Development of a Field Method to Assess Root Parasites of Woody Plants - Ecological Analyses of the Grapevine - Phylloxera Interaction

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by Dipl.-Biol. Mark Hoffmann
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Dean: XXXX

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XXXX, examiner
XXXX, co-examiner

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Sie mussten zusammen singen, aber es wollte nicht recht gehen, denn die wirkliche Nachtigall sang auf ihre Weise, und der Kunstvogel ging auf Walzen.

H.C. Andersen, Die Nachtigall, 1844

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1 Aim and Background of the Work

1.1 Aims

Damages in agriculture, horticulture and forestry caused by plant parasites and microbial pathogens continue to be a global problem in terms of both yield and economic losses. Integrated pest management (IPM) is an approach of crop management to solve specific pest problems in agriculture, aiming a significant reduction of the use of pesticides and managing the impact of pests by ecological methods (Kogan, 1998). These methods must be developed culture, pest and region specific and are performed in three stages: prevention, observation and intervention (Kogan, 1998). The **first aim** of the present was the development of an assessment method to quantify parasite-root interactions under field conditions. Regarding the three stages in IPM, the present work can be assigned to the second stage: observation. Generally an assessment of population structure is connected to questions of representative parameters. The focus in the present work was on the statistical comprehensibility of data, an easy handling of the used methods and the theoretical description of field conditions. Currently, no methods exist to quantify parasite-root interaction on woody plants. But generally, the assessment of the dynamics and damage potential of parasites is important to develop both global and local pest management strategies.
The detection of parasitic insect population dynamics and their impact on plant performance demands fundamental knowledge about parasite host relationships in an ecosystem. The **second aim** of the present work was the validation and prediction of ecological aspects of parasite population development under field conditions.

1.2 Background

In the present work, grape phylloxera (*Daktulosphaira vitifoliae* Fitch) was used as model organism for "small insect pests". In insect pest research, the concept of "small insect pests" was introduced with the intension to point out the high methodical difficulties in controlling plant damaging insects with a diminutive individual body size (Morse and Hoddle, 2006). Aphids (Hemiptera) are considered as small insect pests typically (Moran, 1992). Regarding aboveground small insect pests, for example the observation and quantification of migration procedures caused major problems (Frank, 2010). Considering insect root parasites, the features and complexity of rhizosphere and soil agroecosystems lead to an additional complication in evaluation of insect-plant-interactions (Gunning and Cahill, 2009). Especially perennial root systems typically have an increased biomass and higher branching structures in comparison to annual root systems (section 2.4). Further, they often have complex physiological and genetic properties, belonging to their long exposition to multiple stress factors. Additionally, features of woody root systems complicate the assessment of infections and damages in field. Possibilities of an inhomogeneous distributed population (spatial and on a single plant) as well as simultaneous infections by various pests must be included in the employed assessment or control method as well. Parasites which develop their life cycles close to the permanent organs of plants have to deal with highly changing

environments, due to the dynamics in temporal nutrient stocks in host organs or to the dynamics of physiology in perennial host plants. Having a view on these difficulties, it is not surprising that up to now neither suitable methods to evaluate grape root vitality (considering parasites) nor exemplified models were established.

Multiple reasons led to the selection of grape phylloxera (*Daktulosphaira vitifoliae* Fitch) as model organism. Indeed, many questions regarding impact and ecology of grape phylloxera remain not understood, but due to its high economical impact (section 2.3), biology (e.g. Granett et al. (2001b)), life-cycle (see Forneck and Huber (2009)) and population genetic diversity (e.g. Vorwerk and Forneck (2007)) of grape phylloxera are investigated very well. The life stages are strongly linked to host plant organs (Forneck and Huber, 2009) and grape phylloxera is common on roots of grafted vines in cool climate viticulture. Non oviparous and oviparous larval stages on roots could be distinguished with low magnifications and induced root swellings (nodosities) can be detected visibly. So it should be a likely candidate to develop a method to assess root parasites. Furthermore, many efforts were done to evaluate grape phylloxera infestation by a lot of authors before. Granett et al. (1983) and Granett et al. (1985) introduced an *in vitro* method with excised roots to observe grape phylloxera population development and count grape phylloxera instars. Based on this method, Granett et al. (1985) differentiated grape phylloxera biotypes, collected in Californian vineyards. The structure of grape phylloxera populations were assessed by counting instars and eggs and the dynamics of grape phylloxera population was assessed by generating live tables (Granett et al., 1983, 1985). Although the excised root method is very invasive and highly artificial, it was used to assess variability of biotypes (Granett et al., 1985; Song and Granett, 1990; de Benedictis and Granett, 1992; Kocsis et al., 1997), to investigate effects of grape phylloxera infestation on physiology of grape roots (Granett, 1990; Omer et al., 1995; Turley et al., 1996; Ryan et al., 2000; Omer and Granett, 2000), and to assess host suitability of different grapevine cultivars (Granett et al., 1992; de Benedictis and Granett, 1993; Makee et al., 2004). But Granett et al. (2001a) themselves showed a significantly higher performance of grape phylloxera population on *in vitro* "excised root" system (25 times higher on American rootstock cultivars) than on roots in field. Also Korosi et al. (2010) reported high differences in the development of grape phylloxera population between *in vitro* and field trials. So results achieved on unique base of an excised root system can not be assigned to field conditions without further ado. Due to the deficiency of excised root bioassays, Omer and Granett (2000), Granett et al. (2005) and Roush et al. (2007) assessed grape phylloxera instars on excavated and attached roots. Forneck et al. (1996) introduced an *in vitro* aseptic dual culture method to observe interactions between *Vitis* ssp. cuttings and grape phylloxera. This method conduced to investigate fundamental interactions between grape phylloxera and *Vitis* ssp. (Forneck et al., 1996, 1999). Using this method, Forneck et al. (2001a) showed that performance of a given clonal grape phylloxera lineage changed over successive generations, depending on host plant and phylloxera lineages. But in other systems, especially to investigate interactions between *Vitis* ssp. and fungi, this method is hardly practicable (Hammes, 2008). Rootstock tolerance, evaluated by the

aseptic dual culture method, did not correspond with field observations in all cases (Grezegorczyk and Walker, 1998). Artificial circumstances of the aseptic dual culture method (young plant, no soil, temperature conditions, sterile chamber etc.) never were discussed (Grezegorczyk and Walker, 1998; Forneck et al., 1999). Also evident handicaps of a greenhouse based system to evaluate grape phylloxera performance, introduced by Forneck et al. (2001b), never were discussed. Indeed laboratory studies as well as greenhouse experiments are important to identify possible main factors which could impact plant performance. But such studies cannot replace field trials, because they cannot predict the environmental impact on plant development, particularly in perennial cultures. Root systems are affected by room restriction in pots and greenhouses. Furthermore, differences exist in soil structure (field and pitting soil) as well as in temperature between field and laboratory trials (pots and soil often have room temperature). Several reasons make *Vitis* ssp. a suitable perennial and woody model plant in field: (1) Generally a big part of grapevine root system (and a big part of grape phylloxera population) is located in the topmost meter of soil in field. (2) Further, grapevine species and cultivars show a very fast root growth and a high root production in comparison to other woody plants (pers. comm. D. Eissenstat). In the present work, these reasons enabled a sampling method which was easily practicable.

According to Coleman (1985), holistic approaches are necessary for an understanding of the multiple relationships between plant and (agricultural) soil ecosystems, including investigations of abiotic and biotic factors. Particularly the development of management strategies in agriculture requires a broad knowledge of relationships and interactions within the ecosystem. In grapevine, multiple gene loci and molecular interactions are responsible for the plant's reaction on environmental factors (Chiarappa and Buddenhagen, 1994). Huber (2007) and Porten (unpb) were the first who began to develop new vineyard management strategies on base of holistic long-term investigations in Germany. The soil ecosystem was in the focus of Huber (2007), whereas Porten (unpb) focused on aboveground vitality of grapevines. Until now, only the works of Milko (1961) and Nedov and Guler (1987) also used a holistic approach to understand soil ecosystems in vineyards. Huber (2007) showed that soil ecosystems in cool climate vineyards are highly sensitive, in doing so particularly soil and root microflora are affected by management methods (see also Peterson (1958) and Hammes (2008)). Especially damages in grape phylloxera infested vineyards could be related to fungal populations and manuring, but not directly to grape phylloxera infestation. These results led to the suggestion, that particularly pathogen suppressive soil characteristics can prevent general damages in vineyards (Huber, 2007; Huber et al., 2009), particularly due to infections by soil borne pathogens such as *Sorospheara viticola* (Neuhauser et al., 2009), *Roesleria subterranea* (Weinm.) Redhaed (von Thuemen, 1878; Hoefer, 1993), other fungal species (see Huber (2007)) or nematodes (e.g. Lamberti et al. (1989)). The field site which was sampled in the present work was thought to have pathogen suppressive soil conditions (Huber, 2007) and first studies of grape phylloxera population development were done by Porten (unpb).

Generally, the development of assessment methods is often accompanied by great difficulties, depending on biology of the host and parasite. Plants are mainly damaged chemically by microbial organisms

(Cheng et al., 2010), whereas insects mainly cause feeding damages, open transmission paths for other plant pathogens (such as viruses) or disclose infection paths for microorganisms (e.g. Karban and Baldwin (1997)). The location of infected host organs (aboveground or below ground), size, dispersion and persistence of the parasite, the lifetime of the host plant (annual or perennial) as well as the nature of the parasitized tissue (woody, non woody) are fundamental factors that can influence the development of assessment methods.

2 Introduction

2.1 Root System Ecology

Root systems were often investigated and discussed in terms of their general functions such as water and nutrient uptake (e.g. Kozinka (1992)) or anchorage (e.g. Danjon et al. (1999)). Beside these important functions, roots are often part of complex ecosystems. Questions which factors could be influenced by roots and how roots (growth, physiology, root-microbe interactions etc.) are influenced by abiotic and other biotic factors remain poorly understood, especially in woody roots. But more than 30 % of the earth's land mass is covered by woody plants, storing more than 75 % of carbon in terrestrial ecosystems with up to 40 % of biomass in their root systems (Godbold and Brunner, 2007). Even in other cycles than the carbon cycle plants play a major role in connecting aboveground ecosystems with belowground ecosystems. Plants represent the basic trophic level of all associated biotopes (Newton et al., 2010) and roots play an immanent role as nutrient source in soil ecosystems (Gisi, 1997). Particularly the current understanding of woody root dynamics and their regulation by plant and environmental factors is limited (Comas et al., 2010). Although methods were improved during the last years to explore the mostly hard accessible root ecosystems (section 2.4), the time-consuming and difficult techniques to explore especially complex and big root systems still lead to a lack of knowledge of ecosystems including woody plants (Boehm, 1979; Gregory, 2006a). Generally, roots (incl. rhizosphere) can provide high diversity in soil and are a habitat for animals, protozoa, fungi, bacteria and other plants. Associations (parasitic or symbiotic) between members of the edaphone and roots are essential (Price, 1980; Gregory, 2006a). Many plant-important physiological activities take place in the plant root: production of hormones or amino acids, N-fixing, nutrient absorption and storage of reserve substances. Such root functions are influenced by the above mentioned interactions as well as by factors impacting the aboveground plant organs (leaves and shoots) (Hodge, 2009). In turn, influences on root systems can impact also aboveground ecosystems (Poveda et al., 2006). Regarding agroecosystems, yield quantity and/or quality and aboveground plant vitality can be affected by changing root system patterns (Comas et al., 2005; Huber et al., 2009).

Multiple interactions exist between root system and soil system which depends on soil ecosystems and the plant species (van der Putten et al., 2009). Resendes et al. (2008) showed that the general function of woody roots (long-term anchorage or short-term nutrient supply) is determined in a very early stage of development. But roots have to cope with prevailing conditions in the soil environment (e.g. exploitation of nutrient-rich patches or water-zones), so developmental plans may not be determined absolutely (Hodge, 2009). Comas and Eissenstat (2004) suggested general differences in potential soil exploitation and root defense strategies between species differing in potential root growth rate, thereby root physiological patterns were more tied to general plant physiology. Interactions with soil microorganisms can impact both, the growth of lateral roots and physiological parameters, affecting e.g. hormone mechanisms in the root tissue (Contreras-Cornejo et al., 2009).

Generally works dealing with morphology, dynamics or physiology of grape root systems are not very abundant. In all studies usually cultivated vines were investigated. It is known that root growth and root development are highly dependent on the cultivars (Hofmann, 1957; Perry et al., 1983). Most of the grape roots occur in the top 1 m of soil. Depending on the soil and aquifer roots could reach up to 6 m depth (Richards, 1983). Tillage, mulching and irrigation affect root growth (e.g. Comas et al. (2005)). Also abiotic factors such as nutrient supply, temperature or moisture affect the growth of the root system (e.g. Huang et al. (2005)). Anatomy and morphology of grape roots during primary development is similar to that of many other woody plants. Early roots are usually pale in appearance with a 2 - 4 mm root tip and a root hair zone approx. 2mm above the tip and up to 30 mm long. The cortex consists 8 - 10 layers of parenchyma cells with large intercellular spaces. Young roots suberizes in a zone usually between one and several centimeters away from the tip. Browning of young roots (before secondary development) could happen by oxidation of phenoles released from vacuoles of dead or collapsed epidermal cells, leading to a suberization of the rhizodermal cells (Richards, 1983). The secondary development of grape roots begins with the development of a vascular cambium and a cork cambium. Roots change morphologically and physiologically during secondary thickening (Currle et al., 1983). The production of lateral roots usually occurs above the root hair zone (Richards, 1983). But until now only a few studies focused on the dynamics of grape root systems, especially under consideration of grape phylloxera (e.g. Milko (1961) or Bauerle et al. (2007)). As far as known to the author, the present work represents one of the first long-term data-collections in field, basing on periodical quantitative measurements of grape root system parameters. Only some works before focused on the ecology of grape root systems: Steinberg (1968) investigated the root tips and grape phylloxera infestation of different rootstocks in a two year field study. Similar questions were investigated by Bauerle et al. (2007). In the last decade, especially the development of fresh grape roots was investigated in long-term (2-4 years) field studies (Comas et al., 2005), measuring root age and life-span (Anderson et al., 2003; Volder et al., 2005; Comas et al., 2010) or root death (Comas et al., 2000) and relating it to different impact factors. So the timing of grape root production can be responsive to soil moisture, but not the root life-span (Comas et al., 2010). The life-span of primary grape roots is influenced by soil depth, root diameter and time of birth (near bloom, late-season) (Anderson et al., 2003). Nitrate uptake and respiration of grape rootstocks are declining quickly with increasing root age (Volder et al., 2005). Important questions remain whether and how plant-parasite interactions have an impact on root system development of grapevines.

2.2 Parasites

A parasite is an organism living in or on another living organism, obtaining from it part or all of its nutrients, commonly exhibiting some degree of adaptive structural modification, and causing some degree of damage to its host (Price, 1980). Parasitism is the most common way of life, appearing in hundreds of species in every biological kingdom. The borders between parasitism and browsing, grazing,

saprophytic organisms and even symbiosis could be very porose. However, evolution and development of biological diversity can not be understood completely without regarding parasitism. Particularly the evolution of parasitic organisms with small body sizes base on special ecological concepts (Price, 1980). These organisms are adapted to exploit small, discontinuous environments and must have developed strategies to solve the problems in colonizing widely dispersed resources (even within the same patches), e.g. by mass production of spores and eggs, high dispersal of inseminated females, dispersal in time and/or by dispersal by attaching to a larger organism. Parasites represent the extreme in resource exploitation and they are specialized in only one or a few hosts in the most cases. Population development and dispersion of parasites mainly depend on variability of the environment. Environments which are variable in space and stable in time can be related to local monomorphic populations, whereas environments uniform in space but variable in time are related to polymorphism. Evolutionary rates and speciation rates of parasites can be high. Evidence for such high rates may be seen in the many sibling species, subspecies and races observed in parasitic organisms (Price, 1980). Evolutionary questions are still not answered regarding grape phylloxera (Forneck and Huber, 2009). But some authors have regarded a high genetic variability in grape phylloxera populations on both agricultural and native sites (section 2.3) (Downie and Granett, 1999; Vorwerk and Forneck, 2007). Generally, the degree of the development of adaptive radiation within a parasitic taxon is mainly depending upon host diversity, size of the host target, the evolutionary time available and the selective pressure for co-evolutionary modification (Price, 1980).

As mentioned above, parasites form a large proportion of the diversity of life on earth. The present knowledge of both general patterns of interactions between two different species and mechanisms which could lead finally to parasitism is very poor. Especially a huge number of insects develop parasitic forms at least in one life stage (Price, 1980; Moran, 1992). Aphids (*Aphidoidea*) are an approx. 4000 species including insect super-family within the suborder *Sternorrhyncha* and the order *Hemiptera*. Most families within the Aphidoidea are vivipar, but not the family of *Adelgidae* and the *Phylloxeridae*. Aphids as a group are perhaps 200 million years old (Moran, 1992). All aphids have a proboscis and are feeding and living on plants as parasites. The life cycles of aphids are among the most remarkable of any animal group, accompanied by several unusual phenomena like extensive polyphenism, seasonal alternation between two sets of hosts, soldier castes or sex-ratio control (Moran, 1992). They can be divided into two different groups, the non-host-alternating species (monoecious) and the host-alternating species (heteroecious) (Kawada, 1987). The fact that several subfamilies of the *Aphidoidea* are restricted to old groups of woody host plants as well as corresponding fossil evidences let several authors suggest that ancestral aphids have evolved on woody plants (Moran, 1992).

2.3 Grape Phylloxera

Grape phylloxera (*Daktulosphaira vitifoliae* Fitch, Phylloxeridae, Aphidoidea) is a mesophyll sucking, gall-forming aphid, native to North America (Mississippi Valley), feeding on below- and aboveground

organs of susceptible *Vitis* species (Jacob, 1938; Forneck and Huber, 2009). First it was described as leaf feeding aphid by Fitch (1855), who mistook the economical risk emanating from the insect. Vines in Europe and Asia (*Vitis vinifera*) evolved in the absence of grape phylloxera, whereas the insect co-evolved with the *Vitis* species of North America (Chiarappa and Buddenhagen, 1994). But grapevine cultivation only took place in Europe, Africa and Asia for several thousand years (Weeber, 2005). Due to another newly introduced pest (*Uncinula necator* (powdery mildew)), European botanists and wine growers imported American vine planting material, particularly in the period from 1848 to 1862. Different grape phylloxera introductions via American planting material took place during this time and grape phylloxera spread over whole European winegrowing regions, planted with *Vitis vinifera* cultivars. The big grapevine die off in the late 19th century in Europe is due to grape phylloxera impact. The introduction of grafted grapevines (susceptible European cultivars (scion) grafted on co-evolved American cultivars (rootstocks)) at the beginning of the 20th century could prevent the high losses and led to a recovery of European viticulture. On such grafted grapevines, the life cycle of grape phylloxera takes place on the roots of the American cultivar and should not lead to damages or yield losses (Chiarappa and Buddenhagen, 1994; Forneck and Huber, 2009). But in the last three decades, reports of damages related to soil ecosystems are increasing (a good overview gives Huber (2007)). Especially Huber et al. (2009) showed that such damages can be suppressed with integrated pest management approaches and are not caused directly by grape phylloxera. Against such backgrounds it must be suggested that the impact of grape phylloxera can not be evaluated without considering the complex pathosystems in vineyards (Chiarappa and Buddenhagen, 1994). These considerations lead to the question whether world-wide dispersal and/or quarantine restrictions make sense without regarding holistic approaches. Due to the known association of grape phylloxera with bacteria (Hoffmann, 2006; Vorwerk et al., 2007; Lawo and Forneck, 2010), it can be suggested that grape phylloxera could serve also as a vector for other parasitic microorganisms such as *Sorospheara viticola* (Neuhauser et al., 2009). However, grape phylloxera must be regarded as an permanent impact factor in the most winegrowing regions in cool climate viticulture, and among root sucking insects it could be assumed that grape phylloxera still has the largest economical impact. So its importance is demonstrated by acts and orders concerning dispersal and/or quarantine of *Daktulosphaira vitifoliae* Fitch world-wide.

Generally, aphid reproduction can encompass different strategies, whereas some or all life cycle stages are connected to parthenogenesis. Cyclical parthenogenesis strategies and obligate parthenogenesis strategies could be differed, whereas parthenogenesis is overwhelmingly apomictic (Wilson et al., 2003). The life cycle of grape phylloxera is exclusively linked to *Vitis* ssp. organs and provides a wide range of reproduction strategies: sexually (holocyclic), asexually (anholocyclic) and cyclic parthenogenesis (Forneck and Huber, 2009). On roots of *Vitis* ssp., grape phylloxera induces swelling (nodosities). Nodosities are fleshy galls formed on primary roots by swelling of the root cortex (Ritter and Ruebsaamen, 1900; Granett et al., 2001b; Kellow et al., 2004). In the contrary, swelling induced by grape phylloxera on older (mostly coarse) roots are called tuberosities (Ritter and Ruebsaamen, 1900; Granett et al.,

2001b). The "classical" life cycle of *Daktulosphaira vitifoliae* Fitch shows a cyclic parthenogenesis with temporal polyphenism (Downie and Granett, 1998) and is similar to many members of the superfamiliy Aphidoidea (Wilson et al., 2003). Grape phylloxera individuals reproduce parthenogenetically during spring and summer on leaves and roots of *Vitis* ssp., whereas in late summer the root-feeding wingless females produce winged individuals (sexuparae), which turn to mate and finally lay sexual eggs. Especially the belowground anholocyclic reproduction of grape phylloxera populations can be observed in grafted vineyards, later in season often switching to cyclic parthenogenesis with polyphenism. An obligate anholocycly (lost of ability to reproduce sexually) of grape phylloxera was not demonstrated until now (Forneck and Huber, 2009). In infested German vineyards, often a temporal polymorphism within the root feeding populations is detectable. The wingless female morphs are most commonly observed on anholocyclus on roots. Their growth involves five larval stages, the fifth stage is the adult oviparous individual. Also a development of winged preforms was detected on roots in commercial used vineyards in late summer (e.g. Michaelis (2007)). Due to the geographically dispersion of grape phylloxera outside its native range and its persistence on non native hosts in non natural ecosystems, it can be suggested that the life cycle showed many specifics, but only very few studies concerning diversity and/or life cycle of *Daktulosphaira vitifoliae* Fitch in its native range and on native hosts (Fergusson-Kolmes and Dennehy, 1991; Downie and Granett, 1998, 1999; Lin et al., 1999; Downie, 2000; Downie et al., 2001). So Downie and Granett (1998) described a special life cycle of *Daktulosphaira vitifoliae* Fitch in isolated grape phylloxera populations of some native North American *Vitis* species with holocycle on leaves and a simultaneous absence of root feeding morphs. Already Balbiani (1874) assume the presence of leaf holocycly at *Vitis vinifera*. The major knowledge of grape phylloxera life cycle and the related parasite-host systems is based on investigations made in agricultural ecosystems. A good overview of this knowledge give Huber (2007) and Forneck and Huber (2009).

Grape phylloxera show different performance on different *Vitis* cultivars (Chiarappa and Buddenhagen, 1994). de Benedictis and Granett (1992) observed populations of grape phylloxera with diverse performance on roots of grapes *in vitro* and *in vivo* and classified them as biotypes. Thereby different assessment systems were used (section 1). Later studies also showed high genetic diversity among grape phylloxera populations world-wide (Vorwerk and Forneck, 2007), with genotypes in Australia are more distinct (Herbert et al., 2006; Korosi et al., 2010). Parthenogenesis is suggested to be the predominant reproduction mode on viticulture sites world-wide (Forneck and Huber, 2009). Generally, a switch from asexual to sexual reproduction in aphid populations depends on species, environmental factors and population genetics (Wilson et al., 2003). Environmental changes could also be suggested as switching initiators in grape phylloxera populations, possibly depending on differences between genotypes (Forneck and Huber, 2009). But the factors initiating holocyclic reproduction of grape phylloxera are still unknown. Despite temperature and moisture thresholds (e.g. Turley et al. (1996)), it can be suggested that changes in nutrient concentration/composition of the host tissue could be responsible for switches between life cycles (Kawada, 1987).

2.4 Methodical Introduction

Some methodical problems had to be solved during this work. First of all, the techniques for transportation and mid-term storage of *Vitis* fresh root samples from commercial farmed vineyards did not exist, particularly with regard to a living grape phylloxera population. Also, there was no knowledge about a valid number of sample units (SU) as well as about the material, perimeter and depth of a drill or digging box. Particularly to solve the transport- and storage-problems, some little experiments with different transport solutions preceded this work (not specified here). In cool tap water roots keep fresh and larval stages of grape phylloxera keep alive for about two weeks after sample drawing. To estimate grape phylloxera infestation on roots of grape rootstocks in field, a field method according to Porten and Huber (2003) existed. This method was developed with focus on assessing differences in grape phylloxera population density directly in field and was used during the first year of this work. Although the method led to comprehensible results, it was not used in the present work for population assessment, due to the required distinguishable larval stages of grape phylloxera (see section 4.1.1).
Having managed the basic methodical problems, one *Vitis riparia* x *Vitis berlandieri* cultivar (5C) was investigated on the study site from 2006 to 2007, whereas number of SU, dimension of the digging box and system of grape phylloxera detection were adapted several times. During this development period, root system parameters and grape phylloxera parameters were the only data, which were determined. In the following years (2008 and 2009), the dimension of the digging box and number of SU remained constant. The system of grape phylloxera detection was worked out until the end of 2008. In the beginning of 2008, two variants with two different rootstocks on the same study site were established. The intention was to describe the system more completely and to record possible differences between rootstocks. So, the setup of a new variant was accompanied by the collection of more data in addition to root system parameters and grape phylloxera population. Since 2008 physical and biological soil parameters had been measured, Specific Root Length (SRL) (Fitter, 1979) was determined and root and nodosity occupying fungi were isolated. Also climate data of soil and atmosphere were used.
A great methodical problem was the processing of the digital root system data with regard to an accurate calculation of root system parameters. Newman (1966) and Tennant (1975) introduced the line intersect method to estimate root length per unit soil volume. To reduce the labor involved in this method (needing a calibrated microscope), several researchers have worked on the development of computer based systems for estimating morphological root parameters (Ruark and Bockheim, 1988; Harris and Campbell, 1989; Pan and Bolton, 1991; Berntson, 1992; Kirchhof, 1992; Smit et al., 1994; Tanaka et al., 1995; Dowdy et al., 1998). Thanks to insights of such studies, several programs are available for measuring morphological root parameters on the basis of image analysis. EZ-Rhizo is a new public domain program for the measurement of root system architecture, using a non invasive laboratory method (Armengaud et al., 2009). Older analysis systems, often using commercial scanners to create root system images are Delta-T Scan (Delta-T Devices Ltd, UK) and WinRhizo (Regent Instruments Inc., Canada) (Arsenault et al., 1995). Open Alea (http://openalea.gforge.inra.fr), an open source

project developed mainly by the French functional-structural plant model community, integrates programs for visualization, statistical analysis, fractal analysis, computer graphics and functional-structural models (Pradal et al., 2007). Although accuracy is given within the programs, results obtained by scanning are sensitive to the used protocol. So, potential sources of error are scanning resolution (Bouma et al., 2000), an overestimation of root diameter by shadowing (Pan and Bolton, 1991) and of other measured values by overlapping roots (Harris and Campbell, 1989; Pan and Bolton, 1991; Smit et al., 1994). Bouma et al. (2000) published a widely accepted sample preparation and scanning protocol for Delta-T Scan and WinRhizo. This protocol in combination with Win Rhizo Pro (RegentInstruments, 2005) was used to scan the root samples in the present work.

Particular root systems of woody (mostly perennial) plants (like *Vitis* ssp.) have an increased dimension in comparison to herbaceous species. Large plant root systems involve not only an increase in size, but also an increase in complexity (Fitter, 1994; Eshel, 1998). Also the volume of the soil explored by the roots increased as a result of continuous branching. This goes hand in hand with an increase in root-length-density (Fitter, 1994). This could lead to increasing errors in digital analysis, in particular because of overlapping roots (see above). Additionally, root traits of woody plants are poorly investigated (Comas and Eissenstat, 2009). Only a few works were focused on the root system of *Vitis* ssp. (Babo and Mach, 1881; Gabovic, 1963; Matzuok, 1978; Richards, 1983; Hughes et al., 1995; Moralt and Jacquet, 2003; Bauerle et al., 2007). Even fewer works investigated the root systems of grafted grapevines or grape rootstocks (Steinberg, 1968; Morano and Kliewer, 1994; Zapata et al., 2001; Morlat and Jacqeut, 2003; Schreiner, 2003). And only a handful concerned digital analysis of roots or root fragments of *Vitis* ssp. (e.g. Bauerle et al. (2007)). Further management methods in vineyards have high influences on root development and root growth. According to e.g. Comas et al. (2005), root length (assessed by minirhizothrons and measured by RooTracker) of minimal pruned vines (*Vitis lambrusca* ssp.) generally was higher than root length of heavily pruned vines in early stages of crop development. Peaks in mortality of roots often coincided with fruit development or fruit ripening and pruning also affected root distribution in soil profile (*Vitis lambrusca* on Concord rootstocks) (Comas et al., 2005). But generally, problems probably occurring in digital analysis of big root systems as well as *Vitis* ssp. specific problems are not well documented or totally missing.

However, there have been verified parameters to determine the size of root systems since the 1970s (Newman, 1966; Tennant, 1975; Fitter, 1979; Boehm, 1979). But morphological parameters describing the size of root systems (mainly length and diameter) did not reveal anything about root system branching attributes. So, several models for describing branching of root systems exist. Most approaches based upon the developmental system of root axes and laterals of various orders (Ketipearachchi and Tatsumi, 2000). Others, mostly introduced by Fitter (1986, 1991) are based upon a topological approach and use the links of a root system. A link is a root segment between two branching points. All models describing branching structure of root systems require thorough data for the number and length of axes and laterals (developmental model) or link number and path length for entire link constituting

the whole root system (topological model) (Ketipearachchi and Tatsumi, 2000). So, a concern of researchers was to downsize the quantity of data. Here, great hopes are pinned on the application of principles of fractal geometry to the description of root systems. Since its introduction by Mandelbrot (1983), fractal geometry has been applied to the structures of various biological systems in human physiology and medicine (Lewis and Rees, 1985; Smith et al., 1989; Goldberger et al., 1990), microbiology (Obert et al., 1990; Gee and Warwick, 1994; Mihail et al., 1995) botany and ecology (Morse et al., 1985; Zeide, 1993). Tatsumi et al. (1989) and Fitter and Stickland (1992) introduced the fractal dimension (*FD*) for the characterization of root system architecture. The repetition of branchings of roots leads to a degree of self-symmetry, which is a fundamental characteristic of fractal systems (Mandelbrot, 1983). *FD* has been calculated for whole root systems (Tatsumi et al., 1989), separated branches and planar sections (Nielsen et al., 1997; Eshel, 1998) and *FD* was closely correlated with root topological architecture (Fitter and Stickland, 1992; Nielsen et al., 1997). The determination of *FD* for sections of root systems showed differences between monocots and dicots (Fitter and Stickland, 1992), in genotype, plant age or growth conditions (Izumi et al., 1995; Nielsen et al., 1999). Already 1997, Lynch et al. (1997) developed a geometric simulation model, considering fractal dimension. In fact, researchers today are able to develop 3D-models of woody (Danjon and Reubens, 2008) and non woody root systems (Gregory et al., 2009) by using invasive as well as non invasive techniques. In the present work, planar fragments of root systems were analyzed digitally. Although Nielsen et al. (1997) correlated *FD* of planar sections with *FD* of whole root systems and Walk et al. (2004) suggest, that fractal analysis of planar subsamples may be appropriate for big root systems, no statement of a possible three dimensional expansion will be made in the present work. The used methods to collect and digitize root system data are not suitable for modeling in a three dimensional scale. Moreover, soil compaction in the machine track lead to a limited three dimensional development of grape root systems in the most conventional vineyards.

Altogether, a wide variation of root system morphology and architecture among plant species was shown in recent works. Although root system morphology and architecture are important in relation to physiological functions of plants (Ketipearachchi and Tatsumi, 2000), research in viticulture is several decades back. Only a handful of works world-wide deal with image-based computer-analyze approaches to describe parts of the *Vitis* ssp. root system (Comas et al., 2005; Bauerle et al., 2007). Furthermore, studies regarding fundamental biological issues like trophic pathways (also competitive structures) in soil (e.g. Reuss and Chamberlain (2010)) or above- belowground interactions (e.g. van der Putten et al. (2009)) were done mainly in natural ecosystems. Such results can only be transfered with care, regarding the more productive and statical agricultural ecosystems.

3 Material and Methods

3.1 Study Site and Climate

The study site is located in the wine-growing district of the Upper Rheingau in Germany (between Wiesbaden and Ruedesheim). The Rheingau is subdivided into Upper and Lower Rheingau. The Upper Rheingau is geologically and pedologically part of the Mainz Basin, the lower Rheingau is part of the middle Rhine valley. In the upper Rheingau, in consequence of a long time cultivation of *Vitis* ssp., soils are mainly Rigosols of the category Terrestrial Kultosoles. The cultivation of vine in this area is pursued for over 1200 years (Friedrich and Sabel, 2004).
The study site is part of the location "Burgweg/Moenchspfad" in the boundary Geisenheim. GPS dates are N 49 59.748' and E 007 57,105' on 157 m height (+/- 9,3 m) above German Reference Surface. The parent rock material is formed by alluvial gravel in the subsoil and loess, partly with alluvial gravel in the Rigosolhorizon. In the subsoil, the type of soil is sand, gravel and sandy clay (concentration of lime 0 - 15 %). In the Rigosolhorizon, the type of soil is a sandy to silty clay without a measurable concentration of lime (Friedrich and Sabel, 2004).
The study site is a commercial used vineyard, planted in 1985 with scion "Weisser Reisling" grafted on 5C (*Vitis berlandieri* x *Vitis riparia*) and 125AA (*Vitis berlandieri* x *Vitis riparia*) rootstocks. 5C is planted in rows one to 32, 125AA is planted in rows 33 to 56. Total area of the vineyard is about 2500 sqm, row distance is 200 cm, the distance between vines 135 cm. Until 1997 and since 2005, the vineyard was yearly managed organically based on stall manure (horse, cow or poultry dung). Between the years 1997 and 2004, manuring has changed in the context of previous works (Huber, 2007). For the present work, two variants were arranged on the study site, Variant 5C and Variant 125AA (Fig. 1).

In Germany, viticulture is only possible at preferential climatic and geomorphologic sites. Temperature, air moisture (in Rheingau regulated by the vast water area of the Rhein) and a southern slope (Taunus) are preferential factors for viticulture (Vogt and Goetz, 1979). Therefore, conditions regarding location and climate play a decisive role in cultivation of *Vitis* ssp. and *Vitis* ssp. rootstocks. The weather related parameters of Geisenheim/Rheingau are shown for the investigation period in Figure 2 (monthly mean values of air- and soil temperature (-0.1 m) and monthly sum of precipitation). Data were received from the meteorological station Geisenheim of the DWD (Deutscher Wetter Dienst) (figure 2).

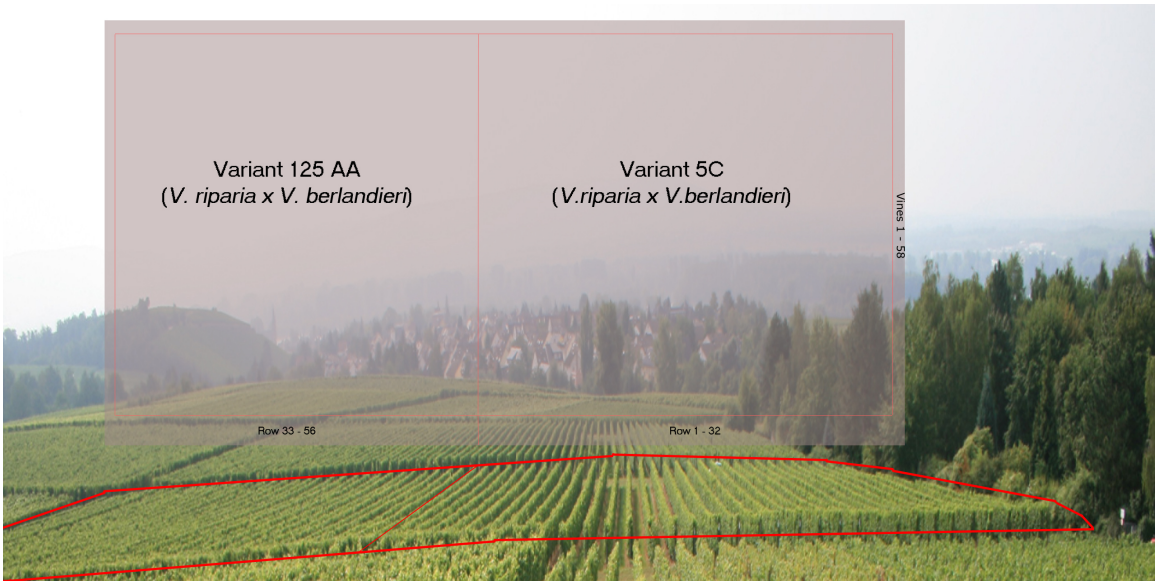


Figure 1: Study Site and Variants

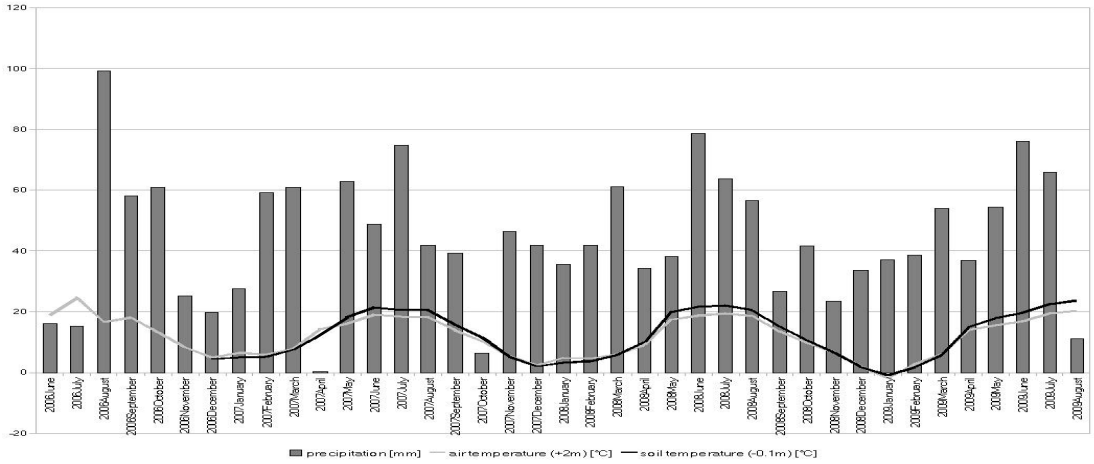


Figure 2: Monthly weather related parameters: mean values of Air Temperature [C] and Soil Temperature (-0.1m) [C], sum of Precipitation [mm]

Figure 3: The sample area under foliage wall. Both soil and root samples were taken in the sample area.

Figure 4: Spatial sectors of the trial field. l = lower sector (low slope); m = mid sector (slope 2-4°); u = upper sector (slope > 4°).

3.2 Examination Techniques

3.2.1 Sample Drawing

Samples were collected from June 2006 to August 2009. To realize a statistical meaningful number of SU and a practicable sample drawing, investigations were limited to the upper 20 cm of soil under the foliage wall. All root samples were taken with a quadrangular mechanic steel digging box, whereas all soil samples were taken with a mesofauna borer (figure 5). Before August 2007, digging boxes with 0.04 sqm surface area and 20 cm depth were used. From August 2007 (31st August 2007), digging boxes were scaled down to 0.01 sqm surface area. These digging boxes were used for sample drawing until August 2009.

Both, root and soil samples were randomly taken under foliage wall (figure 3). Three spatial sectors were classified on the study site, depending on the soil slope: l = lower sector (0-2° slope), m = mid sector (2-4° slope) and u = upper sector (> 4 ° slope) (figure 4).

On variant 5C, root samples were taken from June 2006 to August 2009 continuously. In 2006, the number of SU was n = 8. In the first half of 2007, the number of SU was n = 4, from August 2007 until August 2009, the number of SU was n = 10. From March 2008 until August 2009, variant 125AA was sampled additionally to variant 5C also with n = 10. Additionally, n = 10 soil samples of each variant (5C and 125AA) were taken. From January 2007 until August 2009, the spatial sectors were recorded. Sample dates, number of sample units and variants are listed in table 1.

(a) Field work material

(b) Digging box

(c) Sample 1

(d) Sample 2

Figure 5: A: Field work material. Shown are small and big digging boxes, drill for soil sampling, plastic tubes filled with water for root transportation. **B:** Digging box in soil. **C:** Soil sample for root extraction. **D:** Root extraction from soil.

All roots in a core sample were carefully isolated from soil in the field and were stored in cool water (cool box) in order to be transported. In the laboratory they were stored in water between 4 - 7 °C. Soil samples were taken on variant 5C and 125AA from March 2008 to August 2009 from the first 20 cm under foliage wall. They were packed in plastic bags to transport and stored in laboratory at 4 - 7 °C.

3.2.2 Traits

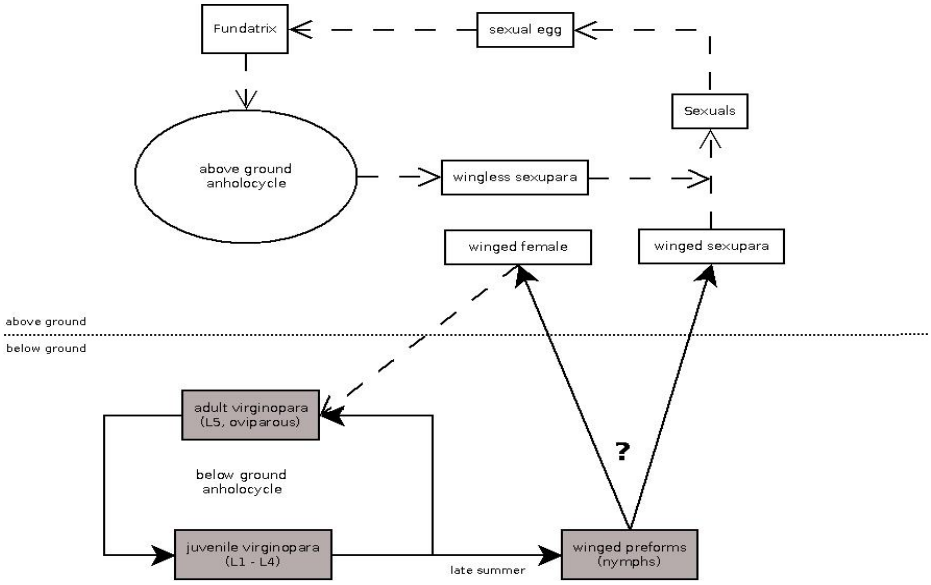
3.2.2.1 Grape Phylloxera Population Density and structure of grape phylloxera population (*Daktulosphaira vitifoliae* Fitch) and nodosities were assessed from 2006 to 2009 at all root samples. The survey of the collected roots - to detect nodosities and larval stages of *Daktulosphaira vitifoliae* Fitch - took place in the laboratory. Before root digitizing, all collected roots were transfered in fresh water and were carefully explored under a ZEISS stereomicroscope on either side. All root swellings and grape

Table 1: Dates, Variants, Number of SU (n) 2006 - 2009

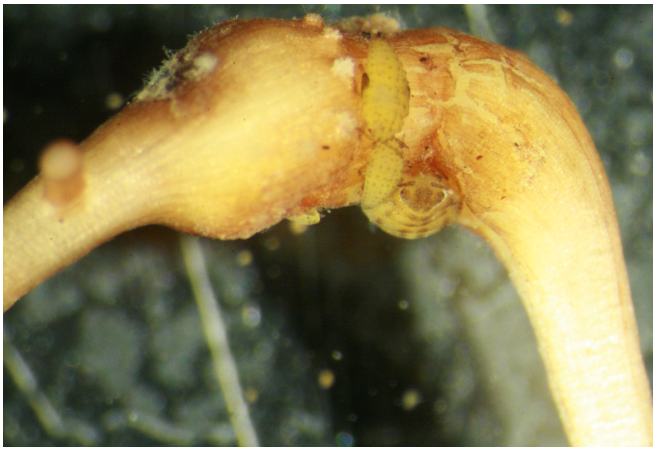
Year	Date	Variant	SU per variant
2006	19.06. 11.07.,24.07. 08.08.,22.08. 05.09.,19.09. 05.10.,24.10. 20.11. 18.12.	5C	8
2007	23.01. 29.03. 24.04. 21.05. 03.07.	5C	4
	31.08. 10.10.		10
2008	22.01. 14.03. 23.04. 14.05. 09.06.,30.06. 21.07. 07.08.,26.08. 16.09. 14.10. 20.11.	5C 125AA	10
2009	20.01. 10.02.	5C	8
	04.03. 06.04. 09.05. 05.06. 13.07. 14.08.	5C 125AA	10

phylloxera instars were recorded. In 2006, a field method according to Porten and Huber (2003) was used additionally.

In the context of the methodical development new attributes were introduced to specify the structure of grape phylloxera population between 2007 and 2009 (section 4.1.1). All grape phylloxera population attributes (instars, nodosities) were classified into **main parameters**, describing the total amount of the attribute classes (one grape phylloxera population class, three nodosity classes). **Sub parameters** describe the number of instars/nodosities in the specific developed sub classes (three grape phylloxera sub classes, nine nodosity sub classes). Grape phylloxera instars were classified in three sub classes: L1-L4 instars (juvenile virginopara), L5 instars (adult virginopara) and nymphal instars (wingend preforms). These classes are common in the grape phylloxera below ground anholocycle (figure 6). The development of the nodosity classification has been described in section 4.1.1.2.



(a) Grape phylloxera life cycle (accord. to Forneck and Huber (2009))



(b) Instars on nodosity

Figure 6: **A:** Grape phylloxera life cycle (partly), according to Forneck and Huber (2009). Larval stages, sampled and classified in the present work are in gray boxes. **B:** All classes of larval stages on a nodosity. Top left: winged preform. Bottom left: L1-L4; center: L5.

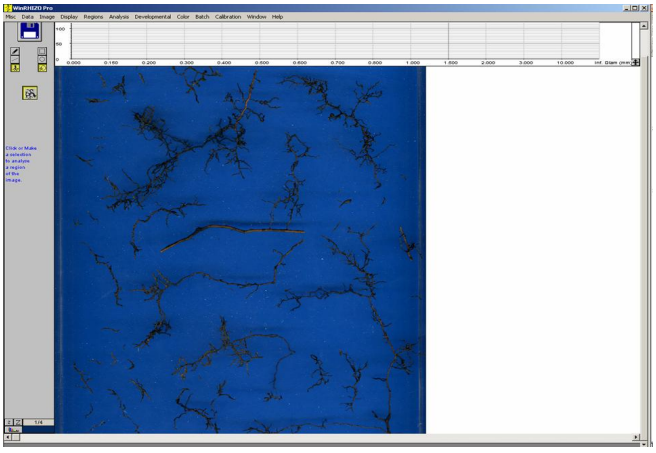
All parameters were used to calculate different population specifications:
The term **RPD** (= Relative Phylloxera Density) describes the abundance of the attributes per soil unit. The reference value was a 10^{-2}sqm soil surface and 20 cm depth (analog to the size of a small digging box). RPD parameters were specified with *attribute per 10^{-2}sqm* .
The term **APD** (= Absolute Phylloxera Density) describes the abundance of attributes without the influence of a variable root quantity. The reference values were root length [cm] or root dry weight (DW, [g]). APD parameters were specified with *attribute per cm* or *attribute per DW*.
The term **CNA** (= Combined Nodosity Attributes) describes a nodosity with all available nodosity attributes. The reference value is a 10^{-2}sqm soil surface and 20 cm depth. For reasons of clarity, CNA parameters were specified without a unit.
The term **NOP** (= Nodosity Occupation Parameters) describes the mean number of instars per nodosity. The parameters were calculated using RPD parameters and do not have a unit. In table 7 all attributes were specified.

3.2.2.2 Root System Traits The root samples were analyszed digitally after the aforementioned detection of the grape phylloxera population (section 3.2.2.1). The root samples were rinsed carefully in floating water to remove adherent soil particles (figure 7A,B). Washed roots were digitized by acquiring 400 dpi 8 bit gray scale images and 400 dpi 24-bit RGB color images with an EPSON Perfect Scan 4490, running on an Intel Pentium IV 1800 (MS Windows XP) (figure 7C,D). An overlapping of root fragments was prevented. 8-bit gray scale images were generated by the scanner TLU (transmitted light unit). 24-bit RGB color images were generated without TLU and with a blue background. All images were saved as TIFF-Files. To determine the *Specific Root Lenght* (SRL [m/g]), root samples were dried at 105 °C for 24 hours (Boehm, 1979) after digital analysis and weighted with an analytical balance. SRL was recorded from June 2008 to August 2009 on both variants (5C, 125AA).

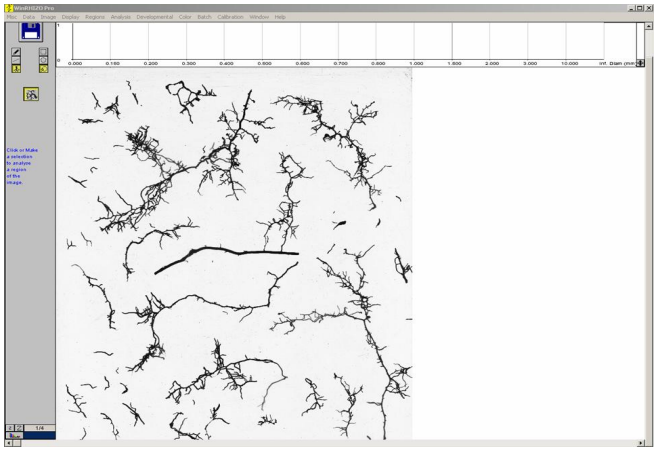
3.2.2.2.1 WinRhizo Pro Created images were analyzed using the program WinRhizo Pro V 2005beta, running on an Intel DualCore 2x 3000 (MS Windows XP). WinRhizo Pro was developed by Regent Instruments (Canada) to acquire parameters describing root systems on the basis of gray- and RGB-scale images. As well in gray scale analysis as in color analysis, these parameters were calculated by using the quantity of pixels related to the measuring scale. Using a normal scanner as in the present work, the measuring scale is predetermined. By the analysis of color images, differences of roots basing on different coloration are considered. In the present work, morphological root properties were analyzed on base of gray scale images. Differences in root coloration were analyzed on base of RGB-color images (figure 7E,F).
Morphological parameters were classified in main- and sub parameters. Root system main parameters were length [cm], surface area [sqcm], projected area [sqcm], diameter [mm], and the number of tips, forks and crossings. Furthermore, the number of crossings and the number of forks were calculated

(a) Root Cleaning 1

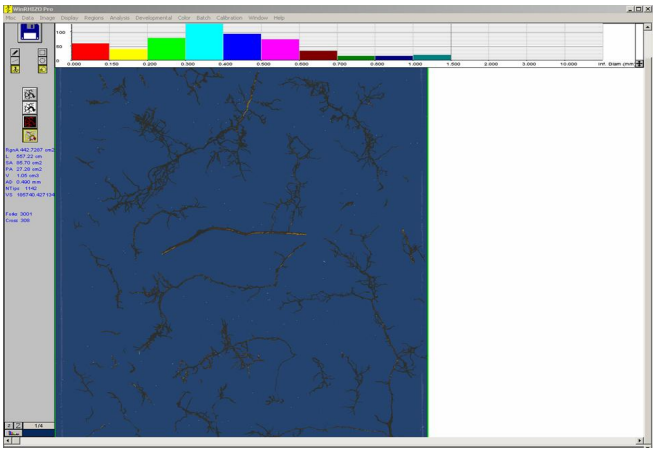
(b) Root Cleaning 2



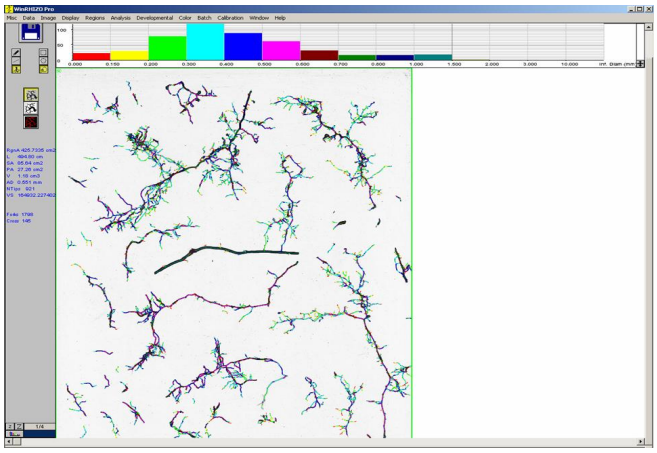
(c) RGB Color Image



(d) Gray Scale Image



(e) Analyzed RGB Color Image



(f) Analyzed Gray Scale Image

Figure 7: A: Equipment for root cleaning **B:** Roots were rinsed under water and were carefully cleaned with a brush. **C:** Roots were digitized with Win Rhizo Pro to RGB color images **D:** and gray scale images. **E:** Color analysis with different color groups shown. **F:** Gray scale analysis for morphological root system parameters. Root samples are from 14.08.2009, variant 5C, sample number 5.

per cm (*crossings per cm* and *forks per cm*). Morphological root system sub parameters were length and surface area in different diameter classes. They were classified according to Boehm (1979) and assigned to 14 individual diameter classes (0 to 0.15 to 0.2 to 0.3 to 0.4 to 0.5 to 0.6 to 0.7 to 0.8 to 1.0 to 1.5 to 2.0 to 3.0 to 10 > 10 mm). Additionally, an analysis of the architecture of the root system by calculating the fractal dimension (*FD*) was performed.
The analysis of RGB color images was done to identify possible differences in dark and pale coloration of root parts. Two different settings were used to describe dark and pale root parts (section 4.1.3).
Win Rhizo Pro is also able to analyze the branching structure of a root system (link analysis). This feature was not used, because in the present work root fragments were used mainly (RegentInstruments, 2005). Table 2 shows the program settings in the single analysis passages, settings in table 3 were adjusted in all analysis passages.

Table 2: Program settings in single analysis passages of Win Rhizo Pro

Type	Scale	Diameter Classes	Settings
Morphological Root System Parameters	Gray	Boehm Classification 14 ind. Diameter Classes	Morphology Diameter interpolation Diameter precision: max
Fractal Architecture	Gray		Pixel size: min 0.2 to 2.0 mm Displaying time: 2s Input: Pixel Classification
Morphological Root System Parameters of pale and dark root parts	Color	14 ind. Diameter Classes	Morphology Diameter interpolation Diameter precision: max defined color classes

Table 3: Permanent settings of Win Rhizo Pro

Menue	Setting
Calibration Method	Intrinsic
Diameter interpolation	max. diameter precision
Pixel Classification	Automaitc
Removal of objects	Area smaller than 0.005 sqcm
Pictures	Dark roots on withe background

3.2.2.3 Fungal Endophytes The isolation of root occupying fungi occured from March 2008 to August 2009 on both variants (5C, 125AA). Before root drying, two nodosities (if present) and two little pieces of roots were taken randomly from every sample with a scalpel. Adherent roots on nodosities were detached. The surface of the extracted root pieces and nodosities was sterilized according to

Schulz et al. (1993), but modified (table 4).

After sterilization, petri dishes with the fungi nutrient deficit medium (NDM) (table 5) and the fungi full culture medium PDA (Potato-Dextrose-Agar, Merck), both at pH 5.4, were inoculated with a single nodosity or rather a root piece respectively. Inoculated dishes were incubated at 24 °C for two weeks. Afterwards, fungal mycelia growing out visibly of the inoculated tissue were separated and incubated again on PDA resp. NDM at 24 °C for one to two weeks (figure 8). All fungi cultures produced in this way were grouped into fungal morphotypes. The fungal morphotypes were sectioned on the basis of color of the mycelium, thickness and ramification of hyphae and form of asexual conidiospores fruiting bodies (if present). Fungal morphotypes should be identified on species-level by Dr. habil. Martin Kirchmair, University of Innsbruck, Institute of Microbiology. Unfortunately, no identification could be done until the release of the present work. So, analyses were only possible on base of morphotype differences.

Table 4: Root and nodosity sterilization steps

Step	Incubation time	Substance
1st step	3 min	HCl 0.5 M
2nd step	2 min	NaOCl 6 %
3rd step	3 min	Ethanol 75 %
4th step	several times	steril demin. H_2O

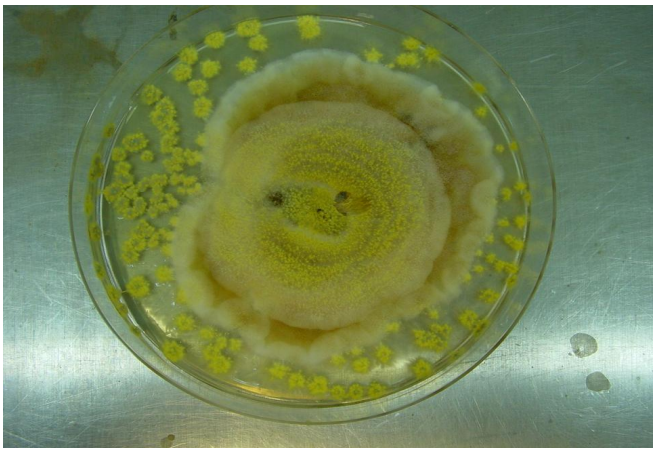
Table 5: Fungi Nutrient Deficient Medium (NDM)

Substance	Concentration
Agar	20 g/l
K_2HPO_4	1 g/l
KNO_3	1 g/l
$MgSO_4 \bullet 7\ H_2O$	0.5 g/l
KCl	0.5 g/l
Glucose	0.2 g/l
Saccharose	0.2 g/l

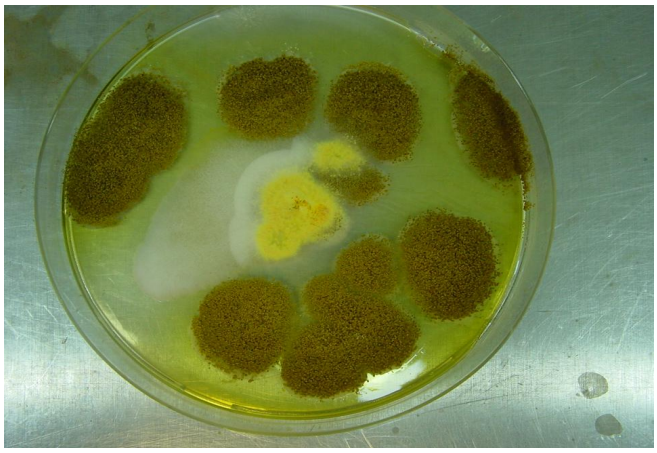
3.2.2.4 Soil Properties Biotic and abiotic soil properties were recorded from March 2008 to August 2009 on with 10 SU per variant (5C, 125AA).

To determine the **Soil Water Content** (SWC), fresh and sieved (5 mm mesh size) soil samples were weighed with a microbalance (n = 10 per variant). Afterwards they were dried in a drying chamber for 24 hours at 105 °C, cooled in an exsiccator and weighed out again. SWC is specified in percent of dry mass of soil [% DM].

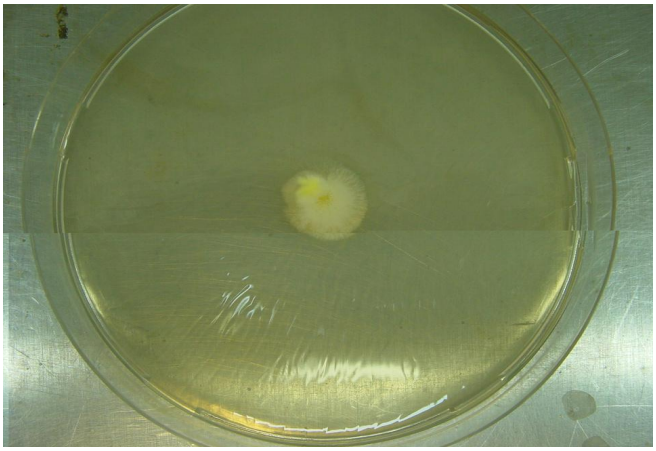
The **pH-value** of soil was determined according to DIN 19684. 10 g of each (n = 10 per variant) air-dried and sieved (5 mm mesh size) soil sample were suspended with 25 ml of 0.01 M $CaCl_2$ solution. After suspension, soil samples were allowed to stand for 30 min. pH-value was measured with a pH meter. The pH meter was calibrated each two months.



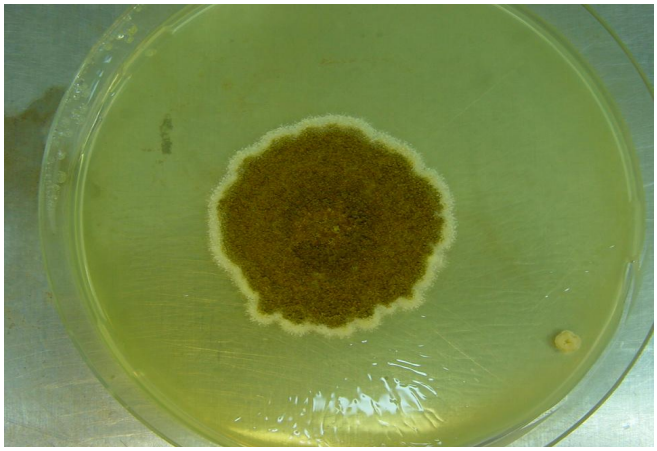
(a) Primary Isolation



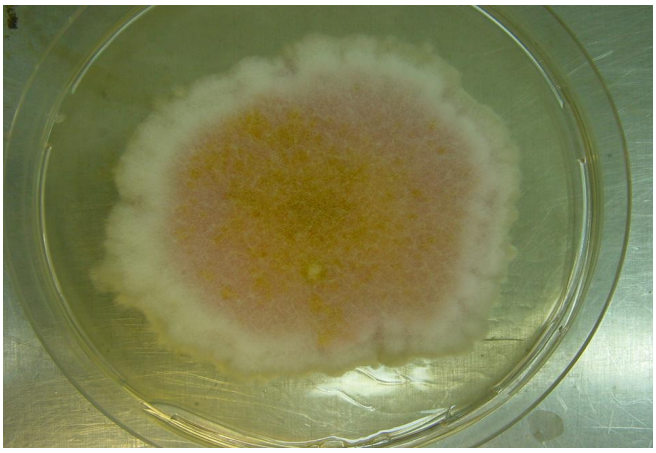
(b) Secondary Isolation



(c) Final Isolation of Morphotype 1



(d) Final Isolation of Morphotype 2



(e) Final Isolation of Morphotype 3

Figure 8: (Sample 5C, 26.08.2008, sample number 3, PDA) Primary isolation of fungal cultures from root tissue after two weeks of growth (**A**). Secondary isolation (one week; **B**). Final isolations of three morphotypes (one week; **C,D,E**)

The determination of the **Soil Organic Matter** (SOM) took place by detection of the loss of ignition (LOI) after the incineration of the soil samples. The sieved (5 mm mesh size) and dried (105 °C, 24 hours) soil samples (n = 10 per Variant) were incubated for 24 hours at 400 °C in a muffle furnace. SOM is specified in percent of dry mass of soil [% DM]
The measurement of the **Basic Soil Respiration** (BSR) took place according to Isermeyer (1952). BSR is a dimension for the activity of soil organisms, whereas the BSR value is based upon the CO_2 production of soil occupying organisms. Under natural conditions, microorganisms and plant roots chiefly produce CO_2 in soil. The soil samples in this work were sieved (5 mm mesh size), so that the bigger parts of plant roots were removed. Thus, the CO_2 produced in the laboratory experiment came mainly from soil microorganisms.
The determination of BSR took place with n = 10 per variant (5C, 125AA) and a blank sample (without soil). Every sample was weighed in a round, transparent glass container (1000 ml) and adjusted with steril sterile demin. H_2O to 40 % moisture content. In every container, a glass shell with 2 ml NaOH (1M) was positioned. Afterwards, the containers were closed airtight and stored for 24 hours at 22°C in darkness. During the incubation time accumulated CO_2 was bond chemically as Na_2CO_3. After incubation, Na_2CO_3 was precipitate as $BaCl_2$ by addition of $BaCO_3$ (saturated). The mass of not transformed NaOH was determined by a volumetric analysis (titration) with HCl (1M) (tracer: Phenolphthalein). BSR is specified in miligram carbon per gram soil dry mass and hour [$\frac{mgCO_2C}{gDM*h}$].

3.2.3 Whole-mount Staining and Light Microscopy

3.2.3.1 Cleaning, Bleaching and Staining To search for a potential staining method in combination with the root digitization, a whole-mount staining with Pianeze was tried. Therefore, at 6th April 2009, some extra roots of Variant 5C were collected and fixed in 75 % Ethanol. Pianeze is a generally used staining method to detect fungal strucures in microscopic preparations of plant tissue. In a normal case, pianeze is used after cutting light microscopical supplements. In the present work, a staining of uncut roots (whole-mount) was tried. All staining and preparation steps were done with friendly support of chemical technical assistant (CTA) XXXXXXXX.
The first step was **tissue cleaning**. Roots were cleaned using two different methods. Method 1 (V1) was a pure chemical cleaning method, according to Koske and Gemma (1989). Tissue was incubated for 30 min in 2.5 % KOH-soultion at 80 °C in a water bath. Method 2 (V2) was a mechanical-chemical method. Roots were incubated twice (each 5 min) in 2.5 % KOH-solution at 80 °C in a ultrasonic bath.
Afterwards roots were washed several times with distiled water. All roots were **bleached** according to Koske and Gemma (1989) for 90 min in an alkaline H_2O_2 solution (1:10 NH_4OH (20 %) : H_2O_2 (3 %)). Then roots were washed several times in distilled water again. For **acidification** roots were incubated in 1 % HCl O/N.
Staining: roots were stained for 60 min with Pianeze-Staining-Solution (25:8.3:1 Water (dest) :

Ethanol (90 %) : Pianeze mixture) at room temperature. As a next step roots were differentiated in Ethanol-Acetic Acid solution (9.9:0.1 Ethanol (90 %) : Glacial Acetic Acid).

3.2.3.2 Light Microscopy In order to test the success of the whole-mount staining, roots were dissected for light microscopy to prepare cross-sections. Therefore, stained roots were subdivided into specimens and dehydrated by an ascending alcohol series, ending in Butanol. Afterwards, specimens were incubated in paraffin wax (Roth) at 60 °C O/N and following at 7 °C for hardening. Specimens were cut on a Leica slide microtome at 8 μm. The cuttings were stretched at 40 °C in a water bath and incubated on a slide at 35 °C in a dry chamber O/N. To free the cuttings from paraffin, they were incubated in Xylol for 20 min. Cuttings were covered with Canada turpentine (mixed with chloroform) and were hardened for seven days at room temperature. The preparations were studied with a light microscope (Leica DLMB). Photos were taken with a Canon EOS 400 D and afterwards arranged with the program Combine ZP (by Alan Hadley).

To relate grape phylloxera traits and root system traits to a greater context, a continuous measurement of additional parameters were done since August 2007 (table 6). The spatial distribution of samples was recorded since August 2007, in March 2008 two root stock variants (5C, 125AAA) were introduced. Simultaneously, the continuous recording of soil properties (*SWC, pH, SOM* and *BSR*, section 3.2.2.4) and fungal endophytes started. Since July 2008 root dry weight (*DW*) and specific root length (*SRL*) were detected. Additionally, new attributes associated to grape phylloxera population were developed from January 2007 until January 2009.

Table 6: Introduction of additional traits since January 2007. Traits were recorded continuous until August 2009

Trait	Unit	Introduction date
Spat. distribution	-	August 2007
Two Variants	-	March 2008
SOM	%	March 2008
pH	-	March 2008
SWC	%	March 2008
BSR	$\frac{mgCO_2C}{gDM*h}$	March 2008
Fungal endophytes	-	March 2008
SRL	m/g	June 2008

3.3 Statistical Analysis

Statistical analyses based on methods according to Sachs and Hedderich (2006), de Sá (2007) and Leyer and Wesche (2008) mainly. If other sources were used, they were specified.

3.3.1 Parameters and Programs

Table 7 shows an overview of all recorded parameters and their mathematical classification. All raw data were stored in Open Office Calc 3.1 (http://www.openoffice.org). Statistical analyses (descriptive, univariate, multivariate) were done with Statistica 9.0 (StatSoft Inc.). Structural equation models (SEM) were calculated with AMOS 17 in SPSS 17.0.1 (SPSS Software GmbH). Macros to rearrange the data for an ensuing statistical analysis were written in R 2.8.1 (R Foundation for Statistical Computing, http://www.r-project.org) with friendly support of Dipl.-Biol. XXXX. The data was interchanged between single programs as ASCII or xls-files.
Plots were drawn in Statistica 9.0 and in Gnuplot 4.2.6 (http://www.gnuplot.info). Charts were done in DIA (http://dia-installer.de), pictures were created in Image-J
(http://rsbweb.nih.gov/ij/index.html). This document was written in LaTeX MikTex 2.7 (http://miktex.org), using TeXnic Center 1 Beta 7.50 (http://toolscenter.org).

Table 7: Recorded parameters and their mathematical classification. APD=Absolute Phylloxera Density. CNA = Combined Nodosity Attributes. DW=Dry weight. non-term=not terminal. NOP = Nodosity Occupation Parameters. Nym=nymphal instars. RPD=Relative Phylloxera Density. Term=terminal.

Trait	main parameter	sub parameters	Scale
Grape Phylloxera Instars	Instars per cm (APD)	L1-L4, L5, Nym	Quantitative
	Instars per 10^{-2}sqm (RPD)	L1-L4, L5, Nym	
	Instars per DW (APD)	L1-L4, L5, Nym	
Grape Phylloxera Nodosities	Color per cm (APD)	sv, lv, uv	Quantitative
	Color per 10^{-2}sqm (RPD)	sv, lv, uv	
	Color per DW (APD)	sv, lv, uv	
	Form per cm (APD)	A, B, C, D	Quantitative
	Form per 10^{-2}sqm (RPD)	A, B, C, D	
	Form per DW (APD)	A, B, C, D	
	Branches per cm (APD)	term, non-term	Quantitative
	Branches per 10^{-2}sqm (RPD)	term, non-term	
	Branches per DW (APD)	term, non-term	
	CNA (per 10^{-2}sqm)	Color+Form+Branches	Quantitative
NOP	Instars per nodosity	Instars per color	Quantitative
		Instars per form	
		Instars per term, non-term	
		Instars per combined parameters	
Root System Traits	Total root length [cm]	Diameter classes	Quantitative
	Total root surface area [sqcm]	Diameter classes	
	Average root diameter [mm]	-	
	Number of Forks	-	
	Number of Crossings	-	
	Fractal Dimension	-	
	Specific Root Length [m/g]	-	
	Root dry weight (DW) [g]	-	
Soil Properties	Soil Water Content [%]	-	Quantitative
	Basic Soil Respiration $[\frac{mgCO_2C}{gDM*h}]$	-	
	Soil Organic Matter [%]	-	
	Soil pH	-	
Endophyte Population	Morphotypes tissue (root/nodosity)	-	Qualitative
	Morphotypes variant (5C/125AA)	-	

3.3.2 Statistical Analysis

3.3.2.1 Method Development To verify the grape phylloxera field assessment method according to Porten and Huber (2003) in terms of an quantitative assessment, it was compared with the laboratory counting of grape phylloxera instars. Here the dataset from 2006 was used. Correlations (r, r^2) between assessment classes and numbers of instars were calculated. To identify possible differences in total numbers of instars and total numbers of assessment classes, a Wilcoxon-test for paired samples (P=0.05) was calculated. To spot the seasonal differences in sensitivity of the field assessment method, a χ^2-test of homogeneity with delta-option was done. Therefore, the logarithm of the data was taken. The introduction and specifications of main and sub nodosity parameters were described. To verify groups of main and sub parameters, an exploratory factor analysis (FA, extraction: principal components) was done. Before, variables were tested for correlations (r). To compare the variables on different measuring scales, the data was standardized by a z-transformation.

To detect effects of the size of the digging box or the number of sample units (SU) on root system main parameters (*total root length, total root surface area* and *average root diameter*), a Kruskal-Wallis ANOVA was calculated (only variant 5C). Afterwards, a Bonferroni post-hoc test (P=0.05) was calculated to identify significances in the single factor levels.
To estimate the impact of overlapping root fragments on failure of digital measurement (gray scale), correlations (r, r^2) and regression lines for *number of crossings*, *crossings per cm* and *total root length* were calculated.

To verify an impact of digital measuring errors at color analysis, a Kruskal-Wallis ANOVA was calculated. Factors were different analysis types (three factor levels (two types of color analysis, one gray scale analysis)) (section 4.1.2). Dependent variables were root system main parameters (total and over diameter classes). Afterwards, a Bonferroni post-hoc test (P=0.05) was done to identify the factor levels with significant differences.

3.3.2.2 Traits The temporal development in a course of a year as well as the spatial distribution of grape phylloxera traits, root system traits or soil properties were given (generally and for each variant). Parameter distributions were charted by mean values and 0.95 * standard error. Temporal development was plotted in the course of a year (monthly). Spatial distribution was described in three different spatial classes, regarding the slope of the trial field. Class "l" = lower part of the trail field (0° - 1°), class "m" = middle part of the trail field (1° - 3°), class "u" = upper part of the trial field (> 3°). Differences in the development of parameters were displayed.
To verify significant differences in the total amount of values, omnibus tests were calculated (factor levels: variant (5C,125AA), months (temporal), spatial classes). To identify significant factor levels,

post-hoc tests followed. Before, parameters were tested on normal distribution (K-S test, P=0.05) and homogeneity of variance (Levene test, P=0.05). Homogeneous and normal distributed parameters were analyzed by an one-way ANOVA (fixed effect model). Afterwards, Tukey HSD post-hoc tests (P=0.05) were done to identify the factor levels according to significant differences. Not homogeneous or not normal distributed parameters were analyzed by a Kruskal-Wallis ANOVA with a following Bonferroni post-hoc test.

Endophyte data was analyzed on base of morphotypes (dataset Mar08-Aug09), considering the variant (5C, 125AA), the nutrient medium (PDA, NDM) and the origin tissue (Root, Nodosity). A general discriminant analysis was done to identify possible distinct morphotypes. The squared Mahalanobis distances between groups were tested on significance by a F-Test (P=0.05).
To identify possible differences related to the amount of isolated morphotypes, a Wilcoxon-test for paired samples (P=0.05) was calculated. Afterwards, a χ^2-test of homogeneity was done to identify possible differences in frequency of isolated fungi, regarding the origin tissue and the rootstock variants.

3.3.2.3 Regressions Models based on different datasets (table 8). In multiple and normal regression models (MLR, NLR), parameters were standardized by a z-transformation to avoid the impact of different measuring scales.

Table 8: Different datasets and number of sample units

Dataset	Months	SU (grape phylloxera)	SU (root system)	SU (soil properties)
2006-2009	39	449	490	-
2007-2009	32	406	406	-
Aug07-Aug09	24	386	386	-
2008-2009	20	356	356	356
Jun08-Aug09	15	296	296	296
Jul08-Aug09	14	256	256	256
2009	8	136	136	136

3.3.2.3.1 Linear Regression Models Relationships between the recorded parameters within single traits (grape phylloxera population and root system traits) were analyzed by multiple linear regression (MLR) models. Different grape phylloxera attributes were predicted by densities of grape phylloxera instars. Diameter sub parameters were predicted by *total root length* or *total root surface area*. As well, root system main parameters were predicted by *fractal dimension*. Settings of different models were described in table 9. Adjusted R^2 values and β coefficients were calculated.

Univariate relationship between soil temperature (-0.1m) or moisture and grape phylloxera traits,

Table 9: Different settings of the multiple linear regression (MLR, NLR) models.

Trait	Dependent variable(s)	Predicted attributes	Table
Grape Phylloxera	*ttl instars per 10^{-2}sqm*	RPD,APD,CNA and NOP	31
	ttl inst per cm	RPD,APD,CNA and NOP	32
	RPD instar sub parameters	RPD,APD and CNA	33
	APD instar sub parameters	RPD,APD and CNA	34
	RPD and APD instar sub par.	NOP (nodosity attr.)	35
	RPD and APD instar sub par.	NOP (instars)	36
Root System	*Total root length*	Length of diam. sub parameters	37
	Total surface area	Surface area of diam. sub parameters	37
	Fractal Dimension	Root system main parameters	38

root system traits or soil properties were charted by scatter plots, shown in section C. To validate the impact of temperature or moisture on grape phylloxera population and on root system development, multiple and normal linear regression (MLR, NLR) models were calculated. Predicted variables were APD parameters, RPD parameters, combined parameters or nodosity occupation parameters. Adjusted R^2 values and β coefficients were calculated.

3.3.2.3.2 Structural Equation Models To estimate a possible general impact of grape phylloxera population on grape root system, structural equation models (SEM) were calculated. In a SEM, predicted relationships are based on theoretical and logical considerations. These relationships were imaged with hypothetical constructs. Latent values depended on measured endogenous values (dependent variables), independent variables were exogenous values (Mitchell, 1992). In the present work, the impact of APD main parameters (*ttl inst per cm, ttl nod per cm*) or RPD main parameters (*ttl inst per 10^{-2}sqm , ttl nod per 10^{-2}sqm*) on *total root length, total root surface area, fractal dimension* and *average root diameter* were modeled under consideration of soil temperature. It was expected, that total densities of grape phylloxera instars and nodosities were related to morphological parameters of grape root system. All calculated models based on the dataset Aug07-Aug09. The calculation of all SEM models based on iteration with asymptotically distribution-free discrepancy. Variables (endogenous and exogenous) and latent values were listed, β coefficients and χ^2/DOF values were calculated.

4 Results

To compare data, different datasets had to be used: the dataset 2006-2009, dataset 2007-2009 (introduction of new nodosity attributes), dataset Mar08-Aug09 (introduction of variants, soil traits, fungal endophytes), dataset Jul08-Aug09 (introduction of *SRL* and new nodosity attributes) and the dataset 2009 (additional introduction of new nodosity attributes). Altogether, a total of 1.7 m^3 soil and 195.5 km roots (average diameter of 0.61 mm) was investigated. 11972 root swellings and 3880 grape phylloxera individuals were counted. After the last development of trait related attributes in the end 2008, the dataset 2009 held nearly 190 parameters per sample.
The aim of the present work is the development of a field method to assess the grape phylloxera - grape root interactions. This aim is reflected by the design of this paragraph, which is divided into three sections. The first section (section 4.1) focus on the methodical development and occurring problems during the work. Especially the development of nodosity parameters or problems during digital analysis of the root system are pointed out. To verify the temporal, spatial and cultivar-specific sensitivity of the method, the second section (section 4.2) focus on the assessed and developed parameters. Especially the sensitivity and propriety of the method regarding the temporal, spatial and cultivar-specific dynamics of the developed and assessed parameters are pointed out. The last section (section 4.3) focus on the general and specific relations in grape phylloxera population and root system dynamics. Here the developed and assessed parameters are selected on base of their relationships in field.

4.1 Method Development

Figure 9: First section of results: description of methodical development and methodical problems

The development and validation of the used methods was mainly influenced by field conditions and observations made in field. Fundamental methodical problems are explained in section 1 and 2.4.
Methodical questions mainly came up regarding a field assessment method according to Porten and Huber (2003). It was compared with a instar counting method in laboratory. Regarding the grape phylloxera population structure, new visual parameter had to be developed. Here, nodosities were used as a visual indicator of a grape phylloxera infestation and quantitative nodosity parameters were introduced (section 4.1.1). Also the adaption of some digital assessed root system parameters were questionable concerning the digitized root fragments or the high branching rate of grape roots (section 4.1.2). At last, the potential application of a root color analysis was verified (section 4.1.3).

4.1.1 Grape Phylloxera Recording

From June 2006 until January 2007, a grape phylloxera assessment method according to Porten and Huber (2003) was adopted besides a counting of grape phylloxera instars in laboratory. In the laboratory method, instars were grouped into three classes: L1-L4 (juvenile virginopara), L5 (adult virginopara), and nymphae (winged preforms). Field assessment and laboratory recording methods were compared. To improve the description of grape phylloxera population on roots, the laboratory counting method was enhanced with different nodosity attributes in the years 2007 and 2008 (section 4.1.1.2).

4.1.1.1 Field Assessment and Counting Method All results shown in this paragraph based on the dataset 2006 (June 2006 to January 2007). All parameters (assessment classes, number of instars) had the used digging box (0.008 m^3) as a reference value. The assessment according to Porten and Huber (2003) focused strength and potential progression of grape phylloxera infestation on the root system. Here, no differentiation of instars was designated. The assessment of infestation density of single nodosities happened by a classification of the number of instars as well as the number of eggs (Porten and Huber, 2003). Class III are nodosities occupied by one individual (no eggs), class V are nodosities occupied by more than one individual (no eggs), class VII are nodosities occupied by more than one individual (only one egg), class IX are nodosities occupied by more than one individual (more than one egg).
The counting method on the other hand focused a quantitative recording of infestation density. Therefore, it was necessary to count single grape phylloxera instars in laboratory. During the treatment of the samples (between field and laboratory), eggs were detached from the roots. So, within the counting laboratory method, eggs could not be regarded. To verify the assumption that the non oviparous juvenile virginopara (L1-L4) append mainly to the classes III and V (no eggs) and the oviparous adult virginopara (L5) append mainly to the classes VII and IX, correlations were calculated (table 10). Although assessed classes and the number of counted instars differ in validity, significant correlations could be calculated. In particular, the classes III and V correlated with the counted L1-L4 and class IX correlated with the counted L5 (table 10).
A Wilcoxon test of paired samples (P=0.05) with total numbers of counted instars and total numbers of assessed classes per month was calculated (table 11). Significant differences appeared in the months October to January, whereas the number of counted instars were higher than the assessed number of classes (figure 10). This depended mainly on different quantities in assessed classes III (and V) and counted numbers of L1-L4 (figure 10A). During the summer months (June-September), the assessed number of classes fit with counted instars.

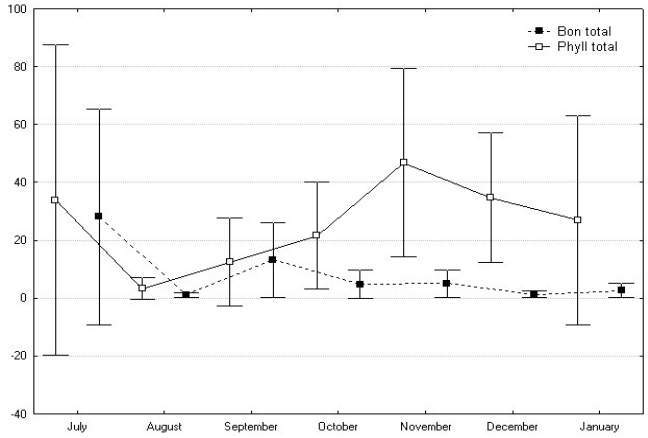
Detected differences between assessment method and laboratory method could be caused by various facts. Because the assessment method detects nodosities and no instars, the number of instars per nodosity (occupation) could be higher in winter than in summer. Furthermore single instars can not be

Table 10: Correlation of assessment classes and counted instars (=parameter). Reference value: digging box. SU=44. *=significant, P=0.05

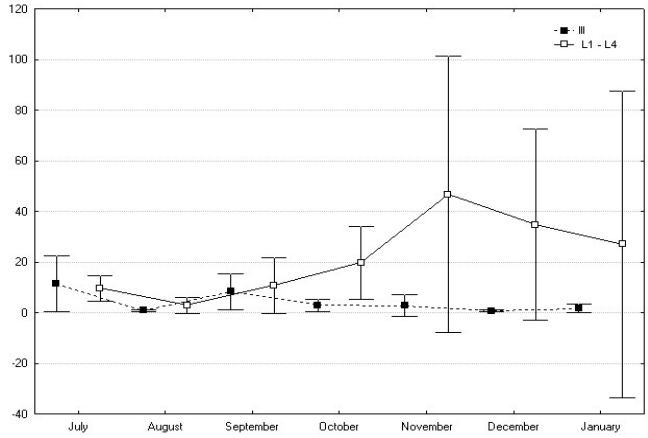
Class/Parameter	L1-L4	L5	Nym	Ttl. inst.
III	0.3*	0.64*	0.46*	0.42*
V	0.49*	0.68*	0.46*	0.61*
VII	0.06	0.41*	0.33*	0.18
IX	0.26	0.78*	0.52*	0.41*
total	0.4*	0.66*	0.46*	0.52*

Table 11: Wilcoxon test of total instars against total assessed classes per month, *=significant, P=0.05

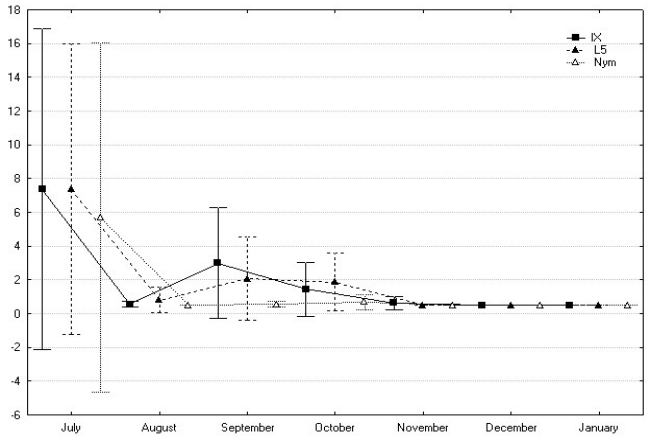
Period	n	Z	p
July 06	8	1.12	0.26
August 06	8	1.46	0.14
October 06	8	2.52	0.01*
Nov 06 - Jan 07	12	3.06	0.001*



(a) Total assessment classes vs. total counted instars



(b) Assessment class II vs. L1-L4



(c) Assessment class IX vs. L5 and Nym

Figure 10: Mean and 1.96 * SD of monthly values of laboratory counting method and monthly values of the field assessment method from July 2006 to January 2007. Reference value: digging box. **A:** All assessment classes and all counted instars. **B:** Assessment class III and L1-L4 instars. **C:** Assessment class IX, L5 and nymphal instars.

assigned to a single class directly and some classes are defined by an occupation with more than one individual.

Therefore, a direct relation of counted number of instars to the number of assessed classes provides only an indication of differences comparing the two methods. Seasonal and total frequencies of assessed classes and counted numbers of instars were compared by a χ^2 test of homogeneity (table 12). Comparing the total frequencies, a high p level (0.99) was found. The grape phylloxera population is highly refelcetd by the assessment method according to Porten and Huber (2003). Comparing seasonal frequencies, no significances were found. But from October to January the p level decreased from 0.99 (September) to 0.09 (January) for total classes and total instars (table 12). So, frequencies in number of instars and assessed classes differed more in those months than in summer. Considering the aim of the present work (quantitative assessment of grape phylloxera population over the whole year), the method according to Porten and Huber (2003) could not be used.

Table 12: χ^2-test of homogeneity of assessed classes against counted instars

Period	n	Pair of Values (Obs,Exp)	χ^2	p
July 06	8	total classes, total instars	2.32	0.94
		class IX, L5	4.13	0.76
		class III, L1-L4	2.4	0.93
August 06	8	total classes, total instars	1.47	0.98
		class IX, L5	2.64	0.91
		class III, L1-L4	1.27	0.98
September 06	8	total classes, total instars	1.19	0.99
		class IX, L5	0.35	0.99
		class III, L1-L4	1.88	0.97
October 06	8	total classes, total instars	5.26	0.63
		class IX, L5	6.63	0.47
		class III, L1-L4	4.05	0.77
November 06	4	total classes, total instars	2.48	0.48
		class IX, L5	-	-
		class III, L1-L4	3.75	0.29
December 06	4	total classes, total instars	6.29	0.09
		class IX, L5	-	-
		class III, L1-L4	7.1	0.07
January 07	4	total classes, total instars	3.35	0.34
		class IX, L5	-	-
		class III, L1-L4	3.96	0.27
Nov 06 - Jan 07	12	total classes, total instars	12.12	0.36
		class IX, L5	-	-
		class III, L1-L4	14.81	0.19
Jul 06 - Jan 07	44	total classes, total instars	22.36	0.99
		class IX, L5	6.2	0.99
		class III, L1-L4	28.37	0.95

Summary: The assessment of grape phylloxera density in field according to Porten and Huber (2003) reflected counted instars of grape phylloxera in summer particularly. But in winter differences

in the frequency of the detected instars and counted classes occurred. This corresponded with significant differences in total sums of counted instars and counted classes. This underestimation of grape phylloxera infestation on roots could be caused by an increased occupation per nodosity.

4.1.1.2 Nodosities The detection of grape phylloxera population was connected to a continuous introduction of new nodosity classes from January 2007 to January 2009. These classes are nodosity color, nodosity form and nodosity branching (= main parameters, figure 11). Nodosity color classes were divided into three sub parameters, nodosity form into four sub parameters and nodosity branching into two sub parameters (table 13).

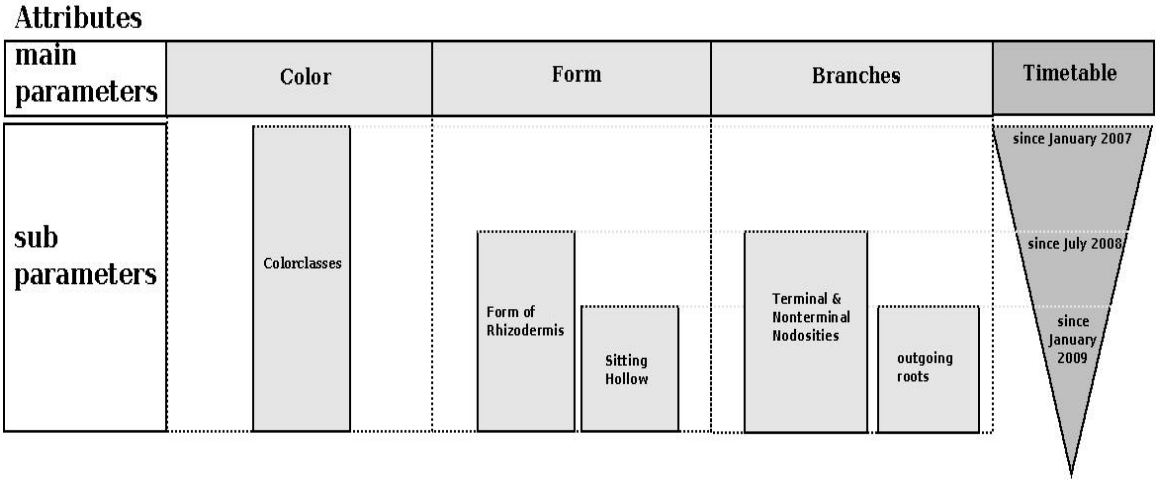


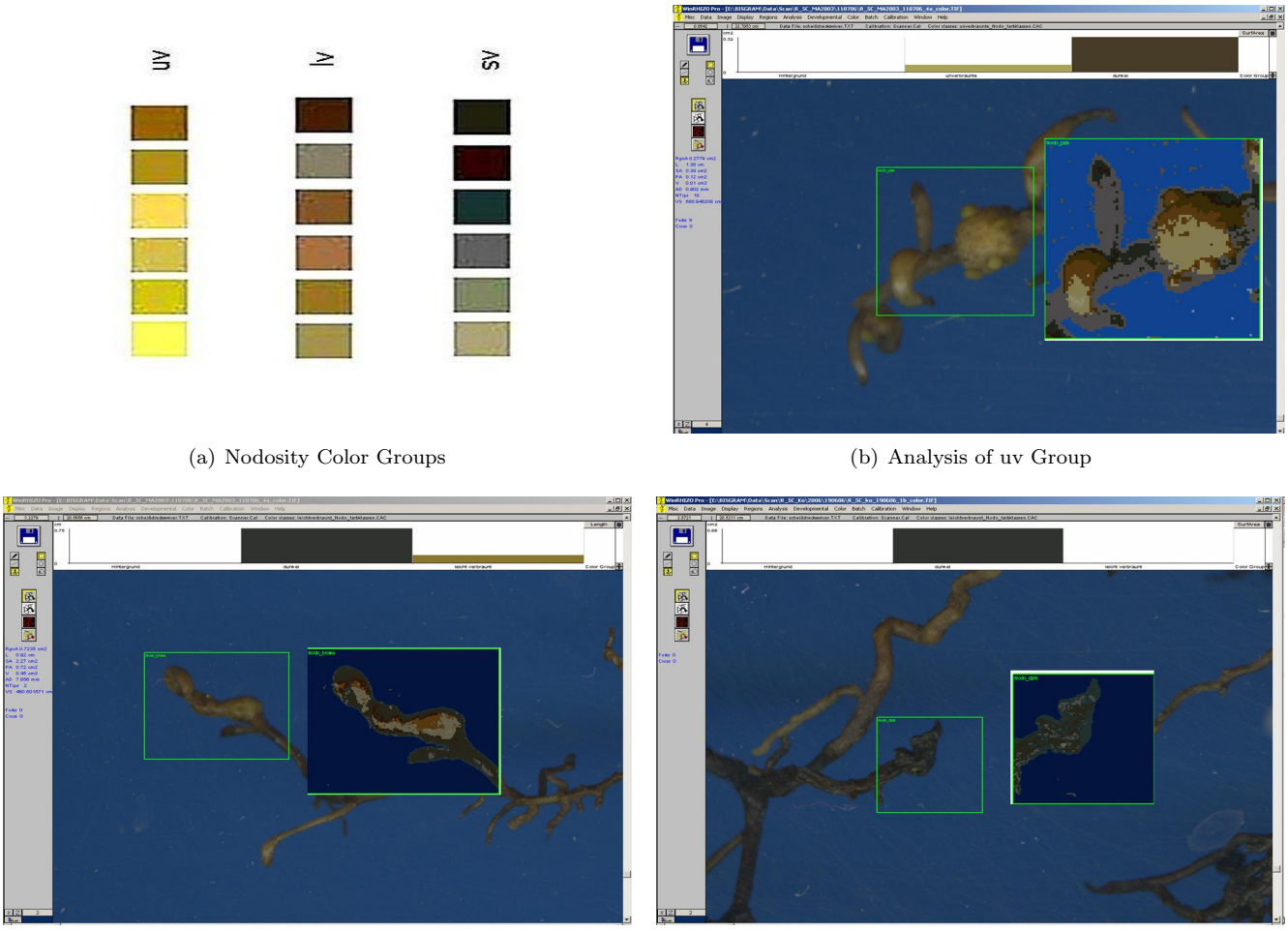
Figure 11: Timetable illustrating the implementation stages of the nodosity attributes related to the phylloxera counting laboratory method.

Table 13: Nodosity main parameters, sub parameters and specification

Main parameter	Sub parameter	Specification
color	sv	dark brownish
	lv	light brownish
	uv	not - little brownish
form	A	bursted epidermis, sitting hollow
	B	bursted epidermis, no sitting hollow
	C	no bursted epidermis, sitting hollow
	D	no bursted epidermis, no sitting hollow (= no feeding site)
branching	terminal (term)	root ends at nodosity
	not terminal (non-term)	root continues after nodosity (= perfoliated)

Since January 2007, all root swellings identified as nodosity were counted. Root swellings occupied by grape phylloxera instars were identified as nodosity. Not occupied root swellings were identified as nodosity by identifying the feeding site. The feeding site was characterized by the presence of a sitting hollow (often with the presence of a burtsed bark). These characteristics were introduced in the

nodosity form parameters in July 2008.
Since January 2007, the nodosity coloration was quantified. RGB color images of the dataset of 2006 were analyzed by Win Rhizo Pro, regarding the color of nodosities. In doing so, color classes were set by hand and tested by a visual comparison of the dispersion of the single color classes within selected images (dataset 2006). Three color classes (sub parameters) were compiled: sv (dark brownish), lv (middle brownish) and uv (light to non brownish) (figure 12).
Since July 2008, the attribute form and the attribute branches were introduced. The form of nodosity was described by its bursted or not bursted epidermis until January 2009. Since January 2009, the occurrence of a fully developed feeding site (sitting hollow) was considered also. The attribute branches were detected by recording terminal (term) and non-terminal (non-term) nodosities since July 2008, describing a normal root growth of all nodosity attached roots. The number of nodosity outgoing roots was detected since January 2009 (figure 11). In figure 13, examples for some nodosity attributes and values are given. In field, many root swellings were found which could not be related to a grape phylloxera occupation. Due to the absence of a feeding site and a bursted epidermis, these root swellings were counted from January 2009 to August 2009 and classified in form class D.



(a) Nodosity Color Groups

(b) Analysis of uv Group

(c) Analysis of lv Group

(d) Analysis of sv Group

Figure 12: **A:** Nodosity color classes (sub parameters) uv, lv, sv created by analysis of RGB images of dataset 2006 by Win Rhizo Pro. These color scheme was used under the stereo microscope to classify nodosity coloration. **B:** uv color group: Nodosity coloration was pale, but sometimes variable (sample: 5C, 11.07.2006, number 8). **C:** lv color group: nodositiy coloration was very variable. **D:** sv color group: nodosity coloration is similar to dark root coloration.

Altogether, four different datasets reflect the stages of the attribute introduction regarding the grape phylloxera population: dataset 2006-2009 (grape phylloxera instars), dataset 2007-2009 (instars, color of nodosities), dataset Jul08-Aug09 (instars, color, form (A,C), branches of nodosities), dataset 2009 (instars, color, form (A-D), branches, outgoing roots of nodosities).

To identify group explaining parts of the total variance within a dataset, four factor analyses were done (extraction: principal components, rotation: varimax, max. number of factors: 3, min. eigenvalue: 1.0). In every dataset, all parameters were integrated (RPD, APD, CNA, NOP): 2006-2009, eight parameters; 2007-2009: 21 parameters; July 2008 - August 2009: 70 parameters; January 2009 to August 2009: 95 parameters (tables 14 and 16). Regarding the dataset 2006-2009, 7 of 8 included parameters could be assigned to a factor level. 71 % of total variance could be explained by two factor levels. But the eigenvalues were very low (table 15). Factor 2 explained a lower part of variance by

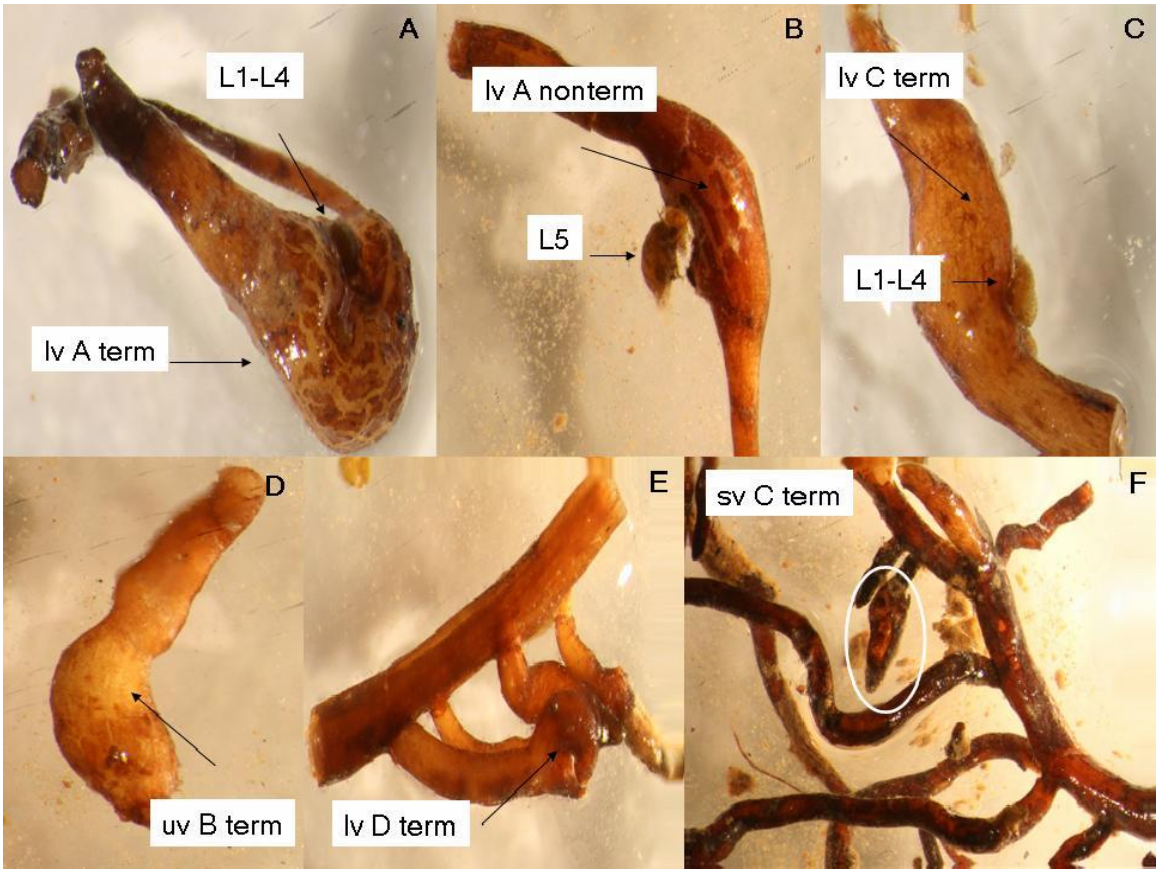


Figure 13: Example Images of different Nodosities.**A:** lv A term nodosity with L1-L4 instars. **B:** lv A non-term nodosity with L5 instar. **C:** lv C term nodosity with L1-L4 instar. **D:** uv B term nodosity, no instars. **E:** lv D term, no instars. **F:** sv C term, no instars.

total number of instars per cm and *number of L1-L4 per cm*. The main part of variance was explained by factor 1 (all other parameters) (table 14). In dataset 2007-2009, only 9 of 21 (42.9 %) included parameters were assigned to a factor level, but 47.8 % of total variance could be explained with higher eigenvalues than in dataset 2006-2009 (table 15). Factor 1 with the highest eigenvalues was built by grape phylloxera instar attributes. Factor 2 was built by nodosity attributes mainly, factor 3 by nodosity occupation parameters (table 14).

Table 14: FA of grape phylloxera traits in dataset 2006-2009 and 2007-2009. Extraction method: Principal Components, Rotation: Varimax, maximum factors: 3. Shown are only parameters with one factor loading ≥ 0.7 (highlighted in bold letters). Incl. Par. = Number of included parameters

Dataset	Num. SU	Incl. Par.	Parameter	Factor 1	Factor 2	Factor 3
2006-2009	449	8	L1-L4 per cm	0.13	**0.93**	-
			Nym per cm	**0.73**	0.12	
			ttl phyll per cm	0.15	**0.92**	
			L1-L4 per 10^{-2}sqm	**0.82**	0.27	
			L5 per 10^{-2}sqm	**0.84**	0.1	
			Nym per 10^{-2}sqm	**0.84**	0.05	
			ttl phyll per 10^{-2}sqm	**0.89**	0.23	
2007-2009	406	21	Nym per cm	**0.78**	0.13	-0.02
			sv per 10^{-2}sqm	0.07	**0.75**	-0.08
			lv per 10^{-2}sqm	0.09	**0.72**	0.08
			L1-L4 per 10^{-2}sqm	**0.7**	0.21	0.47
			L5 per 10^{-2}sqm	**0.79**	0.06	0.1
			Nym per 10^{-2}sqm	**0.81**	0.09	0.06
			ttl phyll per 10^{-2}sqm	**0.78**	0.19	0.41
			ttl nod per 10^{-2}sqm	0.43	**0.75**	0.08
			L1-L4 per uv	0.24	-0,08	**0.73**

Table 15: Eigenvalues of the single factors

Dataset	Factor	Eigenvalue	expl. Variance[%]	cumul. Variance[%]
2006-2009	Factor 1	4.21	52.67	52.67
	Factor 2	1.47	18.39	71.07
2007-2009	Factor 1	6.46	30.77	30.77
	Factor 2	1.91	9.1	39.86
	Factor 3	1.67	7.96	47.83
Jul08-Aug09	Factor 1	18	25.72	25.72
	Factor 2	6.42	9.17	34.89
	Factor 3	4.56	6.51	41.4
2009	Factor 1	22.31	23.49	23.49
	Factor 2	8.49	8.93	32.42
	Factor 3	6.83	7.19	39.61

Regarding dataset Jul08-Aug09, 14 of 70 (20 %) included parameters explained 41.39 % of the total variance in three factor levels (tables 15 and 16). As in dataset 2007-2009, grape phylloxera instar attributes held the factor with the highest eigenvalues and could be divided from nodosity attributes and nodosity occupation parameters (table 16). In dataset 2009 (Jan09-Aug09) 25 of 95 (26.3 %) included parameters explained 39.6 % of the total variance in three factor levels, whereas factor 1 had a high eigenvalue (table 15). Not only instar parameters explained the highest part of variance (factor 1), but also nodosity parameters like *uv per 10^{-2}sqm* , *A per 10^{-2}sqm* or NOP like *L5 per uv* and *L5 per A* held high loadings on factor 1 (table 16). Nodosity attributes (factor 2) could be divided from parameters regarding DW and nodosity occupation parameters (factor 3) (table 16).

The variances in the dataset 2006-2009 could be mainly explained (52.67 %) by the relative instar densities (RPD) per 10^{-2}sqm . Absolute instar densities (APD) could explain another 18.39 % of total

Table 16: FA of grape phylloxera traits dataset Jul08-Aug09 and 2009. Extraction method: Principal Components, Rotation: Varimax, maximum factors: 3. Shown are only parameters with one factor loading ≥ 0.7 (highlighted in bold letters). Incl. Par. = Number of included parameters

Dataset	Num. SU	Incl. Par.	Parameter	Factor 1	Factor 2	Factor 3
Jul08-Aug09	248	70	Nym per cm	**0.77**	0.15	0.09
			C per 10^{-2}sqm	0.12	**0.76**	0.08
			L5 per 10^{-2}sqm	**0.75**	0.07	0.17
			Nym per 10^{-2}sqm	**0.76**	0.03	0.26
			ttl phyll per uv	0.25	0.07	**0.81**
			ttl phyll per A	0.27	0.03	**0.82**
			ttl phyll per term	0.2	0.08	**0.72**
			ttl phyll per non-term	0.39	-0.04	**0.73**
			L1-L4 per lv	-0.05	0.16	**0.7**
			L1-L4 per uv	0.12	0.1	**0.81**
			L1-L4 per A	0.07	0.1	**0.85**
			L1-L4 per term	0.06	0.08	**0.72**
			L1-L4 prt non-term	0.6	0.04	**0.76**
2009	136	95	L5 per cm	**0.72**	-0.11	0.22
			Nym per cm	**0.73**	0.01	0.23
			lv per 10^{-2}sqm	0.14	**0.71**	0.06
			uv per 10^{-2}sqm	**0.89**	-0.01	0.19
			A per 10^{-2}sqm	**0.73**	0.37	0.21
			L5 per 10^{-2}sqm	**0.77**	0.05	0.29
			ttl nod per 10^{-2}sqm	0.44	**0.76**	0.04
			L1-L4 per DW	0.04	0.3	**0.74**
			ttl phyll per DW	0.32	0.08	**0.73**
			ttl phyll per lv	0.08	0.06	**0.76**
			ttl phyll per uv	0.23	-0,03	**0.81**
			ttl phyll per A	0.39	-0.04	**0.8**
			ttl phyll per term	0.17	-0.09	**0.74**
			ttl phyll per non-term	0.42	0.05	**0.74**
			L1-L4 per lv	-0.02	0.02	**0.76**
			L1-L4 per uv	0.12	-0.03	**0.79**
			L1-L4 per A	0.18	-0.01	**0.85**
			L1-L4 per term	0.17	-0.04	**0.74**
			L1-L4 per non-term	0.23	0.02	**0.78**
			L5 per uv	**0.73**	0.04	0.07
			L5 per A	**0.74**	-0.03	0.19
			L5 per non-term	**0.7**	0.05	0.13
			uvAterm	**0.84**	0.08	0.14
			uvCterm	**0.82**	0.05	0.15
			uvAnon-term	**0.75**	0.04	0.28

variance in the dataset. By introducing nodosity parameters (dataset 2007-2009), grape phylloxera instar densities (both APD and RPD) could explain 30.77 % of total variance, whereas the relative nodosity densities (RPD) explained another 9.1 %. Also nodosity occupation parameters explained a part of total variance (dataset Jul08-Aug09; dataset 2009).

Altogether, grape phylloxera population related parameters can be divided into four groups. Group 1 is explained by factor 1 (high eigenvalues). This factor chiefly described the development of grape phylloxera instars and described the main part of the total variance in a dataset. Further, the FA of the dataset 2006-2009 showed that RPD and APD parameters explain different parts of the total variance. The parameters related to the group 2 are explained by factor 2 (lower eigenvalues) and described the development and densitiy of nodosities. The parameters in group 3 are explained by factor 3 (lowest eigenvalues). These are mainly nodosity occupation parameters.

Summary: The main variance in the population density assessed by the introduced parameters can be explained by the grape phylloxera instar development. By introducing nodosity characteristics, additional variances could be explained. Nodosity parameters are important parameters to explain the variance in the occurrence of grape phylloxera population structure. Besides APD and RPD parameters, NOP were able to explain a part of the variance in total grape phylloxera appearance. Grape phylloxera population related parameters could be divided into four groups: grape phylloxera related parameters, nodosity related parameters, nodosity occupation parameters, and those explaining no variance.

4.1.2 Gray Scale Analysis

The root system of *Vitis berlandieri* x *Vitis riparia* was investigated by collecting root fragments of a predetermined soil volume (section 3.2.1). Root fragments were digitized and analyzed by Win Rhizo Pro (section 3.2.2.2). Due to working with root fragments, a possible overestimation of counted root tips can emerge. Furthermore, due to the high complexity of *Vitis* ssp. root system, a possible overestimation of calculated root length by overlapping root fragments can emerge. Before the verification, the effects of the different digging box sizes and number of sample units on root system main parameters were assessed.

4.1.2.1 Number of Sample Units, Size of the Digging Box The number of sample units (SU) as well as the size of the used digging box played an important role, reflecting accurate results. Digging box size (reference sample volume) and the number of SU were in line with the practicability in field and had to be balanced with statistical reproducibility. Altogether, three different approaches were executed from 2006 to 2007. Between June 2006 and October 2006, SU of n=8 with a big digging box (0.04 sqm surface, 20 cm depth; figure 14A) and a simultaneous grape phylloxera assessment in field (section 4.1.1) was done. The high amount of work could be managed with the friendly support of Dipl. Biol. XXXXXX. In consequence of a reduction of manpower, number of SU was decreased to n=4 from November 2006 to July 2007. Because of a lag in statistical validity and a furthermore manageability of the work amount, the number of SU was increased to n=10 and the digging box size was reduced (= small digging box, 0.01 sqm surface, 20 cm depth; figure 14B) since August 2007. All data used to calculate effects of size of the digging box and number of sample units based on variant 5C and were converted to the reference value of a 10^{-2}sqm soil surface (and 20 cm depth).

(a) Big digging box (b) Small digging box

Figure 14: A: Big digging box. **B:** Small digging box.

Table 17: Kruskal Wallis ANOVA (H), Median test (χ^2) and Bonferroni post-hoc test (effect: number of SU). Variant = 5C. Effects: size of digging box, number of SU, dep. var.: total root length, total surface area, average diameter (all per 10^{-2}sqm) Valid n = 319. *=significant

Effect	Parameter	H	p (H)	χ^2	p (χ^2)	sign. post-hoc
digging box size	Length	30.69	0.001*	22.17	0.001*	
	Surface area	27.3	0.001*	19.97	0.001*	
	Average diameter	2.838	0.0921	0.6516	0.415	
Number of SU	Length	9.413	0.009*	11.16	0.0038*	SU=4 and SU=10
	Surface Area	8.02	0.018*	9.27	0.01*	SU=4 and SU=10
	Average Diameter	5.72	0.057	1.386	0.5	-

A Kruskal Wallis ANOVA was calculated to identify significant effects of the digging box size or the number of sample units in root system main parameters (table 17). The number of SU had significant effects on *total root length* and *total root surface area* (per 10^{-2}sqm). To identify significant level of the factor SU, a Bonferroni post-hoc test was done. Between the levels SU=4 and SU=10 significant differences in *total root length* and *total root surface area* occurred (table 17). This could be conditioned seasonally (sample period mainly in winter and spring in SU=4) and/or up to a low total n (24) in SU=4.

Total root length and *total root surface area* (both per 10^{-2}sqm) were affected by size of the digging box significantly. A median test confirmed this results (table 17). *Average root diameter* was not effected by size of the digging box or number of SU. The mean values of *total root length* and *total root surface area* (per 10^{-2}sqm) decreased with a less digging box size. Also an intense decrease of variance could be observed (table 18). Considering monthly values, variances spread mostly with a big digging box size and were lower in the off-peak period than during growth period (not shown here).

Table 18: Variances of some root system main parameters (per 10^{-2}sqm). Variant 5C.. big = 0.04 * 10^{-2}sqm soil surface, small = 0.01 * 10^{-2}sqm soil surface.

Parameter	Digging Box Size	Total n	Mean	Variance
length	big	103	578.51 cm	83980
	small	216	394.56 cm	48179
surface area	big	103	105.56 sqcm	2413.15
	small	216	75.12 sqcm	1625.42
average diameter	big	103	0.596 mm	0.0871
	small	216	0.617 mm	0.01

Summary: Observed significant effects between different numbers of SU could be caused by seasonal differences. But the main parameters *total root length* and *total root surface area* decreased with a smaller size of the digging box significantly. Due to the lower diameter of a small digging box, a lower chance to find parts with high root growth in soil could be a possible reason. Also the variances decreased, whereas variances in off-peak months are lower than within growth period.

4.1.2.2 Root System Parameters Root system parameters are calculated on base of gray level images. This method provides accurate results (Arsenault et al., 1995; Bouma et al., 2000). But in the present work some specifics must be considered, regarding the use of root fragments as well as the high branching structure of *Vitis* ssp. root system. First working with root fragments could cause an overestimation of root tips, if every fragment end is counted as a tip. Second the high branching structure could lead to an overestimation of length by overlapping roots (section 2.4).

Figure 15 shows a gray scale image (generated by a TLU) of a root fragment (5C, 05.06.2009, sample number one). Red points are root tips counted by Win Rhizo Pro. In figure 15 every ending of a fragment is counted as a tip. On a length of 5.54 cm five root tips were counted by Win Rhizo Pro, but in fact the analyzed fragment did not have any root tips. The root fragment in figure 15 exemplified the overestimation of tips demonstratively. Altogether, in morphological root system analyses, the counted number of tips was depended by the number of fragments per image. This led to an not calculable overestimation of root tips.

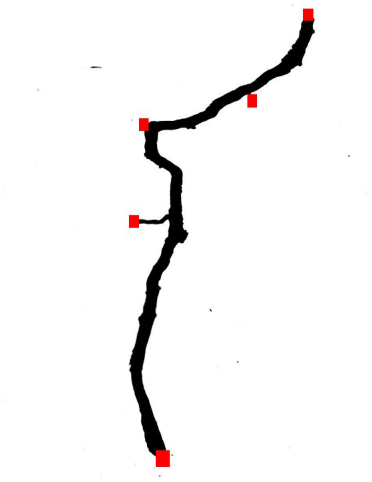


Figure 15: Overestimation of root tips by Win Rhizo Pro related to root fragments. Fragment from sample number one (05.06.2009), variant 5C (original length: 5.54 cm). Red points are digital counted tips. In fact, the root fragment did not have any tips.

Overlapping branches of roots could lead to an overestimation of root length (section 2.4). By extracting and digitizing root fragments, a generally three dimensional root system is reduced to two dimensions. In the present work, images were produced in a manner that root fragments did not overlap. But regarding the complex branching structure of *Vitis* ssp. root system, a root overlapping in fine root parts of high density was normal. This led to an overestimation of the number of crossings and number of forks (figure 16). But an indication of overlapping roots could be an indication of complexity of the analyzed part of the root system. It could be supposed that the more roots in a predetermined soil volume, the higher the complexity is.

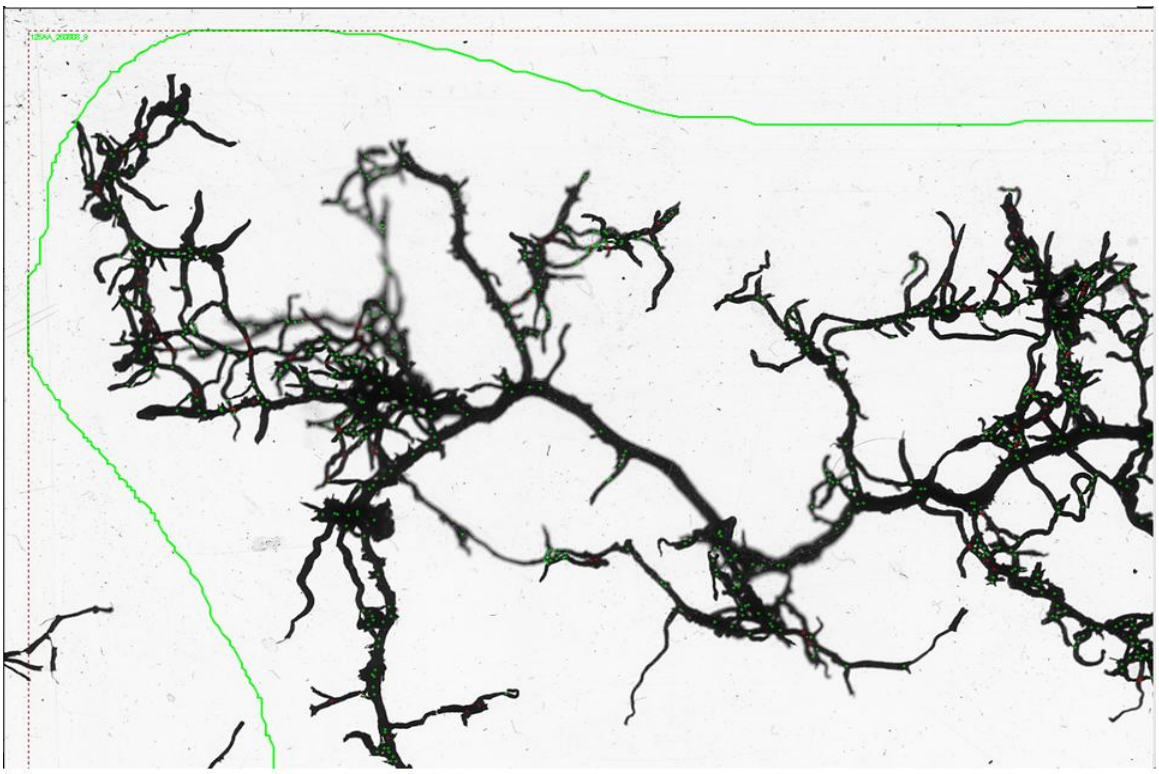


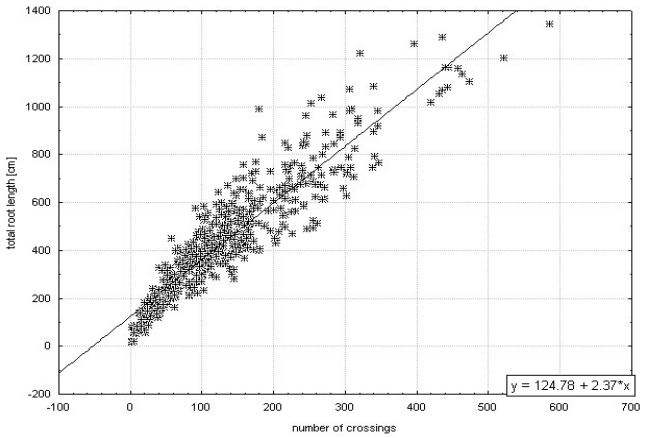
Figure 16: Overestimation of crossings and forks in a typically dense part of *Vitis* ssp. root system. Part of a bigger fragment from sample number nine (26.08.2008), variant 125AA (length of total fragment: 190.5 cm, counted crossings: 105, counted forks: 1144). Red points are digital counted crossings, green points are digital counted forks.

Regarding above mentioned arguments, it could be assumed that *number of crossings* did not reflect the natural number of crossings, but it could be a possible indicator for overlapping roots. Furthermore, *crossings per cm* could give an indication of density of overlapping roots. So, a possible impact of overlapping roots on the calculation of root length was estimated by comparing the calculated *number of crossings* with the calculated *total root length*. If an increased overlapping of highly branched roots had no impact on calculated root length, counted *number of crossings* and the calculated *total root length* should be in a high linear relation. Furthermore, *crossings per cm* should not be in a relation to *total root length*. Correlations between *number of crossings*, *crossings per cm* and *total root length*

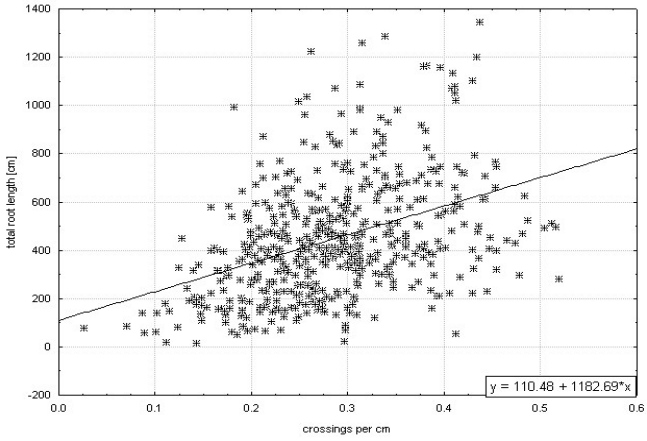
(total data set) were calculated (table 19).

Table 19: Correlations between *number of crossings*, *crossings per cm* and *total root length*. Total data set, n=489. *=significant

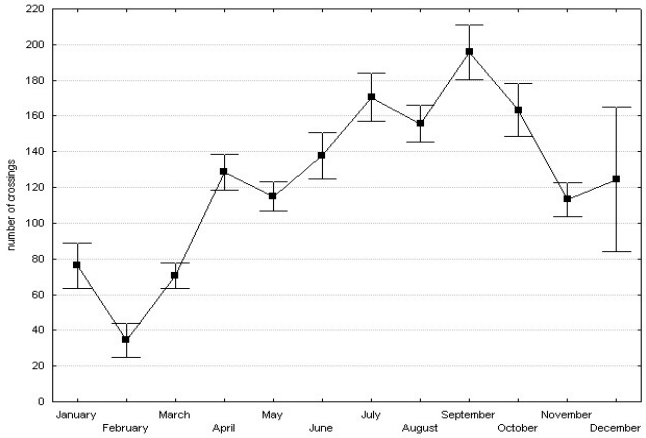
Correlation Pair	r	r^2	y
Total root length, number of crossings	0.92*	0.85	124.78 + 2.37 * x
Total root length, crossings per cm	0.4*	0.16	110.48 + 1182.69 * x



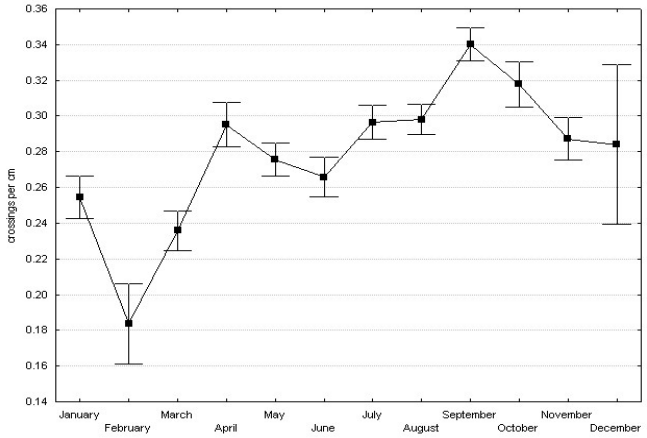
(a) Number of crossings vs. Total root length



(b) Crossings per cm vs. Total root length



(c) Temporal development Number of Crossings



(d) Temporal development Crossings per cm

Figure 17: Dataset: 2006-2009. **A:** Scatterplot *number of crossings* and *total root length*. High correlation. **B:** Scatterplot *crossings per cm* and *total root length*. Low correlation. **C:** Development of *number of crossings* per month (mean and 1.96*SD). **D:** Development of *crossings per cm* per month (mean and 1.96*SD).

A strong linear correlation between *number of crossings* and *total root length* was detected (r=0.92). Against the assumption, correlation between *crossings per cm* and *total root length* was significant, but weak (r=0.4) (table 19). Considering the temporal (mean course of all years) distribution of the parameters, both *number of crossings* and *crossings per cm* showed similar progressions (figure 17). As well, *total root length* showed a similar development in the course of a year (section 4.2.2). Besides

a possible weak influence of overlapping roots on the calculation of *total root length*, also an increased complexity of the root system in summer could be causal for the calculated significance in correlation between *total root length* and *crossings per cm*. *Fractal dimension*, which could conduce also to an indication for complexity, increased between winter and late summer, too (section 4.2.2). So, the increased density of *crossings per cm* could be up to an increased complexity of root system in summer.

Summary: Working with fragments of roots led to a not calculable overestimation of counted tips. Thus, analyzed values for measured tips could not be considered. Although an overlapping of single fragments was prevented, overlapping branches of roots (reducing from three dimensions to two dimensions) lead to a not calculable overestimation of crossings and forks. This has no major effects on the calculation of *total root length* within the present dataset. A weak correlation between density of crossings and root length could be caused by an increased complexity of root system in summer.

4.1.3 Color Analysis

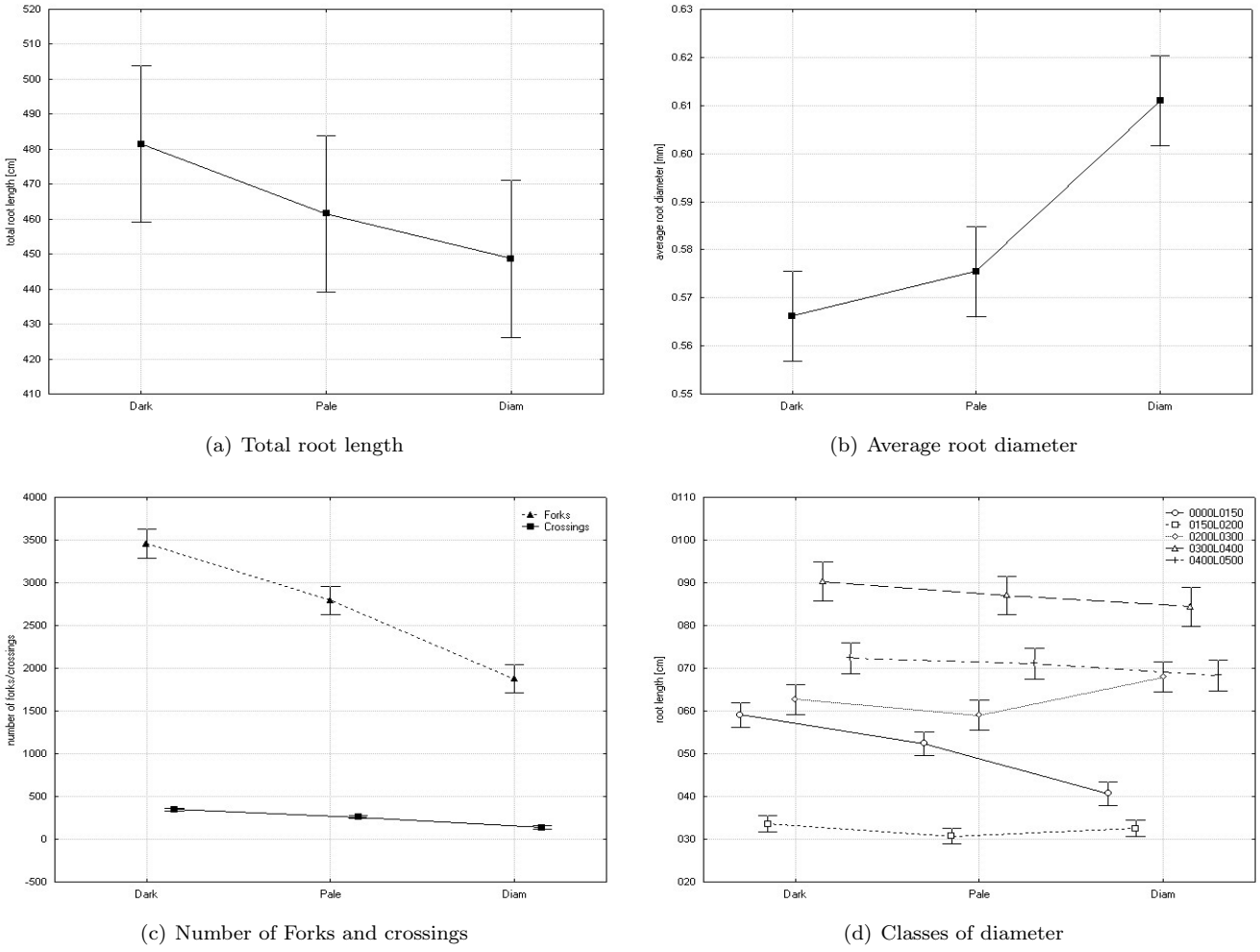
4.1.3.1 Accuracy of Color Analysis The analysis of RGB color images via Win Rhizo Pro should be used to differentiate dark and pale root parts. This could be suitable for an estimation of fresh roots and root turnover consequently. In Win Rhizo Pro, colors are assigned to root parts manually. Three color groups must be defined, each with a maximum of 12 color classes. Not defined colors were approximated to one color group ("Dark" or "Pale"), regarding the color tolerance. The tolerance adjustments of color intensity, color hue and color saturation could be changed in each color class.
Considering the very diverse coloration of *Vitis* ssp. roots, problems have been arisen in assigning different brown colors to the color groups "Dark" or "Pale". In consequence, some almost similar brown tones appeared in "Dark" as well as in "Pale". This led to different measurement results. To verify the impact of the tolerance adjustments, two different settings were introduced: a wide tolerance in all brownish colors in "Dark" (= WDR (wide dark range)) and a wide tolerance in all brownish colors in "Pale" (=WPR (wide pale range)).

To verify an impact of color classes on morphological root system parameters, WDR and WPR were compared to the gray scale analysis (GSA) by a Kurskal-Wallis ANOVA (Factors: type of analysis, three factor levels (WDR, WPR, GSA)) (table 20). Dependent variables were root system main parameters. Afterwards, a Bonferroni post-hoc test was done (P=0.05) to identify significant factor levels. *Number of forks* and *number of crossings* decreased in gray scale analysis significantly (table 20, figure 18C). Thus *average root diameter* was significantly higher at gray scale analysis than in WDR or WPR (table 20, figure 18D). Although no significances could be calculated, a strong increase of *total root length* in color analysis was detected (figure 18A). The main impact of differences occurred in length in diameter classes 0 - 0.5 mm (figure 18D). In *total root surface area* no significant differences were found. This could be up to higher values of *total root length* and lower values of *average root diameter* in WDR.

Table 20: Kurskal-Wallis ANOVA: color analysis: p-values of Bonferroni post-hoc test. *=significant. n=490 per factor level

Parameter	WDR - WPR	WDR - GSA	WPR - GSA
Length	0.44	0.1	0.7
Projected area	0.71	0.99	0.78
Surface area	0.71	0.99	0.78
Average diameter	0.36	0.001*	0.001*
Forks	0.001*	0.001*	0.001*
Crossings	0.001*	0.001*	0.001*

The found significances could be partly caused by shadowing problems, which emerged during the color analyses (figure 19). Color images could not be generated by the TLU, but by an usual below placed luminous source. Due to the scanning method, root fragments with higher diameters as well as with an intense three dimensional orientation caused dark brownish shadows. The coloration of this



(a) Total root length

(b) Average root diameter

(c) Number of Forks and crossings

(d) Classes of diameter

Figure 18: Mean and 1.96*SD of length, average diameter, number of forks and crossings and length in diameter classes 0-0.5mm (all per 10^{-2}sqm surface). Different analysis types: WDR, WPR and GSA. **A:** Total root length. **B:** Average root diameter. **C:** Number of forks and crossings. **D:** Length in diameter classes 0-0.5 mm.

shadows was similar to dark root parts. An automatical differentiation between shadow and root was not possible. An exemplary manually deletion of a shadow reduced the measuring failure (compared with the GSA) (figure 19).

Summary: Two main problems occurred during the color analysis of root fragments. Due to the possibility to change tolerance values of each assigned color class, different measurements of morphological root parameters related to color groups could happen. Secondly, strong differences in coloration of root parts as well as shadows lead to a strong overestimation of *total root length, number of crossings* and *number of forks* as well as a simultaneous underestimation of *average root diameter*.

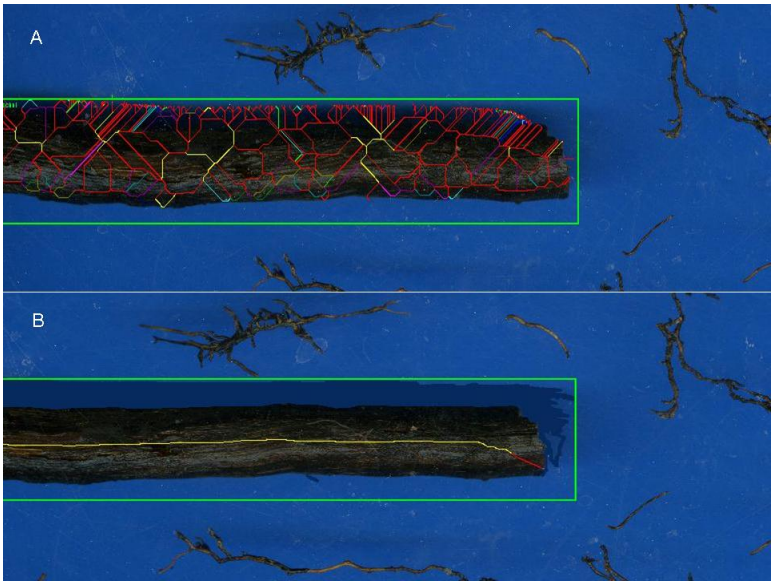


Figure 19: Example for shadowing of a coarse root in color analysis. Shown is calculated total root length **A:** Analysis of root part with shadows. **B:** Manually deletion of shadows.

4.1.3.2 Staining Considering the possibility of color detection in Win Rhizo Pro, a digital identification of fungal infected roots would be suitable. For this purpose, a whole-mount staining method with Pianeze was tried (section 3.2.3). Two different methods of tissue cleaning were applied (V1, V2), whereas V1 accorded to the tissue cleaning method in Koske and Gemma (1989). In V2 an ultrasonic bath was used.
Pianeze whole-mount staining provide matchable results for both tissue cleaning methods. In both variants (V1, V2), no fungal structures could be identified in the stained specimens, but stained tissue within the central cylinder was visible (V1, V2) (figure 20).

Summary: The results let suggest, that a Pianeze whole-mount staining of *Vitis* ssp. roots is practicable. The chemical tissue cleaning methods according to Koske and Gemma (1989) could be replaced by cleaning with an ultrasonic bath.

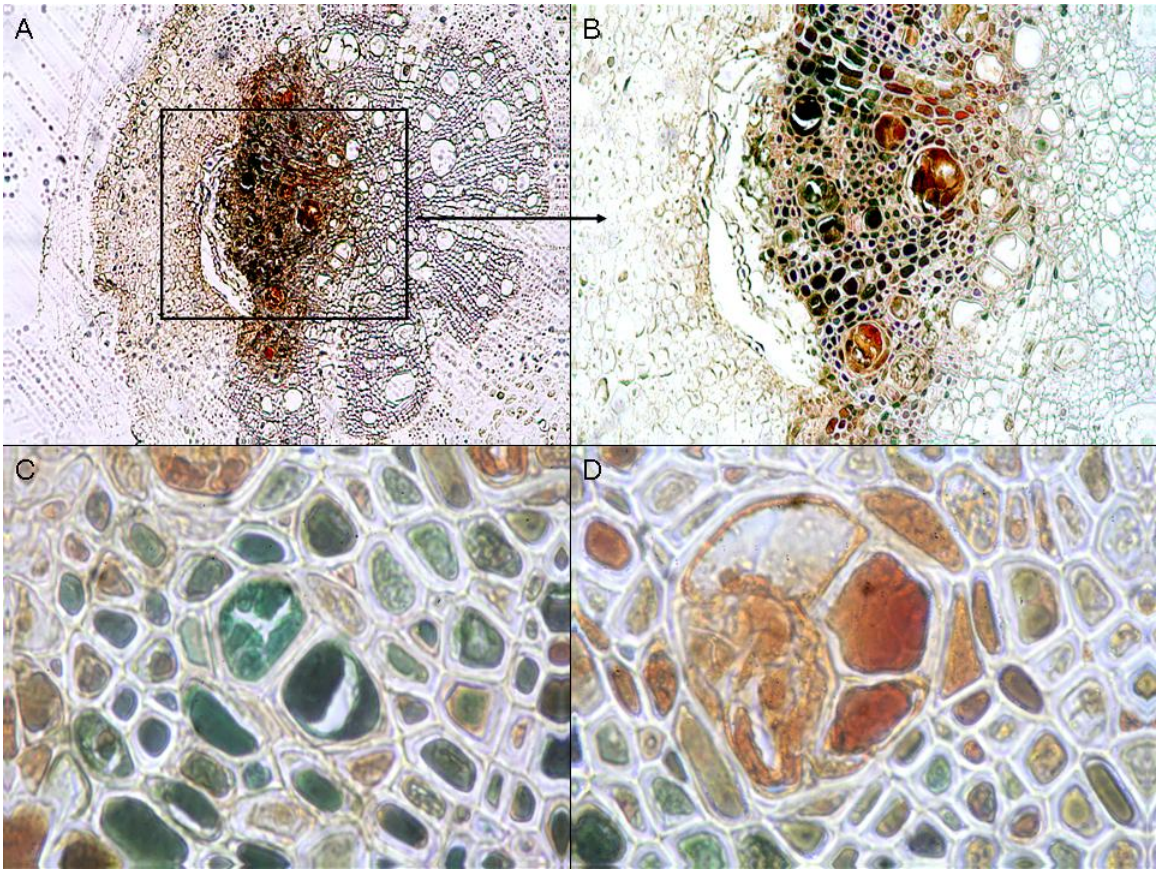


Figure 20: Whole mount staining of lateral roots (5C) with Pianeze. **A:** 50x magnification, staining in central tube of a lateral root (V1). **B:** 100x magnification, xylem and phloem are stained (V1). **C:** 800x magnification, parenchym of central tube (V2). **D:** 800x magnification, xylem (V2).

4.2 Traits

In this section the temporal, spatial and cultivar-specific development of the developed and assessed parameters were described and calculated (figure 21).

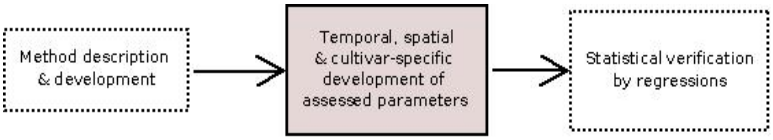


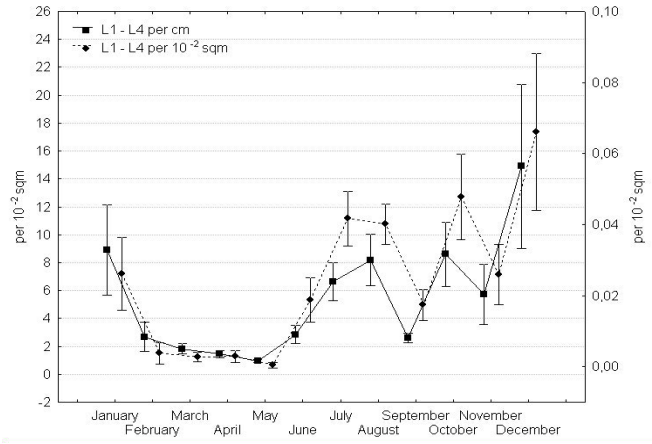
Figure 21: Second section of results: Temporal, spatial and cultivar-specific development of the assessed and developed parameters.

4.2.1 Grape Phylloxera Population

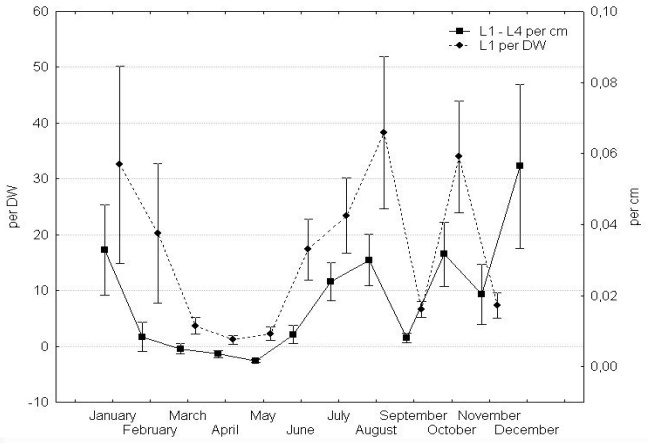
4.2.1.1 General Temporal and Spatial Differences To verify general effects in the variability of the grape phylloxera population structure, regarding temporal and spatial distribution, values of 5C and 125AA were pooled. Differences in single parameters were detected by Kruskal-Wallis ANOVAs using different datasets (2006-2009, 2007-2009, Jul 2008 - Aug 2009, 2009). Factor levels regarding the

temporal distribution were months (January - December), factor levels regarding the spatial distribution on the study site were the classes l,m and u (section 3.3).
Only the RPD parameters *sv per* 10^{-2}*sqm* and *B per* 10^{-2}*sqm* (dataset 2009) showed no significant effect regarding the course of a year. Also the CNA *uvBnon-term, lvCnon-term, lvBnon-term, lvBterm, svAnon-term* and *svAterm* had no significant effects considering the twelve factor levels (months) of temporal distribution. Regarding the NOP, *ttl phyll/sv, L1-L4/sv* and *L5/C* were not significant in any factor level. On the contrary, APD parameters *sv per cm, lv per cm, ttl nod per cm, B per cm, D per cm, outgoing roots per cm, sv per DW, ttl Nod per DW, non-terminal per DW, outgoing roots per DW, terminal per DW, lv per DW* and *A-D per DW* (dataset 2009) were not significant in any factor level.
All other parameters showed high significant effects ($p < 0.001$) between factor levels considering the course of a year (table 21, figures 26, 27, 28). Differences between APD and RPD densities in their temporal development are shown in figures 22 to 25.

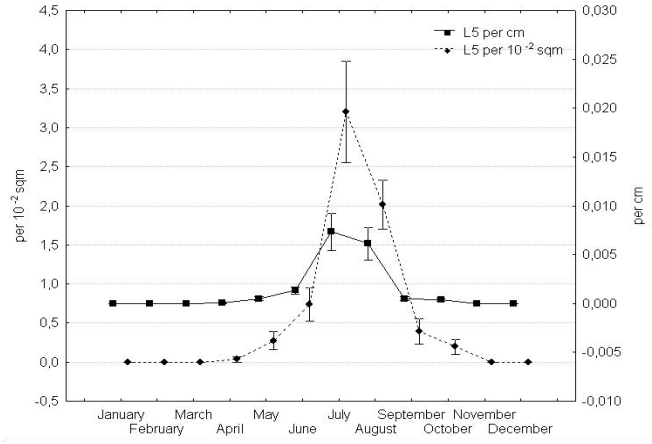
Comparing the temporal development of grape phylloxera densities (*per cm*, *per DW* and *per* 10^{-2}*sqm*), only marginal differences could be shown (figure 22). All parameters show significant differences in the course of a year (table 21) and only marginal significant differences between the months could be calculated (table 22).
But regarding the temporal development of different colored nodosities, especially differences between the peaks of RPD and APD of lv and sv nodosities occurred in late summer (figure 23). These differences could be verified by Bonferronie post-hoc tests (table 22). APD show lower significances than RPD values. Such differences could also be verified for A-formed nodosites and the densities of terminal and non-terminal nodosities (figures 24 and 25).
These results let assume, that especially for an assessment of the densities of nodosities (or in general: infestation patches), a direct relation to root system properties is important for a verification of the impact on the plant.



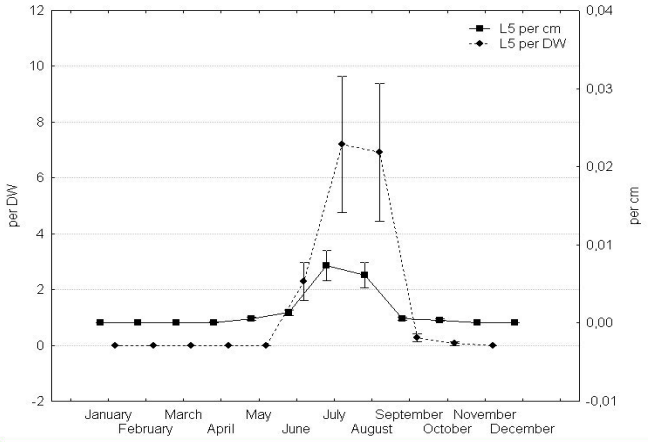
(a) L1-L4 per cm / per 10^{-2}sqm



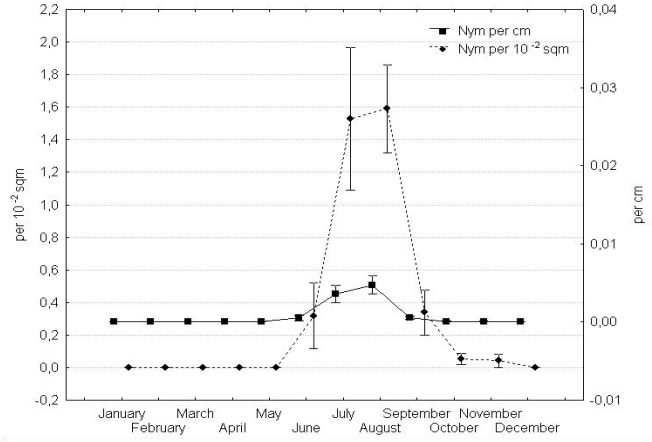
(b) L1-L4 per cm / per DW



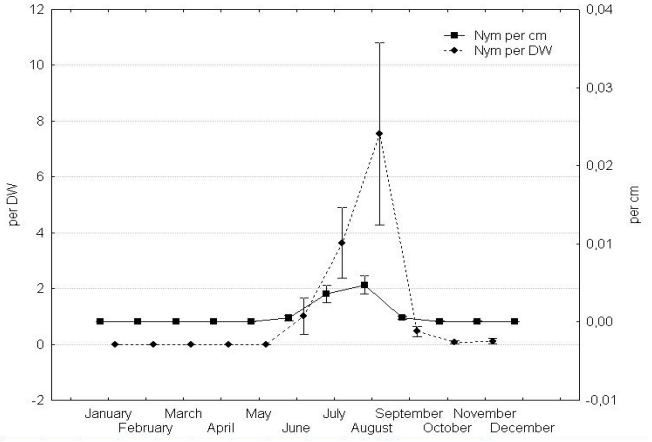
(c) L5 per cm / per 10^{-2}sqm



(d) L5 per cm / per DW

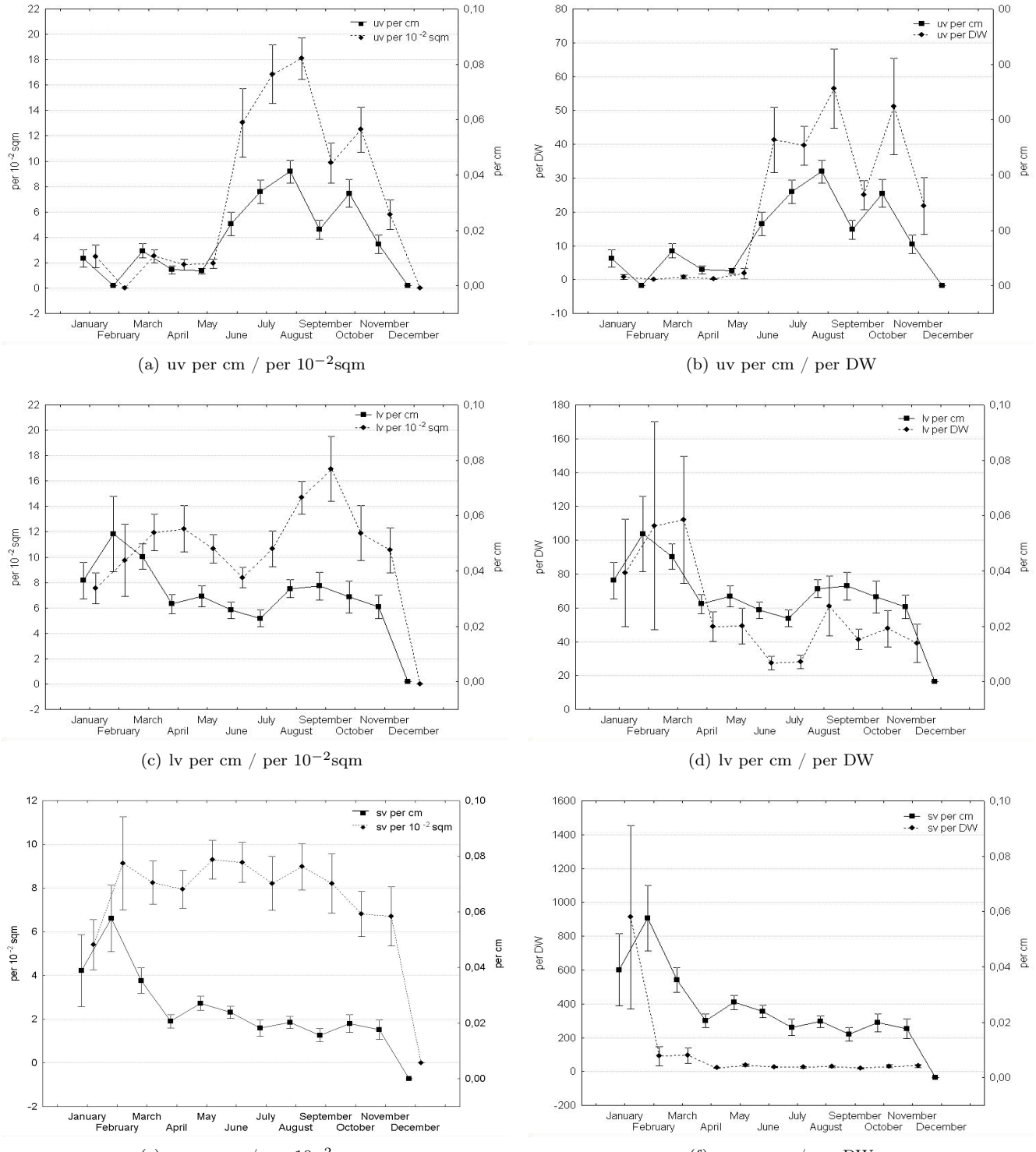


(e) Nym per cm / per 10^{-2}sqm



(f) Nym per cm / per DW

Figure 22: Temporal development (in the course of a year) of RPD and APD of grape phylloxera instar classes, comparing different reference values (10^{-2}sqm , cm ,DW). Plotted are mean values per month and 0.95 * standard error. **A:** L1-L4 per cm vs. per 10^{-2}sqm (dataset 2006-2009). **B:** L1-L4 per cm vs. per DW (dataset Jun08-Aug09). **C:** L5 per cm vs. per 10^{-2}sqm (dataset 2006-2009). **D:** L5 per cm vs. per DW (dataset Jun08-Aug09). **E:** Nym per cm vs. per 10^{-2}sqm (dataset 2006-2009). **F:** Nym per cm vs. per DW (dataset Jun08-Aug09).



(a) uv per cm / per 10^{-2}sqm

(b) uv per cm / per DW

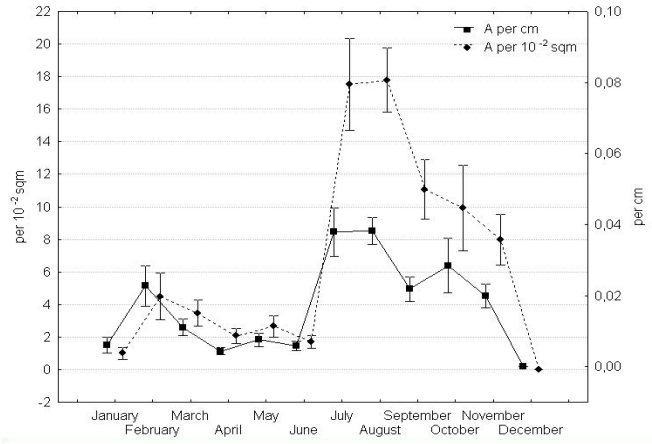
(c) lv per cm / per 10^{-2}sqm

(d) lv per cm / per DW

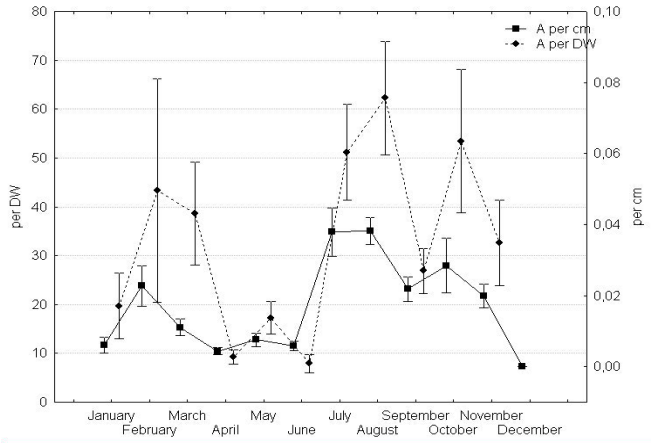
(e) sv per cm / per 10^{-2}sqm

(f) sv per cm / per DW

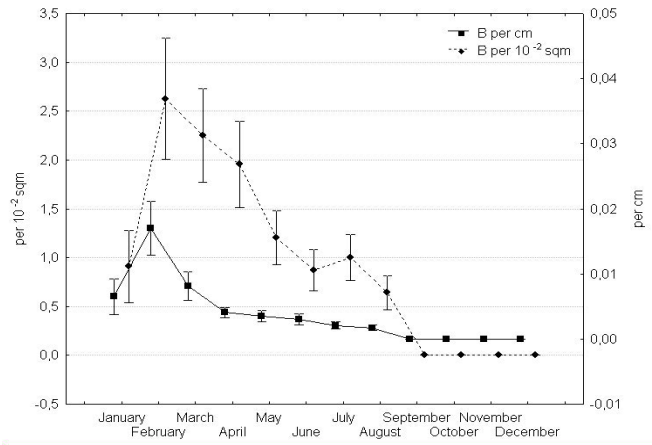
Figure 23: Temporal development (in the course of a year) of RPD and APD of nodosity color classes, comparing different reference values (10^{-2}sqm , cm ,DW). Plotted are mean values per month and 0.95 * standard error. **A:** uv per cm vs. per 10^{-2}sqm (dataset: 2007-2009). **B:** uv per cm vs. per DW (dataset: Jun08-Aug09).**C:** lv per cm vs. per 10^{-2}sqm (dataset: 2007-2009). **D:** lv per cm vs. per DW (dataset: Jun08-Aug09). **E:** sv per cm vs. per 10^{-2}sqm (dataset: 2007-2009). **F:** sv per cm vs. per DW (dataset: Jun08-Aug09).



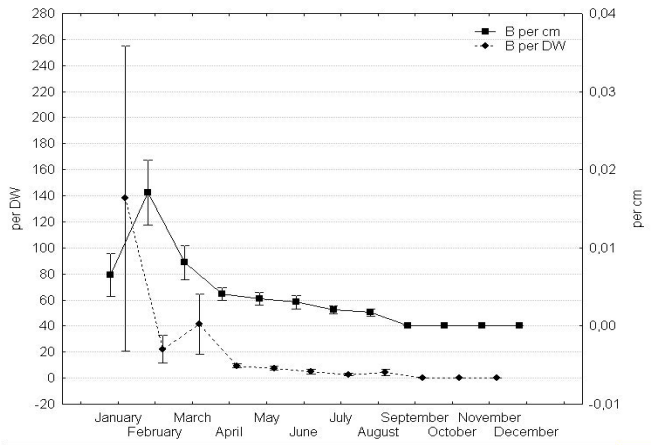
(a) A per cm / per 10^{-2}sqm



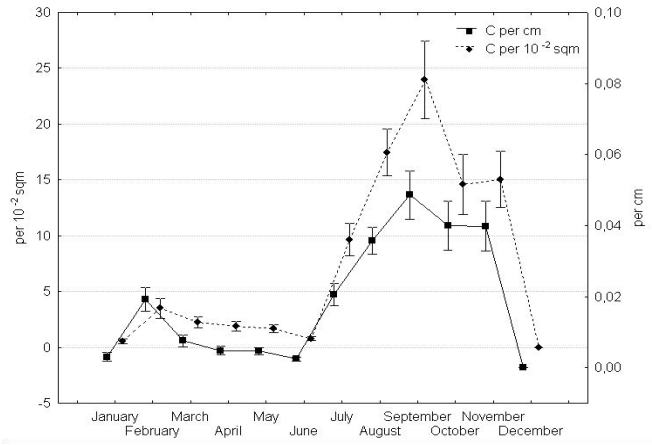
(b) A per cm / per DW



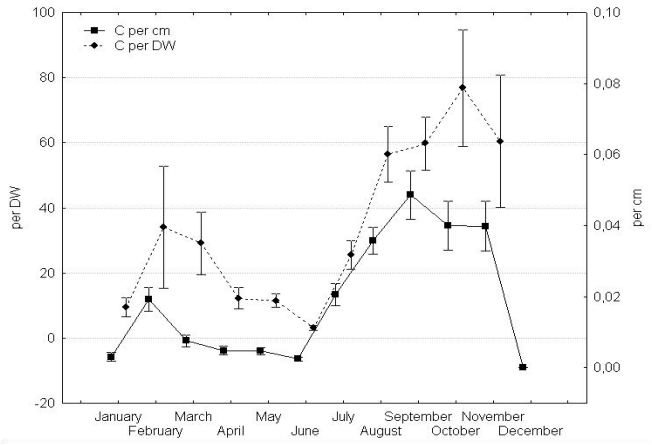
(c) B per cm / per 10^{-2}sqm



(d) B per cm / per DW

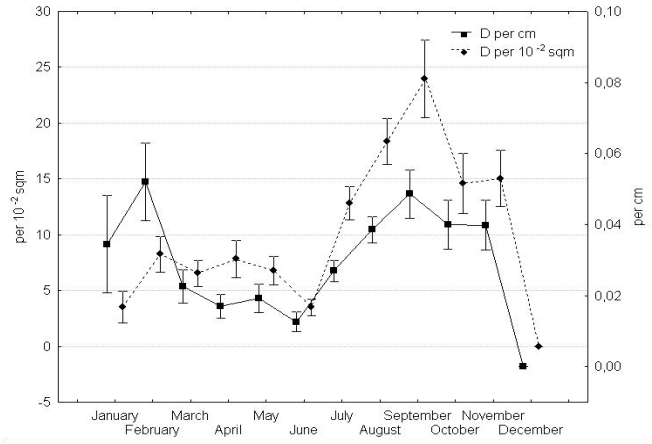


(e) C per cm / per 10^{-2}sqm

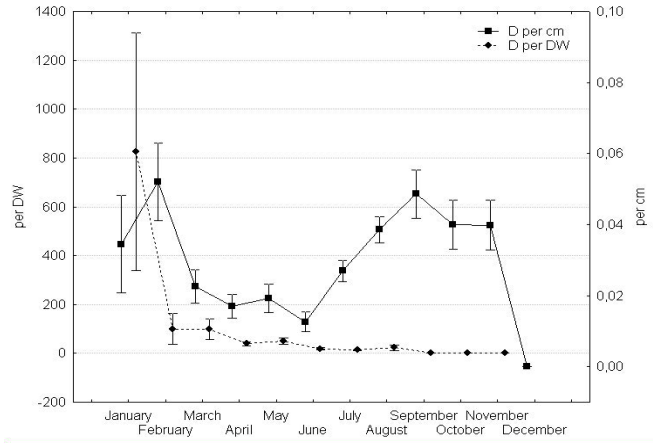


(f) C per cm / per DW

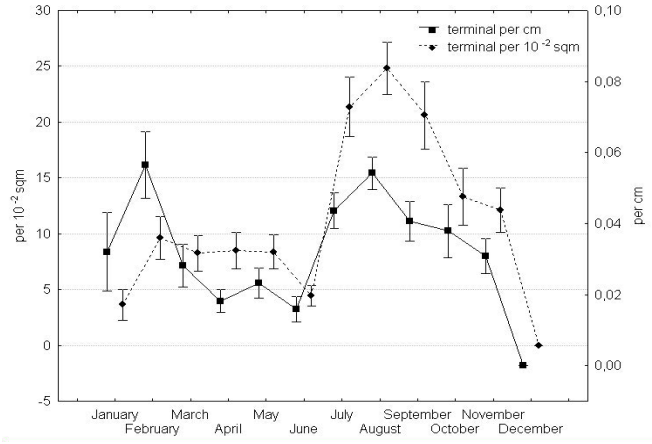
Figure 24: Temporal development (in the course of a year) of RPD and APD of nodosity form classes, comparing different reference values (10^{-2}sqm , cm ,DW). Plotted are mean values per month and 0.95 * standard error (all datasets: Jul08-Aug09). **A:** A per cm vs. per 10^{-2}sqm . **B:** A per cm vs. per DW.**C:** L5 per cm vs. per 10^{-2}sqm . **D:** L5 per cm vs. per DW. **E:** Nym per cm vs. per 10^{-2}sqm . **F:** Nym per cm vs. per DW.



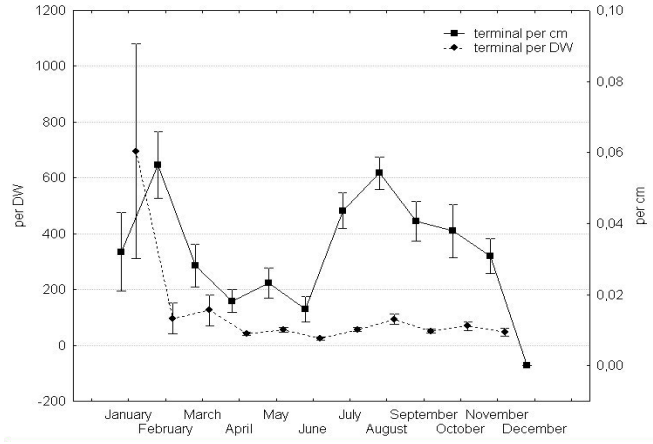
(a) D per cm / per 10^{-2}sqm



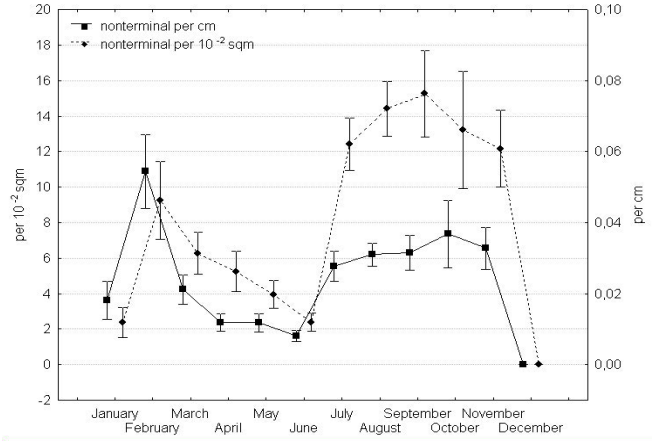
(b) D per cm / per DW



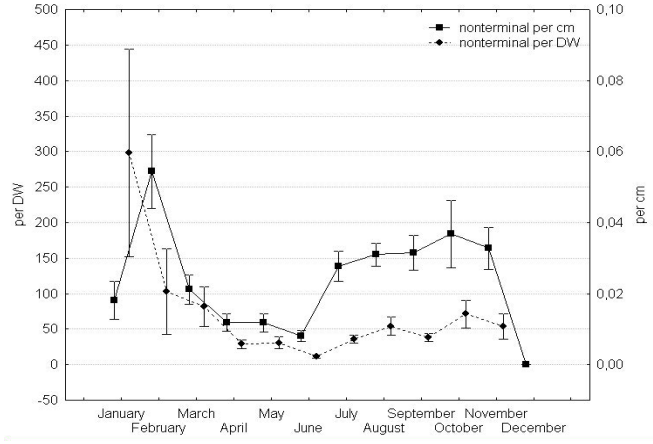
(c) term per cm / per 10^{-2}sqm



(d) term per cm / per DW



(e) non-term per cm / per 10^{-2}sqm



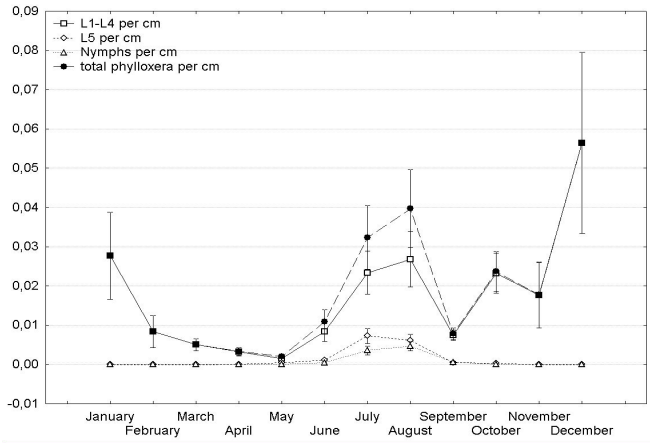
(f) non-term per cm / per DW

Figure 25: Temporal development (in the course of a year) of RPD and APD of nodosity form and branching classes, comparing different reference values (10^{-2}sqm , cm ,DW). Plotted are mean values per month and 0.95 * standard error (all datasets: Jul08-Aug09). **A:** D per cm vs. per 10^{-2}sqm . **B:** D per cm vs. per DW.**C:** terminal nodosities per cm vs. per 10^{-2}sqm . **D:** terminal nodosities per cm vs. per DW. **E:** non-terminal nodosities per cm vs. per 10^{-2}sqm . **F:** non-terminal nodosities per cm vs. per DW.

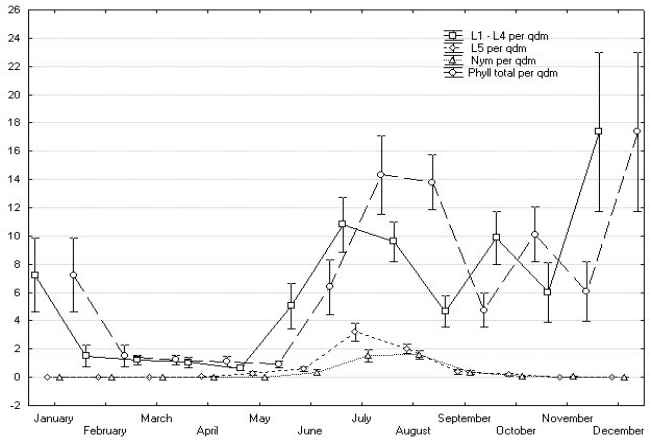
Table 21: Kruskal-Wallis ANOVA, spatial and temporal distribution of grape phylloxera traits. Shown are only significant ($p < 0.05$) parameters. Pooled variants (5C, 125AA). a=2006-2009, b=2007-2009, c=Jul2008-Aug2009, d=2009. *$p < 0.05$. **$p < 0.01$. ***$p < 0.001$

Distribution	Dataset	Parameters
Temporal	a	L1-L4 per cm***, per 10^{-2}sqm ***, per DW(dataset c)***
		L5 per cm***, per 10^{-2}sqm ***, per DW(dataset c)***
		Nym per cm***, per 10^{-2}sqm ***, per DW(dataset c)***
		ttl Phyll per cm***, per 10^{-2}sqm ***, per DW(dataset c)***
	b	lv per 10^{-2}sqm ***
		uv per cm***, per 10^{-2}sqm ***, per DW(dataset c)***
		ttl Nod per 10^{-2}sqm ***
		ttl Phyll per lv***, per uv***
		L1-L4 per lv***, per uv***
		L5 per lv***, per uv***
		Nym per lv***, per uv***
	c	A per cm*, per 10^{-2}sqm ***, per DW***
		C per cm**, per 10^{-2}sqm ***, per DW***
		terminal per cm**, per 10^{-2}sqm ***
		non-terminal per cm*, per 10^{-2}sqm ***
		ttl Phyll per non-term***, per term***, per C***, per A***
		Nym per non-term***, per term***, per C*, per A***
		L5 per non-term***, per term***, per A***
		L1-L4 per non-term***, per term***, per C***, per A***
	d	A per cm***, A per 10^{-2}sqm ***
		D per 10^{-2}sqm *
		uvDterm***, uvDnon-term***, uvCterm***, uvCnon-term***
		uvBterm***, uvAterm***, uvAnon-term***
		lvDterm*, lvDnon-term***, lvCterm**, lvAterm***, lvAnon-term*
		svDnon-term**, svCnon-term***, svBnon-term**
		svDterm**, svCterm***, svBterm***
Spatial	b	sv per 10^{-2}sqm ***
	c	non-terminal per cm**, per 10^{-2}sqm *, per DW*
		terminal per 10^{-2}sqm *
	d	C per cm*
		B per 10^{-2}sqm *
		D per 10^{-2}sqm ***
		svCnon-term*, svCterm*, svDterm**

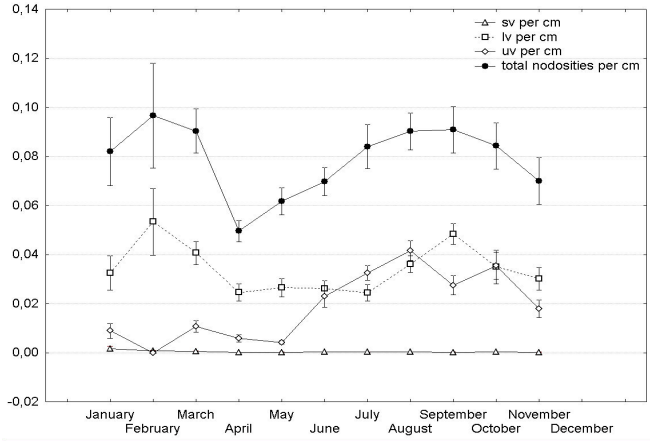
To identify the single factor levels with significant differences, Bonferroni-post hoc tests were done (table 22). Regarding the temporal distribution, most parameters showed significant effects between spring and summer or late summer/autumn months respectively (figures 26, 27, 28). Particular sub parameters of L5 or nymphae (per cm, per 10^{-2}sqm and per DW) showed significant differences between July/August and all other months (figure 26A,B). The sub parameters of non brownish nodosities (uv) (per cm, per 10^{-2}sqm and per DW) showed significances from June to October (figure 26C,D), particularly non brownish CNA showed a high increase in summer (per 10^{-2}sqm) (figure 27E,F). Regarding the forms of nodosities, mainly A forms were influenced by the course of a year. *A per cm* was significant from June to August, *A per 10^{-2}sqm* showed significances in many levels (figure 27A,B). But also *nodosity outgoing roots per 10^{-2}sqm* (RPD) increased from January to March and again



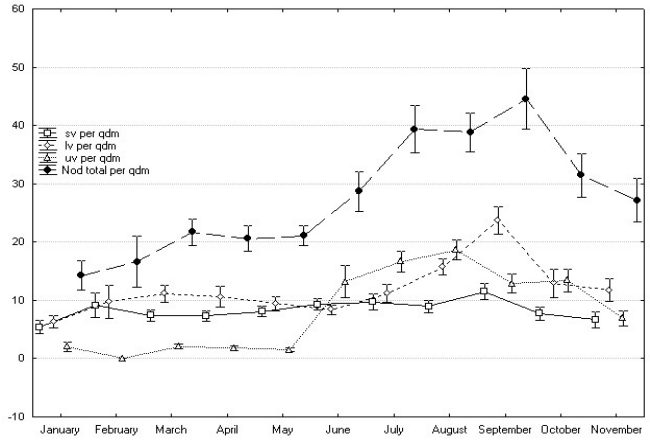
(a) Total instars per cm



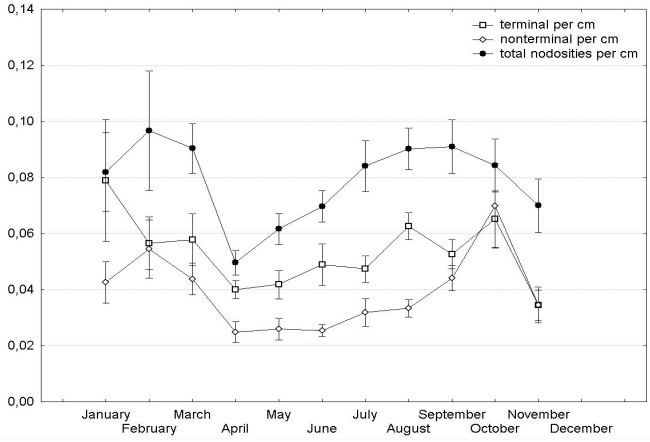
(b) Total instars per 10^{-2}sqm



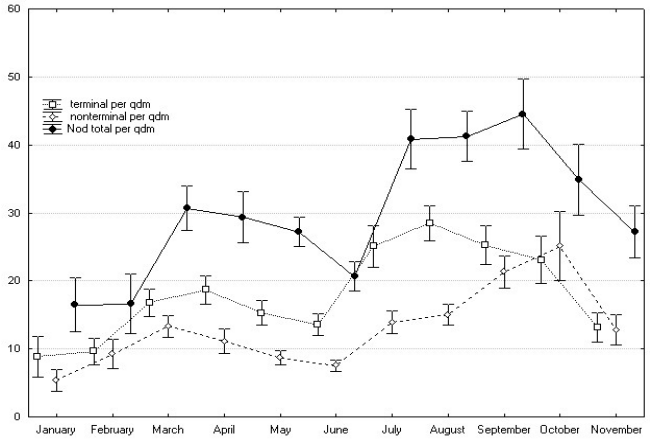
(c) Coloration of nodosities per cm



(d) Coloration of nodosities per 10^{-2}sqm

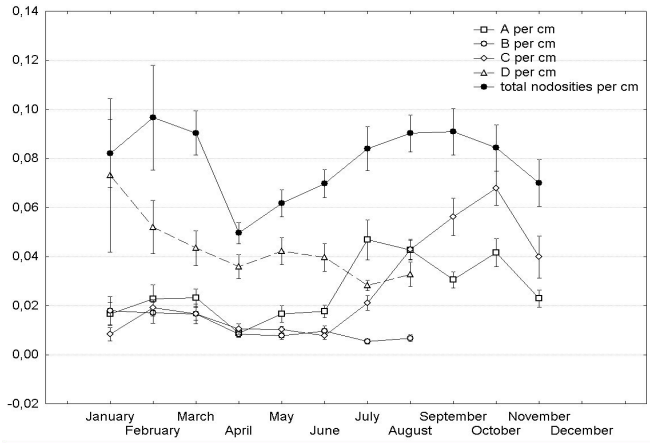


(e) Term/Nonterm nodosities per cm

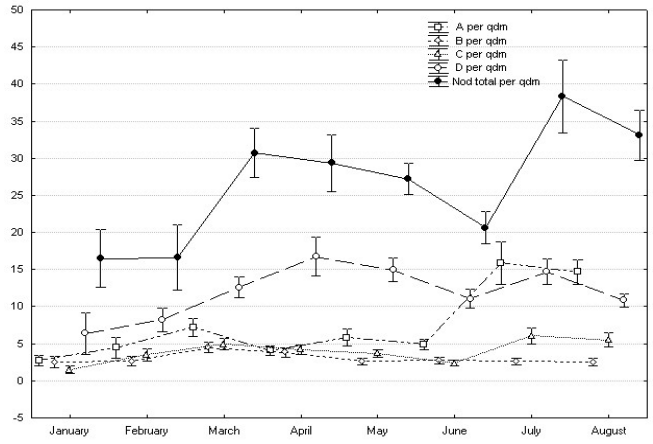


(f) Term/Nonterm nodosities per 10^{-2}sqm

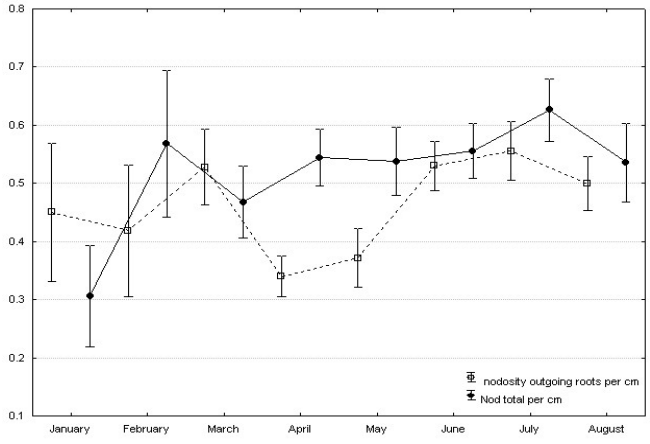
Figure 26: Temporal development (in the course of a year) of RPD and APD grape phylloxera instars, coloration of nodosities and terminal/non-terminal nodosities. Plotted are mean values per month and 0.95 * standard error. **A:** Total grape phylloxera instars per cm (dataset 2006-2009). **B:** Total grape phylloxera instars per 10^{-2}sqm (dataset 2006-2009).**C:** Color of nodosities per cm (dataset 2007-2009). **D:** Color of nodosities per 10^{-2}sqm (dataset 2007-2009). **E:** Terminal/non-terminal nodosities per cm (dataset Jul08-Aug09). **F:** Terminal/non-terminal nodosities per 10^{-2}sqm (dataset Jul08-Aug09)



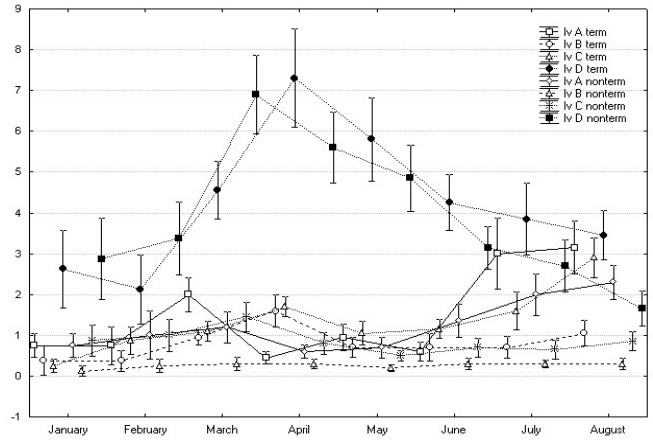
(a) Nodosity form per cm



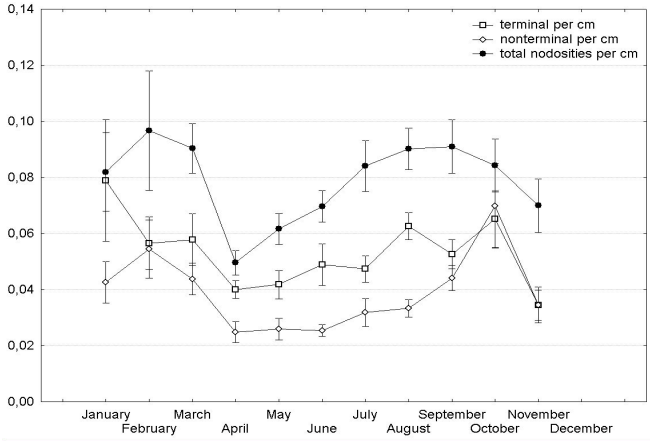
(b) Nodosity form per 10^{-2}sqm



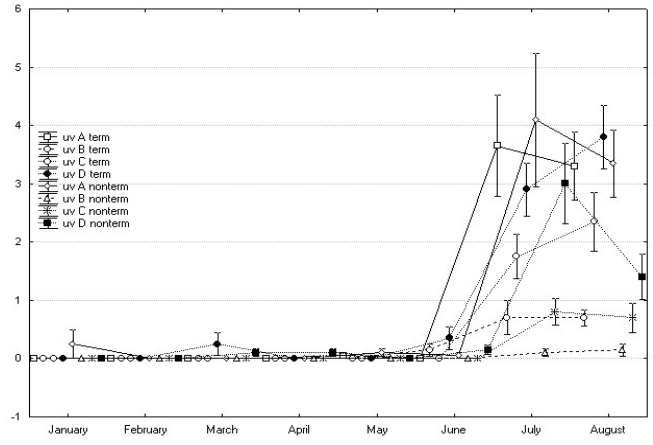
(c) Nodosity outgoing roots per cm



(d) Nodosity outgoing roots per 10^{-2}sqm

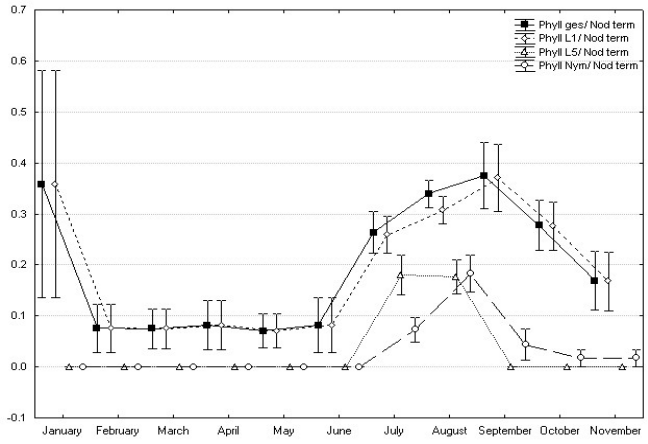


(e) Light brownish nodosities per 10^{-2}sqm

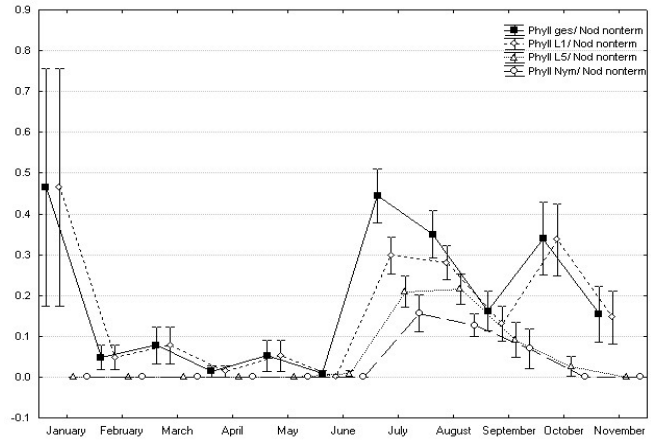


(f) Non brownish nodosities per 10^{-2}sqm

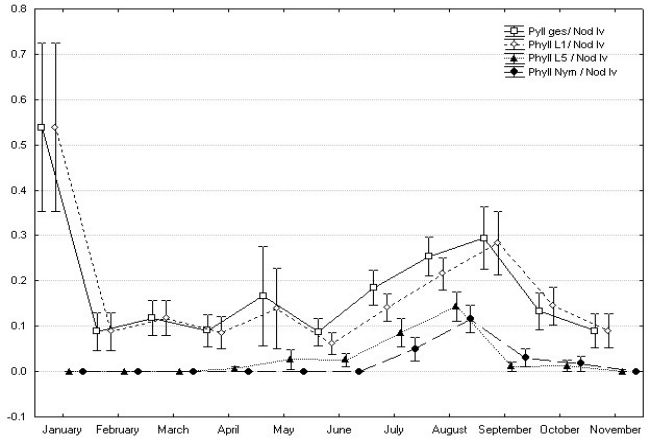
Figure 27: Temporal development (in the course of a year) of RPD and APD form of nodosities, nodosity outgouing roots and CNA. Plotted are mean values per month and 0.95 * standard error. **A:** Nodosity form per cm (dataset 2009). **B:** Nodosity form per 10^{-2}sqm (dataset 2009). **C:** Nodositiy outgoing roots per cm (2009). **D:** Nodosity outgoing roots per 10^{-2}sqm (dataset 2009). **E:** Light brownish nodosities per 10^{-2}sqm (dataset 2009). **F:** Non brownish nodosities per 10^{-2}sqm (dataset 2009)



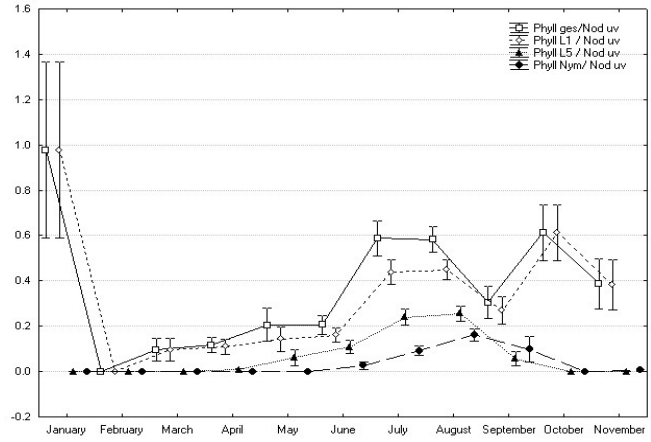
(a) Occupation of terminal nodosities



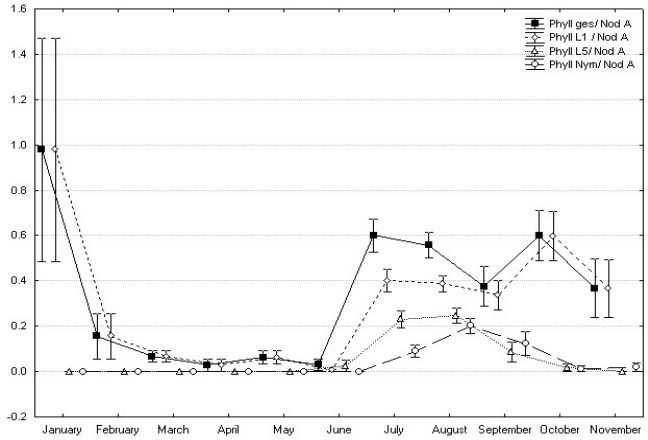
(b) Occupation of nonterminal nodosities



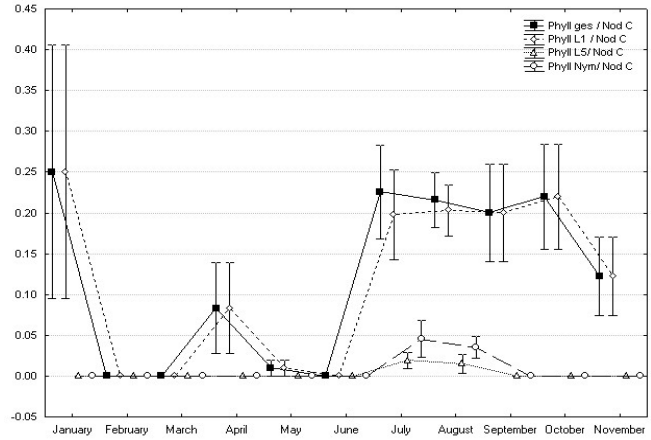
(c) Occupation of lv nodosities



(d) Occupation of uv nodosities



(e) Occupation of A formed nodosities



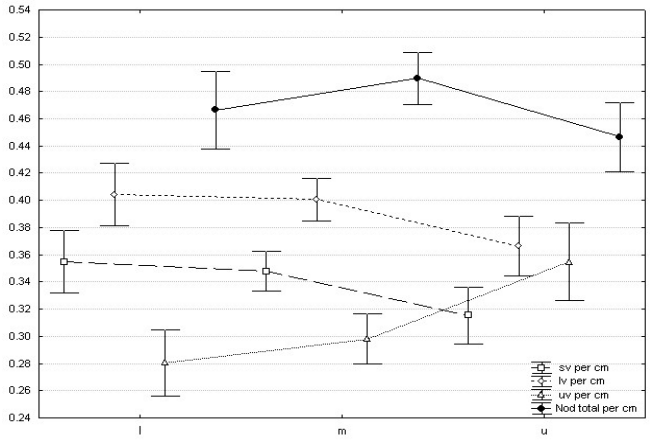
(f) Occupation of C formed nodosities

Figure 28: Temporal development (in the course of a year) of NOP. Plotted are mean values per month and 0.95 * standard error. **A:** Occupation of terminal nodosities (dataset Jul08-Aug09). **B:** Occupation of non-terminal nodosities (dataset Jul08-Aug09). **C:** Occupation of light brownish nodosities (dataset 2007-2009). **D:** Occupation of non brownish nodosities (dataset 2007-2009). **E:** Occupation of A formed nodosities (dataset Jul08-Aug09). **F:** Occupation of C formed nodosities (dataset Jul08-Aug09)

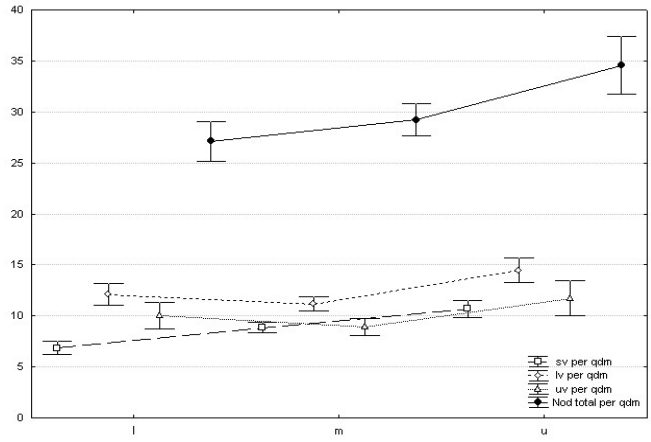
from June to July (figure 27C,D). The density of nodosity occupation (NOP) was positively affected by the course of a year and increased in nearly all classes from spring to summer (figure 28).
Altogether, from spring and early summer to late summer and autumn most of the parameters showed an increase. 60 % APD/NOP and 79 % RPD and CNA parameters showed significances (table 21). Vice versa, 40 % of APD/NOP and 21% of RPD/CNA parameters were not effected by temporal distribution. Most RPD parameters were significant in more than one temporal factor level (table 22), particularly the nodosity attributes color and form.

Table 22: Bonferroni post-hoc tests of some grape phylloxera traits, regarding the temporal distribution. Factor levels are months. *p<0.05, **p<0.01, ***p<0.001

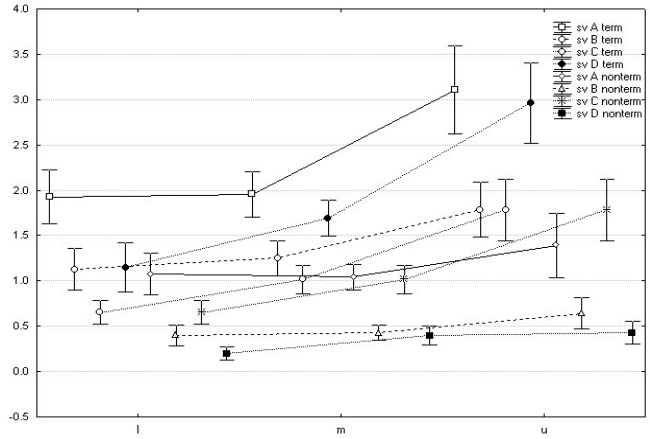
Parameters	Significant factor levels
ttl Phyll per cm	Jul-Apr** Aug-Mar**,Aug-Apr***,Aug-May**,Aug-Jun** Sep-Apr**, Sep-May*
ttl Phyll per 10^{-2}sqm	Jul-Mar***, Jul-Apr***,Jul-May*** Aug-Mar***, Aug-Apr***, Aug-May***
uv per cm	Jan-Aug*, Jan-Oct* Feb-Jun**, Feb-Jul*, Feb-Aug**, Feb-Sep*, Feb-Oct*** Mar-Jun*, Mar-Aug***, Mar-Oct*** Apr-Jun**, Apr-Jul*, Apr-Aug***, Apr-Oct***
uv per 10^{-2}sqm	Jan-Jun*, Jan-Jul**, Jan-Aug*** Feb-Jul*, Feb-Aug** Mar-Jun***, Mar-Jul***, Mar-Aug***, Mar-Oct*** Apr-Jun***, Apr-Jul***, Apr-Aug***, Apr-Oct*** May-Jun***, May-Jul***, May-Aug***, May-Sep***, May-Oct***
A per cm	Jun-Aug*
A per 10^{-2}sqm	Jul-Jan*, Jul-Mar**, Jul-Apr***, Jul-May**, Jul-Jun*** Aug-Jan*, Aug-Mar*, Aug-Apr***, Aug-May**, Aug-Jun***
terminal per 10^{-2}sqm	Aug-Jun*, Aug-Nov**
non-terminal per 10^{-2}sqm	Sep-May*, Sep-Jun* Oct-Jan**, Oct-Apr**, Oct-May***, Oct-Jun***, Oct-Jul*, Oct-Aug*



(a) Nodositiy color per cm



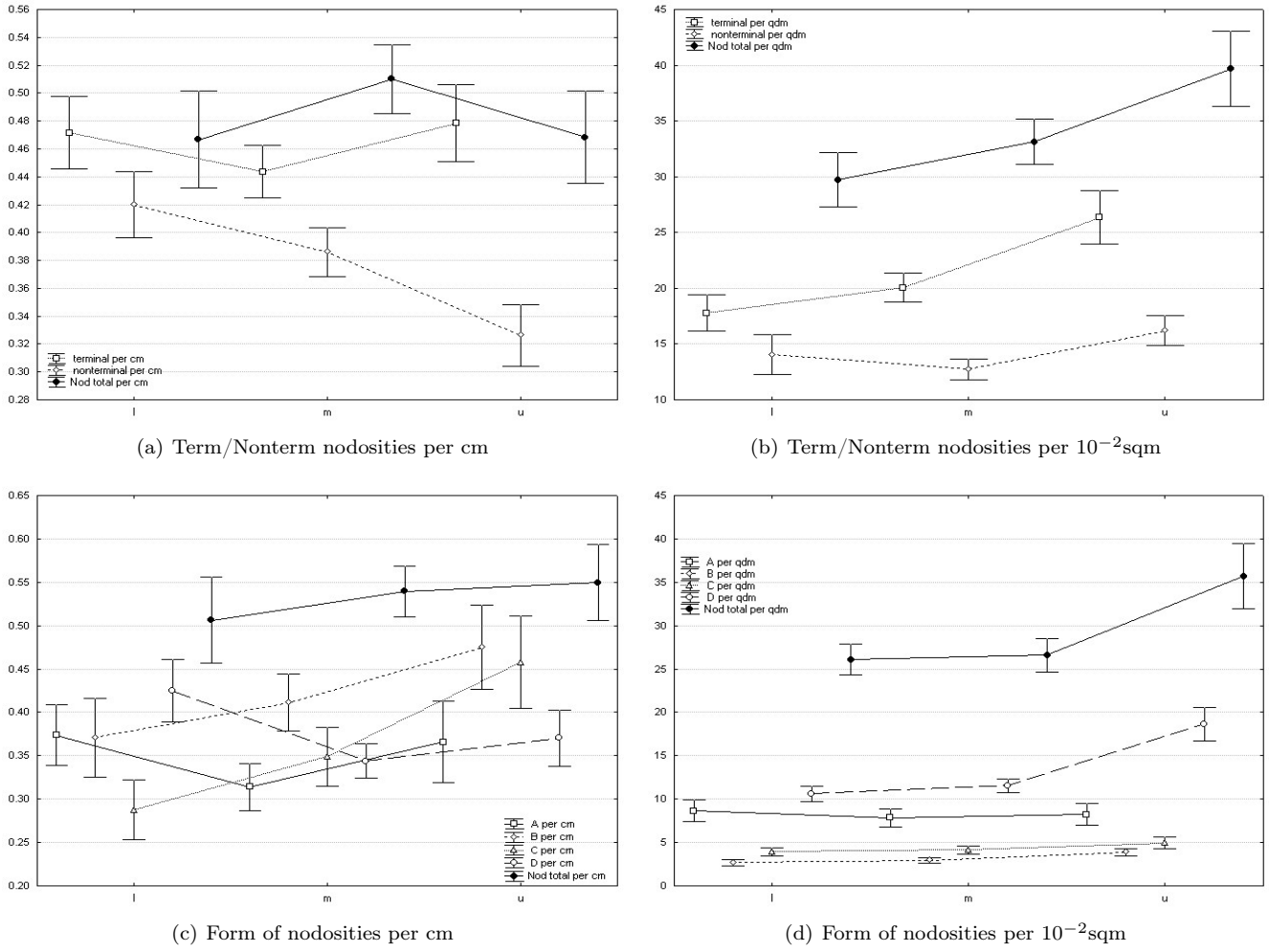
(b) Nodosity color per 10^{-2}sqm



(c) Dark brownish nodosities per 10^{-2}sqm

Figure 29: Spatial distribution of nodosity traits on the study site. Plotted are mean values per class and 0.95 * standard error. **A:** Color of nodosities per cm (dataset 2007-2009). **B:** Color of nodosities per 10^{-2}sqm (dataset 2007-2009). **C:** Dark brownish nodosities (dataset 2009).

Regarding the spatial distribution, only a few parameters differed significantly between factor levels (spatial classes). In particular the occurrence of RPD sub parameter *D per* 10^{-2}*sqm* had a high significant effect ($p < 0.001$) (table 21, figure 30C+D). Altogether, only 6 % APD and 18 % RPD showed significant differences, mostly between level "m" and "u" (table 21). Particularly, the branching attributes of nodosities (terminal, non-terminal) were affected by the location on the study site. So the sub parameters *non-term per* 10^{-2}*sqm* and *term per* 10^{-2}*sqm* showed a significant incresase from class "m" to "u", whereas the absolute density of non-terminal nodosities (*non-term per cm*) was significantly lower on the upper part of the study site (figure 30A+B). Furthermore, a significant higher amount of dark brownish (*sv*) nodosities were found in the upper part of the study site (figure 29).



(a) Term/Nonterm nodosities per cm

(b) Term/Nonterm nodosities per 10^{-2}sqm

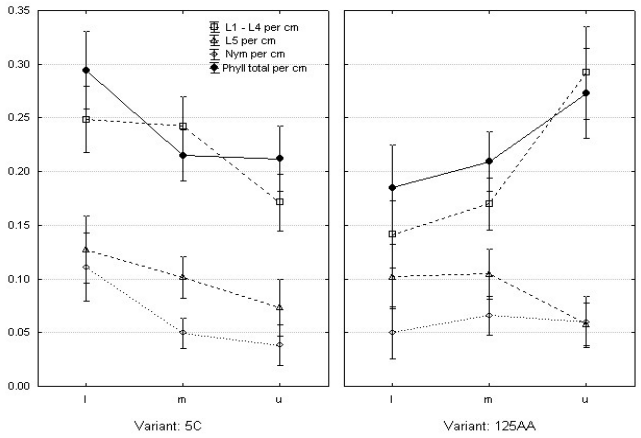
(c) Form of nodosities per cm

(d) Form of nodosities per 10^{-2}sqm

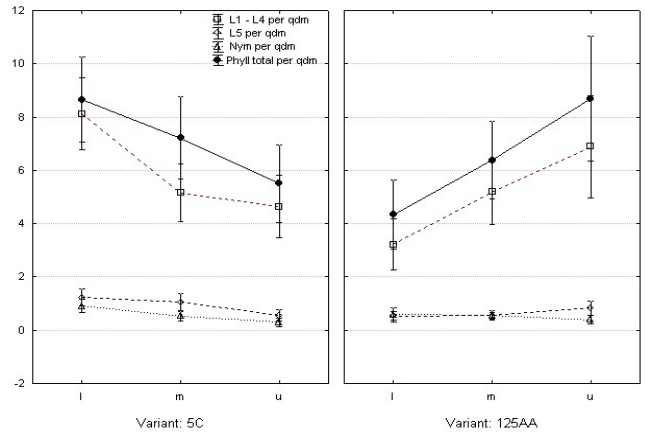
Figure 30: Spatial distribution of nodosity traits on the study site. Plotted are mean values per class and 0.95 * standard error. **A:** Branching of nodosities per cm (dataset Jul08-Aug09). **B:** Branching of nodosities per 10^{-2}sqm (dataset Jul08-Aug09). **C:** Form of nodosities per cm (dataset 2009). **D:** Form of nodosities per 10^{-2}sqm (dataset 2009)

4.2.1.2 Cumulative and Spatial Differences in Variants Variant (5C, 125AA) dependent calculations were done on basis of the dataset Mar08-Aug09 (without Jan-Feb09). This leads to n=340 (170 per variant). To detect differences in the cumulative occurrence of single parameters, a Kruskal-Wallis ANOVA (H test) was calculated (dataset: Mar08-Aug09). The occupation parameter *ttl instars per term* showed significant differences (mean 5C: 0.258, mean 125AA: 0.22, p=0.022). Within the sub parameters (*L1-L4 per term, L5 per term, Nym per term*) no significances could be detected. All other parameters showed no significances in their cumulative values between the variants.

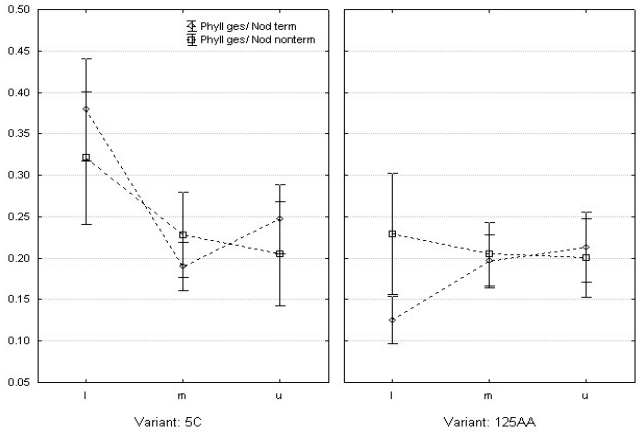
Variant dependent differences in the spatial distribution of grape phylloxera population could lead back to an inhomogeneous occurrence of *Daktulosphaira vitifoliae* Fitch on the study site, in peak population development particularly. On 5C, grape phylloxera instars appeared in the lower part of the study site mostly, whereas on 125AA the highest amounts were detected in the upper part of the



(a) Instars per cm



(b) Instars per 10^{-2}sqm



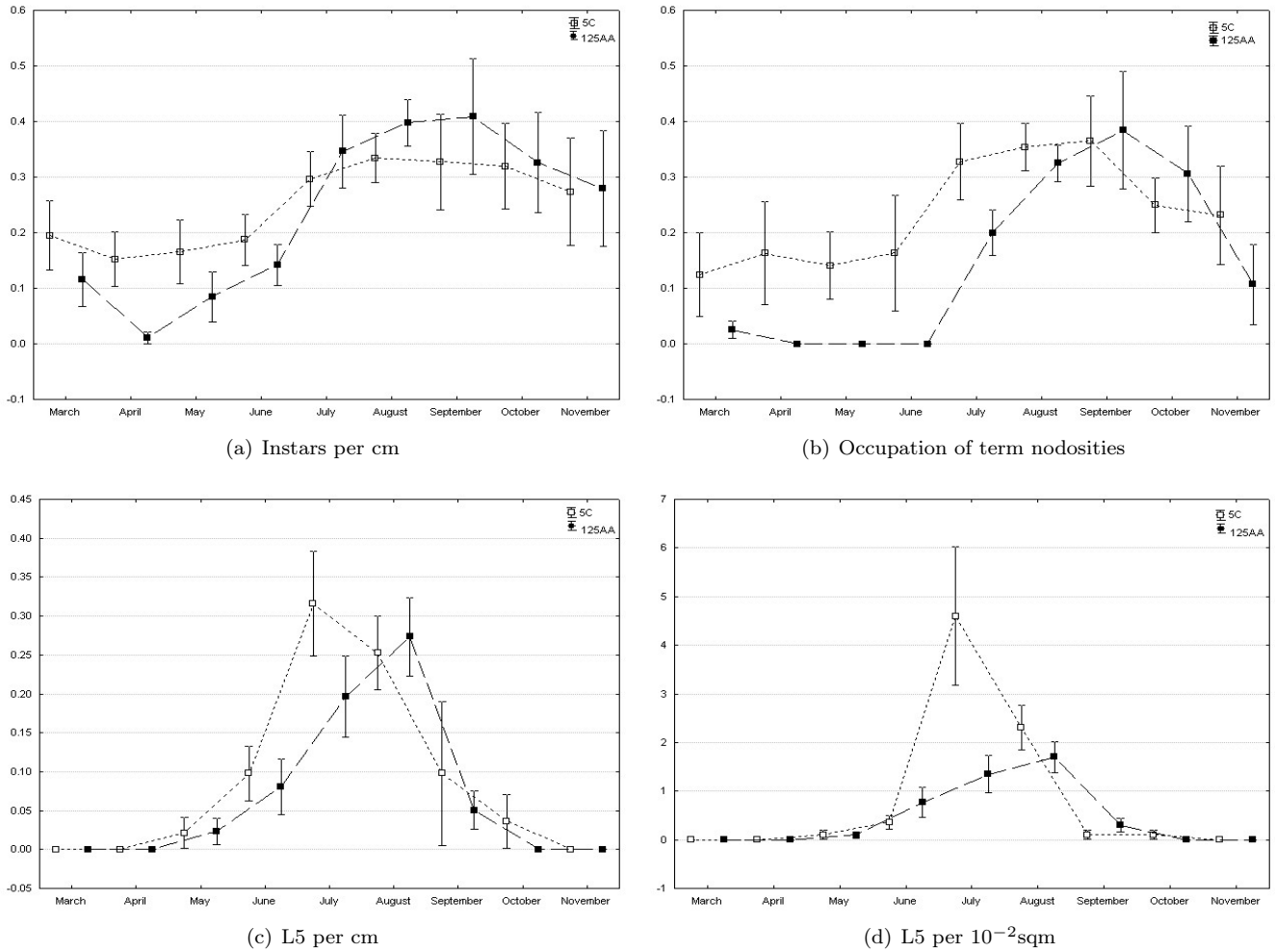
(c) Occupation of term/nonterm nodosities

Figure 31: Spatial distribution of grape phylloxera population on variants. Plotted are mean values per class and 0.95 * standard error. **A:** left: 5C, right: 125AA. Grape phylloxera instars per cm (dataset Mar08-Aug09) **B:** left: 5C. right: 125AA. Grape phylloxera instars per 10^{-2}sqm (dataset Mar08-Aug09). **C:** left: 5C, right: 125AA. Total occupation of terminal and non-terminal nodosities (dataset Jul08-Aug09).

study site (figure 31A+B). On the lower part of the study site (high grape phylloxera densities on 5C), a bigger part of instars occupied terminal nodosities on 5C than on 125AA. On the other hand, no differences occurred in the occupation of terminal nodosities on the upper part of the study site (figure 31C).

4.2.1.3 Temporal Differences in Variants Variant dependent differences in the temporal progress of RPD parameters occurred in the development of oviparous (L5) instars particularly. The peak of instar development on 5C is one month earlier (July) than on 125AA (August). The absolute values (APD) of *L5 per cm* were in the same amount within the variants, but the total amount of L5 instars (RPD) on 5C is much higher than on 125AA (figure 32C+D). The development of nymphal and L1-L4 instars were similar in both variants. Also the development of the nodosity RPD in a course of a year showed

no differences within the variants (not shown here).



(a) Instars per cm

(b) Occupation of term nodosities

(c) L5 per cm

(d) L5 per 10^{-2}sqm

Figure 32: Temporal distribution of grape phylloxera population on variants. Plotted are mean values per class and 0.95 * standard error. **A:** Total grape phylloxera instars per cm (dataset Mar08-Aug09) **B:** Total occupation of terminal nodosities (dataset Jul08-Aug09). **C:** L5 instars per cm (dataset Mar08-Aug09). **D:** L5 instars per 10^{-2}sqm (dataset Mar08-Aug09)

Although there were no differences between the variants in the development of terminal and non-terminal nodosities (APD values), the nodosity occupation behavior of instars differed between the variants highly. On 5C, between 10-25 % of terminal nodosities were occupied during the whole year. On 125AA, only in late summer and autumn, an occupation of terminal nodosities was detected (figure 32A+B). The occupation of non-terminal nodosities was on both variants in the same amount (not shown here).

Summary: General differences in the temporal development (course of a year) of grape phylloxera population structure was recorded. Mainly RPD and CNA parameters showed significant increases from spring/early summer to late summer. Non brownish colored nodosities, L5 an nymphal instars

and nodosity A forms seemed to be highly affected by seasonal conditions in both their absolute and relative densities. Regarding the variants, differences in temporal distribution occured in development of relative amount (RPD) of L5 instars (on 5C earlier) and in the occupation of terminal nodosities. On 125AA only in late summer and autumn terminal nodosities were occupied.
Regarding the spatial dispersion, an inhomogeneous distribution of grape phylloxera population was recorded. On the 125AA study site, main population was recorded in the upper part of the vineyard, whereas on the 5C study site the highest amounts were found in the lower part. General differences in spatial distribution were recorded in RPD parameters of terminal and non-terminal nodosities as well as in APD parameters of non-terminal nodosities.

4.2.2 Root System

Morphological root system parameters were detected by Win Rhizo Pro on base of gray scale images (section 3.2.2.2). The recorded main parameters were *total root length* [cm], *total root surface area* [sqcm], *average root diameter* [mm], *specific root length (SRL)* [m/g], *number of crossings, number of forks, crossings per cm, forks per cm* and *root dry weight (DW)*[g]. Recorded architectural root system parameter was *fractal dimension*. Sub parameters were the distribution of length and surface area in different diameter classes (section 3.2.2.2). All parameters with exception of *SRL* and *DW* were detected from 2006 to 2009. *SRL* and *DW* were detected from June 2008 to August 2009.

4.2.2.1 General Temporal and Spatial Differences To detect significant differences in root system traits regarding their temporal or spatial distribution, values of 5C and 125AA were pooled. Normal distributed parameters with homogeneity in variance were analyzed by an one-factor ANOVA. Not normal distributed parameters were analyzed by a Kruskal-Wallis ANOVA. Factor levels regarding the temporal distribution were months (January - December), factor levels regarding the spatial distribution on the study site were the classes l,m and u (section 3.3).
Because the size of the digging box had an effect on root system main parameters (section 4.1.2.1), calculation based on the dataset Aug07-Aug09 (small size). *Total root length* and *total root surface area* were distributed normally (K-S test) and showed a homogeneity in variances (Levene test).

Table 23: ANOVA and Kruskal-Wallis ANOVA of root system main parameters (all per 10^{-2}sqm soil surface) regarding the temporal or spatial distribution. Factorlevels: months; l, m, u. Dataset: Aug2007-Aug2009. n=386. *=significant

Factor	Parameter	R^2 (adj)	F/H value	p value
Temporal distribution	*Total root length*	0.15	6.8	0.001*
	Total root surface area	0.16	6.9	0.001*
	Average root diameter	-	25.14	0.005*
	Number of Forks	-	67.63	0.001*
	Number of Crossings	-	67.9	0.001*
	Fractal Dimension	-	50.03	0.001*
	SRL (n=276)	-	9.28	0.5
Spatial Distribution	*Total root length*	0.07	15.48	0.001*
	Total root surface area	0.04	9.6	0.001*
	Average root diameter	-	18.9	0.001*
	Number of Forks	-	27.96	0.001*
	Number of Crossings	-	32.65	0.001*
	Fractal Dimension	-	0.69	0.7
	SRL (n=276)	-	9.7	0.008*

Considering the course of a year as well as the spatial distribution, almost all root system main parameters showed significant differences (table 23). In the course of a year, only *SRL* showed no significant differences, though regarding the spatial distribution. *Fractal dimension* showed no significances regarding the spatial distribution. On closer examination of the sub parameters, root length

Table 24: Post-hoc tests (Tuckey HSD, Bonferroni) of temporal distribution of some root system main parameters (all per 10^{-2}sqm soil surface). *p<0.05, **p<0.01, ***p<0.001

Parameter	Significant factor levels
Total root length	Jan -Apr*, Jan-Jun*, Jan-Jul***, Jan-Aug***, Jan-Sep***, Jan-Oct* Feb-Apr*, Feb-Jun*, Feb-Jul**, Feb-Jul**, Feb-Aug** Mar-Jun**, Mar-Jul***, Mar-Aug***, Mar-Sep***, Mar-Oct*
Total root surface area	Jan-Jun**, Jan-Jul***, Jan-Aug***, Jan-Sep** Feb-Jun*, Feb-Jul**, Feb-Aug**, Feb-Sep** Mar-Jun***, Mar-Jul***, Mar-Aug***, Mar-Sep**
Average root diameter	Jun-Nov*
Fractal dimension	Jun-Jan***, Jun-Mar***, Jun-May*, Jun-Oct*, Jun-Nov*** Jul-Jan**, Jul-Mar***, Jul-Nov*** Aug-Jan**, Aug-Mar***, Aug-Nov***
Number of forks	Jan-Jun*, Jan-Jul*, Jan-Aug*, Jan-Sep** Feb-Jun*, Feb-Jul*, Feb-Aug**, Feb-Sep*** Mar-Sep*

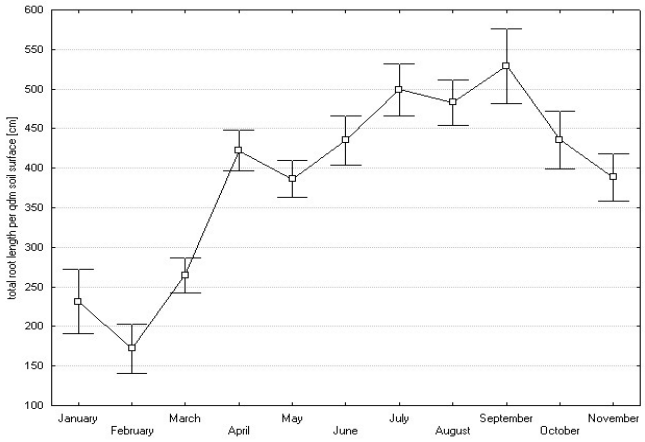
and root surface area across the low diameter classes (14 ind. classes and classes according to Boehm (1979)) showed significant differences, regarding their temporal as well as their spatial distribution. Higher diameter classes ($\geq$ 2mm) showed no differences.

To identify the factor levels with significant differences, post-hoc tests were done. Parameters analyzed by an one-factor ANOVA were tested by a Tuckey HSD post-hoc test, parameters analyzed by a Kruskal-Wallis ANOVA were tested by a Bonferroni post-hoc test. Nearly all root system main parameters showed significances in more than one summer and winter months (table 24). *Total root length*, *total root surface area* (also *number of forks*) showed significant increases from January/ February/ March to June/ July/ August/ September/ October. These increases occurred mainly in the diameter class 0-0.5mm and 0.5-2mm (figure 33A-D). *Average root diameter* decreased lightly by trend within the course of a year with a significant difference between June (0.65mm) and November (0.57mm) (figure 33E). *Fractal dimension* held highest values from June to August ($\approx$ 1.48) and lowest values in winter ($\approx$ 1.43) (figure 33F).

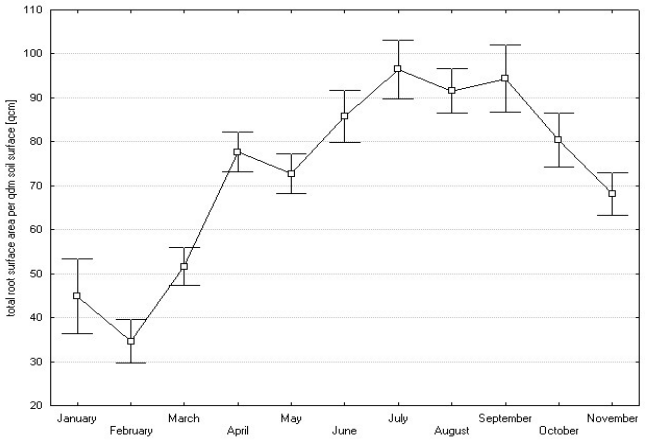
Almost all root system main parameters showed significant differences in low and upper parts of the study site (table 25). *Total root length*, *total root surface area* (also *number of forks*) showed significant increases from low and middle parts of the study site to the upper part. These increases occurred mainly in low diameter classes from 0-1mm (not shown here), whereas *average root diameter* showed a significant decrease from low and middle parts ($\approx$ 0.63mm) to upper parts (0.58mm) of the study site (figure 34A-C). *Fractal dimension* decreased from middle to upper parts lightly, but not significant (figure 34D). *SRL* held highest values in middle and upper parts ($\approx$ 13 m/g) and lowest values in the lower part of the study site ($\approx$ 9 m/g) (figure 34E), root dry weight held highest values in the middle of the study site (figure 34F).

Table 25: Post-hoc tests (Tuckey HSD, Bonferroni) of spatial distribution of some root system main parameters (all per 10^{-2}sqm soil surface). *p<0.05, **p<0.01, ***p<0.001

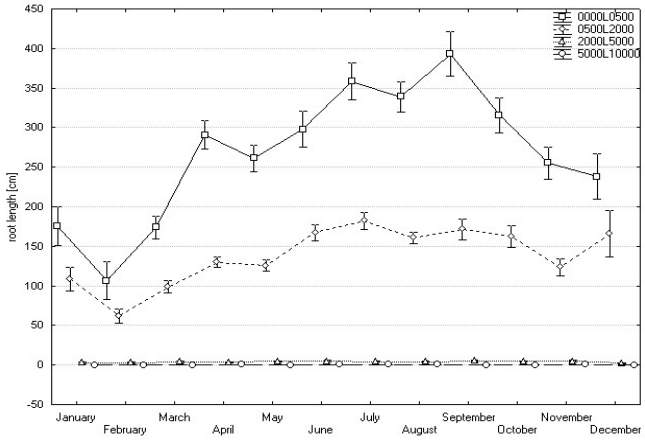
Parameter	Significant factor levels
Total root length	l-u***, m-u***
Total root surface area	l-u***, m-u**
Average root diameter	l-u**, m-u**
SRL	l-m*
Number of forks	l-u***, m-u***



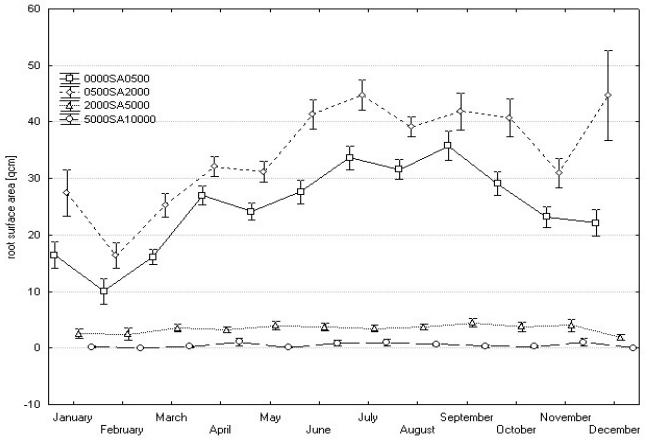
(a) Root length per 10^{-2}sqm



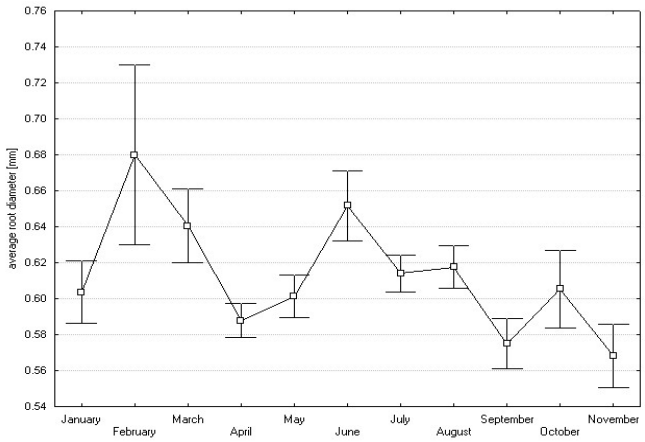
(b) Root surface area per 10^{-2}sqm



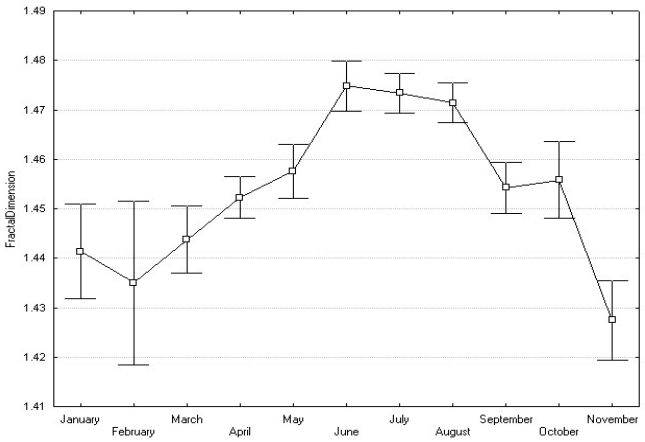
(c) Root length (0-10 mm diameter)



(d) Root surface area (0-10 mm diameter)

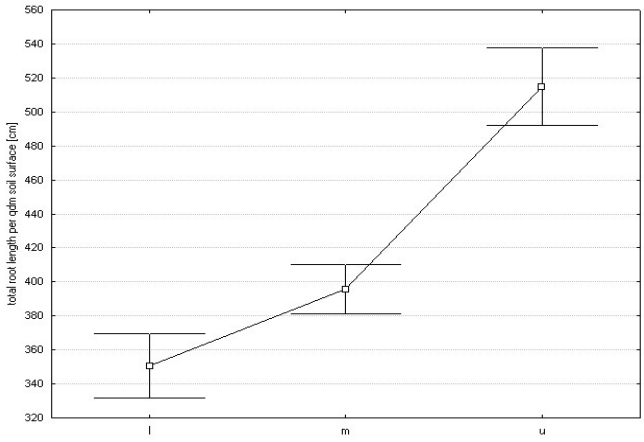


(e) Average root diameter

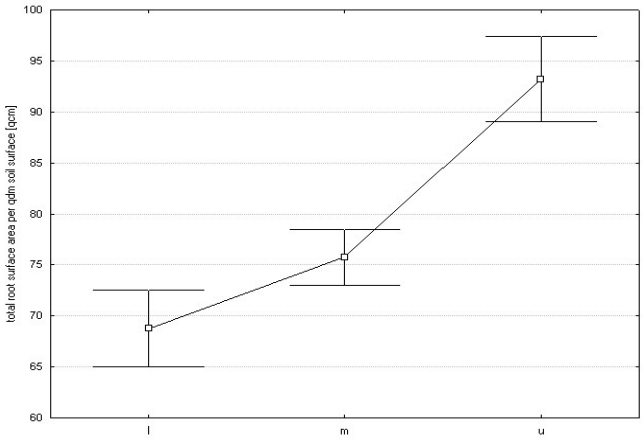


(f) Fractal dimension

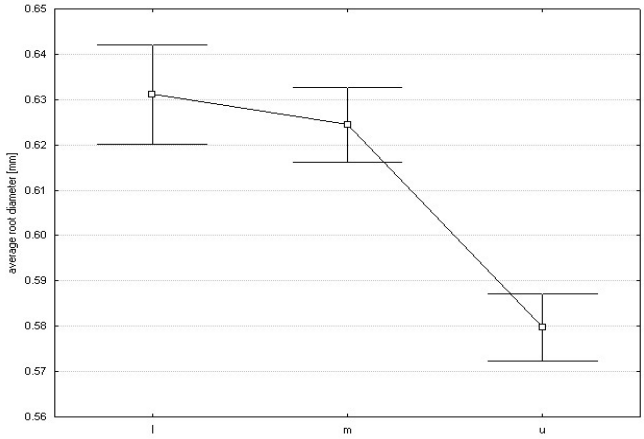
Figure 33: Temporal development (per month) of root system main traits. Plotted are mean values per month and 0.95 * standard error. **A:** Total root length per 10^{-2}sqm [cm]. **B:** Total root surface area per 10^{-2}sqm [sqcm]. **C:** Root length per 10^{-2}sqm in diameter classes 0-10mm (according to Boehm (1979)). **D:** Root surface area per 10^{-2}sqm in diameter classes 0-10mm (according to Boehm (1979)). **E:** Average root diameter per 10^{-2}sqm . **F:** Fractal Dimension.



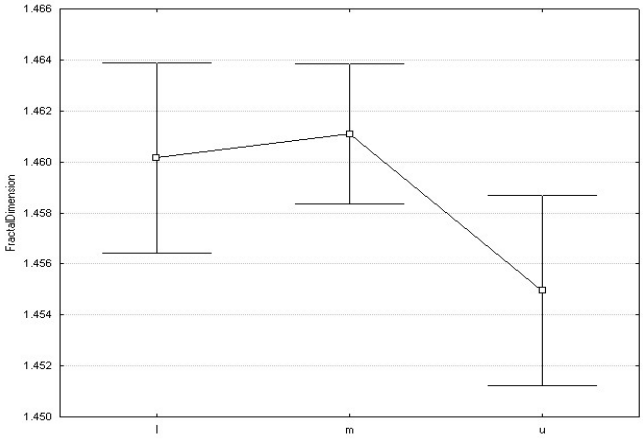
(a) Root length per 10^{-2}sqm



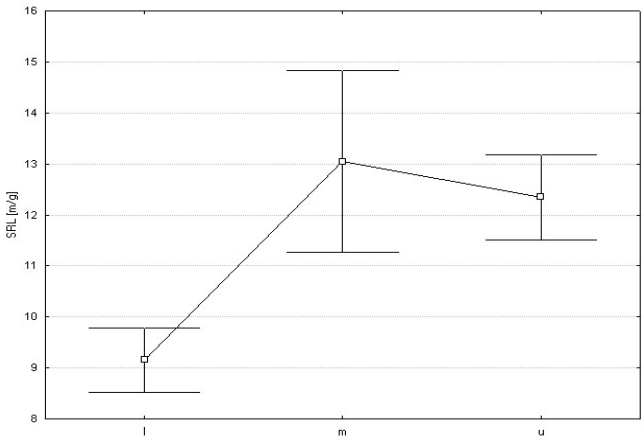
(b) Root surface area per 10^{-2}sqm



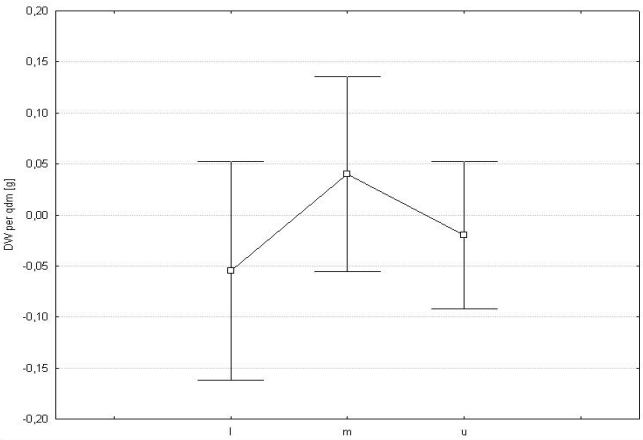
(c) Average root diameter



(d) Fractal dimension



(e) Specific root length (SRL)



(f) Root dry weight

Figure 34: Spatial distribution of root system main traits in classes l, m and u. Plotted are mean per class and 0.95 * standard error. **A:** Total root length per 10^{-2}sqm [cm]. **B:** Total root surface area per 10^{-2}sqm [sqcm]. **C:** Average root diameter per 10^{-2}sqm [mm]. **D:** Fractal Dimension. **E:** Specific root length [m/g]. **F:** Root dry weight per 10^{-2}sqm [g]

4.2.2.2 Cumulative and Spatial Differences in Variants To detect variant (5C, 125AA) dependent differences in cumulative values, an ANOVA with the normal distributed parameters was calculated. Normal distribution could be assumed for *total root length* and *total root surface area* as well as for both parameters in the diameter classes 0.4-0.5, 0-0.5 and 0.5-2 mm (K-S Test, P=0.05). All relevant parameters showed homogeneity of variance (Levene test, P=0.05). Not normal distributed parameters (K-S test) were tested by a Kruskal-Wallis ANOVA.

In almost every parameter, 125AA showed higher mean values. Significant differences between the rootstock variants could be detected in the values of length and surface area in the class *0-0.5mm*, in length and surface area in the classes *0.15-0.2 mm* and *1.5-2 mm*, length in the class *0.2-0.3 mm*, *number of crossings* and *crossings per cm* (table 26 and table 27).

A moderate influence of the rootstock can be supposed to *total root length, total root surface area* and *fractal dimension*. Here p-values were very low (table 26 and table 27). No influence of the rootstock variant was detected in *average root diameter, number of forks, forks per cm* and *SRL*. Also higher diameter classes showed no differences in cumulative values. The distribution of length in surface area in diameter classes did not differ between variants.

Table 26: ANOVA. Factor: variant, Factor levels: 5C, 125AA. mean values, R^2 (adjusted) values and p values. n = 170 per variant, dataset: Mar08-Aug09 (except of January and February 2009). *=significant

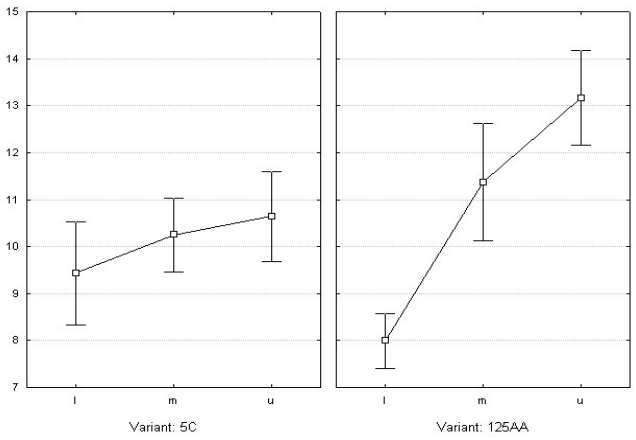
Parameter	mean 5C	mean 125AA	R^2	p value
Total root length	416	438.7	0.007	0.06
Total root surface area	78.8	82.4	0.08	
Length 0-0.5mm	270.1	293.9	0.012	0.021*
Surface area 0-0.5mm	25.4	27.3	0.009	0.037*

Table 27: Kruskal-Wallis ANOVA. Factor: variant, factor levels:5C, 125AA. mean values, H values, p values. n = 340, dataset: March 2008 - August 2009 (except of January and February 2009). * = significant

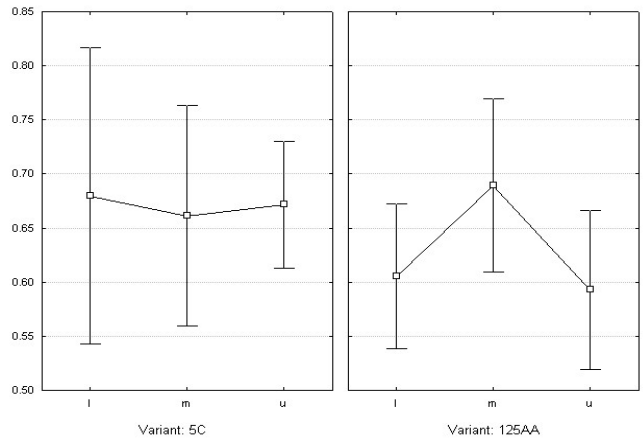
Parameter	mean 5C	mean 125AA	H value	p value
Average root diameter	0.63	0.61	0.81	0.36
Number of crossings	114.48	137.71	6.09	0.014*
Crossings per cm	0.26	0.3	14.15	0.001*
Fractal Dimension	1.46	1.47	3.71	0.06
Length 0.15-0.2mm	28.56	32.67	4.62	0.032*
Length 0.2-0.3mm	61.04	68.9	3.86	0.049*
Length 1.5-2mm	5.3	6.52	4.09	0.043*
Surface area 0.15-0.2mm	1.6	1.83	4.54	0.033*
Surface area 1.5-2mm	2.83	3.5	4.16	0.042*

The spatial distribution of most root system main parameters was similar on 125AA and 5C. *Total root length, total root surface area, number of crossings* and *number of forks* showed highest values on the upper part of the study site on both variants. Also *crossings per cm* and *forks per cm* held higher values on the upper part of the study site. *Average root diameter* decreased from low to upper parts on both variants.

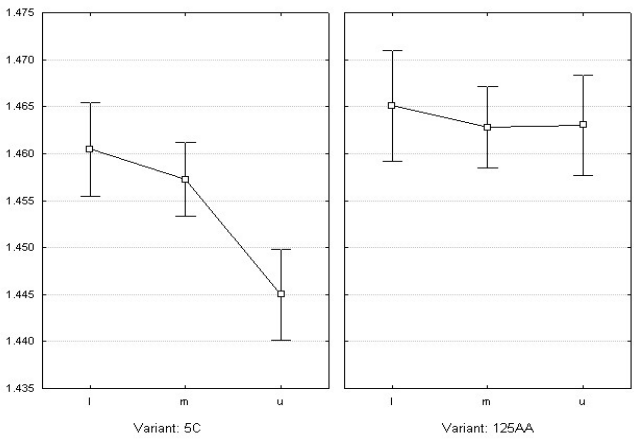
Differences between variants occurred in *SRL*, *DW* and the *fractal dimension* (figure 35). *DW* of 125AA rootstocks showed highest values in the middle part of the study site (figure 35B). Due to an higher root length, *SRL* of 125AA rootstocks showed a highly increase from low to upper part of the study site. In contrast, *DW* of 5C rootstocks did not show spatial differences and *SRL* showed the highest values in the middle part of the study site (figure 35A). Differences in *fractal dimension* between variants were remarkable. *Fractal dimension* of 5C rootstocks decreased from lower to upper parts of the study site, whereas *fractal dimension* of 125AA rootstocks did not show any differences (figure 35C).



(a) Specific root length (SRL)



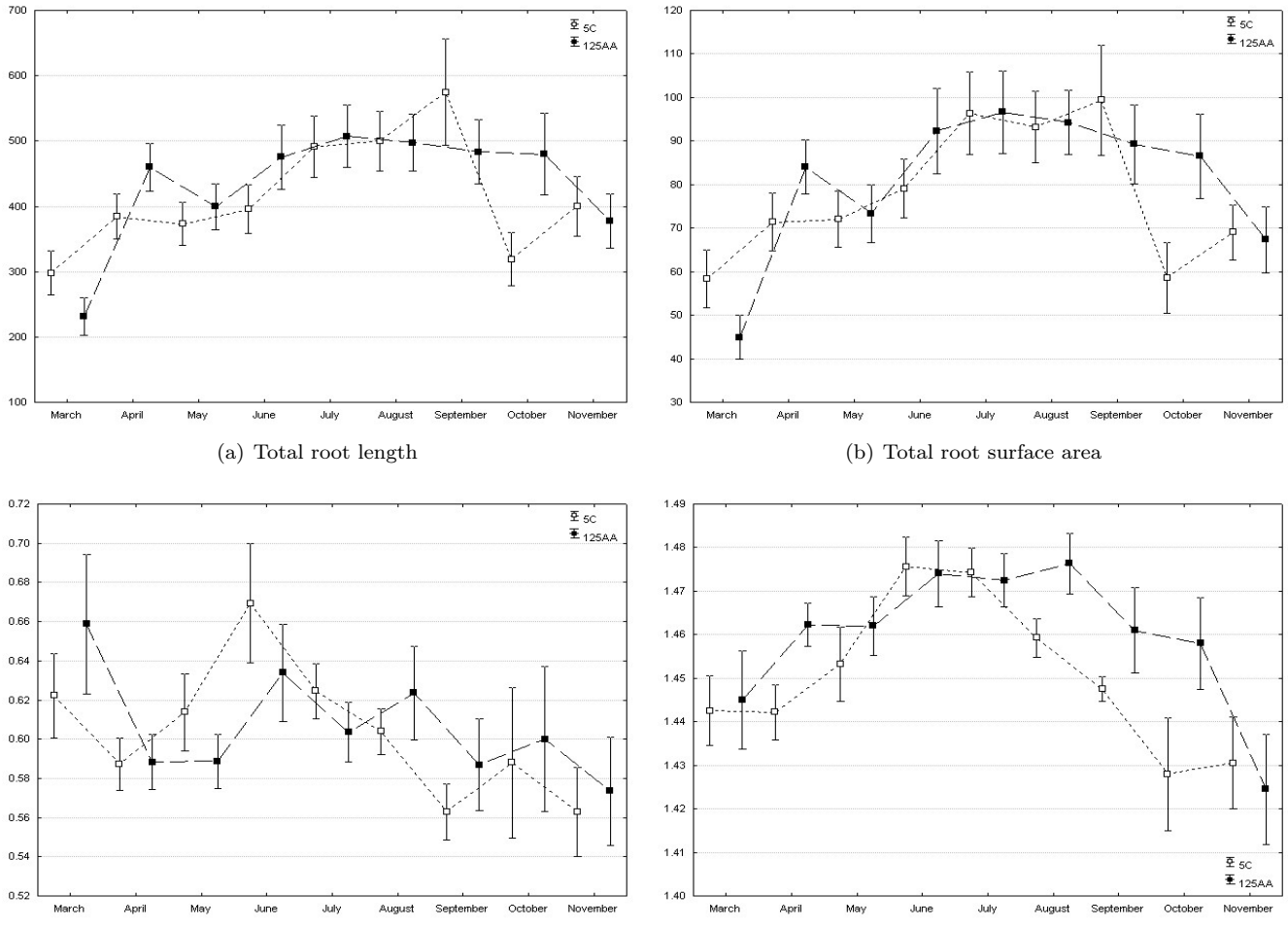
(b) Root dry weight



(c) Fractal dimension

Figure 35: Variant dependent spatial distribution of root system traits in classes l, m and u. Plotted are mean per class and 0.95 * standard error. **A:** Specific root length (SRL) [m/g]. **B:** Root dry weight per 10^{-2}sqm (DW) [g]. **C:** Fractal dimension.

4.2.2.3 Temporal Differences in Variants The distribution of root system parameters in the course of a year on both variants conformed widely to the general temporal development that has been illustrated in figure 33 (section 4.2.2.1). Marginal variant dependent differences occurred in the temporal distribution of *fractal dimension*. Highest values occurred in June and July on both variants, but 125AA rootstocks held high values also in August. 5C showed a decrease in *fractal dimension* from July to October, 125AA from August to November (figure 36).



(a) Total root length

(b) Total root surface area

(c) Average root diameter

(d) Fractal dimension

Figure 36: Variant dependent spatial distribution of root system traits in classes l, m and u. Plotted are mean per class and 0.95 * standard error. **A:** Total root length [cm]. **B:** Total root surface area [sqcm]. **C:** Average root diameter [mm]. **D:** Fractal dimension.

Summary: General differences in the temporal distribution (course of a year) were recorded in almost all morphological root system parameters. Only *SRL* was not affected by seasonal differences. Most morphological parameters and *fractal dimension* (architectural parameter) showed highest values in late summer months. *Average root diameter* lightly decreased from spring to winter. These developments were similar on both rootstocks. Only *fractal dimension* showed rootstock dependent marginal

differences in the seasonal development.
Regarding the general spatial distribution, *total root surface area*, *SRL* and *total root length* showed an increase from lower to upper parts of the study site, whereas *average root diameter* and *fractal dimension* decreased. Most of the spatial distributions were similar on both rootstocks. Differences between rootstocks occurred in spatial distribution of *DW*, *SRL* and *fractal dimension*.

4.2.3 Fungal Endophytes

Fungal endophytes were isolated from nodosity and root tissue using two different culture mediums (section 3.2.2.3). Fungal morphotypes were visually differentiated by the color of the mycelium, thickness and ramification of hyphae and form of asexual conidiospores fruiting bodies (if present) (section 3.2.2.3). Due to unexpected personnel decisions within the Institute of Microbiology at Innsbruck University, the intended species specification of morphotypes could not be performed by Dr. M. Kirchmair.

Although morphotypes could not be classified into fungal pathogen classes according to Huber (2007), a general discriminant analysis (DA) with morphotypes as single groups was done to identify possible distinct differences between origin tissue or rootstock variant. Due to the absent classification of morphotypes, only one factor root could be extracted. A two dimensional image of extracted roots was not possible. To identify the significance of the distances on the factor root, a F-test of the squared Mahalanobis distances was done. Morphotypes were only moderate different between the categories (origin tissue or variant) and distances between the categories were not significant (table 28).

Table 28: Discriminant analysis (DA). F-Test of squared Mahalanobis distances. Only one root could be extracted in the DA.

Medium	Category	F	p
NDM	Nodositiy tissue - Root tissue	1.03	0.52
	5C - 125AA	1.07	0.49
PDA	Nodosity tissue - Root tissue	0.94	0.6
	5C - 125AA	0.98	0.54

Possible differences in the total amount of recorded morphotypes as well as the frequency of single morphotypes could be indications for morphotype dependent differences within the categories (origin tissue, variant). To identify differences in total amount of isolated morphotypes, a Wilcoxon test for paired samples was done (P=0.05) (table 29). No variant or tissue specific significances in the total amount of morphotypes could be detected. The amount of isolated fungi did not differ significantly, considering origin tissue or rootstock.

Table 29: Wilcoxon test of paired samples. Origin tissue: nodosity and roots. Variants: 5C and 125AA. P=0.05

Medium	Category	Z	p
NDM	Origin tissue	0.53	0.59
	Variant	0.48	0.63
PDA	Origin tissue	1.58	0.11
	Variant	0.16	0.87

To identify possible differences in the frequency of morphotypes, a chi^2-test of homogeneity was done (table 30). The morphotypes isolated from roots were classified as the expected group, morphotypes isolated from nodosities were classified as the observed group. Regarding the variants, observed

and expected group were assigned to both rootstocks.
On NDM, frequency of isolated fungi differed significantly, considering the origin tissue (table 30). On PDA, no significant differences could be found. Regarding the variant, on PDA significant differences in the frequency of isolated endophytes were found. On NDM, no differences were detected (table 30).

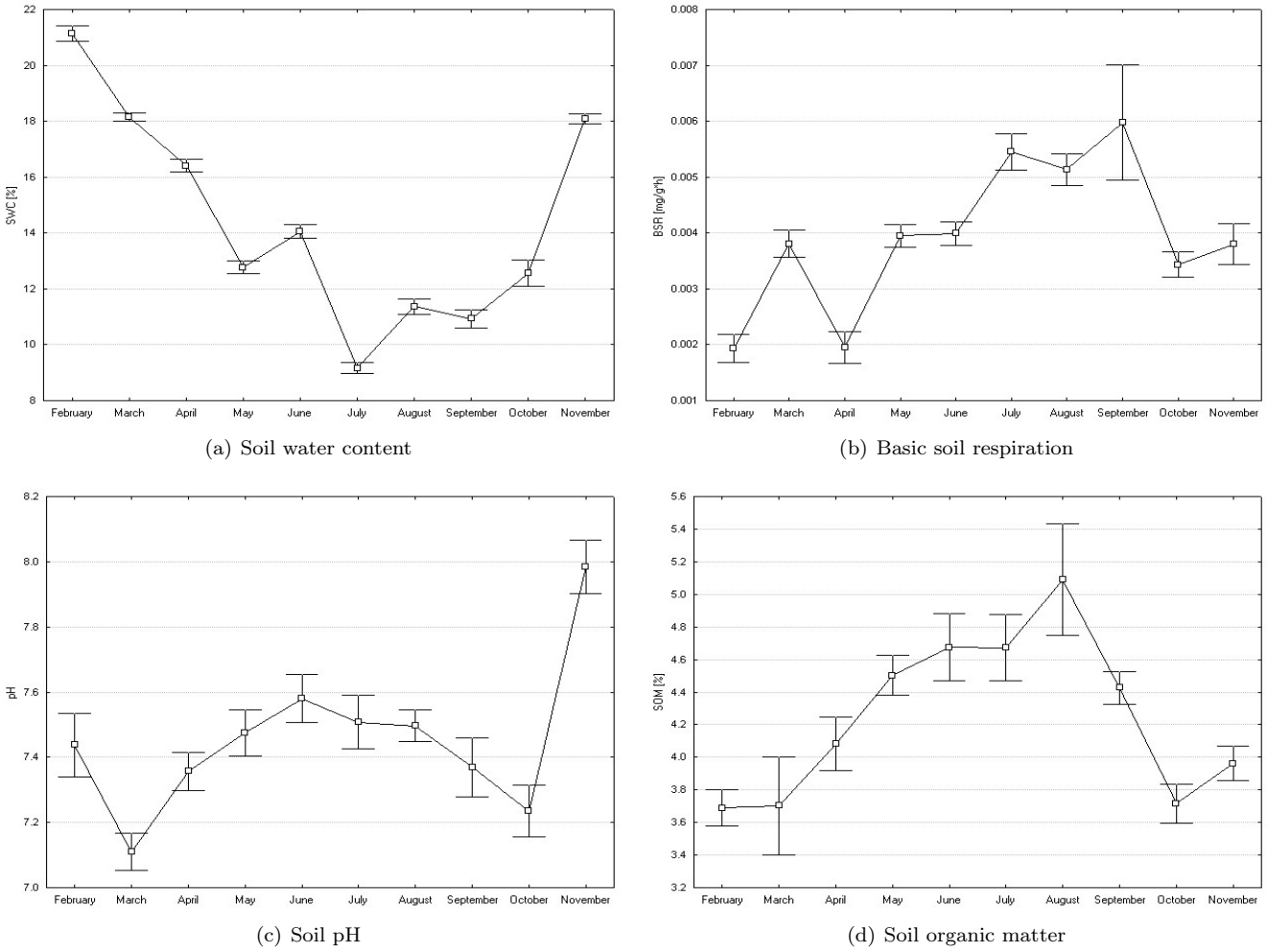
Table 30: chi^2-test of homogeneity of fungal morphotypes. Homogeneity of origin tissue (nodosity or root) and of variant (5C, 125AA) were tested. 1 = 5C expected, 125AA observed. 2 = 125AA expected, 5C observed. * = significant ($p < 0.05$).

Medium	Category	chi^2	p
NDM	Origin tissue	94.31	0.03*
	Variant[1]	78.55	0.25
	Variant[2]	69.27	0.54
PDA	Origin tissue	102.41	0.12
	Variant[1]	135.1	0.001*
	Variant[2]	118.4	0.01*

Summary: Tissue or rootstock dependent difference between fungal morphotypes could not be detected with a discriminant analysis (DA). But the frequency of single morphotypes differed significantly between the both origin tissues (nodosity and root; on NDM) and between the both variants (5C and 125AA; on PDA). The total amounts of isolated morphotypes did not differ significantly.

4.2.4 Soil Properties

The soil parameters *SWC, pH, BSR* and *SOM* were recorded from March 2008 to August 2009 with 10 SU per variant (5C, 125AA) on the study site. The following calculations were based on the dataset Mar08-Aug09.



(a) Soil water content

(b) Basic soil respiration

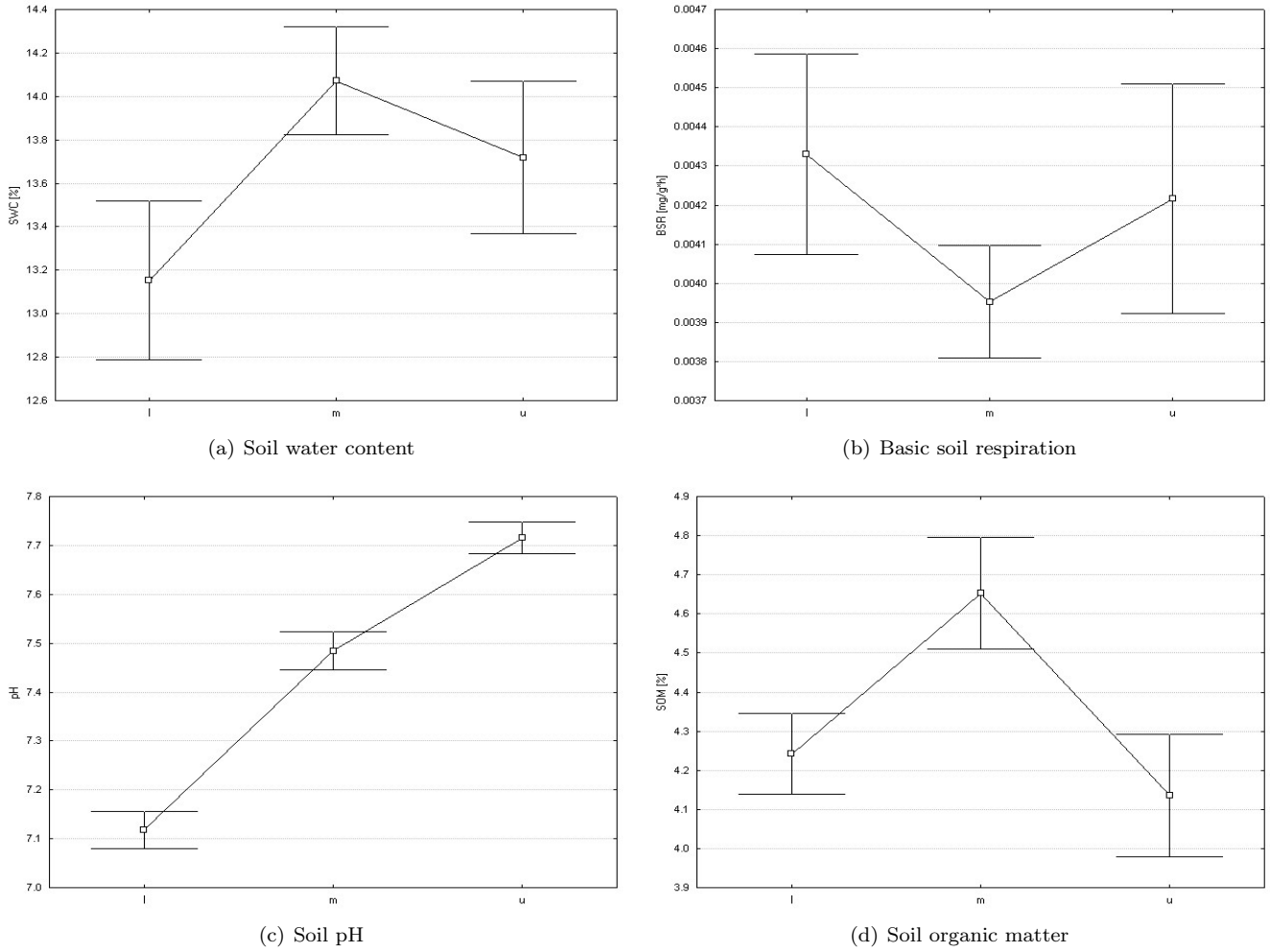
(c) Soil pH

(d) Soil organic matter

Figure 37: Temporal distribution of soil parameters. Plotted are mean values per month and 0.95 * standard error. **A:** Soil water content (soil moisture) (SWC). [%]. **B:** Basic soil respiration [$\frac{mgCO_2C}{gDM*h}$]. **C:** Soil acidity pH. **D:** Soil organic matter (SOM) [%].

4.2.4.1 General Temporal and Spatial Differences To detect general significant differences in the soil properties regarding their temporal or spatial distribution, values of 5C and 125AA were pooled. Regarding the course of a year (temporal distribution), all recorded parameters showed significant differences within the factor levels (months) (Kruskal-Wallis ANOVA). *SWC* on the study site showed significant differences between all months (Bonferroni post-hoc test, P=0.05) with highest mean values in winter (18-21 %) and lowest in summer (July 9%) (figure 37A). *pH* on the study site showed signifi-

cant differences between March (lowest mean value: 7.1) and most other months (Bonferroni post-hoc test, P=0.05). Regarding the course of a year, *pH* showed a first peak during summer months (June: 7.6) and a second, higher peak during winter months (November: 8.0) (figure 37C). *BSR* showed significant differences between early spring months and summer to autumn months (Bonferroni post-hoc test, P=0.05). The lowest values appeared in late winter and early spring with a significant increase in May. Highest peak was recorded in September with 0.006 $\frac{mgCO_2C}{gDM*h}$ (figure 37B). *SOM* showed only a significant difference between March and August (Bonferroni post-hoc test, P=0.05). *SOM* increased from winter to summer with highest values in August (5.1 %) and a strong decrease from summer to winter (figure 37D).



(a) Soil water content

(b) Basic soil respiration

(c) Soil pH

(d) Soil organic matter

Figure 38: Spatial distribution of soil parameters. Plotted are mean values per class and 0.95 * standard error. **A:** Soil water content (soil moisture) (SWC). [%]. **B:** Basic soil respiration [$\frac{mgCO_2C}{gDM*h}$]. **C:** Soil acidity pH. **D:** Soil organic matter (SOM) [%].

Regarding the general spatial distribution (lower, middle and upper parts of the study site), *pH* and *SOM* showed significant differences (Kruskal-Wallis ANOVA) (figure 38C+D). *SOM* showed a peak in

the middle part of the study site (mean value: 4.65 %) and a significant decrease to the upper part of the study site (4.15 %) whereas *pH* increased from the lower part of the study site (mean value: 7.1) to the middle (7.5) and upper parts (7.7) significantly (Bonferroni post-hoc tests, P=0.05) (figure 38A).

4.2.4.2 Differences in Variants To detect variations in the cumulative amount of values, a Kruskal-Wallis ANOVA (H-Test) was calculated. *SWC, pH* and *BSR* showed a light decrease from the 5C part of the study site to the 125AA part. Values of *SWC* showed significant differences (mean values: 5C: 14.2%, 125AA: 13.2 %). The parameter *SOM* showed a light increase from 5C to 125AA (mean values: 5C: 4.35%, 125AA: 4.5%).
Regarding the variants, only marginal differences in temporal or spatial distribution could be detected. Significant differences in soil moisture (*SWC*) occurred in spatial classes. In the lower part of the study site, differences in *SWC* between 125AA area and 5C area were extensive (not shown here). The soil acidity changes on both variants from pH $\approx$ 7 on the lower part to pH $\approx$ 8 on the upper part of the study site.

Summary: *SWC, pH* and *BSR* decreased from 5C area to 125AA area, *SOM* an increase. This could be caused by their different location on the study site. All measured soil properties on the study site were affected by their temporal distribution (course of a year), whereas *SWC* decreases between winter and summer and all other parameters showed peaks during summer months. Regarding the spatial distribution, *pH* values increased significantly from the lower to the upper part of the study site.

4.3 Regressions

4.3.1 Grape Phylloxera Population

4.3.1.1 General Relations General dynamic relationships of grape phylloxera population structure in field was predicted by multiple linear regressions (MLR). The total amount of grape phylloxera instars was related to different population attributes. The MLR based on different datasets, due to the introduction of attributes.

Figure 39: Third section of results: Verification and Relations of assessed parameters by linear regression.

Before MLR were calculated, data was standardized by a z-transformation to avoid difficulties due to the different measuring scales of the parametes (per cm, per DW, per 10^{-2}sqm). In the regressions, different population attributes (RPD, CNA, APD, NOP) should be predicted by the amount of grape phylloxera instars. In all regressions, dependent variables were *ttl instars per* 10^{-2}*sqm* or *ttl instars per cm* respectively (table 31 and 32).

4.3.1.1.1 Dependent variable: $ttl\ inst\ per\ 10^{-2}sqm$ As expected, the prediction values of the different RPD (per 10^{-2}sqm) instar sub parameters (L1-L4, L5, nymphae) were very high. *ttl inst per* 10^{-2}*sqm* was highly reflected by *L1-L4 per* 10^{-2}*sqm* (table 31). Also RPD nodosity attributes were moderately affected by the total amount of grape phylloxera instars, particularly the nodosity attributes "color" and "form". Strong positive relationships could be detected for non brownish colored nodosities as well as for A-forms (both per 10^{-2}sqm). Prediction values for instars and color of nodosities are based on a broad database, whereas prediction values for nodosity forms only base on dataset 2009 (table 31).
Also absolute densities of instars (per cm and per DW) could be positively implicated in *ttl instars per* 10^{-2}*sqm* . Especially the absolute amount of nymphal instars per cm was in a positive relation to the total relative amount of instars per 10^{-2}sqm (table 31).
Regarding nodosity occupation parameters (NOP), high positive relations could be calculated. The occupation of non brownish colored nodosities by L1-L4 and nymphal instars were positively related with the total relative amount of instars (per 10^{-2}sqm). Regarding the nodosity form as well as the combined nodosity attributes (CNA), also high prediction values were achieved. The database of the last mentioned regressions was very weak (dataset 2009) (table 31).

Table 31: Multiple linear regressions. Dependent variable: *ttl instars per* 10^{-2} *sqm* . Predictor: grape phylloxera traits. R^2 (adjusted), β coefficients. * = significant R^2 value. **= significant β coefficients. In CNA and NOP regressions only significant β coefficients were shown, but all relevant parameters were included.

Pred. density	Dataset	Attributes	R^2	Pred. var.	β coeff.
RPD	2006-2009	instars	0.93*	L1-L4	0.75**
				L5	0.17**
				Nymphae	0.14**
	2007-2009	color	0.36*	sv	-0.1
				lv	0.12**
				uv	0.57**
	Jul08-Aug09	branching	0.28*	term	0.45**
				non-term	0.13**
	2009	form	0.47*	A	0.73**
				B	-0.06
				C	-0.04
				D	-0.1
NOP	2007-2009	occupation color	0.6*	*L1-L4 per sv*	0.15**
				L1-L4 per uv	0.4**
				L5 per lv	0.15**
				L5 per uv	0.2**
				Nym per lv	0.14**
				Nym per uv	0.34**
	Jul08-Aug09	occ. branching	0.57*	*L1-L4 per term*	0.14**
				L1-L4 per non-term	0.35**
	2009	occupation form	0.66*	*L1-L4 per A*	0.33**
				L1-L4 per B	0.14**
				L5 per A	0.34**
				L5 per C	0.12**
				Nym per C	0.31**
CNA	2009	all	0.69*	*lvAterm*	0.26**
				uvAterm	0.33**
				uvDnon-term	0.14**
				uvAnon-term	0.72**
APD (per cm)	2006-2009	instars	0.39*	L1-L4	0.22**
				L5	0.21**
				Nymphae	0.41**
APD (per DW)	Jun08-Aug09	instars	0.39*	L1-L4	0.43**
				L5	0.25**
				Nympae	0.04

4.3.1.1.2 Dependent variable: *ttl inst per cm* Generally, regressions with the dependent variable *ttl instars per cm* (APD) showed much lower R^2 values than those with *ttl instars per* 10^{-2}*sqm* as dependent variable. Highest values occurred by the prediction of APD instar subclasses (per cm). The absolute number of L5 instars per cm was not related to the total amount of instars (table 32). Also non of the absolute amounts of nodosity attributes showed a remarkable relation to the absolute number of instars. Highest values show *uv per cm* with β coefficient of 0.24. But prediction strength of the regression was very weak with a $R^2 = 0.06$. Also prediction of relative densities (RPD) of nodosity attributes (per 10^{-2}sqm) showed only low R^2 values (table 32).
Highest R^2 values occurred regarding nodosity occupation parameters (NOP). The total amount of instars per cm was positively related to the occupation of terminal as well as A formed nodosities by L1-L4 instars and weak related to the occupation of non-terminal nodosities by L5 and nymphal instars (table 32). Also the occurrence of non brownish A formed terminal nodosities (CNA) showed a moderate positive relation to *ttl instars per cm*.

Table 32: Multiple linear regressions. Dependent variable: *ttl instars per cm*. Predictor: grape phylloxera traits. R^2 (adjusted), β coefficients. * = significant R^2 value. **= significant β coefficients. In CNA and NOP regressions only significant β coefficients were shown, but all relevant parameters were included.

Pred. density	Dataset	Attributes	R^2	Pred. var.	β coeff.
APD	2006-2009	instars	0.6*	L1-L4	0.72**
				L5	0.05
				Nymphae	0.14**
	2007-2009	color	0.06*	sv	0.004
				lv	0.07
				uv	0.24**
NOP	Jun08-Aug09	occ. branches	0.3*	*L1-L4 per term*	0.43**
				L5 per non-term	0.12**
				Nym per non-term	0.18**
	2009	occupation form	0.28*	*L1-L4 per A*	0.44**
				L5 per A	0.33**
CNA	2009	comb. nod.	0.21*	*svDterm*	-0.14**
				uvAterm	0.32**
				uvDnon-term	0.27**
RPD	2007-2009	color	0.1*	sv	0.04
				lv	0.07
				uv	0.3**
	2009	form	0.1*	A	0.34**
				B	-0.12
				C	0.03
				D	0.03

Summary: Positive relationships between relative density of total instars and absolute densities of instar sub classes (mainly *L1-L4 per cm*) could be calculated. Relative densities of total instars were mostly dependent on relative densities of L1-L4 instars and of non brownish nodosities. Also high positive relationships between occupation of non brownish nodosities by L1-L4 instars and nymphal

instars could be detected. Regarding regressions with weaker databases, particularly relations between A formed nodosities (also NOP and CNA) and the relative densities of total instars were calculated.
Altogether, relationships between the absolute density of total instars and other grape phylloxera population traits were substantially lower. Regarding all instar sub classes, *ttl instars per cm* were highly related to *L1-L4 per cm*. Regarding regressions with weaker database, particularly occupation of terminal nodosities by L1-L4 instars and non-terminal nodosities by L5 and nymphal instars were in a positive relationship with the absolute density of total instars.

4.3.1.2 Detailed Relations Relationships between sub classes of instars and other grape phylloxera population traits were calculated by multiple linear regressions (MLR). Dependent variables were all instar subclasses, predicted variables were relevant grape phylloxera traits. Before, data were z-transformed.
In regressions the dataset 2009 was used, calculated dependencies regarding L5 or nymphal parameters could also be strongly related to their temporal development.

4.3.1.2.1 Dependent variables: RPD instar sub parameters High relations could be calculated regarding the prediction of APD (per cm) instar sub classes by RPD instar sub classes. Particularly the absolute density of nymphal instars (*Nym per cm*) was related to the relative densities of all instar sub classes (table 33). APD (per DW) instar sub classes were highly related to the RPD sub classes (table 33). The prediction of different RPD nodosity color classes by instar sub classes per 10^{-2}sqm showed a high relation of *uv per* 10^{-2}*sqm* to *L1-L4 per* 10^{-2}*sqm* (table 33). Also the relative densities of terminal nodosities (*term per* 10^{-2}*sqm*) could be related to *L1-L4 per* 10^{-2}*sqm* and *L5 per* 10^{-2}*sqm* (table 33).
Regarding the regressions with a weaker database (dataset 2009), A-formed nodosities per 10^{-2}sqm (*A per* 10^{-2}*sqm*) could be highly predicted by all RPD instar sub classes (table 33). Also the CNA regression showed high relations between RPD of instar sub classes and A-formed nodosities. Particularly *uvAnon-term* was highly related to all RPD instar sub classes (table 33).

4.3.1.2.2 Dependent variable: APD instar sub parameters Regarding the prediction of APD (per cm) traits by APD instar sub classes (per cm), no high relations could be detected (not specified here). Also the prediction of instar sub classes per DW by instar sub classes per cm showed moderate R^2 values. *Nym per DW* was dependent on *Nym per cm* (table 34).
The relative densities of A-formed nodosities increased at increasing absolute densities of all instar classes, whereas APD of nymphal instars had the highest relation (table 34). Also the prediction of CNA parameters by absolute instar densities showed higher R^2 values. Especially the occurrence of

Table 33: Multiple linear regression. Dependent variables: RPD sub parameters instars (L1-L4, L5, Nym) per 10^{-2}sqm . Predictor: RPD, APD and CNA grape phylloxera traits. R^2 (adjusted), β coefficients. * = significant R^2 value. **= significant β coefficients. In regressions only significant β coefficients were shown, but all relevant parameters were included.

Pred. density	Dataset	Dep. var.	R^2	Pred. var.	β coeff.
RPD color	2007-2009	*L1-L4 per 10^{-2}sqm*	0.39**	*lv per 10^{-2}sqm*	0.1**
				uv per 10^{-2}sqm	0.59**
		L5 per 10^{-2}sqm	0.15*	*uv per 10^{-2}sqm*	0.38**
		Nym per 10^{-2}sqm	0.17*	*uv per 10^{-2}sqm*	0.4**
RPD branches	Jul08-Aug09	*L1-L4 per 10^{-2}sqm*	0.31*	*term per 10^{-2}sqm*	0.45**
				non-term per 10^{-2}sqm	0.17**
		L5 per 10^{-2}sqm	0.22*	*term per 10^{-2}sqm*	0.44**
		Nym per 10^{-2}sqm	0.11*	*term per 10^{-2}sqm*	0.29**
RPD form	2009	*L1-L4 per 10^{-2}sqm*	0.49*	*A per 10^{-2}sqm*	0.71**
				D per 10^{-2}sqm	-0.13**
		L5 per 10^{-2}sqm	0.51*	*A per 10^{-2}sqm*	0.7**
				B per 10^{-2}sqm	-0.25**
		Nym per 10^{-2}sqm	0.41*	*A per 10^{-2}sqm*	0.71**
APD inst per cm	2006-2009	*L1-L4 per 10^{-2}sqm*	0.31*	*L1-L4 per cm*	0.25**
				L5 per cm	0.19**
				Nym per cm	0.35**
		L5 per 10^{-2}sqm	0.39**	*L1-L4 per cm*	0.09**
				L5 per cm	0.31**
				Nym per cm	0.48**
		Nym per 10^{-2}sqm	0.41*	*L5 per cm*	0.18**
				Nym per cm	0.54**
APD inst per DW	Jun08-Aug09	*L1-L4 per 10^{-2}sqm*	0.34*	*L1-L4 per DW*	0.5**
				L5 per DW	0.17**
		L5 per 10^{-2}sqm	0.45*	*L5 per DW*	0.71**
		Nym per 10^{-2}sqm	0.48*	*L1-L4 per DW*	0.11**
				Nym per DW	0.7**
CNA	2009	*L1-L4 per 10^{-2}sqm*	0.68*	*lvAterm*	0.25**
				uvAterm	0.35**
				uvAnon-term	0.64**
				uvBnon-term	0.09**
		L5 per 10^{-2}sqm	0.72*	*lvAterm*	0.13**
				lvAnon-term	-0.19**
				uvCterm	0.15 **
				uvAnon-term	0.9**
				uvBnon-term	-0.28**
				uvCnon-term	-0.19**
		Nym per 10^{-2}sqm	0.43*	*uvAterm*	0.28**
				uvDterm	0.2**
				uvAnon-term	0.67**
				uvDnon-term	-0.4**

Table 34: Multiple linear regression. Dependent variables: APD sub parameters instars (L1-L4, L5, Nym) per cm, per DW. Predictor: APD, RPD and CNA grape phylloxera traits. R^2 (adjusted), β coefficients. * = significant R^2 value. **= significant β coefficients. In regressions only significant β coefficients were shown, but all relevant parameters were included.

Pred. density	Dataset	Dep. var.	R^2	Pred. var.	β coeff.
APD (per DW)	Jun08-Aug09	*L1-L4 per cm*	0.1*	*L1-L4 per DW*	0.36**
		L5 per cm	0.17*	*L5 per DW*	0.23**
				Nym per DW	0.18**
		Nym per cm	0.25*	*Nym per DW*	0.46**
RPD instars	2006-2009	*L1-L4 per cm*	0.12*	*L1-L4 per 10^{-2}sqm*	0.37**
		L5 per cm	0.26*	*L5 per 10^{-2}sqm*	0.36**
				Nym per 10^{-2}sqm	0.13**
		Nym per cm	0.42*	*L5 per 10^{-2}sqm*	0.23**
				Nym per 10^{-2}sqm	0.44**
	Jun08-Aug09	*L1-L4 per DW*	0.35*	*L1-L4 per 10^{-2}sqm*	0.52**
				Nym per 10^{-2}sqm	0.2**
		L5 per DW	0.44*	*L5 per 10^{-2}sqm*	0.64**
		Nym per DW	0.48*	*L1-L4 per 10^{-2}sqm*	-0.13**
				L5 per 10^{-2}sqm	0.12**
				Nym per 10^{-2}sqm	0.7**
RPD form	2009	*L1-L4 per cm*	0.17*	*A per 10^{-2}sqm*	0.43**
		L5 per cm	0.29*	*A per 10^{-2}sqm*	0.48**
				B per 10^{-2}sqm	-0.28**
		Nym per cm	0.41*	*A per 10^{-2}sqm*	0.57**
				B per 10^{-2}sqm	-0.17**
				C per 10^{-2}sqm	0.16**
				D per 10^{-2}sqm	-0.16**
CNA	2009	*L1-L4 per cm*	0.23*	*svDnon-term*	0.43**
				lvAterm	0.28**
				ucCterm	0.17**
		L5 per cm	0.3*	*uvCterm*	0.2**
				uvDterm	0.35**
				uvAnon-term	0.27**
		Nym per cm	0.38*	*uvAterm*	0.24**
				uvDterm	0.55**
				uvCnon-term	-0.21**
				uvDnon-term	-0.27**
		L1-L4 per DW	0.3*	*lvBnon-term*	0.41**
				uvBterm	0.15**
				uvCterm	-0.16**
				uvAnon-term	0.29**
				uvDnon-term	-0.22**
		L5 per DW	0.29*	*svAnon-term*	0.32**
				lvAnon-term	-0.19**
				uvAterm	-0.16**
				uvAnon-term	0.77**
				uvDnon-term	-0.29**
		Nym per DW	0.16*	*svAnon-term*	0.48**
				uvBterm	0.16**
				uvAnonerm	0.51**
				uvDnon-term	-0.39**

uvAnon-term nodosities could be predicted by the absolute densities of L5 instars (both per cm and per DW), whereas the occurrence of *uvAterm* nodosities was mainly dependent on the absolute density of nymphal instars per cm (table 34). Regarding regressions with the strongest databases (2006-2009), high values were calculated in prediction of relative instar densities by absolute instar densities (table 34). Particularly relative densities of L5 and nymphal instars could be predicted by absolute values (both per cm and per DW) (table 34).

4.3.1.2.3 Prediction of NOP High relations could also be recorded between the occupation of nodosities and densities of instars sub parameters (table 35 and 36). regressions regarding the occupation of different attributed nodosities were shown in table 35. The prediction of the total occupation of different colored nodosities by APD and RPD instar densities based on the strongest dataset (2007-2009). The total occupation of sv, lv and uv nodosities was highly dependent on L1-L4 instar denstity (both APD and RPD). Also, moderate dependencies to absolute densities of L5 or nymphal instars could be detected (table 35). Considering the NOP (branches) regressions, higher densities of instars led to higher occupation of both terminal and non-terminal nodosities. But the occupation of non-terminal nodosities could mainly be predicted by densities of L5 instars (both APD and RPD) (table 35). A weak database was used to calculate the NOP (form) regressions (dataset 2009). Mainly the occupation of A-formed and D-formed nodosities could be predicted by RPD instar sub classes. Also, APD parameters showed moderate relations to nodosity occupation. A very strong relationship could be calculated for prediction of *ttl inst per A* by *L1-L4 per DW* (table 35).
Relations between RPD/APD of instar sub classes and NOP were shown in table 36. The highest R^2 values were reached in prediction of occupation by *L1-L4 per* 10^{-2}*sqm , L1-L4 per DW, L5 per cm, Nym per* 10^{-2}*sqm* and *Nym per cm*. The occupation of non-terminal nodosities by L1-L4 instars was positively dependent on *L1-L4 per* 10^{-2}*sqm* , the occupation of light brownish nodosities negatively. The occupation of terminal and non-terminal nodosities by L5 instars could be predicted by *L5 per cm*. Occupation of A formed nodosities by L1-L4 instars were positively related to *L1-L4 per DW* (table 36). The occupation of terminal nodosities by nymphal instars was highly dependent on *Nym per cm* (table 36). These regressions base on a very weak database (dataset 2009).

Table 35: Multiple linear regression. Dependent variables: APD and RPD sub parameters instars (L1-L4, L5, Nym) per cm, DW or 10^{-2}sqm (all classes). Predictor: Nodosity occupation parameters (NOP) (ttl instars). R^2 (adjusted), β coefficients. * = significant R^2 value. **= significant β coefficients. In regressions only significant β coefficients were shown, but all relevant parameters were included.

Pred. density	Dataset	Dep. var.	R^2	Pred. var.	β coeff.
NOP(color)	2007-2009	*L1-L4 per 10^{-2}sqm*	0.38*	*ttl inst per sv*	0.12**
				ttl inst per lv	0.1**
				ttl inst per uv	0.55**
		L5 per 10^{-2}sqm	0.14*	*ttl inst per sv*	0.16**
				ttl inst per uv	0.32**
		Nym per 10^{-2}sqm	0.14*	*ttl inst per uv*	0.36**
		L1-L4 per cm	0.09*	*ttl inst per uv*	0.27**
		L5 per cm	0.11*	*ttl inst per uv*	0.28**
		Nym per cm	0.09*	*ttl inst per uv*	0.31**
	Jun08-Aug09	*L1-L4 per DW*	0.33*	*ttl inst per lv*	0.19**
				ttl inst per uv	0.47**
		L5 per DW	0.1*	*ttl inst per uv*	0.3**
		Nym per DW	0.13*	*ttl inst per uv*	0.38**
NOP(branches)	Jul08-Aug09	*L1-L4 per 10^{-2}sqm*	0.44*	*ttl inst per term*	0.39**
				ttl inst per non-term	0.53**
		L5 per 10^{-2}sqm	0.19*	*ttl inst per non-term*	0.38**
		Nym per 10^{-2}sqm	0.32*	*ttl inst per term*	0.13**
				ttl inst per non-term	0.5**
		L1-L4 per cm	0.21*	*ttl inst per term*	0.37**
				ttl inst per non-term	0.16**
		L5 per cm	0.2*	*ttl inst per non-term*	0.43**
		Nym per cm	0.12*	*ttl inst per term*	0.17**
				ttl inst per non-term	0.24**
		L1-L4 per DW	0.3*	*ttl inst per term*	0.3**
				ttl inst per non-term	0.35**
		L5 per DW	0.12*	*ttl inst per term*	0.16**
				ttl inst per non-term	0.25**
		Nym per DW	0.2*	*ttl inst per term*	0.2**
				ttl inst per non-term	0.32**
NOP(form)	2009	*L1-L4 per 10^{-2}sqm*	0.59*	*ttl inst per A*	0.47**
				ttl inst per B	0.17**
				ttl inst per D	0.3**
		L5 per 10^{-2}sqm	0.55*	*ttl inst per A*	0.37**
				ttl inst per D	0.49**
		Nym per 10^{-2}sqm	0.35*	*ttl inst per A*	0.25**
				ttl inst per D	0.37**
		L1-L4 per cm	0.27*	*ttl inst per A*	0.38**
				ttl inst per C	0.19**
		L5 per cm	0.35*	*ttl inst per C*	0.35**
				ttl inst per D	0.28**
		Nym per cm	0.23*	*ttl inst per A*	0.3**
				ttl inst per D	0.28**
		L1-L4 per DW	0.56*	*ttl inst per A*	0.7**
		L5 per DW	0.19*	*ttl inst per A*	0.38**
		Nym per DW	0.19*	*ttl inst per A*	0.35**

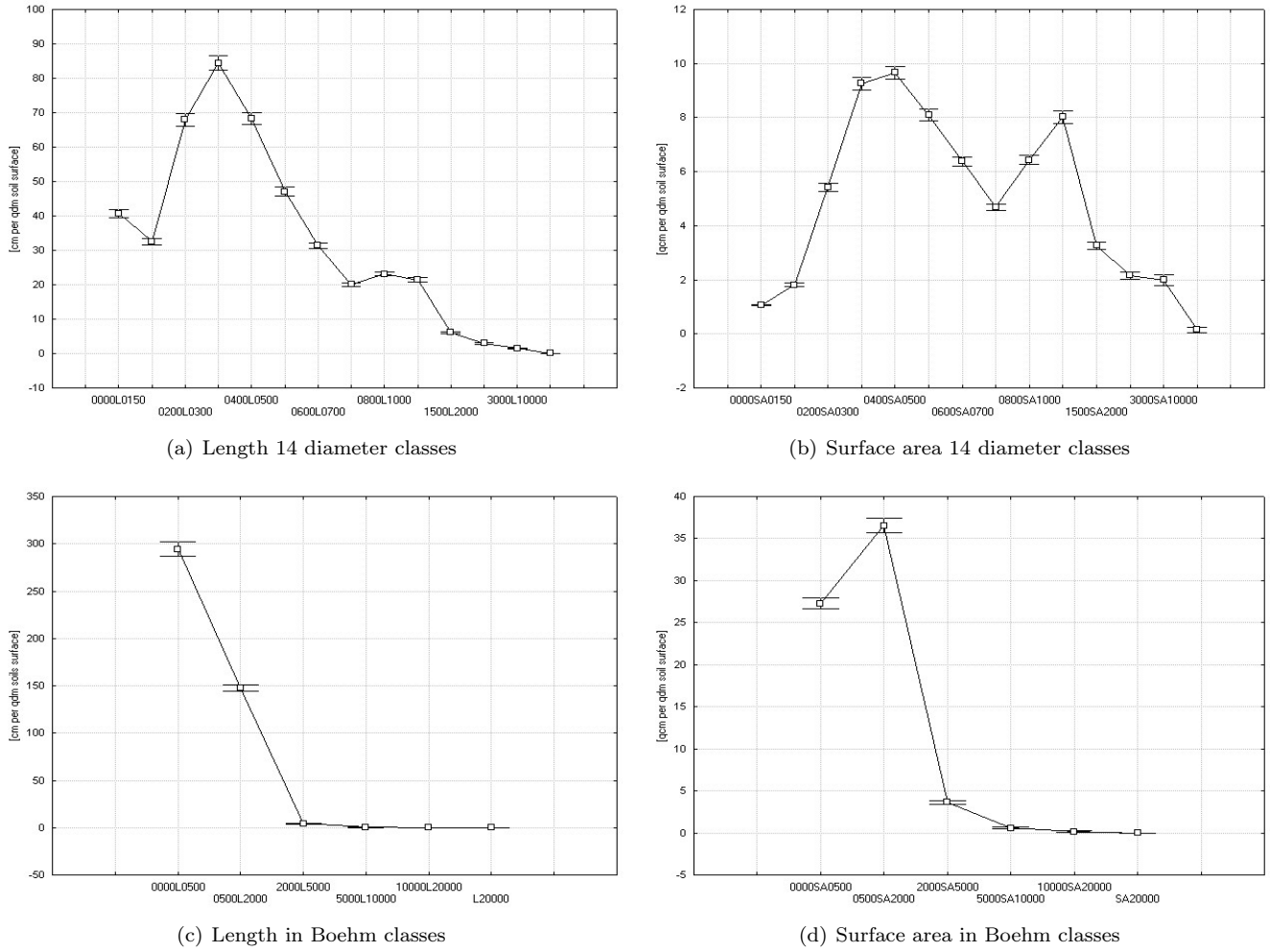
Table 36: Multiple linear regression. Dependent variables: APD and RPD instar sub classes (L1-4, L5 or Nym) (per cm, DW or 10^{-2}sqm). Predictor: Nodosity occupation parameters (NOP) (L1-4, L5 or Nym). Dataset = 2009. R^2 (adjusted), β coefficients. * = significant R^2 value. **= significant β coefficients. In regressions only significant β coefficients were shown, but all relevant parameters were included.

Pred. density	Dep. var.	R^2	Pred. var.	β coeff.
NOP (L1-L4)	*L1-L4 per* 10^{-2}*sqm*	0.58*	*L1-L4 per sv*	0.2**
			L1-L4 per lv	-0.29**
			L1-L4 per D	0.18**
			L1-L4 per term	0.28**
			L1-L4 per non-term	0.41**
	L1-L4 per cm	0.27*	*L1-L4 per A*	0.34**
			L1-L4 per C	0.23**
			L1 per term	0.13**
	L1-L4 per DW	0.53*	*L1-L4 per A*	0.76**
			L1-L4 per C	0.2**
NOP (L5)	*L5 per* 10^{-2}*sqm*	0.43*	*L5 per lv*	0.2**
			L5 per uv	0.2**
			L5 per C	0.32**
			L5 per non-term	0.22**
	L5 per cm	0.61*	*L5 per lv*	0.11**
			L5 per A	0.15**
			L5 per C	0.15**
			L5 per term	0.33**
			L5 per non-term	0.32**
	L5 per DW	0.19*	*L5 per lv*	0.24**
			L5 per uv	0.19**
			L5 per C	0.21**
NOP (Nym)	*Nym per* 10^{-2}*sqm*	0.8*	*Nym per lv*	0.37**
			Nym per uv	0.24**
			Nym per B	0.37**
			Nym per D	0.2**
			Nym per non-term	0.23**
	Nym per cm	0.76*	*Nym per lv*	-0.21**
			Nym per A	0.15**
			Nym per C	-0.31**
			Nym per D	0.09
			Nym per term	0.78**
			Nym per non-term	0.5**
	Nym per DW	0.34*	*Nym per lv*	0.65**
			Nym per uv	0.54**
			Nym per term	-0.29**
			Nym per non-term	-0.27**

Summary: Absolute densities of grape phylloxera instar sub classes could be predicted by relative densities of instar sub classes and vice versa. High relations to relative densities of A-formed and non brownish nodosities were calculated. This could also be recorded regarding absolute values (APD regressions). In 2009, particularly *uvAnon-term* per 10^{-2}sqm increased with higher relative densities of all instar subclasses and higher absolute densities of L5 instars.
Regarding the occupation of different colored nodosities, high dependencies on RPD and APD (per DW) instar sub classes could be calculated. APD (per DW) instar sub classes were highly related to RPD instar subclasses. Regarding the occupation of different branched nodosities, a relation between the total occupation of non-terminal nodosities and the APD/RPD of L5 instars could be calculated. The occupation of terminal nodosities was positively related to APD (per DW) of L1-L4 instars.

4.3.2 Root System Parameters

4.3.2.1 Diameter Classes In figure 40, the general function of root length and root surface area in 14 diameter classes and in classes according to Boehm (1979) was shown. Highest amounts of root length were found in the classes 0.15-0.6mm, whereas root surface area held two peaks between 0.3-0.7mm and between 0.8-1.5mm of root diameter (figure 40A+B). Surface area in the classification according to Boehm (1979) showed one peak (class 0.5-2mm), length decreased from 0-2mm (figure 40C+D).



(a) Length 14 diameter classes

(b) Surface area 14 diameter classes

(c) Length in Boehm classes

(d) Surface area in Boehm classes

Figure 40: Root length and root surface area as function of root diameter. Plotted are mean values per diameter class and 0.95 * standard error. **A:** Root length in 14 diameter classes. **B:** Root surface area in 14 diameter classes. **C:** Root length in classes according to Boehm (1979). **D:** Root surface area in classes according to Boehm (1979)

To assess the relationships between different diameter classes and total root system quantitatively, multiple linear regression regressions were calculated. The root system sub parameters were predicted by one main root system parameter, *total root length* or *total root surface area* respectively (table 37).

Table 37: Multiple linear regression. Dependent variables: *Total root length* or *total surface area*. Predictors: Diameter sub parameters (14 individual classes or according to Boehm (1979)). R^2 (adjusted), β coefficients. * = significant R^2 value. **= significant β coefficients.

Dep. var.	Dataset	Pred. trait	R^2	Pred. var.	β coeff.
Total root length	2006-2009	L ind. diam classes	0.99*	0.0-0.15mm	0.11**
				0.15-0.2mm	0.09**
				0.2-0.3mm	0.17**
				0.3-0.4mm	0.2**
				0.4-0.5mm	0.16**
				0.5-0.6mm	0.11**
				0.6-0.7mm	0.07**
				0.7-0.8mm	0.05**
				0.8-1mm	0.06**
				1-1.5mm	0.06**
				1.5-2mm	0.02**
				2-3mm	0.02**
				3-10mm	0.01**
				$\geq$ 10mm	0.003
		L in Boehm classes	0.86*	0-0.5mm	0.7**
				0.5-2mm	0.32**
				2-5mm	0.005
				5-10mm	0
				10-20mm	0
				$\geq$ 20mm	-0.02
Total root surface area	2006-2009	SA ind. diam classes	0.99*	0.0-0.15mm	-0.01**
				0.15-0.2mm	0.1**
				0.2-0.3mm	0.09**
				0.3-0.4mm	0.12**
				0.4-0.5mm	0.21**
				0.5-0.6mm	0.09**
				0.6-0.7mm	0.09**
				0.7-0.8mm	0.1**
				0.8-1mm	0.1**
				1-1.5mm	0.14**
				1.5-2mm	0.07**
				2-3mm	0.08**
				3-10mm	0.12**
				$\geq$ 10mm	0.06**
		SA in Boehm classes	0.86*	0-0.5mm	0.52**
				0.5-2mm	0.41**
				2-5mm	0.1**
				5-10mm	0.07**
				10-20mm	0.06**
				$\geq$ 20mm	-0.03

regressions regarding the 14 individual diameter classes explained a very high part of variation of total root system main parameters (R^2 values were very high). Altogether, β values were low to moderate and significant in almost all diameter classes. Root **length** in the first six individual diameter classes 0 - 0.06 mm showed highest β values. Roots in low diameter classes had the highest influence on total root length. In contrast, root **surface area** in classes 0.2-1.5 mm (with a peak at 0.4-0.5mm) and in the class 3-10mm showed highest β values (table 37). Finest roots in 0-2mm did not have a high impact on total surface area. Altogether, surface area is influenced mainly by roots in diameter classes 0.2-1.5mm and coarse roots with diameter 3-10mm.
Diameter classification according to Boehm (1979) subsumed the 14 diameter classes into 6 classes. Regressions considering this classification had lower R^2 values. Particularly the two lowest diameter classes (0-0.5mm and 0.5-2mm) showed high β coefficients, considering both length and surface area. Altogether, diameter classifications according to Boehm (1979) had a low resolution regarding the root system of *Vitis* ssp..

Summary: The *total root length* was highly dependent on the roots between 0 and 0.6mm diameter. In contrast, the *total root surface area* was mainly dependent on roots between 0.2 and 1.5 mm diameter and on coarse roots between 3 and 10 mm diameter. This high resolution results could not be reflected by the classification according to Boehm (1979).

4.3.2.2 Fractal Dimension To assess relationships between the architectural parameter *fractal dimension* and the morphological root system parameters, multiple regression regressions were calculated. No relations could be calculated regarding *total root length* or *total root surface area* (not specified here). But *fractal dimension* were highly dependent on the structural morphological parameters *average root diameter*, *crossings per cm* and *SRL* (table 38). An increasing diameter had a strong positive effect on *fractal dimension*. Also the density of crossings was in a positive relation with *fractal dimension*, whereas *SRL* showed a light negative influence.

Table 38: Multiple linear regression. Dependent variable: *Fractal dimension*. Predictors: *Average root diameter*, *SRL* and *Crossings per cm*. R^2 (adjusted), β coefficients. * = significant R^2 value. **= significant β coefficients.

Dep. var.	Dataset	R^2	Pred. var.	β coeff.
Fractal dimension	Jun08-Aug09	0.62*	*Average root diameter*	0.85**
			Crossings per cm	0.45**
			SRL	-0.12**

Summary: The architectural parameter *fractal dimension* showed a high dependency on root diameter and the density of root crossings per cm root length. No dependency was observed, regarding other morphological root parameters (like length or surface area).

4.3.3 Influence of Temperature and Moisture

Soil temperature and soil water content could play a major role in the development of root system related traits. In the present work, most parameters showed significant negative or positive progresses in the course of a year (section 4.2). In this chapter, the direct impact of soil temperature and soil moisture under field conditions was calculated in linear regression. The specifications of attributes were predicted by soil temperature or soil water content (*SWC*) respectively.

4.3.3.1 Grape Phylloxera Population Regarding the plots of soil temperature or soil moisture and single grape phylloxera population attributes, no differences between the variants could be detected (section appendix C).
To estimate the direct impact of temperature and moisture on general grape phylloxera traits under field conditions, multiple linear regression (MLR) were calculated. Predictor variables were APD parameters, RPD parameters, NOP and CNA (different datasets). Every regression included all parameters describing one attribute (e.g. predicted attribute: color per cm (APD): predicted variables were: *sv per cm, lv per cm, uv per cm*). In all regressions, the dependent variable was *soil temperature* (-0.1m) (table 39) or *SWC* (table 40) as moisture parameter respectively. Before multiple linear regressions were calculated, data was standardized by a z-transformation.

Soil temperature had an impact on absolute densities of grape phylloxera instars (per cm), particularly on L5 instar density. *L5 per cm* increased at higher soil temperatures significantly. Regarding the relative densities of instars, R^2 value is very weak, but a positive influence of soil temperature on *L5 per* 10^{-2}*sqm* was also calculated. In this regression, soil temperature influenced *L1-L4 per* 10^{-2}*sqm* negatively. The densities of nymphal instars (per cm, per DW and per 10^{-2}sqm) were not influenced by soil temperature. Also *L1-L4 per cm* was not impacted by soil temperature. The regressions (instarsAPD/instarsRPD) based on a database (2006-2009) of a total of 39 months.
Moreover, absolute and relative densities of nodosity color attributes were impacted by soil temperature. Densities (per cm, per DW and per 10^{-2}sqm) of non brownish nodosities increased significantly under temperature influence in field, whereas *lv per DW* and *lv per* 10^{-2}*sqm* decreased. sv forms of nodosities were not influenced by soil temperature. These regressions based on a 32 month dataset (2007-2009).
Soil temperature also showed an influence on the form of nodosities. Absolute densities (APD) of D-formed nodosities decreased, whereas *C per cm* and *A per cm* increased at higher temperatures. Regarding the relative densities (RPD), soil temperature had a strong influence on *A per* 10^{-2}*sqm* , whereas *B per* 10^{-2}*sqm* decreased. Both regressions were based on a dataset of 8 months (2009) (table 39).
Stronger influence of soil temperature could be detected in regressions regarding the nodosity occupation parameters (NOP). Although soil temperature showed a negative effect on the absolute density

Table 39: Multiple linear regression. Dependent variable: soil temperature (-0.1m). Causal variables (predictor): grape phylloxera traits. Shown are only regressions with significant R^2 values (adjusted) ≥ 0.11. β coefficients. The influence of soil temperature on CNA is illustrated by trend. * = significant R^2 value, $p < 0.05$. ** = significant β value (t test), $p < 0.05$. - = negative influence by trend. + = positive influence by trend.

Attribute	Dataset	Predicted trait	R^2	Predicted var.	β coeff.
Instars APD	2006-2009	Instars per cm	0.14*	*L1-L4 per cm*	0
				L5 per cm	0.36**
				Nym per cm	0.06
Nodosites APD	2007-2009	Color per cm	0.11*	*uv per cm*	0.34**
				lv per cm	0
				sv per cm	0
	Jun08-Aug09	Color per DW	0.13*	*uv per DW*	0.35**
				lv per DW	-0.15**
				sv per DW	-0.1
	2009	Form per cm	0.12*	*A per cm*	0.19**
				B per cm	0.05
				C per cm	0.17**
				D per cm	-0.27**
Instars RPD	2006-2009	Instars per 10^{-2}sqm	0.09*	*L1-L4 per* 10^{-2}*sqm*	-0.15**
				L5 per 10^{-2}*sqm*	0.29**
				Nym per 10^{-2}*sqm*	0.11
Nodosities RPD	2007-2009	Color per 10^{-2}sqm	0.11*	*uv per* 10^{-2}*sqm*	0.34**
				lv per 10^{-2}*sqm*	-0.1**
				sv per 10^{-2}*sqm*	0.03
	2009	Form per 10^{-2}sqm	0.19*	*A per* 10^{-2}*sqm*	0.43**
				B per 10^{-2}*sqm*	-0.25**
				C per 10^{-2}*sqm*	-0.02
				D per 10^{-2}*sqm*	0.16
NOP	2009	Ttl. inst. per form	0.12*	*ttl inst per A*	0.06
				ttl inst per B	-0.11
				ttl inst per C	0.14
				ttl inst per D	0.27**
	2007-2009	L5 per color	0.15*	*L5 per lv*	0.14**
				L5 per uv	0.33**
	2009	L5/Nym per A	0.19*	*L5 per A*	0.38**
				Nym per A	0.14**
	Jun08-Aug09	L5 per branches	0.19*	*L5 per term*	0.3**
				L5 per non-term	0.25**
CNA	2009	sv nodosities	0.18*	*svAterm, svAnon-term*	+
				svBterm, svBnon-term	-
		uv nodos.	0.27*	all	+
		A nodos.	0.15*	*svA, uvAterm*	+
		B nodos.	0.17*	*svBterm, svBnon-term*	-
				uvBterm	+
		D nodos.	0.2*	sv, lv forms	-
				uv forms	+
		Term. nodos.	0.29*	*svBterm*	-
				all other forms	+
		non-term. nodos.	0.2*	B forms	-
				A and D forms	+

(per cm) of D-formed nodosities, a positive effect could be recorded in the regression regarding the occupation of total instars per D-formed nodosity. Also the number of L5 instars per uv colored or A formed nodosities had a strong positive relationship to soil temperature. Also, soil temperature showed effects on occupation of terminal and non-terminal nodosities with L5 instars (table 39).
Highest adjusted R^2 values occurred in the regressions regarding combined nodosity parameters (CNP). In all regressions, soil temperature showed a positive effect on the development of A-formed nodosities and uv colored nodosities, a negative effect on dark brownish (sv) colored nodosities (with exception of A-forms) and all B forms of non-terminal nodosities. Those regressions based on an eigth month database (2009) (table 39).

Soil temperature had a high negative impact on soil water content (*SWC*) (table 42). This impact is reflected by the regressions in table 40. Parameters which were positively influenced by soil temperature were impacted by soil water content negatively in most of the regressions. Therefore effects could not be divided. But in some regressions differences between soil temperature and soil moisture influence could be detected.
Regarding the densities (APD, RPD) of instars, *Nym per cm* and *Nym per* 10^{-2}*sqm* were not impacted by soil temperature positively, but by *SWC* negatively. Temperature regressions based on dataset 2006-2009 (n=449). Calculating the temperature regressions with the dataset Mar08-Aug09 (n=356), the occurred values were analog to temperature regressions on base of dataset 2006-2009 (not specified here). So *SWC* had a weak negative impact on development of nymphal instars under field conditions (table 40).
Regarding nodosity attributes, the appearance of *A per DW* and *B per DW* were influenced by soil moisture, but not by soil temperature. A-forms decreased, whereas B-forms increased. This regression based on dataset 2009 (table 40).
Also nodosity occupation (NOP) was influenced by soil moisture, but not by soil temperature. Occupation of terminal and non-terminal nodisities was influenced negatively by *SWC*. Also the occupation of A-formed nodosities was not affected by soil temperature, but by soil moisture (table 40). Regarding the densitiy of combined nodosity attributes (CNA), most regressions correspond to the soil temperature regressions. Weak differences occurred in lv nodosities and C nodosities (table 40). These regressions based on the dataset 2009.

Summary: Almost all grape phylloxera traits were influenced by soil temperature. But soil temperature explained only a small part of the recorded grape phylloxera variance in field. β coefficients (between 0.35-0.43) and R^2 values (between 0.09-0.19) were moderate. Mainly densities (APD and RPD) of L5 instars, non brownish nodosities and A-formed nodosities were positively affected by soil temperature. Regarding the nodosity occupation (NOP), mainly the occupation by L5 instars is positively influenced by soil temperature. Also soil moisture had moderate, almost negative impacts on

Table 40: Multiple linear regression. Dependent variable: soil water content (*SWC*). Causal variables (predictor): grape phylloxera traits. Shown are only regressions with significant R^2 values (adjusted) ≥ 0.11. β coefficients. The influence of *SWC* on CNA is illustrated by trend. * = significant R^2 value, $p < 0.05$. ** = significant β value (t test), $p < 0.05$. - = negative influence by trend. + = positive influence by trend.

Attribute	Dataset	Predicted trait	R^2	Predicted var.	β coeff.
Instars APD	Mar08-Aug09	Instars per cm	0.13*	*L1-L4 per cm*	-0.1
				L5 per cm	-0.26**
				Nym per cm	-0.12**
Nodosities APD	2009	Form per DW	0.11*	*A per DW*	-0.33**
				B per DW	0.28**
				C per DW	0.12
				D per DW	0.02
Instars RPD	Mar08-Aug09	Instars per 10^{-2}sqm	0.08*	*L1-L4 per 10^{-2}sqm*	0.01
				L5 per 10^{-2}sqm	-0.16**
				Nym per 10^{-2}sqm	-0.19**
Nodosities RPD	2009	Form per 10^{-2}sqm	0.21*	*A per 10^{-2}sqm*	-0.47**
				B per 10^{-2}sqm	0.27**
				C per 10^{-2}sqm	0.07
				D per 10^{-2}sqm	-0.08
NOP	Jun08-Aug09	Ttl. inst. per bran.	0.12*	*ttl inst per term*	-0.19**
				ttl inst per non-term	-0.22**
	Jun08-Aug09	L5 per branches	0.15*	*L5 per term*	-0.22**
				L5 per non-term	-0.27**
	2009	Ttl. inst. per form	0.23*	*ttl inst per A*	-0.39**
				ttl inst per B	0.08
				ttl inst per C	-0.08
				ttl inst per D	-0.14
	2009	L5/Nym per A	0.12*	*L5 per A*	-0.29**
				Nym per A	-0.14**
CNA	2009	sv nodos.	0.14*	B forms	+
		lv nodos.	0.13*	*lvAterm, lvBnon-term*	-
				lvC, lvDnon-term	+
		uv nodos.	0.2*	*uvBnon-term*	+
		B nodos.	0.19*	sv forms	+
				uv forms	-
		C nodos.	0.12*	*lvCnon-term*	+
				all other forms	-
		D nodos.	0.2*	sv forms	+
				uv forms	-
		Term. nodos.	0.23*	*svBterm*	+
				uvC, uvDterm	-
		non-term. nodos.	0.25*	*svB, lvDnon-term*	+
				lvA, uvC, uvDnon-term	-

grape phylloxera traits. Particularly densities of nymphal instars are negative influenced by soil moisture. Altogether, the negative impact of soil temperature on soil water content (*SWC*) was mainly reflected in soil moisture regressions.

4.3.3.2 Morphological Root System Parameters Regarding the plots of soil temperature or soil moisture and single root system traits, no differences between the variants could be detected (section appendix C).
To estimate the direct impact of temperature and moisture on root system traits, multiple and normal linear regression (MLR and NLR) regressions were calculated. Predictor variables were root system main parameters and sub parameters related to length and surface area (different datasets). In all regressions, the dependent variable was *soil temperature* (-0.1m) or *SWC* as the moisture parameter respectively (table 41). Before multiple linear regressions were calculated, data was standardized by a z-transformation.

Table 41: Multiple linear regression / normal linear regression. Dependent variables: soil temperature (-0.1m) or soil moisture (SWC) Causal variables (predictor): root system parameters. Shown are only regressions with significant R^2 values (adjusted) ≥ 0.11. β coefficients. In regressions with diameter classes, only significant β coefficients are shown. L = Length. SA = Surface area. * = significant R^2 value, $p < 0.05$. ** = significant β value (t test), $p < 0.05$.

Dependent var.	Dataset	R^2	Predicted var.	β coeff.
Soil temperature	2006-2009	0.12*	*Total root length*	-0.42
			Total root surface area	0.63**
		0.17*	*Average root diameter*	-0.15**
			Fractal Dimension	0.62**
		0.14*	Boehm: L 0.5-2mm	0.46**
		0.14*	Boehm: SA 0.5-2mm	0.35**
		0.17*	Diam: L 0-0.15mm	-0.54**
		0.16*	Diam: SA 0-0.15mm	-0.54**
Soil moisture (SWC)	2006-2009	0.16*	Diam: L 0.3-0.4mm	0.48**
			Diam: L 0.4-0.5mm	0.91**
		0.16*	Diam: SA 0.3-0.4mm	0.46**
			Diam: SA 0.4-0.5mm	0.95**

Soil temperature had a positive impact on *total root surface area* and *fractal dimension*. *Total root length* was influenced negatively, but not significantly. Also *average diameter* decreased lightly with increasing soil temperature. The length and surface area of coarse roots between 0.5 and 2 mm were positively influenced, whereas length and surface area of finest roots (between 0-0.15mm) were under a strongly negative influence (table 41). Soil moisture showed strong positive effects on length and surface area of roots between 0.3-0.5mm. No influence was detected regarding *SRL* and *DW* (table 41).

Summary: Higher temperatures showed negative effects on the growth of roots with low diameter (0-0.15mm), but positive effects on growth of more coarse roots (0.5-2mm). In contrast, high soil water contents showed positive effects on the growth of roots between 0.3-0.5mm diameter. No influences

could be detected in *SRL* or *DW*.

4.3.3.3 Soil Properties Regarding the plots of soil temperature or soil moisture and soil properties, no differences between the variants could be detected (section appendix C).
To estimate a general impact of temperature and moisture on soil properties, normal linear regression (NLR) were calculated (table 42). Predictor variables were single soil parameters, dependent variable was *soil temperature* (-0.1m) or *SWC* as moisture parameter respectively. Before NLR were calculated, data was standardized by a z-transformation.

Table 42: Normal linear regression. Dependent variables: soil temperature (-0.1m) or soil moisture (SWC). Causal variables (predictor): soil properties. Shown are only regressions with significant R^2 values (adjusted) ≥ 0.11. β coefficients. * = significant R^2 value, $p < 0.05$. ** = significant β value (t test), $p < 0.05$.

Dependent var.	Dataset	R^2	Predicted var.	β coeff.
Soil temperature	Mar08-Aug09	0.39*	SWC	-0.62**
Soil moisture (SWC)	Mar08-Aug09	0.11*	BSR	-0.34**

Increasing soil temperature had a strong negative influence on soil water content (*SWC*), but no significant effects on *BSR, SOM* or *pH*. Though, an increasing soil water content had an moderate negative effect on *BSR*. *SOM* and *pH* were not affected by soil temperature or soil moisture (table 42).

Summary: A negative influence of increasing temperature on *SWC* as well as a negative relation between *SWC* and *BSR* could be detected.

4.3.4 Grape Phylloxera Population and Root Development

In face of a future possibility to predict the impact of small scale root parasites on root systems, structural equation models (SEM) could be very useful. To estimate the impact of general grape phylloxera population structure on grape root system, SEM were calculated. In the present section, two different pre-models were calculated and were fitted by a specification search. The models based on the working hypothesis, that total densities of grape phylloxera instars and nodosities had a relationship to morphological parameters of grape root system. In these models, dataset Aug07-Aug09 was used, and densities of APD and RPD main phylloxera parameters were considered. As an example for possible influenced root system parameters, *total surface area*, *fractal dimension* and *average root diameter* were used. APD main parameters (*ttl inst per cm*, *ttl nod per cm*) or RPD main parameters (*ttl inst per* 10^{-2}*sqm* , *ttl nod per* 10^{-2}*sqm*) were exogenous variables (table 43). All models were calculated by iteration with asymptotically distribution-free discrepancy and based on the dataset Aug07-Aug09. Variables (endogenous and exogenous) and latent values were listed, β coefficients and χ^2/DOF values were calculated.

Table 43: Structural equation models (SEM): latent variables and indicators

regression	Latent variables	Indicators
APD-Surface	**exogenous:**	
	Grape phyll nodosities	*ttl nod per cm*
	Grape phyll instars	*ttl inst per cm*
	Soil temperature	*soil temperature*
	endogenous:	
	Root surface	*total root surface area*
	Root structure	*fractal dimension*
	Root diameter	*average root diameter*
RPD-Surface	**exogenous:**	
	Grape phyll nodosities	*ttl nod per* 10^{-2}*sqm*
	Grape phyll instars	*ttl inst per* 10^{-2}*sqm*
	Soil temperature	*soil temperature*
	endogenous:	
	Root surface	*total root surface area*
	Root structure	*fractal dimension*
	Root diameter	*average root diameter*

In the model **APD-Surface**, indicators of exogenous variables were absolute density main parameters (per cm) and soil temperature (*ttl nod per cm*, *ttl inst per cm*, *soil temperature*). Indicators of endogenous variables were *total root surface area*, *fractal dimension* and *average root diameter*. In **RPD-Surface** indicators of exogenous variables were relative density main parameters (per 10^{-2}sqm) and soil temperature (*ttl nod per* 10^{-2}*sqm* , *ttl inst per* 10^{-2}*sqm* , *soil temperature*). Indicators of endogenous variables were *total root surface area*, *fractal dimension* and *average root diameter* (table 43). In figure 41 the structure of the first pre-SEM (standardized values) were shown.

Absolute densities of nodosities did not have an impact on root system parameters in model APD-Surface pre. The absolute density of instars (per cm) was positively related to root surface area. Highest impact on root system was shown by soil temperature (table 44). Regarding RPD parameters, the amount of nodosities per 10^{-2}sqm soil was related positively to root surface area and negatively to root diameter. In contrast, the relative amount of instars (per 10^{-2}sqm) was related negatively to surface area and positively to root diameter. In the both first models (appendix "pre"), χ^2/DOF values were very high (table 44). To fit the models, a specification search was done, considering the χ^2/DOF value that fitted best.

Table 44: Structural equation models: β coefficients and χ^2/DOF values. ** = significant β coefficient (P=0.05)

Model	χ^2/DOF	Predictor	Dep. var.	β coefficient
APD-Surface pre	12.71	Grape phyll nodosities	→ Root surface area	0.044
			→ Root structure	-0.025
			→ Root diameter	-0.011
		Grape phyll instars	→ Root surface area	0.21**
			→ Root structure	0.15
			→ Root diameter	0.102
		Soil temperature	→ Root surface area	0.345**
			→ Root structure	0.358**
			→ Root diameter	-0.129
RPD-Surface pre	14.3	Grape phyll nodosities	→ Root surface area	0.577**
			→ Root structure	-0.015
			→ Root diameter	-0.649**
		Grape phyll instars	→ Root surface area	-0.127**
			→ Root structure	0.135
			→ Root diameter	0.69**
		Soil temperature	→ Root surface area	0.276**
			→ Root structure	0.331**
			→ Root diameter	-0.147
APD-Surface final	5.27	Grape phyll nodosities	→ Root surface area	-
			→ Root structure	-
			→ Root diameter	-
		Grape phyll instars	→ Root surface area	0.213**
			→ Root structure	0.116
			→ Root diameter	-
		Soil temperature	→ Root surface area	0.336**
			→ Root structure	0.382**
			→ Root diameter	-
RPD-Surface final	5.706	Grape phyll nodosities	→ Root surface area	-
			→ Root structure	-
			→ Root diameter	-
		Grape phyll instars	→ Root surface area	0.89**
			→ Root structure	0.33**
			→ Root diameter	-
		Soil temperature	→ Root surface area	-
			→ Root structure	0.283**
			→ Root diameter	-

Models, after specification search (appendix "final"), were shown in figure 42 (standardized values). In both models, nodosities and diameter were not directly related to a endogenous or exogenous variable. In RPD-Surface model, the relative amount of phylloxera instars is strong positively related to root surface area, whereas soil temperature had an impact on root structure, but not on surface area (table 44). Regarding absolute densities (APD-Surface), absolute amount of grape phylloxera instars was positively related to surface area, soil temperature also had a significant positive impact on root surface (table 44).

χ^2/DOF conduced to a validation of a single model. If χ^2/DOF is $\geq$ 2.5, the model should be refused and modified. The χ^2/DOF values of both, "pre" and "final" models were much higher than 2.5 (table 44). Only some general variables were introduced into the models. It could be assumed, that grape phylloxera main parameters could not explain variability of root system. Additionally, latent variables (endogenous and exogenous) were explained by only one directly monitored parameter (indicator) (table 43). This led to a unlucky ratio of observed and unobserved variables and to a very low degree of freedom (DOF) in the models. An overview of numbers of observed and unobserved variables, DOF and the fitness of the single models was shown in table 45. DOF is calculated by subtracting the number of distinct estimated parameters from the number of distinct sample moments in the model.

Table 45: Structural equation models: number of observed and unobserved variables, degree of freedom (DOF), χ^2 and fitness (χ^2/DOF) of single models

Model	Obs.	Unobs.	DOF	χ^2	χ^2/DOF
APD-Surface pre	6	15	4	50.526	12.71
RPD-Surface pre	6	15	3	42.892	14.3
APD-Surface final	6	15	11	58.044	5.27
RPD-Surface final	6	15	9	51.358	5.706

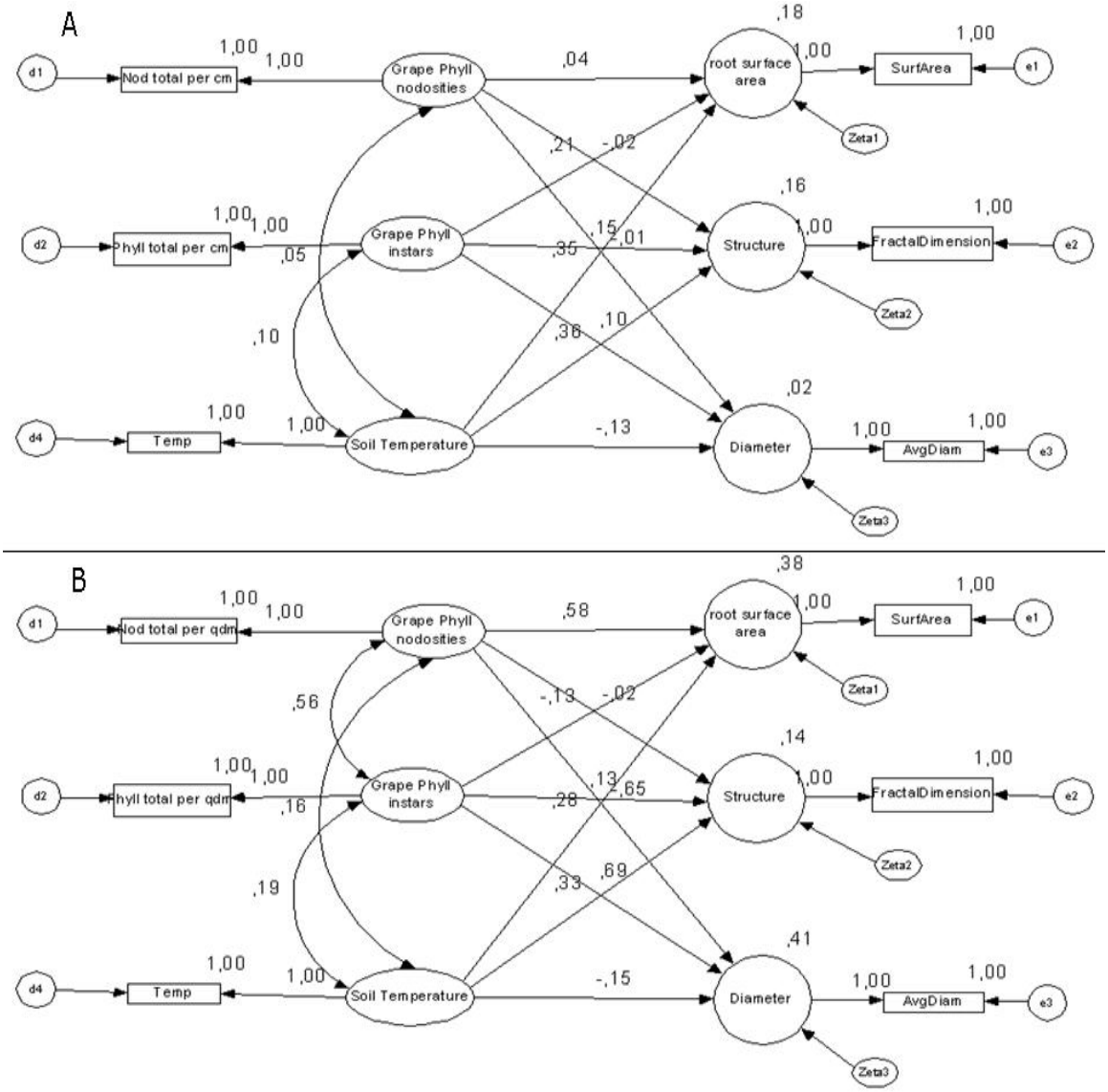


Figure 41: Structural equation pre-models (standardized values). Shown were β coefficients (arrows), covarainces (double arrows) and variances of endogenous variables. **A:** APD-Surface pre. $\chi^2/\text{DOF} = 12.71$. **B:** RPD-Surface pre. $\chi^2/\text{DOF} = 14.3$

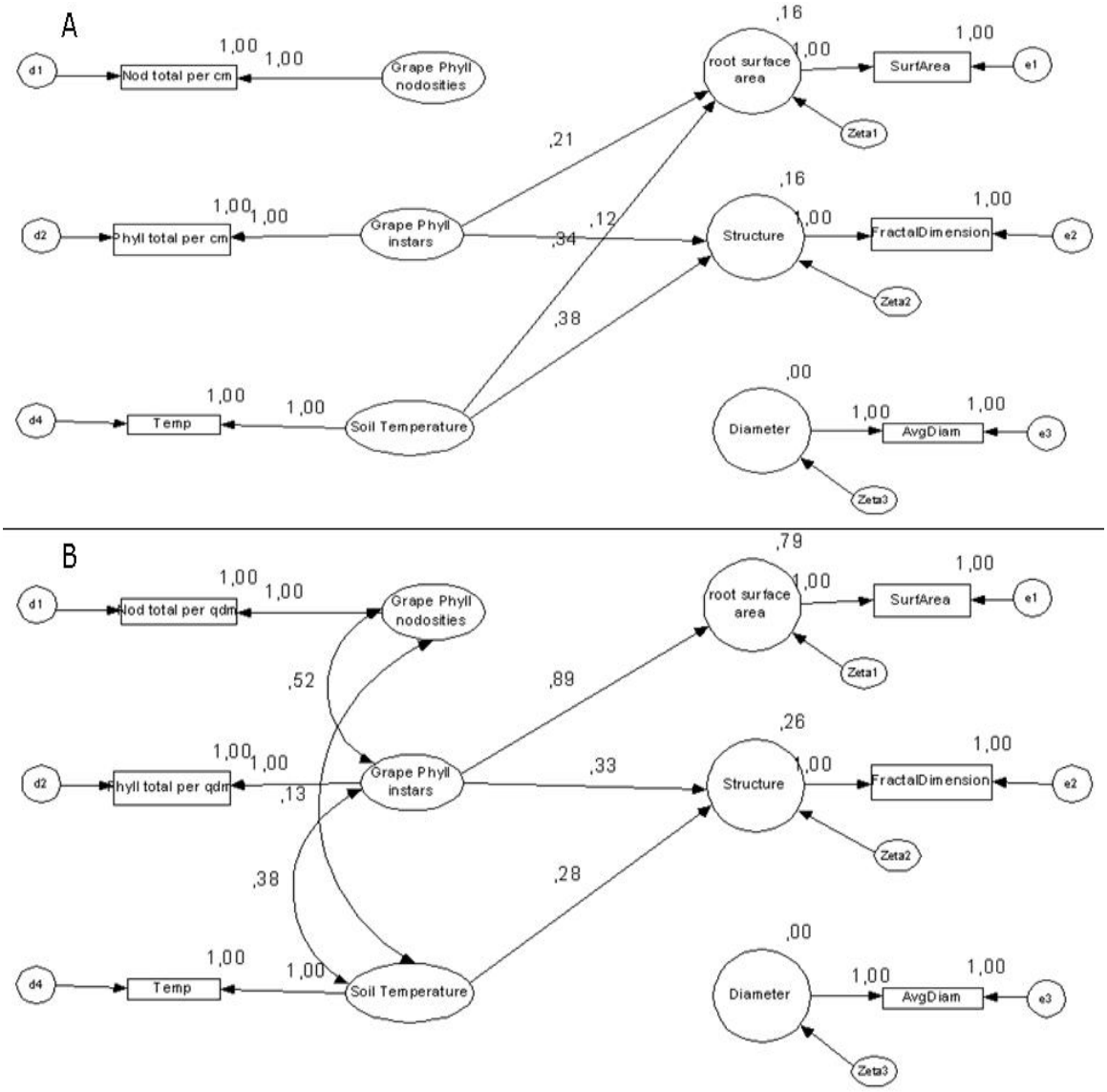


Figure 42: Structural equation "final" models (standardized values). Shown were β coefficients (arrows), covaraiances (double arrows) and variances of endogenous variables. **A:** APD-Surface final. $\chi^2/\text{DOF} = 5.27$. **B:** RPD-Surface pre. $\chi^2/\text{DOF} = 5.706$

Summary: In face of a future possibility to predict the impact of small scale root parasites on root system, structural equation models (SEM) could be very useful. In case of the present work, the variability of grape root system parameters could not be predicted by grape phylloxera main parameters satisfactorily. It could be assumed, that other parameters had more influence on the grape root system. But to estimate an impact of grape phylloxera population amount on grape root system, models should be modified and other parameters should be included.

5 Discussion

Insects and microbial pests cause great losses in agriculture worldwide. Many factors including densities of parasites and pathogens as well as timings of control activities can affect the success of pest management programs (Tang et al., 2010). General theories and classifications of population outbreaks, useful as base of insect outbreak models only recently began to develop (Berryman, 1987). Methods to assess the dynamics and strength of parasite populations are essential for both connecting population structure to recorded damages and avoiding outbreaks by prediction models, embedded in pest management strategies (Porten and Huber, 2003). Especially field investigation methods to record the influence of parasitizing insects in natural and agricultural systems began to develop only recently. Due to the fact that exploration of soil is connected to many difficulties, questions regarding below ground processes are poorly understood in terrestrial ecology.

Pedosphere can not be compared with aboveground habitats in many respects. Habitats in soil are generally characterized by high material density and by boundary layers between abiotic spheres (lithosphere (soil minerals), hydrosphere (soil water), and atmosphere (soil air)) (Gisi, 1997). Due to the attraction plant roots carrying out as nutrient source on most soil organisms at different trophic stages, the density of soil organisms in rhizosphere generally is higher than in blank soil (van der Putten, 2003; Lambers et al., 2009). Species diversity in rhizosphere can be very low (e.g. mycorrhizas; Oehl et al. (2010)). Roots can be regarded as a fourth sphere for the most soil organisms, especially for root parasites (Price, 1980). Soils and plants are important parts in almost all cycles of material, whereas plants play a major rule in connecting atmospheric aboveground systems and pedospheric below ground systems (van der Putten et al., 2009). Many interactions between below ground processes and aboveground processes via plants could be found, with below ground processes influencing biocoenosis (e.g. Young-Mathews et al. (2010)), above and below plant architecture (e.g. Lintunen and Kalliokoski (2010)), plant physiology in general (e.g. Kayler et al. (2010)) as well as plant defense mechanisms (e.g. Poveda et al. (2006)). From the plant's point of view the recognition of root function as well as strategies for nutrient acquisition are very important. But considering plant growth strategies from an ecological point of view, particularly the variation of root traits and their effects on ecological processes are important patterns for an understanding of agricultural soil ecosystems (e.g. Comas and Eissenstat (2009)). A first step to become familiar with the embedding of below ground parasites in such structures is the development of equal assessment methods. Ecological processes must be included into the explanation of root system and parasite dynamics, e.g. the role of plant occupying fungi (Hartley and Gange, 2009) and soil microflora (Alabouvette et al., 1985). Due to its multitrophic properties, also possible interactions between root-parasite system and the aboveground ecosystems must be considered (Comas et al., 2010).

Numerous publications exist, regarding the biology (Blankenhorn and Moritz, 1875; David, 1875; Cornu, 1878; Ritter and Ruebsaamen, 1900; Parniewski, 1963; Granett et al., 2001b, 2005; Michaelis, 2007),

damaging mechanisms (Granett et al., 1983; El-Nady, 2001) and population development (Downie et al., 2001; Vorwerk and Forneck, 2007) of grape phylloxera (*Daktulosphaira vitifoliae* Fitch). Although Bauerle et al. (2007) observed grape phylloxera induced rotting of root tips (*Vitis lambrusca* Concorde) and e.g. Steinberg (1968) found a direct correlation between the number of root tips and the growth of leaves (Riesling/26G), no assumptions could be made on the impact of root infestation by phylloxera regarding grape vitality and aboveground damages (Huber, 2007; Huber et al., 2009). The vigor of grapes in vineyards is influenced by numerous abiotic factors (Anderson et al., 2003), pathogens (e.g. Huber et al. (2006)), pest management (Huber, 2007), canopy management (Comas et al., 2000), grafting combinations (Wolpert et al., 1992), tillage and fertilization methods (Porten, unpb), and suppressive or conductive soil conditions (Huber et al., 2009). Even in vineyards with high grape phylloxera infestation on root system, a direct impact on vitality can not be assumed per se (Huber, 2007).

The present work must be seen against these backgrounds. The biggest part of discussion (section 5.3) focuses on the development of the assessment method. Particularly problems (section 5.3.1) and sensivity (section 5.3.2) of the assessment methods are discussed intensely. Before, in sections 5.1 and 5.2 the development of the observed grape phylloxera population and root system parameters are discussed and related to the assessment.

5.1 Structure and Dynamics of Grape Phylloxera Population

In the present work, grape phylloxera population structure was classified by a visual survey. Larval instar stages were differentiated into three classes (see also Michaelis (2007)). Two classes were assigned to the wingless female morphs (virginoparae aptera), which were commonly found on roots and reproduce parthenogenetically (Forneck and Huber, 2009). The first group covered the non oviparous larval stages of the wingless female morph, larval stage one to four (L1-L4, juvenile virginopara). The second group covered the oviparous larval stage five (L5, adult virginopara). The third group was assigned to the juvenile larval stages three and four of winged preforms (nymphal larval stages). In the third larval stage of the winged preforms, the wing pads are fully developed and presented a valid identifying feature (Forneck and Huber, 2009; Michaelis, 2007). Nodosities (grape phylloxera induced root swellings) were classified by their color, form and branching status (root development of nodosity attached roots) (section 5.3.1.1.2).

Grape phylloxera population structure was specified in abundances based on different reference values: soil volume (20 cm depth and 10^{-2}sqm soil surface; equal to the size of a small digging box) was the reference value for relative phylloxera densities (RPD) and combined nodosity attributes (CNA). Because an unbalanced horizontal and vertical distribution of grape phylloxera population can be assumed in soil, no approximations to a m^3 soil volume or a m^2 soil surface were done. Absolute phylloxera densities (APD) considered the dynamics of root system by including root length or rather root dry weight (DW) as reference value. Values were given per cm or per g. APD, RPD and CNA were calculated on the

same base: the counted numbers of instars and nodosities. In contrast, nodosity occupation parameters (NOP) gave information on mean instar occupation per nodosity. NOP based on the proportion of RPD instar to RPD nodosity values. In this section, the general development of APD, RPD (CNA) and NOP will be discussed.

5.1.1 Seasonal Development of Instars

Seasonal fluctuations in the development of grape phylloxera population on roots of *Vitis* ssp. were observed by many authors in field (Blankenhorn and Moritz, 1875; Cornu, 1878; Omer et al., 1997; Porten and Huber, 2003; Powell et al., 2003; Herbert et al., 2006; Michaelis, 2007). In cool climate viticulture, the increase of population is connected to an increase of both juvenile and adult virginopara in the mid to late vegetation period (Blankenhorn and Moritz, 1875; Cornu, 1878; Omer et al., 1997; Porten and Huber, 2003; Herbert et al., 2006; Michaelis, 2007) and to a short appearance of juvenile winged preforms (nymphae) on roots in the late vegetation period (Huber, 2007; Michaelis, 2007; Forneck and Huber, 2009). The present work generally confirmed these results, regarding the seasonally development of the mean values of the investigation period (2006-2009). But comparing different reference values in the calculation of the abundances (per 10^{-2}sqm , per cm or per DW (2008-2009)), differences in seasonal late population development were observed. This is in accordance to observations made by Balbiani (1874), who already suggested that the development of instars is related to local root and soil conditions. Low RPD and APD values of all larval stages were found in the months February to May and increasing densities from May to October were recorded. Observed differences which have occurred from the use of different reference values in the calculation of the abundances are discussed intensely in section 5.3.

Densities of juvenile virginopara are generally highly related to the total densities of grape phylloxera (see also Powell et al. (2003)). Adult virginopara and nymphal larval stages appeared from April to October with significant increases of total population density. These observations are in accordance to other works (Cornu, 1878; Omer et al., 1997; Porten and Huber, 2003; Herbert et al., 2006; Michaelis, 2007). Already Cornu (1878) described the appearance of adult virginopara in conjunction with increasing abundances on the roots of *Vitis vinifera*. The last generation of juvenile virginopara showed a high resistance against cold temperature conditions: The highest abundances of juvenile virginopara occurred between October and December with $\approx$ 0.4 individuals per cm root length (data: October 2006 - 2008, November 2006+2008, December 2006). Until March a decrease to $<$ 0.1 individuals per cm root length could be observed (data: January 2007 - 2009, February 2009, March 2007 - 2009). Regarding the relative densities of juvenile virginopara, the mentioned high densities (per cm) could partly be due to the decrease of the root system in winter. Anyway, also L1-L4 per 10^{-2}sqm were significant higher in October to December than in spring. Grape phylloxera developed two simultaneous life cycle strategies to survive. First the sexual pathway, producing winged sexuparae and fundatrices (overview in Forneck and Huber (2009)). The second strategy was described long before the present

study (Cornu, 1878; Omer et al., 1997; Granett et al., 2005; Forneck and Huber, 2009) and it seems to take place completely belowground. Considering the data of the present work, grape phylloxera must produce high amounts of asexual eggs at the end of the growing season, shortly after the development of sexual life stages (see also Troitzken (1929)). This high production could be driven by the permanent root development of *Vitis* ssp. until autumn (David, 1875; Anderson et al., 2003; Loxdale, 2010). It can be suggested that these juvenile virginopara serve at least partly for starting a new holocyclus on roots at the beginning of the growing season, particularly on sites with no developed aboveground life cycles (section 5.1.2). According to Price (1980) it can be suggested that the evolutionary background of this intense production of new parthenogenetic generations are beneficial conditions for overwintering. From this point of view, the production of winged sexuparae (which provide a sexual life cycle) shortly before winter (Balbiani, 1874; Troitzken, 1929) consumes high amounts of resources which are needful for production of overwintering juvenile virginopara (which provide a parthenogenetic life cycle). According to Loxdale (2010), sexual as well as asexual reproduction can have advantages and disadvantages for aphids, whereas asexual reproduction has the advantage of conserving favorable alleles which may be locally adapted. Regarding the increase of grape roots until autumn, the asexual reproduction of grape phylloxera also allows quick exploitation of nutrient resources (Loxdale, 2010).

Base for the production of winged sexupara, winged migrants as well as aptere virginopara are oviparous adult virginopara (L5 larval stage) (Forneck and Huber, 2009). In the present work, absolute and relative densities of adult virginopara increased significantly between June and September with a peak in July (5C) and August (125AA). Densities of juvenile winged preforms (nymphae) showed a significant shift in August. Winged preforms could not be clearly identified as winged sexupara or winged migrants (Huber, 2007; Michaelis, 2007). These results are against observations made by e.g. Powell et al. (2003), who observed bimodal population development of grape phylloxera in Australian vineyards. In the present work only moderate direct relations were calculated between instar abundances and abiotic factors like soil temperature or soil moisture (against the assumptions of Granett and Timper (1987), Turley et al. (1996) or Makee (2004); section 5.1.2). It can be suggested that in field other factors are restricting grape phylloxera fertilization (and even activity) much more than temperature does (Troitzken, 1929). Forneck and Huber (2009) suggested biotic factors like nutrients as important influence factors, regarding the works of Henke (1961) and Stoev et al. (1966). Henke (1961) suggested effects of amino acid concentration on development of nodosities and instars. Generally aphids shift from parthenogenetic to sexual reproduction in response to environmental changes, whereas close effects of plant physiology on development and demography of aphid populations could be observed (Kawada, 1987). This is in accordance to Price (1980), who suggested that a further development of close host-parasite relations is mainly driven by biotic (physiological) influence factors. Population dynamics of aphids often related closely by the N-compounds of the host tissue and aphids need high amounts of amino acids for reproduction (Kawada, 1987). Forneck and Huber (2009) suggested also a change in nutrient concentration as a possible trigger for grape phylloxera population development.

The high concentrations of proteolytic enzymes in the spittle of grape phylloxera support this suggestion (Henke, 1961). Observations made by Stoev et al. (1966) showed a high general capacity of grape roots (*Vitis vinifera*) to synthesize and allocate organic substrates. The number and content of amino acids in both, roots of non grafted *Vitis vinifera* and roots of rootstocks changed mainly with the state of vegetation (Stoev et al., 1966). The nutrient accumulation in *Vitis vinifera* roots occurred from a lack in late summer to a peak in winter mainly. The number and composition of amino acids in roots is related to flowering and is decreasing at the begin of flowering. In spring and early summer (until flowering or fruit ripeness), nutrients were displaced to aboveground organs and the content of starch in roots decreased until summer. But in contrast, free sugar concentrations in roots showed a shift in summer. Concentrations were higher in thinner roots than in thicker roots (Stoev et al., 1966). Such seasonal nutrient oscillations in grape roots (as well as root age dependent differences regarded by Volder et al. (2005) on *V. rupestris x V. riparia* (3309 C)) let suggest that changes in amino acid and sugar concentrations in rootstocks could be a trigger for an increasing production of winged sexupara in summer as well as for the high production of juvenile virginopara in late autumn (section 5.1.2).
In any case questions must be asked when the determination of individuals (as virginopara or sexupara) did occur. Downie and Granett (1998) investigated native phylloxera stems parasitizing on *Vitis arizonica* in the southwest of the USA. They assumed that stimuli for determination must be received prior to the reproduction period of the adult mother. Because of high differences in biology and population development of the observed grape phylloxera population in comparison to other investigations, Downie and Granett (1998) also assumed that the observed life cycle has been co-evolved with the host and must be highly adapted on *Vitis arizonica*. But generally, polyphenism is very common among aphids and in some species a single oviparous morph may produce several discrete phenotypes that differ in attributes like morphology or physiology (Moran, 1992). The nodosities surveyed in the present work were often occupied by juvenile winged preforms and juvenile virginopara at the same time, possibly due to a high mobility of juvenile virginopara, migrating to preformed grape phylloxera induced root swellings (Omer et al., 1995). In fact, crowding could be a trigger to produce winged individuals in aphids (Moran, 1992). But regarding the instar abundances per 10^{-2}sqm calculated in the present work, highest densities of adult virginopara were found in July, followed in August by decreasing densities of juvenile virginopara and highest densities of juvenile winged preforms. So it can be possible that the larval production is determined before maturing of the mother like suggested by Downie and Granett (1998). However, it can not be cleared finally if juvenile winged preforms and/or virginopara are moving actively to a nodosity or if they are produced by a single female on this nodosity.

5.1.2 Overwintering

As many authors observed before, juvenile virginopara hibernate without developing to adult virginopara (Cornu, 1878; Stevenson, 1964; Omer et al., 1997; Downie and Granett, 1998; Granett et al., 2005; Forneck and Huber, 2009). Also in the present work hibernating juvenile virginopara were found in

each winter (2006/07, 07/08, 08/09). Basing mainly on the focus of their studies as well as the used methods, up to now most authors found low abundances of hibernating instars (Omer et al., 1997; Porten and Huber, 2003; Granett et al., 2005; Herbert et al., 2006). Extrinsic factors such as temperature, root physiology, and the activity of soil-inhabiting pathogens of grape phylloxera are all thought to have an important impact on population dynamics of grape phylloxera (Omer et al., 1997; Herbert et al., 2006; Huber, 2007). Low temperatures were discussed to have critical impact on activity and survivorship (Granett and Timper, 1987; Turley et al., 1996). There are various descriptions of reproduction and activity of grape phylloxera related to temperature (Blankenhorn and Moritz, 1875; David, 1875; Cornu, 1878; Granett and Timper, 1987; Turley et al., 1996). Temperature was described as a deciding factor for retrieving mobility (Blankenhorn and Moritz, 1875; David, 1875; Cornu, 1878). Turley et al. (1996) published threshold levels in temperature for reproduction (18 °C) as well as for motion and suction activity (7-10 °C). According to Granett and Timper (1987), the survivorship of immature larval stages on *Vitis vinifera* roots is strongly reduced under 16 °C. Also Blankenhorn and Moritz (1875) observed very low abundances of overwintering grape phylloxera on roots. In the present work, high amounts of juvenile virginopara and fresh induced nodosities were observed in each winter, even in the frozen soil of the winter 2008/09. These results are not in accordance to the above mentioned authors (e.g. Granett and Timper (1987) or Turley et al. (1996)). Also the warm winter periods 2006/07 and 2007/08 had no influences on abundances of juvenile virginopara or coloration of nodosities, compared to the cold winter 2008/09. Regarding the calculated influences of temperature or moisture on grape phylloxera APD values in the present work support the suggestion, that abiotic factors like temperature or soil moisture are not the main influence factors of grape phylloxera activity under field conditions. Only APD values of adult virginopara (L5) showed a significant, but moderate positive development with increasing soil temperature. Also soil moisture showed no remarkable impact on most APD values. Only the abundances of juvenile winged preforms decreased with increasing soil moisture. But these special larval forms only appeared from June to September. Consequently, the calculated impact of soil moisture could be artificial. However, already David (1875) reports of a ruggedness of grape phylloxera against low temperatures and high moisture. David (1875) observed living grape phylloxera in the first 20 cm depth of frozen soil. Troitzken (1929) report from high phylloxera related damages in vineyards in regions of Russia with a yearly medium temperature of + 8.7 °C. Also Stevenson (1964) observed in a three year study in Canada high amounts of juvenile virginopara as well as fresh induced nodosities in December. These results correspond with the observations made in the present work, especially in January 2009. Also Cornu (1878) described a continuous appearance of nodosities over several years in cool-temperate soils. Very confusing are reports by Blankenhorn and Moritz (1875), who came from a statical hibernation of grape phylloxera on roots, but who also observed that root infesting larvals do not loose their mobility during winter. Downie and Granett (1998) reported from overwintering instars in Californian vineyards and already Omer et al. (2002) suggested a lower influence of temperature on grape phylloxera population in field as assumed by Granett and

Timper (1987) and Turley et al. (1996) *in vitro*. These results are confirmed for German vineyards by long-term studies (1997-2010) of Porten (unpb).

As explained in section 5.1.1 plant physiology and nutrient composition in roots can be suggested as more limiting than temperature or soil moisture for activity of grape phylloxera. According to this, the regulation of morph production in aphids is often related to the photoperiod and the nutrient status of the host (Kawada, 1987; Moran, 1992). So, especially the high abundances of L1-L4 instars (in fact mainly L1 instars) and nodosities during late autumn and early winter recorded in the present work (section 5.1.1). Cumulating all off peak seasons which were observed during the present work (2006/07, 2007/08, 2008/09), significantly higher APD values of fresh (non brownish) nodosities (per cm) were observed from October to January, accompanied by significant high APD values of juvenile virginopara. Considering the discussion in section 5.1.1, it can be suggested that the development of hibernating L1 larval stages is mainly driven by amino acid and starch accumulations in the roots (Henke, 1961; Stoev et al., 1966). The induction of nodosities by juvenile virginopara is supported by a root development phase from spring until autumn (a significant shift in root length until September could be observed in the present work). Stoev et al. (1966) suggested mainly a production of glutamine and glutamic acid by amination of α-keto glutaric acid. Also in grafted grapevines, the content of amino acids does not change by passing the callus. The production of amino acids in roots increased at NK/PK-fertilization, and the content of amino acids in phloem increased from 2 to 5 after NK/PK-fertilization. Stoev et al. (1966) found contents of starch between 7.75 (summer) to 19 % (winter) in *Vitis vinifera* coarse roots (3-6mm) and between 12.32 (summer) to 23.5 % in roots of 1-3mm. According to the observations made by Stevenson (1964), it can be suggested that the hibernation is accompanied by a crowding of L1 instars on nodosities, leading to the high observed winter occupation rates of non brownish and light brownish nodosities by juvenile virginopara, which were significantly higher than in spring.

Doing a further step, the absence of leafs in winter and constant available nutrients in the roots could be a key to explain the evolutionary development of the belowground life cycle of grape phylloxera. Parasite life cycles are often characterized by host changes. It is suggested that the different hosts provide better conditions for the particular life stage of the parasite (Price, 1980). This means that factors like host defense mechanisms, nutrient availability, host behavior etc. must be suitable for the development of the parasite life stages which are living on or in the new host. Generally gall inducing insects are highly specialized on their hosts (Price, 1980). Downie and Granett (1999) found among some native *Vitis* species no or very low abundances of foliar grape phylloxera as well as very high abundances, depending on the host-species. Regarding the question how grape phylloxera populations and life cycles could have evolved in their native range, it could be suggested that native grape phylloxera populations are developing independently, depending on their native host. Due to the low knowledge of native grape phylloxera populations, these questions are not finally answered yet (Downie and Granett, 1999; Lin et al., 1999; Downie, 2000; Downie et al., 2001). Downie and Granett (1999) found only weak evidences that grape phylloxera populations on different native *Vitis* species are regulated independently. Indeed,

Lin et al. (1999) could find high genetic differences between native grape phylloxera populations on *Vitis riparia* in the East of America and *Vitis arizonica* in the West of America, but especially in habitats with low geographic barriers, gene flow between the populations was not restricted. Downie (2000) and Downie et al. (2001) suggested that there are only small host-mediated effects on the genetic variation of native grape phylloxera populations in their observed areas in the USA. However, only a few works are dealing with native grape phylloxera populations, and most of them focus mainly on evolutionary questions. Only Downie and Granett (1998, 1999) investigated native populations on native *Vitis* species. They did not found root infestations at *Vitis arizonica*, but root infesting instars on *Vitis riparia*, *V. vulpina* and *V. aestivalis*.

In case of grape phylloxera, the belowground life cycle could be evolved as a host change to provide overwintering which could not be realized at the leaves, driven by the nutrient availability in the roots during winter (Lampel, 1968; Kawada, 1987). Already other aphids like e.g. *Pemphigus bursarius* produce cold-adapted females which hibernate on the roots of a secondary host (Judge, 1967; Moran, 1992). But this hypothesis is mainly speculation yet, far from the small knowledge of population development of grape phylloxera on their native hosts and in their native range. But aphids have evolved in temperate zone habitats mainly (Eastop, 1973), in which especially the quality of phloem sap undergoes high seasonal fluctuations (Moran, 1992). This could be also an evidence for the development of a host change during winter. In any case it must be assumed that the hibernation of asexual instars of grape phylloxera, which in fact was observed by many authors (David, 1875; Cornu, 1878; Omer et al., 1997; Downie and Granett, 1998; Granett et al., 2005), must take place with higher abundances and with a higher phylloxera activity as suggested e.g. by Granett and Timper (1987), Turley et al. (1996) or Makee (2004). Consequently, the classical life cycle with only small or none overwintering root populations (Downie and Granett, 1999; Downie et al., 2001) can not be the strategy which is proceeded mainly on cultivated sites in temperate climates.

5.1.3 Nodosity Degeneration and Infestation Rate

Grape phylloxera induces swellings (nodosities) at the roots of grapevines. Nodosities are causing changes in local root physiology and morphology, the root is locally swelling up and starch is accumulating. Good overviews on the histology of nodosites give Hofmann (1957) and Forneck et al. (2002) on roots of rootstocks, and Forneck et al. (2002) and Kellow et al. (2004) on roots of *Vitis vinifera*. Nodosities are primarily induced by the spittle of grape phylloxera, which is containing proteolytic enzymes. Some *Vitis* cultivars based on native American species, are able to supress the activity of the proteolytic enzymes of the spittle of grape phylloxera (Henke, 1961).

Regarding the relations between the amount of nodosities of a different color class and the amount of instars, high differences occurred between absolute (per cm) and relative (per 10^{-2}sqm) values. High relations occurred between relative amounts of nodosities and relative abundances of instars. But such relationships could not be calculated for the abundances which are directly related to the

plants root length (per cm). These results are supported by the calculated low to moderate relations between APD (per cm) and RPD (per 10^{-2}sqm) instar values. Many workers characterized the aggressiveness of biotypes on basis of life span and/or nodosity induction (King and Rilling, 1985; Song and Granett, 1990; de Benedictis and Granett, 1992, 1993) or studied nodosity induction mechanisms (e.g. Forneck et al. (2002); Lawo et al. (2010)). But none of these authors included the questions of nodosity life span or relations between infestation ratio and nodosity degeneration status in their investigations. Nodosity development time (Blankenhorn and Moritz, 1875; Cornu, 1878; Stellwaag, 1928) and degeneration factors (Cornu, 1878; Millardet, 1878) were investigated and discussed intensively in older literature particularly. Although relating grape phylloxera tolerance of rootstocks with nodosity development, Hofmann (1957) already pointed out that nodosity development and life-time must be related to multiple factors. Millardet (1878) suggested that microorganisms could be a main factor for nodosity degeneration. In fact there are many indications that microorganisms (Omer and Granett, 2000), moisture (Comas et al., 2010) and/or plant vitality (Huber et al., 2009) could have a higher impact on nodosity life-span in field than vitality and life-span of grape phylloxera. Furthermore, especially nodosities are discussed to be entrances for secondary root pathogens such as pathogen stems of *Fusarium oxysporum* (Omer and Granett, 2000; Huber, 2007).

Regarding the amounts of different coloration statuses, the nodosity densities per 10^{-2}sqm showed high relations to the instar densities per 10^{-2}sqm . Especially the RPD values of every instar class was highly related to the RPD values of non brownish (fresh) nodosities. This is in accordance to most authors who reported higher amounts of fresh nodosities at higher grape phylloxera abundances (e.g. Cornu (1878); Steinberg (1968); Kocsis et al. (1997); Omer et al. (1997); Granett et al. (2001a); Porten and Huber (2003)). These calculated high relationships can not be suggested in field under consideration of the root system dynamics. Despite significant differences in instar densities between spring and summer, the APD values of fresh nodosities showed only light increases and no dependencies to the amounts of instars (per cm). The APD values (per cm) of light and dark brownish nodosities showed no significant differences during a year. The browning of gall tissue can be used as an indicator for tissue degeneration, basing mainly on microbiological degradation and plant defense mechanisms (Omer and Granett, 2000; Forneck et al., 1996; Roush et al., 2007). So the life-span of nodosities may not be directly related to the life-span of grape phylloxera. This assumption is supported by the high amounts of juvenile larval stages occupied light brownish nodosities, which already showed first signals of degeneration. Some authors suggested that the vitality of grape organs (consequently also nodosity degeneration) depend on the soil- and endophyte flora (e.g. Huber (2007)). According to Hoffmann (2006) and Vorwerk et al. (2007), the presence of a *Pantoea agglomerans* related bacterium (PARB) could also be a degeneration affecting factor. PARB is associated with the cuticula of grape phylloxera instars on roots (Lawo and Forneck, 2010). Also plant defense mechanisms lead to different degeneration rates of root galls (Ludwig-Mueller et al., 2009). According to the present results, it can be assumed that the infestation by grape phylloxera can not be the main impact factor on nodosity

life-span and degeneration in field. High RPD and APD values of perfoliated nodosities support this assumption.

5.1.4 Nodosities, Phylloxera Infestation and Root System

Generally the biology of aphids is closely related to the inducing and forming of galls (Kawada, 1987; Moran, 1992). Also the activity and development of grape phylloxera population must be seen in relation to the induction and persistence of root galls (Kennedy, 1951; Henke, 1961). So, close physiological relations between grape phylloxera and grape roots are assumed by Lawo et al. (2010), who showed that nodosity formation depends on the regulation of putative expansins in *Vitis* ssp.. In the present work, several quantitative parameters were calculated to relate nodosity density to the grape phylloxera population (section 5.3), root system development and abiotic factors. But especially soil temperature or soil moisture did not show big influences on nodosity densities in soil. Although Comas and Eissenstat (2009) found high dependencies between soil temperature and nodosity development in pots, in field soil temperature could have a lower influence on nodosity life-span (Omer et al., 2002). In the present work, NOP were related strongly to the instar densities (RPD and APD). In bioassay trials with excised roots of different *Vitis* ssp. cultivars, Kocsis et al. (1999) found higher population development on tuberosities of *Vitis vinifera* cul. Cabernet Sauvignon than on tuberosities of *Vitis berlandieri* x *Vitis riparia* sorts (SO4, 5C). For nodosities, Kocsis et al. (1999) found higher rates of instars and eggs on *Vitis berlandieri* x *Vitis riparia* sorts (SO4, 5C). In the present work, differences occurred between the relative and absolute densities of grape phylloxera. Absolute densities of L5 instars (both per cm and per DW) showed high positive relations to the occupation of light brownish nodosities, whereas relative densities of L5 instars showed high relations to the occupation of light and non brownish nodosities. A significant decrease in occupation rates of non brownish nodosities could be observed in September, whereas the occupation of light brownish nodosities showed a significant peak in September. Non-terminal nodosities showed significantly highest occupations in June, terminal nodosities in September.

Nodosities are known to change their color into dark brown short after induction (Cornu, 1878). Generally, the coloration of fibrous roots can be related to physiological traits (Baldi et al., 2010b). The coloration status of nodosities is related to the degeneration of nodosity tissue (section 5.3.1.1.2), based on defense mechanisms of the host as well as on colonization by fungi (Milko, 1961). It can be suggested that the absence of instars accelerate the degeneration of nodosites. This suggestion is supported by the association of grape phylloxera with fungicidal bacteria (Hoffmann, 2006; Vorwerk et al., 2007; Lawo and Forneck, 2010). Consequently, in the present work occupation rates of light brownish nodosities were very low and dark brownish nodosities showed no occupations.

Root swellings with the typically nodosity form (fully developed feeding site and broken epidermis) showed the highest occupation rates. Moderate occupation rates were found on B and C formed root swellings. Both showed at least one the above mentioned characteristics. Root swellings without any

of these characteristics were subsumed into the class D. Most of these swellings were not occupied and only a marginal amount of juvenile virginopara was found sporadicly. L5 instars were never found on any D-formed swelling. But the significant increase of light brownish root swellings in the D class in Spring 2009 must be pointed out. No significant increases of D formed root swellings were found in any of the other color classes. This significant increase did not correspond with an increase of grape phylloxera instars and could be caused by a high gall production of nematodes during spring (Ritter and Ruebsaamen, 1900) (section 5.3.1.1.2).

Very interesting is the occupation of terminal and non-terminal nodosities. Both were occupied in equal rates, whereas non-terminal nodosities showed the highest occupation rates in July, terminal nodosities in September. Generally, terminal nodosities were found in a marginal higher amount than non-terminal nodosities. 125AA showed only infestations of terminal nodosities from July - November. Already Hofmann (1957) suggested that the development of terminal and non-terminal nodosities depends on the rootstock cultivar. This suggestion can be supported in view of the fact that 125AA showed other occupation rates of terminal nodosities than 5C. It can be suggested that more infested root tips of 5C than of 125AA show a further root development (perfoliated) at high infestation rates (summer). On the other hand it is possible that parts of 125AA root system, which are able to develop a further root development after nodosity induction, are more able to suppress the larval development of grape phylloxera. Already Balbiani (1874) suggested that different parts of the *Vitis vinifera* root system must have different properties to suppress grape phylloxera.

In the general structural equation models calculated in the present work, the densities of nodosities could not be related to general root development patterns. It could be suggested that on the study site the growth of the grape root system is not primarily influenced by nodosity induction. Huber et al. (2009) observed on the same study site that aboveground damages of vines are not related to the belowground grape phylloxera infestation, but generally aboveground damages were more related to fungal soil and root flora. These results are in accordance to Granett and Walker (2009), who observed low damages on grape phylloxera infested vineyards in California. Also Nedov and Guler (1987) show that vitality even of European vines is not directly dependent on grape phylloxera infestation. Huber et al. (2009) suggested that soil suppressive characteristics as responsible for their observations (Alabouvette et al., 1985; Alabouvette and Steinberg, 2006). Consequently, also the vitality of the root system could be more affected by suppressive soil conditions than by grape phylloxera infestation. However, actually some recent authors suggested that root development is highly affected by (more aggressive) grape phylloxera biotypes or genotypes at "susceptible" rootstocks (Forneck et al., 2001b; Lawo et al., 2010). Steinberg (1968) observed a high impact of grape phylloxera on root tip amount in field. But Bauerle et al. (2007) observed that high grape phylloxera infestations may result in a successive higher root tip production in Californian vineyards (1103P and 101-14 Mgt grafted with *Vitis vinifera* cv. Merlot). Although infested roots showed a shorter lifespan than not infested roots, Bauerle et al. (2007) observed only a low percentage of infested tips in their two year study. In the

present work the mean summer infestation rates were at 0.4 instars per cm and 0.55 nodosities per cm, with mean nodosity occupation rates of 0.6 instars per non brownish and 0.3 instars per light brownish nodosity. So only the half of the observed nodosities were occupied. After all, it can be suggested that grape phylloxera infestation do not have a high impact on root system development of American rootstocks under pathogen suppressive soil conditions.

5.2 Dynamics of Young Grape Roots

In the present work fragments of the root system in the top 20 cm were analyzed with Win Rhizo Pro by measuring root length, root surface area and root diameter. Further, the fractal dimension of the root system was calculated. Although Lehnart et al. (2008) found the major root distribution of 5C rootstocks (grafted with Riesling) in the top 40-100 cm in vineyards in Rheingau, on the study site of the present work both 5C and 125AA rootstocks (both grafted with Riesling) showed high root densities directly beneath the soil surface. Beside cultivar depending differences in root distribution (Perry et al., 1983), soil and nutrient conditions have high impacts on root development (e.g. Comas et al. (2010)). In the present work, the calculated mean root length (whole observation time) per 10^{-2}sqm soil surface differed between $\approx$ 150 cm in late winter and $\approx$ 550 cm in early autumn for the top 20 cm of soil. The root length is depending mainly on roots between 0 - 0.6 mm. 5C and 125AA only show marginal differences in the development of the main root parameters, but significant differences could be observed in the mean values of length and surface area as well as in nearly all fine diameter classes. Thereby, the 125AA rootstock showed significant higher values than 5C rootstock especially in lower diameter classes (Mar08-Aug09). The root density in the top 20 cm soil was higher and with a smaller diameter in 125AA than in 5C. Consequently it can be suggested that 125AA is able to build a higher amount of finer roots, especially on mid-aged vineyards. Further results are discussed in section 5.4.

5.2.1 Grape Root Growth

5.2.1.1 Temporal and Spatial Root Distribution The assessment of root systems and consequently the verification of root system development and root growth was (and already is) very difficult under field conditions and is highly dependent on the investigation methods (e.g. Anderson et al. (2003)). Several approaches were used to verify the growth of grape root systems. Lehnart et al. (2008) e.g. measured the growth of roots as an increase of root length in a defined soil volume (root length density (Boehm, 1979)). Especially the quantitative assessments of turnover in early grape roots were in the focus of research (e.g. Comas et al. (2000)). In the present work, the development of several root system traits were taken to verify root growth: root length, root diameter, fractal dimension (all 2006-2009) as well as root dry weight (DW) and SRL (Jun08-Aug09). These root parameters were taken as indicators for general predictions concerning grape young root system development in the top 20 cm,

related to 10^{-2}sqm soil surface.

Significant increases of root length and root surface area were observed from low levels in spring to high levels in autumn in the present work. These observations are in accordance to Comas et al. (2005), Eissenstat et al. (2006) or Comas et al. (2010), who observed that the main root production in temperate vineyards occurs between flowering and veraison. Comas et al. (2005) observed mainly one peak of young root production at non grafted Concord grapevines (*Vitis labruscana*) in their four-year study. Also Reimers et al. (1994) and Lehnart et al. (2008) did not observe two peaks in root growth in vineyards in the Rheingau, using minirhizotrons. It can be suggested that the longstanding assumptions of two root production peaks (spring and autumn), driven by C-allocation after fruit ripening, are not universal (Richards, 1983; Currle et al., 1983; Mullins et al., 1992). Also content and composition of amino acids in xylem and phloem sap of both, grafted grapevines and non grafted grapevines showed (dependent on vegetation period) variations daily and yearly (Stoev et al., 1966). On the other hand, long-term observations of root tip production in the Rheingau (Riesling on 26G) by Steinberg (1968) showed an increase of tip production until the end of summer with two peaks. He found significant differences in the amount of root tips (*Vitis vinifera* cv. Riesling on 26G) between early spring, early summer and late summer and related the decrease of root tips in summer to the cutting of top shoots. Hofmann (1957) recorded two temporal caused peaks of root growth in potted plants (different American *Vitis* ssp. sorts). But he found the highest root growth activity in late spring and summer. According to Steinberg (1968), depth of soil and the course of a year are main influence factors for building root tips. Steinberg (1972) showed that rough tillage influenced the root tip development negatively (Riesling/26G). Steinberg (1968) found a mean of 270.5 root tips in the topmost 20 cm of 1200 ccm soil (Riesling grafted on 26G, values converted). Considering these results, a mean of 450 root tips could be estimated per sample in the present work (values approximated to the volume of the used small digging box (2000ccm)). Regarding the work of Bauerle et al. (2007) much lower numbers of fresh roots (and tips) were found in the first 30 cm soil in California (Merlot on 1103P and on 101-14 Mgt).

Already Fitter (1994) and Eshel (1998) showed that increasing root length densities led to a higher complexity of root systems. Also in the present work a simultaneous significant increase of root length and fractal dimension was observed from spring to late summer. But beside these observed increases (root length and surface area, root system complexity), significant decreases in average root diameter from summer to winter as well as constant values of SRL over the whole year were observed. So it can be assumed that the observed significant increase in length and complexity of the grape root system is accompanied by a production of finer roots with a lower weight, consequently leading to constant SRL Rates. This corresponds to studies of Comas and Eissenstat (2004, 2009), who investigated different woody plants in natural ecosystems and observed significant inter specific differences in root traits (especially SRL), but no intra specific differences. Also SRL between 5C and 125AA did not differ significantly (section 5.4).

Regarding root growth, high efforts were done to determine the time when early grape roots are changing their color (root age) and to link this pattern to functional root changes and aboveground management systems (Comas et al., 2000). Generally it must be assumed that beside bloom, veraison and harvest, grape root production must be related to multiple factors such as management as well as environmental (biotic and abiotic) soil conditions and aboveground parameters (Comas et al., 2010). Particularly the significant differences in horizontal root distribution calculated in the present work could be an indication that even on very homogeneous study sites high differences in root system patterns can occur. So root length and root surface area increased significantly in the upper parts of the study site, whereas root diameter was decreasing significantly. Also soil conditions changed significantly from the lower to the upper parts of the study site, whereas especially soil acidity was significant higher on the lower parts of the study site. Such inhomogeneous dispersions were suggested by Steinberg (1968) as well, who observed an inhomogeneous dispersion of root tips in field. The root lifespan is influenced by factors such as soil depth and diameter. Coarse roots seem to have higher lifespans (Anderson et al., 2003). According to Anderson et al. (2003) root lifespan must be shorter in the parts of vineyards with significant lower diameters. On the present study site, influences of soil conditions like acidity on root system morphology could be suggested, but not calculated.

5.2.1.2 Impact of Temperature and Moisture Temperature and precipitation are important environmental regulators of plant growth. Consequently also root growth and root physiology are impacted by soil temperature and soil moisture (Kaspar and Bland, 1992). In a temperate natural forest ecosystem, Steinaker and Wilson (2008) could observe that leaf production increased with soil moisture and root production with soil temperature. Between these boundaries such as given by soil temperature and moisture, root growth and root development is impacted by a variety of factors - e.g. the timing of grape root production varies widely among different regions (Comas et al., 2010), as well as among rootstocks and canopy management (Wolpert et al., 1992). In the present work, general significant effects of soil temperature on root diameter, root surface area and fractal dimension could be calculated under field conditions. No assumption could be made if the significances were directly or indirectly related. Especially root diameter was negatively affected by increasing soil temperature, whereas the growth of coarse roots between 0.5 and 2 mm was increasing and the growth of fine roots (0 - 0.15 mm) was decreasing with increasing temperatures. Soil moisture did not affect general root system traits, but was positively related to root length in the classes 0.3 - 0.5 mm. Soil moisture can affect grape root physiology indirectly by affecting soil thermal properties (Comas et al., 2010). The timing of root production can be responsive to soil moisture, whereas grape root life span is not highly affected (because of nocturnal water displacements) (Comas et al., 2010). Also physiological root properties of grapevines such as root respiration are depending on both soil moisture and soil temperature, whereas root respiration accounts for 30-60 % of total soil respiration (Gisi, 1997). Considering the observed basic soil respiration rates (without roots), significant increases from spring to summer could be ob-

served. Already Huber (2007) observed these differences in BSR on the same study site. Especially at moderate temperatures, soil moisture exerts a substantial influence on root respiration (Bryla et al., 2001). The respiration of young roots (lower order or diameter) is much higher and much more sensitive to temperature and moisture than old roots (Desrochers et al., 2002). In pot trials with young Concord grapevines, Huang et al. (2005) found out that root respiration is increasing with short-term increases of soil temperature (1h from 10 to 40 °C). But by slowly increasing temperature (3d from 10°C to 40 °C), root respiration was decreasing after 33°C. Root respiration decreased with the depletion of soil moisture, wherby under drought conditions, root respiration of all different root orders was reduced (Huang et al., 2005). Bouma et al. (1996) connected root respiration to ion-uptake, maintenance and root growth. Generally, temperature and moisture related root growth corresponds to observed higher densities of soil organisms and enhanced N and P mineralization at elevated soil temperatures (Ruess et al., 1999), resulting in soil areas with higher nutrient availability particularly. Regarding the observations made in the present work, soil temperatures on the study site reached maximal daily means of 23 - 24 °C from June to August (2006-2009). Higher soil temperatures showed significantly positive effects in the growth of roots between 0.5 and 2 mm diameter as well as significantly negative effects on the development of finest roots (0 - 0.15 mm). On the contrary, the growth of roots (increase of length and surface area) in the diameter classes 0.3 - 0.5 mm showed significantly positive relations to increasing soil moisture in field. It can be suggested that the observed effects of soil moisture can not be directly related to thermal soil conditions, like assumed by Huang et al. (2005). So e.g. Stock et al. (2007) observed on soil dewatering crops (lucerne) an exceeding of root growth in highly dewatered soils (and an increase of soil penetration strength). Non dewatering crops (wild rye) did not show higher root growth at the same time. In the present work the increased growth of thicker roots at higher soil moisture can be related to the fast exploitation of water resources in the top soil, whereas the decrease of fine root development at higher temperatures could be an effect of a suggested fast decline of root growth at high temperatures (Bouma et al., 1996; Huang et al., 2005).

5.2.2 Grape Root Ecology

Improving the knowledge of root system growth, root functionality and root activity in agroecosystems lead to improved and more precise management approaches and systems (e.g. Comas et al. (2010)). Because of both environmental (e.g. Fader (1966), Weber (1984)) and biological factors (e.g. Bauerle et al. (2007), Resendes et al. (2008)) can affect root dynamics, predictions of root production timing or of root biomass in field and are unlikely to achieve. However, Comas et al. (2010) assumed that only longterm data collections can provide regionally specific ecological models of grape root dynamics and their functioning.

Root length and root surface area as well as root system complexity greatly influence water and nutrient uptake of roots (e.g. root length as deciding factor for P uptake of maize roots (Barber et al., 1989)). So already Richards (1983) underlined the importance of the knowledge of grape root system development

and behavior, concerning the development of future management methods. Perry et al. (1983) observed cultivar dependent differences in root distribution and related the root surface area of four *Vitis* cultivars to the aboveground canopy. Beside abiotic factors, which are responsible for the general ecological conditions, biotic parameters could impact grape root system development particularly. So grape phylloxera has long been thought of haveing direct impact on grape root development. Already Cornu (1878) described a lower level of roots with small diameter on intensive infested roots sytems of *Vitis vinifera*. But roots of American *Vitis* species are characterized by a thickened suberic layer and many thin marrow rays, whereas European *Vitis* species showed mostly thin suberic layers and only a few, thick marrow rays (Parniewski, 1963). Parniewski (1963) studied the relationship between root anatomy and grape phylloxera tolerance of grape rootstocks and correlated the amount of tuberosities with the size of suberic layers and the marrow rays. Roots of American *Vitis* species or rootstocks showed a lower infestation and thicker suberic layers (Parniewski, 1963). Approaches considering the ecology and biology of soil organisms which could influence soil ecosystem, root development and/or plant vitality of grafted grapevines were only in the focus of few authors (e.g. Huber (2007)). However, the evolutionary backgrounds as well as the biological kingdoms of soil borne pests (parasitic and herbivorous) in viticulture are very diverse. Beside insects such as *Daktulosphaira vitifoliae* Fitch, primary fungal pathogens such as *Roesleria subterranea* (Weinm.) Redhaed or plasmodiophorids such as *Sorospheara viticola* are able to infect and damage grape root systems (Kirchmair et al., 2005; Huber et al., 2006, 2007; Kirchmair et al., 2008; Neuhauser et al., 2009; Miles and Schilder, 2009). For parasitic insects, the host plant is home and mating site as well as main nutrient resource (Price, 1980). Consequences of this tight specialization could be linkages between host choice, mating and population development as well as a reduction of gene flow among populations across divergent host plants. Parasite diversification could be driven by host-associated genetic variation (Downie et al., 2001; Wilson et al., 2003). Grape phylloxera hold many characteristics thought to promote host-plant associated divergence (Downie et al., 2001). So it shows genetic variation of fitness on different hosts (Granett et al., 2001b) and it is used as a organism for a host-driven model of genetic divergence by Downie et al. (2001).

In both agricultural and natural ecosystems, roots are almost ubiquitously present in soil. But ecological factors that may influence root development are not very well investigated, especially in perennial and woody plants. There is a preponderance of studies on cereals among crop plants and data on the dynamics of woody root systems are comparatively rare (Gregory, 2006a; Comas and Eissenstat, 2009). In ecosystems, where the main plant biomass is provided by perennial plants (e.g. forests), average root mass per sqm soil surface is suggested between 2 and 5 kg (Gregory, 2006b). Especially the root surface area and the type of root (e.g. fibrous root or pioneer root) are acting differently, influenced by biotic factors (Resendes et al., 2008). In natural ecosystems, the peak root production of woody plants is approx. one and a half months after the peak of leaf production (Steinaker and Wilson, 2008). But there is limited understanding of patterns of variation that exist among root traits of different woody

species, especially under field conditions. So, investigating 11 tree species, Comas and Eissenstat (2004) observed that roots of fast-growing woody species have higher growth and branching rates than those of slow-growing species. Later, Comas and Eissenstat (2009) observed that morphological root parameters such as SRL or branching variety as well as phenolic concentrations varied most widely among woody tree species, and those with ectomycorrhiza had a higher branching intensity than those forming arbuscular mycorrhiza. Consequently it can be suggested, that root system development is highly influenced by root-soil interactions on both evolutionary and directly interactions. But many questions remain how biological factors can influence root system morphology and complexity. Studies with non woody plant models (particularly *Arabidopsis thalliana*) showed that natural humic acids as well as soil microbes have significant effects on root system traits (e.g. Dobbss et al. (2007), Micallef et al. (2009), Contreras-Cornejo et al. (2009)). Baldi et al. (2010a) showed that the life span of peach roots increased significantly by using organic fertilizers. Generally, compounds released by roots in soil can act as a messenger and are able to initiate interactions between roots and soil organisms (Walker et al., 2003). Mathesius (2003) examined the interactions between plants and microbes involved in *Rhizobium*-legume symbiosis, mycorrhizas and nematode-induced galls. She demonstrated that there are substantial overlaps in the signaling pathways underlying these interactions. Similar plant genes control these processes irrespective of the invading organism, and several hundred secondary metabolites from plants and microbes have been identified as involved in plant microbe interactions. Plant roots are able to release compounds to disturb or enhance the communication between microorganisms (Gregory, 2006a). Some bacteria and fungi can promote plant or root growth directly by producing phytohormones or enclosing new nutrient resources (Gregory, 2006a) and indirectly by suppressing potential pathogens in soil (Alabouvette and Steinberg, 2006). The diversity of antagonists in agricultural soils can be influenced by soil management and manuring processes (e.g. Coventry et al. (2005)). Huber (2007) and Huber et al. (2009) showed that the antagonist diversity in vineyard soils is highly dependent on manuring methods. Porten (unpb) demonstrated that growth depressions of grapevines are related to humus management and the C/N proportion in soil (section 5.4). Further it is known that root feeding insects can have impacts on aboveground parasite and herbivore populations (Gregory, 2006a). In the present study, approx. 40 % of the observed nodosities showed one or more roots continuing after the feeding site (perfoliated). Regarding all counted nodosities, the total amount of continuing roots is nearly compensating the number of terminal nodosities over a year (Jun08-Aug09) with exception of the months April and May. A preponderating amount of D-form nodosities was found in spring, which possibly was not related to grape phylloxera impact (section 5.3.1.1.2). Endophytic fungi, as well as secondary pathogens, are knwon to be associated with grape phylloxera infestations (Milko, 1961; Omer and Granett, 2000; Huber et al., 2009). Milko (1961) has observed that soil fungi are intruding into the roots mainly via the bursted rhizodermis of the nodosities. Further, Vorwerk et al. (2007) observed antagonistic bacteria that are associated with grape phylloxera. Milko (1961) suggested that the rot of rootswellings is mainly induced by *Fusarium oxysporum, Cylindrocarpon radiciciola,*

Gliocladium verticilloides and *Fusarium gibbosum*. Huber (2007) showed that the diversity of the mainly antagonistical fungal microflora was very high on the study stite (compared to other manuring and soil management trials) (Hoffmann, 2006; Hammes, 2008). In his investigations in 2000, he could not detect one of the fungal species mentioned by Milko (1961) on the study site, but severaly potantial pathogens (e.g. some species of the genus *Fusarium*). But on other study site in the Rheingau, he detected a much lower diversity of anatgonists (e.g. genus *Trichoderma*) and potential pathogen stems of *Fusarium oxysporum*. So it must be suggested that mainly the absence of a diverse pathogen flora as well as the high diversity of antagonists in soil could influence the root system development, maybe resulting in positive effects on root growth.

5.3 The Assessment Method

Although grape phylloxera population structure represents an important part of many investigations focusing diverse problems (de Benedictis and Granett, 1992; Omer et al., 1995; Grezegorczyk and Walker, 1998; Omer and Granett, 2000; Forneck and Huber, 2009; Bauerle et al., 2007), the importance of comparative research and comprehensible parameters has been disregarded all the years of grape phylloxera research, with exception of the works of Balbiani in 19th century (see Huber (2007)). In recent literature, diverse approaches to develop assessment methods were made. But the methods were often specific to the relative investigation focus, difficult to handle and far away from field reality in many cases. A large part of investigations regarding an assessment of grape phylloxera instars or nodosities were done *in vitro*, in aseptic culture or potted vines (Granett et al., 1985; Song and Granett, 1990; Grezegorczyk and Walker, 1998; Forneck et al., 2001b; Granett et al., 2005).
The big advantage of the *in vitro* and greenhouse methods is their big deficiency at the same time: field conditions were not considered and the results base on *in vitro* methods could not be assigned to field conditions (Granett et al., 2001a). Generally, a development from a focus on investigations *in vitro* to focused field conditions can be recognized in the last 30 years (Granett et al., 1985; Forneck et al., 1996, 2001b; Porten and Huber, 2003). The first approach to evaluate grape phylloxera performance in field was made by Porten and Huber (2003). Consequently, the developmental work is mainly orientated on the works of Porten and Huber (2003), Huber (2007) and Michaelis (2007).

5.3.1 Methodical Problems

Already Hofmann (1957) suggested that rootstock root system parameters (he suggested the vigor of roots) could be beneficial indicators to include into grape phylloxera tolerance assessments. Due to the inhomogeneity of soil structure and dispersion of roots and grape phylloxera population in field, a high number of sample units were required to assess structures statistically. General methodical aspects were described in section 1. A quantitative assessment of the grape phylloxera population structure could only be done by predefined reference values of root and/or soil. An invasive method with a digging box

was used to investigate root system of grapes. Morphological parameters of roots were analyzed by a scanner based image analysis. So observations were limited by the number of sample units, the size and depth of the digging box and the measured parameters.
Observations were made on the study site only in the topmost 20 cm of soil under the foliage wall. According to Steinberg (1972), the main root amount of *Vitis* ssp. occurred in the first meter of soil. The vertical and horizontal development of grape roots depends on many factors like grapevine variety (Geier et al., 2008), planting depth (Wolpert et al., 1992), soil structure, aboveground management techniques (Fader, 1966; Steinberg, 1972) or nutrient amount (Comas et al., 2005). Considering the study site, Huber (2007) recorded a high root development in the first 20 cm of soil. Huber (2007) and Michaelis (2007) found high amounts of grape phylloxera populations in the top 20 cm on the study site. So, it could be suggested that the use of a digging box with 20 cm depth lead to reproductive results.
With the use of a digging box, the work was accompanied by different methodical problems. To the knowledge of the author, there is no experience in analyzing fragments of such big, perennial root systems with scanner based images. Costa et al. (2000) published methods to investigate large root systems with root scanners, but they worked with different *Zea mays* genotypes. Handling of the plant material as well as the size of the root system was not transferable to *Vitis* ssp. root systems. These problems are explained in section 2.4. Due to an absent methodical experience, the size of the digging box and the number of sample units was changed numerous times in the first two years of investigations. The usage of different digging boxesizes in combination with different number of sample units led to significant differences in some root morphological parameters (section 5.3.1.2.1). Using a digging box to extract roots of *Vitis berlandieri* x *Vitis riparia* rootstocks led to a high amount of root fragments. But the protocol to digitize roots according to Bouma et al. (2000) was developed for whole non complex root systems. Potential problems resulting from a digitization of root fragments were specified in section 2.4. In the present work, especially the digital assessment of root tips, forks and crossings led to high overestimations (section 5.3.1.2.2).
Additionally, the development of quantitative visual parameters of grape phylloxera population structure was accompanied by methodical questions, especially the quantitative visual classification of nodosities (section 5.3.1.1.2). At the beginning of the practical work, a method according to Porten and Huber (2003) was used to assess grape phylloxera population in field. Although the method works accurate, it was not developed to record single population structure elements (section 5.3.1.1.1).

5.3.1.1 Grape Phylloxera Population Structure

5.3.1.1.1 Field Assessment and Counting Method Finally, a quantitative assessment of population structure (section 1) means a simple counting or measurement of single structure elements (traits). Porten and Huber (2003) published a method to assess frequency and intensity of grape phylloxera root

infestation via visual properties quickly in field. Single population structure elements could not distinguished visually in field. So Porten and Huber (2003) introduced distinct assessment classes on base of the number of eggs and instars as well as the number of "old" and "new" nodosities. The first aim of their study was a direct validation of grape phylloxera structure in field. Due to the practicability in field, a counting of number of instars, nodosities or eggs was not included in the method developed in the present work. An usage of this field assessment method was very questionable, considering the aim of the present work. In the beginning of the field works in 2006, grape phylloxera population was assessed mainly in field according to Porten and Huber (2003). But to verify the advantages of the field method for the aimed objectives, the instar larval stages were counted manually in laboratory. Results of field assessment and laboratory counting were compared (section 4.1.1). The field assessment method showed a high relation to the quantitative counting of larval stages in laboratory. The correlations of total numbers of counted assessment classes and counted larval stages showed high significances. Also the comparison of the frequencies of assessed classes and counted instars by a χ^2 test proved the accuracy of the assessment method.

The method according to Porten and Huber (2003) showed seasonally caused differences to a quantitative assessment in laboratory. So the relation between the counted number of assessment classes and the counted number of instars showed significant differences in winter and fall. No significances could be recorded regarding the frequencies of assessed classes and larval stages (χ^2), but p-values in fall and winter months were very low. Further, no specific larval stage could be correlated to a specific assessment class. This occurred as a problem in assessment of distinct stages of the life cycle of grape phylloxera.

Although the densities of instars are higher in late summer months, high relations between counted instars and assessment classes occurred in summer and big differences in fall and winter were shown. Several causes could be possible. Assessment classes according to Porten and Huber (2003) did not regard the exact amount of instars per nodosity. If number of instars per nodosity (NOP) is low, the number of assessment classes will be high. So NOP could increase in fall and winter. In the present work the detection of nodosities occurred only from 2007 on, assigning nodosities to calculated color classes. In the years 2007 to 2009, occupation rate of non brownish / light brownish nodosities had highest values in July and August and again in October (only non brownish nodosities). So an increased occupation rate in fall and winter can only be a limited explanation for the differences between field method and laboratory method. Own field observations are confirmed by observations by David (1875) and Cornu (1878), who reported of smaller and brownish larval stages in fall. The bad recognizability of such larval stages in field, especially under bad lighting conditions in the late seasons, in context with possible higher occupation rates could be a reason for the found significances in fall and winter months. In conclusion, the method according to Porten and Huber (2003) is very suitable to assess the infestation of grape roots by grape phylloxera, especially in summer. But to describe the structure of grape phylloxera population on grape roots, a counting of structure elements in laboratory was

necessary.

5.3.1.1.2 Nodosities Reproduction and the development of grape phylloxera population on roots is dependent on the induction of nodosities (Henke, 1961). Root physiology and morphology is changed by an effective induction of nodosities (Kellow et al., 2004), and infection paths for microorganisms were disclosed (Omer and Granett, 2000). Only a few recent works deal with a closer consideration of visual nodosity properties. To point out the importance of a quantitative visual nodosity assessment, two examples will be given. 1.: King and Rilling (1985) described different nodosity forming reactions on potted grapevine sorts (among others *Vitis berlandieri* x *Vitis riparia* sorts). King and Rilling (1985) classified nodosities on the basis of their diameter and their length. 2.: Kellow et al. (2004) used a classification on base of larval stages on nodosites.

Considering the results of the present work, the method according to Kellow et al. (2004) appeared to be problematic in respect of both, the frequently recorded occupation of nodosites by more than one larval stage and the high recorded amount of non occupied nodosities in field. But also the method according to King and Rilling (1985) is not in accordance with the observations made in the present work. No conclusions could be drawn about diameter or length of a nodositiy as a meaningful visual attribute under field conditions. An additional problem is the comparability of different works. If different authors used different self made and not verified classification methods, works could not be compared. Visual nodosity properties could give sensible indications to the status of root physiology and grape phylloxera population (Hofmann, 1957; Forneck et al., 1996; Roush et al., 2007; Forneck and Huber, 2009). Already Cornu (1878) and Ritter and Ruebsaamen (1900) pointed out visual differences of nodosities and described a high variability, particularly of nodosity forms and colors on *Vitis vinifera* roots. Most field working authors found parts of root system with high infestation rates and parts with low or no infestation (Cornu, 1878; Ritter and Ruebsaamen, 1900; Hofmann, 1957; Porten and Huber, 2003; Bauerle et al., 2007), due to local soil and root system differences (e.g. Balbiani (1874)). The confusion of nodosities with other root swellings during a visual survey was problematic. Already Ritter and Ruebsaamen (1900) suggested, that nodosities could be confused with galls induced by endoparasitic nematodes. Although on the study site mainly ectoparasitic nematodes were found (Huber, 2007), the occurrence of endoparasitic nematodes can not be excluded. So, indistinct galls (without specific attributes) were subsumed in one group (form group D) in the present work. The classification of visual nodosity properties acted in accordance to the above mentioned authors and the observations made in field. So coloration (Cornu, 1878; Ritter and Ruebsaamen, 1900; Forneck et al., 1996; Porten and Huber, 2003), form (Cornu, 1878; Ritter and Ruebsaamen, 1900; Kellow et al., 2004) and branching status (Hofmann, 1957) of each single nodosity were recorded during the stereomicroscopical root survey.

Coloration attributes were introduced in January 2007. To differentiate the coloration of nodosities on a more objective base, colors were related to a color group. RGB root images of the year 2006 were

analyzed using the color analyze function of Win Rhizo Pro. The color classes were modified until all nodosities equal to one color group were covered by the according color classes. Due to the variation of colors in the course of a year, the resulting color groups overlapped in some color classes. In summer nodosities on the observed roots generally appeared more pale than in other seasons. Further, due to the inhomogeneous coloration of each nodosity, no single color class could cover the color of a whole nodosity. Altogether, a nodosity was related to a color group visually on base of their entire coloration habitus and with help of the before defined color classes. Due to the complex relationships which influence nodosity development (Hofmann, 1957), browning color groups have to be redefined for every new study site. Nevertheless, the assessment of the nodosity browning status is important for a validation of the grape phylloxera population. Browning could be used as an indicator for nodosity degeneration. Cornu (1878) for example found black and rotten nodosities generally at the end of summer at *Vitis vinifera* roots and described a time related browning of nodosities. These observations were supported by Millardet (1878); Hofmann (1957); Forneck et al. (1996); Porten and Huber (2003) and Bauerle et al. (2007), who also related the degeneration of nodosities to more brownish colors.

Since July 2008, the form of the counted root swellings was recorded. The attributes *sitting hollow* and *bursted epidermis* were subsumed into four classes. A *sitting hollow* is a highly developed grape phylloxera feeding site. This class should represent the development of a feeding site, from fresh induced to highly developed. In contrast, the attribute *bursted epidermis* should describe the development of a grape phylloxera induced root swelling, from a normal epidermis at the beginning to a damaged epidermis at an advanced developmental state. This attribute (*bursted epidermis*) was introduced firstly in the year 2008. The microscopical observations showed that grape phylloxera occupied root swellings could have both bursted and non bursted epidermis, and a bursted epidermis is a common mentioned visual characteristic of nodosities (Cornu, 1878; Kellow et al., 2004; Forneck et al., 1996; Porten and Huber, 2003; Ritter and Ruebsaamen, 1900). For a better differentiation of non phylloxera induced swellings from phylloxera induced swellings, the feeding site was included into the classification. Therefore, the parameter *sitting hollow* was developed and was assessed additionally since January 2009. Ritter and Ruebsaamen (1900) related the formation of a feeding site to the age of the nodosity. In the present work, particularly the relative densities of nodosities with fully developed sitting hollow (A form) showed a high relation to the densities of instars. Nodosities without a sitting hollow (B and D forms) showed a significant decrease at higher amounts of instars. In contrast, coloration as indicator for nodosity aging showed no relation to the nodosity form. Already Cornu (1878) considered that the formation of a sitting hollow is rather dependent by sucking activity of instars than by the age of a nodosity. So, considering this interpretation of feeding sites and sitting hollows, the term "age" was misselected by Ritter and Ruebsaamen (1900). It led to the assumption, that nodosity maturity is generally related to the formation of a sitting hollow. But in the present work, also dark and light brownish nodosities (already occupied by instars) without a sitting hollow were found. So it can be assumed that the development of a feeding site depends more on the activity of the sucking instars

than the time period instars are occupying a nodosity.
During the visual root surveys in 2006 and 2007, high amounts of nodosities with normal growing roots on both ends were noticed. These observations agree with those made by Hofmann (1957). Since July 2008 the branching status of each nodosity has been assessed in two classes: *terminal* (with a pecker-like end) and *non-terminal* (root showed normal growth behind nodosity; perfoliated). Cornu (1878) described the terminal and bent nodosities with a pecker-like end and a bursted bark as very common. But he did not exclude the appearance of other forms. So Cornu (1878) and Ritter and Ruebsaamen (1900) also described and illustrated non-terminal forms of nodosities as well as forms without a bursted bark. Later, Hofmann (1957) recorded terminal and non-terminal nodosities on different rootstocks, but excluded the appearance of non-terminal nodosities at *Vitis vinifera* roots. Also Grezegorczyk and Walker (1998) suggested terminal and non-terminal nodosities in *in vitro* observations.
Already Cornu (1878) suggested that nodosity appearance must be considered for an explanation of grape phylloxera population dynamics. But only in a few works visual nodosity parameters were regarded (Hofmann, 1957; King and Rilling, 1985; Porten and Huber, 2003; Kellow et al., 2004). The present work represents the first approach to introduce quantitative assessable visual nodosity attributes (color, form, branching). Nodosity attributes (different densities) explained parts of the total variability in the structure of grape phylloxera population. Nodosity attribute related parameters could be separated significantly from instar densities by a FA. Nodosity attributes were used to generate occupation parameters (NOP) via dividing the number of instars per 10^{-2}sqm by the number of nodositiy attributes per 10^{-2}sqm . Also NOP could be separated significantly from pure instar densities and pure nodosity densities. So the big groups "instars", "nodosities" and "nodosity occupation" could be separated and could explain parts of the total variability of grape phylloxera population in field.

5.3.1.2 Problems in Assessing Root System Parameters

5.3.1.2.1 Influence of Digging Box Size and Number of Sample Units The size of a digging box had significant influences on measured root parameters. In the year 2007 the digging box size was decreased to enable a higher number of sample units in field. The measured values of root length and root surface area were significantly influenced by the decreased digging box size. Mean values and variances of root length and surface area were lower, if roots were collected with a small digging box. Root diameter did not show significant differences between digging box sizes. Due to the synchronous changes of digging box size and number of sample units, effects could not completely be separated. Although mean values appeared to be high, it can be suggested, that total values of root length and root surface area are rather underestimated than overrated in small digging box sizes.
The variances of measured values decreased with a smaller digging box size and a higher number of sample units. The obvious thighter approximation to natural conditions using a big digging box is connected to higher sample caused variances. Due to this high variances in samples, it can be suggested

that big digging boxes only can be used in combination with a higher number of sample units (more than 8). In contrast, variances are essential lower using the small digging box (half the size of the big digging box) and a comparable number of sample units (10). Regarding the aims of the present works, particularly an assessment of general variability on the study site was important. Local, sample caused variances, may not be of consequences for further analyses. Due to this, using bigger digging box sizes led to the necessity of a very high number of sample units. Following a practicability in field, smaller digging boxes were used in the present work.

5.3.1.2.2 Overestimation of Tips, Forks and Crossings Roots of *Vitis berlandieri* x *Vitis riparia* rootstocks were extracted by using a digging box. Due to this invasive method as well as to the complexity and size of *Vitis* ssp. root systems (Richards, 1983), root fragments were extracted and analyzed. The analysis of root fragments did not influence the calculation of root system parameters like root length, root surface area, average root diameter, and fractal dimension, but it led to a huge overestimation of the number of tips, crossings and forks.
Beside the fractal dimension, the number of crossings per cm root length could be taken as an indicator for the complexity of the root system. This suggestion is supported by the temporal development of both parameters. However, a problem is to characterize branching structure and architecture of the root system with the used sampling method. Working with root fragments prohibits a sensible calculation of a branching structure (Walk et al., 2004). Also the calculation of the linear fractal dimension (FD_1, (Eshel, 1998)) gives only a hint to the architecture of root systems, mainly by comparing it with *FD* values of other root systems. Though three-dimensional fractal dimension (FD_3) correlates with planar (FD_2) and linear (FD_1) fractal dimension (Eshel, 1998), Walk et al. (2004) proposed the use of more than one fractal parameter (fractal abundance (*FA*) particularly) to characterize root system architecture more accurate.

5.3.1.2.3 Root Color Analysis The relationship between root age and root physiology is poorly understood, despite its importance for water and nutrient uptake or root-microbe interactions (Volder et al., 2009; Comas et al., 2010; Baldi et al., 2010b). So, fresh pale roots of peaches (seedlings) showed higher nutrient uptake rates and higher concentrations of N, K, Mg, Mn, Fe and Cu than older, brown root parts (Baldi et al., 2010b). The root coloration conduced as an indication for root age (Cornu, 1878; Comas et al., 2010; Baldi et al., 2010b). Assessing the "old-new-ratio" of grape roots quantitatively by a color analysis would be an important step, regarding breeding activities particularly. Beside non invasive methods to asses root age (via minirhizotrons) (Comas et al., 2010), the technique to analyze colors of roots - implemented in Win Rhizo Pro - offers the opportunity to make statements about root age and fresh root building. Relating these data to other assessed data of the ecosystem, statements about root physiology could be possible.
The color analysis of scanned RGB images in the present work was afflicted with difficulties due to

the inhomogeneous coloration of *Vitis berlandieri* x *Vitis riparia* roots in field and a huge shadowing of coarse root parts. Due to the defined color class settings, different values of all root main parameters were measured. This applied to the two color settings as well as the color settings and the (accurate) gray scale analysis. An inhomogeneous coloration of *Vitis* roots was a problem regarding the settings of manually defined color classes mainly. But significant differences in average root diameter as well as in number of forks and crossing between color analysis (two tested color settings) and gray scale analysis could not be explained by coloration of roots alone. These significances were caused by effects of shadowing. Because shadows have the same coloration as dark root parts, no difference could be made between root and shadow by Win Rhizo Pro. A result of this was an overestimation of root length and a strong underestimation of average root diameter. This should have led to incorrect assumptions about dispersion of dark and pale root parts. Removing the shadows manually, the measured values between color analysis and gray scale analysis were adapted (for the special root parts). Due to the high amount of RGB images (most sample had more than one scan image), an automatically removing of shadows would make sense. Perhaps these problems can be solved in future, for example by the programming of a special image editing software. But this has not been the aim of the present work.
Due to the inaccuracy, color analysis of *Vitis berlandieri* x *Vitis riparia* root fragments could not be used in the present work, but a possible application of color analysis in future could be the detection of fungal hyphae on roots. To detect stained fungal structures, roots had to be be stained whole-mount before scanning. In the present work, roots were stained whole-mount by Pianeze staining solution, using methods to fix, clean and bleach roots according to Koske and Gemma (1989). Koske and Gemma (1989) originally introduced a method to stain roots whole-mount for VAM detection with trypan blue. This method is practicable on most root tissues and a standard method to detect VAM structures. To detect other fungal structures in plant tissue, Vaughan (1914) introduced Pianeze staining to differentiate fungal and host tissue in plants. Pianeze is a pigment mixture, originally developed in late 19th century to detect cancer cells in human tissue (Pianeze (1896), cit. in Vaughan (1914)). Also mycorrhiza structures in pine roots could be detected via Pianeze staining (Wilcox and Marsh, 1964). The investigated tissues in the present work did not show fungal structures. But the successfully staining was clearly detectable. The tissue cleaning method according to Koske and Gemma (1989) was replaced by a method using an ultrasonic bath, which also led to a successful whole-mount staining of roots.
Bleaching and staining roots to detect and assess superficial fungal structures via root scanners could be a possible part of root system validation of grapevines in future. The present work showed, that a whole mount Pianeze staining is practicable. The next step may be a development of a method to assess fungal colonization of roots under consideration of Pianeze stained roots via the scanning method.

5.3.2 Sensitivity of the Assessment Method

Grape phylloxera density parameters (RPD, APD, CNA, NOP) represent mean values per sample. Nevertheless, high differences in instar and nodositiy densities within the same sample were found frequently. Particularly in the off-peak population development, roots found in soil zones with high amount of space (e.g. earthworm canals) or in non frozen soil parts (e.g. slut clutches) showed high infestation densities, whereas roots found in other soil zones were not infested. Such differences within a unique sample could not be reproduced or predicted with the developed assessment method. But the methods used to assess the grape phylloxera population in the present work are very sensitive, regarding spatial differences on the study site as well as differences between different rootstocks. Further it is possible to predict nodosity degeneration and development on base of grape phylloxera instars and to estimate the impact of soil moisture and temperature on population development.

The explanatory power of the grape phylloxera population parameters depends on the potential investigation aims of the study in which they are applied. But it can be supposed that parameters which include the host dynamics (e.g. root system length) are more beneficial to most investigations in viticulture. Even if only comparative studies are aimed, absolute abundances give much more information of the system than relative abundances. But especially in pure field observations with an aimed fast approximation to infestation status of root system (e.g. in Porten and Huber (2003)), root system parameters can not be assessed.

The detection methods used in the present work provide an evaluation of the spatial distribution of grape phylloxera population on the study site (in the top 20 cm soil). To identify horizontal distribution patterns, the study site was classified into three classes on base of their partial slope. Generally the study site is very flat with an increasing slope in the upper parts.

The used methods combined with this simple and rough classification of small slope differences lead to significant differences in grape phylloxera population. Due to the expected inhomogeneous horizontal distribution of the grape phylloxera population (Kocsis et al., 1999; Omer and Granett, 2000; Porten and Huber, 2003), significant horizontal differences in nearly all parameters were expected. But only a few parameters differed significantly. Particularly high degenerated nodosities were found in significant higher amounts in the upper part of the study site (section 5.1.3). But regarding the spatial distribution of the two variants (5C, 125AA) separately, clear differences occur in population abundances between the area planted with 5C and the area planted with 125AA. In variant 5C highest grape phylloxera and nodosity densities were found in the lower part of the study site, whereas in variant 125AA the highest densities were found in the upper part of the study site. Also occupation parameters (based on a weaker dataset) were found to be different in the different areas of the study site. These results must be lead back to a inhomogeneous dispersal of grape phylloxera population on the study site (see also Michaelis (2007)). Areal differences in population densities could be detected. These results show the high sensitivity of the used method to assess population densities of grape phylloxera and to identify spots of high abundances. Every root system main parameter (with exception of *fractal dimension*)

showed significant differences in its horizontal distribution. Especially with a higher slope, root length increased and average diameter decreased significantly (section 5.2). In contrast to grape phylloxera densities, the root system parameters seem to be mostly homogeneous between the variants. So the number of SU were suitable to detect significant differences in length: diameter ratios of the grape root system in field. Highly significant spatial differences could also be detected in all assessed soil parameters. Especially *pH* increased significantly from the lower to the upper area of the study site.
Beside the spatial sensitivity of the assessment, rootstock dependent differences in grape pyhlloxera densities and population development could be detected. Differences were detected e.g. in the monthly mean abundances of adult virginopara as well as in the occupation rates of terminal nodosities (section 5.1.1). In contrast, a wide similarity in root system parameters between the both variants could be detected. *Fractal dimension* showed a faster decrease in 5C than in 125AA in late winter (section 5.2). Also significant decreases in soil parameters between the 5C and 125AA areas of the study site could be detected.
Nodosity densities and occupation rates could be predicted by instar densities on base of the collected data. Due to general low dependencies between the absolute nodosity densities and grape phylloxera infestation rates (section 5.1.3), prediction values were very moderate. But the occupation of light and non degenerated nodosities could be predicted by the relative abundances of instar classes, especially by the relative abundances of juvenile virginopara. This corresponds with the suggestions of Stevenson (1964), who calculated high dependencies between the abundances of the first larval stages and occupation of nodosities, especially in winter. The relative nodosity densities could be predicted by relative instar abundances. So, especially the densities of non degenerated nodosities could be related to relative densities of all instar classes. Relative densities of fresh nodosities could be predicted especially by the relative abundances of juvenile virginopara. Regarding models with a lower database, especially relative densities of A-formed nodosities or relative densities of both branching classes could be predicted by the relative and absolute instar densities. These models based on a very weak database, but generally dependencies between instar and nodosity densities were suggested and observed by many authors (e.g. Parniewski (1963) or Stevenson (1964)).

5.4 Aspects in Viticulture

In the last decade, researchers in viticulture observed dependencies between root system surface area and grapevine canopy area (Anderson et al., 2003), effects of different rootstock cultivars on vegetative and reproductive development of aboveground organs (Tandonnet et al., 2008), relations between grape root production and management methods (irrigation, pruning) (Comas et al., 2005), influence of humus management on aboveground vitality (Porten, unpb), and relations between soil microflora, belowground parasite infestation and aboveground vitality (Huber et al., 2003, 2009). But many questions remain unanswered regarding the relations between belowground and aboveground vitality of grapes in agricultural ecosystems. Regarding the multiplicity of management methods, grafting

combinations or soil and weather conditions in viticulture world-wide, such research could provide enhancements of vineyard management in future by developing local predictive models (Comas et al., 2010). Changes in climate and environmental care (Hasselmann et al., 2003) necessitate such models, particularly considering issues such as optimization of irrigation (e.g. Iacono et al. (1998), Padgett-Johnson et al. (2000)) or the influence, spread and management of (soil borne) pathogens (e.g. Huber et al. (2007), Miles and Schilder (2009)). In the present work, a first approach was done to assess data and implicate it in simple models. On base of the data analysis, some important aspects in viticulture became evident. So differences found especially between the mature rootstocks 5C and 125AA (section 5.4.1) as well as general aspects (section 5.4.2) could have impacts on current research, management and legislation procedures in viticulture.

5.4.1 Special Rootstock Differences

Most rootstocks currently used in cool climate viticulture are *Vitis berlandieri* x *Vitis riparia* cultivars such as Kober 5BB, 5C Geisenheim, SO4 and 125AA. The rootstock 125AA is known as a cultivar with increasing effects on the vigor of scions (Schmid et al., 2003), although the assessment of grapevine vigor characteristics was not evaluated before Porten (unpb) (pers. comm. M. Porten). The present results let assume that mature 125AA produce significant more fine root mass than mature 5C rootstocks (significant differences in root length and root surface area), particularly in the diameter classes 0 - 1 mm. So significant differences in root growth could be found between the two mature *Vitis berlandieri* x *Vitis riparia* rootstocks 5C and 125AA. It must be suggested that factors like humus management, nutrient supply and C/N relations in soil in combination with soil microflora community have high impacts on the rooting behavior in field (Porten, unpb). Also differences in plant physiology depending on the grafting combination (e.g. Geisler (1961), Walker et al. (1998), Iacono et al. (1998), Padgett-Johnson et al. (2000), Walker et al. (2009), Stevens et al. (2010)) may have an impact on root development, regarding the generally high exchange of substances between above and belowground organs in plants (Gregory, 2006a). Such factors as well as genetic rootstock differences can show an impact on grape phylloxera occupation rates on the roots. In the present work differences between 5C and 125AA could be calculated for the occupation of roots with adult virginopara. The development of adult virginopara could be observed one month earlier on 5C than on 125AA. Terminal nodosities on 125AA only were occupied by L5 larval stages between July and September. Although the observed results could be partly reasoned in the inhomogeneous spatial distribution of the main grape phylloxera population on the study site, it can be assumed that different rootstock cultivars must have important effects on the life cycle of different grape phylloxera biotypes. So influence of soil parameters (e.g. soil pH) on occupation behavior of grape phylloxera can not be excluded. But it could be suggested that other fine mechanisms are predominate in 125AA to regulate grape phylloxera infestation than in 5C. In spring no adult virginopara could be detected on terminal nodosities on 125AA, but certainly in 5C. The total number of terminal or non-terminal nodosities did not differ significantly between both

rootstocks. So adult virginopara did not settle or (more supposable) die earlier on terminal nodosities at 125AA. Factors which influence tolerance and defense mechanisms are not clear, but significant differences in the frequency of endophyte morphotypes could be detected between 5C and 125AA in the present work. Although Rilling (1975) concluded that microorganisms did not have a big part on root damaging on *Vitis vinifera* roots, Vannacci et al. (1984) could isolate more fungi from roots infested by grape phylloxera than from not infested roots. An artificial wounding of roots leads to a higher amount of isolated fungi as well (Vannacci et al., 1984). Also mycorrhiza is discussed by some authors as growth promoting factor in grapevines (e.g. Karagiannidis et al. (1997)). In natural ecosystems, Oehl et al. (2010) could classify 53 % of found arbuscular mycorrhizal fungal (AMF) communities to special soil types and/or land use intensities. Thereby plant species compositions had only subordinate influence on the AMF communities. In the context of *Roesleria subterranea* (Weinm.) Redhaed and *Sorospheara viticola* studies on the trial site, mycorrizal infections were observed only in marginal parts of the investigated root material (pers. comm. L. Huber). Generally farming and manuring can have significant effects on soil microflora (Coventry et al., 2005) and on plant-herbivore and plant-pathogen interactions (Poveda et al., 2006). Some endophyte-biota are able to improve plant performance and stress tolerance (see Yuan et al. (2010)) and in viticulture it is known that root and soil inhabitating fungi are related to manuring on the one hand and to grape phylloxera related aboveground damages on the other hand (Huber et al., 2009; Porten, unpb).

5.4.2 General Aspects

Against the current regulations in EU (Regulation(EU)1493/1999 (1999) and Regulation(EU)1227/2000 (2000)) (Manty et al., 2003), the present results show clearly the necessity of rootstock classifications. Significant differences were quantified even between closely related rootstocks, especially in endophyte frequency, root growth and grape phylloxera occupation. High differences in root growth could be detected, depending on low differences in slope and abiotic soil conditions (see also Fader (1966)). It can be suggested that the development of integrated pest management models in viticulture which are adapted to changing conditions (e.g. climate change) must consider belowground as well as aboveground patterns of grapevine vitality (Huber et al., 2003, 2009; Comas et al., 2010). The developed method represents an approach which can provide a sensitive classification of mature grapevines, considering pest control as well as aboveground vitality assessments (see Huber et al. (2003); Porten (unpb)).

World-wide phytosanitary activities in viticulture on base of administration acts (such as §2 Nr.3 ReblV in Germany) must be intensively discussed. In the present work, sensitive cultivar specific differences in the occurrence and settlement of grape phylloxera were demonstrated. It can be suggested that rootstock cultivars have high influences on the development of genotypes and life cycles of grape phylloxera. Assumptions could be made that the nodosity degeneration is not directly related to the grape phylloxera infestation rates of the root system. These results are in accordance to Milko (1961), Nedov

and Guler (1987), Huber et al. (2003), Huber (2007), Huber et al. (2009) and Porten (unpb), who demonstrated that aboveground damages in phylloxera infested vineyards are not directly related to grape phylloxera infestation, but to soil and humus management as well as to soil microbiota. Huber et al. (2003) and Porten (unpb) showed that the vigor of grapevines in grape phylloxera infested vineyards is related to humus C/N relations particularly and N-uptake of grape roots is mainly depending on root age (Volder et al., 2009). Although fresh infested roots of non grafted grapevines (*V. lambrusca* Concord) showed a lower life-span, an increased root growth could be observed after grape phylloxera infestation (Bauerle et al., 2007). In the present work approx. 40 % of the observed nodosities were perfoliated and the attached roots showed a normal growth. Grape phylloxera could be associated with bacteria (e.g. Vorwerk et al. (2007)) and it could be suggested that also other microorganism (especially microbial root pathogens) are associated with grape phylloxera (Neuhauser et al., 2009). Such complex interactions in soil must also be considered in unsolved questions in controling highly impacting soil borne pests in viticulture such as *Roesleria subterranea* (Weinm.) Redhaed (section 6).

6 Future Aspects

The function and development of root systems are influenced by multiple factors (e.g. Comas et al. (2010)). Factors such as tillage, fertilization, irrigation, canopy management, abiotic soil conditions, slope angle as well as diversity of soil microbiota and the persistence of above and belowground pests or herbivory species can influence the root system development of woody root systems significantly. Hence, the management of longstanding agricultural ecosystems such as vineyards must be recognized as a conclusive package of multiple solutions (Kogan, 1998; Huber et al., 2003; Huber, 2007; Porten, unpb), particularly under changing climate conditions. The method developed in the present work must be recognized as a holistic approach to quantify and predict parasite interactions at grape root systems. Especially in evaluating belowground pest control strategies (e.g. Kirchmair et al. (2006)) or connecting above and belowground assessments, this method is conductive to the assessment and prediction of grapevine vitality in local integrated management solutions (Porten, unpb). In the future, the presented method could be transfered to other study sites which show generally other soil conditions, steep slopes and deeper main root distribution, probably by using other techniques to explore root systems (e.g. minirhizotrons or access boxes). Computer based solutions to assess young pale roots as well as stained root material would enhance the presented method. Parameters to describe connections between belowground and aboveground grape vitality (method developed by Porten (unpb)) could be analyzed, particularly against the background of soil borne primary pathogens such as *Sorospheara viticola* (e.g. Neuhauser et al. (2009)) or *Roesleria subterranea* (Weinm.) Redhaed (e.g. Hoefer (1993), Hoffmann (2006), Kayler et al. (2010), Kirchmair et al. (2008) and Miles and Schilder (2009)).
Regarding fundamental research, questions remain whether and how biotic factors can influence root development and root function of woody plants. Particularly to investigate general patterns in the settlement of endophytes and BCAs in young grape roots, the author has applied for a two-year post-doctoral fellowship at Penn State University.

7 Conclusions

In the present work, a general set-up is provided to assess parasite populations on grape root systems in field. Pest specific (grape phylloxera, *Daktulosphaira vitifoliae* Fitch) quantitative parameters could be developed and root system dynamics was included into the assessment procedure. With the presented approach multiple impact factors could be evaluated and high spatial, temporal and cultivar specific sensitivity could be achieved. Simple local prediction models considering pest population structure could be calculated.

The results which could be achieved in the present work demonstrate that grape phylloxera population development is highly affected by grape root system dynamics. Differences occurred also between two closely related *Vitis berlandieri* x *Vitis riparia* cultivars. Grape phylloxera was able to overwinter in both warm and hard winters with high densities of juvenile larval stages. Further it could be demonstrated that general root growth depends only moderately on temperature or moisture conditions, but the root growth in special diameter classes (e.g. 0 - 0.15mm) significantly decreased at high soil temperatures.

8 Abstract / Zusammenfassung

8.1 Abstract

The development and the growth of plants is strongly affected by the interactions between roots, root associated organisms and rhizosphere communities. Methods to assess such interactions are hardly to develop particularly in perennial and woody plants, due to their complex root system structure and their temporal change in physiology patterns. In this respect, grape root systems are not investigated very well. The aim of the present work was the development of a method to assess and predict interactions at the root system of rootstocks (*Vitis berlandieri* x *Vitis riparia*) in field. To achieve this aim, grape phylloxera (*Daktulosphaira vitifoliae* Fitch, Hemiptera, Aphidoidea) was used as a graperoot parasitizing model.

To develop the methodical approach, a longt-term trial (2006-2009) was arranged on a commercial used vineyard in Geisenheim/Rheingau. All 2 to 8 weeks the top most 20 cm of soil under the foliage wall were investigated and root material was extracted (n=8-10). To include temporal, spatial and cultivar specific root system dynamics, the extracted root material was analyzed digitally on the morphological properties. The grape phylloxera population was quantified and characterized visually on base of their larvalstages (oviparous, non oviparous and winged preliminary stages). Infection patches (nodosities) were characterized visually as well, partly supported by digital root color analyses. Due to the known effects of fungal endophytes on the vitality of grape phylloxera infested grapevines, fungal endophytes were isolated from nodosity and root tissue and characterized (morphotypes) afterwards. Further abiotic and biotic soil conditions of the vineyards were assessed. The temporal, spatial and cultivar specific sensitivity of single parameters were analyzed by omnibus tests (ANOVAs) and adjacent post-hoc tests. The relations between different parameters were analyzed by multiple regression models.

Quantitative parameters to assess the degeneration of nodosity, the development nodosity attached roots and to differentiate between nodosities and other root swellings in field were developed. Significant differences were shown between root dynamic including parameters and root dynamic ignoring parameters. Regarding the description of grape phylloxera population and root system dynamic, the method showed a high temporal, spatial and cultivar specific sensitivity. Further, specific differences could be shown in the frequency of endophyte morphotypes between root and nodosity tissue as well as between cultivars. Degeneration of nodosities as well as nodosity occupation rates could be related to the calculated abundances of grape phylloxera population. Further ecological questions considering grape root development (e.g. relation between moisture and root development) and grape phylloxera population development (e.g. relation between temperature and population structure) could be answered for field conditions.

Generally, the presented work provides an approach to evaluate vitality of grape root systems. This approach can be useful, considering the development of control strategies against soilborne pests in viticulture (e.g. grape phylloxera, *Sorospheara viticola*, *Roesleria subterranea* (Weinm.) Redhaed) as

well as considering the evaluation of integrated management systems in viticulture.

8.2 Zusammenfassung

Die Entwicklung und das Wachstum von Pflanzen ist beeinflusst von Interaktionen zwischen der Pflanzenwurzel, wurzelbewohnenden Organismen und den Zönosen in der Rhizosphäre. Bei mehrjährigen, verholzten Pflanzen erschweren die Komplexität der Wurzelsystemstruktur sowie standzeitbedingte Änderungen in der Physiologie die Entwicklung von Methoden zur Erfassung solcher Interaktionen. Insbesondere Rebwurzelsysteme sind in dieser Hinsicht kaum untersucht. Ziel der Arbeit war die Entwicklung einer Methode zur Erfassung und Vorhersage von Interaktionen im Wurzelbereich von Unterlagsreben (*Vitis berlandieri* x *Vitis riparia*). Dazu wurde die Reblaus (*Daktulosphaira vitifoliae* Fitch, Hemiptera, Aphidoidea) als wurzelparasitierender Modellorganismus herangezogen.

Zur Entwicklung dieser Methode wurde ein Langzeitversuch (Juni 2006 - August 2009) auf einer kommerziell genutzten Rebfläche in Geisenheim/Rheingau eingerichtet. In Abständen von zwei bis acht Wochen (n=8-10) wurde den obersten 20 cm des Unterstockbereichs Wurzel- und Bodenmaterial entnommen. Um die jahreszeitliche, räumliche und sortenspezifische Dynamik des Wurzelwachstums mit einzubeziehen, wurden morphologische Eigenschaften der Wurzeln digital ermittelt. Die Erfassung und Charakterisierung der Reblauspopulation erfolgte visuell an Hand der Larvenstadien (unterschieden wurden Ovipare, Nicht-Ovipare und Vorstufen zu geflügelten Individuen). Die Charakterisierung der Infektionsstellen (Nodositäten) erfolgte z.T. mit Hilfe digitaler Farbanalysen des Wurzelmaterials. Da insbesondere bei Reblausbefall wurzelbewohnende Pilze entscheidende Effekte auf die Vitalität von Reben haben können, wurden pilzliche Endophyten aus Nodositäten und nicht infiziertem Wurzelmaterial isoliert und nach Morphotypen eingeteilt. Weiterhin wurden abiotische (pH, OBS, Wassergehalt) und biotische (Basisrespiration) Bodenparameter erfasst. Die jahreszeitliche, räumliche oder variantenabhängige Sensitivität einzelner Parameter wurde mit Omnibus Tests (ANOVAs) und anschließenden Post-hoc Tests analysiert. Zusammenhänge zwischen den Parametern wurden mit multiplen Regressionsmodellen analysiert.

Es konnten quantitative Parameter zur Erfassung der Degeneration der Nodositäten, der Wurzelentwicklung an Nodositäten sowie zur Abgrenzung gegenüber anderen Wurzelschwellungen entwickelt werden. Signifikante Unterschiede ergaben sich zwischen Wurzellänge einbeziehenden und ignorierenden Abundanzberechnungen. Weiterhin konnten hohe temporale (monatliche), räumliche (Hangneigungsunterschiede) und sortenspezifische Sensitivität bei der Darstellung von Wurzelsystem- und Reblausparametern nachgewiesen werden. Signifikante Unterschiede in der Häufigkeit endophytischer Morphotypen zwischen Wurzel- und Nodositätengewebe sowie zwischen den einzelnen Unterlagssorten konnten ebenfalls festgestellt werden. Desweiteren wurden Degenerationserscheinungen von Nodositäten sowie die Dichte der Nodositätenbesiedlung in Zusammenhang mit Dichten der Reblauspopulation gebracht. Darüber hinaus konnten ökologische Fragestellungen zur Rebwurzelentwicklung (z.B. Einfluss von Bodenfeuchtigkeit auf das Wachstum in verschiedenen Dickenklassen) und Reblausökologie (z.B. Temperaturabhängigkeit der Populationsstruktur) beantwortet werden. Die vorgestellte Methode bietet einen Ansatz zur Bewertung von Vitalitätseigenschaften von Rebwurzelsystemen. Damit ist sie im Hinblick

auf die Entwicklung effektiver Kontrollmaßnahmen bodenbürtiger Schaderreger (z.B. Reblaus, *Sorosphaera viticola*, *Roesleria subterranea* (Weinm.) Redhaed) ebenso von Bedeutung wie zur Bewertung und Entwicklung nachhaltiger Bodenmanagementsysteme.

A Shortcuts

APD	Absolute Phylloxera Density (per cm root length or per root DW)
BSR	Basic Soil Respiration
CNA	Combined Nodosity Attributes
DA	Discriminant Analysis
DW	Dry Weight
DOF	Degree of Freedom
FA	Factor Analysis
GSA	Gray Scale Analysis
lv	middle brownish
MLR	Multiple linear Regression
NDM	Nutrient Deficit Medium
NLR	Normal linear Regression
Nod	Nodosity
NOP	Nodosity Occupation Parameters
PCA	Principal Component Analysis
Phyll	Grape phylloxera
RPD	Relative Phylloxera Density (per 10^{-2}sqm surface area and in 20 cm depth under foliage wall)
SD	Standard Deviation
SEM	Structural Equation Model
SOM	Soil Organic Matter
SU	Sample Units
sqm	square meter
sv	dark brownish
SWC	Soil Water Content
TLU	transmitted light unit
ttl	total
unpb	unpublished
uv	light to non brownish
WDR	Wide Dark Range
WPR	Wide Pale Range

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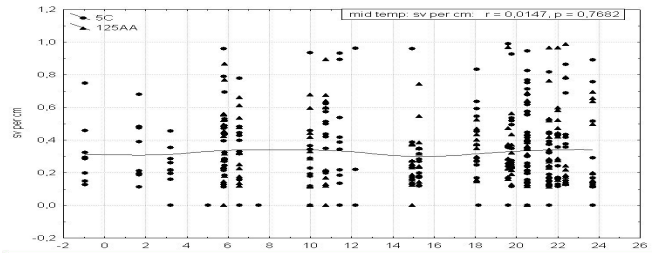
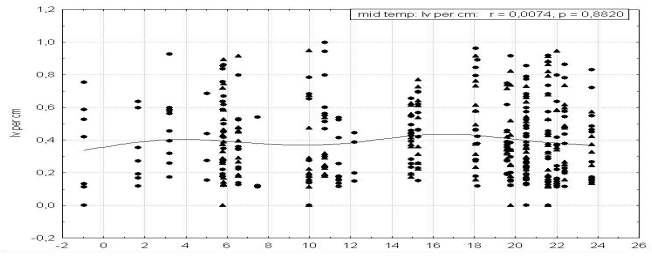
C Appendix



(a) sv per cm



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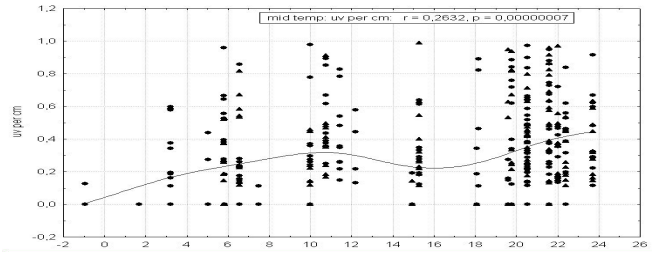

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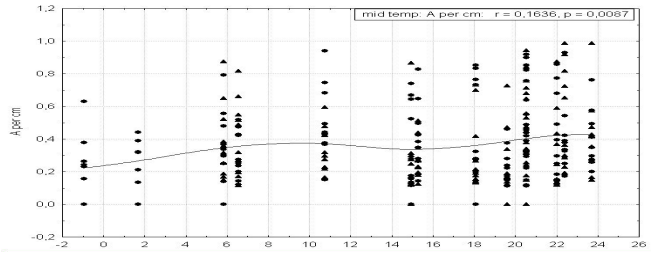
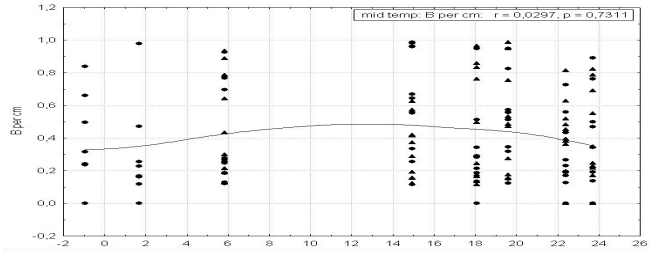
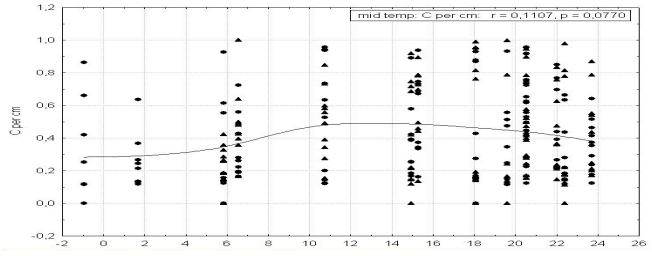


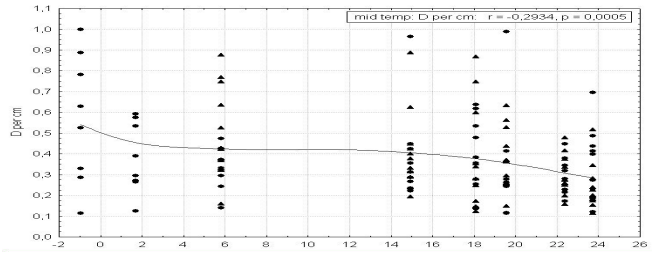
(d) A per cm



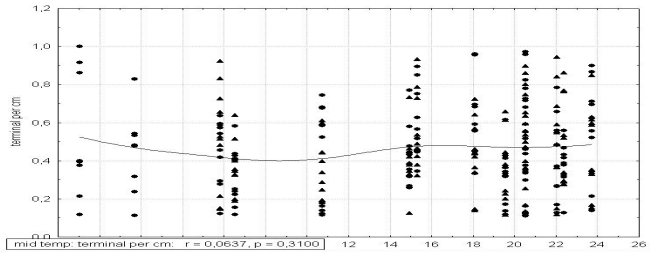
(e) B per cm



(f) C per cm

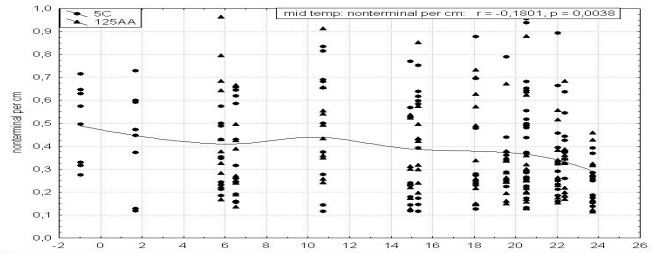


(g) D per cm

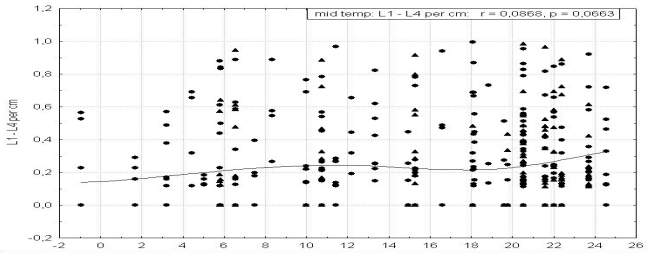


(h) term per cm

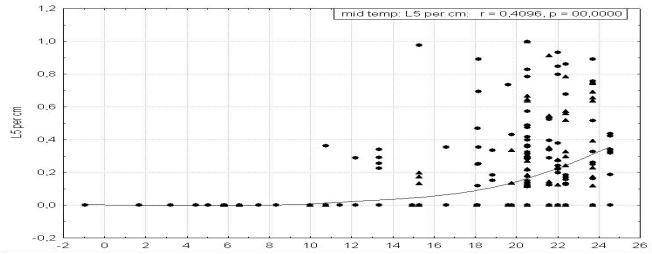
Figure 43: Grape Phylloxera Traits - Soil Temperature. **A:** sv per cm. **B:** lv per cm. **C:** uv per cm. **D:** A per cm. **E:** B per cm. **F:** C per cm. **G:** D per cm. **H:** terminal per cm.



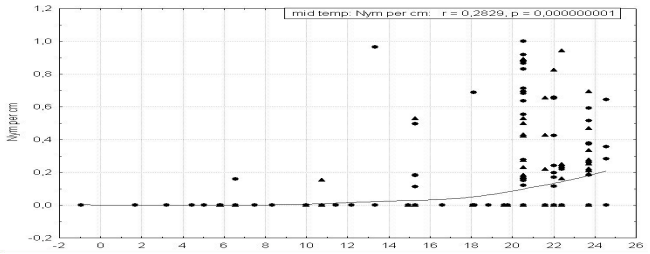
(a) non-term per cm



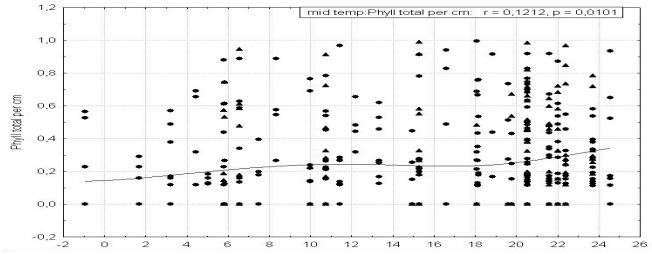
(b) L1-L4 per cm



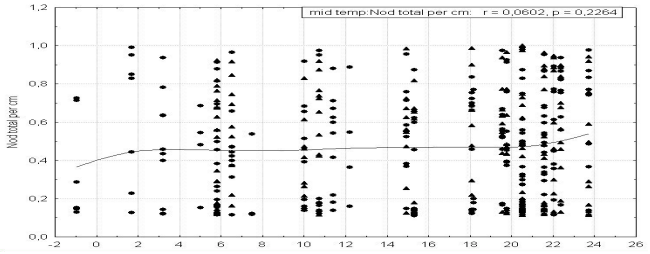
(c) L5 per cm



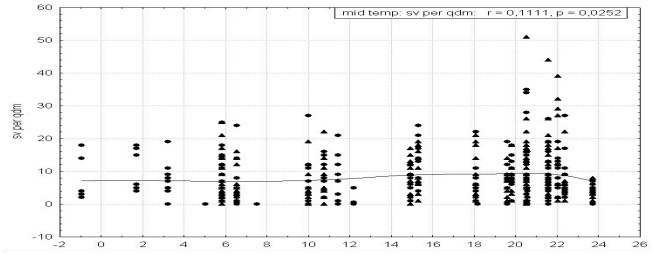
(d) Nym per cm



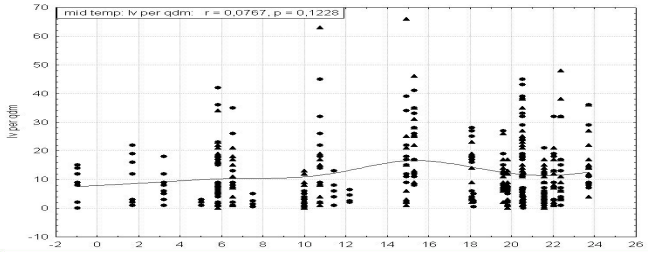
(e) ttl inst per cm



(f) ttl nodos per cm

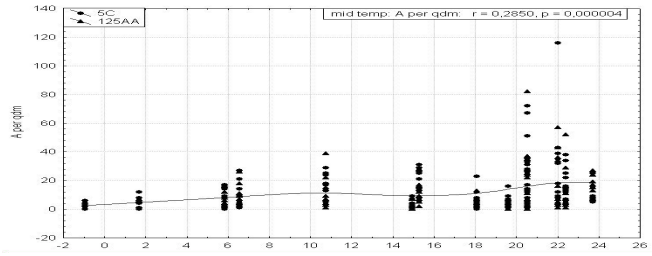


(g) sv per 10^{-2}sqm

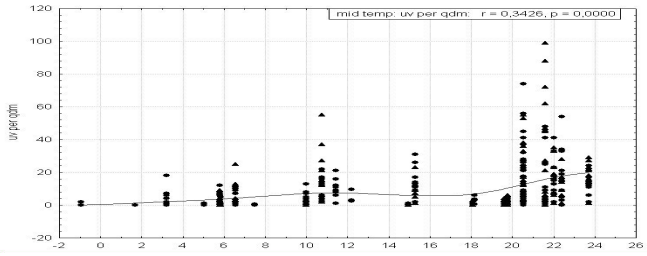


(h) lv per 10^{-2}sqm

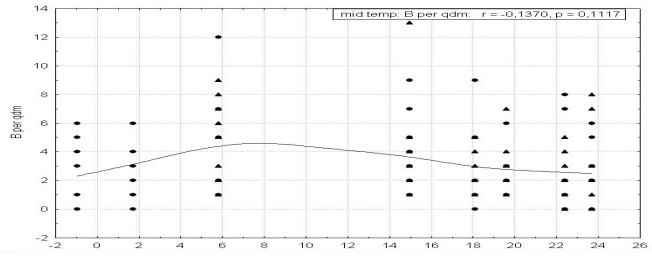
Figure 44: Grape Phylloxera Traits - Soil Temperature. **A:** non-terminal per cm. **B:** L1-L4 per cm. **C:** L5 per cm. **D:** Nym per cm. **E:** Total instars per cm. **F:** Total nodostities per cm. **G:** sv per 10^{-2}sqm . **H:** lv per 10^{-2}sqm .



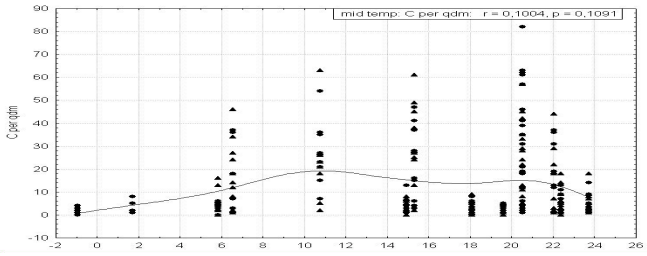
(a) A per 10^{-2}sqm



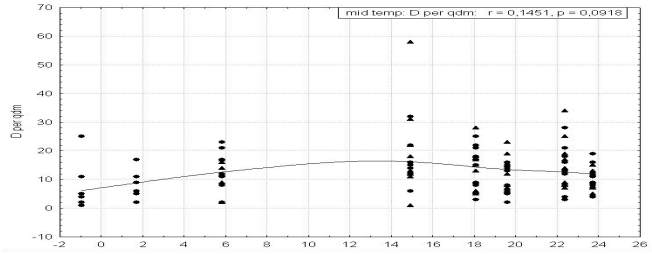
(b) uv per 10^{-2}sqm



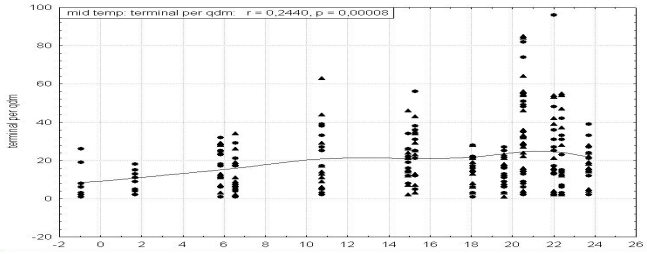
(c) B per 10^{-2}sqm



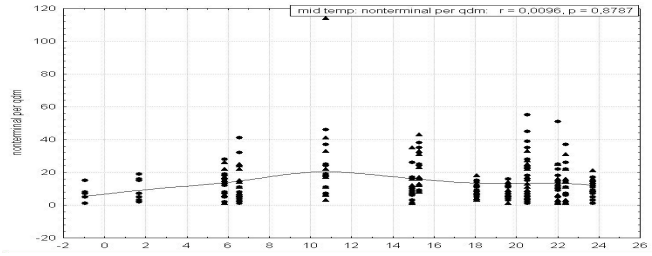
(d) C per 10^{-2}sqm



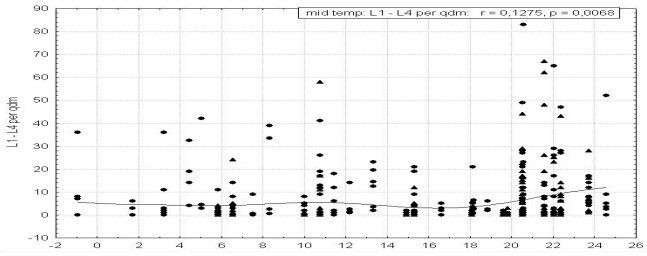
(e) D per 10^{-2}sqm



(f) term per 10^{-2}sqm

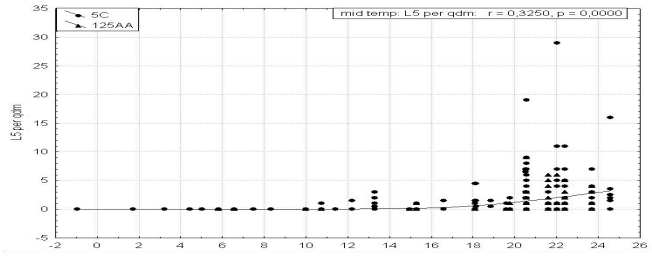


(g) non-term per 10^{-2}sqm

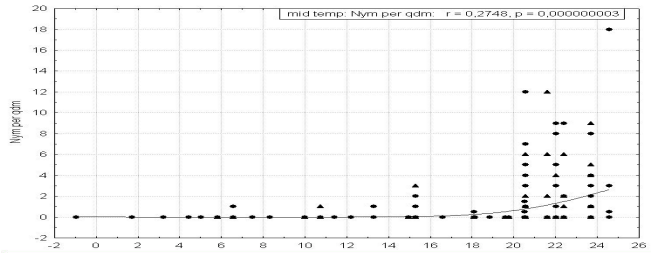


(h) L1-L4 per 10^{-2}sqm

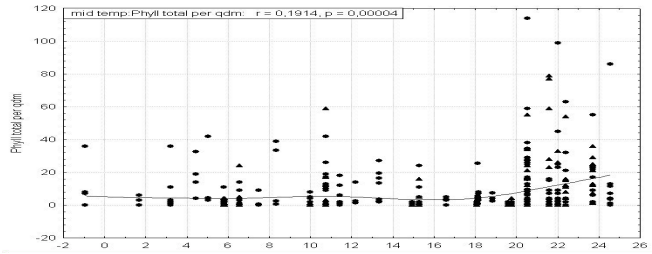
Figure 45: Grape Phylloxera Traits - Soil Temperature. **A:** A per 10^{-2}sqm . **B:** uv per 10^{-2}sqm . **C:** B per 10^{-2}sqm . **D:** C per 10^{-2}sqm . **E:** D per 10^{-2}sqm . **F:** terminal per 10^{-2}sqm . **G:** non-terminal per 10^{-2}sqm . **H:** L1-L4 per 10^{-2}sqm .



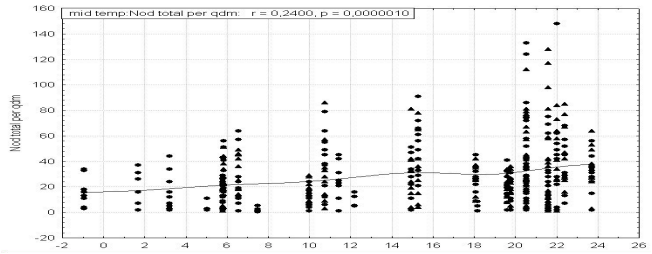
(a) L5 per 10^{-2}sqm



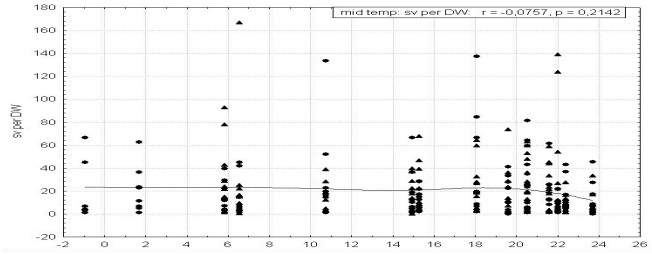
(b) Nym per 10^{-2}sqm



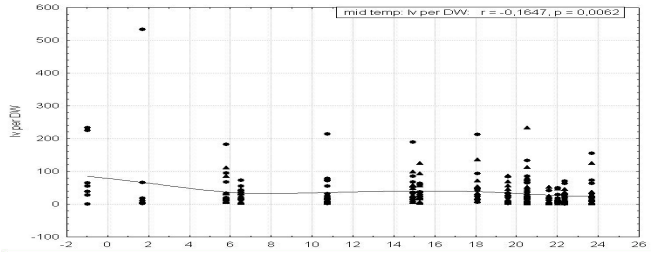
(c) ttl inst per 10^{-2}sqm



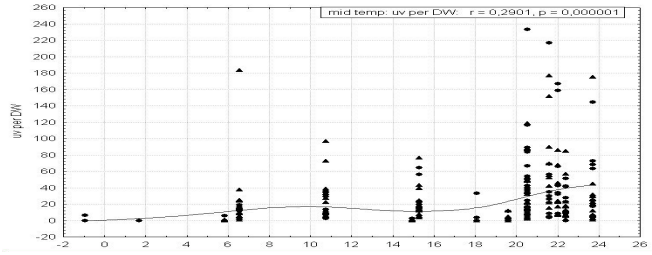
(d) ttl nod per 10^{-2}sqm



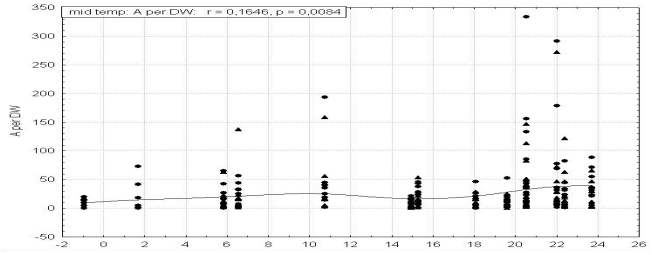
(e) sv per DW



(f) lv per DW

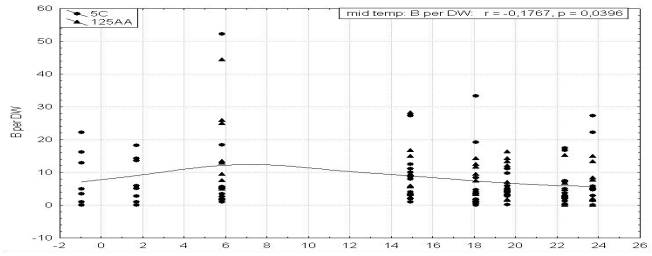


(g) uv per DW

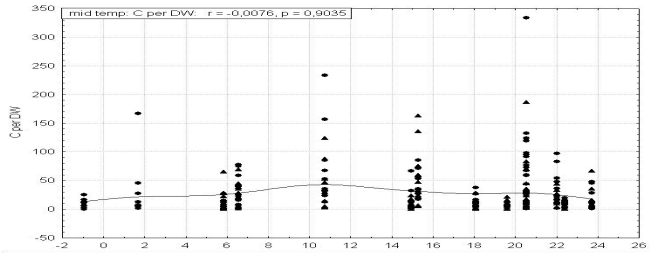


(h) A per DW

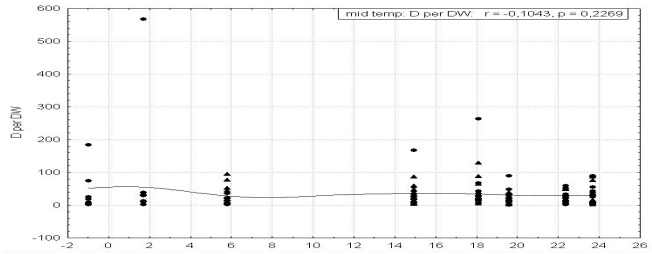
Figure 46: Grape Phylloxera Traits - Soil Temperature. **A:** L5 per 10^{-2}sqm . **B:** Nym per 10^{-2}sqm . **C:** Total instars per 10^{-2}sqm . **D:** Total nodosities per 10^{-2}sqm . **E:** sv per DW. **F:** lv per DW. **G:** uv per DW. **H:** A per DW.



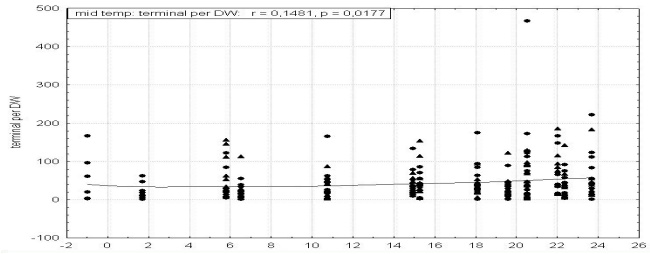
(a) B per DW



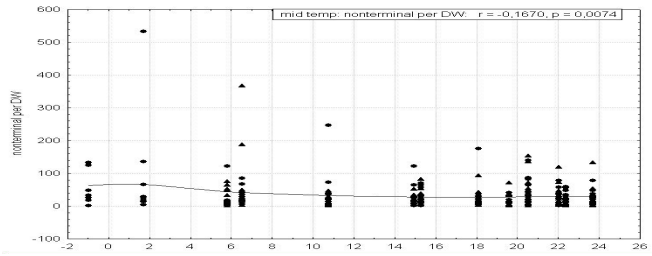
(b) C per DW



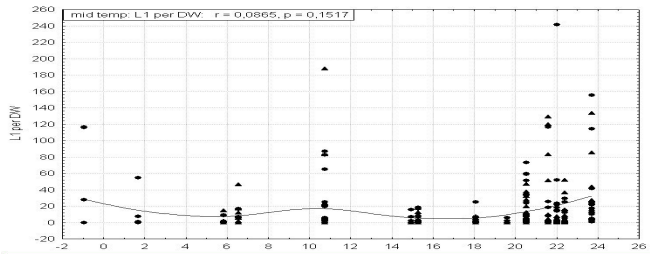
(c) D per DW



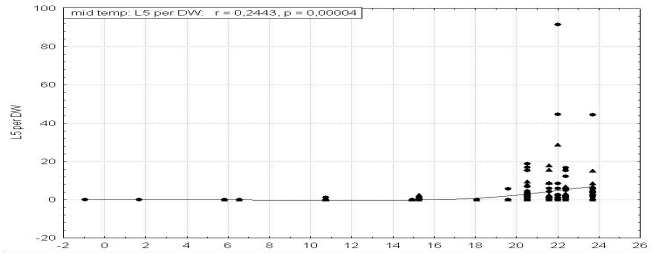
(d) term per DW



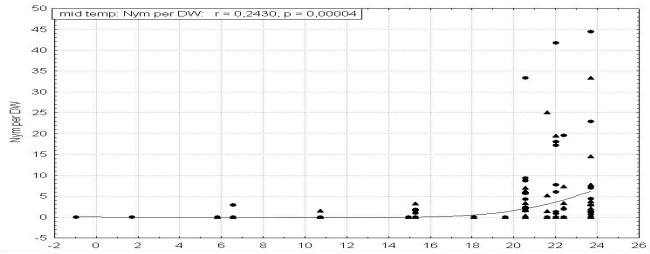
(e) non-term per DW



(f) L1-L4 per DW

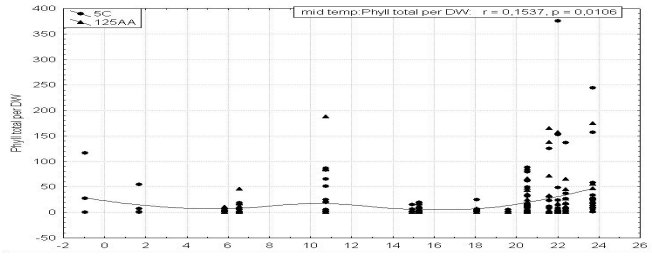


(g) L5 per DW

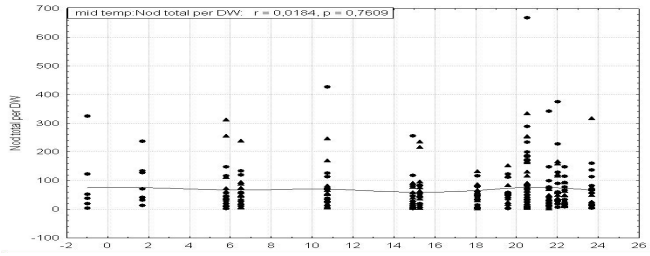


(h) Nym per DW

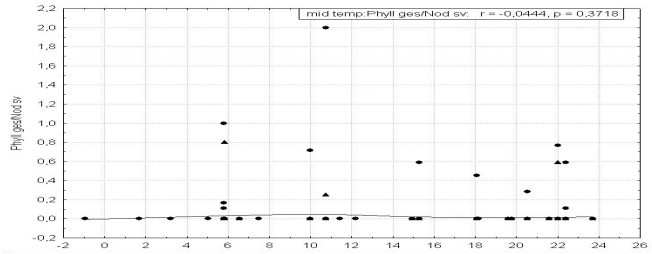
Figure 47: Grape Phylloxera Traits - Soil Temperature. **A:** B per DW. **B:** C per DW. **C:** D per DW. **D:** terminal per DW. **E:** non-terminal per DW. **F:** L1-L4 per DW. **G:** L5 per DW. **H:** Nym per DW.



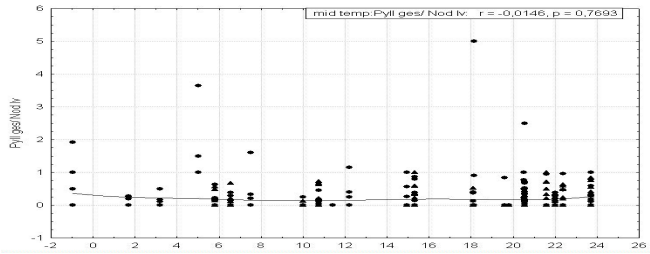
(a) ttl inst per DW



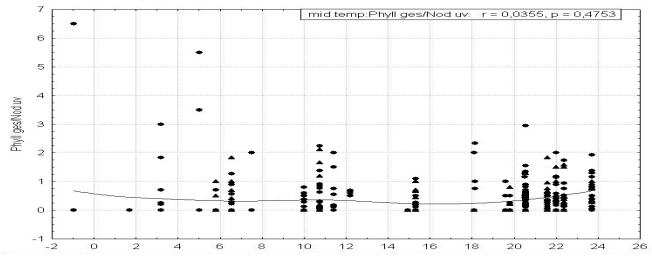
(b) ttl nod per DW



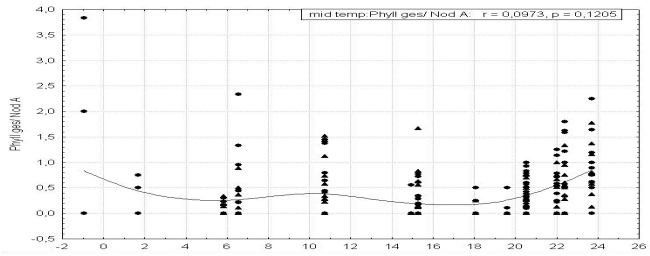
(c) ttl inst per sv



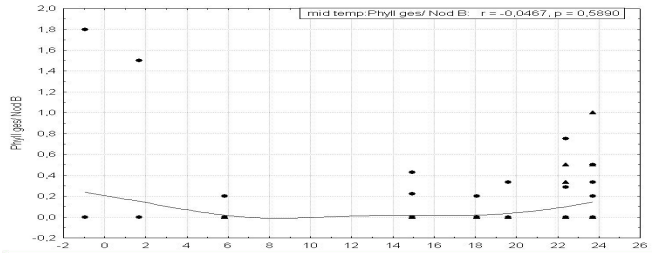
(d) ttl inst per lv



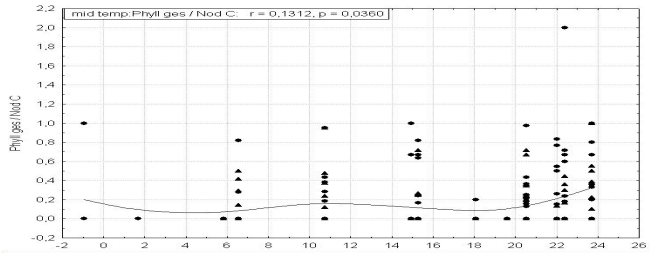
(e) ttl inst per uv



(f) ttl inst per A

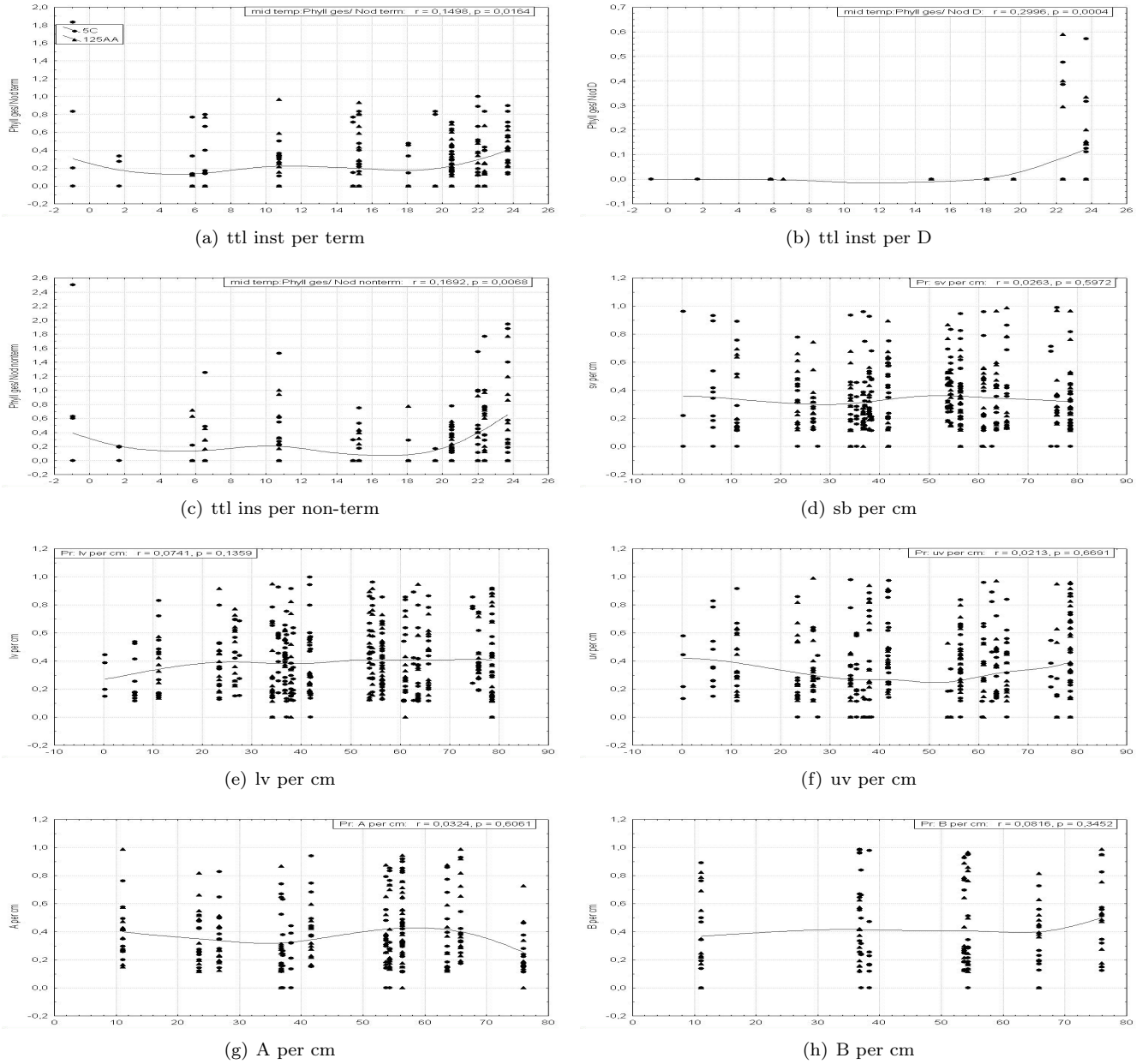


(g) ttl inst per B



(h) ttl inst per C

Figure 48: Grape Phylloxera Traits - Soil Temperature. **A:** Total instars per DW. **B:** Total nodosities per DW. **C:** Ttl. inst. per sv. **D:** Ttl. inst. per lv. **E:** Ttl. inst. per uv. **F:** Ttl. inst. per A. **G:** Ttl. inst. per B. **H:** Ttl. inst. per C.



(a) ttl inst per term

(b) ttl inst per D

(c) ttl ins per non-term

(d) sb per cm

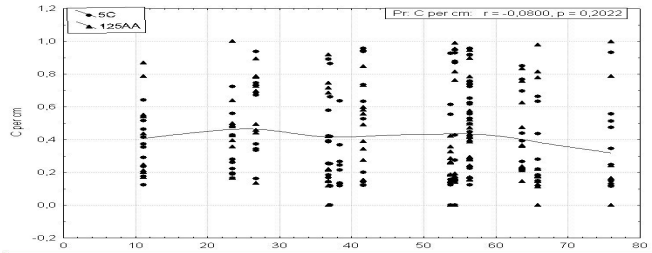
(e) lv per cm

(f) uv per cm

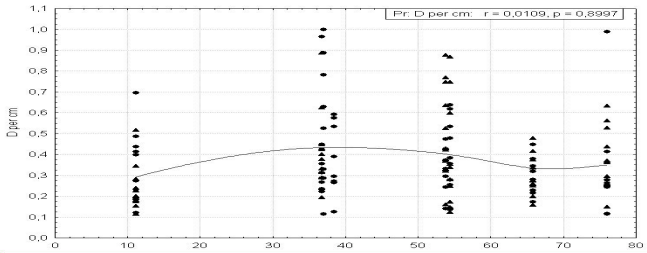
(g) A per cm

(h) B per cm

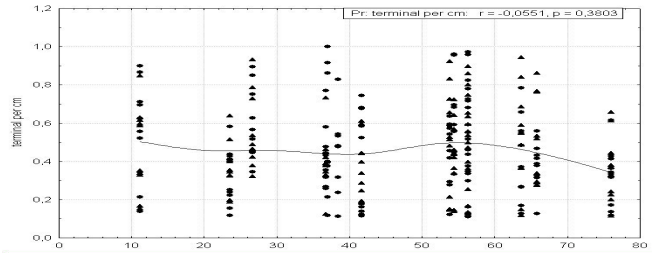
Figure 49: Grape Phylloxera Traits - **A-C:** Soil temperature. **D-H:** Soil Moisture**A:** Ttl. inst. per term. **B:** Ttl. inst. per D. **C:** Ttl. inst. per non-term. **D:** sv per cm (moisture). **E:** lv per cm (moisture). **F:** uv per cm (moisture). **G:** A per cm (moisture). **H:** B per cm (moisture)



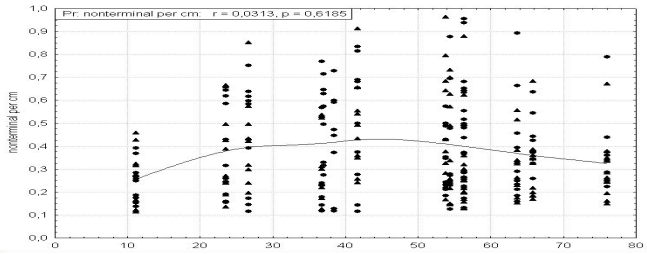
(a) C per cm



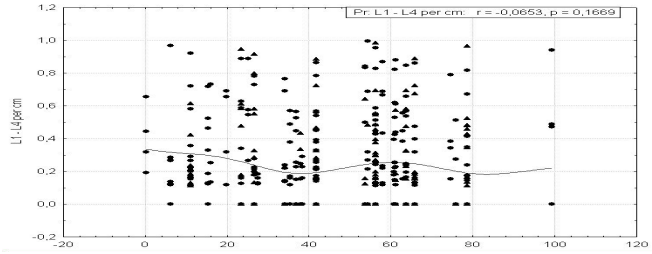
(b) D per cm



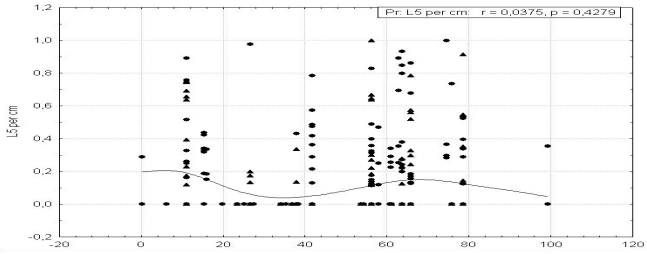
(c) term per cm



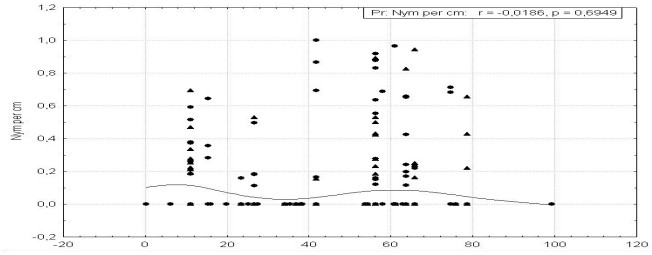
(d) non-term per cm



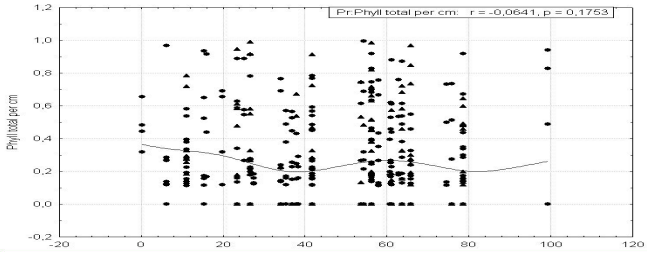
(e) L1-L4 per cm



(f) L5 per cm

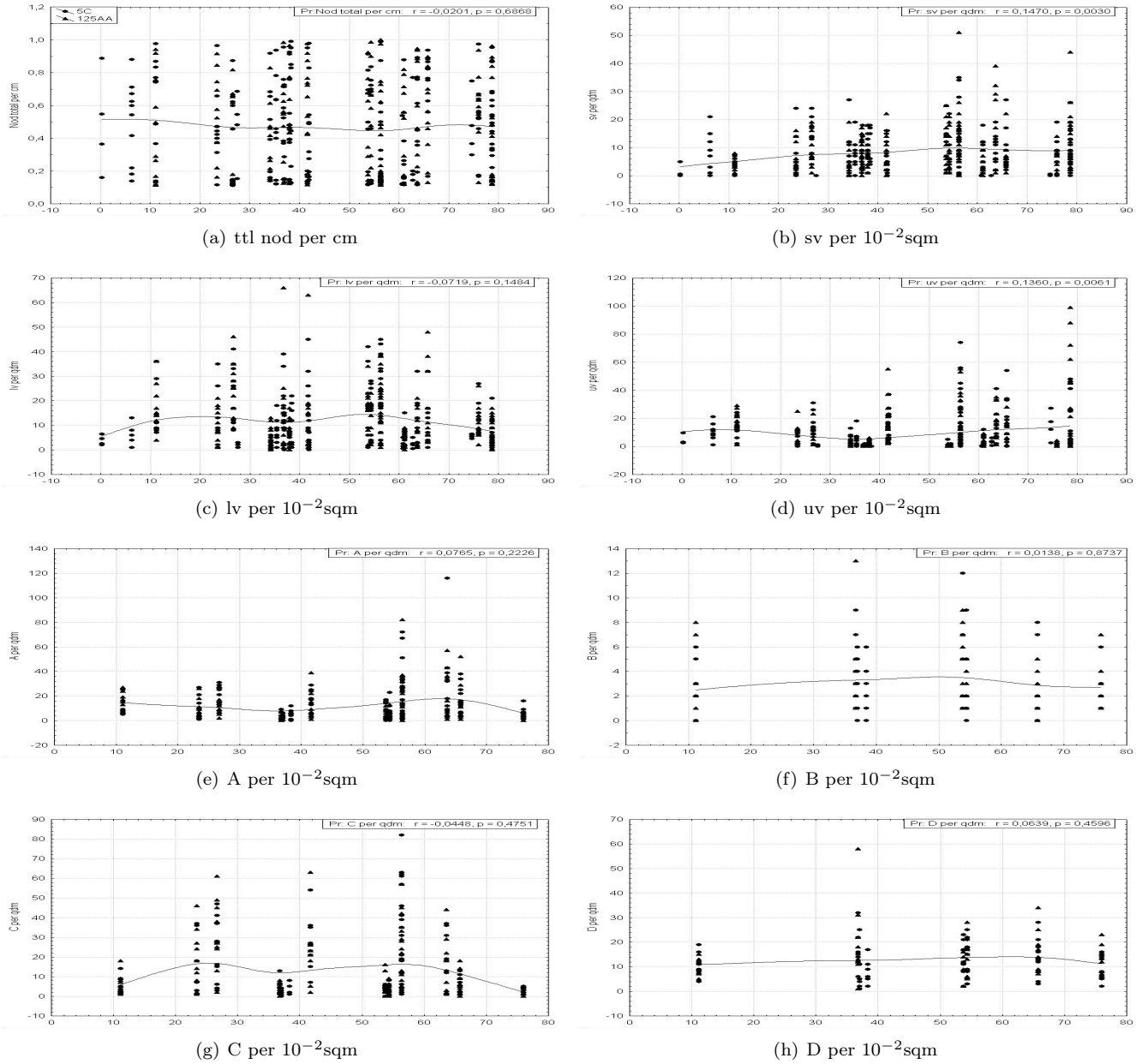


(g) Nym per cm



(h) ttl inst per cm

Figure 50: Grape Phylloxera - Soil Moisture. **A:** C per cm. **B:** D per cm. **C:** terminal per cm. **D:** non-terminal per cm. **E:** L1-L4 per cm. **F:** L5 per cm. **G:** Nym per cm. **H:** Total instars per cm.



(a) ttl nod per cm

(b) sv per 10^{-2}sqm

(c) lv per 10^{-2}sqm

(d) uv per 10^{-2}sqm

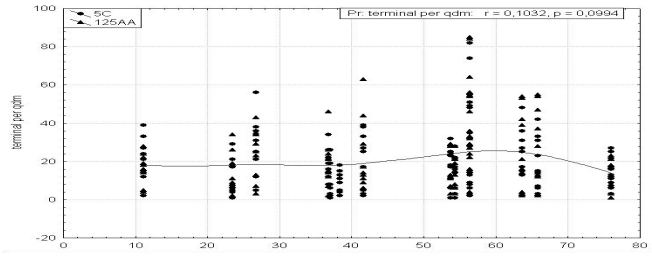
(e) A per 10^{-2}sqm

(f) B per 10^{-2}sqm

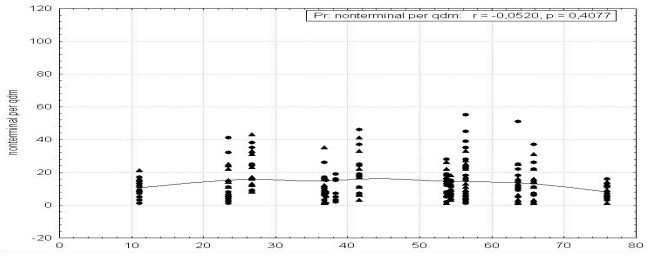
(g) C per 10^{-2}sqm

(h) D per 10^{-2}sqm

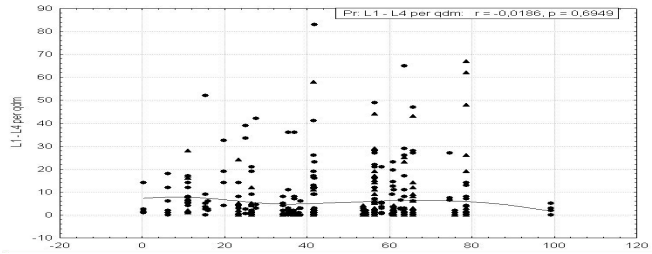
Figure 51: Grape Phylloxera - Soil Moisture. **A:** Total nodosities per cm. **B:** sv per 10^{-2}sqm . **C:** lv per 10^{-2}sqm . **D:** uv per 10^{-2}sqm . **E:** A per 10^{-2}sqm . **F:** B per 10^{-2}sqm . **G:** C per 10^{-2}sqm . **H:** D per 10^{-2}sqm .



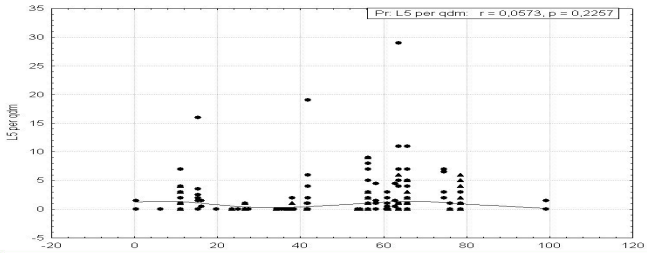
(a) term per 10^{-2}sqm



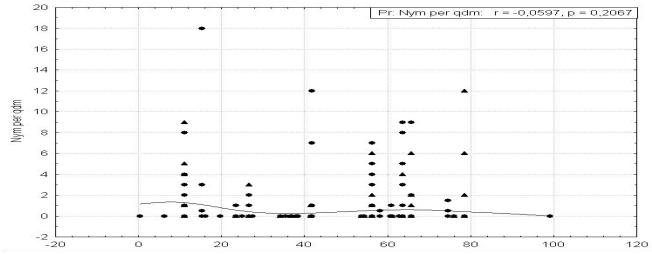
(b) non-term per 10^{-2}sqm



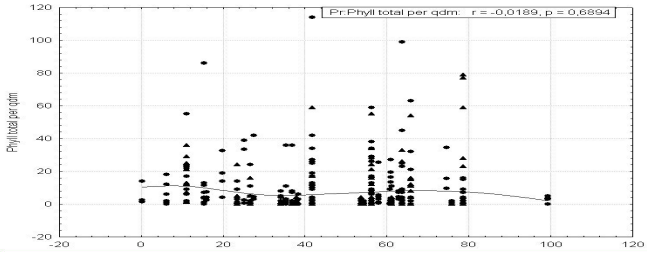
(c) L1-L4 per 10^{-2}sqm



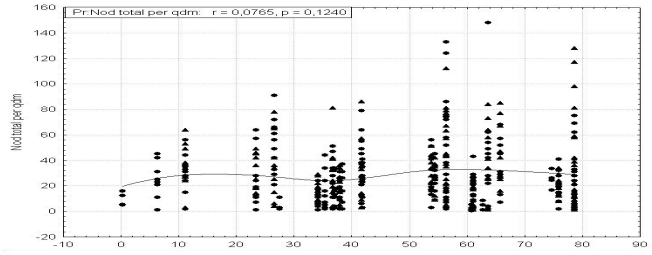
(d) L5 per 10^{-2}sqm



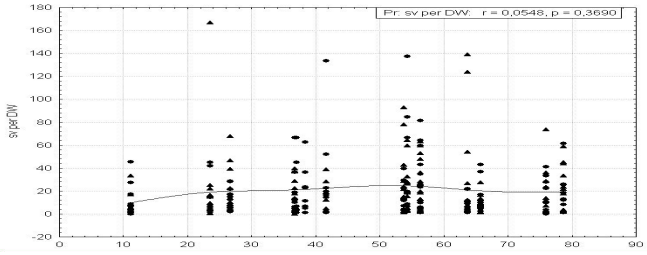
(e) Nym per 10^{-2}sqm



(f) ttl inst per 10^{-2}sqm

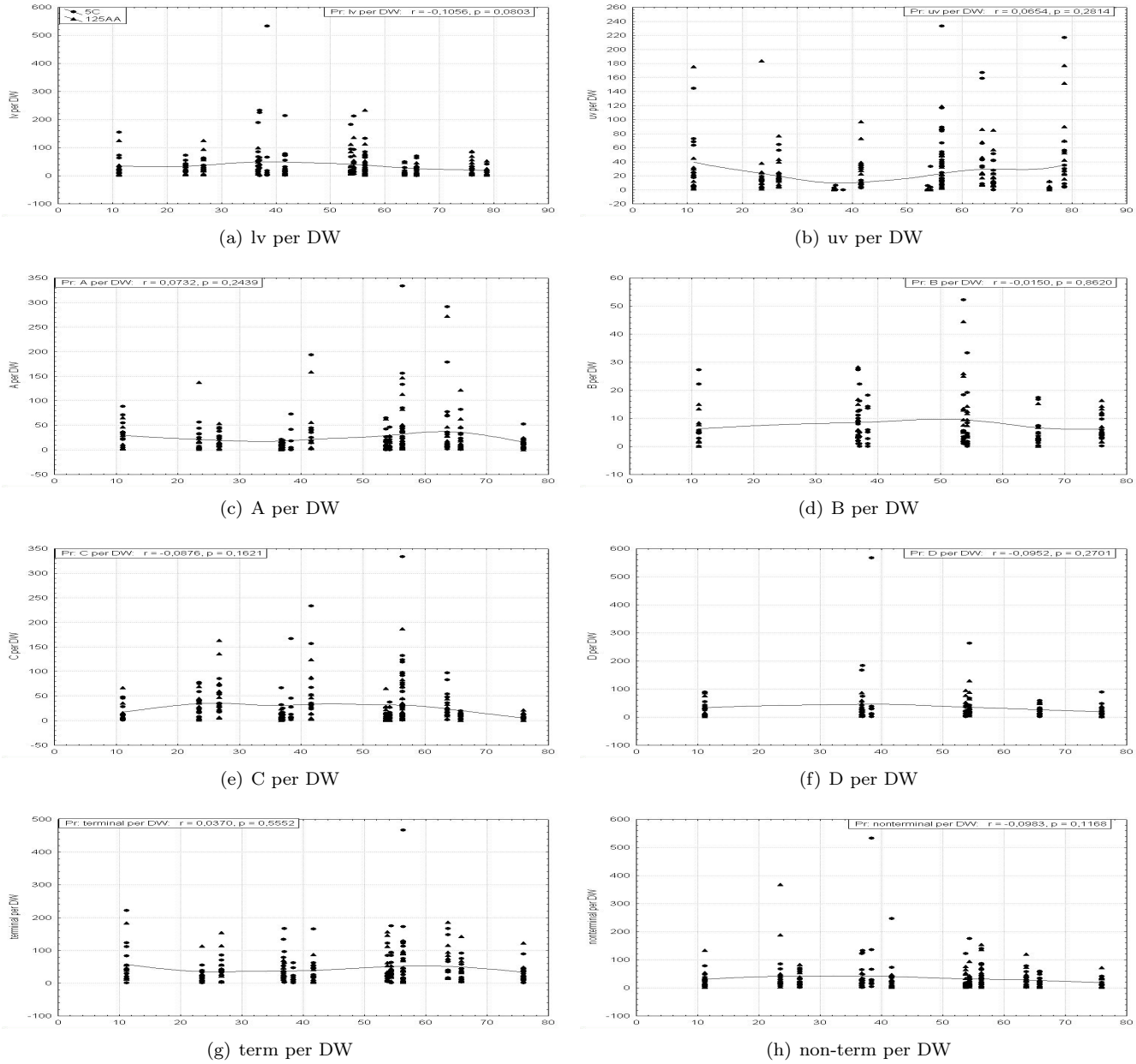


(g) ttl nod per 10^{-2}sqm



(h) sv per DW

Figure 52: Grape Phylloxera - Soil Moisture. **A:** terminal per 10^{-2}sqm . **B:** non-terminal per 10^{-2}sqm . **C:** L1-L4 per 10^{-2}sqm . **D:** L5 per 10^{-2}sqm . **E:** Nym per 10^{-2}sqm . **F:** total instars per 10^{-2}sqm . **G:** total nodosities per 10^{-2}sqm . **H:** sv per DW.



(a) lv per DW

(b) uv per DW

(c) A per DW

(d) B per DW

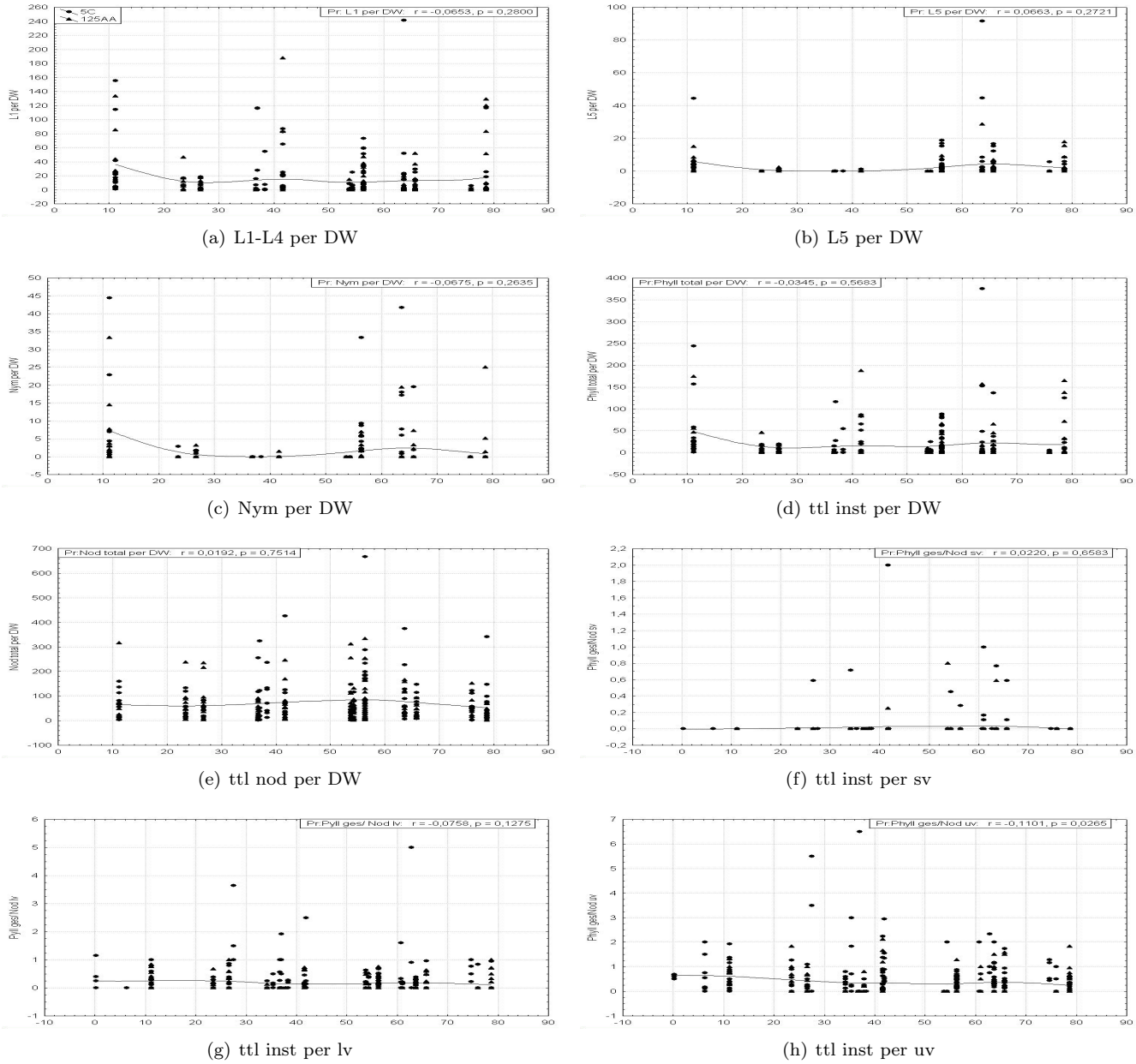
(e) C per DW

(f) D per DW

(g) term per DW

(h) non-term per DW

Figure 53: Grape Phylloxera - Soil Moisture. **A:** lv per DW. **B:** uv per DW. **C:** A per DW. **D:** B per DW. **E:** C per DW. **F:** D per DW. **G:** terminal per DW. **H:** non-terminal per DW.



(a) L1-L4 per DW

(b) L5 per DW

(c) Nym per DW

(d) ttl inst per DW

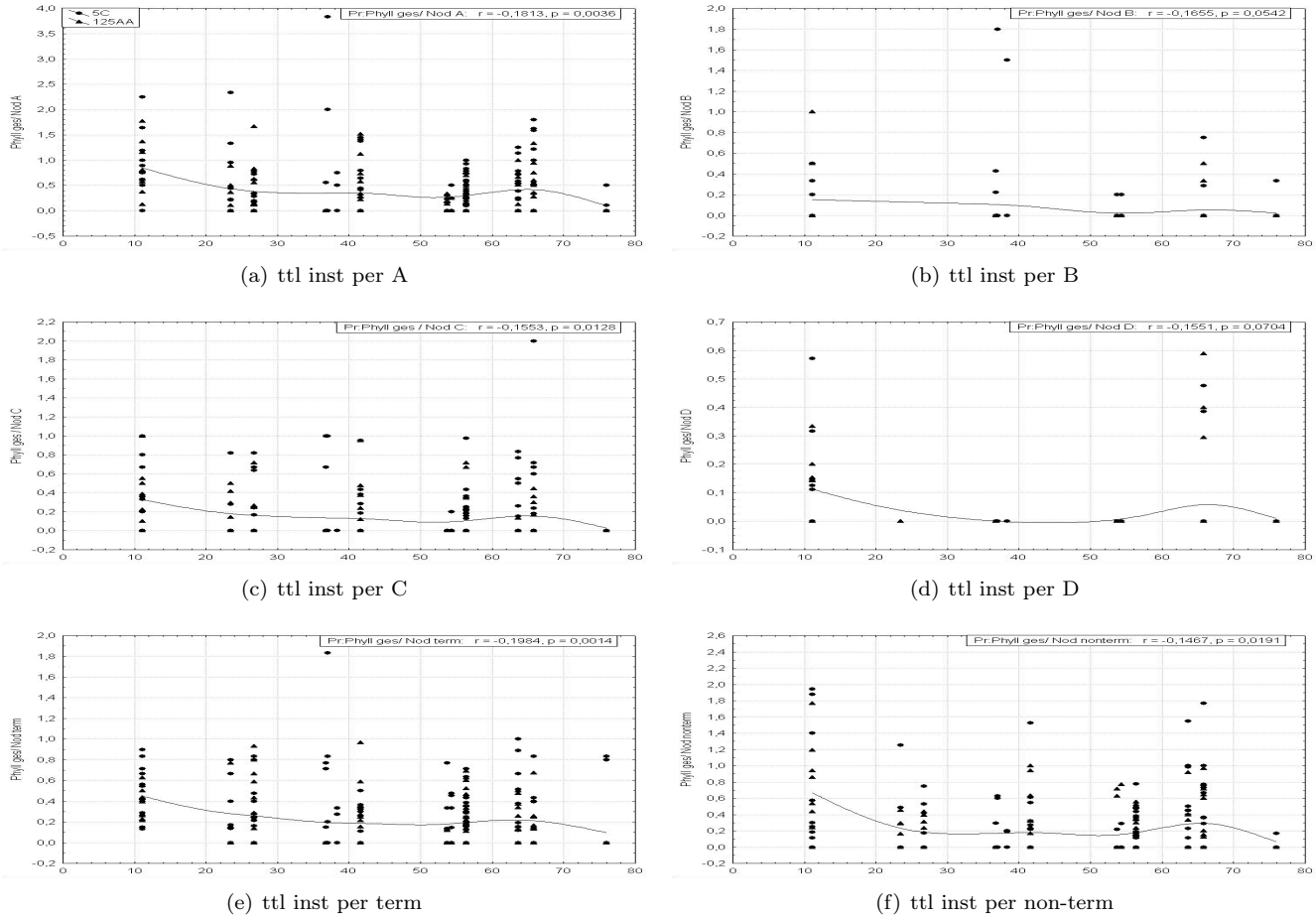
(e) ttl nod per DW

(f) ttl inst per sv

(g) ttl inst per lv

(h) ttl inst per uv

Figure 54: Grape Phylloxera - Soil Moisture. **A:** L1-L4 per DW. **B:** L5 per DW. **C:** Nym per DW. **D:** total instars per DW. **E:** total nodosities per DW. **F:** Ttl. inst. per sv. **G:** Ttl. inst. per lv. **H:** Ttl. inst. per uv.



(a) ttl inst per A

(b) ttl inst per B

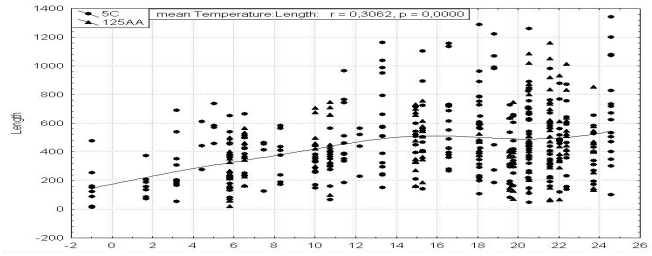
(c) ttl inst per C

(d) ttl inst per D

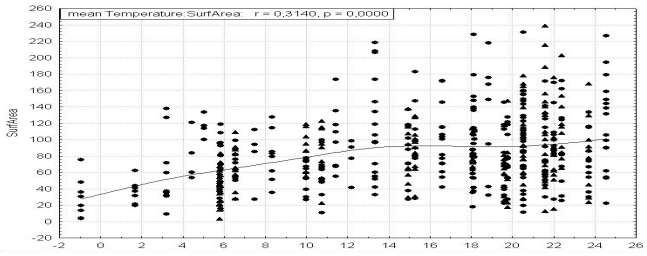
(e) ttl inst per term

(f) ttl inst per non-term

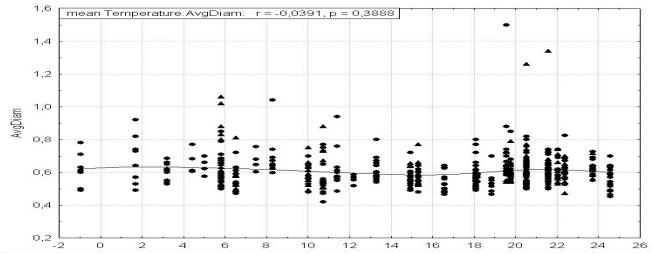
Figure 55: Grape Phylloxera - Soil Moisture. **A:** Ttl. inst. per A. **B:** Ttl. inst. per B. **C:** Ttl. inst. per C. **D:** Ttl. inst. per D. **E:** Ttl. inst. per term. **F:** Ttl. inst. per non-term.



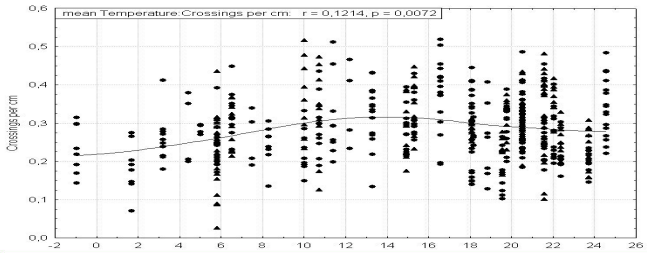
(a) Length



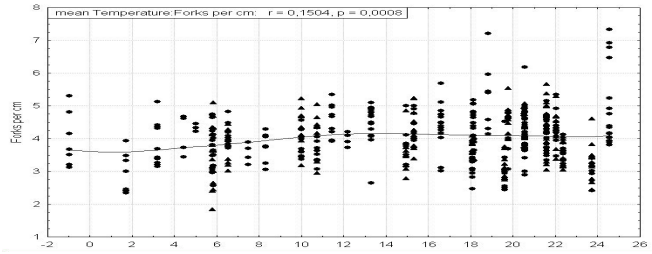
(b) Surface Area



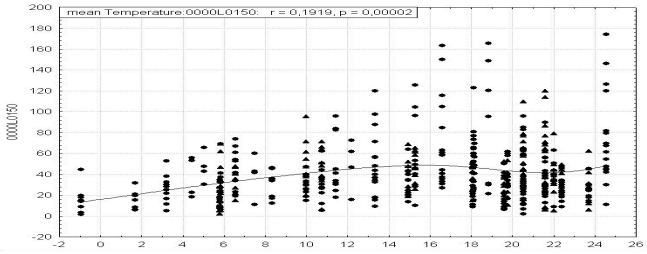
(c) Average Diameter



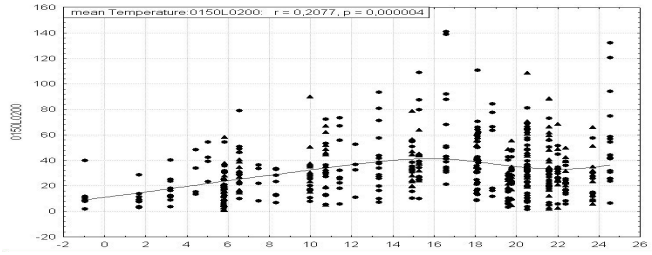
(d) Cross per cm



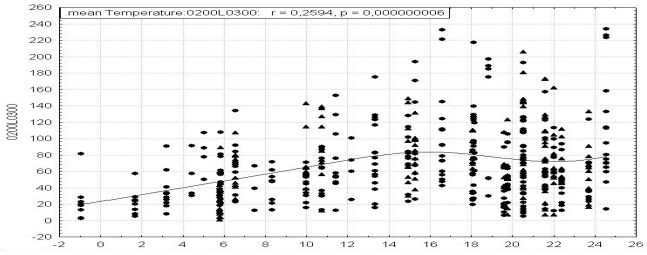
(e) Forks per cm



(f) Length 0-0.15mm

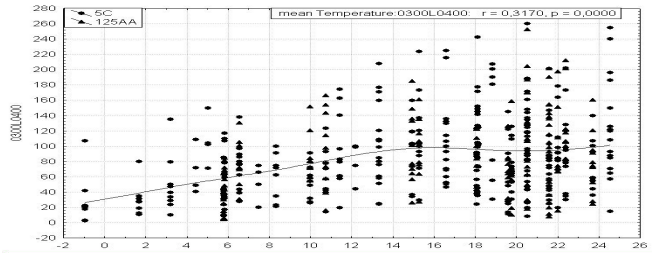


(g) 0.15-0.2mm

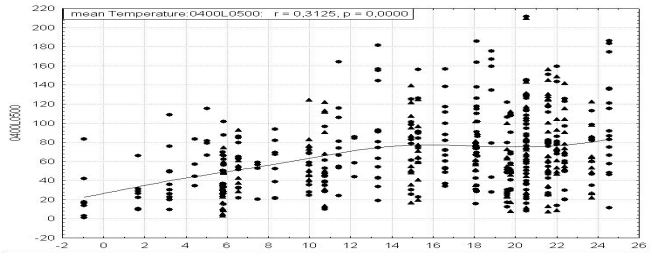


(h) 0.2-0.3mm

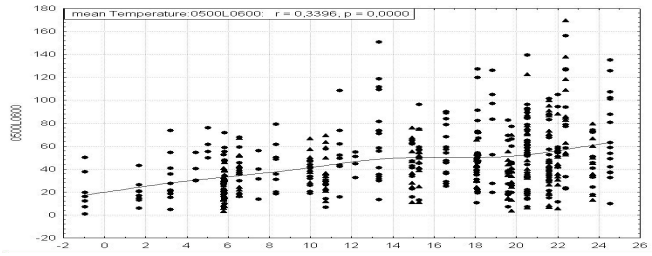
Figure 56: Root System Traits - Soil Temperature. **A:** Length. **B:** Surface area. **C:** Average diameter. **D:**Crossing per cm. **E:** Forks per cm. **F:** Length 0-0.15mm. **G:** Length 0.15-0.2mm. **H:** Length 0.2-0.3mm.



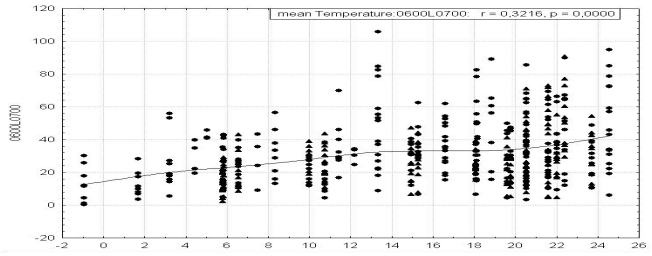
(a) Length 0.3-0.4mm



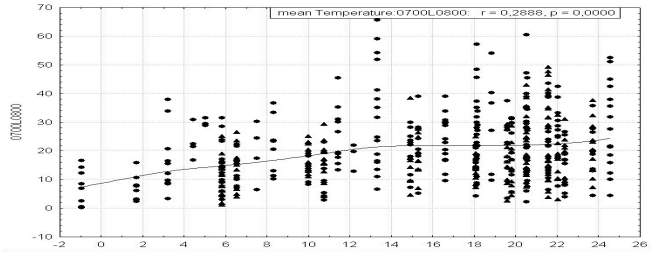
(b) 0.4-0.5mm



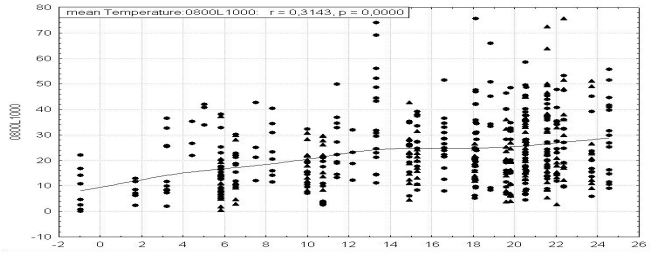
(c) 0.5-0.6mm



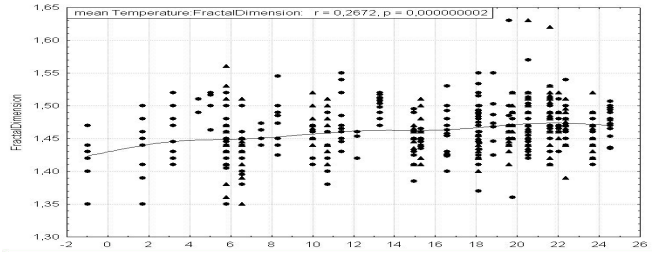
(d) 0.6-0.7mm



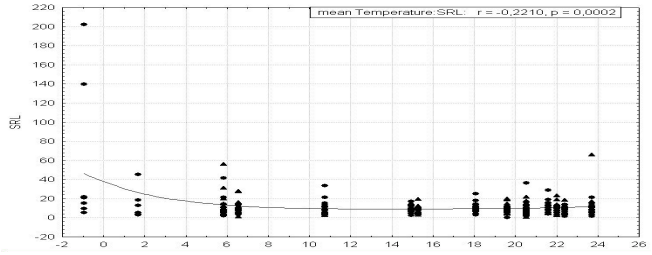
(e) 0.7-0.8mm



(f) 0.8-1mm

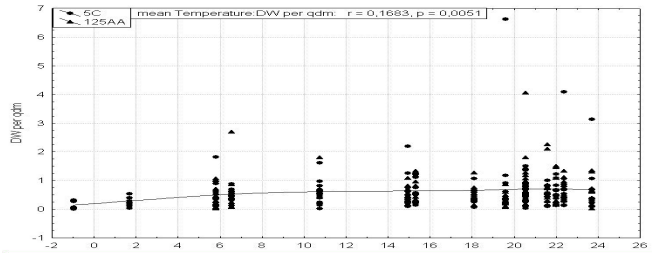


(g) Fractal Dimension

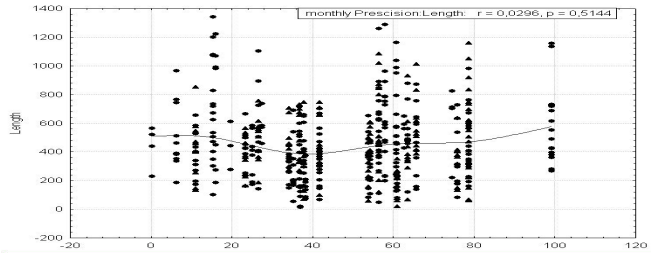


(h) SRL

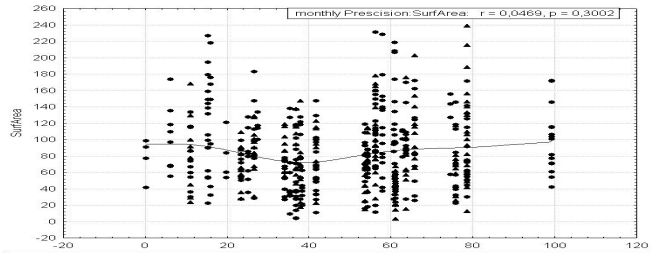
Figure 57: Root System Traits - Soil Temperature. **A:** Length 0.3-0.4mm. **B:** Length 0.4-0.5mm. **C:** Length 0.5-0.6mm. **D:** Length 0.6-0.7mm. **E:** Length 0.7-0.8mm. **F:** Length 0.8-1mm. **G:** Fractal dimension. **H:** SRL.



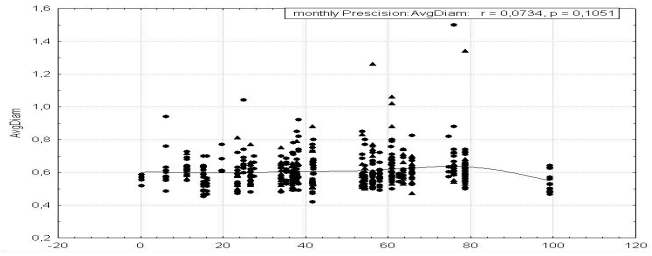
(a) DW (vs. soil temp)



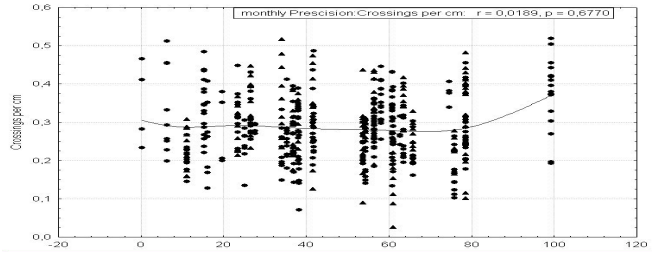
(b) Length



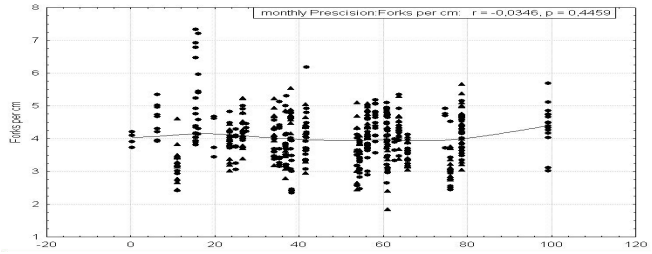
(c) Surface Area



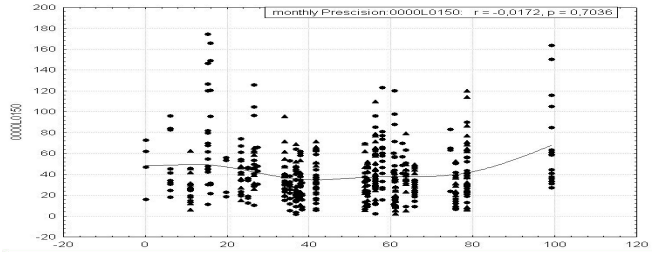
(d) Average Diameter



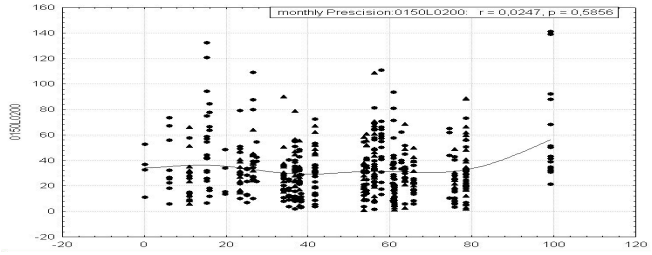
(e) Crossing per cm



(f) Forks per cm

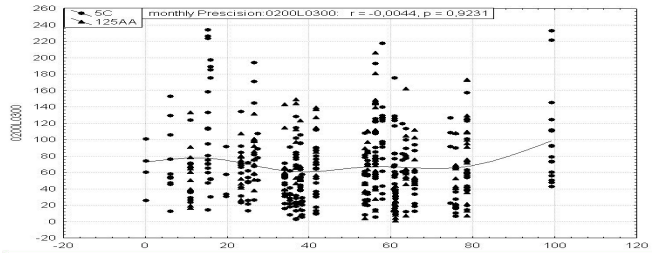


(g) Length 0-0.15mm

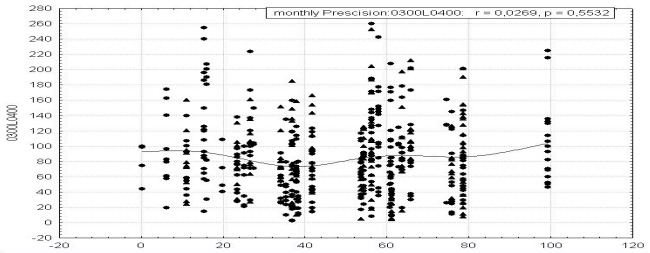


(h) 0.15-0.2mm

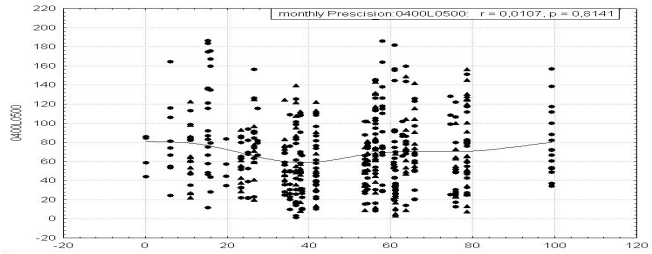
Figure 58: Root System Traits - Soil Moisture. **A:** DW (vs. soil temperature). **B:** Length. **C:** Surface area. **D:** Average diameter. **E:** Crossings per cm. **F:** Forks per cm. **G:** Length 0-0.15mm. **H:** Length 0.15-0.2mm.



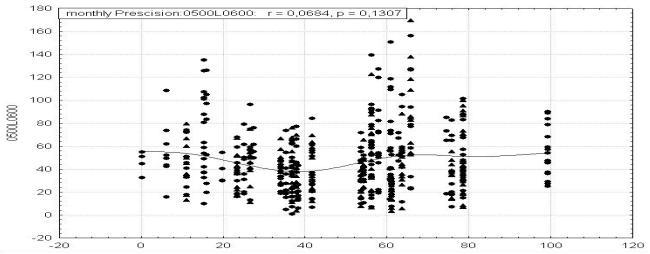
(a) Length 0.2-0.3mm



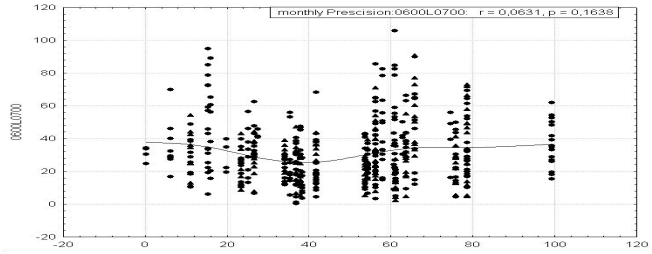
(b) 0.3-0.4mm



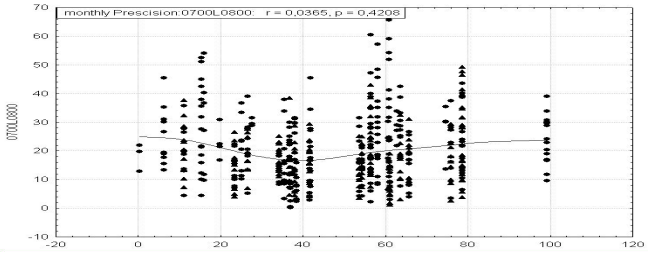
(c) 0.4-0.5mm



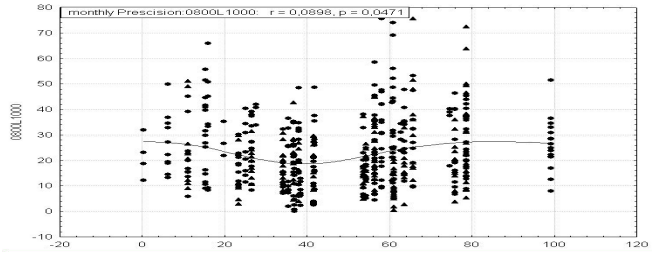
(d) 0.5-0.6mm



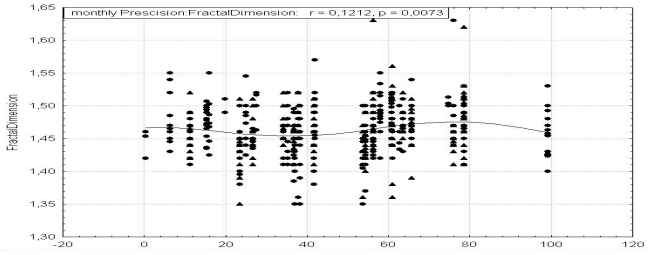
(e) 0.6-0.7mm



(f) 0.7-0.8mm

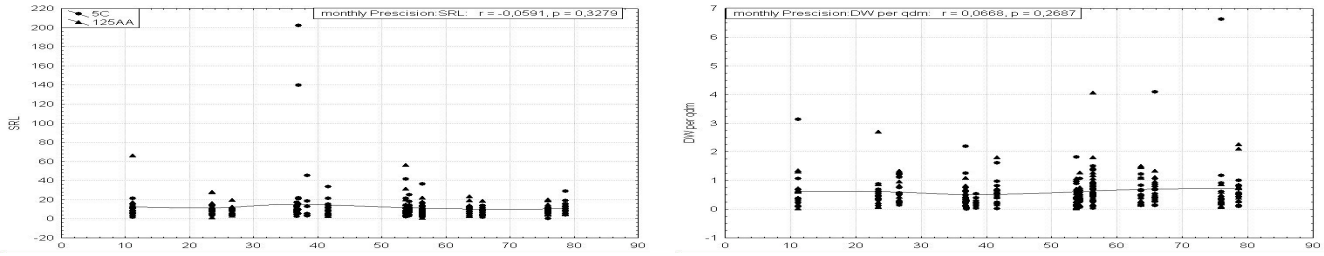


(g) 0.8-1mm



(h) Fractal Dimension

Figure 59: Root System Traits - Soil Moisture. **A:** Length 0.2-0.3mm. **B:** Length 0.3-0.4mm. **C:** Length 0.4-0.5mm. **D:** Length 0.5-0.6mm. **E:** Length 0.6-0.7mm. **F:** Length 0.7-0.8mm. **G:** Length 0.8-1mm. **H:** Fractal dimension.



(a) SRL (b) DW

Figure 60: Root System Traits - Soil Moisture. **A:** SRL. **B:** DW per 10^{-2}sqm .

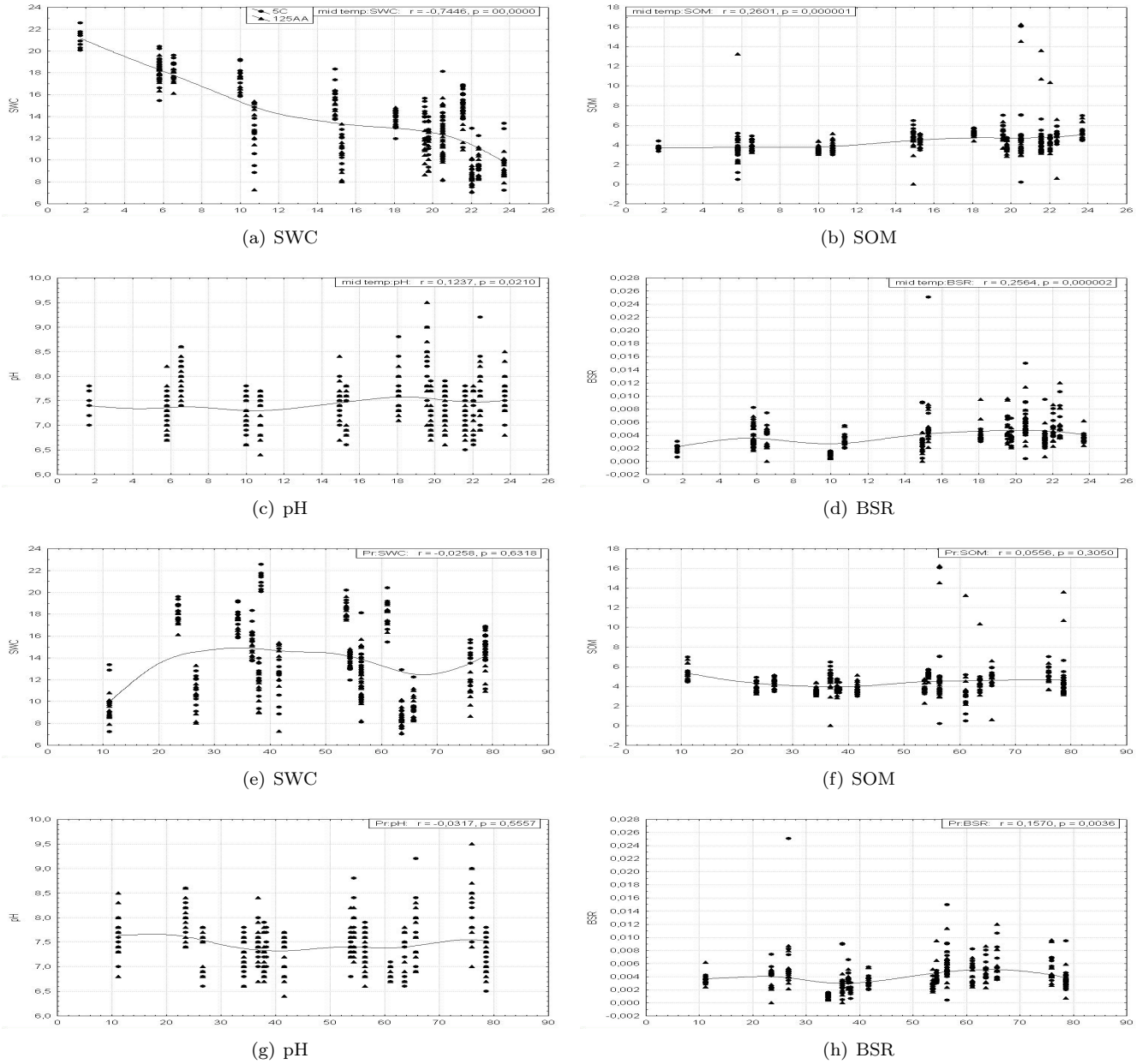


Figure 61: Soil properties vs. **A-D:** Soil temperature. **E-H:** Soil moisture. **A:** SWC. **B:** SOM. **C:** pH. **D:** BSR. **E:** SWC. **F:** SOM. **G:** pH. **H:** BSR.