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ECOPHYSIOLOGY OF HORSE CHESTNUT (*AESCULUS HIPPOCASTANUM* L.) IN DEGRADED AND RESTORED URBAN SITES

ABSTRACT: We explored changes in growth, phenology, net CO_2 assimilation rate, water use efficiency, secondary defense compounds, substrate and foliage nutrient concentration of a degraded urban horse chestnut (*Aesculus hippocastanum* L.) site restored for three years using mulching (tree branches including foliage) and fertilization (primarily nitrogen addition). Prior to restoration, this site was characterized by high pH (ca. 8), low foliage and substrate N, and high Na and Cl concentration. Our data indicated that in untreated plots NaCl used for road deicing is the decisive factors that may be responsible for the decrease of foliar N concentration (*via* a reduction in NO_3^- uptake), for the decrease in photosynthesis (through high concentrations of Na and Cl in the leaves) and for increased senescence of the leaves. After three years of treatment, total nitrogen concentration in substrate increased by 3- to 4-fold and calcium concentration decreased by more than 50% in relation to pretreatment levels. Treatment significantly increased seed production (from less than 12 to more than 100 seeds per tree), individual leaf mass (from 1.8 to 3.3 g/leaf), CO_2 assimilation rate (by 21 to 30 %), improved leaf C:N ratio, and increased foliage life span by as much as six weeks. The beginning of leaf fall in untreated control trees started in mid-July and those of mulched and fertilized trees in late October. Applied treatment also eliminated visible symptoms of leaf damage due to high sodium and chlorine levels, indicating the possible role of other factors in the development of necroses. After three years of treatment, pH of most degraded plots declined from 8.2 to 7.8. That decline was accompanied by an increase in foliar Zn, Cu, and Pb concentration in the mulched and fertilized plants. In addition, treatment lowered foliage phenolics making these plants potentially more vulnerable to insect herbivory. Our study indicates that stable carbon isotope discrimination is of little value as an indicator of cumulative salinity and urban environment stress in *A. hippocastanum* due to pronounced differences in leaf phenology and ontogeny. The results of our study show that street tree recovery can take as little as two to three years after application of fertilization and mulching.

KEY WORDS: horse chestnut, urban environment, CO_2 exchange, stable carbon isotopes, restoration

1. INTRODUCTION

The environments of large urban industrial agglomerations are characterized by many common features including soil and air pollution, "heat islands", and disruption of water relations and nutrient cycling (Craul 1999, Unger *et al.* 2001). In older cities, soils of urban centers (also known as a "cultural layer") consist of construction debris, municipal waste, and other materials deposited by humans (Pickett *et al*. 2001). The thickness of such substrates increases with increasing age of the settlement and can vary from 1 to more than 20 m (Schleuss *et al*. 1998, Alexandrovskaya and Alexandrovskiy 2000). Usually urban soil substrates are alkaline with intrusions of construction and household rubbish, parent material, industrial wastes, and buried pockets of more fertile soil. This substrate generates widely varying growing conditions. Moreover, all of the above mentioned features alter tree physiology, productivity, and vigor. Those factors are responsible for the creation of sites with a mosaic of environmental conditions. Therefore, due to the diverse and complex interactions between the urban environment and plants, studies in this environment have never been easy or attractive for ecological and applied studies.

One of the most popular tree species planted in urban environments is horse chestnut (*Aesculus hippocastanum* L.). This species is native to the mountains of northern Greece, Albania, and Bulgaria and was brought from Constantinople to Vienna in 1576 by Clusius, then the director of the Holy Roman emperor's garden (Krüssman 1984). From there *A. hippocastanum* was distributed over all of western and central Europe. Horse chestnut was introduced to city plantings in the 17th century and is widely cultivated as a large shade and street tree. In 1633 John Gerard provided the following description of this tree species "*The Horse Chestnut growth likewise to be a very great tree, spreading his great and large armes or branches far abroad, by which meanes it maketh a very good coole shadow*" (Gerard 1633).

There are conflicting reports regarding *A. hippocastanum* tolerance to present-day urban conditions. For example, most U.S. references consider this species to be resistant to salinity, which is considered one of the most significant factors influencing tree growth in urban environments (Johnson and Sucoff 1999), whereas many European scientists regard this species as highly sensitive (Braun *et al*. 1978, Suchara 1982).

The main aim of our study was to identify how substrate structure and chemistry affects foliage morphology, physiology, and seed production of horse chestnut in the urban and industrial agglomeration of Poznań in Western Poland. In addition, we investigated to what extent the adverse substrate effect on horse chestnut can be alleviated by improvement of substrate trophic conditions. In this study we explored the effects of key limiting factors for tree biology in urban environments including substrate pH, Ca, and Cl concentration and their effects on tree physiology and phenology. Therefore, it is likely that these results are indicative for physiological responses of *Aesculus hippocastanum trees*, and perhaps other similar tree species, in urban areas.

In recent years, rapid spread of a newly described species of the horse chestnut leaf miner *(Cameraria ohridella* Deschka & Dimic, Lepidoptera: Gracillaridae) was noted in many parts of Europe, including Poland. Due to the fast colonization rate and population density growth with up to five generations per year of *C. ohridella* and up to several hundred mines per leaf, this species is causing severe damage to horse chestnut trees in urban areas (Skuhravy 1999). Therefore, in our study we also tested whether nutritional changes in horse chestnut foliage will affect its defense traits against insects.

2. MATERIAL AND METHODS

2.1. Site and plant description

The study was conducted in the city of Poznań, Poland (51° 40' N, 22° 19' E, 158 m altitude). Poznań was established prior to the 10th century and currently has a population of 589,000 people. Besides a long settlement history, Poznań has various industrial plants. Among them, 13 are considered to be especially harmful to the environment (Krysiak *et al*. 2000), nine of which have no gaseous pollution reduction equipment. Total emission from stationary emitters in 1994 averaged more than 26800 t yr^{-1} of SO_2 and 7200 t yr^{-1} NO_2. In the mid 1990s, the estimated atmospheric SO_2 concentration in winter months was 80 μg m^{-3}, ranging in different locations from 44 to 140 μg m^{-3} (Pikhart *et al*. 2001).

The mean annual precipitation of Poznań is 522 mm with extremes of 768 mm maximum (1967) and minimums of 275 (1982) and 356 mm (1983). In addition, up to 85% of the surface water is exported from the city by storm drains (Schleuss *et al.* 1998) thereby exacerbating water deficit in drought years. Catastrophic droughts in 1982, 1983, and 1992 caused massive tree decline in the city center (S. Łukasiewicz, personal observation). In general, all sites within the Poznań city center are considered highly xeric. The mean monthly temperature of Poznań is 25.0 ˚C for the warmest month and –6.1 ˚C for the coolest month (with mean daily extremes 37.2 ˚C 1983, –30.2 ˚C 1984–1985). The soil cultural layer varies between 2 and 10 m in depth (Karolczak 1993).

The study site was established at one of main streets i.e. Aleja Wielkopolska Street in a 10 m wide median between two opposing lanes of traffic with an electric streetcar track line in the northern site. This site was part of the old Warta River valley that consists of clay and silt soil layers. The local terrain is sloping to the Warta River, but Aleja Wielkopolska Street is flat suggesting that grading and heavy soil disturbance occurred when the street was established. Aleja Wielkopolska Street was established at the turn of the 19 and 20th century. The first plantings of horse chestnut were established at that time.

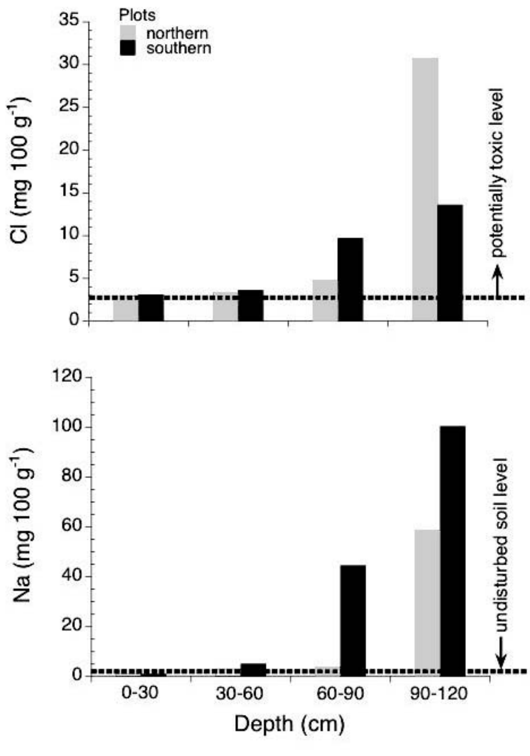

Fig. 1. Differences in substrate concentration of Cl and Na in an *Aesculus hippocastanum* site in city of Poznań, Poland in relation to distance from street. Trees at the south plot were planted 0.5 to 1 m and those at the north plot, planted 4 to 5 m from the street. Potentially toxic Cl level after Marschner (1995), and undisturbed soil level after Nowosielski (1974, 1988).

A permanent experimental area (90 m long by 10 m wide) with 50 to 70 year old *Aesculus hippocastanum* trees was established in 1996. At the same year, tree phenology and soil analysis observations were initiated. The site was divided into two replicated blocks each with four plots (two northern and southern). In two plots of each block mulch plus fertilizer and control treatments were applied. The treatment was designed to elevate the severe nitrogen deficit in the soil and mulching was designed to retain soil moisture (annual precipitation in Poznań in dry years could be < 400 mm) and provide additional necessary nutrients (see below). Division into south and north plots was necessary because trees on the south plots of the median were planted at a distance of 0.5 to 1 m from the street and therefore are more exposed to higher concentrations of NaCl used for road deicing than those at the north site, planted 4 to 5 m from the street. These differences in distance from the street resulted in a significantly higher accumulation of Cl and especially Na in substrate (Fig. 1). Trees on the southern side of the street are also exposed to higher insolation compared to those on the northern side of the street. The site is likely to be extremely unfavorable for tree growth due to a substrate of rubbish with unfavorable chemical composition and high pH (Table 1).

On each plot six trees were present with a mean height of 13.6 ± 3.4 m (SD), a mean diameter of 0.53 m ± 0.11 (SD), and a mean tree crown diameter of 5.5 ± 1.4 m (SD). Due to poor soil conditions in the plot there was only sparse grass cover and frequent tree decline over the past 20 years.

The mulch consisted of chipped coniferous and deciduous tree branches with foliage that was stored for one year prior to application. Average mulch nutrient concentration was as follow: N – 0.91% of dry mass, P – 0.11%, K – 0.22%, Ca – 1.53%, Mg – 0.15%, S – 0.09%, Fe –201 ppm, Mn – 45 ppm, Zn – 103 ppm, and Cu – 75 ppm. The mulch was applied to an average depth of 8 cm (from 5 to 10 cm).

During the first two years of treatment (1999 and 2000), NH_4-NO_3 fertilizer was applied monthly between 15 April and 15 September at a rate of 17 g N m^{-2} $month^{-1}$. In 2001, due to a significant improvement of substrate N concentration and in order to elevate low S concentration of the upper soil layer we applied $(NH_4)_2SO_4$ monthly between 15 April and 15 September at a rate of 7 g N and 16 g S m^{-2} $month^{-1}$.

2.2. Soil and foliar chemical analysis

Soil chemical analyses (macro- and micronutrients and pH) were conducted in 1997 and 2001 from, depth of 0–30 cm where most of fine roots of *A. hippocastanum* are distributed (A. Łukasiewicz, personal observations). Before onset of the experiment, in 1997, additional soil samples were collected from four depths (0–30, 30–60, 60–90, and 90–120 cm) for Cl and Na analyses, soil chemical analyses of soluble nutrients were conducted in Lindsey solution in 0.03 N CH_3COOH according to the protocol described by Nowosielski (1974, 1988) at the Department of Crop Fertilization of the Agricultural University in Poznań. Soil structural properties were conducted according to methods described by Mocek *et al.* (1997).

Foliar chemical analysis was determined on sunlit foliage sampled in August 1997, 2000, and 2001. Each treatment was represented by two equally-weighted composite samples containing foliage from four trees. Total foliar nitrogen concentration was determined in 1997 and 2000 on ground dried leaves (65 °C) digested by the micro-Kjehldal method and processed using a BÜCHI distillation unit B-323 (BÜCHI Analytical, Inc., Switzerland), and in 2001 was determined using ground dried leaves (65 °C) on a Carlo-Erba C, H, and N Analyzer Series NA1500 (Milan, Italy).

Analyses of foliar concentrations of P, K, Ca, Mg, Mn, Fe, Cu, Zn, B, Pb, Cd, Na, and Cl were conducted simultaneously with an Inductively Coupled Plasma Emission Spectrometer (ICP-AES, model ARL 3560) at the University of Minnesota Research Analytical Laboratory, St. Paul, MN, USA. The standard dry ashing method of sample preparation for the ICP analysis used in this study may not give complete recovery of Fe. However, it should not affect the relative differences in concentration of Fe in different years. Total S concentration was determined by infrared absorption of evolved SO_2 by dry combustion on a LECO Sulfur Determinator Model No. SC-132.

Levels of soil and foliar nutrients were compared to data from an undisturbed site at the Adam Mickiewicz University Botanical Garden in Poznań located 2.3 km from the site. Foliage at the Botanical garden was sampled in August 1997. Trees in the Botanical garden were not watered or fertilized and of the same age as those in the experiment.

2.3. Analysis of foliar carbon isotope, nitrogen and phenols

The carbon isotope ratio ($^{13}C/^{12}C$) was determined for homogeneous foliage samples and was conducted using the same methodology as Kloeppel *et al.* (1998). All data were reported relative to PDB, a standard limestone fossil of *Belemnitella americana* from the Cretaceous Pee Dee formation in South Carolina, USA. Analyses for ^{13}C, total C, and total N were conducted on subsamples (24 trees, 1 leaf per tree) of leaves used for the CO_2 assimilation measurements described below. Results for total N were calibrated against a wide range (0.70 to 2.94 % total N) of plant tissue standards (National Bureau of Standards, Gaithersburg, MD, USA) that were digested to insure that the sample digestion was complete. The objective of the carbon isotope analysis was to explore the effects of treatment on long-term water use efficiency of *A. hippocastanum*.

The content of total soluble phenols (TPh) was determined in a 0.25 g sample of leaf dry mass after a boiling extraction for 15 minutes in 10 cm^{-3} 95% ethanol and 10 minutes in 10 cm^{-3} boiling 80% ethanol. The analyses were performed using the spectrophotometric method described by Singelton and Rossi (1965) using the Folin-Ciocalteu phenol reagent. The leaves were sampled after the first, second, and third season of treatment in August 1999, 2000, and 2001. The concentration of total phenols in *A. hippocastanum* was determined on leaves collected from 1–3 branches per tree × 3 to 4 trees × 2 treatments × 2 plot directions (north and south). The content of total phenols was expressed in μmol of chlorogenic acid per g of dry mass.

2.4. CO_2 assimilation and foliar morphology measurements

A calibrated open infrared gas analyzer system (Model LCA-4, Analytical Development System, Herts, England) was used to measure foliar CO_2 assimilation. Light-saturated net photosynthesis (A) was measured on fully-expanded undetached leaves from sun shoots under field conditions using the Parkinson leaf chamber (PLC-B). Assimilation was calculated on a dry-mass (A_{mass}) or area basis (A_{area}). All CO_2 measurements were taken under clear sky conditions in August 2000 under saturating natural light conditions. During measurements, air temperature averaged 28°C, and relative humidity 61%. Gas exchange was measured on 24 trees (two leaves per tree) of *A. hippocastanum* including six trees for each treatment (control *vs*. mulched and fertilized) and plot (north *vs*. south) combination. After measurement, foliar tissue was refrigerated until leaf area was measured (within 48 hours) and was dried (65 ˚C) to a constant mass and weighed. Projected leaf area was determined using an image analysis system and the WinFOLIA Pro Software (Regent Instruments, Quebec, Canada). Specific leaf area (SLA, defined as projected leaf area divided by leaf mass) was calculated for the same leaves used for gas exchange analysis.

The objectives of the net photosynthesis measurements were to: 1) examine the maximum instantaneous assimilation rates of *A. hippocastanum* and 2) to compare the effects of treatment, foliar structure, and chemistry on CO_2 assimilation of *A. hippocastanum*.

2.5. Phenological observations and statistical analyses

Phenological observations were conducted based on methods described by Łukasiewicz (1984). A total of four vegetative (leaf budding, green foliation, autumn coloration, and leaf fall) and five generative (flower budding, flowering, seed maturation, mature seed, and seed fall) stages were observed. Observations were conducted every 4 to 7 days. Seed production was calculated by counting green husks on all trees. Values are presented in relation to the reference site at the Botanical Garden of the Adam Mickiewicz University in Poznań.

For all variables, differences among treatments were calculated using analysis of variance (GLM procedure). For presentation, correlation analyses were used, but we do not assume that direct causal relationships are involved. All statistical analyses were conducted using JMP software (version 3.1.5, SAS Institute, Cary, NC).

3. RESULTS

3.1. Substrate and foliar chemistry

Substrate pH before the onset of treatment was basic ranging from 7.7 in northern to 8.0 in southern plots (Table 1). In all plots total nitrogen (N_{TOT}, 0.4 to 0.04 mg 100 g^{-1}) and phosphorus (1.1 to 1.4 mg 100 g^{-1}) concentrations in the topsoil were low. Only potassium concentration (6.4 to 10.4 mg 100 g^{-1}) was comparable with undisturbed soil (Table 1). Calcium concentration was ≈750 mg 100 g^{-1} and exceeded by more than 10-fold those values found in natural conditions. Both sodium and chloride concentration increased with depth in the substrate profile reaching the highest level in the 90 – 120 cm depth with values of 58 to 108 and 14 to 31 mg 100 g^{-1}, respectively (Fig. 1). All micronutrients (Fe, Mn, Zn, Cu, and B) were in the range of values common to natural conditions.

Three years after treatment substrate pH decreased by up to 0.4 units. Total nitrogen concentration increased by 3- to 4-fold and calcium concentration decreased by more than 50% in relation to pretreatment levels. Available S-SO_4 concentration increased from trace amounts before treatment to 0.9 mg 100 g^{-1} after three years of treatment (Table 1). Plots location has significant effect on substrate pH, P, Mg, Fe, Zn, Cu, Pb

Table 1. Nutrient concentration at substrate depth of 0–30 cm, pH (water), and conductivity in substrate before (1997) and three years (2001) after treatment in the *Aesculus hippocastanum* site in Poznan, Poland. All nutrient values are in mg 100 g^{-1} of soil. Electrical conductivity (EC) values are in μS cm^{-1}. 0 – concentration lower that detection limit.

Time after treatment	Plots orientation	Mul–ching	pH	N–NH_4	N–NO_3	N_{TOT}	P	K	Ca	Mg	Na	Cl	S–SO_4	Fe	Mn	Zn	Cu	B	Pb	Cd	EC
before	northern	no	7.7	0.7	0.7	1.4	1.1	10.4	746	12.7	1.1	2.9	0.0	5.2	1.3	1.7	0.77	0.03	1.33	0.05	217
	southern	no	8.0	0	0.4	0.4	1.4	6.4	747	8.8	1.0	3.1	0.0	4.2	1.4	1.3	0.48	0	1.88	0.04	154
3 years	northern	no	7.9	0	0.17	0.17	1.3	6.5	502	15.0	2.5	0.8	0.9	7.1	1.4	1.9	1.04	0.12	1.29	0.01	197
	northern	yes	7.8	0.1	5.0	5.1	1.1	14.7	483	21.2	2.4	0.7	0.9	6.5	1.4	1.8	1.03	0.12	1.20	0.02	398
	southern	no	8.2	0	0	0	1.1	6.6	505	10.6	2.5	0.7	0.7	5.5	1.5	1.0	0.50	0.08	0.91	0.002	157
	southern	yes	7.8	0.28	3.8	4.1	2.2	11.7	460	15.0	3.4	0.7	0.9	3.4	1.4	1.0	0.55	0.11	0.81	0.01	365
ANOVA	plots (P)		0.01	0.39	0.59	0.63	0.005	0.53	0.11	0.0007	0.20	0.86	0.69	0.0001	0.62	0.001	0.0001	0.30	0.002	0.003	0.33
effects	mulching (M)		0.0001	0.08	0.005	0.004	0.01	0.01	0.0002	0.0007	0.31	0.71	0.38	0.005	0.78	0.84	0.76	0.66	0.37	0.0001	0.0001
P > F	S × M		0.01	0.39	0.68	0.73	0.005	0.52	0.06	0.47	0.25	0.44	0.48	0.30	0.93	0.67	0.65	0.63	0.95	0.93	0.93
Reference, Botanical Garden, ±SE			7.05 ±0.15	1.05 ±0.35	1.15 ±0.35	2.2 ±0.70	3.6 ±0.70	9.1 ±1.3	46.5 ±0.4	5.9 ±0.9	0.45 ±0.15	2.0 ±0.1	1.0 ±0.0	7.7 ±0.02	4.5 ±0.3	3.1 ±0.3	0.44 ±0.175	0.02 ±0.0	1.04 ±0.27	0.025 ±0.005	176 ±13.5

Table 2. Foliar nutrient concentration before (1997) and three years (2001) after mulching and fertilization in the *Aesculus hippocastanum* site in Poznan, Poland. Fully developed leaves were sampled from the sunlit portion of the tree crowns in August.

Time after treatment	Plot orientation	Mul-ching	N	P	K	Ca	Mg	S	Na	*Cl*	B	Fe	Mn	Zn	Cu	Pb	Cd
			%						ppm								
before	northern	no	1.70	0.23	2.45	2.08	0.27	0.14	650	6660	51.3	200	19.5	13.2	9.8	5.5	0.3
	southern	no	1.68	0.19	1.83	1.87	0.26	0.15	59	6120	48.7	325	21.0	20.7	26.7	10.9	0.3
3 years	northern	no	1.59	0.178	0.729	1.899	0.233	0.10	75	2975	61.1	157	12.7	12.7	6.1	5.5	1.9
	northern	yes	2.78	0.175	0.781	2.168	0.239	0.16	34	2400	28.1	191	33.9	17.9	8.5	6.2	1.9
	southern	no	1.54	0.238	1.394	1.694	0.174	0.09	256	5000	53.7	155	14.5	11.4	5.8	5.6	1.7
	southern	yes	2.56	0.168	1.084	2.647	0.289	0.17	483	4750	34.7	179	39.6	21.6	7.7	7.7	2.3
ANOVA effects P > F	plot (P)		0.31	0.11	0.0005	0.64	0.55	1.0	0.02	0.0002	0.93	0.53	0.47	0.76	0.46	0.35	0.66
	mulching (M)		<0.0001	0.03	0.23	0.056	0.07	0.0003	0.45	0.33	<0.0001	0.03	0.0007	0.06	0.01	0.09	0.22
	P × M		0.53	0.04	0.10	0.26	0.10	0.46	0.28	0.70	0.11	0.70	0.71	0.52	0.71	0.37	0.28
Botanical Garden*			2.20	0.16	0.92	1.68	0.18	0.17	26	2100	39.1	115	90.5	13.2	5.9	2.5	0.31

*Adam Mickiewicz University Botanical Garden, undisturbed soil, distance 2.3 km from the study site (Aleja Wielkopolska Street)

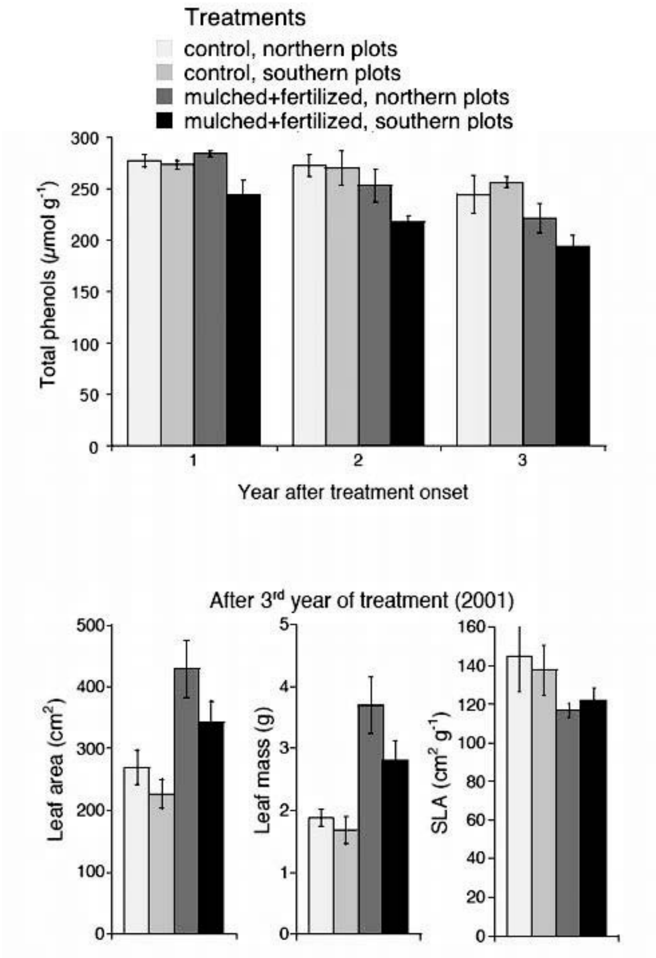

Fig. 2. *Upper panel*: Leaf total phenol concentration after one, two, and three years of mulching and fertilization treatment of horse chestnut (*Aesculus hippocastanum*) trees grown in a degraded street site in city of Poznań, Poland. *Lower panel*: Leaf area, leaf mass, and specific leaf area (SLA) after three years of treatment. For all traits leaves were sampled in August. Error bars indicate ± standard error of the mean.

and Cd. Mulching and fertilisation affected all soil traits except Na, Cl, S-SO_4, Mn, Zn, Cu, B and Pb (Table 1).

Foliar nutrient analyses before and after the initiation of the mulching plus fertilization treatment presented in Table 2 indicate that after three years of treatment average foliar N concentration increased by 36 % from 1.7 to 2.7 %, reaching or in fact exceeding values comparable with those for *A. hippocastanum* foliage at a relatively undisturbed site at the Botanical Garden. There were no plot location effects on foliar-N%. Despite slightly deficient substrate P concentrations, both untreated control and mulched and fertilized plants have foliar P concentrations comparable with those in the Botanical Garden (Table 2).

There was a highly significant effect of plot location on K concentration with trees in southern plots having 65% higher foliar K concentration than those in northern plots (1.2 *vs*. 0.75% K, respectively). Foliar Ca concentration in treated plants was 33 % higher than in control (2.4 *vs*. 1.8% Ca, respectively, Table 2).

Foliage in southern plots exhibited significantly higher concentration of Cl and Na and there were no differences between treatments or interaction between plot location and treatments (Table 2). On average, foliar Cl concentration in southern plots was 81% higher than those in northern plots (0.49 *vs*. 0.27% Cl, respectively). Overall, Cl concentration in southern plots was more than two-fold higher in comparison with data from the Botanical Garden. Similarly, foliar sodium concentration was significantly higher in southern than northern plots (369 *vs*. 54 ppm Na, respectively). Treatment significantly increased S, Fe, Mn, and Cu, and decreased B concentrations in *A. hippocastanum* foliage (Table 2).

Total foliage phenolic concentration in treated trees was lower than those in control in all years of observation, decreasing slightly (by 11%, but not statistically significant) after the first year, and by 36% after the second year (P = 0.01), and 43% after the third year (P = 0.006, Fig. 2). The greatest difference in phenolic concentration was recorded in southern plots, where average concentration reached 256 μmol g^{-1} in leaves of control plants and 193 μmol g^{-1} in treated plants (Fig. 2). Overall, plot differences and the treatment × plot interaction were statistically significant ($P \leq 0.05$).

3.2. Foliar gas exchange and stable carbon isotopes

Two years after treatment, both A_{area} and A_{mass} increased significantly by *ca*. 21 to 30 % when comparing mulched and fertilized to control sites (Table 3). Location as well as the interaction between plot location (north vs. south plot) and treatment were not significant ($P \geq 0.10$). Overall, there was a significant correlation between SLA and A_{area} (r^2 = 0.18, $P = 0.003$).

Significant differences were observed between sites and plots in foliar stable carbon isotope composition two and three years after treatment (Table 3). There were also differences between two years of observation. In 2000, average $\delta^{13}C/^{12}C$ was –27.6 and in 2001 was –28.4 (Table 3). Two years after treatment (2000), $\delta^{13}C/^{12}C$ on the mulched plus fertilized site was –26.9 whereas on the control site it was –28.3. These differences further increased after the third year of treatment (2001) and $\delta^{13}C/^{12}C$ reached –27.5 on the mulched plus fertilized site and –29.3 on the control site. In both years of observation, statistically significant differences between northern and southern plots were observed, with plants in southern plots having higher (less negative) values of $\delta^{13}C/^{12}C$ (Table 3). Especially pronounced differences in $\delta^{13}C/^{12}C$ were observed in treated and control plants in southern plots, when the average value of mulched plus fertilized trees was –26.8 and control trees was –28.9.

3.3. Foliar structural properties and tree phenology

Three years after treatment, individual leaf mass of treated trees increased by 82% in comparison with those for untreated trees (3.3 vs. 1.8 g $leaf^{-1}$, respectively; P = 0.0004). There was a marginally significant (P = 0.1) plot effect with leaves from northern plots having 20% heavier foliage, and no plot × treatment interaction (Fig. 2). Similarly, individual leaf area of treated plants was 56 % higher than those of control plants (386 vs. 248 cm^2 $leaf^{-1}$, respectively; P = 0.002); a marginally significant (P = 0.08) plot effect was observed (Fig. 2). On average, specific leaf area (SLA) of treated plants was 15% lower than those for control plants (119 vs. 141 cm^2 g^{-1}, respectively; P = 0.09). No significant differences were found for plot effect or for the interaction of treatment × plot ($P \geq 0.60$).

On average, green leaves remained on trees for 130 days in control and for 175 days in mulched and fertilized trees (Fig. 3). The beginning of leaf fall in untreated control trees started 100 days earlier (in mid-July) than those of mulched and fertilized trees (end of October). On October 7, all control trees were leafless, whereas on treated trees foliage remained until November 16, 2000 (Fig. 3). In all years preceding the treatment, trees produced less than twelve seeds per tree. During the same year when treatment was initiated (1999), the treated trees developed more than 100 seeds per tree before any other visible changes in foliage condition took place. However, the level of seed production in treated trees was only half of that observed in trees of comparable age growing in undisturbed soil at the Botanical Garden (Table 3).

Table 3. Leaf traits, seed production and ANOVA effects of Aesculus hippocastanum in sites treated and untreated with mulching and fertilizer in Poznan, Poland. Leaf N, C and $^{13}C/^{12}C$ PDB were sampled after second (2000) and third (2001) year of treatment, photosynthesis (Aarea and Amass) and seed production in 2000.

Plot orientation	Mulching	Aarea (mmol m^{-2} s^{-1})	Amass (nmol g^{-1} s^{-1})	Leaf N (%)		Leaf C (%)		Leaf C:N ratio		$^{13}C/^{12}C$ PDB (o/oo)		Seed production* (%)
				2000	2001	2000	2001	2000	2001	2000	2001	
northern	no	5.0	57	1.45	1.59	47.5	48.5	33.6	31.4	–29.0	–29.8	25
northern	yes	6.9	83	3.24	2.78	48.1	48.6	15.4	17.6	–27.2	–28.2	69
southern	no	5.9	78	1.58	1.54	47.4	48.6	30.0	31.7	–27.7	–28.9	11
southern yes		7.4	81	2.65	2.56	48.2	47.0	18.3	18.6	–26.4	–26.8	50
ANOVA effects P>F												
plot (P)		0.26	0.16	0.20	0.31	0.98	0.21	0.78	0.72	<0.0001	<0.0001	0.14
mulching (M)		0.007	0.04	<0.0001	<0.0001	0.04	0.21	<0.0001	<0.0001	<0.0001	0.0027	0.0008
P x M		0.76	0.10	0.05	0.53	0.79	0.16	0.03	0.87	0.20	0.52	0.82

*calculated in relation to the reference site (Botanical Garden of the Adam Mickiewicz University)

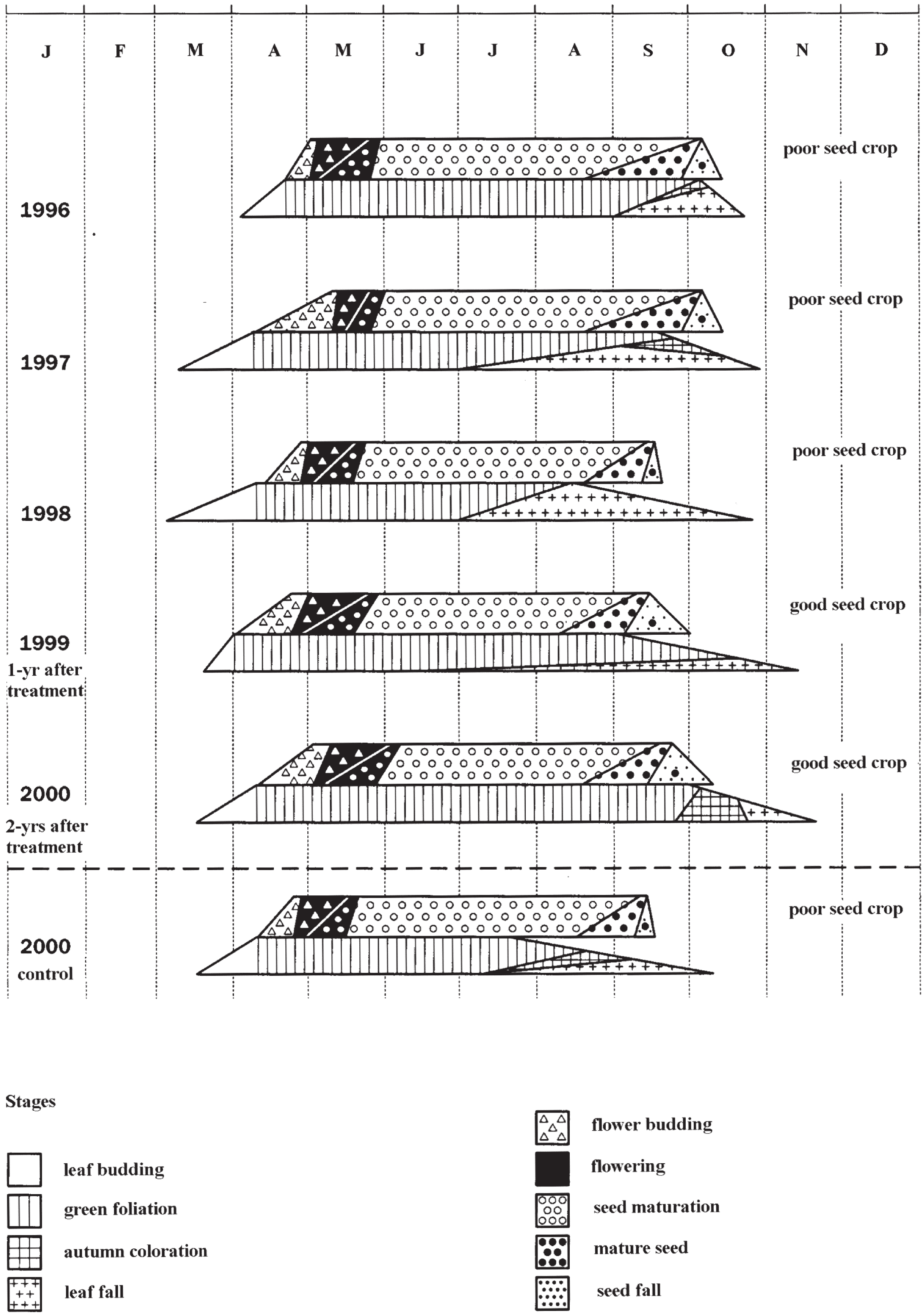

Fig. 3. Phenological diagrams showing foliage and generative stages of horse chestnut (*Aesculus hippocastanum*) trees grown in a degraded street site in city of Poznań, Poland prior to and after restoration treatment. See text for more explanation.

4. DISCUSSION

4.1. Substrate environment

Chemical analyses of substrate preceding the onset of treatment has shown potentially toxic levels of both Cl^- and Na^+ ions and very low concentrations of N and P and high concentration of Ca (Table 1, Fig. 1). The major source of high Na and Cl concentration in urban substrates is road-deicing treatments. In the study site, up to a seven-fold higher than safe level for sensitive plant species (*ca.* 3 mg 100 g^{-1} substrate) was observed in the lower substrate layer indicating potential toxic levels for *A. hippocastanum* (Fig. 1). Elevated soil Na and Cl concentration are among the major factors responsible for poor vitality of horse chestnut in urban environments (Fuhrer and Erismann 1980). Its alleviation should be considered a prerequisite for restoration of urban soil and vegetation. Results of investigations on health conditions of *A. hippocastanum* and other street trees after discontinued use of de-icing salts on streets in Berlin indicated that discontinuation of the use of NaCl in less than six years resulted in revitalization of even seriously damaged street trees (Leh 1990).

Observed pH values of untreated plots (7.9 to 8.2) are significantly higher than optimal values (6.6 to 7.2) recommended for *A. hippocastanum* (Puchalski and Prusinkiewicz 1975). Basic substrate pH can lead to lower nutrient availability for plants, depression of microbial activity, and micorrhizal decline (Theodorou and Bowen 1969, Stroganova *et al.* 1998). High soil pH is often observed in urban environments and is considered as one of the leading factors responsible for constraints to growth and poor tree health (Fostad and Pedersen 1997, Stroganova *et al.* 1998). High pH is a result of anthropogenic soil and air input of alkalizing elements, including calcium, potassium, and magnesium (Tagaki *et al.* 1997). The main source of calcium is mortar from demolished building materials and coal ash from household heating and industrial sources.

In urban environments, basic pH may also have a positive effect by immobilizing the potential threat of trace metal toxicity (Ge *et al.* 2000). Our data indicate increases in foliar Zn, Cu, and Pb concentration in the mulched and fertilized plants when substrate pH decreased (Table 2). Current foliar concentrations of heavy metals are in the range observed in control site. However, further investigation is needed to determine if increased availability of heavy metals due to decreased soil pH will be detrimental. It is especially crucial in urban areas since the amount of bioavailable trace metals is low in comparison to those in stable forms (Ge *et al.* 2000).

Pretreatment substrate was deficient in total nitrogen for plant growth, significantly limiting CO_2 assimilation rate of *A. hippocastanum* (Table 3). Average nitrogen concentration in normal arable soils is three to five time higher than those observed at the study site (*ca.* 1 mg 100 g^{-1} in our site *vs.* 3 to 5 mg 100 g^{-1} in rural environments (Breś *et al.* 1997, Nowosielski 1988). In addition, the relatively high amount of $N\text{-}NO_3^-$ *vs.* $N\text{-}NH_4^+$ is unfavorable for plant uptake in study sites due to the substrate high basic pH since $N\text{-}NO_3^-$ uptake is optimal at low pH (Barber 1984). It is also likely that nitrate uptake by *A. hippocastanum* in our site is impaired by high sodium levels (Lorenzo *et al.* 2000), especially in the southern plots.

Available phosphorus concentration is below the concentration for undisturbed soils (Table 1). In addition, basic pH may adversely affect the ability of plants to uptake phosphorus. High calcium concentration binds phosphorus and converts it to unavailable compounds. Jim (1998) identified that the low phosphorus concentration in urban substrates may be due to alkaline conditions related to the release of carbonate from calcareous construction waste. In general, urban substrates differ widely in phosphorus concentrations. Some authors reported significantly elevated levels of phosphorus (Alexandrovskaya and Alexandrovskiy 2000, Birke and Rauch 2000, Zhang *et al.* 2001), whereas others reported deficits (Hiller 2000). Those differences originate most likely from parent material, prior site use, and other factors.

4.2. Foliar nutrition, physiology, and phenology

Mulching and fertilization significantly improved foliage nutritional status including nitrogen concentrations that reached values found at a relatively undisturbed site at the Botanical Garden in Poznań (Table 2). Along with the improvement of foliage N and S supplied with fertilization, the concentrations of several other elements such as Ca, Mg, Fe, Mn, Zn, and Cu increased after treatment. That increase was most likely a result of increased root density due to elimination of the grass competition, additional medium for root growth, and improvement of soil moisture in the substrate surface layers. Within three years of treatment, mulch was almost entirely mineralized providing trees with other elements.

Calcium concentration in control, treated, and reference foliage (Botanical Garden) was more than two times higher than that observed in *A. hippocastanum* in forest conditions (Guha and Mitchell 1966). This is the result of both basic substrate pH as well as the high rates of dry deposition of basic elements in an urban environment. Control trees exhibited a 34% lower calcium concentration in comparison with mulched and fertilized trees. This is most likely a result of differences in foliage lifespan among treatments. As evidence from phenological observations, trees in control plots started to senesce and developed necroses in July, more than two months earlier than those in the treated site (Fig. 3). The ions of alkaline earths, especially Ca, accumulate steadily in the leaves at the end of translocation routes (Larcher 1995). Therefore, under comparable calcium levels in substrate the concentration of Ca is directly related to the age of leaves (Oleksyn *et al.* 2000, 2002).

Southern plots compared to northern plots exhibited a 60% higher concentration of potassium. The southern plots are also exposed to higher insolation and substrate NaCl level compared with those on the northern plots (Fig. 1, Table 2) and high potassium concentration is most likely a response to long-term high evaporation demands. Since K+ play a key role in stomatal regulation of the osmotic potential in the vacuoles and contributes to water adsorption at the cell and whole plant level (Hsiao and Läuchli 1986), the increased leaf K+ concentration may lead to a better hydration of foliage which may improve water balance of trees. At the same time, a two to 10 fold lower leaf K/Na ratio in southern than northern sites, may indicate potentially higher risk of salinity-induced growth reduction (Asch *et al.* 2000).

Foliar Cl concentration was 2 to 3 times higher in the study site than in the reference site (Botanical Garden) reflecting the differences in substrate chlorine concentration related to the application of NaCl as a street deicer. In 1997, foliar Cl concentration exceeded by as much as 43 % the toxic level of 3.5 mg g^{-1} for sensitive trees and herbs (Marschner 1995, Breś *et al.* 1997). However, our observations were lower than the 9 to 14 mg Cl g^{-1} dry mass associated with 25% leaf necroses of *A. hippocastanum* trees grown along the streets of Berne, Switzerland (Fuhrer and Erismann 1980). Development of salinity necroses and premature foliar loss (Fig. 3) in control plants at chlorine concentration of 3 to 5 mg g^{-1} indicates that the susceptibility of horse chestnut to Cl is likely also related to local edaphic and/or microclimatic factors. These data explain apparent differences in opinion among researchers regarding *A. hippocastanum* sensitivity to salinity (see Introduction).

The greater foliar carbon concentration in treated than control plots (Table 3) and lower SLA (Fig. 2) is most likely related to longer leaf life span in the treated site (Fig. 3). For unstressed trees leaf area development in broad-leafed species is completed one to two months earlier than that of leaf mass (Oleksyn *et al.* 2000). At the control site, leaf area expansion stopped in early June (data not shown), less than three weeks before the onset of leaf fall (Fig. 3).

Observed differences among treatments in foliar N concentration explained well the differences of light-saturated net photosynthesis in foliage from mulched and fertilized plots versus those from the control one (Table 3). A significant increase of net photosynthesis underlines the importance of nitrogen as a limiting factor in urban environments.

Significantly lower concentration of phenolics in foliage of mulched and fertilized trees may indicate that enhancement of tree vigor by improvement of substrate fertility and water conditions in urban environments can lead to a higher investment of carbon in growth and lower investment to secondary defense compounds such as phenolics (Fig. 2). Therefore, this supports the idea of a negative association between plant growth and chemical defense (Inbar *et al.* 2001). This is important due to observations in the last few years of the rapid spread in European countries of the *Cameraria ohridella* leafminer and its effects on growth and physiology of horse chestnut (Salleo *et al.* 2003, Thalmann *et al.* 2003, Raimondo *et al.* 2003).

4.2.1. Is foliar stable carbon isotope composition a good indicator of water stress of trees in urban environment?

We initially expected that mulching, by increasing substrate water availability due to minimizing surface evaporation, will increase water-use efficiency of *A. hippocastanum* foliage in comparison with those of control plants. However, results of stable carbon isotope obtained after the second and third year of treatment showed consistently lower water use efficiency of mulched plants (Table 3). The phenological differences in foliage among treated and control plots are most likely responsible for this phenomenon. Due to a much shorter leaf life-span, foliage of control trees accumulate less mass and has lower SLA than those from mulched plots (Fig. 2). In addition, most foliage growth in control plots occurs in early spring when water availability is higher than during late spring and summer when a substantial portion of leaf mass (Oleksyn *et al.* 2000) was added in mulched and fertilized trees. Therefore, characterization of plant stress in an urban environment based on ^{13}C discrimination may not be a good integrated index of stress history in sites where leaf phenology and ontogeny is significantly affected.

In summary, the results of this study show that in untreated plots NaCl is likely the decisive factor that may be responsible for the decrease of foliar N concentration (*via* a reduction in NO_3^- uptake), for the decrease in photosynnthesis (through high concentrations of Na and Cl in the leaves) and for enhanced senescence of the leaves. Presented data idicated that rapid improvement of *Aesculus hippocastanum* vigor can be accomplished even in severely degraded urban sites using mulching and fertilization. Despite a degraded substrate with high levels of potentially toxic compounds such as Cl^- and Na^+ and the high pH thereby lowering nutrient availability for plants, trees in treated plots significantly increased individual leaf mass and area, foliage nutritional status, CO_2 assimilation, seed production, and foliage life-span by as much as six weeks. However, these positive changes were accompanied by lowered foliage phenolics indicating that these plants are potentially better hosts for the horse chestnut leaf miner (*Cameraria ohridella*) that recently affected many parts of Europe. Our studies also indicate that due to pronounced differences in leaf phenology and ontogeny it is exceedingly difficult to use ^{13}C discrimination as a tool for characterization of plant stress in urban environments. The isotopic analyses of horse chestnut foliage may also provide explaination of uncharacteristic results of other attempts to use stable carbon isotopes as a biomarker of urban pollution (Alessio *et al.* 2002).

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