108	0.0508223	0.0297531	0.0297531
	Leaf 8, grape	0.0000000	0.0000476	0.1599391	0.0012059	0.0014274	0.0025214	0.1196593	0.0330143	0.2597331	0.0001095	0.0341593	0.2527526	0.5818345	0.7414187	0.4933937	0.0854968	0.1717283	0.1717283
	Both leaves, grape	0.0000000	0.0003095	0.0000186	0.0000001	0.0001055	0.0001859	0.1749111	0.0788294	0.5126241	0.0000000	0.0809878	0.3940220	0.0064332	0.0178730	0.1610182	0.0650084	0.0529257	0.0529257
	Both leaves, no grape	0.0000000	0.0003292	0.0000460	0.4493284	0.0002342	0.9428602	0.6478889	0.1460646	0.5761291	0.1672899	0.1473691	0.0606813	0.2084890	0.0074782	0.0057958	0.0572206	0.0225541	0.0225541
	Leaf 3	0.0000000	0.0000054	0.0000000	0.0087621	0.0000004	0.2629275	0.8266256	0.0471581	0.5572743	0.0005254	0.0480925	0.0024504	0.1355594	0.0000167	0.0000244	0.0111273	0.0028530	0.0028530
	Leaf 8	0.0000000	0.0000006	0.0000001	0.0000000	0.0000002	0.0000197	0.1183783	0.0219146	0.43002239	0.0000000	0.0224900	0.1415849	0.0000626	0.0017206	0.0226192	0.0100040	0.0046267	0.0046267
	All plants	0.0000000	0.0000005	0.0000000	0.0001909	0.0000002	0.0225224	0.6087669	0.0251211	0.9982367	0.0000030	0.0259437	0.0640386	0.1267827	0.0004162	0.0059746	0.0093866	0.0033800	0.0033800
	Within Chamber																		
Control	Leaf 3 - Leaf 8, no grape	0.8847072	0.0000010	0.0345577	0.6095736	0.0223085	0.9397056	0.9573114	0.0001212	0.9630418	0.5173069	0.0001261	0.0002062	0.2901387	0.0013791	0.0026994	0.0002015	0.0015060	0.0015060
	Leaf 3 - Leaf 8, grape	0.1623934	0.0000001	0.7559718	0.0000150	0.0000256	0.0000002	0.0000595	0.0000002	0.0004646	0.0848232	0.0000002	0.0003159	0.0011128	0.0399876	0.0419398	0.0000007	0.0000030	0.0000059
	Leaf 3 - Leaf 8	0.2118796	0.0001283	0.7768464	0.8236975	0.0073081	0.6678633	0.9059185	0.0023997	0.9810909	0.1917003	0.0023764	0.1066066	0.2177596	0.4629510	0.5276029	0.0018819	0.0027637	0.0000030
	Grape - no grape	0.1011804	0.0122948	0.0838176	0.5885079	0.0495979	0.3648570	0.2319814	0.1507484	0.2055349	0.1626986	0.1489695	0.3516549	0.2449129	0.0826201	0.0669591	0.0769346	0.0540017	0.0000232
	Leaf 3 grape - Leaf 3 no grape	0.4979637	0.0467510	0.9600142	0.2972660	0.4107421	0.2897184	0.2725677	0.1889794	0.2716491	0.4801329	0.1910831	0.9212887	0.3013795	0.8196546	0.7369334	0.1272425	0.0812929	0.0027637
	Leaf 8 grape - Leaf 8 no grape	0.1318596	0.0051087	0.0022677	0.0250503	0.0123784	0.4701697	0.6191208	0.2097949	0.4278887	0.1328980	0.2043788	0.0517837	0.0102694	0.0038546	0.0058458	0.1174947	0.1328171	0.0057204
Phylloxera	Leaf 3 - Leaf 8, no grape	0.2899274	0.0000058	0.7439745	0.0002014	0.0000001	0.0000074	0.0084627	0.0000050	0.03207741	0.24275695	0.0000049	0.0059968	0.0005659	0.2618508	0.0589205	0.0000020	0.0000059	0.0540017
	Leaf 3 - Leaf 8, grape	0.6758630	0.0000000	0.0002315	0.0420732	0.0009545	0.0002669	0.0010024	0.0000000	0.00166958	0.60706603	0.0000000	0.0000001	0.8085696	0.0000001	0.0000406	0.0000008	0.0000232	0.3448106
	Leaf 3 - Leaf 8	0.3842987	0.0016792	0.4098580	0.0071810	0.0031347	0.0014756	0.0681780	0.0012275	0.14854725	0.02632552	0.0012161	0.0101299	0.1521821	0.1048321	0.0505967	0.0019782	0.0057204	0.0812929
	Grape - no grape	0.0937069	0.3069859	0.3904968	0.0057033	0.0694356	0.0286498	0.9285855	0.2026042	0.66341794	4.2109E-05	0.2018477	0.7009713	0.2019023	0.7064234	0.9115001	0.2081155	0.3448106	0.0379343
	Leaf 3 grape - Leaf 3 no grape	0.0556974	0.1164147	0.0205814	0.0035519	0.0089301	0.0723906	0.4366979	0.1219135	0.20628679	0.00258358	0.1218824	0.1709535	0.0051055	0.0092172	0.0075225	0.0796893	0.0379343	0.1328171
	Leaf 8 grape - Leaf 8 no grape	0.6835770	0.6097790	0.5147779	0.1213257	0.8048019	0.0553514	0.5185117	0.3755785	0.73658835	0.00606198	0.3728909	0.1957261	0.5952213	0.2989448	0.2943510	0.5796563	0.9584139	0.9584139

Table 30: p-values as results of statistical analysis of the 3rd PAM measurement with red values being significantly different (<0.05)

	Ch 195 vs Ch 213	Temp.	ETR	Fm'	Fo'-Calc	Fo'-measured	F	F/Fo	Fm=Fm'	Fv	Fv/Fm	'(II)-measure	Y(II)	Y(NPQ)	Y(NO)	NPQ	qN	qP	qL
Control vs Phylloxera	leaf 3 no grap	0.9210605	0.0171849	0.7942418	0.3733255	0.1390905	0.3738166	0.4160321	0.6479306	0.4559916	0.2161463	0.0111728	0.0117404	0.8917207	0.3463934	0.7331907	0.7082827	0.0157114	0.0286179
	Leaf 3. grape	0.2720516	0.6110507	0.4202275	0.7075396	0.7922429	0.6275678	0.4012561	0.7141463	0.8460268	0.2437862	0.5529845	0.5410489	0.2598672	0.4021598	0.3434008	0.2845391	0.6556836	0.9104003
	Leaf 8, no gra	0.6238148	0.5498415	0.0357175	0.0621905	0.5587198	0.0666357	0.2589406	0.0954339	0.0980425	0.4262457	0.5769454	0.5816591	0.3635730	0.3385257	0.2529370	0.2608905	0.4794661	0.2974862
	Leaf 8, grape	0.4690797	0.4267654	0.5170877	0.9637630	0.8360415	0.8458143	0.5404116	0.5030697	0.5046735	0.8700704	0.3150870	0.3118111	0.1738431	0.6118152	0.3661549	0.3541849	0.4220273	0.6073859
	Both leaves,	0.1901827	0.4902082	0.3836848	0.8042806	0.9899961	0.7020575	0.3582277	0.5338872	0.6130137	0.3648084	0.4030382	0.3957561	0.0772400	0.4140891	0.2074419	0.1702080	0.5126085	0.7146447
	Both leaves,	0.6621169	0.1510454	0.1192473	0.1318899	0.2140436	0.0965308	0.2890739	0.5166382	0.6596128	0.4953543	0.1370372	0.1398550	0.6566104	0.1756520	0.2903321	0.2928652	0.1044235	0.0813074
	Leaf 3	0.3231566	0.1274395	0.4004699	0.1593235	0.3810921	0.2487408	0.1707576	0.4593241	0.6341760	0.2702848	0.1085440	0.1090795	0.8008563	0.3976811	0.6520630	0.7197879	0.0933863	0.0935278
	Leaf 8	0.1794361	0.3622144	0.6915607	0.3241454	0.8376966	0.5538073	0.1983018	0.2058480	0.2277395	0.8941549	0.3139827	0.3106923	0.3738068	0.9650917	0.7480934	0.7072862	0.3356640	0.3413289
	All plants	0.2239987	0.1305154	0.6384817	0.2429891	0.4265506	0.3682565	0.1664622	0.3660152	0.4976491	0.3235488	0.0999356	0.0998014	0.4197610	0.6171006	0.9226748	0.9168768	0.1064094	0.1281770
	within chambers																		
Control	Leaf 3 - Leaf 8	0.2043889	0.0000001	0.2657521	0.0000055	0.0001178	0.0049809	0.0000487	0.0006921	0.0157610	0.0322860	0.0000001	0.0000001	0.0733704	0.1593622	0.5846864	0.6018887	0.0000004	0.0000238
	Leaf 3 - Leaf 8	0.6700618	0.0000403	0.0002916	0.0005738	0.0000090	0.0000295	0.0066693	0.0273165	0.0478075	0.0245945	0.0000320	0.0000329	0.7909958	0.0006573	0.0696185	0.0771981	0.0000151	0.0025020
	Leaf 3 - Leaf 8	0.3290770	0.0005127	0.0730479	0.0081991	0.0083125	0.0080480	0.0210712	0.1598495	0.3178598	0.0238799	0.0004948	0.0005065	0.6970062	0.0298697	0.4325832	0.4729873	0.0004667	0.0000142
	Grape - no gr	0.2427335	0.0274956	0.2814183	0.1466738	0.3833730	0.1159811	0.2556137	0.9664399	0.6408773	0.0349209	0.0305692	0.0314020	0.7845573	0.0693898	0.3505906	0.3278655	0.0297131	0.0000018
	Leaf 3 grape -	0.1764255	0.0012667	0.7949382	0.4026899	0.4347474	0.5068977	0.2895672	0.9915031	0.7023377	0.0813452	0.0009131	0.0009849	0.3835613	0.4727862	0.6270847	0.7480852	0.0016871	0.0009235
	Leaf 8 grape -	0.7453297	0.2024713	0.0452050	0.0496305	0.4216969	0.0387481	0.4734667	0.8477782	0.6832971	0.1561334	0.2419524	0.2443854	0.1517570	0.0456363	0.0818768	0.0791736	0.2144889	0.0291901
Phylloxera	Leaf 3 - Leaf 8	0.4016822	0.0006871	0.0025613	0.0000083	0.0001347	0.0005912	0.0000049	0.0000205	0.0001330	0.7137893	0.0005944	0.0006051	0.1865269	0.2003481	0.7926376	0.8622046	0.0009773	0.0404252
	Leaf 3 - Leaf 8	0.4155721	0.0000001	0.0004360	0.0000935	0.0003567	0.0000141	0.0003782	0.0007299	0.0016106	0.4071751	0.0000000	0.0000000	0.7760958	0.0014996	0.0747388	0.0656158	0.0000001	0.7290092
	Leaf 3 - Leaf 8	0.5197306	0.0058498	0.2418878	0.0210533	0.0631287	0.0702755	0.0069105	0.0442152	0.0889473	0.2047286	0.0046589	0.0046084	0.2739525	0.3097792	0.9949880	0.9916413	0.0093564	0.0074447
	Grape - no gr	0.6867444	0.1961411	0.1736544	0.7805628	0.7494742	0.5947017	0.2286885	0.9513948	0.6750174	0.0196326	0.1778842	0.1738991	0.0464679	0.6522668	0.1684295	0.1571818	0.3337339	0.9603091
	Leaf 3 grape -	0.7251807	0.2316332	0.1818434	0.4765109	0.3032356	0.4602585	0.0462268	0.3428306	0.5733119	0.1656182	0.2509203	0.2447452	0.0483989	0.3008116	0.0820472	0.0821090	0.4510578	0.1576442
	Leaf 8 grape -	0.8274446	0.2549117	0.3847038	0.8017874	0.6969406	0.8593717	0.9333856	0.2668171	0.1755651	0.0503182	0.2006106	0.1983207	0.4991260	0.7357468	0.7762071	0.8130367	0.3390593	0.6460484

ANNEX

Table 31: p-values as results of statistical analysis of the 4th PAM measurement with red values being significantly different (<0.05)

	Ch 195 vs Ch 213	Temp.	ETR	Fm'	Fo'-Calc	Fo'-measured	F	F/Fo	Fm=Fm'	Fv	Fv/Fm	Y(II)-measured	Y(II)	Y(NPQ)	Y(NO)	NPQ	qN	qP	qL
Control vs Phylloxera	leaf 3 no grape	0.084634769	0.170570475	0.000144652	0.001589487	0.96362363	0.000378816	0.105373287	0.00060694	0.00016881	0.05510855	#DIV/0!	0.127550867	0.2878937	0.106967004	0.11626437	0.094764798	0.052591743	0.024351213
	Leaf 3. grape	0.053849515	0.718499197	2.94777E-06	1.17982E-05	0.000374152	1.24706E-06	0.002816387	0.01212022	0.03569313	0.53835984	#DIV/0!	0.000118789	0.95267158	0.000338206	0.00198426	0.002289314	6.55207E-05	7.39927E-05
	Leaf 8, no grape	0.218609964	0.167266474	0.472704852	0.395415682	0.080673446	0.217431181	0.561099316	0.55473638	0.3877683	0.139127	#DIV/0!	0.001187938	0.88990588	0.139298183	0.49901333	0.351126106	0.004532746	0.014638746
	Leaf 8, grape	0.000376038	0.782999968	0.00348264	0.170796364	0.328826365	0.007458282	0.141443684	0.06207546	0.05734552	0.57991723	#DIV/0!	0.566705633	0.00564644	0.000944117	0.00047193	0.000541218	0.117503412	0.034796015
	Both leaves, grape	6.21869E-05	0.748834783	2.82007E-07	5.45605E-05	0.002092551	7.78779E-07	0.148618042	0.36380002	0.50992632	0.44975596	#DIV/0!	0.00080798	0.07671223	6.43354E-06	4.6744E-06	8.43271E-06	6.29018E-05	1.12345E-05
	Both leaves, no grape	0.033812902	0.165072785	0.111216954	0.093933883	0.092970308	0.281901165	0.375767762	0.00394318	0.00118544	0.01507383	#DIV/0!	0.201726625	0.47630928	0.8144012	0.57986963	0.858378377	0.407015785	0.587563117
	Leaf 3	0.004421443	0.166490296	0.000241514	7.12361E-05	0.592823463	0.000680006	0.01880484	0.00011988	9.6234E-05	0.13360064	#DIV/0!	0.075323166	0.55795171	0.075771026	0.01820751	0.092506397	0.025028526	0.009604317
	Leaf 8	5.66527E-05	0.2953004	0.003073578	0.003718176	0.739426214	0.007371459	0.411805736	0.27408354	0.28616541	0.69515105	#DIV/0!	0.137060925	0.18096678	0.030508859	0.00100919	0.026989976	0.028017763	0.005981431
	All plants	1.44181E-05	0.21288914	2.76476E-05	6.32607E-05	0.764451547	0.000206018	0.103771914	0.00549687	0.00418651	0.18965325	#DIV/0!	0.098667825	0.09496862	0.011159583	0.00036675	0.007209959	0.018843974	0.003931407
							within chambers												
Control	Leaf 3 - Leaf 8, no grape	0.385908651	4.07524E-07	4.25768E-06	3.67813E-06	8.3484E-07	5.07866E-07	0.00389292	0.00072396	0.00127292	0.86964361	#DIV/0!	1.93522E-07	0.43968655	0.000313429	0.00790269	0.007652445	1.50636E-07	6.01134E-07
	Leaf 3 - Leaf 8, grape	0.009276549	6.1911E-07	0.000548116	0.0000573	9.36677E-05	3.71768E-05	5.29022E-05	0.00082904	0.00874601	0.0455125	#DIV/0!	3.93259E-07	0.14166528	0.002710493	0.0738518	0.097935077	2.81885E-07	2.53211E-07
	Leaf 3 - Leaf 8	0.051055918	0.00088631	0.004193797	0.015713102	0.012753719	0.001562129	0.071211715	0.00646474	0.00725886	0.62113141	#DIV/0!	0.000795897	0.27303081	0.027425178	0.14696885	0.136317578	0.000568033	0.000508503
	Grape - no grape	0.033620429	0.096537624	0.16429548	0.686866428	0.662388077	0.125310537	0.849049386	0.04362919	0.01155957	0.00557222	#DIV/0!	0.100189394	0.33058946	0.413322908	0.67983922	0.581710912	0.07408256	0.058798061
	Leaf 3 grape - Leaf 3 no grape	0.53130603	0.071176611	0.117326238	0.895858182	0.627319491	0.098423135	0.404221824	0.04449385	0.01326673	0.0128178	#DIV/0!	0.083993947	0.8313161	0.41997793	0.54452907	0.446928164	0.043487418	0.02049831
	Leaf 8 grape - Leaf 8 no grape	0.018341208	0.107491777	0.393911055	0.451598402	0.747557423	0.156580061	0.570195587	0.17826392	0.13268636	0.21085811	#DIV/0!	0.092332161	0.18402436	0.602756639	0.93395301	0.889045663	0.083852689	0.089778279
Phylloxera	Leaf 3 - Leaf 8, no grape	0.350381905	5.50913E-05	0.580283083	0.737320786	0.640578236	0.753071397	0.939566895	0.78464564	0.75423217	0.9128586	0.111477035	0.148893144	0.34126446	0.842381313	0.89695578	0.629587229	0.196141714	0.250388049
	Leaf 3 - Leaf 8, grape	0.73981181	1.21197E-06	0.636567087	0.791270166	0.123101305	0.610613132	0.694164937	0.16455707	0.12222942	0.10709118	0.718747289	0.763188419	0.24572768	0.213988497	0.23368142	0.202509595	0.752000148	0.88007155
	Leaf 3 - Leaf 8	0.88772968	0.018059609	0.270718879	0.317708834	0.258999023	0.121215191	0.946678094	0.50457576	0.43597236	0.47240625	0.138851201	0.009551979	0.88749432	0.074231565	0.08332689	0.196317411	0.010142849	0.015061577
	Grape - no grape	0.880381923	0.753597466	0.001443876	0.007947344	0.002275447	3.32455E-05	0.902637011	0.60135094	0.5019326	0.42450501	1.37041E-07	1.7334E-10	0.9086046	1.66991E-05	0.00014524	0.001171123	1.54803E-10	5.90488E-10
	Leaf 3 grape - Leaf 3 no grape	0.575829789	0.398446096	0.01292104	0.119268853	0.000611007	0.00606023	0.869572854	0.92069327	0.94417604	0.98182491	0.003873356	5.78227E-05	0.16229376	0.00193102	0.02871707	0.02571802	5.27382E-05	8.19775E-05
	Leaf 8 grape - Leaf 8 no grape	0.447435588	0.916340133	0.029909951	0.034809824	0.047278042	0.007958604	0.985277481	0.35665414	0.27874264	0.25549533	7.88437E-07	1.17244E-07	0.45230278	0.00314048	0.00232481	0.017976249	2.33703E-07	7.65699E-07

ANNEX

Table 32: Mean values and standard deviations of 1st HandyPEA measurement, both chambers (ch 195 & 213)

mean ch 195 (01.07.)																
Leaf	**Fo**	**Fm**	**Fv**	**Fv/Fm**	**Tfm**	**Area**	**F1**	**F2**	**F3**	**F4**	**F5**	**Dark Offset**	**RC / ABS**	**Fv / Fo**	**(1-Vj) / Vj**	**PI**
3rd	**666.20**	**3643.32**	**2977.12**	**0.82**	**407.32**	**54925.59**	**768.49**	**863.73**	**1235.68**	**1983.00**	**3264.61**	**14.34**	**1.09**	**4.48**	**0.56**	**2.75**
StaDev	34.59	172.06	153.39	0.01	397.86	11408.15	44.11	56.51	104.27	200.29	168.59	0.76	0.15	0.24	0.06	0.64
8th	**619.08**	**3459.40**	**2840.33**	**0.82**	**341.25**	**59787.78**	**703.33**	**780.28**	**1096.10**	**1792.90**	**3015.60**	**14.33**	**1.24**	**4.61**	**0.59**	**3.42**
StaDev	289.24	1433.27	343.42	1432.32	181.61	30536.37	30357.92	77.49	183.60	377.86	633.91	1513.18	6.61	1.72	2.05	1.52
3rd (grape)	**672.88**	**3661.96**	**2989.08**	**0.82**	**460.00**	**56762.76**	**776.72**	**872.92**	**1244.36**	**1978.32**	**3271.08**	**14.44**	**1.09**	**4.45**	**0.56**	**2.78**
StaDev	34.50	150.72	136.38	0.01	504.65	13633.21	43.71	56.74	106.34	213.11	146.49	0.82	0.16	0.26	0.07	0.71
3rd (no grape)	**655.75**	**3614.19**	**2958.44**	**0.82**	**325.00**	**52055.00**	**755.63**	**849.38**	**1222.13**	**1990.31**	**3254.50**	**14.19**	**1.09**	**4.51**	**0.55**	**2.71**
StaDev	33.10	202.77	179.92	0.01	52.92	5937.83	42.92	54.80	102.85	185.00	203.17	0.66	0.13	0.22	0.05	0.54
8th (grape)	**622.04**	**3449.83**	**2827.79**	**0.82**	**339.17**	**59744.46**	**708.00**	**786.50**	**1106.13**	**1799.04**	**3003.17**	**14.54**	**1.22**	**4.58**	**0.58**	**3.33**
StaDev	61.21	154.50	158.04	0.02	97.26	9303.58	73.49	87.71	127.40	186.26	148.16	0.66	0.15	0.46	0.05	0.76
8th (no grape)	**614.63**	**3473.75**	**2859.13**	**0.82**	**344.38**	**59852.75**	**696.31**	**770.94**	**1081.06**	**1783.69**	**3034.25**	**14.00**	**1.28**	**4.66**	**0.59**	**3.55**
StaDev	30.14	137.19	124.19	0.01	68.31	8149.25	37.15	45.09	72.57	143.82	121.32	1.03	0.15	0.25	0.05	0.77
ALL	642.93	3552.49	2909.57	0.82	374.69	57326.67	736.31	822.52	1166.75	1889.12	3141.64	14.33	1.16	4.54	0.57	3.08
StaDev	49.14	183.91	163.37	0.01	289.57	10411.27	62.25	77.34	126.87	207.57	197.71	0.81	0.17	0.33	0.06	0.77
mean ch 213 (01.07.)																
Leaf	**Fo**	**Fm**	**Fv**	**Fv/Fm**	**Tfm**	**Area**	**F1**	**F2**	**F3**	**F4**	**F5**	**Dark Offset**	**RC / ABS**	**Fv / Fo**	**(1-Vj) / Vj**	**PI**
3rd	**680.07**	**3565.05**	**2884.98**	**0.81**	**325.35**	**50916.93**	**790.19**	**892.26**	**1300.74**	**2104.07**	**3213.70**	**14.79**	**0.98**	**4.30**	**0.50**	**2.29**
StaDev	83.94	208.64	218.96	0.03	78.48	12911.33	107.77	134.32	211.23	294.28	204.86	0.74	0.21	0.58	0.09	0.89
8th	**614.21**	**3359.63**	**2745.42**	**0.82**	**332.56**	**57439.49**	**699.16**	**776.40**	**1101.16**	**1817.35**	**2937.37**	**14.70**	**1.19**	**4.51**	**0.56**	**3.11**
StaDev	61.73	161.62	167.24	0.02	61.45	10810.13	78.28	95.54	152.87	232.54	158.61	0.71	0.18	0.48	0.07	0.81
3rd (grape)	**661.64**	**3548.56**	**2886.92**	**0.81**	**304.40**	**53955.88**	**765.64**	**861.84**	**1253.76**	**2046.60**	**3163.76**	**14.96**	**1.04**	**4.43**	**0.52**	**2.56**
StaDev	79.72	186.21	207.98	0.03	37.20	12442.07	105.77	135.21	218.96	292.11	194.91	0.73	0.22	0.59	0.10	0.93
3rd (no grape)	**705.67**	**3587.94**	**2882.28**	**0.80**	**354.44**	**46696.17**	**824.28**	**934.50**	**1366.00**	**2183.89**	**3283.06**	**14.56**	**0.90**	**4.14**	**0.48**	**1.91**
StaDev	85.09	240.09	239.51	0.03	108.26	12683.09	103.85	124.56	186.54	286.17	203.21	0.70	0.17	0.54	0.09	0.70
8th (grape)	**606.96**	**3353.08**	**2746.12**	**0.82**	**320.40**	**59715.00**	**691.24**	**766.64**	**1086.68**	**1791.72**	**2911.60**	**14.68**	**1.20**	**4.57**	**0.57**	**3.23**
StaDev	60.65	122.72	145.70	0.02	46.23	11258.50	75.32	91.35	139.44	207.41	112.76	0.75	0.18	0.51	0.07	0.85
8th (no grape)	**624.28**	**3368.72**	**2744.44**	**0.81**	**349.44**	**54279.06**	**710.17**	**789.94**	**1121.28**	**1852.94**	**2973.17**	**14.72**	**1.16**	**4.43**	**0.55**	**2.94**
StaDev	63.53	207.66	197.80	0.02	76.12	9569.37	83.13	102.15	171.89	265.61	204.62	0.67	0.19	0.44	0.07	0.74
ALL	**647.14**	**3462.34**	**2815.20**	**0.81**	**328.95**	**54178.21**	**744.67**	**834.33**	**1200.95**	**1960.71**	**3075.53**	**14.74**	**1.08**	**4.41**	**0.53**	**2.70**
StaDev	80.38	212.34	206.00	0.02	70.16	12283.06	104.22	129.69	208.97	300.51	229.09	0.72	0.22	0.54	0.09	0.94

ANNEX

Table 33: Mean values and standard deviations of 2nd HandyPEA measurement, both chambers (ch 195 & 213)

mean ch195 (14.07.)																
Leaf	Fo	Fm	Fv	Fv/Fm	Tfm	Area	F1	F2	F3	F4	F5	Dark Offset	RC / ABS	Fv / Fo	(1-Vj) / Vj	PI
3rd	795.46	3998.00	3202.54	0.80	218.34	40399.11	0.05	921.54	0.10	1040.77	0.30	1490.06	2.00	2349.74	30.00	3745.49
StaDev	113.55	215.92	205.31	0.03	167.54	18498.49	0.00	145.90	0.00	181.04	0.00	284.10	0.00	419.31	0.00	287.43
8th	711.67	3835.67	3124.00	0.81	333.61	63075.36	0.05	805.00	0.10	892.00	0.30	1235.97	2.00	1979.03	30.00	3374.58
StaDev	77.67	231.64	206.60	0.02	131.15	15642.63	0.00	93.90	0.00	113.21	0.00	164.97	0.00	247.50	0.00	267.84
3rd (grape)	768.53	4043.37	3274.84	0.81	179.00	45517.68	0.05	888.11	0.10	999.16	0.30	1437.05	2.00	2306.47	30.00	3729.63
StaDev	105.05	65.59	102.92	0.03	106.73	20141.32	0.00	138.71	0.00	174.34	0.00	274.40	0.00	371.34	0.00	212.33
3rd (no grape	827.44	3944.13	3116.69	0.79	265.06	34320.81	0.05	961.25	0.10	1090.19	0.30	1553.00	2.00	2401.13	30.00	3764.31
StaDev	118.22	307.92	261.44	0.02	213.71	14705.02	0.00	148.55	0.00	181.72	0.00	291.21	0.00	477.36	0.00	363.96
8th (grape)	690.11	3828.16	3138.05	0.82	298.95	67048.21	0.05	779.05	0.10	861.37	0.30	1192.37	2.00	1916.37	30.00	3327.16
StaDev	59.08	278.97	229.73	0.01	82.05	10775.74	0.00	65.08	0.00	73.21	0.00	95.64	0.00	160.67	0.00	287.59
8th (no grape	735.76	3844.06	3108.29	0.81	372.35	58635.12	0.05	834.00	0.10	926.24	0.30	1284.71	2.00	2049.06	30.00	3427.59
StaDev	90.01	172.29	183.04	0.02	164.41	19103.43	0.00	113.27	0.00	140.16	0.00	210.86	0.00	308.36	0.00	241.29
ALL	752.97	3915.69	3162.72	0.81	276.79	51896.93	0.05	862.45	0.10	965.34	0.30	1361.23	2.00	2161.77	30.00	3557.42
StaDev	105.16	236.97	208.28	0.02	160.01	20467.49	0.00	134.88	0.00	167.15	0.00	263.02	0.00	388.42	0.00	332.98
mean ch213 (14.07.)																
Leaf	Fo	Fm	Fv	Fv/Fm	Tfm	Area	F1	F2	F3	F4	F5	Dark Offset	RC / ABS	Fv / Fo	(1-Vj) / Vj	PI
3rd	823.59	4051.91	3228.32	0.80	150.35	33808.62	0.05	951.50	0.10	1074.74	0.30	1523.53	2.00	2387.74	30.00	3839.35
StaDev	126.88	90.71	149.82	0.03	103.70	14549.03	0.00	163.36	0.00	204.82	0.00	326.37	0.00	487.21	0.00	194.70
8th	736.74	3830.69	3089.38	0.81	307.19	59300.06	0.05	840.72	0.10	935.03	0.30	1300.75	2.00	2092.19	30.00	3388.97
StaDev	134.87	188.12	243.07	0.04	117.84	18833.67	0.00	166.48	0.00	195.59	0.00	274.59	0.00	368.89	0.00	230.30
3rd (grape)	808.11	4032.50	3224.39	0.80	158.61	38227.56	0.05	922.67	0.10	1032.78	0.30	1434.28	2.00	2227.06	30.00	3765.39
StaDev	95.62	122.10	149.62	0.02	100.77	15128.29	0.00	123.77	0.00	154.74	0.00	258.82	0.00	452.22	0.00	209.61
3rd (no grape	862.65	4074.00	3211.35	0.79	134.35	27359.59	0.05	1012.53	0.10	1158.35	0.30	1679.18	2.00	2629.88	30.00	3931.71
StaDev	175.73	15.22	173.94	0.04	109.51	13559.11	0.00	225.11	0.00	281.81	0.00	425.84	0.00	524.05	0.00	140.88
8th (grape)	746.28	3853.06	3106.78	0.81	293.33	59943.50	0.05	848.61	0.10	944.72	0.30	1319.61	2.00	2125.39	30.00	3406.06
StaDev	155.12	170.75	226.84	0.04	85.20	20691.18	0.00	185.08	0.00	218.24	0.00	309.43	0.00	423.59	0.00	219.35
8th (no grape	726.65	3814.41	3087.76	0.81	338.24	59430.71	0.05	820.41	0.10	909.59	0.30	1257.41	2.00	2032.53	30.00	3376.12
StaDev	113.48	203.01	254.00	0.04	152.69	15774.21	0.00	133.44	0.00	155.94	0.00	215.29	0.00	279.63	0.00	237.74
ALL	785.67	3943.47	3157.80	0.80	230.99	46321.63	0.05	900.61	0.10	1010.71	0.30	1421.31	2.00	2251.50	30.00	3618.84
StaDev	145.54	181.57	209.57	0.04	141.40	21415.42	0.00	183.29	0.00	225.48	0.00	344.44	0.00	477.28	0.00	310.54

ANNEX

Table 34: Mean values and standard deviations of 3rd HandyPEA measurement, both chambers (ch 195 & 213)

mean ch195 (05.08.)																
Leaf	Fo	Fm	Fv	Fv/Fm	Tfm	Area	F1	F2	F3	F4	F5	Dark Offset	RC / ABS	Fv / Fo	(1-Vj) / Vj	PI
3rd	639.97	3439.29	2799.31	0.81	566.57	63347.57	0.05	734.54	0.10	824.29	0.30	1158.77	2.00	1817.23	30.00	3096.37
StaDev	58.72	233.56	203.71	0.01	579.26	44336.55	0.00	76.18	0.00	98.03	0.00	149.88	0.00	187.81	0.00	237.58
8th	587.34	3184.26	2596.91	0.82	429.71	70554.49	0.05	659.97	0.10	727.60	0.30	996.83	2.00	1585.63	30.00	2758.54
StaDev	52.86	313.90	266.26	0.01	450.71	85420.05	0.00	59.32	0.00	67.07	0.00	97.30	0.00	169.42	0.00	267.24
3rd (grape)	643.33	3386.28	2742.94	0.81	640.56	72205.61	0.05	734.17	0.10	820.89	0.30	1142.06	2.00	1780.61	30.00	3038.56
StaDev	65.65	227.29	197.17	0.02	695.95	58272.53	0.00	78.51	0.00	95.15	0.00	118.81	0.00	150.53	0.00	211.41
3rd (no grape)	636.41	3495.41	2859.00	0.82	488.24	53968.47	0.05	734.94	0.10	827.88	0.30	1176.47	2.00	1856.00	30.00	3157.59
StaDev	52.16	233.50	198.80	0.01	430.98	19685.50	0.00	76.04	0.00	103.79	0.00	179.13	0.00	218.57	0.00	254.38
8th (grape)	576.11	3117.28	2541.17	0.81	346.11	56878.78	0.05	647.11	0.10	713.22	0.30	976.28	2.00	1543.72	30.00	2674.72
StaDev	68.74	399.63	333.21	0.01	131.20	15331.08	0.00	77.08	0.00	86.49	0.00	122.16	0.00	199.36	0.00	317.94
8th (no grape)	599.24	3255.18	2655.94	0.82	518.24	85034.65	0.05	673.59	0.10	742.82	0.30	1018.59	2.00	1630.00	30.00	2847.29
StaDev	24.99	171.17	159.66	0.01	630.23	121746.98	0.00	27.98	0.00	33.66	0.00	57.33	0.00	121.24	0.00	167.58
ALL	613.66	3311.77	2698.11	0.81	498.14	66951.03	0.05	697.26	0.10	775.94	0.30	1077.80	2.00	1701.43	30.00	2927.46
StaDev	61.47	303.20	256.46	0.01	519.80	67655.10	0.00	77.49	0.00	96.55	0.00	149.62	0.00	212.43	0.00	303.23
mean ch213 (05.08.)																
Leaf	Fo	Fm	Fv	Fv/Fm	Tfm	Area	F1	F2	F3	F4	F5	Dark Offset	RC / ABS	Fv / Fo	(1-Vj) / Vj	PI
3rd	634.71	3443.57	2808.86	0.82	446.00	59257.51	0.05	728.00	0.10	815.46	0.30	1150.20	2.00	1829.14	30.00	3077.71
StaDev	35.31	221.43	199.54	0.01	440.64	30473.54	0.00	48.94	0.00	63.67	0.00	109.73	0.00	165.89	0.00	202.78
8th	591.00	3163.60	2572.60	0.81	384.86	56603.66	0.05	665.71	0.10	734.17	0.30	1009.14	2.00	1601.71	30.00	2727.60
StaDev	20.36	171.02	167.63	0.01	151.57	10229.92	0.00	24.73	0.00	30.35	0.00	57.28	0.00	103.44	0.00	125.94
3rd (grape)	628.61	3470.33	2841.72	0.82	522.22	64431.72	0.05	718.50	0.10	803.61	0.30	1128.83	2.00	1794.56	30.00	3083.94
StaDev	30.75	152.67	126.01	0.00	604.73	39495.07	0.00	41.13	0.00	54.51	0.00	93.82	0.00	139.73	0.00	167.35
3rd (no grape)	641.18	3415.24	2774.06	0.81	365.29	53778.94	0.05	738.06	0.10	828.00	0.30	1172.82	2.00	1865.76	30.00	3071.12
StaDev	39.48	278.87	255.41	0.01	102.84	15937.08	0.00	55.53	0.00	71.64	0.00	123.20	0.00	186.99	0.00	239.86
8th (grape)	589.11	3201.89	2612.78	0.82	355.56	58633.72	0.05	665.06	0.10	734.33	0.30	1011.28	2.00	1596.56	30.00	2735.89
StaDev	21.74	156.13	147.99	0.01	118.13	8678.93	0.00	25.24	0.00	30.46	0.00	57.95	0.00	113.89	0.00	132.78
8th (no grape)	593.00	3123.06	2530.06	0.81	415.88	54454.18	0.05	666.41	0.10	734.00	0.30	1006.88	2.00	1607.18	30.00	2718.82
StaDev	19.25	181.26	180.84	0.01	178.89	11524.19	0.00	24.92	0.00	31.18	0.00	58.26	0.00	94.31	0.00	121.71
ALL	612.86	3303.59	2690.73	0.81	415.43	57930.59	0.05	696.86	0.10	774.81	0.30	1079.67	2.00	1715.43	30.00	2902.66
StaDev	36.10	241.77	218.23	0.01	328.54	22604.04	0.00	49.65	0.00	64.24	0.00	112.23	0.00	178.75	0.00	243.24

Table 35: p-values as results of statistical analysis of the 1st HandyPEA measurement with red values being significantly different (<0.05)

			fo	fv	fm	fvfm	PI
control	no grape	Leaf 3-Leaf 8	**0.001**	**0.029**	0.079	0.093	**0.000**
	with grape	leaf 3-leaf 8	**0.001**	**0.000**	**0.000**	0.430	**0.012**
phylloxera	no grape	Leaf 3-Leaf 8	**0.003**	**0.006**	0.068	0.123	**0.000**
	with grape	Leaf 3-Leaf 8	**0.002**	**0.000**	**0.008**	0.397	**0.010**
control	grape- no grape		0.255	0.738	0.972	0.288	0.636
phylloxera	grape- no grape		0.081	0.576	0.945	0.155	**0.022**
Control vs Phylloxera			0.685	**0.004**	**0.001**	**0.037**	**0.005**

Table 36: p-values as results of statistical analysis of the 2nd HandyPEA measurement with values printed in bold type being significantly different (<0.05)

			fo	fv	fm	fvfm	PI
control	no grape	Leaf 3-Leaf 8	**0.017**	0.254	0.915	**0.039**	**0.000**
	with grape	leaf 3-leaf 8	**0.007**	**0.002**	**0.023**	0.119	**0.000**
phylloxera	no grape	Leaf 3-Leaf 8	**0.012**	**0.000**	0.108	0.143	**0.000**
	with grape	Leaf 3-Leaf 8	0.159	**0.001**	0.075	0.565	0.276
control	grape- no grape		**0.041**	0.448	0.057	**0.005**	**0.011**
phylloxera	grape- no grape		0.620	0.974	0.753	0.623	0.178
Control vs Phylloxera			0.128	0.436	0.889	0.172	0.611

Table 37: p-values as results of statistical analysis of the 3rd HandyPEA measurement with values printed in bold type being significantly different (<0.05)

			fo	fv	fm	fvfm	PI
control	no grape	Leaf 3-Leaf 8	**0.012**	**0.002**	**0.003**	0.499	**0.039**
	with grape	leaf 3-leaf 8	**0.005**	**0.018**	**0.034**	0.227	**0.000**
phylloxera	no grape	Leaf 3-Leaf 8	**0.000**	**0.001**	**0.003**	0.629	**0.010**
	with grape	Leaf 3-Leaf 8	**0.000**	**0.000**	**0.000**	0.174	0.135
control	grape- no grape		0.585	0.089	0.059	0.088	0.693
phylloxera	grape- no grape		0.344	0.250	0.151	**0.006**	**0.019**
Control vs Phylloxera			0.925	0.860	0.855	0.851	0.473

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Table 38: CF mean values of CIRAS, 1st measurement; red lines represent fructifying plants

Messtermin	Treatment	Pflanze	Trieb	Blatt	Fo (Fv/Fm)	Fm (Fv/Fm)	Fv (Fv/Fm)	FvFm (Fv/Fm)	Fs (phiPS2 DA)	Fs (phiPS2 LA)	Fm' (phiPS2 DA)	Fm' (phiPS2LA)	PS2 (phiPS2 DA)	PS2 (phiPS2 LA)	qP (phiPS2 DA)	qP (phiPS2 LA)	qNP (phiPS2 DA)	NPQ (phiPS2 DA)	ETR (phiPS2 DA)	ETR (phiPS2 LA)	Fo' (Fo Prime LA)	Fv' (Fo Prime LA)
08.07.2014	Control	4	1	8	453	1693	1240	0.7324	1048	718	1076	733	0.0260	0.0205	0.0449	0.0377	0.4976	0.5734	10.8310	8.5862	335	398
08.07.2014	Control	7	1	8	465	1721	1256	0.7298	930	981	969	1068	0.0402	0.0815	0.0774	0.1265	0.5987	0.7761	16.8026	34.2135	380	688
08.07.2014	Control	8	1	8	406	1630	1224	0.7509	676	716	746	834	0.0938	0.1415	0.2059	0.2511	0.7222	1.1850	39.4102	59.6027	364	470
08.07.2014	Control	18	1	8	435	1690	1255	0.7426	1172	831	1200	919	0.0233	0.0958	0.0366	0.1522	0.3904	0.4083	9.8000	40.3383	341	578
08.07.2014	Control	12	1	8	421	1630	1209	0.7417	1036	861	1073	912	0.0345	0.0559	0.0567	0.0890	0.4607	0.5191	14.4538	23.5573	339	573
08.07.2014	Control	22	1	8	457	1718	1261	0.7340	963	770	999	907	0.0360	0.1510	0.0664	0.2455	0.5702	0.7197	15.1654	63.6302	349	558
08.07.2014	Control	27	1	8	467	1721	1254	0.7286	885	771	911	833	0.0285	0.0744	0.0586	0.1276	0.6459	0.8891	11.9868	31.4481	347	486
08.07.2014	Control	30	1	8	451	1693	1242	0.7336	807	938	825	1078	0.0218	0.1299	0.0481	0.1972	0.6989	1.0521	9.1636	54.4364	368	710
08.07.2014	Control	36	1	8	437	1682	1245	0.7402	704	949	735	1089	0.0422	0.1286	0.1040	0.1947	0.7606	1.2884	17.6434	53.9405	370	719
08.07.2014	Control	37	1	8	428	1710	1282	0.7497	771	871	790	1045	0.0241	0.1665	0.0525	0.2551	0.7176	1.1646	10.0609	69.6533	363	682
08.07.2014	Control	40	1	8	392	1758	1366	0.7770	813	759	838	827	0.0298	0.0822	0.0561	0.1453	0.6735	1.0979	12.5674	34.5345	359	468
08.07.2014	Control	41	1	8	415	1730	1315	0.7601	731	872	785	1068	0.0688	0.1835	0.1459	0.2689	0.7186	1.2038	28.8917	77.4640	339	729
08.07.2014	Phylloxera	44	1	8	380	1533	1153	0.7521	806	641	867	708	0.0704	0.0946	0.1253	0.1618	0.5776	0.7682	29.5502	39.5470	294	414
08.07.2014	Phylloxera	46	1	8	332	1413	1081	0.7650	708	629	769	749	0.0793	0.1602	0.1396	0.2740	0.5957	0.8375	33.4826	67.4243	311	438
08.07.2014	Phylloxera	49	1	8	385	1718	1333	0.7759	676	617	733	749	0.0778	0.1762	0.1638	0.2940	0.7389	1.3438	32.8563	74.8329	300	449
08.07.2014	Phylloxera	51	1	8	357	1586	1229	0.7749	572	563	646	634	0.1146	0.1120	0.2561	0.2205	0.7648	1.4551	48.1115	47.1288	312	322
08.07.2014	Phylloxera	56	1	8	327	1322	995	0.7526	684	512	737	568	0.0719	0.0986	0.1293	0.1905	0.5879	0.7938	30.0827	41.2428	274	294
08.07.2014	Phylloxera	62	1	8	349	1579	1230	0.7790	626	568	669	704	0.0643	0.1932	0.1344	0.3417	0.7398	1.3602	27.0765	81.2175	306	398
08.07.2014	Phylloxera	66	1	8	327	1482	1155	0.7794	569	549	612	659	0.0703	0.1669	0.1509	0.3005	0.7532	1.4216	29.5393	70.0361	293	366
08.07.2014	Phylloxera	73	1	8	326	1347	1021	0.7580	635	764	660	907	0.0379	0.1577	0.0749	0.2379	0.6729	1.0409	15.9568	66.0859	306	601
08.07.2014	Phylloxera	77	1	8	339	1435	1096	0.7638	591	675	638	784	0.0737	0.1390	0.1572	0.2500	0.7272	1.2492	30.9095	58.1593	348	436
08.07.2014	Phylloxera	79	1	8	328	1451	1123	0.7739	623	588	665	709	0.0632	0.1707	0.1246	0.3079	0.6999	1.1820	26.3672	71.5351	316	393
08.07.2014	Phylloxera	80	1	8	332	1490	1158	0.7772	654	614	704	782	0.0710	0.2148	0.1344	0.3478	0.6788	1.1165	29.7102	90.2302	299	483
08.07.2014	Phylloxera	84	1	8	378	1612	1234	0.7655	562	561	602	686	0.0664	0.1822	0.1786	0.3434	0.8185	1.6777	27.7674	76.7602	322	364

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Table 39: CF mean values of CIRAS, 2nd measurement; red coloumns represent fructifying plants

Messtermin	Treatment	Pflanze	Trieb	Blatt	Fo (Fv/Fm)	Fm (Fv/Fm)	Fv (Fv/Fm)	Fv/Fm (Fv/Fm)	s (phiPS2 DA	Fs (phiPS2 LA)	Fm' (phiPS2 DA)	Fm' (phiPS2LA)	PS2 (phiPS2 DA)	PS2 (phiPS2 LA)	P (phiPS2 DA	qP (phiPS2 LA)	NP (phiPS2 D	PQ (phiPS2 D	TR (phiPS2 D	TR (phiPS2 LA	' (Fo Prime L	' (Fo Prime L
23.07.2014	Control	7	1	8	344	1432	1088	0.7598	604	865	629	1008	0.0397	0.1419	0.0877	0.2141	0.7381	1.2766	16.7099	59.7621	340	668
23.07.2014	Control	27	1	8	348	1526	1178	0.7720	585	702	619	853	0.0549	0.1770	0.1255	0.3139	0.7699	1.4653	22.8849	74.7954	372	481
23.07.2014	Control	12	1	8	373	1616	1243	0.7692	722	605	766	683	0.0574	0.1142	0.1120	0.2086	0.6838	1.1097	24.2942	48.1567	309	374
23.07.2014	Control	18	1	8	309	1488	1179	0.7923	561	560	579	683	0.0311	0.1801	0.0667	0.3106	0.7710	1.5699	13.1223	76.0151	287	396
23.07.2014	Control	36	1	8	299	1538	1239	0.8056	555	532	603	631	0.0796	0.1569	0.1579	0.2929	0.7546	1.5506	33.4328	65.8295	293	338
23.07.2014	Control	40	1	8	323	1485	1162	0.7825	565	506	605	639	0.0661	0.2081	0.1418	0.3878	0.7573	1.4545	27.7686	87.3304	296	343
23.07.2014	Control	8	1	8	338	1513	1175	0.7766	710	613	736	701	0.0353	0.1255	0.0653	0.2217	0.6613	1.0557	14.8815	52.8829	304	397
23.07.2014	Control	4	1	8	358	1610	1252	0.7776	682	631	732	698	0.0683	0.0960	0.1337	0.1777	0.7013	1.1995	28.7459	40.0733	321	377
23.07.2014	Control	22	1	8	341	1441	1100	0.7634	469	493	518	637	0.0946	0.2261	0.2768	0.4390	0.8391	1.7819	39.8092	95.5147	309	328
23.07.2014	Control	30	1	8	318	1559	1241	0.7960	465	500	497	624	0.0644	0.1987	0.1788	0.3780	0.8558	2.1368	27.0152	83.1277	296	328
23.07.2014	Control	37	1	8	310	1441	1131	0.7849	537	616	563	743	0.0462	0.1709	0.1028	0.2974	0.7763	1.5595	19.5125	72.2208	316	427
23.07.2014	Control	41	1	8	305	1431	1126	0.7869	589	559	623	660	0.0546	0.1530	0.1069	0.2928	0.7176	1.2970	23.0589	64.2085	315	345
23.07.2014	Phylloxera	49	1	8	298	1417	1119	0.7897	665	603	705	662	0.0567	0.0891	0.0983	0.1715	0.6363	1.0099	24.0204	37.2074	318	344
23.07.2014	Phylloxera	44	1	8	310	1473	1163	0.7895	600	561	647	639	0.0726	0.1221	0.1395	0.2161	0.7102	1.2767	30.5100	51.1138	278	361
23.07.2014	Phylloxera	56	1	8	305	1402	1097	0.7825	621	475	667	583	0.0690	0.1852	0.1271	0.3661	0.6700	1.1019	28.9366	77.4932	288	295
23.07.2014	Phylloxera	79	1	8	301	1375	1074	0.7811	498	566	533	669	0.0657	0.1540	0.1509	0.2791	0.7840	1.5797	27.4970	64.7930	300	369
23.07.2014	Phylloxera	62	1	8	303	1446	1143	0.7905	609	594	646	710	0.0573	0.1634	0.1079	0.2829	0.6999	1.2384	23.9595	68.8256	300	410
23.07.2014	Phylloxera	84	1	8	295	1436	1141	0.7946	489	474	534	604	0.0843	0.2152	0.1883	0.3927	0.7905	1.6891	35.1101	90.3974	273	331
23.07.2014	Phylloxera	51	1	8	310	1496	1186	0.7928	585	564	624	626	0.0625	0.0990	0.1242	0.1902	0.7352	1.3974	26.1450	41.6806	300	326
23.07.2014	Phylloxera	46	1	8	299	1425	1126	0.7902	578	508	613	598	0.0571	0.1505	0.1115	0.2885	0.7211	1.3246	23.7886	62.9579	286	312
23.07.2014	Phylloxera	73	1	8	308	1514	1206	0.7966	680	592	712	741	0.0449	0.2011	0.0792	0.3311	0.6650	1.1264	18.9330	84.1156	291	450
23.07.2014	Phylloxera	66	1	8	300	1412	1112	0.7875	558	550	583	659	0.0429	0.1654	0.0883	0.2876	0.7455	1.4220	17.9743	69.1910	280	379
23.07.2014	Phylloxera	80	1	8	288	1343	1055	0.7856	471	539	503	665	0.0636	0.1895	0.1488	0.3549	0.7962	1.6700	26.7998	79.5789	310	355
23.07.2014	Phylloxera	77	1	8	315	1576	1261	0.8001	622	541	675	670	0.0785	0.1925	0.1472	0.3525	0.7145	1.3348	33.0437	81.1083	304	366

Table 40: CF mean values of CIRAS, 3rd measurement; red cells represent fructifying plants

Messtermin	Treatment	Pflanze	Trieb	Blatt	Fo (Fv/Fm)	Fm (Fv/Fm)	Fv (Fv/Fm)	FvFm (Fv/Fm	s (phiPS2 DA	s (phiPS2 LA	n' (phiPS2 D	m' (phiPS2LA	S2 (phiPS2 D	S2 (phiPS2 LA	P (phiPS2 DA	P (phiPS2 LA	NP (phiPS2 D	PQ (phiPS2 D	TR (phiPS2 DA	TR (phiPS2 LA	' (Fo Prime L	" (Fo Prime L
21.08.2014	control	4	1	8	523	2147	1624	0.7564	918	861	966	907	0.0497	0.0507	0.1084	0.0985	0.7272	1.2226	20.8070	21.2797	440	467
21.08.2014	control	7	1	8	515	2254	1739	0.7715	970	785	1018	873	0.0472	0.1008	0.0954	0.2028	0.7108	1.2141	19.7837	41.9981	439	434
21.08.2014	control	8	1	8	459	2107	1648	0.7822	921	678	981	808	0.0612	0.1609	0.1149	0.3163	0.6833	1.1478	25.0459	67.3715	397	411
21.08.2014	control	30	1	8	478	2234	1756	0.7860	720	710	762	801	0.0551	0.1136	0.1479	0.2370	0.8383	1.9318	23.1033	47.7631	417	384
21.08.2014	control	27	1	8	482	2107	1625	0.7712	777	771	824	861	0.0570	0.1045	0.1374	0.2036	0.7895	1.5570	24.0282	44.0780	419	442
21.08.2014	control	12	1	8	531	2171	1640	0.7554	870	741	910	844	0.0440	0.1220	0.1055	0.2482	0.7689	1.3857	17.9631	51.4097	429	415
21.08.2014	control	41	1	8	474	2056	1582	0.7695	766	862	837	979	0.0848	0.1195	0.1956	0.2241	0.7705	1.4564	35.6272	49.8427	457	522
21.08.2014	control	40	1	8	489	2189	1700	0.7766	825	683	907	800	0.0904	0.1462	0.1962	0.3103	0.7541	1.4135	37.8194	61.6093	423	377
21.08.2014	control	37	1	8	498	2170	1672	0.7705	845	753	900	864	0.0611	0.1285	0.1368	0.2600	0.7596	1.4111	25.5127	53.5806	437	427
21.08.2014	control	36	1	8	492	2154	1662	0.7716	845	782	884	878	0.0441	0.1093	0.0995	0.2192	0.7641	1.4367	18.5294	46.0603	440	438
21.08.2014	control	22	1	8	478	2172	1694	0.7799	661	593	724	727	0.0870	0.1843	0.2561	0.4467	0.8548	2.0000	36.5470	77.1044	427	300
21.08.2014	control	18	1	8	489	2149	1660	0.7725	768	722	803	820	0.0436	0.1195	0.1115	0.2487	0.8108	1.6762	18.3064	50.1951	426	394
21.08.2014	phylloxera	44	1	8	519	2274	1755	0.7718	1058	892	1123	984	0.0579	0.0935	0.1076	0.1704	0.6558	1.0249	24.3585	39.5039	444	540
21.08.2014	phylloxera	73	1	8	476	2060	1584	0.7689	816	836	867	998	0.0588	0.1623	0.1304	0.3640	0.7532	1.3760	24.5329	68.3809	553	445
21.08.2014	phylloxera	71	1	8	487	2101	1614	0.7682	699	611	761	721	0.0815	0.1526	0.2263	0.3537	0.8302	1.7608	34.0813	64.2699	410	311
21.08.2014	phylloxera	66	1	8	489	2031	1542	0.7592	774	762	834	833	0.0719	0.0852	0.1739	0.1788	0.7763	1.4353	30.0647	35.6551	436	397
21.08.2014	phylloxera	49	1	8	518	2266	1748	0.7714	854	872	911	957	0.0626	0.0888	0.1450	0.1641	0.7752	1.4874	26.2788	37.0056	439	518
21.08.2014	phylloxera	46	1	8	441	1804	1363	0.7555	722	611	812	667	0.1108	0.0840	0.2426	0.1986	0.7278	1.2217	46.1793	35.1918	385	282
21.08.2014	phylloxera	51	1	8	483	2059	1576	0.7654	762	772	803	901	0.0511	0.1432	0.1281	0.2727	0.7970	1.5641	21.0800	60.2535	428	473
21.08.2014	phylloxera	79	1	8	466	1998	1532	0.7668	756	755	826	882	0.0847	0.1440	0.1944	0.3031	0.7650	1.4189	35.5220	60.7181	463	419
21.08.2014	phylloxera	77	1	8	492	2120	1628	0.7679	800	710	866	837	0.0762	0.1517	0.1765	0.3167	0.7703	1.4480	31.1450	63.6639	436	401
21.08.2014	phylloxera	62	1	8	460	2066	1606	0.7773	806	771	859	876	0.0617	0.1199	0.1328	0.2215	0.7516	1.4051	25.6547	50.3425	402	474
21.08.2014	phylloxera	84	1	8	465	2105	1640	0.7791	724	578	764	705	0.0524	0.1801	0.1338	0.4006	0.8177	1.7552	21.8576	75.8109	388	317

Table 41: p-values as results of statistical analysis of CIRAS measurements (only CF data); xxx meaning no significant difference (<0.05)

		First measurement CIRAS																	
		Fo	Fm	Fv	FvFm	Fs 1	Fs 2	Fm´1	Fm´2	PS2 1	PS2 2	qP1	qP2	qNP	NPQ	ETR1	ETR2	Fo'	Fv'
control	Grape - no grape	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
phylloxera	Grape - no grape	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
	Control - phylloxera	0.000	0.000	0.002	0.000	0.000	0.000	0.000	0.000	0.000	0.016	0.002	0.002	0.082	0.029	0.000	0.017	0.000	0.000
		Second measurement CIRAS																	
control	Grape - no grape	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
phylloxera	Grape - no grape	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
	Control - phylloxera	0.002	0.025		0.035	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	0.025	xxx
		Third measurementCIRAS																	
control	Grape - no grape	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
phylloxera	Grape - no grape	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx
	Control - phylloxera	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx	xxx

Affidavit

Last Name: Pfisterer

First Name: Jo

Date of Birth: 10/01/1987

I declare under penalty of perjury that I have produced this work independently, without unauthorized assistance of third parties and without the use of any other than the specified resources. Data and concepts, acquired directly or indirectly from other sources are identified by indicating the references. This also applies to figures, graphs, illustrations and the like, as well as, to Internet sources and unpublished sources.

Bozen, 12.02.2016 _____

Jo Pfisterer