



## Seasonal Growth, Physiological and Biochemical Characterization of Five *Prunus spinosa* Ecotypes

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### Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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### ABSTRACT

*Prunus spinosa* species is distributed across wide range of geographical areas which are subject to climatic, edaphic factors and long-term divergent selection. This could lead to local adaptation hence ecotypes in terms of morphological, physiologically and or biochemical inclination to their local environment. To investigate whether the species (*Prunus spinosa*) has been influenced by their local environmental conditions and whether populations (ecotypes) are adapted to local conditions, cuttings from different demarcated areas of origin in Germany and Italy were sourced and cultivated optimally in common container area. Growth, bud sprout and bud set were evaluated in spring, summer and autumn respectively. Soluble sugars (Glucose, fructose, sucrose and starch), N, P, K, and proline concentrations were analysed in spring and autumn for three years. The findings indicated that plants grown from different locations mostly differed in N, P, K, soluble sugars and starch in spring. Nonetheless, these geographic variations were hardly observed either in summer or in autumn. On phenology, German populations did not differ at all in phenology (flushing and growth cessation) while the Italian population always sprouted earlier and ceased growth later. The results indicate that the German populations are not differentiated by climatic variations across latitude or altitude. In contrast the Italian population is differentiated from

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German population Brandenburg mostly by latitudinal differentiation. Nevertheless, their inherent ability to sprout earlier and late growth cessation might expose the population to frequent frost damage when transplanted to more northern latitude.

**Keywords:** *Ecotypes; growth; nutrient concentration; phenology; proline; seasons; sugars.*

## 1. INTRODUCTION

Being sessile, plants have a suit of functional traits that capacitate them to inhabit, compete and survive in wide range of environment. It could occur that some populations of a given species would adapt, through natural divergent selection pressure to apt ecological conditions [1–3]; and transferring them to other areas could compromise their survival and performance [4]. Nevertheless, high rate of gene flow counters the efficiency of such divergent selection as genetic material is exchanged among populations [5]. The exchange of genetic material (through gene flow) increases genetic variation within populations, reduces variations among populations and increases fitness against abiotic stresses [6].

In nature, phenology shift per 100 m increase in altitude has been demonstrated [7–9]. This shift has also been shown in common garden experiments for some plant species e.g., ash, oak, pine and attributed to genetic differentiation [10].

To be able to determine whether different populations of *Prunus spinosa* 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 distribution of *Prunus spinosa* shrub is wide range across many habitats [11]. Thus, like many perennial shrubs its distribution could be assumed to be determined by their capacity to survive cold extremes in the north or at high altitudes, and their ability to compete with drought adapted species from the south or of low altitudes like other plants. It is cultivated as a landscaping plant but could also be utilized for its fruits and as dwarfing rootstock for plum [12,13].

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 [14]. Its hardiness, like other shrubs and trees in the temperate, could be attributed to deciduousness [15,16] and carbohydrates reserves that supply energy for respiration and offer protection during climatic extremes [17–19]. Nevertheless, origin has been shown to affect phenology and performance of *Prunus spinosa* [20]. But according to [21], differences among populations were levelled out within three years of co-cultivation. In this article we endeavoured to assess the influence of area of origin, seasonal N, P, K, soluble sugars and proline variation in four German and one northern Italian populations, and evaluate differences in bud sprout and bud set of these five populations.

## 2. MATERIALS AND METHODS

*Prunus spinosa* cuttings were collected by [22] supported by local forest research centres which helped in identifying native ecotypes along local forest edges of ostensibly autochthonous ecotypes in Germany and Italy (ITA). From this collection, four populations associated with four German federal states were selected: Brandenburg (BB), Niedersachsen (NDS), Hessen (HES) and Rheinland-Pfalz (RPF). The populations' origin differ in soil, climate and topography. Among them, RPF and ITA is the most heterogeneous in terms of topography and climate varying in few kilometres. Brandenburg (BB) and Niedersachsen (NDS) are less heterogeneous. Thus specific climatic data from a single nearby station is not representative for the situation in RPF, and ITA rather a range is provided (Table 1) to have a comparable database. Some ecological data of the populations is presented in the Table 1 below.

In 2009, four German population's cuttings were sourced and potted. They included Brandenburg (BB), Hessen (HES), Niedersachsen (NDS) and Rheinland-Pfalz (RPF). These plants were used to evaluate bud phenology, height and shoot number. From new shoots and roots of BB, and NDS soluble sugars (Glucose, fructose, sucrose and starch) and proline were analyzed. (Table 2).

**Table 1. 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). Air temperatures and rainfall data [Italian (ITA)] are 12 years' average [2000 - 2012]. (HTTP://WWW.DWD.DE/BVBW/APPMANAGER/BVBW/DWDWWWDESKTOP?\_NFPB=TRUE&\_WINDOWLABEL=T38600134241169726338086&\_URLTYPE=ACTION&\_PAGELABEL=\_DWDWWW\_KLIMA\_UMWELT\_UEBERWACHUNG\_DEUTSCH.)**

Origin	Altitude (M ) a.s.l	latitude	longitude	Precipitation (mm)				Air Temperature (°C)			
				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

**Table 2. Overview of the populations used for seasonal characterization of *Prunus spinosa*. Abbreviations: BB = Brandenburg, NDS = Niedersachsen, HES = Hessen, RPF = Rheinland-Pfalz and ITA = Italy**

Origin/ population	Cutting year	Parameter and when (time) evaluated including replicates			
		Height <sup>x</sup> and RCD <sup>w</sup>	phenology	Dry mass	Nutrients <sup>y</sup> and Biochemical <sup>z</sup> concentration
BB, NDS, NRW, RPF	2009	autumn 2011, spring 2012, autumn 2012	spring 2013: 56(BB) 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 and RPF) autumn 2012: n = 6 (BB and NDS), 8(HES), spring 2013: n = 12 (BB), 8 (RPF)	
BB, RPF	2010	Summer and autumn 2012	autumn 2012 summer: n = 9 (BB and RPF); autumn : n = 8(BB), 6(RPF)	Summer and autumn 2012	
BB, RPF, ITA	2011	autumn 2012 spring and autumn 2013, spring 2014: n = 71 (BB), 90 (ITA),3 (RPF); autumn 2013 and spring 2014: n = 35 (BB), 54 (ITA),		Summer 2013 and spring 2014	spring and autumn 2012 summer 2013, spring 2014 spring and autumn: n =15 (BB, ITA), 6 (RPF); summer 2013 and spring 2014: n =9 (BB, ITA)

<sup>x</sup>height = height of the longest shoot; <sup>z</sup>Biochemical = glucose, fructose, sucrose, starch and proline; <sup>y</sup>Nutrients = Nitrogen, Phosphorus and Potassium (N,P,K); <sup>w</sup>RCD = Root collar diameter (mm)

In 2010 and in 2011, two German population cuttings were sourced and potted respectively. They included Brandenburg (BB) and Rheinland-Pfalz (RPF). Additionally, in 2011, one north Italian (ITA) population cuttings were sourced and potted. These plants were used to evaluate for bud phenology, growth, soluble sugars and N, P, K analyses (Table 2).

## 2.1 Bud Sprouting Scoring Scheme

In spring between the month of March- April 2012 and 2013, bud sprouting was evaluated according to the stage of development as follows (Fig. 1): Buds are dormant and brownish in colour (1); Buds are swollen and tinged with greenish colouration (2); Buds are dehiscent and leaf tips are visible (3); Leaf tips start to be separated(4); Single leaves are visible with slightly yellow to brown stipules (5); Leaves are totally unfolded and are dark green in colour (6).

## 2.2 Bud Set Scoring Scheme

In autumn bud between the month of September – November, bud set was evaluated according to the stage of senescence as follows (Fig. 2): No terminal or lateral buds is visible, new leaves are visible from apical and lateral shoots (1); terminal buds are rudimentary visible and are greenish to brown in colour (2); terminal bud are as big as the lateral buds and are coloured brown or reddish in colour (3); terminal buds are bigger than lateral buds, red-brown in colour and fringy (4) and when terminal buds have smaller adjacent brown coloured buds and hard to incise with finger nail (5).

## 2.3 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 [23] protocols with minor modifications as follows: triethanolamine buffer (14 g triethanolamine + 0.25 g MgSO<sub>4</sub> dissolved in 100 ml water, pH 7.6) and NaOH was used instead of TRIS buffer and KOH respectively).

After extraction of GFS, the remnant pellet was re-suspended in 1.5 ml NaOH (0.5 M) and incubated at 60 °C for 30 minutes. After cooling, 475 µl glacial acetic acid was added to adjust the pH for amylase action. After centrifugation at 5000 rpm for 10 minutes, 10 µl supernatant and 20 µl amyloglucosidase (4.5 mg dissolved in 2 ml citrate buffer) was placed in a microplate and incubated for 60 minutes at 30 °C. The enzyme amyloglucosidase hydrolyzed starch to glucose. Starch concentration was quantified by glucose assay and expressed on dry weight basis (µg g<sup>-1</sup> DW).

## 2.4 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 1500 rpm 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.



Fig. 1. Bud sprouting scheme used for rating bud break in spring for *Prunus spinosa*



**Fig. 2. Bud setting scheme used for rating *Prunus spinosa* in autumn**

## 2.5 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.

### 2.5.1 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 (Ammonium molybdate/ammonium vanadate mixed reagent) and pipetted into a microplate. Absorbance was read with a photometer (VERSAmax®) at 470 nm wavelengths.

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 read with a spectrophotometer (Perkin Elmer Analyst 300).

## 2.6 Statistical Analysis

Data from all variables was subjected to multivariate analysis of variance (MANOVA) to test population differences at  $p \leq 0.05$ . Data was first log transformed for normal distribution prior to analyses. Where there were significant effects ( $p \leq 0.05$ ), population means were separated by Tukey test. All statistical analyses were

performed with R 3.1.3 [24].

## 3. RESULTS

### 3.1 Bud Break and BUD Set

From the results, the 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. 3 and Fig. 4 A). The Italian population was statistically different in its phenology in that it significantly sprouted earlier (Fig. 4 B) and tended to delay their bud set (Fig. 5).

### 3.2 Growth Characterization

The German populations (cutting year 2009) did not vary (with some exception in spring 2011) in most growth parameters (Fig. 6 and 7A). However, they differed in fruit count where HES had significantly higher fruit number than the other (BB, NDS and RPF) populations. (Fig. 7B). The Italian population had significant higher number of shoots and were taller than the German BB population (Fig. 7 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.

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

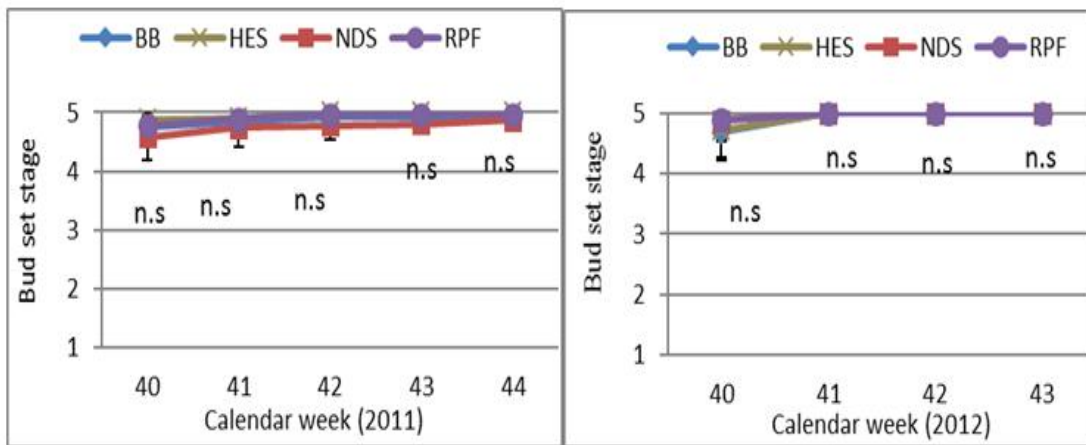
In autumn 2012, the German populations (cutting year 2009) did not differ in any of the analysed parameters in new shoots (Table 3). Contrary, they differed in roots' glucose and in leaves' fructose, sucrose and phosphorus concentration. Nevertheless, these differences were inconsistency with neither 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 3). In roots BB had higher glucose, fructose starch and potassium concentration than RPF. Rheinland-Pfalz (RPF) had higher root's 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 3). N, P, and K was also higher in spring than in autumn (Table 3).

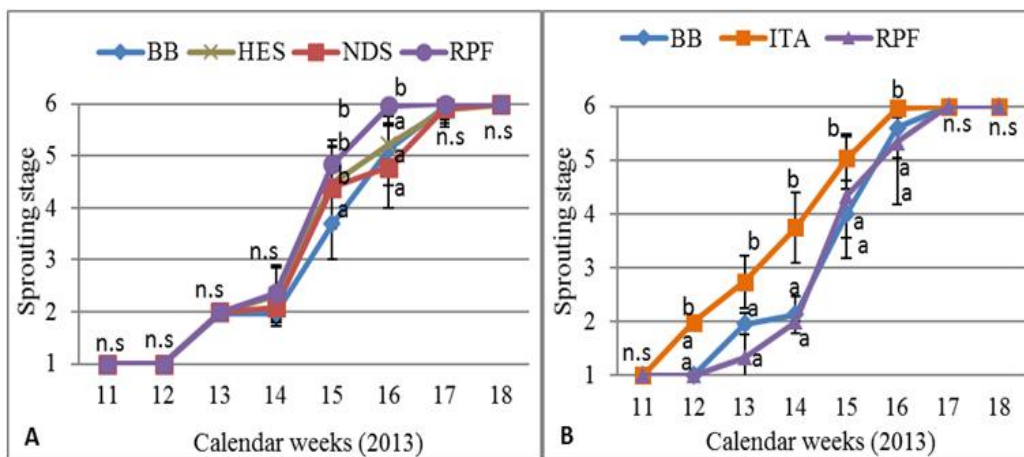
From cutting year 2010, the two German populations (BB and RPF) did not differ in most

parameter (except in autumn roots' glucose) in summer and autumn 2012 (Table 4).

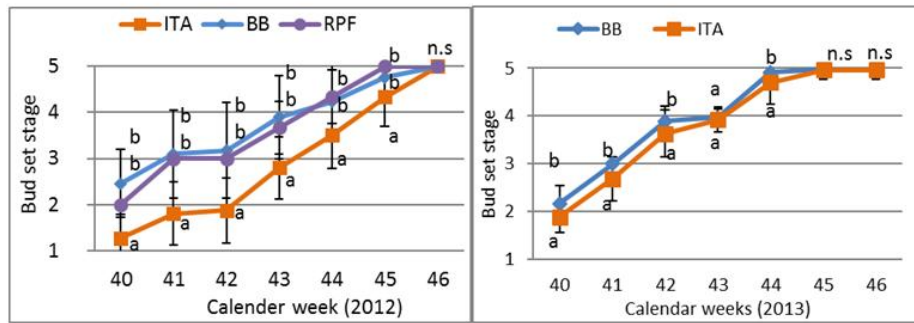
From cutting year 2011, the German populations had significant higher concentration of new shoots' sucrose (BB and RPF), roots' proline (BB and RPF) new shoots' and roots' nitrogen (RPF) and new shoots' and roots' phosphorus (RPF) than the Italian population in spring 2012 (Table 5). During this period (spring 2012), the German populations differed in roots' nitrogen and potassium and in new shoots' and roots' phosphorus (Table 5] where RPF had higher concentration than BB.



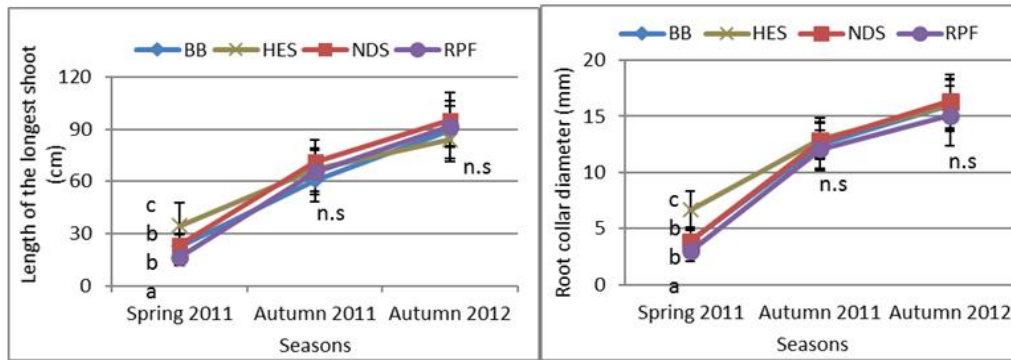
**Fig. 3. Bud setting phenology of four populations of *Prunus spinosa* in autumn 2011 and 2012**  
 Means  $\pm$  SD; n = 56 (BB), 44 (HES), 32 (NDS), 27 (RPF) from cutting year 2009. n.s indicates no significant differences among populations



**Fig. 4. Bud sprouting phenology of *Prunus spinosa* populations in spring 2013**  
 Different letters show significant differences among populations. n.s indicates no significant differences among populations. Mean  $\pm$  SD, n = 56 (BB), 44 (HES), 32 (NDS), 27 (RPF) from cutting year 2009; n = 71 (BB), 90 (ITA), 3 (RPF) from cutting year 2011

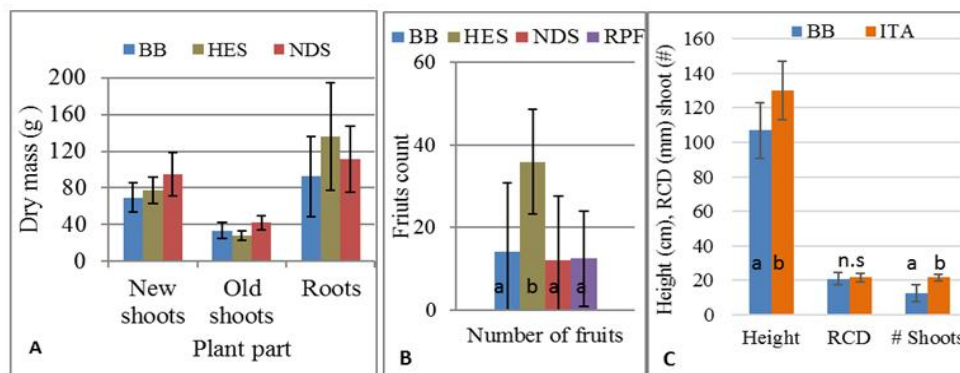


**Fig. 5. Bud setting phenology of *Prunus spinosa* populations in autumn 2012 and autumn 2013**  
 Different letters show significant differences among populations. n.s indicates no significant differences among populations. Means  $\pm$  SD; n = 36 (BB), 54 (ITA) from cutting year 2011



**Fig. 6. 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 indicates no significant differences among population. n.s indicate no significant differences among populations. Mean  $\pm$  SD, n = 56 (BB), 44 (HES), 32 (NDS), 27 (RPF) from cutting year 2009



**Fig. 7. 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 populations n.s indicates no significant differences among population. Mean  $\pm$  SD, n = 56 (BB), 44 (HES), 32 (NDS), 27 (RPF) from cutting year 2009; n = 9 (BB and ITA) from cutting 2011

In summer 2013, the Italian and the German population (BB) differed only in leaves starch concentration. But, in spring 2014, there were more differences between the Italian and the German populations (Table 5): The 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 the Italian population. Conversely, the Italian population had higher roots' fructose and potassium concentration than the German (BB) population (Table 5).

Among the German populations, only BB and RPF had plants 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. Conversely, proline concentration was affected by cutting year.

When all the data were evaluated for similarity, there was is a segregation among the German population (Fig. 8) where RPF and BB (2010 cuttings) obtained from the same region as those of 2009 especially when the leaves physiochemical characteristics are concerned. The Italian ecotype is not different as would have been imagined despite their geographical and

ecological differences to those of the German ecotypes.

#### 4. DISCUSSION

Phenology has been shown to be affected by genetics [25], latitude, longitude, latitude, altitude [26]. When we consider the German populations, there is no significant influencing factors to their phenology as these populations did not differ in their bud sprouting and bud setting phenology. Our results suggest that these populations' responses to decreasing temperature and day length is similar. This is probably due to the fact that their climate of origin is not different from each other (Table 1). The German populations from higher altitude (HES and RPF) depicts that these populations are adapted to heterogeneous environment and although they originate from cooler areas, they can adjust quickly. It demonstrates that they could be utilised in low altitude without any compromise in their bud phenology rhythm. Our result, in part, tend to contrast result of [21] 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 unrelated to origin and was highly variable (Bud set) on year to year climatic conditions.

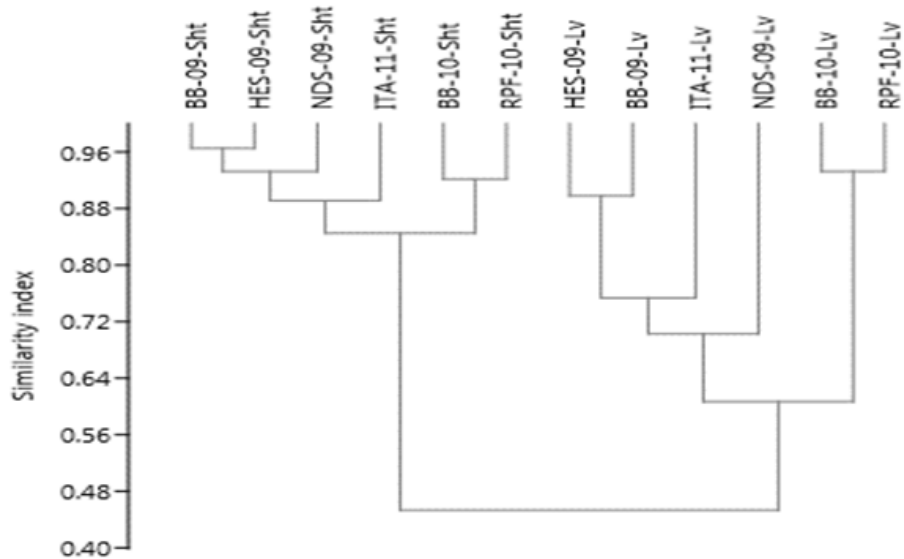


Fig. 8. Dendrogram of the UPGMA cluster analysis based of similarity index of glucose, fructose, sucrose, starch, proline N, P, K in Leaves (Lv) and shoots (Sht)



**Table 3. Glucose, fructose, sucrose, starch, nitrogen, phosphorus potassium (% dry mass) and proline ( $\mu\text{g g}^{-1}$ ) in various parts of *Prunus spinosa* populations. Different letters show significant differences among populations. Mean  $\pm$  SD; n = 6 (BB), 8 (HES), 6 (NDS) in autumn 2012; n = 12 (BB), 8 (RPF) in spring 2013 from cutting year 2009**

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

Time of evaluation and part evaluated						
		Autumn (2012)			Spring (2013)	
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. Glucose, fructose, sucrose, starch, nitrogen, phosphorus potassium (% dry mass) and proline ( $\mu\text{g g}^{-1}$ ) in various parts of *Prunus spinosa* populations. Different letters show significant differences among populations. Mean  $\pm$  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

**Table 5. Glucose, fructose, sucrose, starch, nitrogen, phosphorus potassium (% dry mass) and proline ( $\mu\text{g g}^{-1}$ ) in various parts of *Prunus spinosa* populations. Different letters show significant differences among populations. Mean  $\pm$  SD; n = 15 (BB and ITA), 6 (RPF) in spring 2012; n = 9 (BB and ITA) in summer, autumn 2012 and spring 2014 from cutting year 2011**

Parameter	Origin	Time of evaluation and part evaluated					
		Spring (2012)		Summer (2013)		Spring (2014)	
Glucose	BB	New shoots	Roots	Leaves	Roots	New shoots	Roots
	ITA	0.93 $\pm$ 0.23a	2.03 $\pm$ 1.48a	1.00 $\pm$ 0.24a	1.04 $\pm$ 0.35a	0.25 $\pm$ 0.12b	0.34 $\pm$ 0.08a
	RPF	0.93 $\pm$ 0.21a	1.42 $\pm$ 1.16a	1.00 $\pm$ 0.43a	1.04 $\pm$ 0.28a	0.12 $\pm$ 0.10a	0.43 $\pm$ 0.15a
Fructose	BB	0.89 $\pm$ 0.13a	1.74 $\pm$ 2.01a				
	ITA	0.92 $\pm$ 0.32a	0.41 $\pm$ 0.26a	0.16 $\pm$ 0.07a	0.94 $\pm$ 0.38a	1.48 $\pm$ 0.42b	0.19 $\pm$ 0.09a
	RPF	0.57 $\pm$ 0.11a	0.38 $\pm$ 0.16a	0.23 $\pm$ 0.17a	0.83 $\pm$ 0.4a	0.13 $\pm$ 0.07a	0.47 $\pm$ 0.27b
Sucrose	BB	0.73 $\pm$ 0.13a	0.29 $\pm$ 0.07a				
	ITA	1.16 $\pm$ 0.25b	0.97 $\pm$ 0.26a	2.42 $\pm$ 0.56a	0.77 $\pm$ 0.32a	0.99 $\pm$ 0.37b	1.14 $\pm$ 0.5b
	RPF	0.54 $\pm$ 0.1a	0.93 $\pm$ 0.40a	2.08 $\pm$ 0.64a	0.6 $\pm$ 0.25a	0.22 $\pm$ 0.12a	0.51 $\pm$ 0.32a
Starch	BB	1.02 $\pm$ 0.44b	1.37 $\pm$ 0.84a				
	ITA	1.01 $\pm$ 0.52a	2.12 $\pm$ 1.12a	0.55 $\pm$ 0.19b	2.53 $\pm$ 2.38	0.88 $\pm$ 0.31a	4.08 $\pm$ 2.45b
	RPF	1.00 $\pm$ 0.54a	1.42 $\pm$ 0.78a	0.26 $\pm$ 0.03a	1.16 $\pm$ 1.68	0.68 $\pm$ 0.07a	2.45 $\pm$ 1.09a
Proline	BB	1.09 $\pm$ 1.24a	2.28 $\pm$ 1.94a				
	ITA	1162 $\pm$ 618a	440 $\pm$ 173b	383 $\pm$ 217a	66 $\pm$ 47a	284 $\pm$ 108a	145 $\pm$ 40b
	RPF	930 $\pm$ 108a	285 $\pm$ 131a	203 $\pm$ 141a	38 $\pm$ 15a	234 $\pm$ 59a	103 $\pm$ 12a
Nitrogen	BB	1599 $\pm$ 665a	601 $\pm$ 227b				
	ITA	1.72 $\pm$ 0.71a	2.08 $\pm$ 0.48ab	4.21 $\pm$ 0.58a	1.07 $\pm$ 0.33a	1.39 $\pm$ 0.33b	1.73 $\pm$ 0.39b
	RPF	1.49 $\pm$ 0.26a	1.9 $\pm$ 0.38a	4.02 $\pm$ 0.58a	0.70 $\pm$ 0.15a	0.99 $\pm$ 0.14a	0.96 $\pm$ 0.17a
Phosphorus	BB	2.66 $\pm$ 0.38b	2.5 $\pm$ 0.37b				
	ITA	0.08 $\pm$ 0.02a	0.1 $\pm$ 0.02a	0.53 $\pm$ 0.11a	0.25 $\pm$ 0.06a	0.17 $\pm$ 0.04b	0.26 $\pm$ 0.06b
	RPF	0.08 $\pm$ 0.03a	0.09 $\pm$ 0.03a	0.56 $\pm$ 0.10a	0.16 $\pm$ 0.03a	0.13 $\pm$ 0.02a	0.18 $\pm$ 0.03a
Potassium	BB	0.13 $\pm$ 0.02b	0.13 $\pm$ 0.01b				
	ITA	0.63 $\pm$ 0.17a	0.73 $\pm$ 0.1a	2.76 $\pm$ 0.49a	0.32 $\pm$ 0.11a	0.33 $\pm$ 0.06a	0.33 $\pm$ 0.08a
	RPF	0.88 $\pm$ 0.19b	1.09 $\pm$ 0.21b	2.66 $\pm$ 0.49a	0.29 $\pm$ 0.13a	0.46 $\pm$ 0.10b	0.44 $\pm$ 0.09b

When we contrasted the German population BB and RPF with that of Italian population, we found significant differences in phenology (bud sprouting and bud setting). Ecologically, Italian population originates from a warmer climate and more south than the German populations (Table 1). Our 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 [25]. The tendency of southern populations to sprout early is similar to that reported for *Quercus petraea* [27] and *Fagus sylvatica* [26]. It also agrees with the results of [21] who reported earlier sprout behaviour of a Hungarian *Prunus spinosa* population. According to [4] population originating from south delays bud set while those of northern latitude will cease their growth early. If this is the case, our Italian population (originating 45° N) perfectly fit this expectation. Nevertheless, 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 [28]. 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 [29].

Sprouting in spring diminished carbohydrates and nutrient reserves as they are remobilised for growth [30]. 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 for the observed differences.

When contrasted, these two German populations (BB and RPF) from cutting year 2010 (in summer and autumn) and cutting 2011 (spring), there was a little difference 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 nutrient allocation in leaves, shoots and roots did not differ among the German populations except for a few cases where BB differed from RPF in K (Table 2 and Table 4-spring 2012/2013) and P (Table 4-spring 2012). However, the differences were never consistent with other seasons and could not therefore be attributed to ecological factors from areas of origin. The similarity in nutrients concentration among the German populations grown under the same conditions partially agrees with literature that found nutrient acquisition and allocation to various organs to be similar for oak (*Quercus variabilis*) populations grown in a similar environment [31]. Comparing nutrient concentration of our plants 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 [32].

When we contrast the German BB and Italian populations, cutting 2011, we found that nutrient allocation to various organs could be related to latitude (Table 4). According to our results, the German population BB tended to have higher roots and new shoot, N concentration than the 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 [33].

## 5. CONCLUSION

Our results show high variability within and among the 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 despite its

robust growth, 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.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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