Reactions of different populations of *Corylus avellana* L. and *Prunus spinosa* L. to drought and frost stress under controlled conditions

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Summary

Germany is a member of the International Convention on Biological Diversity. It is committed towards protecting biodiversity for sustainable development. Consequently, Germany implemented a law (Federal Nature Conservation Act § 40) which aims at conserving genetic diversity in open landscapes even below a species level. This law obliges use of autochthonous plant material (trees and shrubs) in open landscape for all plant species. However, data on the genetic diversity and local adaptation among plant populations used is barely available.

Hence this study was in pursuit of basic exemplary scientific information on adaptability of two species (*Corylus avellana* and *Prunus spinosa*) populations to drought and frost stress under controlled conditions. The experiments were carried out with rooted cuttings from various origins. For *Corylus avellana* the cuttings came from Brandenburg (BB), Niedersachsen (NDS), Nordrhein-Westfalen (NRW) and Rheinland-Pfalz (RPF). For *Prunus spinosa* cuttings came from Brandenburg (BB), Niedersachsen (NDS), Hessen (HES) and Rheinland-Pfalz (RPF) and northern Italy (near Lake Garda). All the plants were cultivated under the same optimum conditions in a common container area at Leibniz University, Hannover (52°23'34" N; 9°42'13" E; 53 m a.s.l.

These plants were exposed to frost (early and late frost) and drought stress in two consecutive years. Frost experiments were carried out in climate chambers and drought stress was carried out in a greenhouse. In frost stress, relative electrolyte leakage (REL) was used as a measure of damage while in drought stress, predawn leaf water potential, relative water content (RWC) and stomatal conductance were used. In all stress experiments, biomarkers (glucose, fructose, sucrose, starch and proline) were analysed. In addition, from all stress experiments some plants per treatment were included for regeneration and evaluation of further growth. Moreover, plants from the populations were characterized in spring, summer and autumn for phenology, growth (height and dry matter), glucose, fructose, sucrose, starch, proline and N, P and K concentration.

The biochemical composition and N, P, K concentration of characterization plants per population fluctuated with seasons and cuttings' year. However, there were no consistent trends with origin or differences among the studied German populations in both *Corylus avellana* and *Prunus spinosa*. Phenology and growth was not different among the studied German populations for both species

For *Corylus avellana* in early and late frost experiments, there were no consistent differences in the frost-related damage and in the analysed biomarkers. All populations suffered up to 30% REL in the lowest early frost treatment (-27 °C) while in late frost they sustained up to 60% REL in -12 °C treatment. But the regeneration plants from these treatments survived and sprouted though their apical dominance was broken.

In drought stress, populations endured leaf water potential of -3.2 MPa. The evaluated German populations did not differ in any physiological parameter. The concentration of the analysed biomarkers

(sugars and proline) did not consistently differ among the populations. But, the plants from Brandenburg, in contrast to the other populations, did not increase most of their leaves biomarkers. Probably this population is inclined to drought stress as it receives the lowest summer and annual rainfall amount at its place of origin.

In contrasting the German BB and the Italian *Prunus spinosa,* the Italian sprouted early in spring and delayed its bud set in autumn. It was significantly taller than the German BB population. During the spring time 2014, it had the lowest shoot concentrations of most of the analysed sugars (glucose, fructose, sucrose) and N and P than plants from the German population. In the late frost investigations, the Italian population had a higher frost damage (ca. 85% REL) related to sprouting stage. However, the two populations (German BB and Italian) did not differ in most of the analysed biomarkers. When the two German populations BB and RPF were exposed to late frost treatment, they suffered similar frost damage of up to about 84% REL in -12 °C although concentration of the analysed sugars and proline was often higher in RPF than that of BB. The high REL values did not translate to death as regeneration plants treated in the same manner resumed growth.

In the drought stress experiments, the two sloe German populations BB and RPF did not show any differences either in physiological or in biochemical responses. When the German population BB and the Italian population were drought stressed, plants from Italy reacted quickly to drought stress by sharply decreasing their stomatal conductance. The rapid closure of stomata by the Italian plants is a typical strategy of drought avoidance. This was seen as a characteristic of origin since the Italian plants originated from area of higher precipitation than the German population BB. However, despite their rapid stomata closure, the Italian population did not differ in stomata conductance with the German population as drought stress progressed. This was attributed to the uncontrolled water loss from immature fruits since the Italian population had higher fruit-load than those from Brandenburg. In both *Prunus* drought experiments, plant endured leaf water potential of -3.7 MPa.

In most of the evaluated aspects the Italian *Prunus spinosa* clearly separated itself from the German populations despite reports of low genetic differentiation among the *Prunus spinosa*. Hence growing Italian population, it should be avoided in the areas of frequent late frost and drought. According to literature, its early sprouting and late bud setting phenology, high growth rate nature suggests its vulnerability to frost.

Being aware that for both species only a part of the geographical and climatic conditions in Germany were represented by the populations investigated, overall they depicted greater plasticity and adaptability to new environment and to stressing conditions.

Key words: Area of origin, biomarkers, Federal Nature Conservation Act, phenology, frost damage, populations, predawn water potential, relative water content, stomata conductance

Zusammenfassung

Deutschland ist Mitglied der Biodiversitätskonvention. Es hat sich verpflichtet, die Biodiversität für eine nachhaltige Entwicklung zu schützen. Daher hat Deutschland ein Gesetz verabschiedet (Bundesnaturschutzgesetz, § 40), das das Ziel hat, die genetische Diversität in der freien Landschaft auch innerhalb der Pflanzenarten zu erhalten. Das Gesetz schreibt unabhängig von der Art die Verwendung von gebietseigenem Pflanzenmaterial (Bäume und Sträucher) in der freien Landschaft vor. Allerdings sind nur wenige Daten zur genetischen Diversität und zur lokalen Anpassung verschiedener Vorkommen vorhanden.

Daher sollen mit der vorliegenden Arbeit für verschiedene Vorkommen von *Corylus avellana* und *Prunus spinosa* beispielhaft grundlegende wissenschaftliche Informationen über deren Anpassungsfähigkeit an Frost und Trockenheit unter kontrollierten Bedingungen gegeben werden. Die Versuche wurden mit bewurzelten Stecklingen verschiedener Vorkommen durchgeführt. Für *Corylus avellana* kamen die Stecklinge aus Brandenburg (BB), Niedersachsen (NDS), Nordrhein-Westfalen (NRW) und Rheinland-Pfalz (RPF). Für *Prunus spinosa* kamen sie aus Brandenburg (BB), Niedersachsen (NDS), Hessen (HES) und Rheinland-Pfalz (RPF) sowie aus Norditalien (aus der Nähe des Garda-Sees). Alle Pflanzen wurden unter denselben optimalen Bedingungen auf einer gemeinsamen Containerstellfläche der Leibniz Universität Hannover kultiviert (52°23'34" N; 9°42'13" E; 53 m ü. NN).

In zwei aufeinander folgenden Jahren wurden diese Pflanzen Früh- und Spätfrost sowie Trockenstress ausgesetzt. Die Frostversuche wurden in Klimakammern, die Trockenstressversuche in einem Gewächshaus durchgeführt. In den Frostversuchen wurde der Relative Elektrolytverlust (REL) als Maß für den Schaden ermittelt; in den Trockenstressversuchen wurden das Wasserpotenzial vor Sonnenaufgang, der relativer Wassergehalt und die stomatäre Leitfähigkeit verwendet. In allen Stressversuchen wurden Biomarker (Glucose, Fructose, Saccharose, Stärke und Prolin) analysiert. Zusätzlich enthielten alle Stressversuche einige Pflanzen je Variante, die nach Beendigung der Versuche regenerieren konnten und deren weiteres Wachstum ermittelt wurde (Regenerationspflanzen). Zusätzlich wurden die Pflanzen der verschiedenen Vorkommen im Frühjahr, Sommer und Herbst bezüglich Phänologie, Wachstum (Höhe, Trockensubstanz) und der Konzentration an Glucose, Fructose, Saccharose, Stärke und Prolin sowie N, P und K charakterisiert. Bei der Charakterisierung der Pflanzen schwankten die biochemische Zusammensetzung und die Konzentrationen von N, P und K je Vorkommen in Abhängigkeit von der Jahreszeit und dem Jahr der Stecklingsgewinnung. Für die untersuchten deutschen Vorkommen gab es jedoch weder für *Corylus avellana* noch für *Prunus spinosa* Unterschiede oder eindeutige Trends bezüglich ihrer geografischen Herkunft. Auch die Phänologie und das Wachstum unterschieden sich bei beiden Arten nicht zwischen den untersuchten deutschen Vorkommen.

Bei den Früh- und Spätfrostversuchen mit *Corylus avellana* gab es bezüglich des frostbedingten Schadens und der analysierten Biomarker keine schlüssigen Unterschiede zwischen den Vorkommen. In den Frühfrostversuchen traten bei der Variante mit der tiefsten Temperatur (-27 °C) bei allen Vorkommen REL-Werte bis 30% auf; bei den Spätfrostversuchen wurden bei der niedrigsten Temperatur (-12 °C) REL-Werte bis 60% gemessen. Die Regenerationspflanzen aller Varianten der Frostversuche überlebten und trieben wieder aus, allerdings war die apikale Dominanz gebrochen.

In den Trockenstressversuchen haben die Pflanzen der untersuchten Vorkommen von *Corylus avellana* Wasserpotenziale bis -3,2 MPa ausgehalten. Die untersuchten deutschen Vorkommen unterschieden sich in keiner der ermittelten physiologischen Größen. Auch die Konzentration der analysierten Biomarker (Zucker, Prolin) zeigte keine konsistenten Unterschiede zwischen den Vorkommen. Allerdings reagierten die Pflanzen des Vorkommens aus Brandenburg – im Gegensatz zu den anderen Vorkommen – bei Trockenstress überwiegend nicht mit einem Anstieg der Biomarker in den Blättern. Vermutlich ist dieses Vorkommen an Trockenstress gewöhnt, da verglichen mit den anderen Vorkommen an seinem Herkunftsort die geringsten Sommer- und Jahresniederschläge fallen.

Das italienische Vorkommen von *Prunus spinosa* zeigte im Vergleich mit dem deutschen Vorkommen aus Brandenburg einen früheren Austrieb und einen späteren Triebabschluss. Die Pflanzen des italienischen Vorkommens waren signifikant größer als diejenigen aus Brandenburg. Im Frühjahr 2014 wiesen die Pflanzen des italienischen Vorkommens geringere Konzentrationen der analysierten Zucker (Glucose, Fructose, Saccharose) und von N und P auf als die des deutschen Vorkommens. Im Spätfrostversuch wiesen die Pflanzen des italienischen Vorkommens einen höheren Frostschaden (REL ca. 85%) auf als die des deutschen Vorkommens aus Brandenburg; der höhere Frostschaden beruhte auf dem Austriebsstadium. Das italienische und das deutsche Vorkommen unterschieden sich jedoch nicht in den meisten der analysierten Biomarker. Bei einem Vergleich der deutschen Vorkommen aus Brandenburg und Rheinland-Pfalz erlitten beide im Spätfrostversuch bei -12 °C einen ähnlichen Schaden mit einem REL-Wert von ca. 84%, obwohl die Konzentration der analysierten Zucker und Prolin bei den Pflanzen des Vorkommens aus Rheinland-Pfalz höher war als bei denen aus Brandenburg. Die hohen REL-Werte waren nicht letal; alle Regenerationspflanzen wuchsen nach dem Spätfrost weiter.

In den Trockenstressversuchen mit *Prunus spinosa* zeigten die beiden deutschen Vorkommen aus Rheinland-Pfalz und Brandenburg keine Unterschiede in ihren physiologischen und biochemischen Reaktionen. Bei einem Vergleich des deutschen Vorkommens aus Brandenburg mit dem aus Italien reagierten die Pflanzen des italienischen re bei Trockenstress schnell mit einer starken Abnahme der stomatären Leitfähigkeit. Das schnelle Schließen der Stomata ist eine typische Vermeidungsstrategie bei Trockenstress. Dieses Verhalten wird als charakteristisch für das italienische Vorkommen angesehen, da die Region durch höhere Niederschläge gekennzeichnet ist als bei dem Vorkommen

aus Brandenburg. Trotz des schnellen Schließens der Stomata der Pflanzen des italienischen Vorkommens unterschieden sich bei weiter fortschreitendem Trockenstress die beiden Vorkommen nicht in ihrer stomatären Leitfähigkeit. Die Ursache wird in dem unkontrollierten Wasserverlust der unreifen Früchte gesehen, da die Pflanzen des italienischen Vorkommens mehr Früchte hatten als diejenigen aus Brandenburg. In beiden Trockenstressversuchen mit *Prunus spinosa* wurden Wasserpotenziale bis -3,7 MPa ertragen.

Obwohl in der Literatur von geringen genetischen Unterschieden zwischen den Vorkommen von *Prunus spinosa* berichtet wird, unterschied sich das italienische Vorkommen in den meisten untersuchten Eigenschaften von den beiden deutschen Vorkommen. Die Verwendung des Vorkommens aus Italien sollte daher in Gebieten mit häufigem Spätfrost und Trockenheit vermieden werden. Aufgrund von Angaben aus der Literatur weisen der frühe Austrieb, der späte Triebabschluss und das starke Wachstum auf Frostempfindlichkeit hin.

Bei beiden Arten konnte nur ein Teil der in Deutschland vorhandenen Vorkommen berücksichtigt werden. Insgesamt zeigten die untersuchten Vorkommen von *Corylus avellana* und *Prunus spinosa* eine große Plastizität und Anpassungsfähigkeit an eine neue Umgebung und an Stressbedingungen.

Schlagwörter: Biomarker, Bundesnaturschutzgesetz, Frostschäden, Phänologie, predawn-Wasserpotenzial, relativer Wassergehalt, stomatäre Leitfähigkeit,Vorkommen, Vorkommensgebiete

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Abbreviations

§	Paragraph
#	Number or Count
AFLP	Amplified fragment length polymorphism
a s l	Above sea level
BB	Brandenburg
BLE	Bundesanstalt für Landwirtschaft und Ernährung
BMELV	Bundesministerium für Ernährung Landwirtschaft und Verbraucherschutz
	Changed to BMEL (Bundesministerium für Ernährung und Landwirtschaft)
BMU	Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit
C	Celcius
cm	Centimeters
CsCl	Caesium chloride
DNA	Deoxyribonucleic acid
cpDNA	Chloroplast DNA
DM	Dry matter
DW	Dry weight
EC	Electric conductivity
F	Fahrenheit
FSaatG	Forstsaatgutgesetz
FW	Fresh weight
GFS	glucose, fructose and sucrose
HES	Hessen
Hrs	hours
ITA	Italian
K	Potassium
K_2O	Potassium oxide

Lv	Leaves
M	Molar
Mg	Magnesium
MgO	Magnesium oxide
Min	Minutes
mm	Millimetre
MPa	Megapascal
N	Nitrogen
NDS	Niedersachsen
$N\ L^{-1}$	Nitrogen per litre
nm	Nanometre
N-P-K	Nitrogen, phosphorus and potassium
NRW	Nordrhein-Westfalen
P	Phosphorus
REL	Relative electrolyte leakage
RH	Relative humidity
RPF	Rheinland-Pfalz
RWC	Relative water content
SC	Stomatal conductance
Sht	Shoots
SSR	Simple sequence repeat
SW	Saturated weight
µg	Microgram
µl	Microlitre
UPGMA	Unweighted pair-group method with arithmetic mean
WP	Water potential

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1 General introduction

Historically most northern European trees and shrubs are described to have migrated post glaciation from the southern refugia. Their natural distribution and genetic structure is therefore dependent on the number of refugia they might have originated from, the migratory route taken post glaciation, and the past and present human activities (Willis 1996, Aguinagalde et al. 2005).

Several studies have related spatial genetic structure of forest trees and shrub species to the number and approximate locations of glacial refugia and their expansion to the central and northern part of Europe thus yielding substantial invaluable information concerning common ancestry (Petit et al. 2002, 2003, Persson et al. 2004). Trees and shrubs' life span and high genetic diversity are phylogenetic inertia against evolutionary factors such as isolation, cross pollination, timing and mechanism of migration. They therefore provide a link among them to their common origin in southern Europe. However, although slow, environmental perturbations and anthropogenic disturbances have prompted mutation, natural selection, genetic drift and recombination that might have resulted to ecotypes that are well adapted to certain ecological niches (Hamrick et al. 1992, Donoghue and Edwards 2014). Although these ecotypes are rich in genetic biodiversity within and among them (Petit et al. 2004), cultivating geographically distant populations might introduce a maladaptation to local populations creating a bottleneck for their biodiversity. This prompted governments and conservationists (both local and international) to intervene and issue conservation guidelines to protect genetic biodiversity. These guidelines obligate use of autochthonous propagules in the forestry with the argument that they are better adapted to biotic and abiotic factors. For forestry in Germany, it is required by law since 1979 (FSaatG 1979) that progeny of autochthonous trees should preferably be used in reforestation. However, this was not so for landscaping trees and shrubs, even though they are of high economic importance. Therefore, for decades, plants materials were sourced from low production cost European countries with sustained conflicts of conserving genetic resources and allowing potentially maladapted, economically interesting landscape trees and shrubs (Kleinschmit et al. 2008). This was the case until 2010 when Germany implemented a law (Federal Nature Conservation Act § 40) which aims at conserving genetic diversity in open landscapes even below the species level. The law obliges use of autochthonous plant material (trees and shrubs) in open landscape for all woody plant species. The assumption of the law is that through the natural regeneration and interaction with both biotic and abiotic agents, local ecotypes that are well adapted to their areas of origin could have resulted. In this regard, Germany was subdivided into six areas of origin (Fig. 1.1.1). This zonation presents a problem because data on the genetic diversity and local adaptation within and among populations of woody plant species used in open landscape is barely available.

Figure 1.1.1: Defined areas of origin for woody plants used in free nature (on basis of basic ecological units). Sourced from Bundesministerium für Umwelt, Naturschutz und Reaktorsicherheit 2012.

Since there is no scientific data on genetic diversity and no systematic provenance trials in regard to geographical or local adaptation as basis for the Federal Nature Conservation Act, the Federal Ministry of Food, Agriculture and Consumer protection (BMLEV) through the Federal Office for Agriculture and Food (BLE) funded a project that comprised three groups (Fig. 1.1.2) that sort to answer questions related to:

(1) genetic composition and differentiation of *Corylus avellana* and *Prunus spinosa* populations within Germany,

(2) populations characterization under natural conditions through reciprocal garden and

(3) populations characterization and adaptation to abiotic stress under controlled conditions

For broader comparison, a population from Hungary and Italy was included.

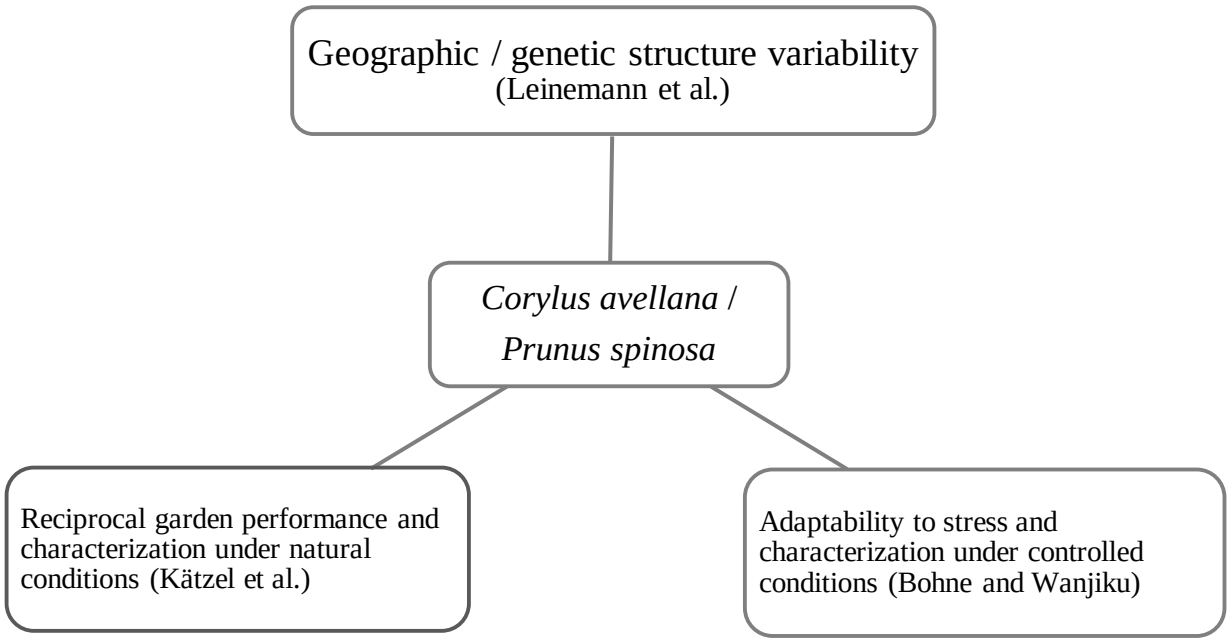

Figure 1.1.2: Schematic overview of the whole project.

This thesis is restricted to findings of *Corylus avellana* L. and *Prunus spinosa* L. populations' adaptability to stress (drought, early and late frost) and seasonal characterization of various populations under controlled conditions. The experimental design permits characterization of plants' seasonal fluctuations as well as their responses to drought and frost reactions. The two shrubs were selected on the basis of being widely used landscape plants which are propagated in masses. *Corylus avellana* is wind pollinated while *Prunus spinosa* is insect pollinated. This means that pollen for both shrubs could be transported long distances and perhaps over several demarcated areas. Owing to the propagation and pollination nature of these two shrubs, there is a considerable influence concerning adaptation and genetic variation within and among the populations. Therefore, the study of these two species might yield substantial exemplary information concerning the Federal Conservation Act.

1.1 Description of species used in this project

1.1.1 *Corylus avellana*

Hazelnut (*Corylus avellana* L.) belongs to Betulaceae family. It is an important multi-stemmed, landscaping shrub with a wide range distribution in Europe. It is reported to have originated from southern Europe before spreading all over with limited distributions to fewer parts outside Europe (Kasapligil 1942, Palmé and Vendramin 2002). Hazelnut is wind pollinated with male and female flower organ maturing at different times. Hazelnut seeds are dispersed by animals such as rodents. Human impact on hazelnut has spanned over Mesolithic era, as it is known to have been cultivated by the Romans (Tallantire 2002). This suggests that hazelnut has been heavily impacted by humans and probably one of the reasons why it is described as genetically similar through isozymes and chloroplast DNA (Persson et al. 2004). However, according to the results from Amplified Fragment Length Polymorphism (AFLP) and cpDNA-SSR by Leinemann et al. (2013b), some of the populations found in Germany are ca. 3.5% genetically differentiated. Accordingly, the populations used for this research were genetically different.

1.1.2 *Prunus spinosa*

Prunus spinosa L. is commonly known as blackthorn or sloe. It belongs to Rosaceae family. It is a deciduous shrub native to Europe, north Africa, and west Asia (Eimert et al. 2012) often used in open landscape in Germany. It has also been naturalised in other parts of the world such as America, Australia and New Zealand (http://www.gbif.org/species/113651614). It is insect-pollinated, animal-dispersed shrub, propagated by seed; stem cuttings and root suckers (Guitián et al. 1993). There is little genetic differentiation among populations in Germany attributed to vegetative propagation and common ancestry (Mohanty et al. 2002, Eimert et al. 2012). However, Leinemann et al. (2014) depicted up to 15% genetic differentiation of sloe using AFLP.

1.2 Influence of origin on selected performance parameters

1.2.1 Phenology

Phenology is a study of natural recurrent biological phenomena occurring at specific times in the lifespan of plants or animals (Forrest and Miller-Rushing 2010). Environmental cues like day length and temperatures are argued to be the most important cues for both bud set or bud sprout (Rohde et al. 2011). Bud phenology could be plastic but consequently might lead to adaptation as a survival strategy (Mortazavi et al. 2004, Rohde et al. 2011). In temperate climate, early bud sprout or later hardening of trees and shrubs could make a plant more susceptible to spring and fall frost damage (Morin et al. 2007, Muffler et al. 2016). Spring frost could damage the young leaves and this will affect overall growth and fitness. Late fall frost damage on the other hand could reduce carbohydrates storage, kill flower and leaf primordia thus affecting next year's survival and growth.

Literature has cited that geographic variations influence spring sprouting and autumn bud setting (Chmura and Rożkowski 2002, Jensen and Deans 2004). Investigating different populations along altitudinal gradient, Vitasse et al. (2009) reported that populations from low altitude sprouted earlier than those of high altitude in ash and oak species. They, however, also reported an opposing trend in beech populations where populations from high altitude flushed earlier than those of low altitude. Latitudinal variation in the critical photoperiod for growth cessation is documented in many species. Naturally, plants are adapted to local photoperiodicity and transferring them either to the north or to the south will alter their norm perception of photoperiodicity (Rohde et al. 2011) and might expose them to frost damage. Similarly, altitude transfer either to low altitude or to high altitude could be fatal (Ovaska et al. 2005). Consequently, plants exhibit various phenological variations along altitudinal or latitudinal gradients.

Both autumn's bud set and spring bud sprout phenology are important to ensure survival and growth. Autumn's bud setting and dormancy stage is entered when growth is prevented even under favourable conditions, while in the spring the ontogenetic development begins as the growth conditions are favourable (Heide 1993) influencing the survival and growth of plant species.

1.2.2 Survival and performance

Plant fitness and distribution is affected by many abiotic and biotic factors. Interaction with both agents could result to local ecotypes that are well adapted to particular ecological area of origin leading to enhanced performance. Kreyling et al. (2014) reported high survival rate to mid-winter and spring frost of *Fagus slyvatica* L. populations originating from colder areas than those originating from warm areas in two common garden experiments.

Conversely, local adaptation influences have not been found in a number of research attributed to immigration history of populations and wide ecological adaptation (Arend et al. 2011); whereas another study has shown better performance of non-natives in comparison to natives (Schreiber et al. 2013a). Schreiber et al. (2013a) demostrated ability of transferred populations to be better adapted than the native provenance populations further supporting importance of environmental cues to bud setting and sprouting.

1.2.3 Plants responses to abiotic stress

Being sessile, plants are faced with plethora of stresses. Among others, low temperature (frost) and drought are major abiotic stresses and will continue to exacerbate with climate change. Nevertheless, trees and shrubs are reported to have a wide ecological adaptation, vast genetic diversity and enormous plasticity that could enhance their adaptability potential, survival and ensure reproduction (Sultan 2000). Genetic diversity among and within populations and high plasticity could improve survival chances to a new or stressing environment (Hamrick 2004, Vitasse et al. 2010).

Although some responses to abiotic stress are distinct, genomic studies have shown considerable similarity in response to cold and drought abiotic stress (Kaur and Gupta 2005, Yamaguchi-Shinozaki and Shinozaki 2006). This is because they trigger cell dehydration in both cases. In response to abiotic stress, various genes are up-regulated (while others are down-regulated), which could initiate various changes that mitigate the effects of stress leading to adjustment and adaptability (Wang et al. 2003). With declining temperatures in autumn hence onset of cold stress, plants in the temperate shed their leaves, set their buds and become dormant until the adverse conditions are over (Kozlowski and Pallardy 2002). During the acclimatization process, there are physical, physiological and biochemical alterations of the cell membranes through accumulation of lipids, proteins and cryoprotective solutes that lower the water freezing point preventing ice formation that could lyse the cells (Weiser 1970). In spring the plants are no longer dormant and hence more susceptible to late frost damage (Guy 1990). But delayed bud sprout (Muffler et al. 2016) and accumulation of cryoprotective substances (Guy et al. 2008), depending on the available resources, might be a strategy to respond to late frost events.

With drought, plants are challenged with a combination of stresses including dehydration, heat and light stress. Of all, dehydration is most challenging as plants may lose water to the environment through roots (Oliver et al. 2010). In response, plants will endeavour to maintain water content by preventing water loss through stomata closure. They also accumulate compatible solutes like proline and sugars to lower their cell osmotic potential and maintain water within the cell (Chaves et al. 2003). Dissipation of excess light, cuticle thickening, cell wall adjustments, slowing growth rate,

losing chlorophyll, adjustment of organs' water content, accumulation of abscisic acid, and starch degradation are among other responses (Wang et al. 2003, Kaur and Gupta 2005).

These plant responses are not mutually exclusive and a combination of strategies are used by plants while adapting to cope with abiotic stresses.

1.3 How can influence of origin be evaluated?

1.3.1 Phenology evaluation

Phenology has been shown to be influenced by photoperiodicity and temperature (Myking and Heide 1995, Rohde et al. 2011). Leaf emergence of deciduous plant signifies the transition from dormancy to growing season in most temperate trees and shrubs. Leaf sprouting marks the beginning of growth and the earlier it begins the longer the growing season (Menzel and Fabian 1999). Bud set marks the end of growing season and onset to dormancy.

Although bud phenology (bud sprout and bud set) may be observed in nature to evaluate the differences among populations (Menzel et al. 2006), common garden evaluation is employed. Bud phenology is usually evaluated to test among populations differences in leafing out (bud sprout) and or in growth cessation (bud set). Phenological evaluation is important in monitoring adaptation plasticity or possible abiotic risks (frost) of a transferred population to that of a local population.

1.3.2 Physiological evaluation

Transferring plants from their native growing conditions may expose the plant to various abiotic and biotic stresses other than what they encounter in their original habitat (Ovaska et al. 2005). When plants are confronted with abiotic stress, they respond through various physiological and biochemical mechanisms that are driven by genetic diversity or by modified gene expression that result to avoidance or tolerance. Physiological responses in combination with other mechanisms lower the risks and enhance the plant to respond to stressing factors and have been demonstrated to vary with the origin as a result of local adaptation (Ennos et al. 1998, Kleinschmit et al. 2004, Bsoul et al. 2006, Savolainen et al. 2007).

Over the course of the year, plants in temperate show dramatic change in their ability to survive freezing temperatures. In preparation to these extremes, environmental cues trigger various physiological changes in plants that capacitate them to tolerance. In particular, plants reduce their water content, fortify their cell membranes and accumulate cryoprotective compounds such as proteins and carbohydrates (Guy 1990). The cryoprotective compounds plays a role in lowering the freezing temperature thus minimize the formation and growth of intra / extra-cellular ice. They also stabilize membrane and enzymes during low temperatures. Otherwise cell membrane would be overwhelmed

by ice leading to cell lysis. Frost damage occurs when ice forms inside the plants tissues which ruptures the cells. Damaged cell membranes can be quantified by relative electrolyte leakage (REL). REL is often used parameter of evaluating stress induced damage since abiotic stress could induce cell membrane damage (Verslues et al. 2006). Since the integrity of the cell is compromised, the cell contents diffuse into an ion free solution and could be measured as a change in electrical conductivity. No sooner the freezing stress is over and plants are sprouted than drought stress sets in. Some of the physiological responses are discussed below and how they can be useful tool in evaluating populations.

Stomata closure is categorized as the earliest physiological responses of plants to drought stress as a result of increased abscisic acid (Chaves et al. 2003, Harb et al. 2010). It minimises leaf transpiration (reduced stomatal conductance) and prevents the development of excessive water deficit in plant tissues (Chaves et al. 2003, Harb et al. 2010, Belko et al. 2012). Inability to regulate stomata will lead to cascading effects of reduced water potential, reduced water content and damage of membranes. Thus water potential may be a useful tool to screen populations (Gebre et al. 1998, Cole and Pagay 2015).

Relative water content (RWC)is expressed as a ratio of the amount of water present in an organ at sampling, relative to when the organ is fully turgid. It therefore indicates the deficit amount of water to reach artificial saturation (González and González-Vilar 2001). Low relative water content could be lethal although RWC threshold is species specific (Dichio et al. 2006, Yin et al. 2009). Investigating two populous species under water stress, Yin et al. (2009) found that a populous originating from high altitude maintained a higher RWC while that of low altitude had a significantly lower RWC.

1.3.3 Biochemical evaluation

Like physiological responses, plants employ various biochemical reactions when confronted with abiotic stress some of which are herein discussed with regard to origin.

Typically, plants store their carbohydrate in form of starch in twigs, stems, bark and roots (Kempa et al. 2008, Jie et al. 2010). During stress period, starch has been reported to be hydrolysed, hence decrease, to reducing sugars which sustain respiration, cell turgor among other functions (Kempa et al. 2007, Jie et al. 2010). The ability and rate of conversion of starch to sugars is dependent on the level and duration of stress and differs among species (Quick et al. 1992), age (Bansal and Germino 2009), latitude (Lei et al. 2013) and altitude of the plant being investigated (Hoch et al. 2002). Carbohydrates enhance plants' capability to mitigate frost and drought through counteracting cells dehydration and thereby maintaining the integrity and functioning of membranes and to stabilize proteins (McDowell 2011, Krasensky and Jonak 2012).

Proline accumulation in leaves, shoots and roots of stressed plants is yet another important parameter to characterize plants ecotypes under stress. Proline has been shown to increase with stress to alleviate or avoid loss of activity of many enzymes (Lei et al. 2006, Chen et al. 2014). Investigating differences in some morphological, physiological, and biochemical responses to drought stress in two contrasting populations of *Populus przewalskii*, Lei et al. (2006) found that proline accumulation increased due to drought besides other amino acids.

Peuke et al. (2002) reported some populations' differences in *Fagus sylvatica* due to drought stress induced increase in sucrose, glucose and fructose concentration. They also reported an opposing distribution of sugar in the provenances tested, that is, provenances with high contents of hexoses (glucose and fructose) had low levels of sucrose and vice versa. In this experiment, proline concentration did not vary correspondingly. An increase of glucose, fructose and sucrose has also been demonstrated to increase with frost tolerance driven by temperature and altitude of origin (Koch 2004, Morin et al. 2007).

Considering the importance of these parameters, analysis of various physiological and biochemical analyses is paramount and will therefore enable comparative study on adaptability of different populations of *Corylus avellana* and *Prunus spinosa* under defined condition. When evaluated under abiotic stresses, the biochemical (glucose, fructose, sucrose starch and proline) will be termed as biomarkers but when analysed for seasonal biochemical composition, they will be referred to as biochemical.

1.4 Research questions

This research study endeavoured to answer the following questions in regards to the populations of each species:

- Are populations obtained from different geographical areas of origin diverse in their phenology, morphology, physiology and biochemical constitution?
- Are the differences or similarities above related to origin?
- Do these populations differ in their responses to frost (early and or late) and drought under controlled conditions?
- Are these differences or similarities related to climatic conditions?

To answer these questions potted plants from different populations established from cuttings were grouped into various evaluation units (Fig. 1.4.1). Those that are coinciding, for instance spring or autumn characterization and late and early frost respectively, were simultaneously evaluated. The following chapters will elucidate how the materials were collected and raised and later it will expound on what was evaluated per each species and the time when each evaluation was feasible.

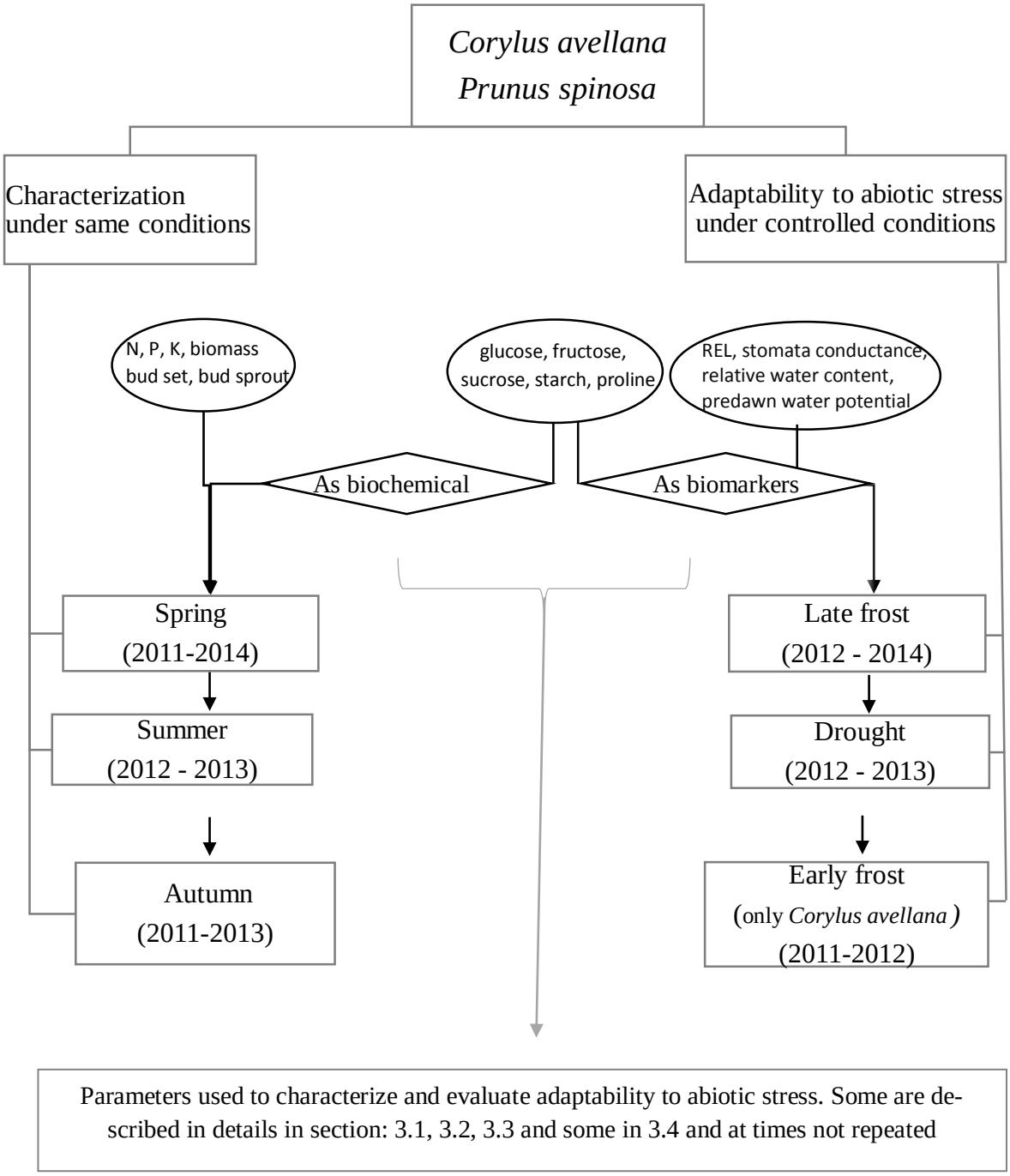

Figure 1.4.1: Overview of the research project (diagram structuring the main experiments of this study).

2 Material, cultivation and some ecological data

2.1 Material collection and climate data

The study material used, for all experiments and seasonal characterization, was part of the material collected by Leinemann et al. (2013a) and (Leinemann et al. 2014) for genetic structure investigations for respective *Corylus avellana* and *Prunus spinosa*. In order to ascertain autochthony, local forest research centres gave assistance. They were collected as cuttings from different localities as indicated by circles in the maps below (Fig. 2.1.1 and Fig. 2.1.2).

For *Corylus avellana* the following populations associated with federal states were used in our study: Brandenburg (BB), Niedersachsen (NDS), Nordrhein-Westfalen (NRW), Rheinland-Pfalz (RPF). They were collected and rooted in two batches tagged with year collected and rooted (2009 and 2011). The four federal state climatic data and map coordinates are given below (Table 2.1.1).

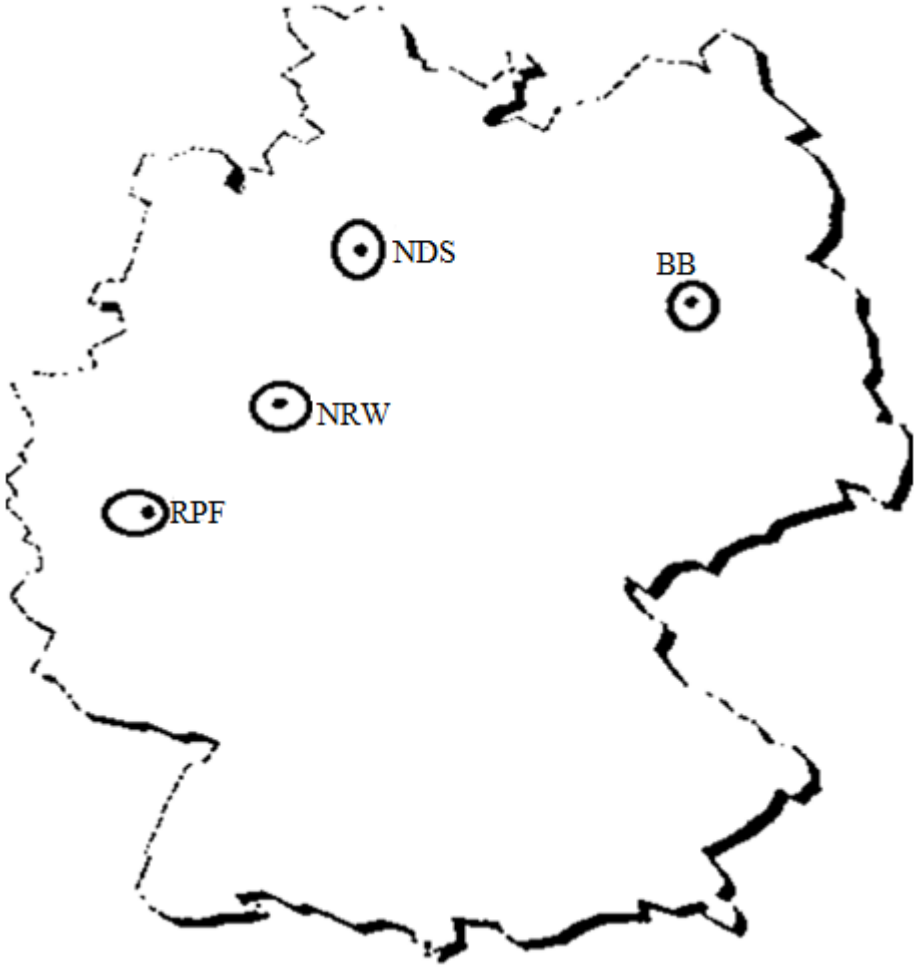

Figure 2.1.1: Geographic locations of the investigated German *Corylus avellana* populations (circled): Brandenburg Niedersachsen (NDS), Nordrhein-Westfalen (NRW) and Rheinland-Pfalz (RPF). Modified from Leinemann et al. (2013a).

Table 2.1.1: Map coordinates of populations *Corylus avellana and Prunus spinosa* sampled and some ecological data. Air temperatures and rainfall data are 30 years averages (1961- 1990) from Klimaatlas Bundesrepublik Deutschland: map 1.12 to 1.16 (temperature); map 2.12 to 2.16 (precipitation). BB = Brandenburg, HES = Hessen, NDS = Niedersachsen, NRW = Nordrhein-Westfalen, RPF = Rheinland-Pfalz and ITA = Italian http://www.dwd.de/bvbw/appmanager/bvbw/dwdwwwDesktop?_nfpb=true&_windowLabel=T38600134241169726338086&_urlType=action&_pageLabel=_dwdwww_klima_umwelt_ueberwachung_deutschland. Air temperatures and rainfall data [Italian (ITA)] are 12 years' average (2000 - 2012).

Origin	Altitude (m a.s.l)	Latitude	Longitude	Precipitation (mm)				Air Temp. (°C)			
				Spring	Summer	Fall	Annual	Spring	Summer	Fall	Annual
BB [x z]	38[x] / 44[z]	52°38'07.2"	12°58'08.3"	120 - 140	160 - 180	100 - 120	475 - 550	8 - 9	17 - 18	9 - 10	8.5 - 9
HES [z]	283	50°57'56.9"	9°51'43.4"	160 - 240	180 - 240	100 - 240	750 - 850	5 - 8	14 - 17	8 - 10	7 - 9
NDS [x z]	63	52°23'27.1"	9°31'45.2"	120 - 160	200 - 240	100 - 120	600 - 700	8 - 9	16 - 17	9 - 10	8 - 9
NRW [x z]	115	51°45'20.5"	9°22'05.4"	160 - 240	180 - 240	100 - 240	700 - 900	5 - 8	16 - 17	8 - 10	7 - 9
RPF [x z]	464	50°17'22.5"	7°00'15.8"	120 - 240	180 - 240	100 - 240	700 - 1000	5 - 9	14 - 17	7 - 9	7 - 9
ITA [z]	330 - 920	45° 43′	10° 52′	120 - 237	268 - 278	150 - 280	607 - 1008	7 - 19	16 - 29	8 - 18	7 - 18

[x] for *Corylus avellana*

[z] for *Prunus spinosa*

For *Prunus spinosa* plants the following populations associated with federal states were collected: Brandenburg (BB), Niedersachsen (NDS), Hessen (HES) and Rheinland-Pfalz (RPF). Additional plants were collected from Italy (ITA) and included in this study (Fig. 2.1.2). They were collected and rooted in three batches tagged with year collected and rooted (2009, 2010 and 2011). Due to the limited number of available plants, not all populations were included in all experiments. For seasonal characterization of German populations, plants rooted in 2009 (cutting 2009) were used. They included: Brandenburg (BB), Niedersachsen (NDS), Hessen (HES) and Rheinland-Pfalz (RPF). From this batch (cutting 2009), only plants from Brandenburg (BB) and Rheinland-Pfalz (RPF) from this cutting year were used for late frost experiment in 2013.

For drought experiments, plants from Brandenburg (BB) and Rheinland-Pfalz (RPF) rooted in 2010 (cutting 2010) were used. This experiment was repeated with plants from Brandenburg (BB) and Italy (ITA) rooted in 2011 (cutting 2011). Some climatic and ecological data is given in table 2.1.1.

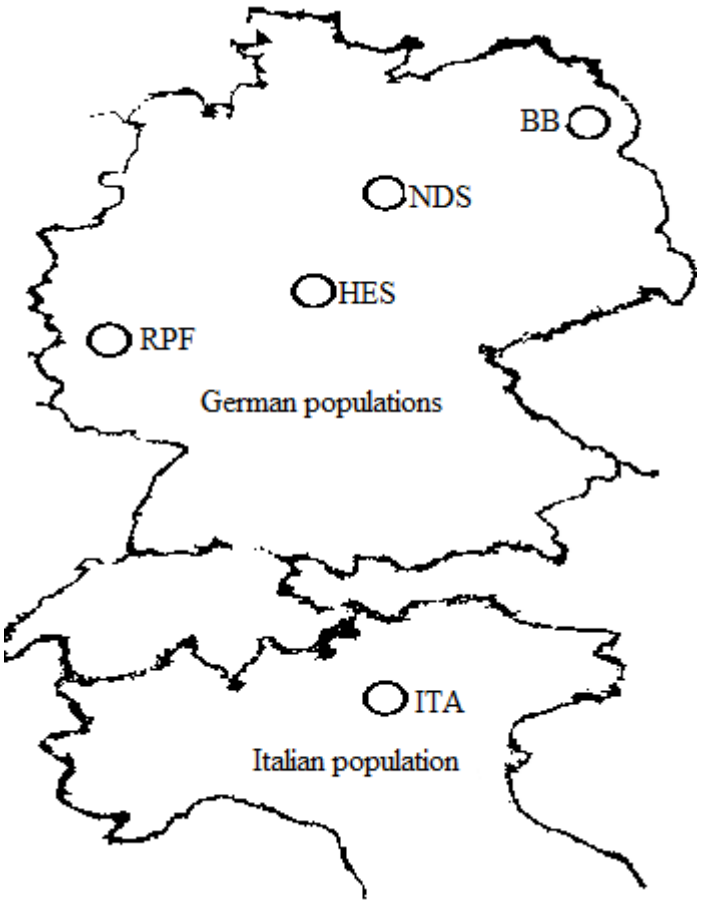

Figure 2.1.2: Geographic locations of the investigated populations (circled) of *Prunus spinosa* from Germany: Brandenburg (BB), Niedersachsen (NDS), Hessen (HES) and Rheinland-Pfalz (RPF) and Italy (ITA).

2.2 Cultivation

All plant cuttings were collected and rooted in separate years (see Table 2.2.1) in mist chamber under high relative humidity (RH ≥ 95%). There was no heating but the temperature was ca. 35 °C. These cuttings were successfully rooted in the Department of Woody Plants and Propagation Physiology at Leibniz Universität Hannover. After rooting the plants were potted in 3 L-container and later into 5 L-containers after one year using Klasmann-Deilmann Peat TS 4® potting substrate premixed with Osmocote® (15% N: 9% P_2O_5:11% K_2O: 2% MgO + trace elements) at a rate corresponding to 0.8 g N L^{-1}. Some plants (rooted elsewhere) were also received from two project partners to kick start the project as rooting was somehow difficult at the beginning.

During the growing season (May to November) plants were cultivated in a common container area and drip irrigated. The common container area's annual average temperature was 10.6 °C (spring = 10.1 °C, summer = 18 °C, autumn = 10.9 °C and winter = 2.94 °C) and sum rainfall was on average 621.6 mm (spring = 126.9 mm, summer = 197.3mm, autumn = 123.6 mm and winter = 173.7 mm) for the three years (2011-2013) these plants were cultivated (climate data from Institute of Meteorology und Climatology (MuK)- Leibniz Universität, Hannover).

During the cold months (December to April) plants were protected from frost by moving them in a poly tunnel. All available plants were assessed for autumn (bud set) and spring (bud sprout) phenology, each according to species' developed bud scoring scheme.

For each experiment, plants from each cutting year were randomly assigned to different drought or frost treatments. Additionally, regeneration plants were included among the stress treatment. These regeneration plants were allowed to recover and regenerate after stress in order to evaluate regeneration capacity among the populations. This however was not always feasible due to limited number of plants per cutting year. Moreover, each population per species was sampled in spring and autumn to determine the concentrations of glucose, fructose, sucrose, starch, proline, nitrogen, phosphorus and potassium.

In the following chapters detailed experimental layouts, results and discussion are expounded in details per experiment and species. However, a general overview of which cuttings used, when and for what purpose they were used is given below (Table 2.2.1).

Table 2.2.1: General overview of which cutting year (rooting year) and populations were used, when they were used in various experiments, phenology and characterization.

			When and where they were used in				
Species	Cutting year	Origin	Early frost	Late frost	Drought	Phenology	Characterization[z]
Corylus avellana	2009	BB, NDS, NRW, RPF	2011	2012	2012	autumn 2011 spring 2012	November 2011 April 2012
	2011	BB, NRW, RPF	2012 (BB, NRW)	2013	2013 (BB, NRW)	autumn 2012 spring 2013	November 2012 April 2013
Prunus spinosa	2009	BB, NDS, HES, RPF	not evaluated	2013 (BB, RPF)	not evaluated	autumn 2011 autumn 2012 spring 2013	November 2011 November 2012 April 2013 (BB, RPF)
	2010	BB, RPF	not evaluated		2012	autumn 2012	November 2012
	2011	BB, ITA	not evaluated	2014	2013	autumn 2012, 2013 spring 2013, 2014	April 2012, 2014
	2011	RPF	not evaluated			autumn 2012 spring 2013	April 2012

[z]Characterization = growth (height, root collar diameter and biomass), N, P, K, glucose, fructose, sucrose, starch and proline.

BB = Brandenburg, HES = Hessen, Nordrhein-Westfalen (NRW), NDS = Niedersachsen, RPF = Rheinland-Pfalz and ITA = Italy

3 *Corylus avellana* populations' reactions to frost, drought and seasonal characterization

3.1 Early frost reactions of different populations of hazelnut (*Corylus avellana* L.)

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3.1.1 Abstract

In Germany, planting of trees and shrubs in an open landscape is regulated by law (Federal Nature Conservation Act § 40) because of supposed genetic differences and regional adaptations to habitat conditions (mainly climatic and soil). Therefore, for trees and shrubs to be planted in the open landscape, Germany is divided into six officially designated regions of origin, often referred to as provenances. Propagation and use of plants must be carried out according to these provenances. To evaluate whether plants from different populations differ in their physiological and biochemical reactions, two years old cuttings of *Corylus avellana* from four populations with different climatic and soil conditions were evaluated in an early frost experiment under controlled conditions. Relative electrolyte leakage as a measure of damage due to frost increased with decreasing temperatures. Selected biomarkers (glucose, fructose, sucrose, starch and proline) were used to assess biochemical reactions of these populations. Increases due to frost were small and in most cases reflected the ranking of the unstressed plants of the populations. Only few statistical differences were found. There were no clear, consistent trends in spite of varied climatic conditions and geographical distance between the populations. Plants from all populations survived frost until -27 °C. Hence there are no severe risks in populations' transfer within these latitude and altitude margins with regards to early frost.

Key words: altitude, climate, cryoprotective compounds, latitude, relative electrolyte leakage, stress

3.1.2 Significance of this study

What is already known on this subject?

The distribution and colonization of many temperate trees and shrubs species in nature are determined by their adaptive capacity to withstand and survive extreme weather conditions like early frosts in their place of origin. It is assumed, therefore, that transferring trees and shrubs from their places of origin to places with differing ecological conditions may jeopardize their adaptation and survival. Despite the fact that it is agreed not to import plants from outside but rather to use native trees and shrubs in free nature, Germany went further to restrict utilization of these plants in free nature to six

officially defined areas of origin through a Federal Conservation Act §40. These defined areas of origin are independent on species.

What are the new findings?

Our results from four German populations of *Corylus avellana* L. exposed to early frost under controlled conditions showed that there is no risk involved in transferring these populations within their latitude (50°N – 52°N) and altitude (38–454 m a.s.l.) margins with respect to early frost. This is because of the reason that these populations were physiologically and biochemically similar during the time we conducted an early frost experiment.

What is the expected impact on horticulture?

Putatively, there are no physiological and or biochemical reasons that could hinder utilization of these populations as negated by the act. With regard to early frost, nurseries collection of propagation materials and sale of such propagules, regardless of the defined area of origin in these margins, does not jeopardize their survival. Moreover, plants from different sources might enhance genetic biodiversity and thus their survival in the wake of rapid climate change.

3.1.3 Introduction

Frost may perturb plants physiological and biochemical processes and may result in permanent injuries that finally bring about death (Inouye 2000, Schreiber et al. 2013b). Even within a given species, differences in its sensitivity to chilling and freezing stress can occur particularly if it is distributed across broad geographic regions (Morin et al. 2007). However the impacts of frost are dependent on the acclimation status of the plant as well as the season of occurrence (Morin et al. 2007). Often, plants in the temperate climate exhibit deciduousness accompanied by winter dormancy as a strategy to survive frost.

Generally, bud set and dormancy correlate strongly with the place of origin. Plants from high latitude and or high elevation heritably set bud early. Thus, they may develop better bud hardiness against early frost injuries than those of low latitude and or low elevation (Rohde et al. 2011). It is also reported in literature that they physiologically synthesise more sugars (Hoch et al. 2002) and, although for herbs, more proline (Bano et al. 2009). These might enable them to cope and withstand low temperatures. This implies that plants are adapted to local photoperiodicity and transferring them either to the north or to the south will alter their norm perception of long day or short day and growth period relative to local population (Rohde et al. 2011). This might increase the risk of frost damage (Cavender-Bares 2007, Morin et al. 2007).

Consequently, nature conservationists advocate the use of native provenances in reforestation as well as in landscaping, with the argument that they are better adapted to the climate in the officially designated region of origin than non-native provenances (Jones et al. 2001, Cavender-Bares 2007). Conversely, trees and shrubs have been demonstrated to possess greater variability to environmental changes (Kramer 1995, Vitasse et al. 2010). They exhibit huge plasticity to environmental changes even though they may be in non-native provenances. Moreover, they even might be better adapted than the native provenance (Schreiber et al. 2013a). There is an ongoing debate on the use of local ecotypes in landscapes restoration (Johnson et al. 2004). But so far there are no substantial agreements perhaps due to little biological evidence. Imperatively, only general guidelines and recommendations, like in the U.S.A, are given (Bower et al. 2014). Despite these un-clarified non-conclusive arguments, Germany has enacted a law (Federal Nature Conservation Act § 40) that confines use of native provenances of trees and shrubs within officially designated regions of origin; due to supposed genetic differences between them as a consequence of adaptations. The act also aims at conserving biodiversity even below species level (BMU 2012). Furthermore, it is postulated that the officially designated regions native populations are more adequately adapted than non-native. Thus, the use of non-native provenances for trees and shrubs is not permitted even in landscaping. Historically most populations in Germany migrated from southern Europe (Willis 1996).

Aguinagalde et al. (2005) questioned if the chloroplast DNA (cpDNA) and genetic structure of the current populations reflect equilibrium between current patterns of gene flow and genetic drift but instead are still the result of the conditions of post-glacial colonisation. Therefore, the issue of adaptation to officially designated regions in Germany in regards to physiology is of concern. In order to gain an insight of adaptability in terms of physiological and biochemical reactions, especially under stress conditions, an experiment was set up using hazelnut (*Corylus avellana* L).

Hazelnut is an important, deciduous, landscaping shrub in Germany. It is a multi-stemmed, wind pollinated plant whose seeds are dispersed by animals. Hazelnut has a common ancestry in the southern part of Europe during the ice age before spreading to other areas (Mehlenbacher 1991, Persson et al. 2004). Genetically, European hazelnut populations have been described to be similar using cpDNA as well as using iso-enzymes (Persson et al. 2004). Nevertheless, Leinemann et al. (2013b) described some of the populations in Germany as genetically different using amplified fragment length polymorphism (AFLP). However, hazelnut is described to inhabit wide ecological areas (Mehlenbacher 1991), therefore, they could proliferate over a wide range of climatic conditions. In this regard, an early frost experiment was conducted to evaluate different populations' physiological and biochemical reactions. Late frost and drought were also investigated in further experiments.

3.1.4 Material and Methods

Our study plant materials came from four German federal states. These were Brandenburg (BB), Niedersachsen (NDS), Nordrhein-Westfalen (NRW) and Rheinland-Pfalz (RPF). The plant cuttings were collected by Leinemann et al. (2013b) assisted by local forest research centres which helped in identifying native (presumably autochthonous) populations. They were part of the populations analysed for genetic variation (Leinemann et al. 2013b). Thus the four populations evaluated in this experiment are genetically different. The four federal states differ in soil, climate and topography. RPF is the most heterogeneous of all, in terms of topography and climate varying in few kilometres followed by NRW whereas BB and NDS are less heterogeneous. Thus especially for RPF and NRW specific climatic data from a single nearby station is not representative for the situation and therefore is not used but rather instead a range is provided (Table 3.1.1). To have a comparable database it is also done for BB and NDS.

All plants were cultivated at Leibniz University, Hannover (52°23'34 N; 9°42'13" E; 53 m a.s.l) under the same environment and irrigation regimes. Our cultivation site was located in the federal state NDS hence population NDS was more or less experiencing nativity as to the temperature and photoperiod. During the two year cultivating period, we rated their bud setting. After full bud set, an early frost experiment was conducted.

Table 3.1.1: Map coordinates of populations sampled, and some ecological data.

				Precipitation (mm)				Air Temperature (°C)			
Origin	Altitude	Latitude	Longitude	Spring	Summer	Fall	Annual	Spring	Summer	Fall	Annual
BB	38	52°38'07.2"	12°58'08.3"	120 - 140	160 - 180	100 - 120	475 - 550	8 - 9	17 - 18	9 - 10	8.5 - 9
NDS	63	52°23'27.1"	9°31'45.2"	120 - 160	200 - 240	100 - 120	600 - 700	8 - 9	16 - 17	9 - 10	8 - 9
NRW	115	51°45'20.5"	9°22'05.4"	160 - 240	180 - 240	100 - 240	700 - 900	5 - 8	16 - 17	8 - 10	7 - 9
RPF	464	50°17'22.5"	7°00'15.8"	120 - 240	180 - 240	100 - 240	700 - 1000	5 - 9	14 - 17	7 - 9	7 - 9

Abbreviations: BB = Brandenburg, NDS = Niedersachsen, NRW = Nordrhein-Westfalen, RPF = Rheinland-Pfalz. Air temperatures and rainfall data are 30 years averages (1961- 1990) from Klimaatlas Bundesrepublik Deutschland: map 1.12 to 1.16 (temperature); map 2.12 to 2.16 (precipitation). http://www.dwd.de/bvbw/appmanager/bvbw/dwdwwwDesktop?_nfpb=true&_windowLabel=T38600134241169726338086&_urlType=action&_pageLabel=_dwdwww_klima_umwelt_ueberwachung_deutschland

Early frost experiment

In November 2011, four weeks after bud set (visually scored following a scheme developed by Rump 2002), early frost experiments were conducted with four populations of *Corylus avellana* with six or eight replicates per treatment. Plants were randomly allocated to frost treatments. Three shoots per plant were cut (≈ 30 cm long) and immediately placed in a plastic bag. These bags containing three shoots per plant were frozen for eight hours, either at -27 °C and -17 °C respectively. Branched roots (up to 7 mm in diameter) were carefully cleaned with soft brushes, placed in plastic bags (three roots per plant) and treated at -6 °C. For both shoots and roots treated at 5 °C served as control. Samples were frozen and thawed at a rate of 5 °C h^{-1}. This early frost experiment was repeated in November 2012 with only two populations (BB and NRW) but with 13 and 14 replicates respectively.

Relative electrolyte leakage (REL)

Frost affects cells membrane integrity therefore releasing its content; which can then be determined electrically and calculated as relative electrolyte leakage (REL). Frost damage was considered as an increase in REL (Verslues et al. 2006).

Electrolyte leakage tests involve measurement of the electrical conductivity (EC) of double distilled water in which detached plant samples have been placed after a freeze-thaw cycle. Shoot tips and fine root tips (≈ 3 cm long) were cut and placed (one tip per vial) in 30 ml of distilled water. The samples infused at room temperature for 24 h after which electrolyte leakage was measured as electrical conductance (EC). The samples were incubated in the oven at 70 °C for another 24 h and a second EC was taken. Relative electrolyte leakage was calculated as:

$$\text{Relative electrolyte leakage} = \frac{\text{First electric conductance}}{\text{Second electric conductance}} \times 100$$

Carbohydrates determination

Treated shoots and roots (without 3 cm used for REL) were cut into small pieces and quickly microwaved for 2 min to arrest enzymatic reactions (Hoch et al. 2002); then oven dried at 70 °C for three days. Each sample was finely ground to pass through a 3 mm mesh. Glucose, fructose and sucrose (GFS) were determined as soluble sugars while starch was hydrolysed to glucose by amyloglucosidase enzyme before determination. For GFS and starch determination, 20 – 30 mg of ground material was weighed in Eppendorf tubes and all other procedures followed that of Zhao et al. (2010) with minor changes (triethanolamine buffer (14 g triethaloamine + 0.25 g $MgSO_4$ dissolved in 100 ml water, pH 7.6) and NaOH were used instead of TRIS buffer and KOH respectively).

Proline determination

Proline determination followed the protocol laid by (Bates et al. 1973). 200 mg of finely ground (as above) material was homogenized with 10% sulfosalicylic acid and incubated on ice. After 30 min., the homogenates were vortexed and centrifuged (14462 x g) for 20 min. Precisely, 0.3 ml of the supernatant was treated with 100% acetic acid and acid-ninhydrin (6.25 g ninhydrin powder in 60% acetic acid + 85% orthophosphoric acid at a volume ratio of 83.8 to 16.2), then boiled for 45 min. The homogenate was cooled; 3 ml toluene added then vortexed. 2 ml coloured phase absorbance was determined by a photometer at 520 nm. Proline concentration was calculated on the basis of dry weight.

Regeneration plants

To assess survival and regeneration capacity after the freezing treatment, five plants per treatment from each of the four populations were randomly selected and placed in a basin filled with peat to protect the root system. These plants served either as control or were treated at -27 °C and -17 °C. After treatment the plants were out-planted in the following year in the field to assess the impact of freezing temperature on the four populations.

3.1.5 Statistical analysis

The experiments were conducted using a completely randomized design with six (BB) and eight (NDS, NRW and RPF) replicates in 2011 and 13 (BB) and 14 (NRW) replicates in 2012. A logarithmic transformation of the data was performed to meet the requirement of a normal distribution of data for the model prior to analyses. All data collected were subjected to multiple analysis of variance (MANOVA) using R 3.0.3 (2014) program according to Pipper et al. (2012). Since there were no interactions (i.e. treatment x population), treatments and population means at $P \leq 0.05$ were separated using the Tukey test. Results in figures and tables are presented as means ± standard deviation (SD).

3.1.6 Results

Relative electrolyte leakage

Estimated damage by relative electrolyte leakage (REL) increased with decreasing temperatures (Table 3.1.2). REL varied greatly within populations tested as indicated by high standard deviations. In shoots (2011), there was a tendency of RPF having relatively higher REL than the other populations across all treatments, however being significant only to BB at control and at -17 °C. Repetition in 2012 yielded similar results in shoots of BB and NRW. Roots REL was higher than in shoots in both years (Table 3.1.2). Populations did not differ either in the control or in -6 °C treatment.

Table 3.1.2: Shoots' and roots' relative electrolyte leakage (REL%) of four populations of *Corylus avellana* (BB, NDS, NRW, and RPF) in early frost experiments (November 2011, November 2012).

		Shoots (REL %)			Roots (REL %)	
		Treatments				
Year/Population		5 °C	- 17 °C	- 27 °C	5 °C	- 6 °C
2011						
	BB	11 ± 1.7Aa	16 ± 3.6 Ba	28 ± 6.4 Ca	44 ± 11 Aa	58 ± 6.3 Ba
	NDS	14 ± 1.9 Aab	18 ± 3.6 Bab	29 ± 7.2 Ca	38 ± 5.3 Aa	56 ± 11 Ba
	NRW	13 ± 2.9 Aab	18 ± 2.9 Bab	30 ± 8.6 Ca	42 ± 8.9 Aa	60 ± 7.0 Ba
	RPF	14 ± 1.8 Ab	22 ± 4.0 Bb	32 ± 7.3 Ca	36 ± 7.4 Aa	55 ± 6.2 Ba
2012						
	BB	11 ± 1.7 Aa	17 ± 2.7 Ba	26 ± 4.1 Ca	42 ± 8.7 Aa	54 ± 7.1 Ba
	NRW	11 ± 1.5 Aa	15 ± 2.3 Ba	27 ± 3.5 Ca	44 ± 9.8 Aa	56 ± 6.4 Ba

Abbreviations: BB = Brandenburg, NDS = Niedersachsen, NRW = Nordrhein-Westfalen, RPF = Rheinland-Pfalz. Different letters show significant differences: small letters among populations within a treatment; capital letters among treatments of each population. Mean ± SD, n = 6 (BB), n = 8 (NDS, NRW, RPF) in 2011; n = 13 (BB), n = 14 (NRW) in 2012.

Biomarkers (glucose, fructose, sucrose, starch and proline)

When unstressed (controls), in 2011 populations did not differ in either glucose (Fig. 3.1.1), proline (Fig. 3.1.2) or in starch (Table 3.1.3) concentration in their shoots. However, populations differed significantly in fructose and sucrose concentration (Fig. 3.1.1).

In 2011 BB, NRW and RPF populations had a tendency to have accumulated significantly higher concentration of fructose and sucrose than NDS. On the contrary, NDS had a tendency to accumulate higher proline when untreated although not statistically different (Fig. 3.1.2).

When stressed, glucose and fructose increased marginally. Only RPF increased its glucose and fructose concentration upon frost. Fructose concentration increase followed a trend similar in amplitude to the basic level of the controls (Fig. 3.1.1). BB and NDS sucrose concentration remained static while that of NRW and RPF declined with decreasing temperatures (Fig. 3.1.1). Proline concentration in shoots increased marginally at -17 °C but not at -27 °C (Fig. 3.1.2). At -17 °C, NDS proline concentration was significantly higher than that of BB. However, neither of them differed with either NRW or RPF. There were no significant changes in proline due to the frost treatment and the marginal

increase followed the order of the basic level of the respective controls. Starch concentration did not react significantly with decreasing temperature and neither did it differ among the populations.

In roots, there were no significant differences among the populations studied in all the biomarkers analysed (data not shown). However, they had higher concentrations of glucose, starch and proline compared to the shoot. Shoots on the other hand had higher concentration of fructose and sucrose than the roots.

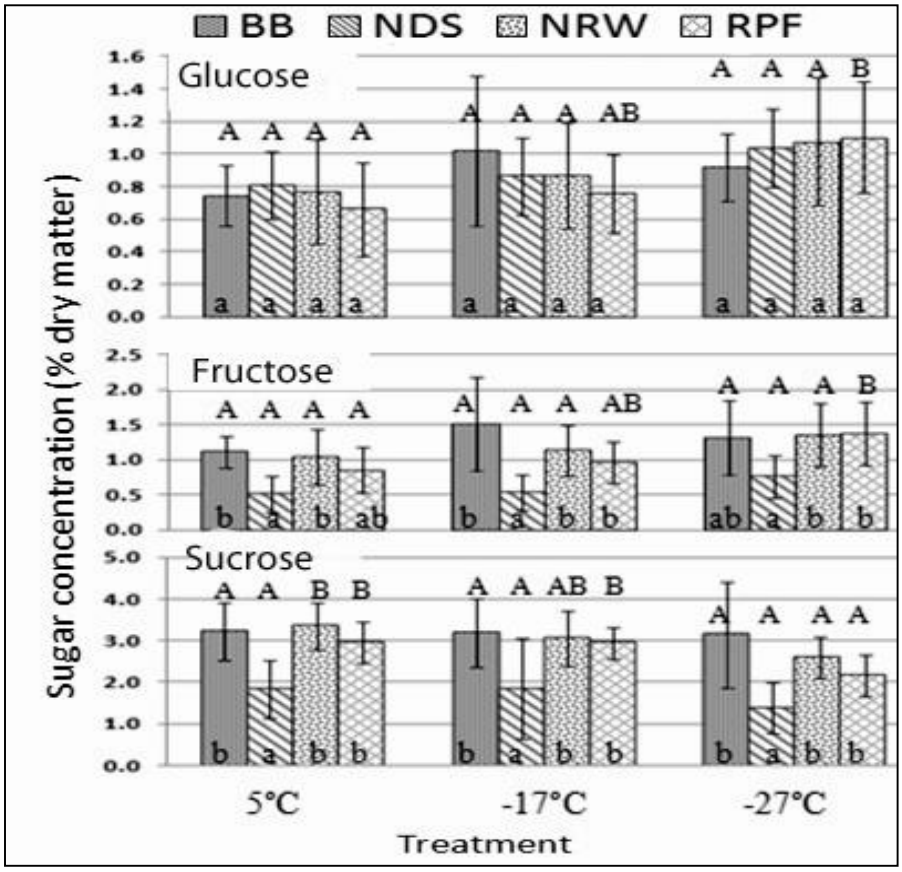

Figure 3.1.1: Glucose, fructose and sucrose (% dry matter) in shoots of four populations of *Corylus avellana* (BB, NDS, NRW, and RPF) in early frost experiments (November 2011). Different letters show significant differences: small letters between populations within a treatment; capital letters between treatments of each population. Mean ± SD, n = 6 (BB), n = 8 (NDS, NRW, RPF). Abbreviations: BB = Brandenburg, NDS = Niedersachsen, NRW = Nordrhein-Westfalen, RPF = Rheinland-Pfalz.

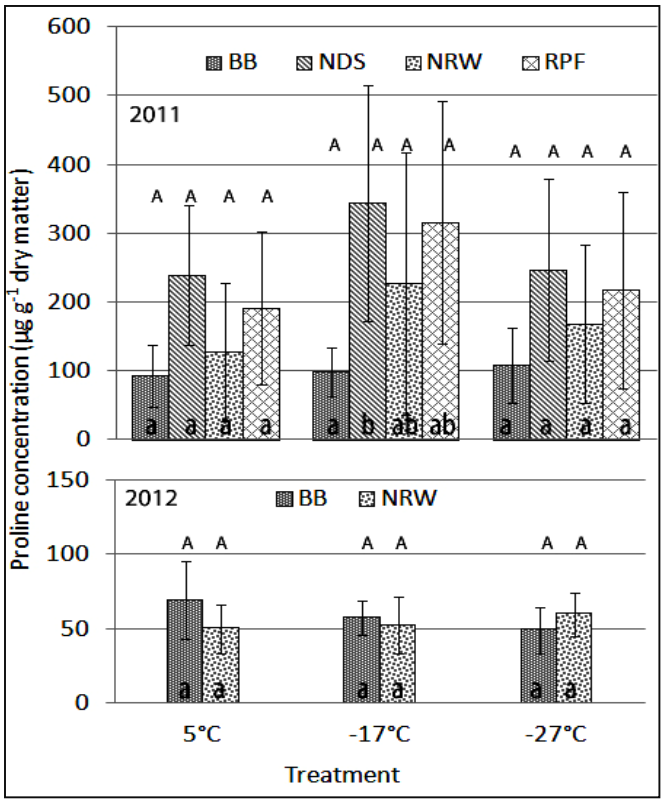

Figure 3.1.2: Proline concentration (µg g^{-1}) in shoots of four populations of *Corylus avellana* (BB, NDS, NRW, and RPF) in early frost experiments (November 2011, November 2012). Different letters show significant differences: small letters between populations within a treatment; capital letters between treatments of each population. Mean ± SD, n = 6 (BB), n = 8 (NDS, NRW, RPF) in 2011; n = 13 (BB), n = 14 (NRW) in 2012. Abbreviations: BB = Brandenburg, NDS = Niedersachsen, NRW = Nordrhein-Westfalen, RPF = Rheinland-Pfalz.

Table 3.1.3: Shoots starch concentration (% dry matter) of four populations of *Corylus avellana* (BB, NDS, NRW, and RPF) in early frost experiments (November 2011, November 2012).

	Starch (% dry matter)		
	Treatments		
Year/Population	5 °C	- 17 °C	- 27 °C
2011			
BB	1.63 ± 0.4 Aa	0.99 ± 0.4 Aa	0.92 ± 0.3 Aa
NDS	1.63 ± 0.8 Aa	0.65 ± 0.3 Aa	0.78 ± 0.3 Aa
NRW	1.90 ± 0.7 Aa	0.97 ± 0.4 Aa	1.0 ± 0.3 Aa
RPF	1.98 ± 0.8 Aa	0.98 ± 0.3 Aa	0.82 ± 0.2 Aa
2012			
BB	2.65 ± 0.7 Aa	2.44 ± 0.6 Aa	2.33 ± 0.5 Aa
NRW	2.28 ± 0.6 Aa	2.37 ± 0.7 Aa	2.44 ± 0.6 Aa

Abbreviations: BB = Brandenburg, NDS = Niedersachsen, NRW = Nordrhein-Westfalen, RPF = Rheinland-Pfalz. Different letters show significant differences: small letters among populations within a treatment; capital letters among treatments of each population. Mean ± SD, n = 6 (BB), n = 8 (NDS, NRW, RPF) in 2011; n = 13 (BB), n = 14 (NRW) in 2012.

A repetition of the early frost experiment in 2012 with BB and NRW populations yielded similar results for shoots in that the two populations did not differ in most of the biomarkers apart from fructose upon frost. In terms of absolute levels, in 2011 proline was remarkably high and starch low compared to 2012 (Fig. 3.1.2 and Table. 3.1.3). Unlike 2011, in roots, BB and NRW differed in glucose, sucrose and starch concentrations (data not shown). BB was high in glucose while NRW was high in both sucrose and starch.

Regeneration

In 2011 and 2012, all treated plants survived freezing temperatures and were able to regenerate. However, apical dominance was compromised in most frost treated plants shoots. This resulted to more shoots sprouting from either the base of the plant or from the lateral buds of the affected shoots. More shoots sprouted in the following order: -27 °C > -17 °C > 5 °C. Reverse order was also true in terms of height (data not shown).

3.1.7 Discussion

Relative electrolyte leakage

A decrease in photoperiod and cold temperature serve as environmental cues for initiating growth cessation, bud setting, dormancy and frost hardening (Mortazavi et al. 2004, Rohde et al. 2011). At the time of our frost experiments, all populations had set bud. Obviously the transfer of the more southern populations RPF and NRW to the experimental site in NDS did not influence bud setting.

Ion leakage has often been correlated with frost hardiness. A higher REL is assumed to indicate a higher damage (Yildiz et al. 2014). Apart from RPF all populations had similar REL, which could have been expected because of the same stage of bud setting. Surprisingly, at control and at -17 °C RPF showed higher REL, although originating from a higher altitude. Frost hardiness is expected to increase with increasing altitude (Gansert et al. 1999, Rumpf 2002). In the literature, phenology and cold hardiness often are closely linked, but it is a highly plastic trait (Vitasse et al. 2010). This implies that it may not always reflect the degree of frost hardiness as indicated by RPF population. There were no differences between RPF and the other populations at -27 °C. The results are in disparity with examples from the literature. For instance, Rumpf (2002) when comparing winter frost hardiness of two *Corylus* populations from the same provenance but different elevation, observed that a population from high elevation (300 m a.s.l) was more frost hardy than a population from low elevation (49 m a.s.l). The reason for our diverging result is not apparent as we expected the RPF population to be the most frost hardy. Since this population is from a high elevation site that is of heterogeneous environment and topography, it may be adapted to an environment where there is a flow of cold air current from up to down the slopes as argued by Larsen (1978). Thus, a local climate which is strongly related to topography can influence frost hardiness. Nonetheless, even the REL level reached by the

RPF is considerably low compared to that obtained by Chozinski (1995) when investigating winter cold hardiness of *Corylus* cultivars. Hence, it may not be lethal. This was confirmed by the ability of the regeneration plants to sprout in the following spring similarly to those of the other populations. This implies that the investigated populations coming from geographically different locations in Germany with different climates were adequately hardy to withstand early frost events up to -27 °C.
The frost treatment had a significant effect on the roots from all populations. This indicates roots to be more prone to frost damage than shoots as it was reported in literature (Calmé et al. 1994, Bigras and Dumais 2005). It could be possible that the roots were not hardened by the time we carried out our experiment and were still growing (Bigras and Dumais 2005). Data reported here are in accordance with data in literature found for roots (i.e. Repo and Ryyppö 2008, Yildiz et al. 2014). There were no significant differences in the root relative electrolyte leakage between the four populations tested.

Biomarkers

When unstressed, at the end of the growing season, the four populations originating from different latitudes (50.2° N – 52.4° N) and altitudes (38 m – 464 m) differed significantly in the biomarkers fructose and sucrose analyzed despite hardening under the same climatic conditions at the experimental site. Concerning altitude, literature reports increasing concentrations of soluble carbohydrates during the growing season for higher elevations (i.e. Hoch et al. 2002, Shi et al. 2006). However, Poirier et al. (2010) found no differences in starch, glucose, fructose, sucrose for two walnut varieties from two different altitudes. Yildiz et al. (2014) concluded from their experiments that the relationship between altitude and soluble sugars were not consistent. Concerning latitude, for different populations of *Pinus sylvestris* between 49° N and 60° N Oleksyn et al. (2000) found higher soluble carbohydrate concentrations in 1-year-old needles in low-latitude populations compared to high-latitude populations. Lei et al. (2013) stated no differences in the concentration of soluble sugars in leaves of *Quercus variabilis* seedlings from latitudes between 25° N and 40° N, but higher concentrations in stems of plants from higher latitude. Interestingly, two years after transplanting the plants from different latitudes into a common garden (30.5° N), the differences in the concentration of soluble carbohydrates did not persist any more. The differences of fructose and glucose found in our populations did not show a trend with regard to latitude and altitude. Populations from the same latitude (BB, NDS) showed significant differences, while those from different latitudes (BB, RPF) did not differ. The concentration in the populations from the higher elevations (RPF, NRW) in most cases was not different from those of lower elevations (BB, NDS). One reason might be that the difference in latitudes was only small compared to examples quoted from literature. Furthermore, like in the experiment of Lei et al. (2013), the plants (apart from NDS) were not cultivated under the conditions

of their geographical origin. Concerning altitude our results are in line with Poirier et al. (2010) and Yildiz et al. (2014). For the amino acid proline only little information concerning latitudinal and altitudinal trends is available. For herbaceous plants, Bano et al. (2009) found increasing concentrations with increasing altitude. Our results showed a tendency of highest concentration for the NDS population, which is from a lower altitude compared to NRW and RPF. To conclude, differences in the concentration of selected biomarkers in the hardened state before the frost experiment could not be related to latitude and altitude, hence geographical origin. Similarly, they were not as a result of the dry matter of the plants, since the differences in dry matter were not significant (data not shown). The plants used in our experiments are a mixture of different genotypes taken from natural stocks in the landscape. However, such mixtures of genotypes are the material used for cultivation in tree nurseries and thereafter for landscaping. Also for cultivars of hazel grown in the same region Okay et al. (2005) found different concentrations of soluble sugars in their bark tissues.

Freezing treatment caused little or no effects on most of the biomarkers analysed. However, the little changes in these biomarkers due to the frost treatment still reflected the order of the initial values of each population; thus retaining their high and low concentrations respectively. RPF was the only population that increased its glucose and fructose concentration. Surprisingly, following the frost treatment, sucrose concentration did not change (BB, NDS) or declined (NRW, RPF) but rather showed a similar order to that of the control of the respective population. The decline of sucrose in NRW and RPF contrasts with much literature (e.g., Kasuga et al. 2007, Morin et al. 2007).We stipulate that this sugar could have been converted to other more putative cryoprotective sugars like raffinose and or stachyose (Guy et al. 2008, Yuanyuan et al. 2009). It could also be possible that the failure of sucrose to increase may have been competitively hampered by other higher energy consuming processes. Proline increased only marginally at -17 °C frost treatment and not at -27 °C. Failure of proline to increase at -27 °C may have been due to other energy overriding processes or possible denaturation of proteins (i.e. enzymes) necessary for proline biosynthesis due to low temperature (Guy et al. 1998). A repetition of the early frost experiment with BB and NRW in 2012 confirmed the results for REL from 2011 for these populations. However, fructose and starch concentrations in shoots were significantly higher compared to the previous year (2011), while proline was lower. The differences between the two years can be explained by the heterogeneity of the populations used where mother plants might have been different genetically hence the dissimilarity in constitutive proline biosynthesis as in the case of walnut genotypes (Aslamarz et al. 2011). Furthermore, variation in the biosynthesis of sugars and starch was also observed for poplar trees investigated in three years by Sauter and van Cleve (1994) and attributed to the respective weather conditions in these years.

The presence of biomarkers like soluble sugars and proline often are reported to contribute to freezing tolerance (Sauter and van Cleve 1994, Généré et al. 2004, Morin et al. 2007, Yuanyuan et al. 2009).

In our experiments, neither the differences in biomarkers between the populations in 2011 nor between 2011 and 2012 for the populations BB and NRW affected REL, hence early frost hardiness. Similarly, Yildiz et al. (2014), working with provenances from different altitudes, only found trends between soluble carbohydrates and cold hardiness. In a semi-physiological model for the prediction of cold hardening Poirier et al. (2010) found that soluble sugars did not have the highest correlation with cold hardiness (LT_{50}). These results indicate that a clear relation between frost hardiness and soluble sugars must not necessarily be expected.

3.1.8 Conclusion

According to our findings, although populations' origin had some statistical significant effects on most of the biomarkers investigated, the results were inconsistent within early frost treatments and in both years. For some set of the populations biomarkers investigated were physiologically and biochemically different while in another set they were similar in spite of the geographical distance and climatic differences. In addition, we did not observe any clear altitudinal or latitudinal trend when comparing the various populations. Compared to results from literature where such trends have partly been observed, the geographical range of the investigated populations could have been too small. Moreover, concluding from RPF, originating from a high altitude and heterogeneous environment, micro-site conditions could have a big impact on the physiological and biochemical constitution of the plants. However, all populations investigated were able to survive early frost stress within the range tested. Genetical differences between the populations found by Leinemann et al. (2013b) were not reflected in their early frost reaction. Most parameters showed high degree of variability (high standard deviations) indicating high heterogeneity among the populations. This implies that the populations are diverse and are probably able to initiate adaptation to other ecological zones than the ones they are currently occupying and which is prescribed by § 40 of the Federal Nature Conservation Act.

3.2 Late frost reactions of different populations of hazelnut (*Corylus avellana* L.)

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3.2.1 Abstract

In Germany, Federal Nature Conservation Act § 40 was enacted in 2010 to regulate trees and shrubs in an open landscape due to postulated genetic differences and regional adaptations to soil and climate. Propagation and utilization of plants must therefore be in accordance to the Act. However, trees and shrubs are reported to possess considerable adaptation plasticity and can inherently perform over wide ecological units. In this study we evaluated plasticity of four populations of *Corylus avellana* from different places of origin to late frost stress. After cultivation on the container area of Leibniz University of Hannover, plants were treated with temperatures of -12 °C and -6 °C under controlled conditions. Relative electrolyte leakage as a measure of damage due to frost increased with decreasing temperatures and sprouting stage. Glucose; fructose, sucrose (GFS) and starch declined with sprouting while proline increased. Starch and proline did not react to late frost treatment while GFS were variable between treatments, years and time of the year. Populations differed consistently only in their proline concentrations. Sprouting stage was the most significant factor influencing both frost-induced electrolyte leakage and biomarkers. In conclusion, there were no clear, consistent differences between the tested populations in spite of varied climatic conditions and geographical distance between their places of origin. Hence no late frost consequences in populations' transfer with regards to latitude and altitude will be expected within the range investigated here.

Key words: altitude, sprouting, climate, cryoprotective compounds, latitude, relative electrolyte leakage, stress

3.2.2 Significance of this study

What is already known on this subject?

Trees and shrubs are reported to have enormous adaptive capacity to endure and proliferate in extreme climatic conditions in nature. However, it is agreed not to source plants from extreme climatic differences, but rather to use native trees and shrubs. In Germany use of trees and shrubs in free nature has been further restricted to six officially defined areas of origin through Federal Nature Conservation Act §40. These defined areas of origin are independent of species.

What are the new findings?

Phenological monitoring during bud sprouting as well as physiological and biochemical results from four German populations of *Corylus avellana* L. exposed to late frost under controlled conditions

demonstrated them to be similar despite climatic differences in their place of origin and also genetic differences. Deductively, there is no risk involved in transferring these populations within the latitude (50°N – 52°N) and altitude (38 m – 454 m a.s.l.) margins tested with respect to late frost.

What is the expected impact on horticulture?

Related to late frost, genetic differences, which are the base of the German Federal Nature Conservation Act, could not be translated to physiological and biochemical reactions. Accordingly, with regard to late and early frost (reported earlier), collection of propagation materials and trade of propagates by nurseries could be done without compromising their survival within the margins given above. Moreover, plants from different sources enhance genetic biodiversity and thus their adaptability, especially due to rapid climate change.

3.2.3 Introduction

Recent widespread anthropogenic global warming events have potentially increased the prevalence of erratic and sporadic frost events (Hänninen 2006; Gu et al. 2008; Kim et al. 2014). Consequently, inevitable and irreversible earlier bud break events have often been reported in many temperate climates (Hänninen 2006; Kim et al. 2014). Early sprouting increases the risk of frost damage to sprouting leaves (Taschler et al. 2004).

Consequently, timing of bud break is essential for adaptations of deciduous trees and shrubs to avoid or minimize late frost damage (Guy et al. 2008, Kim et al. 2014). Native populations' bud breaks are generally better adapted to their natural habitats and are thus reported to synchronize with the local temperatures (De Frenne et al. 2011, Kreyling et al. 2012). It has been predicted that transferring a population either to the north or to the south or either to a different altitude will alter their norm perception of their natural habitat especially air temperatures relative to native population (Rohde et al. 2011). This might increase the risk of frost damage especially if the non-native population originated from a more southern or from a lower altitude area of origin (Morin et al. 2007). It is also presumed that plants synthesize adequate carbohydrates (Hoch et al. 2002) and, although for herbs and semi shrubs, proline (Bano et al. 2009, Unal et al. 2013) inherently to their native habitat. These serve as energy reserves and cryoprotectant. This implies that transferring them to their non-native habitat might endanger them to extinction due to frequent late frost damage.

To protect native plants and to conserve genetic, species and ecosystem biodiversity, various national and international efforts have been made. Following Rio de Janeiro convention in 1992, the Federal Republic of Germany decreed a conservation law: Federal Nature Conservation Act § 40. This law came to effect in 2010 confining utilization of native species within officially designated areas of origins (BMU 2012). This law is however hypothetical as most species found in Germany came from southern Europe and are therefore alleged to be polyphyletic (Willis 1996).

Furthermore, based on the pattern of local adaptation manifested over the course of postglacial recolonization, trees and shrubs have been depicted to have wide ecological adaptation and enormous plasticity that enhances their performance in varying environmental conditions (Kramer 1995, Vitasse et al. 2010).

For physiochemical adaptability insight, especially under stress conditions, an exemplary experiment was set up using hazelnut (*Corylus avellana* L.). Hazelnut are polyphyletic from southern part and are native European species (Mehlenbacher 1991, Persson et al. 2004). Ecologically, in Germany it is an important deciduous landscaping plant; sexually as well as asexually propagated; wind pollinated; multi-stemmed and its seeds dispersed mostly by animals. Additionally, it provides food and shelter for wildlife. Genetically, several studies on European hazelnut populations have reported similarity using chloroplast DNA (cpDNA) and iso-enzymes (e.g., Persson et al. 2004). However, recently Leinemann et al. (2013b) reported some genetic variations using amplified fragment length polymorphism (AFLP) on some German populations.

In this study, therefore, our major objective was to evaluate adaptability of four German *Corylus avellana* population to different environmental stress under controlled conditions. Additionally, since our populations are genetically different (Leinemann et al. 2013b), we also evaluate whether the reported genetical variations would be manifested in their physiochemical reactions to late frost, early frost and drought. In this paper we restrict our findings to late frost experiments.

3.2.4 Materials and Methods

Our study was conducted at Leibniz University, Hannover (52°23'34 N; 9°42'13" E; 53 m a.s.l) where in total four German federal states' hazelnut populations were investigated. Plant material from Brandenburg (BB), Niedersachsen (NDS), Nordrhein-Westfalen (NRW) and Rheinland-Pfalz (RPF) were collected from diverse areas (Fig.3.2.1) by Leinemann et al. (2013a), supported by local forest research centres in identifying native populations. Populations studied were partial materials of which population genetics was conducted (Leinemann et al. 2013b). Hence, in Fig. 3.2.1, genetically similar groups are bordered with big circles according to Leinemann et al. (2013b); while the populations used in this experiment are in small circles, thus our populations were different genetically. The four populations' origins vary in topography, soil and climate. Ecologically, RPF is most heterogeneous of all in topography and climate. NRW on the other hand is less heterogeneous in magnitude compared to RPF whereas BB and NDS have little heterogeneity. Thus for RPF and NRW specific climatic data from a single nearby station cannot be representative, hence a range is given (Table 3.1.1) for all populations studied to provide a comparable data base. All plants were raised under the same environment and irrigation regimes. During the two years of our experiments, sprouting was monitored followed by late frost experiment.

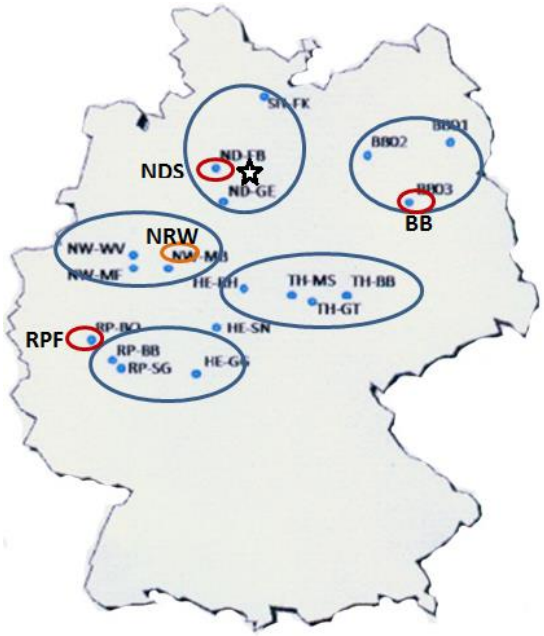

Figure 3.2.1: Geographic locations of the investigated populations of *Corylus avellana* in Germany. Each big circle denotes genetically similar groups in different localities according to Leinemann et al. (2013a) and small circles denote the populations used in our experiments: Brandenburg (BB), Niedersachsen (NDS), Nordrhein-Westfalen (NRW) and Rheinland-Pfalz (RPF).

Bud sprouting

The timing of vegetative bud sprout of two-year old plants in spring 2012 and one year plants in spring 2013 was monitored. Each plant's most advanced shoot (mostly the longest shoot) was evaluated once per week starting in February. We scored shoots' apical buds status from dormant winter bud to leaf unfolding status according to Rump (2002) using a 1 to 6 grading scale (Fig. 3.2.2).

Figure 3.2.2: Bud sprouting scheme used for rating bud break in spring for *Corylus avellana*.

1: All buds dormant, colored brown, length of terminal bud 4-7 mm long.

2: Buds are swollen, with green coloration.

3: Buds dehiscent, leaf tips visible.

4: Leaves begin to unfold.

5: First leaves fully developed and separated, shoot extension begins.

6: Almost all leaves have unfolded.

Late frost experiment

In April 2012, late frost experiments were conducted with four populations of *Corylus avellana* with 6 or 8 replicates per treatment. Plants were randomly allocated to -12 °C and -6 °C frost treatments respectively while 5 °C served as control. Three shoots per plant were cut (≈ 30 cm long, including buds and emerging leaves) and immediately placed in a plastic bag. This content was then treated either with -12 °C or -6 °C for eight hours and later thawed to room temperature. Temperature decrease and later increase was at the rate of 5 °C h^{-1}. After eight hours of treatment (also control), about 3 cm shoot's tip (with buds and emerging leaves) was used for REL determination while the remaining part (three shoots per plant, including buds and emerging leaves) were shredded and microwaved for two minutes and then oven dried at 70 °C.

This late frost experiment was repeated in April 2013, with only three populations (BB, NRW and RPF) and 14 replicates. The two late frost stress treatments were separated each in its own week due to logistic reasons of increased number of replications from 8 to 14. During week 16, plants batched as to receive -12 °C treatment and half of the controls were treated. In week 17, plants batch as -6 °C group and the other half of the controls were treated.

Relative electrolyte leakage (REL)

Relative electrolyte leakage test entails determining the electrical conductivity (EC) of double distilled water (DDW) in which detached plant samples have been bathed after a freeze-thaw cycle. Shoot tips (3 cm each) were severed and immersed in 30 ml of DDW vials. After 24 h infusion at room temperature, shaken and first EC was measured. The vials were then oven heated at 70 °C for another 24 h and a second EC was taken. REL was calculated as:

$$\mathrm{REL} = \frac{\text{First EC}}{\text{Second EC}} \times 100$$

Carbohydrates determination

After samples (without the 3cm used for REL) were shredded, microwaved and dried at 70 °C they were pulverized to fine powder. Ca. 30 mg of ground material, was used to extract glucose, fructose and sucrose (GFS) determinations following Zhao et al. (2010) protocols with minor modifications [triethanolamine buffer (14 g triethanolamine + 0.25 g $MgSO_4$ dissolved in 100 ml water, pH 7.6] and NaOH were used instead of TRIS buffer and KOH respectively).

Starch was first hydrolysed to glucose using amyloglucosidase enzymes then quantified using glucose assay.

Proline determination

About 50mg of ground material, was homogenized with 1.8 ml sulfosalicylic acid (3%) and incubated on ice for 30 min. The homogenates were vortexed and centrifuged at 14462xg for 15 min. Precisely 150 µl of the supernatant was treated with 90 µl acetic acid and 90 µl acid-ninhydrin (6.25 g ninhydrin powder in 60% acetic acid + 85% orthophosphoric acid at a volume ratio of 83.8 to 16.2), then boiled for 45 min. After cooling, 1.5 ml toluene (99.9%) was added then vortexed and 0.2 ml coloured phase absorbance was determined at 520 nm using Versamax® Tuneable –Microplate reader photometer.

Regeneration plants

To assess survival and growth ability after freezing treatment, five plants per treatment from each population were randomly selected and placed in a basin filled with peat to protect the root system. These plants were treated in -12 °C, -6 °C or served as control at the same time as the frost treatment described above. After treatments the plants were allowed to regenerate in their container in the container area (outside). Regeneration plants were available only in 2013.

3.2.5 Statistical analysis

All data collected per population and treatment was subjected to multivariate analysis of variance (MANOVA) to test for treatment, sprouting and population main effects or interactions between and among them. MANOVA is an extended version of ANOVA with the advantage that multiple variables could be evaluating together with multivariate general linear models (Langsrud 2002). A logarithmic transformation of the data (REL, proline, glucose, fructose, sucrose and starch was performed to meet the requirement of a normal distribution prior to analyses. Where there were no interactions, treatments and population means at $p \leq 0.05$ were separated by Tukey test (Pipper et al. 2012). Pearson correlations were also conducted to assess relationship of sprouting on other parameters. All statistical analyses were performed using R 3.0.3 (R Development, 2014)

3.2.6 Results

Sprouting

In 2012, bud sprouting began in calendar week 8 and progressed slowly. On average, it was in stage 5 on week 15, when we started the late frost experiment (Fig. 3.2.3A). However, from the few remaining plants, sprouting progressed up to calendar week 17 (2012) when most plants had fully developed leaves (data not shown). We did not find the place of origin to have a consistent significant influence among the four tested populations. However, there was a clinal tendency of one lower altitude population (NDS) sprouting earlier than those of higher altitude (NRW and RPF) in 2012.

In 2013, there was a delay in bud sprouting which began in week 11 (Fig. 3.2.3B). But by week 17, as in 2012, all tested population had fully developed leaves. Interestingly, a significant reversed order

in bud sprout and origin was observed, where the high altitude populations (RPF and NRW) sprouted earlier than those of lower altitude (BB). However, in 2012 bud swelling began earlier and was spread over 8 weeks while in 2013 bud swelling began in week 11 and sprouting was compressed over a period of 6 weeks. Additionally, at the time of controlled late frost experiment, bud development was more advanced in year 2013 (plants were in stages 5 and 6) than in 2012 (plants ranged almost stage 3 to 6).

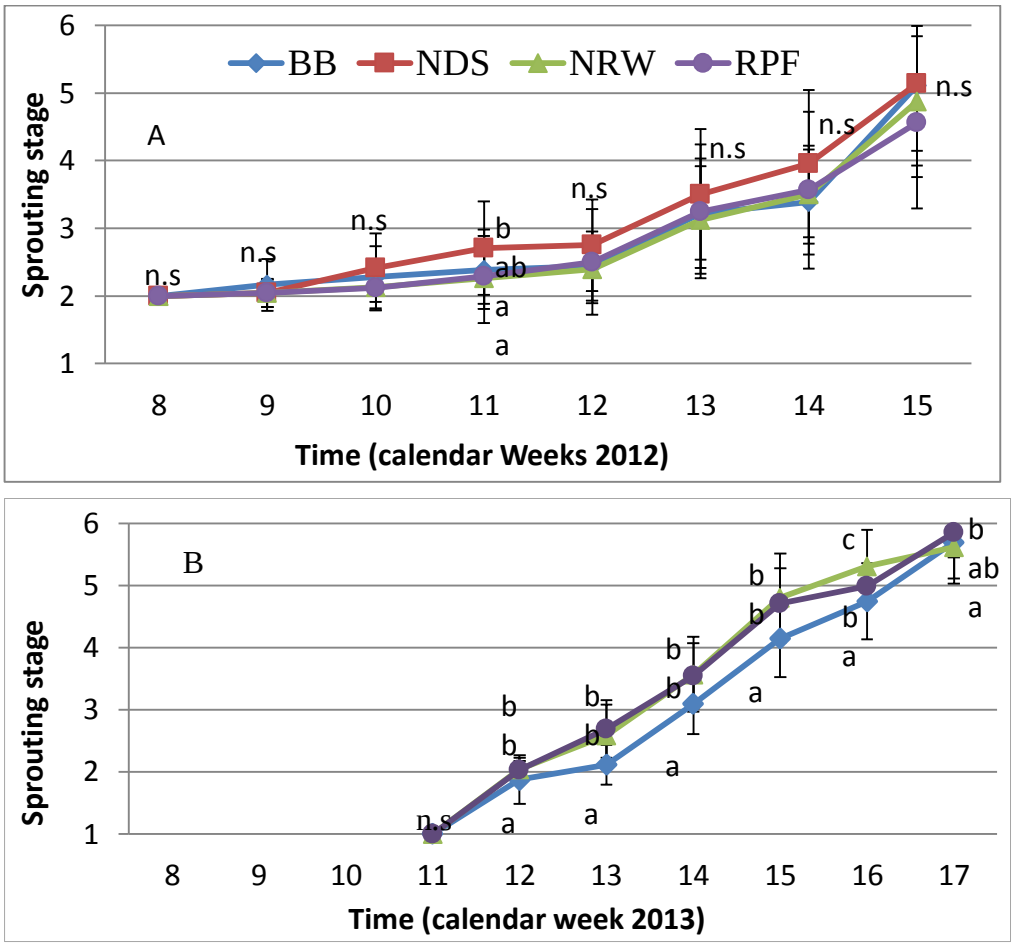

Figure 3.2.3: Bud sprouting phenology of four populations of *Corylus avellana* in spring 2012 (A) and three populations in spring 2013 (B). Different letters show significant differences among populations within each week. n.s = no significant difference. Mean ± SD, n = 24 (BB = 18) in 2012; n = 67 (BB), 101 (NRW), 90 (RPF) in 2013.

Relative electrolyte leakage

Freezing injury, measured as electrolyte leakage, increased with decreasing temperature as expected, excluding RPF (2012). However, there were great variations within the tested populations as indicated by high standard deviations (Table 3.2.1). REL of the evaluated populations did not differ significantly although NRW had the least REL in 2012.

Interestingly, sprouting significantly influenced REL level both when the plants were unstressed and under stress conditions (Table 3.2.2). Higher REL was noted on plants with high sprouting note than those of lower sprouting note as indicated by strong significant correlations (Table 3.2.2).
In 2013, REL results were similar to the previous year on the three populations tested in that they varied with sprouting (Table 3.2.2). Among the tested populations, BB and NRW differed in REL in week 17 after treatment (Table 3.2.1). Astonishingly, and the most intriguing result was how REL drastically increased within one week of development on plants serving as control (Table 3.2.1).

Table 3.2.1: Shoots relative electrolyte leakage (REL %) of four populations of *Corylus avellana* (BB, NDS, NRW, and RPF) in late frost experiments (April 2012, April 2013). Different letters show significant differences: small letters between populations within a treatment; capital letters between treatments of each population. Mean ± SD, n = 6 (BB), n = 8 (NDS, NRW, RPF) in 2012; n = 14, n = 7 (control) in 2013.

			Shoots (REL %)		
Year	Time	Origin	Treatments		
			5 °C	- 6 °C	- 12 °C
2012	Week 15	BB	27 ± 11 Aa	38 ± 15 ABa	53 ± 18 Ba
		NDS	26 ± 16 Aa	35 ± 13 ABa	49 ± 17 Ba
		NRW	19 ± 07 Aa	26 ± 10 ABa	38 ± 11 Ba
		RPF	28 ± 15 Aa	39 ± 22 Aa	47 ± 20 Aa
2013	Week 16				
		BB	27 ± 10 Aa		55 ± 09 Ba
		NRW	30 ± 14 Aa		59 ± 11 Ba
		RPF	26 ± 05 Aa		55 ± 10 Ba
	Week 17				
		BB	50 ± 12 Aa	63 ± 12 Ba	
		NRW	57 ± 11 Aa	74 ± 06 Bb	
		RPF	53 ± 09 Aa	69 ± 9 Bab	

Table 3.2.2: Pearson's correlation coefficient obtained by relating bud sprouting stages (1 to 6) and various parameters from late frost treatments in April 2012 and 2013 of four populations of *Corylus avellana*. Significant correlations ($p \leq 0.05$) are given in bold. n = 6 (BB), n = 8 (NDS, NRW, RPF) in 2012; n = 14, n = 7 (control) in 2013.

			Treatment	Correlation coefficient of sprouting stages (1 to 6) with:					
Year	Time	Population	(°C)	REL	Proline	Glucose	Fructose	Sucrose	Starch
2012	Week 15	BB	5	**0.85**	0.50	-0.76	-0.93	-0.85	-0.94
			-6	**0.76**	0.63	-0.76	-0.82	-0.54	-0.73
			-12	**0.95**	0.61	-0.96	-0.95	-0.12	-0.94
		NDS	5	**0.68**	0.18	-0.73	-0.7	-0.75	-0.50
			-6	0.45	0.36	-0.73	-0.49	-0.53	-0.47
			-12	**0.69**	**0.68**	0.12	0.17	-0.65	-0.63
		NRW	5	**0.68**	0.01	-0.59	0.23	-0.42	0.14
			-6	**0.90**	0.22	-0.45	-0.26	-0.65	0.42
			-12	0.51	0.39	-0.66	-0.52	0.30	-0.10
		RPF	5	**0.86**	**0.63**	-0.55	-0.48	-0.78	-0.67
			-6	**0.87**	**0.78**	-0.69	-0.83	-0.48	-0.48
			-12	**0.62**	**0.89**	-0.40	-0.53	-0.83	-0.71
2013	Week 16	BB	5	**0.9**	-0.02	-0.42	-0.11	-0.51	-0.77
			-12	**0.84**	-0.09	-0.21	-0.06	-0.73	-0.67
		NRW	5	**0.75**	-0.2	-0.38	0.58	-0.55	-0.91
			-12	**0.77**	0.14	0.37	0.04	-0.8	-0.76
		RPF	5	0.06	-0.2	-0.01	0.40	0.10	0.42
			-12	**0.91**	0.2	-0.23	-0.40	-0.81	-0.54
	Week 17	BB	5	**0.93**	**0.76**	-0.16	-0.31	-0.41	-0.56
			-6	**0.88**	**0.58**	-0.1	-0.24	-0.48	-0.64
		NRW	5	0.47	**0.69**	-0.37	-0.06	-0.01	-0.72
			-6	**0.51**	0.09	-0.27	-0.23	0.10	0.07
		RPF	5	**0.68**	0.51	0.06	-0.05	-0.32	-0.52
			-6	**0.75**	0.36	-0.62	-0.15	-0.39	-0.31

Carbohydrates

Carbohydrates were distinctly reduced by sprouting as indicated by glucose, fructose, sucrose and starch showing negative correlations (few exceptions) with sprouting stage (Table 3.2.2). Early sprouting stages had significantly higher concentrations than sprouting stage 6 (data not shown). Nevertheless, the four populations tested in this experiment (2012) did not differ from each other ($p = 0.2$) in the analyzed biomarkers (Fig. 3.2.4).

Upon treatment, glucose, fructose, sucrose and starch concentration in most cases were not significantly affected by frost treatment (Fig. 3.2.4).

A repetition of the same experiment in 2013, although different in that treatments were split into two weeks, yielded remarkable insight on the magnitude of change which can occur within a week.

During week 16 and week 17, the populations did not vary significantly in their sugar and starch concentrations when untreated (control) (Fig. 3.2.4). However, upon treatment, some significant differences were detected. In week 16, treatment (-12 °C) significantly affected glucose, fructose and sucrose (Fig. 3.2.4). Frost treatment increased the concentration of glucose and fructose while it declined the concentration of sucrose. Starch did not react with the treatment (Fig. 3.2.4). When comparing the three populations tested, BB had higher fructose and starch concentration (after treatment) than RPF but neither of them differed from NRW (Fig. 3.2.4).

During week 17 (-6 °C), treatment significantly affected glucose and fructose concentrations but not sucrose and starch (Fig. 3.2.4). When contrasting the three populations after treatment, BB and NRW differed only in sucrose concentrations in that BB had lower sucrose concentration than NRW and neither of them differed with RPF (Fig. 3.2.4). Only in week 16 there was a significant effect of sprouting on sucrose and starch (data not shown). The populations' origin, sprouting and treatment interaction was never significant on carbohydrates concentration in both years. Hence each factor had an independent influence.

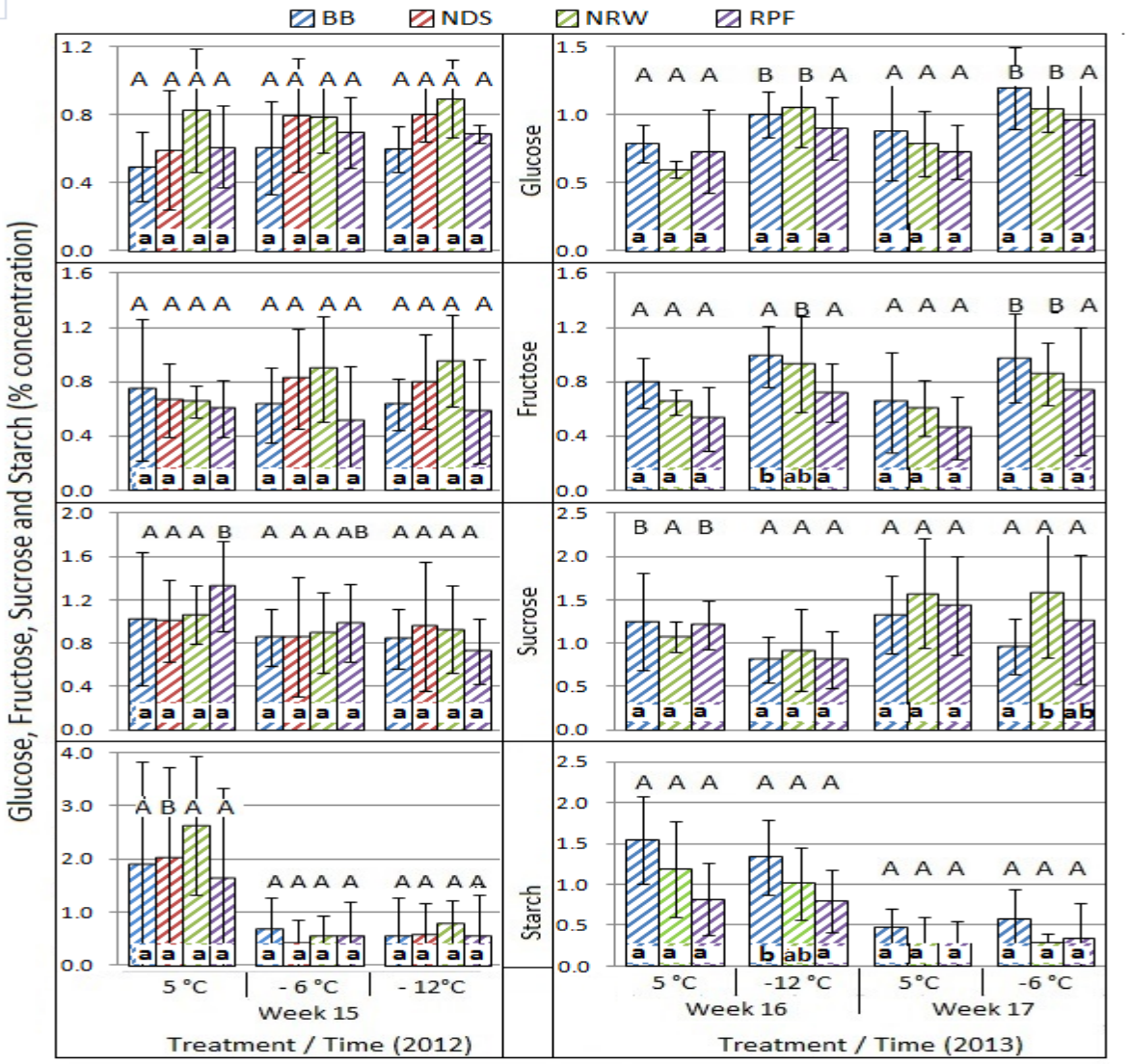

Figure 3.2.4: Glucose, fructose, sucrose and starch concentration (% dry matter) in shoots of four populations of *Corylus avellana* (BB, NDS, NRW, and RPF) in a late frost experiment (April 2012/2013). Different letters show significant differences: small letters among populations within a treatment; capital letters among treatments of each population. Mean ± SD, n = 6 (BB); n = 8 (NDS, NRW, RPF); n = 14, n = 7 (control) (2013).

Proline

Proline concentration mainly increased with sprouting stages, as indicated by positive correlation with significant influence in some cases (Table 3.2.2). Conversely, proline did not react with exposure to low temperatures (Fig. 3.2.5). Among the tested populations, proline concentration differed significantly, where NDS and RPF had significantly higher proline concentration than BB but they (NDS and RPF) did not differ significantly with NRW in 2012.

A repetition of the same experiment in 2013 with BB, NRW and RPF populations yielded similar results for proline concentration in that it was more influenced by sprouting stage than frost treatment as it can be seen on control plants between week 16 and 17 (Table 3.2.2, Fig. 3.2.5, 2013). Contrasting

three populations, again RPF had higher proline concentrations and differed significantly with BB and NRW across all treatments. In terms of absolute levels, populations plants evaluated in 2012 had higher proline concentrations than those tested in 2013 (Fig. 3.2.5).

Regeneration

Sprouting buds were visibly damaged in both frost treatments. Twigs were slightly damaged at -6 °C but were highly damaged at -12 °C. This treatment (-12 °C) resulted in shoot die-back compromising apical dominance and in more shoots sprouting from either the base of the plant or from the lateral buds of the affected shoots. However, all treated plants survived freezing temperatures and were able to regenerate. The number of sprouting shoots was highest on plants treated in -12 °C and least in control plants (5°C). However, in terms of height, control plants were the tallest while the -12 °C were the shortest (data not shown). There were no differences in the three populations whether in height, root collar diameter, number of shoot or in bud setting at the end of the vegetation period.

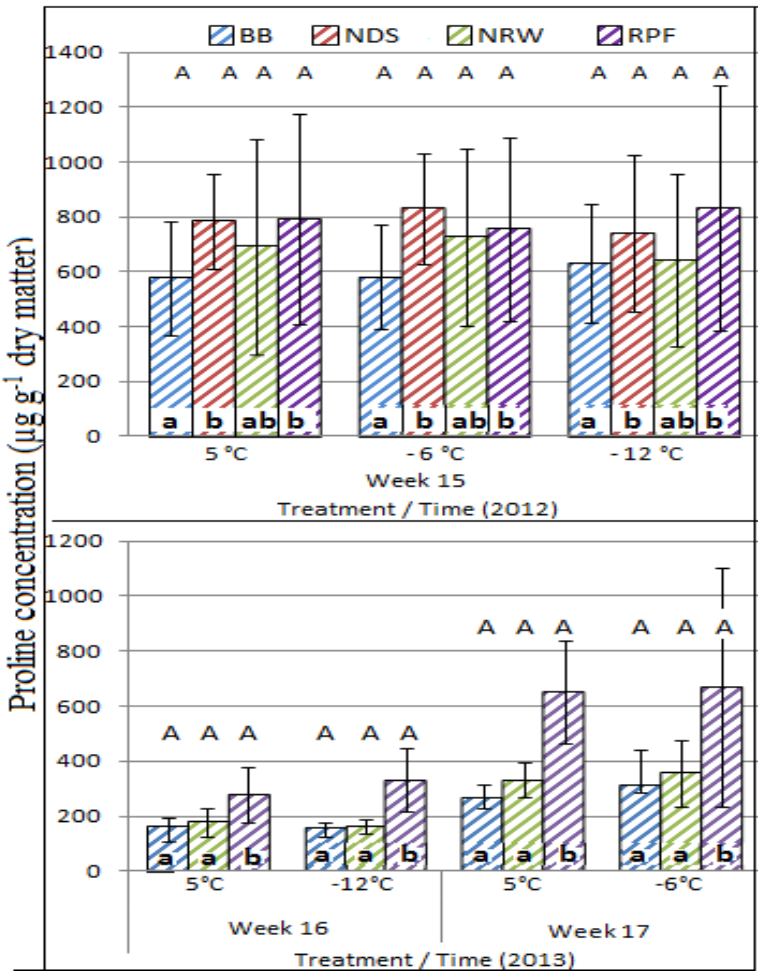

Figure 3.2.5: Proline concentration (µg g^{-1} dry weight) in shoots of four populations of *Corylus avellana* (BB, NDS, NRW, and RPF) in a late frost experiment (April 2012/2013). Different letters show significant differences: small letters between populations within a treatment; capital letters between treatments of each population. Mean ± SD, n = 6 (BB); n = 8 (NDS, NRW, RPF); n = 14 (2013).

3.2.7 Discussion

Late frost experiment in 2013 turned out not only to serve as a repetition of 2012 late frost experiment, but also as an insight of how physiology and biochemical changes can occur within a week. Differences between the two years sprouting and the compressed sprouting in 2013 could be attributed to the temperature difference between the two years. In 2012 temperature (week 8 to week 15) was higher and sprouting started earlier though with a slower rate than in 2013. In 2013, population started to sprout later because the temperature remained low (even below 0 °C) and this delayed sprouting up to week 12. However, in week 15, for example, temperature rose up rapidly where some days' maximum temperature was up to 19 °C (MuK 2013), hence a rapid sprouting rate. Temperature has been shown to have a major influence on sprouting and sprouting rate of *Corylus* (Wesołowski and Rowiński 2006). Our results showed that in 2013 sprouting started late but the rate of sprouting was fast and developmental stages changed rapidly from stage 3 in week 16 to sprouting stage 6 in week 17 as the temperature increased rapidly. This high rate of development gave a high rise in relative electrolyte leakage from control plants where REL rose from 30% in week 16 to 57% in week 17 due to still unlignified leaves' cell wall (Boudet et al. 1995). Although populations did not differ either in untreated state or in severe stress (-12 °C) in REL, they differed in week 17 after a less severe stress (-6 °C). This difference in the level of REL between BB and NRW, was just significant ($p = 0.04$) and may not be termed as a difference since their regeneration plants were similar in their development. This asserts that they were not physiologically different and therefore questions the informative role of a single frost experiment at this time of the year.

Interestingly, biomarkers (glucose, fructose, sucrose, starch and proline) also changed tremendously within a week of development. In all these changes, the population's origin had no influence. A similar phenomenon of soluble sugar and starch change during bud break was also reported in blueberry cultivars (Lee et al. 2012). Proline has also been reported to increase with sprouting (Walton et al. 1998). This depicts how versatile *Corylus* plants can be in their physiological and biochemical reactions within a short period of time.

It was also surprising how applied late frost stress on week 16 caused a remarkable change in glucose, fructose and sucrose compared to week 17 where only glucose and starch reacted. With this background information, it is imperative to carefully interpret our results obtained for the two years.

Overall, the four evaluated populations did not differ consistently in sprouting and only a trend was observed. This contrasted to several studies which had reported influence of latitude and altitude on the flushing trend on deciduous trees (Chmura and Rożkowski 2002, Ovaska et al. 2005). However, their geographical range was bigger than our populations' range, hence the contrasting results. Furthermore, the observed trend concerning sprouting was reversed in the following year. This can be explained by the fact that in our experiments plants cuttings were taken from different mother plants.

Hence such differences in sprouting may be expected due to an influence of different genotypes within the population (Howe et al. 2003). This implies that genetic diversity is high in every population and propagation of *Corylus avellana* will continually consist of different genotypes. These genotypes could in turn interact continually with the environment resulting in phenotypically differing plants. This could further trickle down to nurseries in that their material propagated is never consistent.

Sprouting stage has been shown to affect spring frost damage. In our experiment regardless of population's origin or treatment, sprouting stage 6 had significantly higher REL than stage 2 (the range being considered in our experiments). The increase in REL (especially in control) with sprouting stage could have been caused by unlignified leaves, hence compromised membrane integrity as a result of rapid expansion rate as reported in literature (Taschler et al. 2004). This high REL in control plants was not related to lethality since all control regeneration plants did not show any frost damage related symptoms. For the treated plants, the expanding leaves are reported to be the most susceptible stage and frost hardiness is unlikely (Taschler et al. 2004, Augspurger 2009). However, some literature (e.g., Taschler and Neuner 2004) has also cited clinal frost hardiness with increasing altitude for similar phenological stage which was not evident in our experiment.

Carbohydrates pool, especially the starch and sucrose, was diminished by sprouting. This was used to support bud development as indicted by decreasing concentration with sprouting (Lee et al. 2012). Sprouting stage in this study had a significant influence on the level and concentration of carbohydrates. Sprouting stage 6 had the lowest concentration of starch, glucose, fructose and sucrose (data not shown). This is because bud break and sprouting consume energy and reserve material stored in the stems of hardwood trees (Sauter and van Cleve 1994, Maurel et al. 2004). Hence carbohydrates degradation, although a complex process, during spring might render the plant susceptible to frost damage (Pagter et al. 2011). In the present study, when untreated, most of the carbohydrates in all the tested population declined with sprouting as in other woody plants (Lehmann et al. 2010, Lee et al. 2012). Frost treatments had profound effects on glucose, starch and sucrose concentration. Starch and sucrose decline with temperature in this late frost experiment may be attributed to increased respiration or other energy consuming processes as plants strove to withstand low temperature (Pagter et al. 2011). For instance, a decrease in soluble sugars in our study is negatively correlated with sprouting and increased frost damage upon treatment. This indicates that sprouting consumed carbohydrates reserves that could have otherwise been used to withstand frost. Among the evaluated populations there were no differences between them either in 2012 or in 2013.

Unlike carbohydrates, sprouting was positively influenced proline concentration. This increase in proline concentration could be due to its speculative role in breaking bud dormancy and supplying energy during bud break (Walton et al. 1998). Investigating several herbaceous plants from Hunza

valley Pakistan, Bano et al. (2009), found an increasing proline concentration with altitude. To the contrary, our populations were inconsistent regarding altitude, although the highest altitude population RPF accumulated significantly more proline than other populations in 2013.

In our study, proline concentration was not influenced by treatment (Fig. 3.2.5). This was similar to what we found during the early frost experiment (Wanjiku and Bohne 2015). This contrasts literature that often report proline to be a major player in stress tolerance (Lehmann et al. 2010, Aslamarz et al. 2011). Higher proline concentration in 2012 than 2013 plants could be attributed to the mother plants genetics; hence the dissimilarity in constitutive proline biosynthesis as reported by literature investigating genotypes (Aslamarz et al. 2011).

3.2.8 Conclusion

To conclude, late frost induced various physiological and biochemical perturbations depending on the level of sprouting. This damage however can only partially be related to the level of relative electrolyte leakage. This is because REL proved to change with development stage and not exclusively with damage. Lack of significant influence of origin in these populations tested does not only show wide ecological adaptation of *Corylus* plants but also demonstrates high plasticity of these populations within the ecological range limit tested in this experiment. Hence the reported genetic differences by Leinemann et al. (2013b) were never consistently manifested in either physiological or biochemical reactions during late frost. This raises the question concerning better adaptability of native populations assumed in the Federal Nature Conservation Act.

3.3 Drought reactions of different populations of hazelnut (*Corylus avellana* L.)

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3.3.1 Abstract

Outplanting performance of trees and shrubs cultivated in tree nurseries is assumed to be better, if propagation material is sourced from the designated areas of future growth. However, this requires a local nursery to produce that cultivar, which might reduce the availability of that species. In this study we evaluated drought reactions of 2.5 year-old hazelnut (*Corylus avellana* L.) from four populations origin. After container cultivation, plants were subjected to drought by irrigating 25% (fast stress) or 50% (slow stress) of the lost water. Control plants were well irrigated. Depending on stress development and hence stress duration, different physiological (stomatal conductance, predawn water potential, relative water content) and biochemical (glucose, fructose, sucrose, starch, proline) responses to drought were found. Independent of stress development, only few differences among populations were found. These differences were mostly not related to precipitation in their area of origin, suggesting no local adaptation within the ecological range investigated.

Index words: area of origin, conservation act, osmolytes, stress

3.3.2 Significance to the Horticulture Industry

Recommendations and regulations concerning the use of local ecotypes for landscape plants add logistical problems regarding sourcing of liners and cultivation of plants in the nursery might unnecessarily increase the cost of production. Further, it might hamper landscape management as some areas may not have adequate propagules. Ensuing from our results on drought reactions of four hazelnut populations, such procedures could be eased if the species considered has a wide ecological range and populations come from ecologically similar areas.

3.3.3 Introduction

Commercial nurseries' task is to collect and propagate plant species required to restore and establish landscapes. Recently, demand for locally grown plants has increased due to the notion that local ecotypes of propagules might grow best. However, the challenge on nurseries is where quantities of liners with acceptable quality could be obtained (Mortlock 2000). When local populations' propagules are not adequate or feasible, use of a non-native population had been recommended, provided they are ecologically and functionally similar (Jones 2013).

Use of native populations has sparked a yet to end debate with two perspectives. Ecologically, local ecotypes closely match local conditions which could enhance survival and performance (Jones et al. 2001). Geneticists support the use of local ecotypes to preserve genetic biodiversity (Leinemann et al. 2013b) that might further support adaptation to both biotic and abiotic factors. The contrary argument is that, use of native populations may not always guarantee better performance (Schreiber et al. 2013b) and might be unfit to restore a landscape when the original conditions have been altered.

In Germany, there is an enduring debate principally because of the Federal Nature Conservation Act § 40 (BNatSchG 2010), which aims at conserving regional genetic structures at the population level (transition period until 2020). The underlying principal assumptions of the act are that, adaptations to local growing conditions have an advantage, and that genetic differences among populations are significant and should be preserved. Accordingly, six officially designated areas of origin referred to as provenances for open landscape (not forestry) plants have thus been demarcated. However, one must consider that growing conditions often differ within a provenance, and conditions in one provenance may not differ significantly from adjacent areas.

Moreover, rapid climate change is a threat across ecosystems and amplifies the question of using currently locally adapted plant populations (Thomas et al. 2014). Concerning drought, trees and shrubs possess physiological and biochemical mechanisms to cope with such stresses. These includes stomata closure and accumulation of compatible solutes like sugars and proline (Chaves et al. 2003). It could be assumed that these mechanisms ensure adaptation and survival of populations not only within their local nativity but also in ecologically similar areas.

Our study explored physiological and biochemical effects of drought on four populations of hazelnut (*Corylus avellana* L.) from Germany. Hazelnut is an ecologically important deciduous landscaping plant as it provides food and shelter to many insects, birds and rodents (Mehlenbacher 1991, Tallantire 2002). Leinemann et al. (2013b) classified some German populations as genetically different using amplified fragment length polymorphism (AFLP).

We therefore, attempt to evaluated adaptability of four hazelnut German populations to drought stress under controlled conditions. We also compared physiological responses observed within these four ecotypes to their reactions to drought.

3.3.4 Material and Methods

Hazelnut cuttings were collected by Leinemann et al. (2013b) assisted by local forest research centres in identifying native (presumably autochthonous) populations. From this collection, four populations associated with four German federal states were selected: Brandenburg (BB), Niedersachsen (NDS), Nordrhein-Westfalen (NRW) and Rheinland-Pfalz (RPF) (Table 3.1.1). The four federal states differ partly in summer climate as shown in Table 3.1.1.

Experimental design. In summer 2012, plants were assigned to control or to drought treatments (slow or fast stress) using a completely randomized design with 6 (BB) and 8 (NDS, NRW and RPF) replicates. The experiment was carried out in a greenhouse with an average temperature of 25 ± 4 °C (77 ± 39 °F).

All containers with plants were saturated before moving them into the greenhouse where the weight of each container was taken. Stressed plants were placed on spacers while control plants sat on the table. Every two days, stressed plants were weighed and separately irrigated with either 50% (slow stress) or 25% (fast stress) of the lost weight. The control plants were irrigated twice a day by ebb and flow irrigation. The experiment was terminated when half of the plants in each drought treatment withered. The following parameters were evaluated:

Predawn water potential (WP). At predawn (5:00 to 6:00 a.m.) on every other day, leaf water potential was determined from three randomly chosen plants per treatment per population using a Scholander bomb (PMS Instruments, Corvallis, Ore). At the end of each treatment, leaf water potential was determined from all drought plants and from three control plants per population. For each measurement the topmost fully expanded leaf was used.

Relative leaf water content (RWC). The same leaf excised for WP was used to determine RWC. The fresh weight of each leaf (FW) was recorded. Each leaf was soaked for 24 hrs in water at room temperature under darkness after which saturated weight (SW) was recorded. Leaves were oven dried for 24 hrs at 70 °C (158 °F) before dry weight (DW) was recorded. Then RWC was calculated as: $RWC = [(FW - DW)/(SW - DW)] \times 100$

Stomatal conductance (SC). Each day (11 a.m. to noon) SC was measured with a steady-state AP4 Porometer (Delta-T Devices, Cambridge, United Kingdom) from topmost fully expanded leaf from all plants.

Regeneration. Five additional plants per treatment were included in the drought stress experiment and re-irrigated at the end of the drought period. After re-watering, these plants were transplanted into the field for regeneration under natural precipitation. Regeneration was scored by number of shoots, height and root collar diameter five months after drought treatment (end of autumn).

Sampling at the end of the experiment. From each plant, samples for biomarker (glucose, fructose, sucrose, starch and proline) analysis were taken from the uppermost leaves (3 leaves per shoot). These samples were microwaved for two minutes, to deactivate enzymes, and later dried at 70 °C for 72 hrs. Each sample was pulverized to fine powder before analysis.

Carbohydrates analysis (Microplate (MP) method). About 35 mg of ground material was used to extract soluble GFS (glucose, fructose and sucrose) using 4.5 ml (3 times using 1.5 ml each time, 15 min each). 80% ethanol in warm water bath. Pellet was saved for starch analysis. Glucose, fructose and sucrose were determined enzymatically as detailed by Zhao et al. (2010).

Starch analysis. The pellet (saved above) was suspended with 0.5 ml NaOH (0.5 M). It was then gelatinized by incubating it at 60 °C (140 °F) for 30 min in a shaker at 150 rpm. After cooling, 475 µl and 25 µl of water and acetic acid were added respectively, vortexed and centrifuged (1180 x g) for 5 min. In triplicates, 10 µl supernatant was placed in MP. Starch was hydrolyzed to glucose by adding 20 µl amyloglucosidase enzyme (4.5 mg dissolved in 2 ml citrate buffer for one MP) to the sample then incubating for 30 min at 60 °C and gentle shaking after 10 min. Starch was quantified as glucose assay above.

Proline analysis. Approximately 50 mg of ground material was suspended in 1.8 ml sulfosalicylic acid (3%) and incubated on ice for 30 min. The homogenate was vortexed and centrifuged (14462 x g) for 15 min. Thereafter 150 µl of the supernatant was treated with 90 µl acetic acid and 90 µl acid-ninhydrin followed by boiling in a water bath for 45 min. After cooling, 1.5 ml toluene was added and vortexed. The coloured toluene phase (200 µl) was pipetted in MP (triplicates) and absorbance at 520 nm was determined using a photometer (VERSAmax® Molecular Device, Sunnyvale, California USA). Acid ninhydrin prepared as described by Bates et al. (1973).

Statistical analysis. Data from all parameters was subjected to multivariate analysis of variance (MANOVA) to test treatment and population main effects or interactions between them (Treatment x populations). Data was log transformed for normal distribution prior to analyses. Where there were no interactions, treatments and population means, at $p \leq 0.05$, were separated by Tukey test. All statistical analyses were performed with R 3.1.3 (R Development 2014).

3.3.5 Results

Drought stress did not influence most physiological parameters during the first six days after drought initiation. During this period, treated plants retained similar stomata conductance (Fig. 3.3.1), predawn water potential and relative water content values as those of controls (data not shown). At day 9 (D9) for fast and 13 (D13) for slow stress, over 50% of the treated plants were severely wilted, marking the end of drought experiment. Only then was stomatal conductance decreased significantly differing compared to the control (Fig. 3.3.1). At this time (D9 and D13), control plants had retained high WP ($\geq$ -0.9 MPa) while the treated plants had significantly lowered their WP, up to -3.2 MPa irrespective of origin (Table 3.3.1). Fast stress induced lower WP than slow stress. At D9, RWC of all fast stress treatment plants differed significantly from the control. This was not the case for plants in the slow stress treatment at D13 (Table 3.3.1). For all physiological parameters, there were no significant population differences. Although this was the case, NRW plants in both slow and fast stress treatment tended to close their stomata more quickly compared to the other populations.

Drought stress (slow and fast) varying effects on glucose, fructose and sucrose in the leaves (Table 3.3.1). Slow stress did not affect glucose concentration among the four ecotypes. It did, however,

increased fructose concentration in NDS and NRW but not in BB and RPF. Sucrose concentration was decreased in BB and NDS, but NRW and RPF concentration was not affected by slow stress (Table 3.3.1). Fast stress prompted an increase in leaves' glucose and fructose concentration in NDS, NRW and RPF, but not in BB.

Sucrose concentration in leaves was not affected by fast stress in NDS, NRW and RPF, but was declined in BB compared to the control (Table 3.3.1).

Leaves' starch concentration declined significantly (except NRW in fast stress) compared to control irrespective of drought stress level and origin (Table 3.3.1).

Proline increased in leaves similarly in the two stress treatments (Table 3.3.1). In slow stress, RPF had the lowest of proline concentration and differed significantly from NDS.

Combining results from slow and fast stress treatments at the end of drought, decreasing WP significantly decreased SC, RWC but increased proline concentration in all populations. This is supported by some strong significant linear correlations between these three parameters and predawn water potential (Table 3.3.2). Starch and GFS were least affected by decreasing water potential. However, in NRW, GFS were significantly influenced by WP (Table 3.3.2).

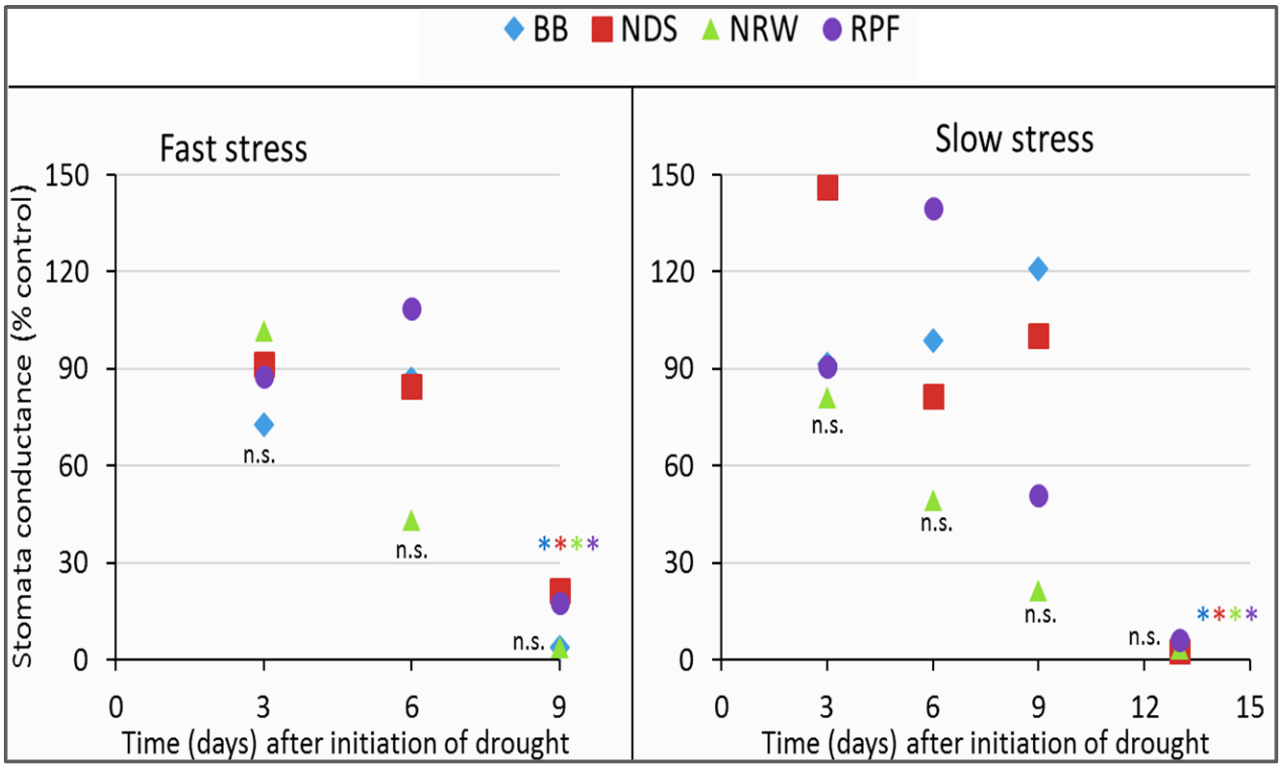

Figure 3.3.1: Stomatal conductance (% control) of four populations of *Corylus avellana* during drought treatment. Mean; n = 3 during the drought period; n = 6 Brandenburg (BB), n = 8 Niedersachsen (NDS) Nordrhein-Westfalen (NRW) and Rheinland-Pfalz (RPF) on the last day. **** = significant differences between each population's stress treatment and its control (raw data). n.s. = no significant difference among populations. Tukey test, $p \leq 0.05$.

Table 3.3.1: Leaves' WP, RWC, GFS, starch, and proline of four *Corylus avellana* populations at the end of fast stress (D9) and slow stress (D13) treatment [z].

Treatment	Origin	WP (MPa)	RWC (%)	Glucose (% dm)	Fructose (% dm)	Sucrose (% dm)	Starch (% dm)	Proline (µg g^{-1})
Control	BB	-0.7 ± 0.1 Ba	80 ± 04 Ba	0.97 ± 0.1 Aa	0.88 ± 0.1 Aa	4.43 ± 0.3 Ca	0.63 ± 0.4 Ba	42 ± 19 Aa
	NDS	-0.9 ± 0.1 Ba	73 ± 04 Ba	1.15 ± 0.4 Aa	0.88 ± 0.3 Aa	4.33 ± 0.6 Ba	0.49 ± 0.2 Ba	38 ± 13 Aa
	NRW	-0.7 ± 0.1 Ba	81 ± 02 Ba	1.02 ± 0.2 Aa	0.83 ± 0.4 Aa	4.04 ± 0.9 ABa	0.41 ± 0.4 Ba	38 ± 24 Aa
	RPF	-0.7 ± 0.2 Ba	75 ± 04 Ba	1.08 ± 0.2 Aa	0.98 ± 0.3 Aa	4.01 ± 0.6 ABa	0.65 ± 0.3 Ba	52 ± 13 Aa
Slow stress	BB	-2.0 ± 0.9 Aa	60 ± 14 Aa	1.35 ± 0.2 Aa	1.30 ± 0.3 Aa	**2.97 ± 0.4 Aa**	0.08 ± 0.03 Aa	138 ± 35 Bab
	NDS	2.1 ± 1.3 Aa	72 ± 14 ABa	1.44 ± 0.2 ABa	1.43 ± 0.2 Ba	3.34 ± 0.4 Aab	0.07 ± 0.02 Aa	**159 ± 47 Bb**
	NRW	1.7 ± 0.7 Aa	71 ± 08 Aa	1.25 ± 0.2 Aa	1.30 ± 0.2 Ba	3.18 ± 0.3 Aab	0.08 ± 0.06 Aa	142 ± 48 Bab
	RPF	-2.1 ± 1.1 Aa	71 ± 14 ABa	1.34 ± 0.4 ABa	1.22 ± 0.2 ABa	**3.73 ± 0.5 Ab**	0.07 ± 0.02 Aa	**93 ± 26 Ba**
Fast stress	BB	3.1 ± 0.2 Aa	58 ± 09 Aa	1.30 ± 0.5 Aa	1.26 ± 0.5 Aa	3.70 ± 0.6 Ba	0.05 ± 0.02 Aa	162 ± 33 Ba
	NDS	2.9 ± 0.9 Aa	59 ± 11 Aa	1.59 ± 0.5 Ba	1.52 ± 0.6 Ba	4.49 ± 0.7 Ba	0.20 ± 0.3 Aa	184 ± 64 Ba
	NRW	-3.1 ± 0.7 Aa	57 ± 09 Aa	1.81 ± 0.5 Ba	1.39 ± 0.5 Ba	4.66 ± 1.3 Ba	0.23 ± 0.2 ABa	168 ± 45 Ba
	RPF	3.2 ± 0.7 Aa	55 ± 10 Aa	1.63 ± 0.1 Ba	1.39 ± 0.2 Ba	4.85 ± 0.9 Ba	0.06 ± 0.03 Aa	193 ± 114 Ca

[z]Mean ± SD, . n = 6 Brandenburg (BB), n = 8 Niedersachsen (NDS), Nordrhein-Westfalen (NRW), and Rheinland-Pfalz (RPF). Different letters show significant differences: capital letter among the treatments within a population while small letters among the populations within a treatment

Table 3.3.2: Pearson's correlation coefficients obtained by relating WP with various parameters of four *Corylus avellana* populations [z].

Year	Origin	SC	RWC	Glucose	Fructose	Sucrose	Starch	Proline
2012	BB	**0.65**	**0.79**	-0.16	-0.22	0.11	0.56	**-0.83**
	NDS	**0.52**	**0.83**	-0.37	-0.33	-0.03	0.18	**-0.78**
	NRW	**0.57**	**0.91**	**-0.72**	**-0.42**	**-0.58**	0.19	**-0.66**
	RPF	**0.45**	**0.61**	-0.25	-0.33	-0.36	0.58	**-0.72**

[z]Significant correlations ($p \leq 0.05$) are given in bold. n = 6 Brandenburg (BB), n = 8 Niedersachsen (NDS), Nordrhein-Westfalen (NRW), and Rheinland-Pfalz (RPF).

After the drought treatments, plants suffered up to 25 cm shoots' dieback, especially plants in fast stress (data not shown). However, they were able to equally regenerate and by the end of the growing season, the differences in height with the control plants were not statistically different (data not shown).

3.3.6 Discussion

Stomata react to soil and atmospheric induced drought stress. Stomatal closure is often reported as the first drought avoidance mechanism in plants (Harb et al. 2009). In our experiment this was not demonstrated significantly before the end of the drought stress. Among others, water potential in the guard cells regulates stomatal behavior. Air humidity between 55 to 65% in the greenhouse and water supply (although decreasing per each irrigation) might have minimized water losses of the growing medium. In both drought treatments, the stress signal may have been weak or too short to cause a reaction. In a different experimental setup, Schulze and Küppers (1979) found that short-term changes in leaf water potential of *Corylus avellana* L. had little influence on stomatal conductance. This response was also found for *Prunus armeniaca* L. (Schulze et al. 1974) and attributed to an optimization of carbon gain versus water loss. As demonstrated in this experiment with fast stress development, further decreasing the water supply led to a sudden closure of stomata, but plants leaves' had reached the turgor loss point. The tested populations did not differ in their response to the abrupt water deficit, although BB comes from a low precipitation area.

Although stomata closed late, slow-stress plants maintained similar RWC to the control, while WP declined significantly. Lower WP can be achieved by decreasing water content, osmotic adjustment and/or elasticity of the cell wall (Ɛ) (Bartlett et al. 2012). In slow stress, osmotic adjustment was low (Table 3.3.1). The effect of Ɛ is discussed controversially in literature. According to Bowman and Robert (1985) high Ɛ (decreasing cell wall elasticity) results in a quick decrease in WP for a given change in water content, which favors further water uptake from a drying soil. This was supported by Savé et al. (1993) with strawberries subjected to mild water stress and could also be a reason for our results. It can only be assumed that our fast stress treatment did not allow such adaptions. Both RWC and WP were more affected in fast than in slow stress. Loss of water through stomata and passive/active accumulation of solutes led to very low RWC (55 to 59%). Read et al. (2010) reported for deciduous *Nothofagus cunninghamii* (Hook.) Oerst. that RWC of 55% caused leaf damage as measured by electrolyte leakage. Although thresholds for RWC seem to be species dependent (Lawlor and Cornic 2002, Dichio et al. 2006), this range applies also for the leaves of hazelnut in our fast stress treatment. The experimental setup resulted in different possibilities of physiological reactions to drought. While slow stress probably allowed adjustments in cell wall elasticity, fast stress did not. But independent on stress development, there were no differences in population's physiological reactions, suggesting a range of potentials against any assumed local adaptation.

Osmotic adjustment in terms of accumulating compatible solutes like proline, glucose, fructose and sucrose is reported as a mechanism for plants to tolerate drought (Chaves et al. 2003). Differences related to stress development and among populations (although not always) were found for these compounds. Proline concentration increased the most, mainly increasing independently of the stress

level and (with one exception) populations' origin. Among biochemical reactions, only proline concentration strongly correlated with water potential (Table 3.3.2). This agrees with literature where proline is reported to increase with water stress (Guo et al. 2010, Boussadia et al. 2013). The reaction of GFS differed for slow and fast stress. While there were few increases in glucose and fructose at the end of slow stress, there were significant glucose and fructose increases due to fast stress compared to the control, except for population BB. Concerning sucrose, (slow and fast stress), BB decreased its concentration significantly while this was not the case for the other populations, (except NDS slow stress). However, the sucrose concentration neither increased in these populations. The stress treatments also had different stress duration. Boussadia et al. (2013), investigating two cultivars, found glucose and fructose concentration partially increased after 10 days of drought and decreased after 20 days compared to the control. Either longer drought duration could not sustain higher concentration of these osmolytes (glucose, fructose, and sucrose) or they were used up for other sugars or sugar alcohols synthesis like mannitol and inositol. The latter was shown by Boussadia et al. (2013) for two cultivars of olive (*Olea europaea* L.), but even the cultivars tested reacted differently. From our data we could not detect if the decrease of a single sugar was due to metabolism, resulting in possible other osmolytes. Among the populations, only NRW GFS concentration was significantly correlated with WP (Table 3.3.2). The reason remains open but cannot be attributed to climate, since the one for NRW is not much different from NDS and RPF.

Starch degrading enzymes are often reported to increase with water stress (Chaves et al. 2003, Harb et al. 2010). These enzymes may be associated with a decline in starch concentration in both slow and fast stress treatment.

In summary, as for physiological reactions, results for GFS were only partially different within the populations tested. Moreover, the few differences found could not be linked to population's climatic conditions (Table 3.1.1), if at all for BB, coming from a low precipitation area compared to others. This agrees with the findings from Peuke et al. (2002) for beech seedlings, who found no distinct influence of provenance on drought-related physiological and biochemical parameters, and no consistent relation to areas of origins' rainfall amount. Additionally, few differences among our hazelnut populations during drought were not reflected in regeneration, which was equally good for all populations.

3.3.7 Conclusion

Mainly insignificant differences found in the drought reactions of the investigated populations suggest no local adaptation. This attests that hazelnut is adapted to wide ecological conditions (Mehlenbacher 1991) attributed to common ancestry (Willis 1996). The genetic differentiations reported by Leinemann et al. (2013b) were not featured by the species reaction to drought. Consequently, if only adaptation to growing conditions is considered, for a species with wide ecological adaptations

the efforts of discrete sourcing of propagation material and cultivation of plants in the nursery concerning the area of origin may be alleviated. This would facilitate nursery cultivation and supply of adapted species, which might not be available if a certain area of origin of the species is prescribed.

3.4 Seasonal characterization of different *Corylus avellana* populations

3.4.1 Abstract

The aim of seasonal characterization was to determine whether there was origin (local adaptation) variations in leaf phenology (sprouting and setting), growth, N, P, K and biochemical composition (glucose, fructose, sucrose, starch and proline) among the four *Corylus avellana* L. populations raised under the same conditions in a common-cultivation area. The cuttings were sourced from four distinct populations, rooted and cultivated under similar conditions. Phenology was scored in spring (March to April) and in autumn (October to November) for bud sprout and bud set respectively. The plants were sampled twice per year in spring (April) and in autumn (November) for biochemical and nutrient analyses. There were no significant differences found in phenology and growth among populations. Mostly, bud sprout was related to local temperature and varied with years. For most nutrients and biochemical parameters, there were no significant differences among the populations. Finally, this study highlighted the fact that both bud set timing and growth pattern were consistent from year to year. Bud sprout was dependent on local temperature and thus not consistent among the years. The results demonstrated that despite the reported genetic differences and distance among the populations in their natural areas there was no local differentiation. These plastic capacities among the population could allow populations transfer within the range tested.

3.4.2 Introduction

Fossil and genetic structure studies have highlighted common southern ancestry of hazelnut populations of the northern Europe trees and shrubs (Willis et al. 2000, Persson et al. 2004). This suggests that migration was the drive behind the current distribution ranges. However, according to Persson et al. (2004), historical bottlenecks during the species' postglacial range-expansion may have resulted to genetic drifts which could result to possible population differentiation (Howe et al. 2003, Krauss and He 2006). At present some governments and some conservationists are restricting intermixing of populations in a bid to protect genetic biodiversity. This presents a challenge as there are no sufficient scientific data of these populations concerning their adaptive capacities and the basis for enforcing conservation acts for using them in restoration and establishment of landscape. There are several parameters that could be explored in pursuit to generate exemplar information on differences among population in which this study endeavoured to explore.

Leaf phenology in temperate climates is of paramount importance as it influences fitness and survival capabilities of trees and shrubs. Early-sprouting or later setting of vegetative buds are more susceptible to spring or late fall frost damage (Taschler et al. 2004). Spring frost may kill the young leaves and exhaust reserves for growth in the season. This damage could considerably affect survival fitness and growth performance (Taschler et al. 2004, Menzel et al. 2015). In fall, frost damage could result

to die backs thereby reducing carbohydrate storage, break apical dormancy and consequently affect growth (Crow 1992).

Carbohydrate reserves are the main source of energy for the metabolic processes. They accumulate during the growing season in the perennial organs like stems and roots. During the dormant period and spring budburst, part of the starch reserves is hydrolyzed into soluble sugars for respiration and cryoprotection (Sivaci 2006). Carbohydrates reserves have been shown to differ with origin in some species (Körner 2003).Therefore, the carbohydrate reserves could affect survival.

Proline has also been shown to serve as a crucial cryoprotective (Lehmann et al. 2010) and although for herbs, it is affected by altitude (Bano et al. 2009). The higher the ability to accumulate these compounds the better for survival and growth.

Leaf senescence may also affect growth because it is associated with remobilization of nutrients and storage of assimilates (Norby et al. 2003, Keskitalo et al. 2005). Nitrogen has been shown to prolong senescence in fall and could result in increased storage of assimilates but can also increase the risk of incomplete nutrients remobilization, owing to frost damage to functional leaves (Keskitalo et al. 2005).

Growth traits can have a strong impact on survival, biotic interactions, long-term establishment and recovery after exposure to extreme environmental conditions (Brodribb et al. 2010, Menzel et al. 2015). Growth traits are therefore appropriate to study populations' inherent differentiations and adaptive capacities.

Since species displays wide range of morphological, and biochemical variations (Körner 2003), the among populations study could improve the basis of Federal Nature Conservation Act §40 on local adaptations to environmental factors. This study explored to answer whether populations sampled at different seasons are differentiated in the evaluated parameters. It also evaluated whether the previously reported genetic differences among populations (Leinemann et al. 2013b) affect these parameters.

3.4.3 Material and Methods

For this study material was collected from four German populations in 2009 and three populations in 2011 whose climatic data is given in table (3.1.1). The cuttings rooted in 2009 were evaluated for bud set in 2011 and bud break in 2012. The three populations rooted in 2011 were evaluated in 2012 for bud set in 2012 and for bud break in 2013. All populations from the two cutting years were evaluated for various parameters. An overview is given in table 3.4.1. The common container area's annual average temperature was 10.6 °C (spring = 10.1 °C, summer = 18 °C, autumn = 10.9 °C and winter = 2.94 °C) and sum rainfall was on average 621.6 mm (spring = 126.9 mm, summer = 197.3mm,

autumn = 123.6 mm and winter = 173.7 mm) for the three years (2011-2013) these plants were cultivated (Climate data from Institute of Meteorology und Climatology- Leibniz Universität, Hannover).

Table 3.4.1: Overview of the populations used for seasonal characterization of *Corylus avellana*. Abbreviations: BB = Brandenburg, NDS = Niedersachsen, NRW = Nordrhein-Westfalen, RPF = Rheinland-Pfalz.

Origin	Cutting year	Parameter and when (time) evalutated including replicates (n)		
		Height[x] RCD[y]	Phenology	Biomass, N, P, K and biochemical[z] concentration
BB, NDS, NRW, RPF	2009	autumn 2011 Autumn 2011: n = 57 (BB), 74 (NDS), 89 (NRW), 72 (RPF) spring 2013: n = 18 (BB), 24 (NDS, NRW, RPF)	autumn 2011, spring 2012	autumn 2011, spring 2012, summer 2012 n = 18 (BB), 24 (NDS, NRW, RPF)
BB, NRW, RPF	2011	autumn 2012 Autumn 2012: n = 161 (BB), 285 (NRW), 97 (RPF) spring 2013: n = 67 (BB), 101 (NRW), 90 (RPF)	autumn 2012, spring 2013	autumn 2012, spring 2013, summer 2013 (BB, NRW) autumn 2012: n = 13 (BB), 14 (NRW), RPF (7) spring 2013: n = 14 (BB, NRW, RPF) summer 2013: n = 14 (BB, NRW)

[z] Biochemical concentration includes: glucose, fructose, sucrose, starch and proline

[y] RCD = root collar diameter in mm [x] Height = height of the longest shoot in cm

Bud setting phenology

For bud set, plants were scored once per week in autumn (2011 - 2013) from calendar week 40 until calendar week 48 when buds were very hard to incise with finger nails. The four populations evaluated in 2011 included: Brandenburg (BB), Niedersachsen (NDS), Nordrhein-Westfalen (NRW) and Rheinland-Pfalz (RPF). For 2012 but setting phenology was scored from plant cutting 2011, Niedersachsen (NDS) population was lacking from this cutting year. Bud set was scored according to Fig. 3.4.1 (adopted from Rumpf 2002) scoring from 1 to 5.

Figure 3.4.1: Bud setting scheme used for rating *Corylus avellana* in autumn.

1 No buds visible, new leaves are visible from apical and lateral shoots.

2 Rudimental development of buds, partly development of new leaves from apical bud, axil buds begin to develop (< 1 mm).

3	Terminal bud is green - reddish in colour, bud scales visible, pruinose visible, axial buds are 2-3 mm and stipules are visible.
4	Buds at shoot tip and in axials: fully developed, 4-6 mm, colour of buds greenish to reddish, bud scales still not distinct reddish, buds still quite soft.
5	Buds are hard to incise with finger nail, no stipules, colour of bud green or red with distinct reddish bud scales.

Bud break phenology is described in section 3.2.4 above

Growth measurement

Morphological measurements were taken twice per year at the beginning (April) and at the end of the growing season (November). These included: plant height, root collar diameter (RCD) number of shoots and dry mass. For dry mass (DW) determination, several plants were randomly chosen per population, and each plant was separated into new shoots, old shoots and roots. These are placed into different bags and are dried at 70 °C for 72 hrs before dry weight can be determined

Carbohydrate and proline measurement

The plants selected for DW were sampled for carbohydrates (glucose, fructose, sucrose and starch) and proline determination. From each plant part (leaves, new shoot and roots (3 to 7 mm)). **Other procedures are as described in section 3.2.4 above**

Nitrogen (N) determination.

Nitrogen concentration was determined using Vario MAX C N analyser (Elementar, Hanau, Germany). Sample (1 g) was placed on Vario MAX crucibles and burnt at 900 °C with oxygen (Dumas method). The Vario MAX C N elementary analyser works on the principle of catalytic column oxidation with a supply of oxygen at high temperatures. The burning gases are purified from foreign gases to exclude interference

Nitrogen concentration was directly computed by Vario MAX software.

Determination of phosphorus (P) and potassium (K)

Approximately 0.1 g per sample was weighed and placed in crucibles. The content was then heated overnight in a muffle furnace at 480 °C. After ashing and cooling down, the ash was dissolved in 4 ml, 0.5 M HCl. The solution was then filtered into a test tube and afterwards used to determine P and K.

For phosphorous determination, 0.8 ml of the solution was mixed with 5 ml mixed reagent and pipetted into a microplate. Absorbance was made by photometer (VERSAmax®) at 470 nm wavelength

For potassium determination, 0.1 ml solution was diluted with 9.9 ml CsCl (Caesium chloride) buffer in a smaller reagent glass and atomic absorption was made with spectrophotometer (Perkin Elmer Analyst 300).

3.4.4 Statistical analysis

All data collected was subjected to statistical analysis using R 3.1.1 program. The analysis of variance was used to evaluate the statistical significance. If the means of the samples differed they were separated using the "Tukey" test, $p \leq 0.05$. Data in figures and tables are presented as mean, with standard deviation (± SD).

3.4.5 Results

Bud set

Although all plants were cultivated under similar environmental conditions, in 2011 plants from the northern and lower elevation (BB and NDS) lagged behind in their phenology while those of southern high (NRW and RPF) quickly responded (Fig. 3.4.2) to the environmental cues of decreasing photoperiod and decreasing temperatures. However, at the end of the calendar week 44 (2011), these differences were no longer visible as all plants attained similar phenology.

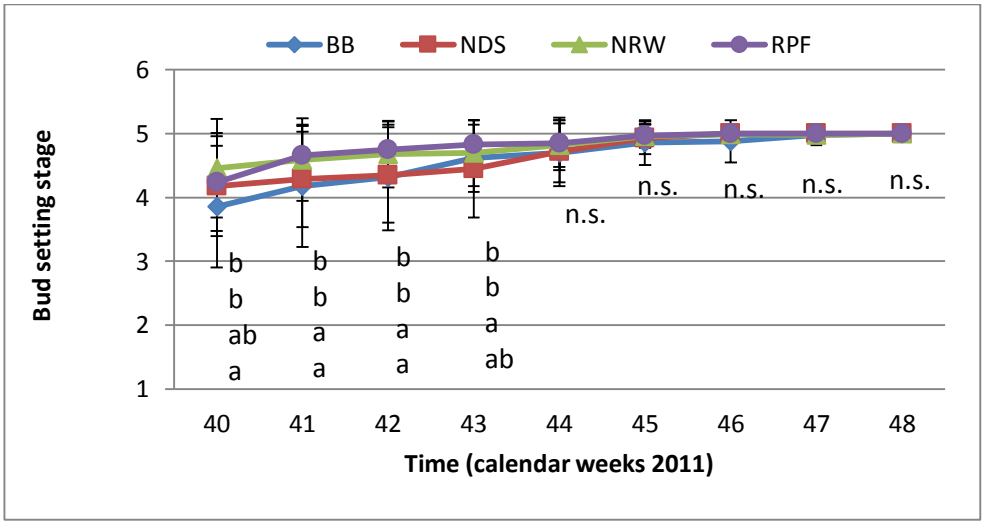

Figure 3.4.2: Bud setting phenology of four populations of *Corylus avellana* in autumn 2011. Mean ± SD, n = 57 Brandenburg (BB), n = 74 Niedersachsen (NDS), 89 Nordrhein-Westfalen (NRW) and 72 Rheinland-Pfalz (RPF) from cutting year 2009. Different letters show significant differences among populations. n.s. = not significant.

Similarly, in 2012, bud set differed in week 42 to 44 where the higher altitude population started setting their buds earlier like in the previous year but these differences were no longer visible from week 44 (Fig. 3.4.3). In 2013, bud setting did not differ among the populations evaluated.

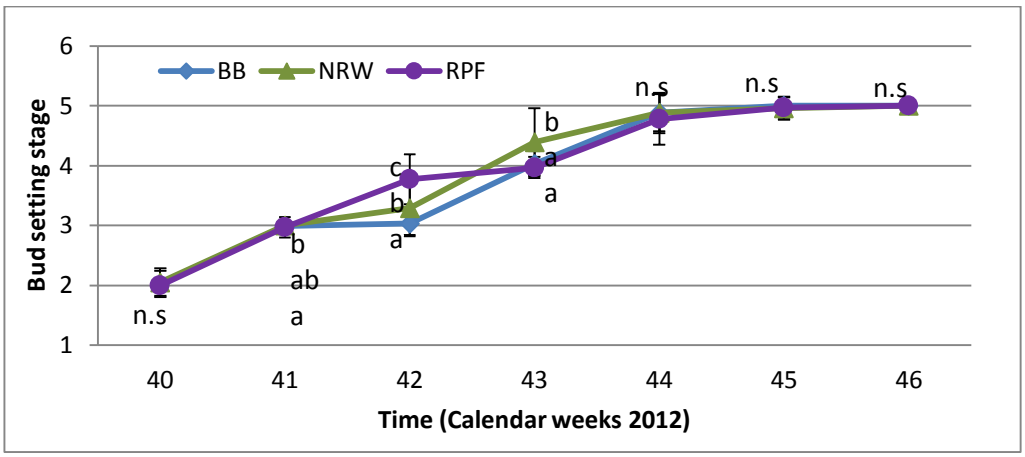

Figure 3.4.3: Bud setting phenology of three populations of *Corylus avellana* in autumn 2012. Mean ± SD, n = 161 Brandenburg (BB), 285 Nordrhein-Westfalen (NRW) and 97 Rheinland-Pfalz (RPF) from cutting year 2011. Different letters show significant differences among populations. n.s. = not significant.

Bud break results are presented in section 3.2.4 above.

Growth characterization

Growth, measured as biomass, height and root collar diameter (RCD), did not statistically differ among the populations (cuttings 2009) evaluated in all seasons (Table 3.4.2, Table 3.4.3). The plants (cutting 2009) were used up by the end of summer 2012 for drought experiment (section 3.3). The cuttings that were sourced in 2011 were used to characterize three populations (BB, NRW and RPF). During the autumn 2012, these populations did not differ in terms of height and RCD. They however differed in terms of shoot number and root mass (Table 3.4.2 - 3). Population NRW had higher number of shoots per plant. In fall, RPF had significantly lower root mass than BB and NRW. In the following year (spring 2013), the population RPF had significant low shoot mass than NRW. It also had low root mass than both BB and NRW. BB differed with NRW in spring's and summer's shoot mass but not in root mass (Table 3.4.2). RPF plants were used up by spring 2013.

N, P, K and biochemical (glucose, fructose, sucrose, starch and proline) concentration

Various plant parts from plants of both cutting years (2009 and 2011) were analysed. In autumn 2011, plants differed in new shoots' fructose, sucrose, starch and potassium concentration. These differences did not follow any order in terms of origins' altitude, latitude or precipitation (Table 3.4.4). These differences were however soon levelled off and by spring only two populations that differed in new shoots' glucose concentration (NDS and RPF) which neither differed with the other two populations (BB and NRW). By summer 2012, these two populations (NDS and RPF) differed no longer in any shoots' parameter but in roots' starch concentration. Again neither of them differed with the other two populations (Table 3.4.4).

Table 3.4.2: Shoot dry mass (old + new shoot, summer: including leaves) and root dry mass (g plant^{-1}) of *Corylus avellana* population. Different letters show significant differences among populations. Mean ± SD, n = 6 (BB), n = 8 (NDS, NRW, RPF) in fall 2011, spring 2012 and summer 2012 for cutting year 2009; n = 13 (BB), 14 (NRW) 7(RPF) in fall 2012; n = 14 (BB, NRW, RPF) in spring 2013; n = 14 (BB and NRW) in summer 2013 for cutting year 2011.

	Time of evaluation					
Origin	Fall (2011 / 2012)		Spring (2012 / 2013)		Summer (2012 / 2013)	
Cutting year	Shoots	Roots	Shoots	Roots	Shoots	Roots
BB-2009	58 ± 17 a	28 ± 13 a	63 ± 17 a	19 ± 05 a	177 ± 32 a	131 ± 28 a
NDS-2009	48 ± 16 a	19 ± 10 a	78 ± 16 a	16 ± 05 a	132 ± 46 a	109 ± 51 a
NRW-2009	58 ± 15 a	18 ± 10 a	71 ± 12 a	19 ± 06 a	136 ± 28 a	112 ± 26 a
RPF-2009	56 ± 14 a	16 ± 08 a	74 ± 8 a	19 ± 10 a	152 ± 28 a	107 ± 30 a
BB-2011	74 ± 24 a	132 ± 21 b	75 ± 17 a	108 ± 46 b	75 ± 21 a	57 ± 18 a
NRW-2011	81 ± 22 a	126 ± 21 b	100 ± 16 b	105 ± 42 b	117 ± 19 b	77 ± 15 a
RPF-2011	99 ± 23 a	80 ± 23 a	62 ± 21 a	67 ± 21 a	no plants available	

Table 3.4.3 Height of the longest shoot, RCD and shoot number (#) of four *Corylus avellana* populations. Different letters show significant differences among populations. Mean ± SD: n = 57 (BB), 74 (NDS), 89 (NRW) and 72 (RPF) in fall 2011 for cutting year 2009; n = 161 (BB), 285 (NRW): n = 97 (RPF) in fall 2012 for cutting year 2011.

Origin/cutting year	height (cm)	RCD (mm)	Shoot #
BB-2009	104 ± 15 a	16 ± 03 a	not counted
NDS-2009	86 ± 19 a	16 ± 03 a	not counted
NRW-2009	104 ± 16 a	19 ± 05 a	not counted
RPF-2009	101 ± 18 a	17 ± 03 a	not counted
BB-2011	124 ± 27 a	16 ± 02 a	04 ± 02 a
NRW-2011	128 ± 22 a	18 ± 03 a	06 ± 02 b
RPF-2011	124 ± 27 a	16 ± 03 a	04 ± 02 a

Table 3.4.4: Glucose, fructose, sucrose, starch, nitrogen, phosphorus potassium (% dry mass) and proline (µg g^{-1}) in various parts of *Corylus avellana* populations. Different letters show significant differences among populations. Mean ± SD: n = 6 (BB), 8 (NDS, NRW, RPF) from cutting year 2009.

		Time of evaluation					
		Autumn (2011)		Spring (2012)		Summer (2012)	
Parameter	Origin	New shoots	Roots	New shoots	Roots	Leaves	Roots
Glucose	BB	0.63 ± 0.14 a	0.75 ± 0.14 a	0.62 ± 0.11 ab	0.68 ± 0.16 a	0.97 ± 0.12 a	0.83 ± 0.33 a
	NDS	0.47 ± 0.18 a	0.77 ± 0.09 a	0.59 ± 0.16 a	0.66 ± 0.28 a	1.15 ± 0.43 a	0.74 ± 0.24 a
	NRW	0.49 ± 0.16 a	0.8 ± 0.11 a	0.84 ± 0.21 ab	0.63 ± 0.25 a	1.02 ± 0.23 a	0.67 ± 0.12 a
	RPF	0.43 ± 0.04 a	0.69 ± 0.04 a	0.88 ± 0.21 b	0.59 ± 0.20 a	1.08 ± 0.15 a	0.67 ± 0.12 a
Fructose	BB	0.89 ± 0.07 c	0.36 ± 0.07 a	0.57 ± 0.10 a	0.82 ± 0.22 a	0.88 ± 0.13 a	1.26 ± 0.72 a
	NDS	0.59 ± 0.17 ab	0.35 ± 0.12 a	0.61 ± 0.20 a	0.78 ± 0.47 a	0.87 ± 0.28 a	0.96 ± 0.37 a
	NRW	0.82 ± 0.19 bc	0.36 ± 0.17 a	0.70 ± 0.25 a	0.59 ± 0.35 a	0.82 ± 0.37 a	0.85 ± 0.25 a
	RPF	0.45 ± 0.15 a	0.4 ± 0.17 a	0.63 ± 0.24 a	0.65 ± 0.31 a	0.98 ± 0.26 a	1.02 ± 0.40 a
Sucrose	BB	4.18 ± 0.42 b	1.6 ± 0.12 a	0.78 ± 0.20 a	1.33 ± 0.73 a	4.43 ± 0.25 a	1.72 ± 0.67 a
	NDS	3.53 ± 0.20 ab	1.74 ± 0.49 a	1.05 ± 0.31 a	1.44 ± 0.83 a	4.33 ± 0.63 a	1.93 ± 0.52 a
	NRW	4.19 ± 0.19 b	1.80 ± 0.48 a	0.96 ± 0.14 a	1.50 ± 0.98 a	4.04 ± 0.84 a	2.25 ± 0.29 a
	RPF	2.73 ± 0.24 a	1.96 ± 0.71 a	0.81 ± 0.18 a	1.38 ± 0.99 a	4.01 ± 0.60 a	1.75 ± 0.61 a
Starch	BB	1.24 ± 0.69 a	1.17 ± 0.48 a	2.43 ± 2.16 a	4.07 ± 1.63 a	0.63 ± 0.41 a	8.20 ± 0.75 ab
	NDS	1.80 ± 0.49 ab	1.20 ± 0.51 a	2.96 ± 1.79 a	3.28 ± 1.10 a	0.49 ± 0.09 a	8.76 ± 0.92 b
	NRW	2.27 ± 0.37 b	1.63 ± 0.61 a	3.74 ± 1.60 a	3.45 ± 1.08 a	0.40 ± 0.39 a	7.84 ± 1.50 ab
	RPF	2.13 ± 0.70 b	1.09 ± 0.34 a	2.71 ± 2.04 a	4.40 ± 0.98 a	0.65 ± 0.27 a	6.92 ± 1.60 a
Proline	BB	100 ± 47 a	633 ± 153 a	465 ± 096 a	1580 ± 405 a	42 ± 20 a	48 ± 21 a
	NDS	184 ± 43 a	541 ± 295 a	615 ± 100 a	1510 ± 426 a	38 ± 13 a	30 ± 09 a
	NRW	198 ± 93 a	605 ± 224 a	585 ± 250 a	2030 ± 354 a	38 ± 18 a	29 ± 12 a
	RPF	190 ± 125 a	670 ± 354 a	614 ± 190 a	1693 ± 513 a	52 ± 14 a	41 ± 15 a
Nitrogen	BB	1.42 ± 0.13 a	1.46 ± 0.10 a	1.51 ± 0.22 a	1.75 ± 0.16 a	1.96 ± 0.13 a	0.34 ± 0.03 a
	NDS	1.45 ± 0.39 a	1.56 ± 0.24 a	1.82 ± 0.17 a	1.86 ± 0.16 a	1.70 ± 0.28 a	0.34 ± 0.06 a
	NRW	1.77 ± 0.36 a	1.56 ± 0.30 a	1.63 ± 0.32 a	1.94 ± 0.30 a	1.82 ± 0.24 a	0.34 ± 0.03 a
	RPF	1.69 ± 0.42 a	1.68 ± 0.23 a	1.77 ± 0.53 a	1.80 ± 0.26 a	1.69 ± 0.20 a	0.35 ± 0.05 a
Phosphorus	BB	0.23 ± 0.02 a	0.26 ± 0.04 a	0.25 ± 0.03 a	0.30 ± 0.05 a	0.22 ± 0.07 a	0.15 ± 0.02 a
	NDS	0.24 ± 0.04 a	0.29 ± 0.03 a	0.26 ± 0.04 a	0.34 ± 0.03 a	0.23 ± 0.05 a	0.22 ± 0.03 a
	NRW	0.22 ± 0.05 a	0.25 ± 0.03 a	0.23 ± 0.03 a	0.35 ± 0.05 a	0.23 ± 0.03 a	0.17 ± 0.04 a
	RPF	0.24 ± 0.05 a	0.28 ± 0.04 a	0.26 ± 0.06 a	0.35 ± 0.04 a	0.23 ± 0.03 a	0.17 ± 0.04 a

Potassium	BB	0.51 ± 0.11 b	0.34 ± 0.06 a	0.50 ± 0.16 a	0.39 ± 0.03 a	0.91 ± 0.09 a	0.30 ± 0.05 a
	NDS	0.45 ± 0.09 ab	0.36 ± 0.06 a	0.47 ± 0.06 a	0.35 ± 0.08 a	0.86 ± 0.17 a	0.27 ± 0.06 a
	NRW	0.36 ± 0.05 a	0.39 ± 0.06 a	0.42 ± 0.07 a	0.34 ± 0.04 a	0.75 ± 0.09 a	0.27 ± 0.04 a
	RPF	0.48 ± 0.05 b	0.41 ± 0.13 a	0.45 ± 0.08 a	0.33 ± 0.05 a	0.76 ± 0.06 a	0.3 ± 0.03 a

In the following seasons populations were characterized by plants from different cutting year (2011). Mostly the populations differed consistently in proline and nitrogen concentrations (Table 3.4.5). The high altitude population RPF had higher concentrations across the season than BB and NRW. In other biochemical analyses, whenever there were differences between the populations RPF had the highest concentration except in shoot' starch (Table 3.4.5).

Table 3.4.5: Glucose, fructose, sucrose, starch, nitrogen, phosphorus potassium (% dry mass) and proline (µg g^{-1}) in various parts of *Corylus avellana* populations. Different letters show significant differences among populations. Mean ± SD; n = 13 (BB), 14 (NRW) 7(RPF) in autumn 2012; n = 14 (BB, NRW, RPF) in spring 2013; n = 14 (BB and NRW) in summer 2013 from cutting year 2011.

		Autumn (2012)		Spring (2013)		Summer (2013)	
Parameter	origin	New shoots	Roots	New shoots	Roots	Leaves	Roots
Glucose	BB	0.73 ± 0.23 a	0.73 ± 0.17 b	0.64 ± 0.14 a	1.04 ± 0.72 a	0.57 ± 0.28 a	0.84 ± 0.22 a
	NRW	0.86 ± 0.24 a	0.6 ± 0.10 a	0.62 ± 0.13 a	1.04 ± 0.73 a	0.74 ± 0.14 b	0.90 ± 0.15 a
	RPF	1.02 ± 0.15 b	1.24 ± 0.17 c	0.54 ± 0.07 a	1.19 ± 0.75 a		
Fructose	BB	0.72 ± 0.26 a	0.47 ± 0.16 a	0.44 ± 0.15 a	0.55 ± 0.17 a	0.39 ± 0.19 a	0.56 ± 0.22 a
	NRW	0.73 ± 0.21 ab	0.39 ± 0.11 a	0.44 ± 0.12 a	0.50 ± 0.21 a	0.52 ± 0.12 b	0.58 ± 0.17 a
	RPF	1.02 ± 0.16 b	1.18 ± 0.19 b	0.35 ± 0.11 a	0.43 ± 0.21 a		
Sucrose	BB	1.95 ± 0.66 a	2.36 ± 0.78 a	0.63 ± 0.25 a	1.65 ± 0.42 a	2.5 ± 0.50 a	0.78 ± 0.46 a
	NRW	1.74 ± 0.96 a	2.63 ± 0.5 a	0.47 ± 0.27 a	1.36 ± 0.36 a	2.23 ± 0.24 a	1.56 ± 0.63 b
	RPF	1.79 ± 0.78 a	2.02 ± 0.48 a	0.47 ± 0.28 a	1.41 ± 0.44 a		
Starch	BB	2.87 ± 0.76 ab	5.19 ± 0.57 a	1.61 ± 0.66 b	2.71 ± 0.93 a	0.07 ± 0.03 a	2.02 ± 1.42 a
	NRW	2.81 ± 0.88 a	5.18 ± 0.41 a	1.26 ± 0.78 b	2.79 ± 1.35 a	0.08 ± 0.03 a	2.73 ± 1.53 a
	RPF	3.93 ± 0.64 b	10.36 ± 2.38 b	0.60 ± 0.42 a	1.98 ± 0.86 a		
Proline	BB	72 ± 64 a	511 ± 177 a	299 ± 90 a	1215 ± 375 a	105 ± 58 b	439 ± 404b
	NRW	64 ± 17 a	529 ± 184 a	320 ± 80 a	1199 ± 262 a	49 ± 34 a	187 ± 275a
	RPF	209 ± 113 b	369 ± 151 a	725 ± 398 b	1693 ± 469 b		

Nitrogen	BB	0.74 ± 0.13 a	0.91 ± 0.19 a	0.94 ± 0.12 a	1.38 ± 0.24 a	3.86 ± 1.17 b	0.89 ± 0.46 a
	NRW	0.79 ± 0.10 a	0.98 ± 0.18 a	1.04 ± 0.25 a	1.35 ± 0.20 a	2.84 ± 0.43 a	0.53 ± 0.20 a
	RPF	1.28 ± 0.33 b	1.44 ± 0.39 b	1.34 ± 0.25 b	1.87 ± 0.26 b		
Phosphorus	BB	0.16 ± 0.02 a	0.23 ± 0.03 a	0.21 ± 0.04 a	0.3 ± 0.04 a	0.47 ± 0.11 b	0.30 ± 0.03 a
	NRW	0.15 ± 0.02 a	0.25 ± 0.03 a	0.24 ± 0.07 a	0.31 ± 0.04 a	0.33 ± 0.06 a	0.30 ± 0.04 a
	RPF	0.17 ± 0.04 a	0.26 ± 0.02 a	0.23 ± 0.06 a	0.31 ± 0.05 a		
Potassium	BB	0.42 ± 0.07 ab	0.36 ± 0.04 b	0.70 ± 0.10 ab	0.35 ± 0.08 b	1.98 ± 0.31 b	0.41 ± 0.16 a
	NRW	0.47 ± 0.08 b	0.39 ± 0.05 b	0.80 ± 0.16 b	0.32 ± 0.07 ab	1.45 ± 0.17 a	0.48 ± 0.08 a
	RPF	0.38 ± 0.05 a	0.25 ± 0.07 a	0.67 ± 0.13 a	0.28 ± 0.06 a		

3.4.6 Discussion

When certain critical day length is reached and certain temperature, plants cease growth and begin to set bud (Li et al. 2003, 2005, Rohde et al. 2011) Since this study was carried out in a common container area, plants from all populations experienced similar temperature and day length. However, at the beginning of bud formation up to calendar week 43, populations varied significantly in both years (Fig. 3.4.1-2). The populations from the higher altitude (NRW and RPF) started to set their buds earlier than the two populations from low altitude (BB and NDS). This may be seen as an adaptation of these populations to cease growth earlier to avoid frost damage than those of lower altitude. Nonetheless, these differences were levelled out by week 44. Consequently, there was no substantial advantage for earlier or late bud cessation for these populations.

According to literature, populations from low altitude are reported to grow faster and longer than those of the high altitude in a common garden (Vitasse et al. 2009). This could be associated with ability to take advantage of warm environment and nutrients (especially N). Concomitantly, late growth cessation is reported to have advantage over earlier bud cessation in that it increases re-translocation time of leaves' photosynthates and nutrients (Norby et al 2003). But according to the results this was not demonstrated by the populations tested. The possible reason could be that these populations' altitudinal / latitudinal differences are not large enough to influence any growth parameter evaluated.

On biochemical concentration, there are reports that plants from different altitudes, climate, latitudes vary in proline (Bano et al. 2009) and carbohydrates concentration in their natural habitats (Shi et al. 2006, Hoch and Körner 2012, Lei et al. 2013). However, when grown on a common environment, these differences (except a few) are levelled out as shown by Lei et al. (2013). Accordingly, since the

populations were originating from different areas of origin, they differed inconsistently in the first year in some of the analysed sugars and proline in autumn and spring but these differences were greatly reduced (except very few cases) with time of cultivation.

Nutrient concentrations (N, P, K) have been reported to be affected by soil and altitude (Jian et al. 2009, Lei et al. 2013), latitudinal increase (Oleksyn et al. 2002). According to Jian et al. (2009), *Quercus liaotungensis* leaves' N, P, K increased with altitude. Contrary, according to Lei et al. (2013), there was a tendency to decline N, P, K with increasing elevation for *Quercus variabilies* species. When cultivated under similar environment, the four *Corylus avellana* populations did not depict any trend consistent with altitude in N, P and K concentration. The only tendency of RPF to have higher N concentration in the following years (autumn 2012 and spring 2013) might be associated to different mother plants. This shows that these populations are similar in acquiring nutrients from the same environment. Under conventional fertilization, it is generally expected that nutrient dilution to occur as the plant biomass increases (Bohne et al. 2014). However, this was not the case (except for root N) for the populations which rather maintained a constant or increasing nutrient concentration with time. Gauging from literature, leaves are the most appropriate reliable indicator of nutrient status plants (Miletic et al. 2001). Referencing from various literature, Milosevic et al. (2009), gave a range of 1.20 - 3.54%, 0.10 - 0.60% and 0.36 - 3.00% for N, P and K respectively fluctuating with genotypes and environment. Milosevic et al. (2009) also reported values of N (1.83 ± 0.07%), P (0.43 ± 0.09%) and K (1.77 ± 0.04%) from three cultivars. In their comparative study on soil fertility and macro-elements in *Corylus* species, Miletic et al. (2001) also gave a range (N = 1.66, 1.86 and 2.08%; P = 0.19, 0.25 and 0.20% and K = 0.42, 0.74 and 0.70%). Hence, the populations in this study were sufficiently nourished from the leaves' nutrient concentration (N, P, K). The concentration of N P and K in new shoots are also comparable to that found in new shoots of *Philadelphus inodorus* var. *grandiflorus* shrub (Röber and Rohde 1984).

3.4.7 Conclusion

Although the populations evaluated here had been shown to differ genetically (Leinemann et al. 2013b), their performance was similar in all seasons since parameters evaluated did not consistently differ. This may be due to the reported wide ecological adaptation of this species (Mehlenbacher 1991). The ability of the populations to set their bud at the same time and to level out physiological differences with time indicates that this species has a high plasticity and flexibility to a range of environmental conditions.

3.5 Conclusion for the populations of *Corylus avellana*

From the three stress experiments and from seasonal characterization data of different populations of hazelnut, this study has gathered some insight on the adaptability of the *Corylus avellana*. This information could be useful for the nursery industry and for further use of hazelnut.

Under similar growing conditions, populations that were from different altitude and latitude (for BB and RPF), did not differ (highly similar) when the plants were under no stress. Conversely, population that were close in range (BB and NDS) were at times different. This could be seen also on the following cluster dendrogram (Fig. 3.5.1). In phenology the influence of origin was rare and environmental cues especially temperature was mostly the drive for bud sprouting. When stressed, there were some biochemical and physiological differences among the evaluated populations either in early frost, late frost or drought but these differences were inconsistent with latitude, altitude and climate data of origin. The lack of significant influence of origin in these populations tested does not only show wide ecological adaptation of *Corylus* plants but also demonstrates high plasticity and quick adaptability of these populations. These results suggest that nurseries could obtain planting material from similar climatic region when feasible material within the given demarcated area of origin is not available without compromising their survival and growth performance.

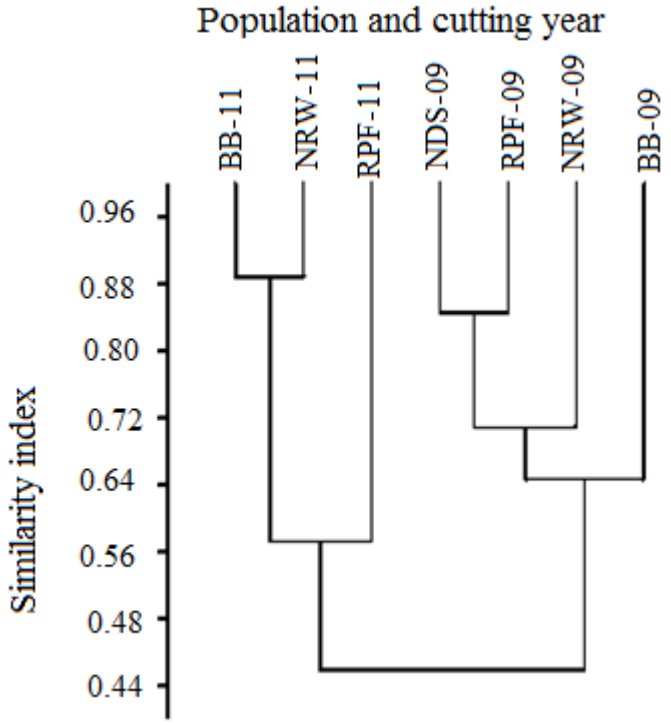

Figure 3.5.1: Dendrogram of the UPGMA cluster analysis based on similarity index of glucose, fructose, sucrose, starch, proline N, P and K.

4 *Prunus spinosa* populations' reactions to frost, drought and seasonal characterization

4.1 Introduction

Sloe (also blackthorn) is a deciduous shrub with a characteristic of growing as hedges along forest edges and open landscapes. The species is widely distributed in Europe, northern Africa and part of Asia (Eimert et al. 2012, Leinemann et al. 2014). According to Mohanty et al. (2002), northern Europe *P. spinosa* populations were derived from localized southern refugia . It has also been naturalized in New Zealand and North America (http://www.gbif.org/species/113651614). It is an important landscaping plant that provides food and shelter to wild animals. It is also used by humans for various purposes, for example fruits for Vitamin C and antioxidants (Ruiz-Rodríguez et al. 2014). In horticulture it is utilised as a dwarfing rootstock for plum (Maas et al. 2014). It propagates through seed, root sucker and stem cuttings (Eimert et al. 2012). It is pollinated by insects (Gyan and Woodell 1987). The mode of pollination might promote gene flow among populations, probably levelling out differences (Baliuckas et al. 2005). However, vegetative regeneration might promote genetic differentiation among populations (Leinemann et al. 2014).

Although its ecological information is scanty, it has been described as hardy shrub due to its ability to inhabit habitats that are challenged by drought and frost (Mohanty et al. 2002). Its hardiness, (like discussed in section 1.2.3 above), could be attributed to deciduousness (Weiser 1970, Lee et al. 2012) and carbohydrates reserves that supply energy for respiration and offer cryoprotection during climatic extremes (Piper et al. 2006, Pagter et al. 2008, Shin et al. 2015). Nevertheless, origin (like discussed in section 1.3) has been shown to have influence, for example, on phenology and performance (Vollrath 2004). But according to Fronia (2009), differences among populations could decrease with time on cultivating them in a common garden.

In this section, the study endeavoured to evaluate the influence of population's origin on: (1) late frost reactions in spring (2) drought responses in summer and (3) growth responses, bud sprout and bud set in spring and autumn respectively. During these three seasons (spring, summer and autumn), seasonal concentration of N, P, K, glucose, fructose, sucrose, starch and proline were analysed.

4.2 Material and methods

Prunus spinosa cuttings were collected by Leinemann et al. (2014) and rooted in different years. All plants were raised under the same environment and irrigation regimes (as previously described in chapter 2). Populations evaluated from Germany included: Brandenburg (BB), Hessen (HES), Niedersachsen (NDS) and Rheinland-Pfalz (RPF). Additionally, plants from Italy were included. The

climatic conditions of origin of these populations partially overlap (Table 4.2.2). These plants were sourced in different years and were used for various experiments as summarized in Table 4.1.1. Due to limited number of plants, not all populations were allocated for all treatments.

Table 4.2.1: Overview of the *Prunus spinosa* populations used for experiments (late frost and drought) and seasonal characterization. Abbreviations: BB = Brandenburg, NDS = Niedersachsen, HES = Hessen, RPF = Rheinland-Pfalz and ITA = Italy.

		When (time) and what was evaluated with which cuttings' year with replicates (n)					
		Experiments		Seasonal characterization			
Origin	Cutting year	Late frost	Drought	Growth (RCD[w], height[x])	Bud set	Bud sprout	Biomass, N, P, K and biochemical[z] concentration
BB, NDS, NRW, RPF	2009	2013 (BB, RPF) n = 12 (BB), 8 (RPF)	not done	2011 - 2013 n = 56 (BB), 44 (HES), 32 (NDS), 27 (RPF)			autumn 2012, spring 2013 autumn 2012: n = 6 (BB), 8 (HES), 6 (NDS) spring 2013: n = 12 (BB), 8 (RPF)
BB, RPF	2010	not done	2012 n = 9 (BB, RPF)	autumn 2012 n = 8 (BB) 6 (RPF)		not done	summer 2012, autumn 2012 summer: n = 9 (BB, RPF) autumn: n = 8 (BB) 6 (RPF)
BB, ITA, RPF	2011	2014 n = 9 (BB, ITA)	2013 n = 9 (BB, ITA)	2012 - 2013 n = 71 (BB), 90 (ITA), 3 (RPF)			spring 2012, summer 2013, spring 2014 spring 2012: n = 15 (BB, ITA), 6 (RPF) summer 2013; spring 2014: n = 9 (BB, ITA)

[z]Biochemical = glucose, fructose, sucrose, starch and proline [x]Height = height of the longest shoot in cm
[w]RCD = root collar diameter in mm

In the following sections (4.2.1 to 4.2.3) each experimental set-up or seasonal characterization material and procedures will be described in details and later results and discussion (4.3.1 to 4.3.3) will be presented per each respectively.

All analyses of glucose, fructose, sucrose and starch were done as described above in section 3.2.4 above. N, P, K analyses were done as described in section 3.4.3.

Table 4.2.2: Populations' map coordinates with some ecological data. Air temperatures and rainfall data are 30 years' averages (1961 - 1990) from KlimaatlasBundesrepublik Deutschland: Karte 1.12 to 1.15 (temperature); Karte 2.12 to 2.15 (rainfall). http://www.dwd.de/bvbw/appmanager/bvbw/dwdwwwDesktop?_nfpb=true&_windowLabel=T3860013424116972633808 6&_urlType=action&_pageLabel=_dwdwww_klima_umwelt_ueberwachung_deutschland. Air temperatures and rainfall data [Italian (ITA)] are 12 years' average (2000 - 2012).

Origin	Altitude	Latitude	Longitude	Precipitation (mm)				Air Temp. (°C)			
	(m a.s.l)			Spring	Summer	Fall	Annual	Spring	Summer	Fall	Annual
BB	44	52°38'07.2"	12°58'08.3"	120 - 140	160 - 180	100 - 120	475 - 550	8 - 9	17 - 18	9 - 10	8.5 - 9
NDS	96	52°20'23.0"	10°44'45.5"	120 - 160	200 - 240	100 - 120	600 - 700	8 - 9	16 - 17	9 - 10	8 - 9
HES	283	50°57'56.9"	9°51'43.4"	160 - 240	180 - 240	100 - 240	750 - 850	5 - 8	14 - 17	8 - 10	7 - 9
RPF	464	50°17'22.5"	7°00′15.8"	120 - 240	180 - 240	100 - 240	700 - 1000	5 - 9	14 - 17	7 - 9	7 - 9
ITA	330 - 920	45° 43′	10° 52′	120 - 237	268 - 278	150 - 280	607 - 1008	7 - 19	16 - 29	8 - 18	7 - 18

4.2.1 Late frost experiment

Late frost experiment was carried out in two separate years: (1) April 2013 with two German populations namely Brandenburg (BB) and Rheinland-Pfalz (RPF). These plants were from cutting year 2009; (2). April 2014 with a German population Brandenburg (BB) and an Italian population (ITA). These plants were from cutting year 2011 (Table 4.2.1).

In April 2013 and in April 2014, late frost experiments were conducted after plants were randomly allocated to either two freezing treatments (-12 °C and -6 °C) or a non-freezing (control) treated at 5 °C. Three shoots per plant were cut (≈ 25 cm long, including buds and emerging leaves) and immediately placed in a plastic bag. Flower petals were removed whenever present. These plant parts were then placed in various chambers according to the treatment allocated (-12 °C or -6 °C). All other procedural details and REL determination follow procedure as explained in section 3.2.4 above.

Regeneration plants

Additional plants to evaluate survival and regeneration were treated in -12 °C, -6 °C and 5°C (control) at the same time as the frost treatment described above. After treatments these plants were allowed to regenerate in their respective containers in the container area (outside). In 2013, only BB had regeneration plants since RPF plants were used up in frost experiment. In 2014, both BB and ITA had five plants per treatment as regeneration plant.

4.2.2 Drought experimental

Drought experiment was carried out in two separate years: (1). In summer 2012 with two German populations BB and RPF. These plants were from cutting 2010. (2). In summer 2013 with a German population Brandenburg (BB) and an Italian population (ITA). These plants were from cutting year 2011 (Table 4.2.1).

In the respective experimental year, plants were assigned to either control or to two drought treatments (slow or fast stress) using a completely randomized design with nine replicates each. All other procedures are and parameters (physiological and biomarkers) evaluated are as described in section 3.4.3.

A wilting scheme was used for scoring wilting symptoms (Fig 4.2.1). The scale ranged from 0 -5 (0: no wilting symptoms; 1: shoot tips bending; 2: shoot tips are severely wilted and few leaves are drooping/cupping; 3: more leaves are drooping and some are rolled up; 4: more leaves are rolled up and lower leaves are yellowing and 5: some leaves are crunchy and or many leaves are yellowing). The experiment was terminated when half of the plants in each drought treatment had withered to scale ≥ 3 (Fig. 4.2.1) or when more than 50% plants per each treatment had 3.8 MPa in 2012 when the plants did not show wilting symptoms.

Figure 4.2.1: *Prunus spinosa* wilting symptoms used to rate morphological wilting during drought experiment under controlled conditions.

Regeneration plants

An additional six (BB and ITA) and four (RPF) plants per treatment were incorporated in the drought stress and later re-watered at the end. These plants were transplanted into the field and evaluated for regeneration without further irrigation apart from natural rainfall. Regeneration was recorded as number of shoots per plant, height and root collar diameter.

4.2.3 Seasonal characterisation of different *Prunus spinosa* populations

Seasonal characterisation was done in three seasons: spring, summer and autumn (Table 4.2.1). Nevertheless, not all populations were samples always due to limited number of plants. The summary is given in table (Table 4.2.3) of parameter evaluated and when they were evaluated using which plants (cutting year).

Table 4.2.3: Overview of the *Prunus spinosa* populations and parameters used for seasonal characterization. Abbreviations: BB = Brandenburg, NDS = Niedersachsen, HES = Hessen, RPF = Rheinland-Pfalz and ITA = Italy.

		Parameter and when (time) evalutated including replicates (n)		
Origin	Cutting year	Height[x] and RCD[w]	Phenology	Biomass, N, P, K and biochemical[z] concentration
BB, NDS, NRW, RPF	2009	autumn 2011, spring 2012, autumn 2012, spring 2013 autumn 2011; spring 2012: n = 56 (BB), 44 (HES), 32 (NDS), 27 (RPF) spring 2013: n = 44 (BB), 35 (HES), 23 (NDS), 25 (RPF)		autumn 2012, spring 2013 (BB, RPF) autumn 2012: n = 6 (BB), 8 (HES), (6) NDS spring 2013: n = 12 (BB), 8 (RPF)
BB, RPF	2010	summer 2012, autumn 2012	autumn 2012	summer 2012, autumn 2012
		summer: n = 9 (BB, RPF); autumn: n = 8 (BB), 6 (RPF)		
BB, ITA, RPF	2011	autumn 2012, spring 2013, autumn 2013, spring 2014 autumn 2012; spring 2013: n = 71 (BB), 90 (ITA), 3 (RPF) autumn 2013; spring 2014: n = 35 (BB), 54 (ITA),		spring 2012[y], summer 2013, spring 2014 spring 2012: n = 15 (BB, ITA), 6 (RPF) summer 2013; spring 2014: n = 9 (BB, ITA)

[z] Biochemical = glucose, fructose, sucrose, starch and proline [y] biomass was not determined

[x] Height = height of the longest shoot in cm [w] RCD = root collar diameter in mm

All analyses of glucose, fructose, sucrose and starch were done as described above in section 3.2.4. Growth parameters were determined and N, P, K analyses were done as described in section 3.4.3. However, phenological evaluation schemes used for respective bud sprout and bud sprout are explained below.

Bud sprouting scoring scheme

In March-April 2012 and 2013, bud sprouting was evaluated according to the following scheme developed by Schmitt (2003).

Figure 4.2.2: Bud sprouting scheme used for rating bud break in spring for *Prunus spinosa*. Pictures by Salomon (2011)

1	Buds are dormant and brownish in colour.
2	Buds are swollen and start with greenish colouration.
3	Buds are dehiscent and leaf tips are visible.
4	Leaf tips start to be separated.
5	Single leaves are visible with slightly yellow to brown stipules.
6	Leaves are totally unfolded and are dark green in colour.

Bud set scoring scheme developed by Schmitt (2003).

Figure 4.2.3: Bud setting scheme used for rating *Prunus spinosa* in autumn. Picture stages 4 and 5 by Salomon (2011)

1	No terminal or lateral buds are visible, new leaves are visible from apical and lateral shoots.
2	Terminal buds are rudimentary visible and are greenish to brown in colour.
3	Terminal bud are as big as the lateral buds and are coloured brown or reddish in colour.
4	Terminal buds are bigger than lateral buds, red-brown in colour and fringy.
5	Terminal buds have smaller adjacent brown coloured buds.

4.3 Statistical analysis

Using R 3.1.1 program, the statistics were carried out as in section 3.4.4.

4.4 Results and discussion

4.4.1 Late frost reactions of three *Prunus spinosa* populations

When the plants were sampled for late frost experiment in 2013, the two German populations were at a similar sprouting stage as shown on the figure below (Fig. 4.4.1). However, in spring 2014, the German and Italian population were at completely different stages since the Italian population plants sprouted two weeks before the German (BB) population (Fig. 4.4.2).

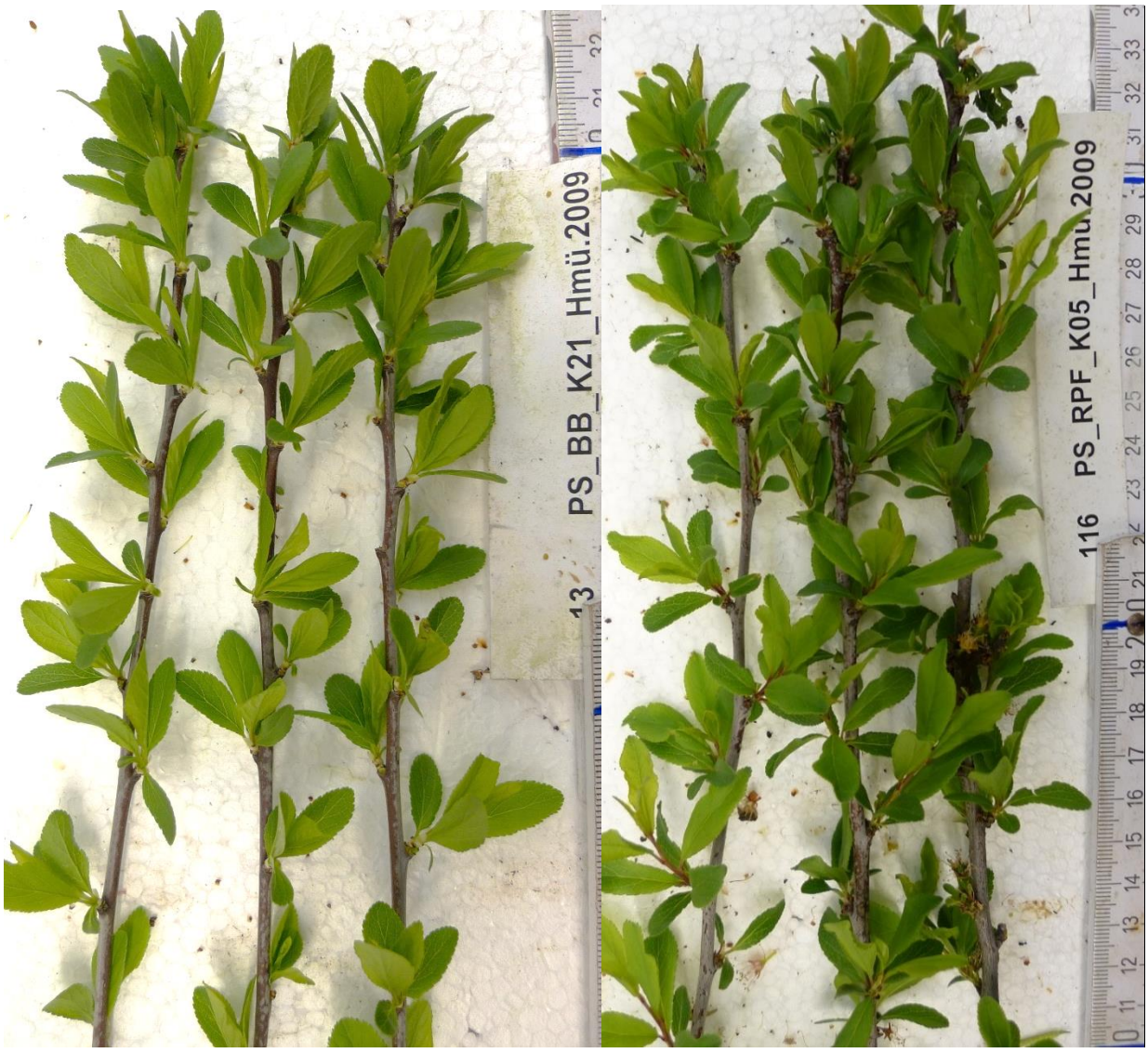

Figure 4.4.1: Shoot tips of two German populations (BB on the left and RPF on the right) of *Prunus spinosa* cutting year 2009 in April 2013 before the late frost experiment.

Figure 4.4.2: Shoots of a German (BB on the left) and an Italian (ITA on the right) population of *Prunus spinosa* cutting year 2011 in April 2014 before the late frost experiment.

Relative electrolyte leakage

Shoots were increasingly damaged, measured as electrolyte leakage, with decreasing treatment temperature in 2013. However, in 2014, the two treatment temperatures caused similar damage (Fig. 4.4.3). The two evaluated German populations (BB and RPF) did not differ significantly in 2013 after treatment. Contrary, the Italian population (ITA) suffered higher leakage than German population BB in the following year (2014) whether treated or not (Fig. 4.4.3). When comparing the BB population in both years, the BB population in 2013 (cutting year 2009), had lower level of REL despite having advanced bud sprout compared to that of 2014 (cutting year 2011).

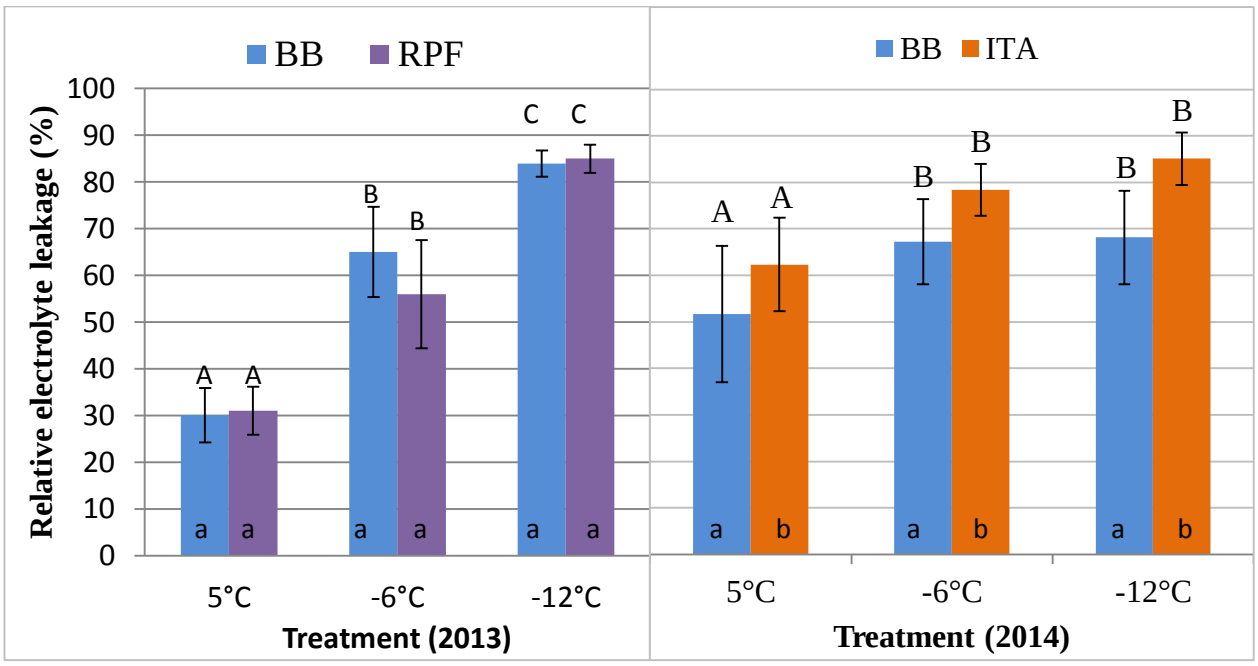

Figure 4.4.3: Relative electrolyte leakage (%) from shoots of two German (BB and RPF) populations in April 2013 and from shoots of a German (BB) and an Italian (ITA) population in April 2014 of *Prunus spinosa* in late frost experiments. Different letters show significant differences: small letters between populations within a treatment; capital letters among the treatments of each population. Mean ± SD, n = 8 (BB and RPF) in 2013; n = 9 (BB and ITA) in 2014.

Biomarkers

When unstressed the German populations BB and RPF did not differ in any of their biomarkers (glucose, fructose, sucrose, starch and proline) concentration (Table 4.4.1). However, when they were treated (-6 °C and -12 °C), the RPF population increased its glucose, fructose and sucrose concentration while the BB population did not react in any of the sugars except a tendency to decline its sucrose concentration. Starch in this experiment did not react to any late frost treatment (Table 4.4.1). The two German populations differed significantly in glucose and sucrose concentration where RPF had higher concentration than BB (Table 4.4.1).

Proline concentration increased marginally but not significant at -6°C compared to the control in both German populations BB and RPF. At -12 °C, the population BB significantly increased its proline concentration compared to that of the control while RPF declined its proline concentration (Table 4.2.1). Due to this opposing trend at -12 °C the populations differed significantly with BB having up to 2.5 times higher proline concentration than that of RPF.

Table 4.4.1: Glucose, fructose, sucrose, starch (% dry mass) and proline (µg g^{-1}) in shoots of *Prunus spinosa* populations in April 2013. Different letters show significant differences: small letters between populations within a treatment; capital letters among the treatments of each population. Mean ± SD, n = 8 in 2013 from cutting year 2009.

Biomarker	Treatment	Population	
		BB	RPF
Glucose	5 °C	0.44 ± 0.13 Aa	0.39 ± 0.10 Aa
	-6 °C	0.40 ± 0.08 Aa	0.60 ± 0.16 Bb
	-12 °C	0.48 ± 0.07 Aa	0.66 ± 0.12 Bb
Fructose	5 °C	0.41 ± 0.13 Aa	0.39 ± 0.21 Aa
	-6 °C	0.38 ± 0.11 Aa	0.47 ± 0.12 ABa
	-12 °C	0.39 ± 0.20 Aa	0.60 ± 0.09 Ba
Sucrose	5 °C	0.82 ± 0.31 Ba	0.90 ± 0.41 Aa
	-6 °C	0.72 ± 0.23 ABa	1.25 ± 0.30 Ab
	-12 °C	0.56 ± 0.35 Aa	0.92 ± 0.25 Ab
Starch	5 °C	0.12 ± 0.11 Aa	0.22 ± 0.31 Aa
	-6 °C	0.22 ± 0.19 Aa	0.38 ± 0.30 Aa
	-12 °C	0.36 ± 0.43 Aa	0.34 ± 0.34 Aa
Proline	5 °C	371 ± 143 Aa	331 ± 90 ABa
	-6 °C	442 ± 334 Aa	572 ± 263 Ba
	-12 °C	659 ± 177 Ba	267 ± 143 Ab

Comparing the German (BB) and the Italian (ITA) population, in the following year (2014): when the population were unstressed, they differed only in starch concentration where BB had higher concentration than ITA (Table 4.4.2). Subsequently after -6 °C treatment, there are no reactions in any of the tested biomarkers in both populations. Still, the difference in starch between the populations was maintained. Upon treating the populations at -12 °C, both populations (BB and ITA) significantly increased their glucose and fructose concentration. Additionally, the Italian population increased its sucrose and starch concentration (Table 4.4.2). Populations differed no more in starch concentration but rather in sucrose and fructose concentration. In both incidences ITA had the highest concentration of fructose and sucrose.

Proline had the tendency to marginally increase with declining temperature treatments. Nonetheless, the German population significantly differed with the Italian population at -6 °C treatment (Table 4.4.2).

Comparing the population BB in both years, in 2013 the population had higher GFS and proline -12 C and lower starch compared to 2014 (Table 4.4.1 and 4.4.2). The REL was higher in 2014 despite the bud being less developed compared to 2013.

Regeneration

In both years, treated twigs did not show severe sign of damage at a glance (Fig. 4.4.4). However, most of those shoots suffered die back with -12 °C inflicting most fatality. However, all treated plants survived freezing temperatures and were able to regenerate. However, apical dominance was heavily compromised in most plants irrespective of origin. This resulted in more shoots sprouting from either the base of the plant or from the lateral buds of the affected shoots. More shoots sprouted in Italian population than the German population (Fig. 4.4.5) and in the following order irrespective of origin: -12°C ≥ -6°C > 5°C. Regeneration plants in 2013 were only available for BB population. Nevertheless, they were comparable to control in height after five months of regeneration.

Table 4.4.2: Glucose, fructose, sucrose, starch (% dry mass) and proline (µg g^{-1}) in shoots of German (BB) and Italian (ITA) population of *Prunus spinosa* in April 2014. Different letters show significant differences: small letters between populations within a treatment; capital letters among the treatments of each population. Mean ± SD, n = 9 from cutting year 2011.

		Population	
Biomarker	Treatment	BB	ITA
Glucose	5 °C	0.14 ± 0.10 A a	0.16 ± 0.10 A a
	-6 °C	0.14 ± 0.06 A a	0.19 ± 0.09 AB a
	-12 °C	0.26 ± 0.07 B a	0.33 ± 0.21 B a
Fructose	5 °C	0.11 ± 0.04 A a	0.1 ± 0.07 A a
	-6 °C	0.14 ± 0.05 AB a	0.11 ± 0.06 A a
	-12 °C	0.19 ± 0.05 B a	1.22 ± 0.79 B b
Sucrose	5 °C	0.2 ± 0.10 A a	0.21 ± 0.11 A a
	-6 °C	0.22 ± 0.09 A a	0.18 ± 0.09 A a
	-12 °C	0.21 ± 0.09 A a	0.91 ± 0.57 B b
Starch	5 °C	0.73 ± 0.05 A b	0.64 ± 0.05 A a
	-6 °C	0.72 ± 0.08 A b	0.65 ± 0.06 A a
	-12 °C	0.74 ± 0.14 A a	0.93 ± 0.3 B a
Proline	5 °C	312 ± 155 A a	260 ± 85 A a
	-6 °C	352 ± 68 A b	283 ± 65 A a
	-12 °C	369 ± 124 A a	335 ± 74 A a

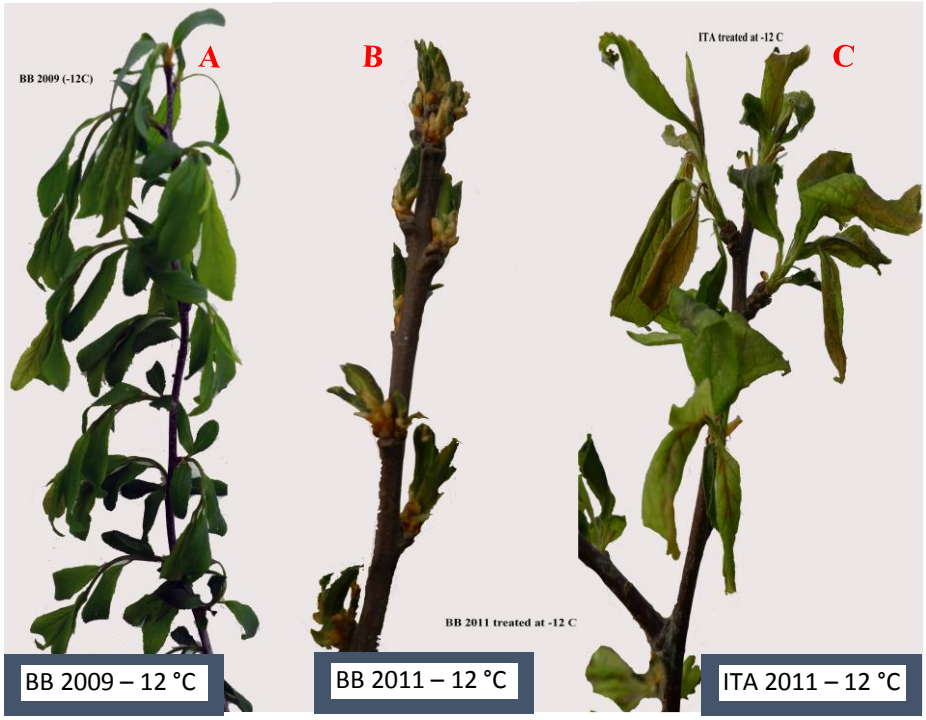

Figure 4.4.4: Morphological outlook of a German(A and B) and an Italian (C) population twigs after frost treatment with -12 °C in April 2013 (A) and April 2014 (B and C) respectively.

Figure 4.4.5: Morphological outlook of German BB and Italian ITA population in September 2014 (five months regeneration) following late frost (-12 °C) treatment in April 2014.

In a common garden, ecotypes from higher altitude and or higher latitude are expected to be harder than those from low altitude or latitude since they are able to tolerate lower temperature of origin (Taschler and Neuner 2004). Imperatively plants originating from higher altitude or latitude ought to have lower electrolyte leakage in spring due to their delayed bud sprouting. However, when considering the two German populations (BB and RPF) evaluated for late frost damage in 2013, these expectations were not conformed as their ion leakage was similar. This could be explained by one reason: their origins' climatic conditions in spring are not much different to have had a considerable influence on their late frost hardiness. This could be supported by their similarity in phenological development at the time of late frost experiment (Fig. 4.4.1). It is important to note that in spring developing plants tissues are less hardy than in winter and therefore prone to have high REL from expanding leaves tissues (Arora et al. 2004, Taschler et al. 2004). Hence the high REL value even from the control plants from expanding leaves. Hypothetically, the high REL values obtained in this experiment did not imply lethality as supported by the ability of regeneration plants to grow after exposure to the lowest temperature (Fig. 4.4.5). When the German (BB) and the Italian (ITA) population are considered, evaluated the following year (2014), the influence of either altitude or latitude on REL is apparent. However, the influence of altitude, although seemingly apparent, was excluded since it did not affect REL between the German populations. The influence of latitude on REL could be explained indirectly by phenology. Since the Italian population is originating from lower latitude its chilling requirement or its thermal requirement to bud break could be low as elucidated for apricot (Campoy et al. 2011). Consequently, it sprouted earlier and advanced its bud phenology faster than the German population BB. Its fast expanding leaves were hence vulnerable to late frost damage as expounded by literature (Taschler et al. 2004, Wanjiku and Bohne 2016). Expanding leaves have been demonstrated by literature to have a tendency to leak more ions due to their un-lignified leaves' cell wall (Boudet et al. 1995, Wanjiku and Bohne 2016). This was also demonstrated in these experiments where the German BB in 2014 when unstressed had higher REL than the BB in 2013 (Fig. 4.4.3). Similarly, the advanced leaf phenology of the Italian population had higher ion leakage compared to that of German population in 2014 (Fig. 4.4.3) late frost experiment even when the plants were unfrozen (control).

Carbohydrates reserves are important carbon pool for growth and protection (Morin et al. 2007, Lee et al. 2012). When unstressed, the German populations BB and RPF did not significantly differ from each other. However, when they were treated with low temperature (-6 °C and -12 °C), RPF significantly increased its glucose and sucrose concentration compared to BB. However, the increase in these two sugars seemed to a play a very minor role in constraining damage (Fig. 4.4.3). The failure of these two German populations (especially BB) to increase their soluble carbohydrates, though speculative, could be as result of unavailable reserves. Most probably they had been used up for

sprouting as demonstrated by literature (Mohamed et al. 2012). Proline in these two populations did not seem to follow any trend with temperature. For BB, it increased at -12 °C while for RPF it decreased. The reason for this opposing trend is not clear.

Comparing the German (BB) and Italian population, when untreated they did not differ in GFS and proline. The starch concentration of the Italian population was low. This difference could be explained by the fact that the Italian plants were more advanced in sprouting than German (BB) population and hence the starch concentration could have been hydrolysed to support new growth. Frost treatments had profound effects only at -12 °C. The German (BB) population increased its glucose and fructose concentrations, while the Italian population increased its glucose, fructose, sucrose and starch concentration. These increases could have helped the plants to withstand, some extent, lower temperature at -12 °C as they maintained similar REL with that of -6 °C (Fig. 4.4.3). However, the increases were not sufficient to contain the late frost damage. The plants lost most of their twigs as can be seen from the regeneration plants (Fig. 4.4.5). The Italian population starch increase (-12 °C) may not be due to frost per se but could be related to few plants, which by chance, had high starch concentration as can be supported with high standard deviation. It could be that some leaves were already photosynthetically active. The evaluated German and Italian populations differed in fructose and sucrose which could be speculated in terms of leaves developmental stage, where the Italian population could be already photosynthesizing.

In this year (2014), proline concentration in both populations (German and Italian) was not significantly influenced by late frost treatments as only a tendency to increase was observed (Table 4.2.2). This was similarly found with *Corylus avellana* late frost experiment (Wanjiku and Bohne 2016). Proline on these two German populations was higher than that of Italian population. This could be due to its speculative role in breaking bud dormancy and supplying energy during bud break (Walton et al. 1998) where the Italian population was beyond this phenological stage.

The dissimilarities in German (BB) population in 2013 (cutting year 2009) and 2014 (cutting year 2011) in some biomarkers concentration could be attributed to phenological stage, age, and genetics composition of the mother plants as reported by literature investigating genotypes (Aslamarz et al. 2011).

Although these plants were greatly affected by the late frost treatments, they were able to regenerate by sprouting more shoots from the crown and by the end of the growing season (autumn), the Italian population had attained a similar height as the dead leader shoot. The German population's regenerated shoots were also proximal in length to the damaged leader shoot. The Italian population was taller than the German population.

Summing up, late frost could cause devastation of plants at developmental stages. This damage however could only partially be related to the level of relative electrolyte leakage. This is because REL

has proved to change with phenological stage and not exclusively with temperature and the level of damage. Similarity in responses to late frost stress between German populations BB and RPF proposes no local adaptation or adaptability to wide ecological range tested in this experiment. This suggests they could be substituted for another. However, the Italian behavior to sprout earlier could be maladapted for use in Germany as it might frequently be frost damaged.

In the following sub-section, responses to drought will be discussed concerning two German populations and between German BB and Italian (ITA) population.

4.4.2 Drought reactions of three *Prunus spinosa* populations

It is noteworthy that plants in 2012 had no fruits while those of 2013 had varying fruit-load with the ITA having a significantly higher fruit-load (Table 4.4.3). The Italian population also differed from the German population (BB) in height, number of shoots and dry mass (Table 4.4.3).

Table 4.4.3: Growth parameters (height, RCD, shoot number, plant dry mass and fruit dry mass) per plant of two German (BB, RPF) and one Italian (ITA) population of *Prunus spinosa* (2012 and 2013). Mean ± SD, n = 9. Different letters show significant differences between population per year.

Origin	Year	Height (cm)	RCD (mm)	Shoots no.	DM (g)	Fruit DM
BB	2012	107 ± 17 a	15 ± 3 a	12 ± 5 a	107 ± 43 a	No fruits
RPF		97 ± 23 a	14 ± 4 a	14 ± 7 a	94 ± 31 a	No fruits
BB	2013	107 ± 16 a	21 ± 4 a	13 ± 5 a	112 ± 40 a	5 ± 8 a
ITA		130 ± 17 b	22 ± 2 a	22 ± 2 b	162 ± 26 b	15 ± 9 b

Stomata conductance (SC) responses due to drought stress

Drought declined stomata conductance significantly in the experiments of both years. The German populations tested (BB and RPF) did not differ in their SC behaviour (data not shown). The scenario was different in the following year with German and Italian population. Pants in both slow and fast stress declined their SC sharply and differed significantly with the control from day three (D3) on-wards. ITA sharply declined its SC significantly differing with BB at D3 in fast stress. In slow stress ITA also had tendency to sharply decline its SC but did not differ with BB population (Fig. 4.4.6).

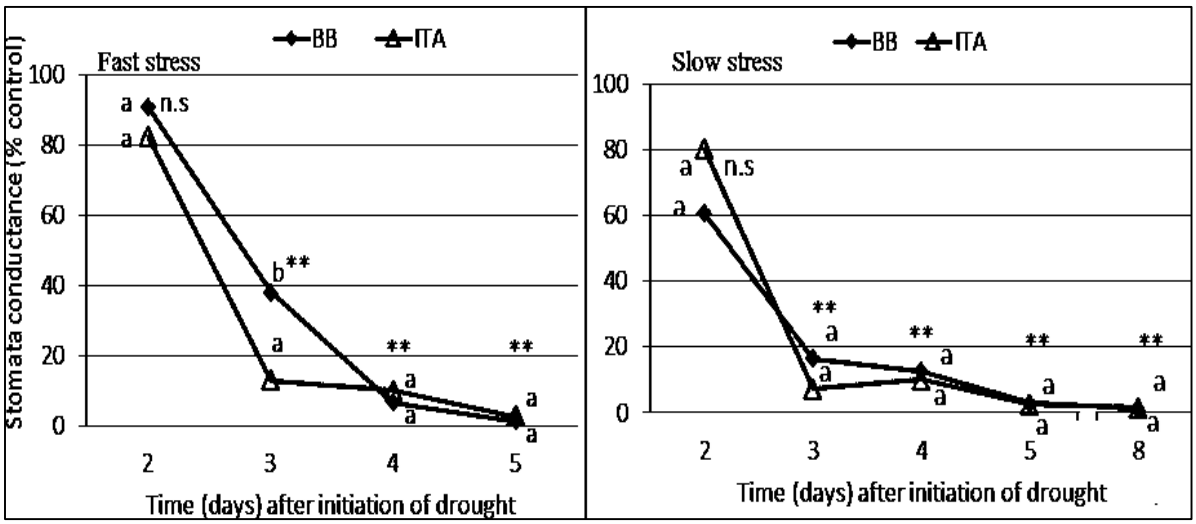

Figure 4.4.6: Stomatal conductance (% control) of German (BB) and Italian (ITA) population of *Prunus spinosa* during drought treatment. Different letters show significant differences between populations within a treatment; n.s = not significant while ** = significant differences between population's stress treatment and the control. Mean; n = 3 during the drought period; n = 9 on the last day.

Relative water content (RWC) responses due to drought stress

All populations in fast stress treatment had significantly lower RWC than the control at the last day of the experiment (Table 4.4.4). In the first experimental year, the German populations in slow stress did not differ with those of the control. However, in the following year, the German as well as the Italian population had significant lower RWC than the control. The Italian population differed statistically with the German population only in control RWC (Table 4.4.4).

Morphological responses due to drought

All stressed plants (slow and fast stress) showed varying wilting symptoms. At some point there were selective leaf yellowing and leaf drop. When the populations were compared: German populations were not different from each other; but the Italian population tended to have more yellow leaves and shed more leaves than the German BB population. The fruit from the drought treated plants were shrivelled with time but were retained on the plants (data not shown).

Table 4.4.4: Leaves' RWC, GFS and proline of two German (BB, RPF) and one Italian (ITA) population of *Prunus spinosa* at the end of drought experiments (2012 and 2013). Mean ± SD, n = 9. Different letters show significant differences: capital letters among treatments within a population per year and small letters between populations per year.

Origin	Year	Treatment	RWC %	Glucose % dm	Fructose % dm	Sucrose % dm	Proline µg g^{-1}dm
BB	2012	Control	92 ± 04 Aa	0.68 ± 0.27 Aa	0.30 ± 0.09 Aa	2.83 ± 0.35 Aa	130 ± 46 Aa
		Slow stress	85 ± 07 Aba	0.96 ± 0.33 Aa	0.70 ± 0.18 Ba	2.72 ± 0.73 Aa	1213 ± 495 Ba
		Fast stress	71 ± 14 Ba	0.92 ± 0.24 Aa	0.70 ± 0.22 Ba	2.30 ± 0.88 Aa	1707 ± 717 Ba
RPF	2012	Control	92 ± 02 Aa	0.76 ± 0.13 Aa	0.26 ± 0.14 Aa	2.43 ± 0.56 Aa	113 ± 52 Aa
		Slow stress	87 ± 10 ABa	0.94 ± 0.25 Aa	0.51 ± 0.11 Ba	2.47 ± 0.43 Aa	907 ± 396 Ba
		Fast stress	80 ± 06 Ba	0.80 ± 0.21 Aa	0.59 ± 0.09 Ba	2.29 ± 0.42 Aa	1713 ± 493 Ba
BB	2013	Control	94 ± 04 Aa	1.00 ± 0.24 Aa	0.15 ± 0.08 Aa	2.41 ± 0.54 Aa	383 ± 217 Aa
		Slow stress	63 ± 12 Ba	1.97 ± 0.54 Ba	0.77 ± 0.27 Ba	3.67 ± 0.53 Bb	1404 ± 635 Ba
		Fast stress	64 ± 10 Ba	1.56 ± 0.23 Ba	0.84 ± 0.13 Ba	3.52 ± 0.66 Bb	2081 ± 374 Ba
ITA	2013	Control	90 ± 02 Ab	1.01 ± 0.41 Aa	0.23 ± 0.19 Aa	2.07 ± 0.63 Aa	203 ± 141 Aa
		Slow stress	54 ± 16 Ba	2.50 ± 1.00 ABa	1.03 ± 0.42 Ba	2.12 ± 0.91 Aa	1357 ± 465 Ba
		Fast stress	56 ± 06 Ca	1.55 ± 0.62 Ba	0.77 ± 0.29 Ba	2.90 ± 0.55 Aa	3389 ± 981 Cb

GFS responses due to drought stress

Concerning the German populations, drought had little effects on GFS as only fructose significantly increased in leaves due to drought independent of stress development and origin (Table 4.4.4).

Comparing the German (BB) and the Italian (ITA) population, in the following year, fructose concentration in leaves increased independent of origin and stress treatment (Table 4.4.4). Additionally, the German population (BB) significantly increased its glucose and sucrose concentration in both stress treatments, while the Italian population increased its leaves' glucose due to slow stress only. ITA leaves' sucrose did not react to stress treatment. German population (BB) had significantly higher sucrose concentration than ITA in both stress treatments (Table 4.4.4).

Proline responses due to drought stress

Proline increased due to drought stress treatment in both years (Table 4.4.4). The German populations similarly increased their proline independent of stress treatment (Table 4.4.4). However, for ITA, proline increase was dependent on treatment with fast stress having the highest proline concentration (Table 4.4.4). The Italian population significantly differed with German population in fast stress proline concentration. ITA had higher proline concentration than BB (Table 4.4.4). There was also significant interaction between population x treatment that affected proline concentration between the German and Italian populations unlike GFS.

Regeneration after drought

By re-watering regeneration plants in both experiments, plants were able to equally regenerate to produce new leaves irrespective of origin and stress treatment. Retained fruits were able to regain lost water and were ripe by the end of the growing season. The German and Italian populations' growth (number of shoots, height of the longest shoot and fruit mass) differences were sustained. There were no differences between the German populations in all evaluated parameters by the end of the growing season (data not shown).

When exposed to drought, plants may minimize water loss through closure of stomata and shedding of leaves. Stomata closure and leaf shedding were observed to some extent in these experiments. The German populations did not differ in their SC behaviour although it was expected that the RPF coming from a higher precipitation area would be more sensitive. This might be explained by the fact that *Prunus spinosa,* as a species, is drought tolerant (Nardini et al. 2013). Hence the narrow margin between the two populations (BB and RPF) was of no influence when considering drought.

The response of SC by Italian and German population (BB) evaluated in the following year, was quick in that they drastically lowered their stomata conductance by D3. Although ITA tended to close its stomata earlier, it did not have an advantage in survival as both populations wilted at the same time and at similar RWC (Table 4.4.4). However, ITA was disadvantaged in that it had a significantly higher fruit load than BB which could have resulted in uncontrollable water loss due to dysfunctional stomata as cited by literature (Knoche et al. 2000).

Stomata closure has been shown to trigger abscisic accumulation which further causes leaf senescence as a result of water stress (Tombesi et al. 2015). In both experiments, leaf senescence (yellowing) and selective shedding was observed mostly in slow stress irrespective of origin. This was a stress avoidance mechanism which strategically reduces transpiration area and recycles nutrients before leaf loss (Munné-Bosch and Alegre 2004, Milla et al. 2005). Leaf shedding contributed to low water loss resulting to similar water status in slow stress by German populations as supported by similar RWC between the control and slow stress (Table 4.4.4). But heavy leaf shedding by both German (BB) and Italian populations (slow stress) was not accompanied by similar RWC with the control but rather a declining RWC (Table 4.4.4) This could partly be due to uncontrollable water loss from the unripe drupes as supported by literature (Bondada and Shutthanandan 2012). It could also be the reason why BB had higher RWC than the ITA in the control.

Plants can accumulate compatible solutes, sugars and proline, when confronted with drought (Regier et al. 2009, Xiao et al. 2009). These compatible solutes assist in osmotic adjustment and reduce oxidative stress effects. In this study, the German populations (BB and RPF) increased leaves' fructose only due to drought (Table 4.4.4). This suggests that there was no osmotic adjustment or one that could not be sustained, and the plants resorted to leaf shedding as an avoidance strategy (Tyree et al. 1993). Accumulation of sugars by both German (BB) and Italian population evaluated show that there was some osmotic adjustment but again it did not seem to be sufficient and leaf shedding was inevitable to combat drought stress. The difference in sucrose concentration in leaves might have been due to fruit load where much of sucrose could have been sunk to maintain important metabolic processes especially for the embryo development (Koch 2004).

Proline accumulation was independent (except one case) of treatment and origin. Proline accumulation in plants has been elucidated as beneficial due to the role it plays in osmotic adjustment in plants (Yancey 2005, Boussadia et al. 2013). Overall from the results, German populations did not differ in their capacity to accumulate this biomarker and are thus equally competent against drought (Table 4.4.4). But the Italian population had significantly higher proline concentration than the German population (Table 4.4.4). This may be attributed to fruit load where proline may have an additional role to protect embryo (in fruit) development against stress (Lehmann et al. 2010).

The regeneration ability of all plants from all treatments and origins demonstrated similar capabilities to recover after drought. More intriguing was how the fruits that were shrivelled due to drought were able to regain water and eventually ripen by the end of the vegetation period. The results with fruit recovery are similar to those obtained by Bondada and Shutthanandan (2012) from recovering grapevines drupes after drought stress.

In summary, the German populations (BB and RPF) did not differ in any aspect, which is attributable to marginal climatic conditions differences in their areas of origin, hence no local adaptation. The

Italian population significantly differed with the German population (BB) probably due to the fact that it originates from a higher precipitation area. Accordingly, it responded to drought by closing stomata earlier. But its early stomata closure did not enhance its avoidance strategy due to fruit load. The fruit load in this case, was an additional stress factor due to loss of much water. This made further comparison of the populations difficult as closure of stomata by ITA could not be track whether would have postponed wilting. It however made clear that vulnerability to drought stress must not only be related to climatic conditions in the area of origin, but also size and phenological stage, in this case the fruit-load.

In the above two sections (4.4.1 and 4.4.2), the German populations did not show any disparity in late frost and drought responses. However, when a German population BB was contrasted with Italian population, there was significance disparity between them. In the following section (4.4.3) four German populations are contrasted over the seasons to find out whether they differ in any of the evaluated parameters with origin. Italian population was also included as an example of a distant population origin with different climate. Parameter evaluated included: Bud set, bud sprout, N, P, K and biochemical composition.

4.4.3 Seasonal characterisation of different *Prunus spinosa* populations

From the results, German populations (from different cutting years) did not statistically differ from one another in their phenology (bud sprout and bud set) in all the three years they were evaluated (Fig. 4.4.7 and Fig. 4.4.8 A). The Italian population was however statistically different in its phenology in that it significantly sprouted earlier (Fig. 4.4.8 B) and tended to delay their bud set (Fig. 4.4.9).

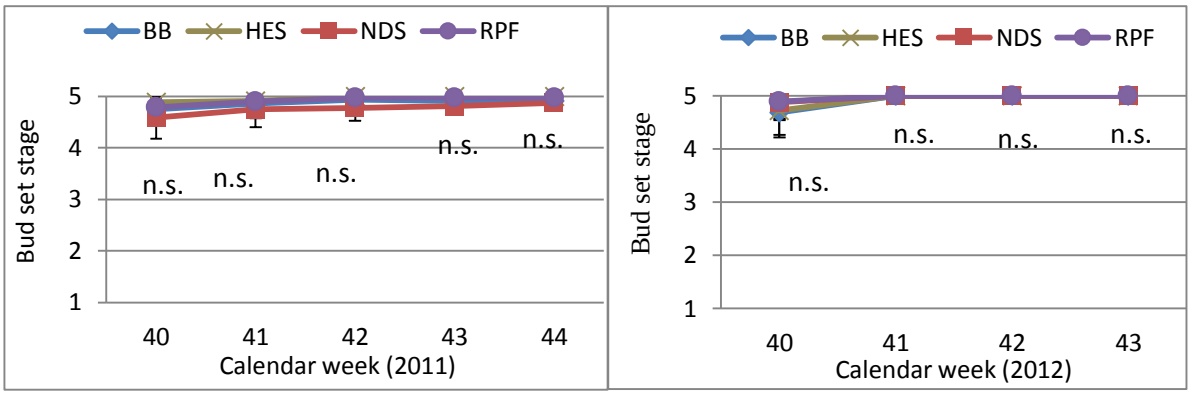

Figure 4.4.7: Bud setting phenology of four populations of *Prunus spinosa* in autumn 2011 and 2012. Means ± SD; n = 56 (BB), 44 (HES), 32 (NDS), 27 (RPF) from cutting year 2009. n.s. = no significant differences among the populations.

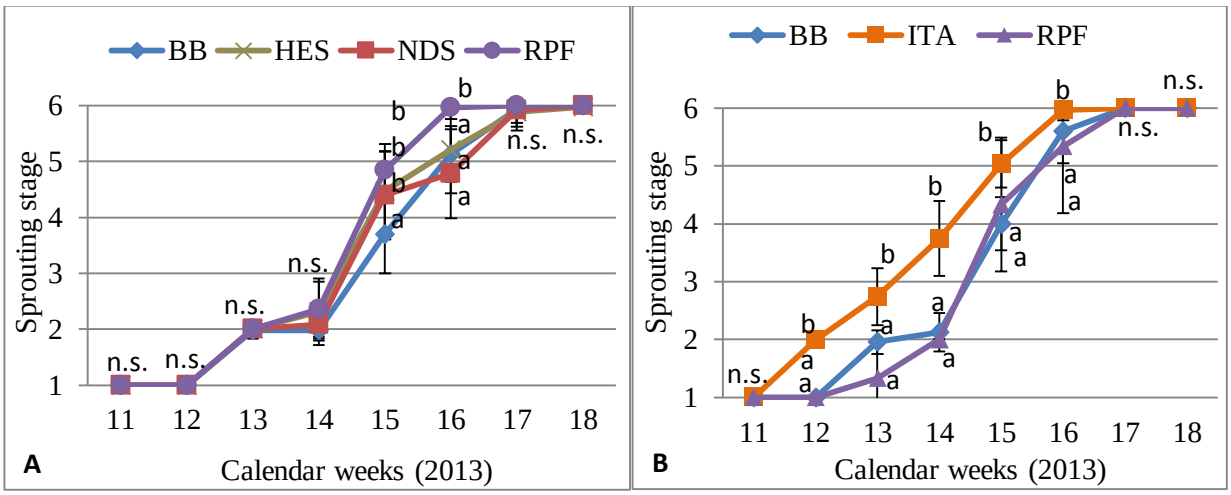

Figure 4.4.8: Bud sprouting phenology of *Prunus spinosa* populations in spring 2013. Different letters show significant differences among populations. n.s. = no significant differences among the populations. Mean ± SD, n = 56 (BB), 44 (HES), 32 (NDS), 27 (RPF) from cutting year 2009 (A); n = 71 (BB), 90 (ITA), 3 (RPF) from cutting year 2011 (B).

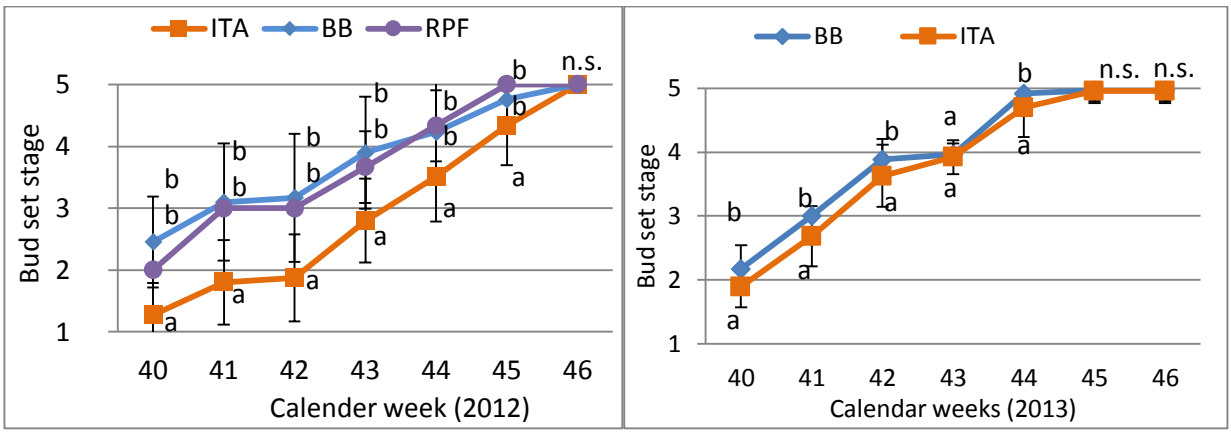

Figure 4.4.9: Bud setting phenology of *Prunus spinosa* populations in autumn 2012 and autumn 2013. Different letters show significant differences among the populations. n.s. = no significant differences among the populations. mean± SD; n = 71 (BB), 90 (ITA), 3(RPF) from cutting year 2011in autumn 2012; mean± SD; n = 36 (BB), 54 (ITA) from cutting year 2011in autumn 2013.

Growth characterization

German populations (cutting year 2009) did not vary (with exception of spring 2011) in most growth parameters (Fig. 4.4.10 and 4.4.11A). However, they differed in fruit count where HES had significantly higher fruit number than the other (BB, NDS and RPF) populations. (Fig. 4.4.11B). The Italian population had significant higher number of shoots and were taller than the German BB population (Fig. 4.4.11 C). Although only observed, the Italian plants tended to have more thorns than the German populations. Plants from cutting year 2011 also bore varying number of fruits but the Italian population had a significant fruit load (Table 4.4.3).

N, P, K and biochemical (glucose, fructose, sucrose, starch and proline) concentration

In autumn 2012, the German populations (cutting year 2009) did not differ in any of the analysed parameters in new shoots (Table 4.4.5). They however differed in roots' glucose and in leaves' fructose, sucrose and phosphorus concentration. Nevertheless, these differences were inconsistent with neither latitude nor altitude. In spring the two populations (BB and RPF, cutting year 2009) analysed differed in new shoots' proline and starch concentration, where BB had higher proline concentration and low starch concentration than RPF respectively (Table 4.4.5). In roots BB had higher glucose, fructose, starch and potassium concentration than RPF. RPF had higher roots 'proline concentration. Comparing seasons for BB new shoots and roots, sucrose and starch were generally higher in autumn while in spring glucose, fructose and proline were higher (Table 4.4.5). N, P, and K was also higher in spring than in autumn (Table 4.4.5).

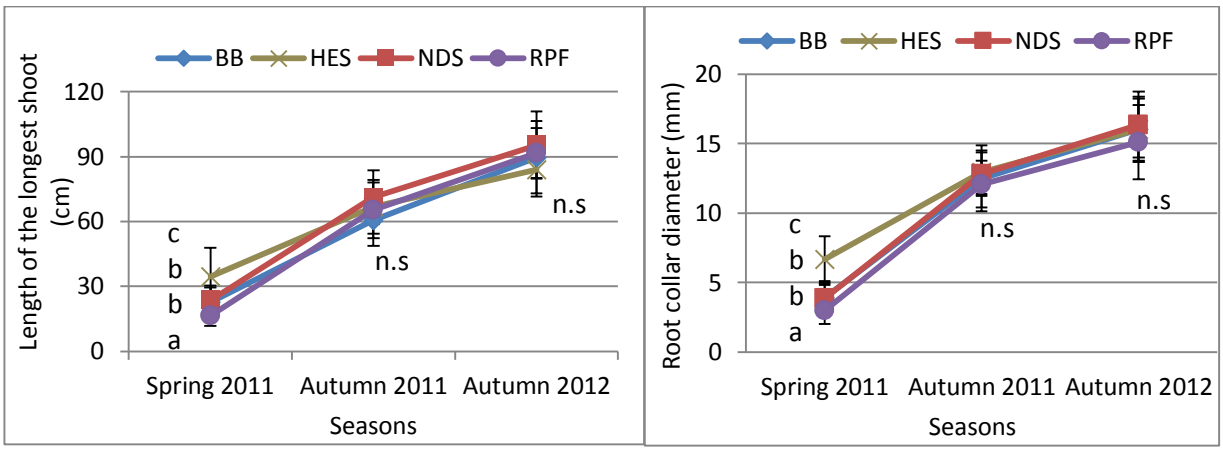

Figure 4.4.10: Seasonal height progression of the longest shoot and root collar diameter (RCD) of four populations of *Prunus spinosa*. Different letters show significant differences among populations n.s = no significant differences among the populations. Mean ± SD, n = 56 (BB), 44 (HES), 32 (NDS), 27 (RPF) from cutting year 2009.

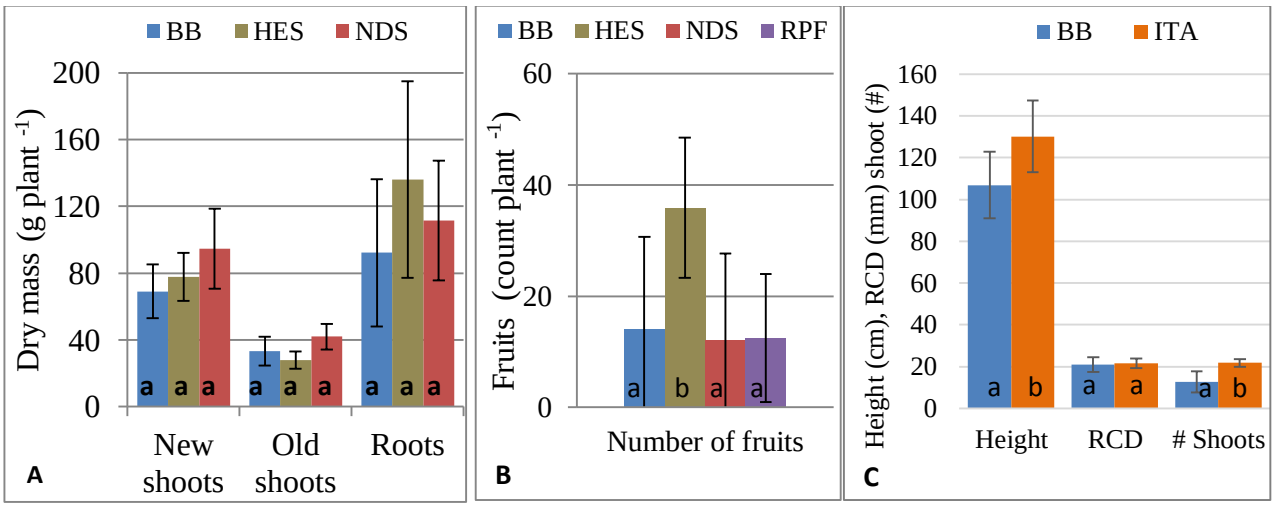

Figure 4.4.11: Dry mass (A) and fruit number (B) of *Prunus spinosa* populations in autumn 2012; height of the longest shoot, RCD and shoot number (# shoot) of German and Italian population (C). Different letters show significant differences among the populations. Mean ± SD, n = 56 (BB), 44 (HES), 32 (NDS), 27 (RPF) from cutting year 2009; n = 9 (BB and ITA) from cutting 2011.

Table 4.4.5: Glucose, fructose, sucrose, starch, nitrogen, phosphorus potassium (% dry mass) and proline (µg g^{-1}) in various parts of *Prunus spinosa* populations. Different letters show significant differences among populations. Mean ± SD; n = 6 (BB), 8 (HES), 6 (NDS) in autumn 2012; n = 12 (BB), 8 (RPF) in spring 2013 from cutting year 2009.

		Time of evaluation and part evaluated				
		Autumn (2012)			Spring (2013)	
Parameter	Origin	Leaves	New shoots	Roots	New shoots	Roots
Glucose	BB	0.85 ± 0.5a	0.42 ± 0.07a	0.43 ± 0.08ab	0.52 ± 0.14a	1.25 ± 0.34b
	HES	0.66 ± 0.07a	0.39 ± 0.09a	0.53 ± 0.12b		
	NDS	1.74 ± 0.36a	0.45 ± 0.03a	0.39 ± 0.09a		
	RPF				0.49 ± 0.12a	0.8 ± 0.18a
Fructose	BB	0.57 ± 0.15a	0.27 ± 0.05a	0.46 ± 0.12a	0.5 ± 0.15a	1.47 ± 0.59b
	HES	0.62 ± 0.2ab	0.28 ± 0.06a	0.59 ± 0.26a		
	NDS	1.13 ± 0.35b	0.34 ± 0.05a	0.49 ± 0.15a		
	RPF				0.46 ± 0.11a	0.95 ± 0.32a
Sucrose	BB	1.56 ± 0.15ab	1.26 ± 0.18a	1.28 ± 0.17a	0.36 ± 0.12a	0.99 ± 0.32a
	HES	2 ± 0.18b	1.13 ± 0.24a	1.16 ± 0.35a		
	NDS	1.41 ± 0.24a	1.34 ± 0.2a	1.42 ± 0.1a		
	RPF				0.35 ± 0.2a	0.73 ± 0.3a
Starch	BB	0.06 ± 0.02a	4.23 ± 0.72a	6.4 ± 0.54a	0.29 ± 0.08a	1.84 ± 1.31b
	HES	0.23 ± 0.22a	4.51 ± 0.61a	6.3 ± 0.32a		
	NDS	0.18 ± 0.03a	4.69 ± 0.6a	6.44 ± 0.32a		
	RPF				0.56 ± 0.34b	0.82 ± 0.56a
Proline	BB	188 ± 48a	160 ± 41a	78 ± 28a	371 ± 143b	125 ± 38a
	HES	219 ± 96a	178 ± 116a	72 ± 45a		
	NDS	254 ± 79a	238 ± 107a	100 ± 38a		
	RPF				332 ± 90a	189 ± 62b
Nitrogen	BB	1.47 ± 0.12a	0.73 ± 0.08a	0.89 ± 0.28a	1.23 ± 0.31a	1.04 ± 0.32a
	HES	1.73 ± 0.16a	0.75 ± 0.18a	0.73 ± 0.25a		
	NDS	1.36 ± 0.14a	0.86 ± 0.15a	0.85 ± 0.2a		
	RPF				1.17 ± 0.28a	1.17 ± 0.3a
Phosphorus	BB	0.59 ± 0.08b	0.1 ± 0.01a	0.17 ± 0.08a	0.21 ± 0.06a	0.2 ± 0.08a
	HES	0.61 ± 0.06b	0.12 ± 0.04a	0.14 ± 0.06a		
	NDS	0.32 ± 0.06a	0.12 ± 0.03a	0.16 ± 0.03a		
	RPF				0.19 ± 0.04a	0.22 ± 0.06a
Potassium	BB	1.76 ± 0.6a	0.36 ± 0.02a	0.31 ± 0.1a	0.72 ± 0.21a	0.33 ± 0.06b
	HES	1.93 ± 0.15a	0.33 ± 0.05a	0.29 ± 0.04a		
	NDS	1.92 ± 0.34a	0.37 ± 0.04a	0.39 ± 0.09a		
	RPF				0.7 ± 0.19a	0.26 ± 0.05a

Table 4.4.6 Glucose, fructose, sucrose, starch, nitrogen, phosphorus potassium (% dry mass) and proline (µg g^{-1}) in various parts of *Prunus spinosa* populations. Different letters show significant differences among populations. Mean ± SD; n = 9 (BB and RPF) in summer 2012; n = 6 (BB), 4 (RPF) in autumn 2012 from cutting year 2010.

		Time of evaluation and part evaluated				
		Summer (2012)		Autumn (2012)		
Parameter	Origin	Leaves	Roots	Leaves	New shoots	Roots
Glucose	BB	0.68 ± 0.27a	0.05 ± 0.05a	0.55 ± 0.14a	0.33 ± 0.13a	0.6 ± 0.09b
	RPF	0.76 ± 0.13a	0.09 ± 0.04b	0.65 ± 0.21a	0.28 ± 0.07a	0.3 ± 0.17a
Fructose	BB	0.3 ± 0.09a	0.7 ± 0.1a	0.39 ± 0.14a	0.52 ± 0.14a	0.51 ± 0.51a
	RPF	0.26 ± 0.14a	0.62 ± 0.2a	0.56 ± 0.24a	0.39 ± 0.03a	0.47 ± 0.14a
Sucrose	BB	2.83 ± 0.35a	0.63 ± 0.16a	2.57 ± 0.53a	1.36 ± 0.41a	1.23 ± 0.29a
	RPF	2.43 ± 0.56a	0.69 ± 0.29a	2.67 ± 0.12a	1.32 ± 0.58a	1.76 ± 0.45a
Starch	BB	0.06 ± 0.02a	3.41 ± 1.12a	0.38 ± 0.16a	4.24 ± 0.88a	6.77 ± 0.82a
	RPF	0.05 ± 0.04a	4.22 ± 1.18a	0.38 ± 0.15a	4.83 ± 0.41a	6.73 ± 0.92a
Proline	BB	184 ± 166a	86 ± 41a	490 ± 292a	465 ± 340a	298 ± 262a
	RPF	114 ± 52a	83 ± 26a	504 ± 387a	398 ± 144a	262 ± 66a
Nitrogen	BB	3.69 ± 0.58a	1.17 ± 0.19a	2.74 ± 0.34a	0.82 ± 0.38a	1.34 ± 0.51a
	RPF	3.53 ± 0.71a	1.15 ± 0.27a	2.67 ± 0.2a	0.71 ± 0.15a	1.29 ± 0.45a
Phosphorus	BB	0.34 ± 0.06a	0.23 ± 0.03a	0.33 ± 0.07a	0.13 ± 0.06a	0.24 ± 0.05a
	RPF	0.34 ± 0.09a	0.23 ± 0.04a	0.28 ± 0.02a	0.11 ± 0.03a	0.23 ± 0.07a
Potassium	BB	2.73 ± 0.06a	0.52 ± 0.04a	2.38 ± 0.25a	0.35 ± 0.04a	0.41 ± 0.14a
	RPF	2.77 ± 0.04a	0.49 ± 0.07a	2.48 ± 0.26a	0.31 ± 0.05a	0.39 ± 0.11a

From cutting year 2010, the two German populations (BB and RPF) did not differ in most parameter (except in autumn roots' glucose) in summer and autumn 2012 (Table 4.4.6).

From cutting year 2011, the German populations had significant higher concentration of new shoots' sucrose (BB and RPF), roots' proline (BB and RPF), new shoots' and roots' nitrogen (RPF) and new shoots' and roots' phosphorus (RPF) than the Italian population in spring 2012 (Table 4.4.7). During this time period (spring 2012), the German populations differed in roots' nitrogen and potassium and in new shoots' and roots' phosphorus (Table 4.4.7) where RPF had higher concentration than BB.

In summer 2013, the Italian and the German population (BB) differed only in leaves starch concentration. However, in spring 2014, there were more differences between the Italian and German (Table 4:4.7): German population (BB) had higher glucose (new shoot), fructose (new shoots), sucrose (new shoots and roots) starch (roots), proline (roots) and phosphorus (new shoots and roots) than Italian population. Conversely, Italian population had higher roots' fructose and potassium concentration than the German (BB) population (Table 4.4.7).

Table 4.4.7: Glucose, fructose, sucrose, starch, nitrogen, phosphorus potassium (% dry mass) and proline (µg g^{-1}) in various parts of *Prunus spinosa* populations. Different letters show significant differences among populations. Mean ± SD; n = 15 (BB and ITA), 6 (RPF) in spring 2012; n = 9 (BB and ITA) in summer and spring 2014 from cutting year 2011.

		Time of evaluation and part evaluated					
		Spring (2012)		Summer (2013)		Spring (2014)	
Parameter	Origin	New shoots	Roots	Leaves	Roots	New shoots	Roots
Glucose	BB	0.93 ± 0.23a	2.03 ± 1.48a	1.00 ± 0.24a	1.04 ± 0.35a	0.25 ± 0.12b	0.34 ± 0.08a
	ITA	0.93 ± 0.21a	1.42 ± 1.16a	1.00 ± 0.43a	1.04 ± 0.28a	0.12 ± 0.10a	0.43 ± 0.15a
	RPF	0.89 ± 0.13a	1.74 ± 2.01a				
Fructose	BB	0.92 ± 0.32a	0.41 ± 0.26a	0.16 ± 0.07a	0.94 ± 0.38a	1.48 ± 0.42b	0.19 ± 0.09a
	ITA	0.57 ± 0.11a	0.38 ± 0.16a	0.23 ± 0.17a	0.83 ± 0.4a	0.13 ± 0.07a	0.47 ± 0.27b
	RPF	0.73 ± 0.13a	0.29 ± 0.07a				
Sucrose	BB	1.16 ± 0.25b	0.97 ± 0.26a	2.42 ± 0.56a	0.77 ± 0.32a	0.99 ± 0.37b	1.14 ± 0.5b
	ITA	0.54 ± 0.1a	0.93 ± 0.40a	2.08 ± 0.64a	0.6 ± 0.25a	0.22 ± 0.12a	0.51 ± 0.32a
	RPF	1.02 ± 0.44b	1.37 ± 0.84a				
Starch	BB	1.01 ± 0.52a	2.12 ± 1.12a	0.55 ± 0.19b	2.53 ± 2.38	0.88 ± 0.31a	4.08 ± 2.45b
	ITA	1.00 ± 0.54a	1.42 ± 0.78a	0.26 ± 0.03a	1.16 ± 1.68	0.68 ± 0.07a	2.45 ± 1.09a
	RPF	1.09 ± 1.24a	2.28 ± 1.94a				
Proline	BB	1162 ± 618a	440 ± 173b	383 ± 217a	66 ± 47a	284 ± 108a	145 ± 40b
	ITA	930 ± 108a	285 ± 131a	203 ± 141a	38 ± 15a	234 ± 59a	103 ± 12a
	RPF	1599 ± 665a	601 ± 227b				
Nitrogen	BB	1.72 ± 0.71a	2.08 ± 0.48ab	4.21 ± 0.58a	1.07 ± 0.33a	1.39 ± 0.33b	1.73 ± 0.39b
	ITA	1.49 ± 0.26a	1.9 ± 0.38a	4.02 ± 0.58a	0.70 ± 0.15a	0.99 ± 0.14a	0.96 ± 0.17a
	RPF	2.66 ± 0.38b	2.5 ± 0.37b				

Phosphorus	BB	0.08 ± 0.02a	0.1 ± 0.02a	0.53 ± 0.11a	0.25 ± 0.06a	0.17 ± 0.04b	0.26 ± 0.06b
	ITA	0.08 ± 0.03a	0.09 ± 0.03a	0.56 ± 0.10a	0.16 ± 0.03a	0.13 ± 0.02a	0.18 ± 0.03a
	RPF	0.13 ± 0.02b	0.13 ± 0.01b				
Potassium	BB	0.63 ± 0.17a	0.73 ± 0.1a	2.76 ± 0.49a	0.32 ± 0.11a	0.33 ± 0.06a	0.33 ± 0.08a
	ITA	0.88 ± 0.19b	1.09 ± 0.21b	2.66 ± 0.49a	0.29 ± 0.13a	0.46 ± 0.10b	0.44 ± 0.09b
	RPF	0.93 ± 0.16b	0.97 ± 0.21b				

Among the German populations, only BB and RPF plants were available in all cutting years (2009 - 2011). Of both BB was mostly analysed in every season. When comparing the cutting years for BB, the concentration of new shoots' and roots' glucose, fructose sucrose, starch, N, P, and K concentrations were similar within a season. However, proline concentration affected by cutting year.

Phenology has been shown to be affected by genetics (Howe et al. 2003), latitude, longitude, altitude (Chmura and Rożkowski 2002). When considering the German populations, there was no significant influencing factor to their phenology as these populations did not differ in their bud sprouting and bud setting phenology. The results suggest that these populations' responses to decreasing temperature and day length are similar. This is probably due to the fact that their climate of origin is not different from each other (Table 4.2.2). German populations from higher altitude (HES and RPF) depicted that these populations are adapted to heterogeneous environment and although they originate from cooler areas, they can quickly adjust to new conditions. It also demonstrated that they could be utilised in low altitude without any compromise in their bud phenology rhythm. These results in phenology, in part, tend to contrast the results of Fronia (2009) who reported some differences in phenology of seven German *Prunus spinosa* populations during the first two years of establishment in a common cultivation area. Nevertheless, his grouping was unrelated to origin and was highly variable (bud set) on year to year climatic conditions.

When the German populations BB and RPF are contrasted with the Italian population, there were significant differences in phenology (bud sprouting and bud setting). Ecologically, Italian is originating from a warmer climate and more south than the German populations (Table 4.2.2). The results depicted Italian population flushing earlier and delaying bud set. Flushing early is characteristic inherent to southern populations whereby they want to maximize growth before the onset of summer drought while those of the northern origin are cautious to lower the risk of late frost damage (Howe et al. 2003). The tendency of southern populations to sprout early was similar to that reported for *Quercus petraea* (Ducousso et al. 1996) and *Fagus sylvatica* (Chmura and Rożkowski 2002). It also

agrees with the results of Fronia (2009) who reported earlier sprout behaviour of a Hungarian *Prunus spinosa* population. According to Rohde et al. (2011) population originating from south delays bud set while those of northern latitude cease their growth early. If this is the case, the Italian population (originating 45° N) perfectly fits this expectation. However, for the German populations this is not fitting as they did not differ in bud setting phenology. This could be explained by the fact that there is a small latitudinal (50° and 52° N) differences between them.

Early flushing - late senescing populations are likely to take photosynthetic advantage due to available nutrients and water, since they start growing earlier and stop growing late (Menzel and Fabian 1999). This could partly explain why the Italian population had high biomass and were taller than the German population (BB). Additionally, population from the south have been shown to have a higher growth rate than those of the north since those of the north invest more of their photosynthetic reserves for protection than those of the southern origin (Loehle 1998).

Sprouting in spring diminished carbohydrates and nutrient reserves as they are remobilised for growth (El Zein et al. 2011). Since Italian population sprouted earlier, they must have remobilised their reserves earlier than the German population. This could explain why the Italian population predominantly had lower concentration of carbohydrates and nutrients (nitrogen and sometimes phosphorous) in spring than the German populations; and not at any other time. For the German populations (BB and RPF), cutting year 2009, spring's carbohydrate (glucose fructose and starch) concentration differences was mainly in roots where BB had higher concentration than RPF. On the other hand, RPF had higher proline and potassium concentration in the roots than BB. At the moment there is no clear explanation why.

When contrasted, these two German populations from cutting year 2010 (in summer and autumn) and cutting 2011 (spring), there were little differences between them in terms of carbohydrates and nutrients concentration. This suggests that these populations are not different despite the distance and climatic differences between them.

Pattern of nutrients allocation in leaves, shoots and roots did not differ among the German populations except for a few cases where BB differed with RPF in K (Table 4.4.5 and Table 4 4.7-spring 2012/2013) and P (Table 4.4.7 - spring 2012). However, the differences were never consistent with other seasons and could not therefore be attributed to ecological factors from the areas of origin. The similarity in nutrients concentration among the German populations grown under the same conditions partially agrees with literature that found nutrients acquisition and allocation to various organs to be similar for oak (*Quercus variabilies*) populations grown in a similar environment (Lei et al. 2013). Comparing nutrients concentration of the plants in this study with that obtained for *Prunus* rootstocks (N: 1.69 - 2.56%, P: 0.18 - 0.3% and K: 0.97 - 4.29%), all plants were sufficiently nourished (García et al. 2005).

When the German BB and Italian population are contrasted, cutting 2011, the nutrients allocation to various organs could be related to latitude (Table 4.4.7). According to the results, German population BB tended to have higher roots and new shoot N concentration than Italian population in spring and not in summer. This could be explained by the fact that the Italian population sprouted earlier and therefore remobilised N for growth. This could have resulted to N dilution as supported by literature (Oleksyn et al. 2002) .

Inferably, the results presented here show high variability within and among German populations in growth, phenology, N, P, K and carbohydrates concentration over the seasons. This suggests that these populations are not physiologically and biochemically differentiated. Consistently their climatic conditions are not much differentiated to induce local adaptation. Conversely the Italian population may not be fit for the German conditions since their early flushing and late senescing behaviour may jeopardize their survival could there be repeated frost (late and early) incidences.

4.5 Conclusion for the populations of *Prunus spinosa*

The natural populations of blackthorn studied here cover a range of different edaphic, topography and climatic conditions. From the seasonal characterization data of this study, the German populations studied did not show any substantial or consistent differences when cultivated under same condition either in phenology, biochemical composition and analysed N, P and K. Populations that were expected to be different (e.g., BB and RPF) showed high similarity (Fig. 4.5.1).

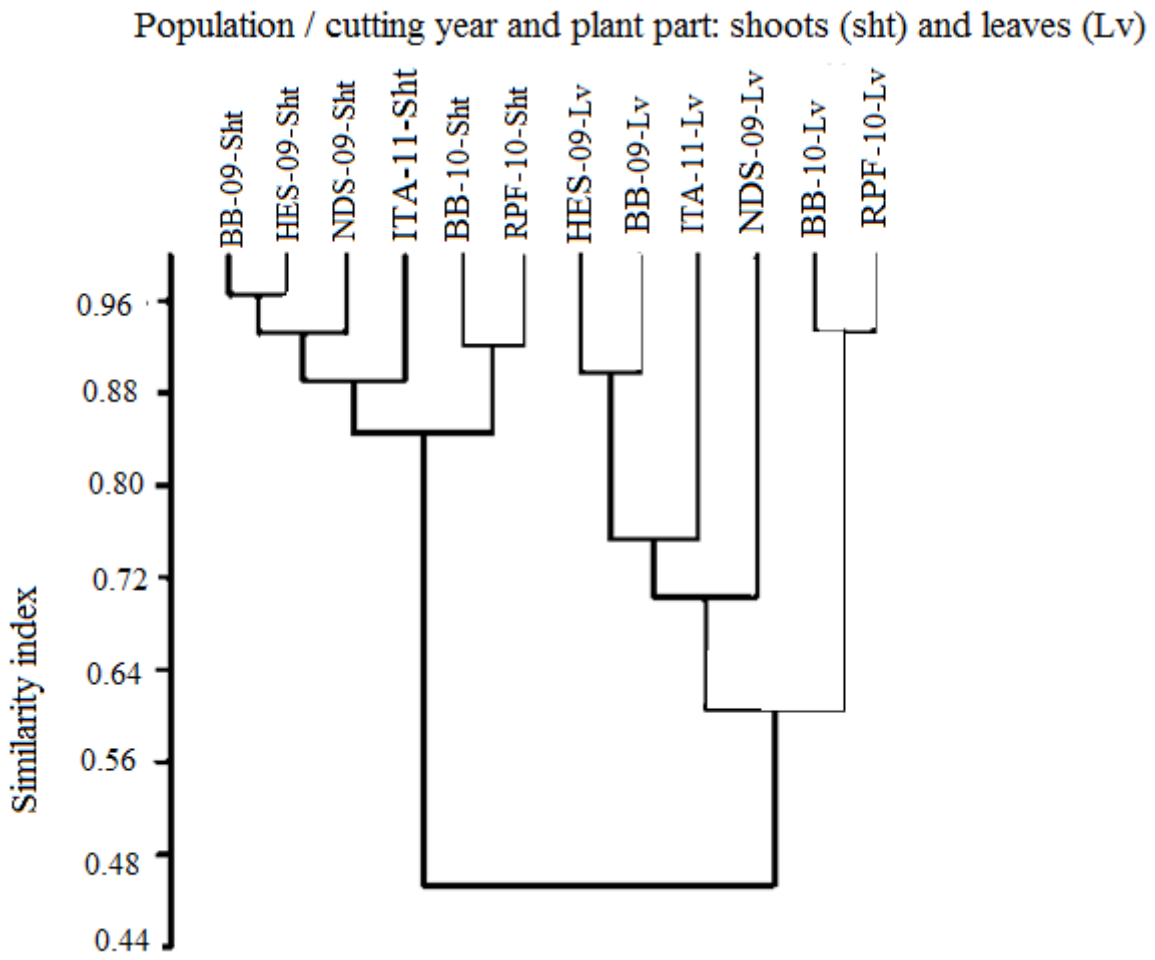

Figure 4.5.1: Dendrogram of the UPGMA cluster analysis based on similarity index of glucose, fructose, sucrose, starch, proline N, P and K.

This was similar to what was reported previously by Fronia (2009), although using different set of parameters (phenology, basal branching, vitality e.t.c.). He reported that populations that originated from wide geographical distances, for example Sachsen and Südbayern, were similar in phenology while those that were close geographically, for example Schorfheide and

Märkisch-Oderland, differed. This suggests that these populations are not genetically differentiated or their differentiation to geographical region is low (Fronia 2009). This was confirmed by Leinemann et al. (2014) who reported weak geographical and genetical pattern among the German populations and reported similarity of up to 85% among the German populations. When exposed to drought, the German populations BB and RPF did not differ in any physiological and biochemical reactions. Similarly, when exposed to frost, they did not differ in frost damage (REL) and in most biochemical reactions. Thus they are highly adaptive when cultivated away from home. Therefore, the issue of demarcating Germany to six areas of origin concerning this species could not be justified at this point in time similar to what was reported by Fronia (2009) concerning *Prunus spinosa*. This implies that nurseries could be allowed to source their propagation material from various populations provided they adhere to locations with similar climatic conditions.

Considering the German and Italian populations, there was substantial similarity dossier when the plants were clustered using the analysed glucose, fructose, sucrose, starch, proline N, P, K data in autumn 2012 (Fig. 4.5.1). This was re-confirmed when considering genetic structure similarity (up to 85%) reported by Leinemann et al. (2014). Nonetheless, in phenology, there was delay in bud set and early emergence in bud sprouting characteristics that could expose the plants to frequent damage by frost. In drought the Italian population responded quickly by early closure of stomata and lowering of water potential and RWC. However, the results were non-conclusive due to an interfering influence by fruit-load. Nevertheless, the Italian population might be inappropriate for use on landscapes that experiences drought stress. Concerning late frost damage and phenology, the results are clear that the Italian population could be maladapted for German landscapes. High growth rate of the Italian population would be commendable for full utilization of the growth season and quick vegetative cover for utilization in open landscapes. Nonetheless due to outcrossing nature through pollination in *Prunus spinosa* (Guitián et al. 1993), it would be wise to limit its utilization in Germany as it might interbreed with native populations and spread maladaptation.

5 General discussion

Plants being sessile have a suit of functional traits that capacitate them to inhabit, compete and survive in wide range of environment. It however occurs that some populations of a given species would adapt, through natural divergent selection pressure to apt ecological conditions (Joshi et al. 2001, Hereford 2009, Lázaro-Nogal et al. 2016); and transferring them to other areas could compromise their survival and performance. However, high rate of gene flow counters the efficiency of such divergent selection as genetic material is exchanged among populations (Perdereau et al. 2014). The exchange of genetic material (through gene flow) increases genetic variation within populations, reduces among populations and increases fitness against abiotic stresses (Hamrick et al. 1992).

This research project's plant material, *Corylus avellana*, is wind pollinated while *Prunus spinosa* is insect pollinated. Putatively, gene flow among *Corylus* populations would be expected to be high over long distances compared to that of *Prunus spinosa* otherwise ecotypes would be expected. Also the fruits dispersal in both species is mainly by small animals hence no long distance transfer of genetic material is foreseen unless by human intervention.

The choice of these two shrubs was on the basis of their ecological importance in Germany landscapes as well as in providing shelter and food for a number of mammals and insects. Therefore, the findings from this project are vital not only in landscapes but also in nature decision making. The findings would also contribute exemplar scientific data for conservation or proper landscape management and maintenance of biodiversity.

To be able to determine whether different populations are phenologically, physiologically or biochemically different, plants were sourced from different populations as cuttings and cultivated under same conditions. Use of cutting, though cumbersome, would ensure genetic identity and characteristics of the parent while cultivation under the same conditions would level out any differences that would normally occur in their natural habitats - probably due to rainfall pattern, nutrients availability or other factors. The differences, if any, would be presumed to be of local adaptation.

The results from this study were highly variable with season, mother plants and experimental year. The values had high standard deviations indicating high variability (diversity) within populations. This suggests that for a given population, phenology, physiological and biochemical responses are highly inconsistent at any given time.

In nature, phenology shift per 100 m increase in altitude has been demonstrated (Ziello et al. 2009, Pellerin et al. 2012, Schieber 2014). This shift has also been shown in common garden experiments for some plant species e.g., ash, oak, pine and attributed to genetic differentiation

(Vitasse et al. 2009). Nevertheless, German populations of both species (*Corylus avellana* and *Prunus spinosa*) did not demonstrate this differentiation either in bud set or bud sprout. In *Corylus*, a tendency of high altitude populations (NRW and RPF) to precede bud set and to lag bud sprout was seen in some instances but only for few weeks and inconsistent between cutting years. In *Prunus*, no differences among German populations were seen. It could be that these populations are highly plastic to changing environment when it comes to phenology which is majorly triggered by temperature and day length (Rohde et al. 2011, Basler and Körner 2012). This is partially supported by tendency to sprout early in 2012 and delay in 2013 when the seasons were warm and cold respectively as discussed in section 3.2. Delay in bud set in 2013 was due to relatively warmer autumn than 2012 (MuK 2011 - 2013). From autumn data (Table 2.1.1), temperatures of origin are not very different to have had a significant influence on phenology.

When transferred to new environment or when exposed to abiotic stress, plants will employ various mechanisms to cope and survive the stressing factor. Some of these responses are majorly steered by minimum genetic diversity while others are but temporal response (Lande 2009). Since the evaluated populations have been shown to be genetically differentiated (Leinemann et al. 2013b, 2014), and ecotypes have been shown to respond differently to abiotic stress (Jensen and Deans 2004, Reinhardt et al. 2011, Yildiz et al. 2014), it would be expected that they respond appropriately and differently to stressor stimuli.

However, results from seasonal characterization (biomass, N, P, K, biochemical composition) of the German populations in both species did not show any association to genetical, altitudinal or latitudinal conditions. They showed high variation within populations and years which could indicate high plasticity or high gene flow among the populations (Hamrick et al. 1992) and different composition of mother plants (Howe et al. 2003). The responses of these populations when exposed to various stresses (early frost, late frost and drought) demonstrated high resilience of these populations to environmental changes. Generally, populations did not significantly vary in most of their physiological and biochemical reactions to these stresses. This could be associated with little differences in their climatic conditions. This implies that they could be substituted for one another whenever the propagation material of one population is not feasible, compromised or whenever quantity demanded is high.

When drought is considered, *Corylus avellana* is depicted as drought sensitive species (Bignami et al. 2009) and *Prunus spinosa* as a drought tolerant species (Nardini et al. 2013). A statement confirmed in the drought experiments. Whereas plants of *Corylus* started to show wilting symptoms at -1.5 MPa, *Prunus* would slightly show wilting at -2 MPa. Also when the fast drought

treatment in 2012 was terminated the *Prunus spinosa*, most plants had no wilting symptoms even at -4 MPa (the max our device is calibrated). Partly, this could be attributed to leaf size and the fact that *Corylus* is an understorey species (Persson et al. 2004) its cuticle could be thin than that of *Prunus*. In *Corylu*s drought experiment, though no significant differences among populations were seen, BB was least physiologically and biochemically sensitive to drought conditions imposed. It maintained relatively higher stomata conductance and rarely did it increase significantly its soluble sugars. It thus, somehow, separated itself from the rest of the populations. These characteristics fairly fits its nativity of low precipitation; hence could be used as alternative population in landscapes that are more likely to experience drought. *Prunus spinosa* populations on the other hand were equal in drought responses and it would not matter which population is utilised in which area of origin.

Principally, the Italian *Prunus spinosa* population is separated from the German populations by its phenology and growth rate. For this species, natural selection must have led to selection of populations that flush early and cease growth late in the south to make maximum use of growth season. This has been shown to occur with other species (e.g., *Quercus petraea* and *Picea sitchensis*) where low latitude populations end their season later than those of higher latitude (Deans and Harvey 1996, Holliday et al. 2010). However, this behaviour is associated with danger of frost damage as plants tend to invest their carbon for growth rather than protection (Loehle 1998). This was demonstrated by this Italian population to steer growth early, more and longest shoots and to suffer high damage (measured by REL). This would render this population unfit for use in higher latitude where frost is most likely to damage them, where they could suffer heavily than population native to these latitudes (in this case BB). The strong phenological and growth rate differences between the German and the Italian populations contrast their genetical (Leinemann et al. 2014), seasonal biochemical and nutrient similarities (Fig. 4.5.1). However, such stark contrasts were reported on phenology and genetic structure of *Populus tremula* (Hall et al. 2007).

While climate is rapidly changing where extreme events are expected (e.g., drought and frost), some of the conditions tested in these experiments were extreme. Nevertheless, the populations evaluated were able to "fight" by employing diverse physiological and biochemical mechanisms to overcome the stressor stimuli. The ability of plants exposed to extreme treatments to regenerate, depicts these populations capacity to adjust to new or changing environment. Nonetheless, the capacity to survive and adapt to abiotic stresses is expected to better with age as literature elucidate for some species (Chen and Li 1978, Cavender-Bares and Bazzaz 2000, Lim

et al. 2014). This implies that mature plants in nature in respective areas of origin could probably withstand harsher treatment than administered, since there was no threshold reached. However, caution need to be exercised as there are disjunction between controlled conditions and field conditions especially due to the fact that in container grown plants, roots are constrained in a fixed volume that could hamper full potential response. For instance, plants exposed to frost experiment in situ, have been shown to tolerate lower temperatures than ex situ excised plant parts (Taschler and Neuner 2004). Another example would be drought, where plant in nature or in bigger containers (Boussadia et al. 2013) would explore broader substrate volume than in the 5 litre container. The latter was demonstrated by the ability of regeneration plants (planted after experiment) to survive up to 3 weeks without natural precipitation in the field. Nonetheless, the experimental setup was similar to that carried out by others for example Rumpf (2002) due to logistics and control of growth conditions.

6 General conclusion

In review to the research questions which this project aimed to answer, that is:

- Are populations obtained from different geographical areas of origin diverse in their phenology, morphology, physiology and biochemical constitution?
- Are the differences or similarities above related to origin?
- Do these populations differ in their responses to frost (early and or late) and drought under controlled conditions?
- Are these differences or similarities related to climatic conditions?

The answer to these research questions is NO when considering German populations of both species, since there was no any consistent trend where the origin of the population had an influence.

However, when the German and Italian *Prunus spinosa* population is considered, it would be concluded that latitude had a significant influence on bud set, bud sprouting and growth rate. In addition, concerning the responses to late frost the answer would be YES since the Italian population had higher REL than German population BB. This was attributed to developmental bud stage which could be at times advantageous if the leaves are past vulnerable stage.

In the case of drought, the fruit-load on the Italian population had a significant influence on responses to drought. Therefore, the comparison may be in favour of Italian population that showed quick response to drought. Nevertheless, its characteristics of high shoot number and heavy fruit-load may cause the population to be more vulnerable to drought.

The genetic differentiation reported among the studied German populations of both species by Leinemann et al. (2013a, 2014) was never demonstrated to have any significant cascading influence on phenological, morphological, physiological and biochemical parameters. These parameters depicted great plasticity and perhaps adaptability of these populations to new environment and to stressing conditions. Hence the demarcation to six areas of origin according to Federal Nature Conservation Act could be scaled down to just a few for the northern populations of Germany up to the range (altitude and latitude) where the evaluated populations were obtained.

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Curriculum vitae

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