Effect of the outbreak of horse-chestnut leaf miner (*Cameraria ohridella* Deschka & Dimić) on tree-ring width in common horse-chestnut (*Aesculus hippocastanum* L.)

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Research Article

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Abstract

Common horse-chestnut is frequently infested by the insect pest horse-chestnut leaf miner [HCLM; *Cameraria ohridella* (Deschka & Dimić, 1986), Gracillariidae, Lepidoptera]. The larvae, feeding on leaf parenchyma, cause browning and dehydration of leaves, which may be shed as early as in summer. The major aims of this study were: (1) to assess the effect of infestation by HCLM on ring-width dynamics in common horse-chestnut; (2) to determine the date of invasion of the pest; and (3) to compare the growth-climate response in the period before and after the invasion of HCLM. In 2017 in north-western Poland, samples from 30 horse-chestnut trees for the dendrochronological analysis were taken with help of a Pressler increment borer. The ring-width chronology was developed using standard dendrochronological methods. Dendroclimatological analyses were made in 2 periods: before the determined date of HCLM invasion (till the year 1999) and after the invasion (in 2000–2016). In 2000, in spite of favourable weather conditions, a reduced growth rate was observed in 91% of the analysed trees. The period of strong reductions lasted till 2010. Before the invasion, radial growth rate was dependent on temperature and precipitation in May and June of the current year, whereas after the invasion, the growth-climate response was dependent on temperature and precipitation in the preceding year and the correlation was stronger. Surprisingly, in recent years (2011–2016), in spite of infestation by HCLM every year, the health condition of the analysed trees has improved and tree-ring width has increased.

Introduction

Common horse-chestnut (*Aesculus hippocastanum* L.) was introduced to Poland nearly 400 years ago. It was then, and still is, planted in gardens near palaces and in urban areas: along streets, in tree lines, parks, and in housing estates (Seneta 1991; Seneta and Dolatowski 2004; [Spasić et al. 2011](#)).

Until recently, horse-chestnut trees were rarely affected by diseases and pests. However, for a few decades in Europe we have observed an increase in frequency of Guignardia leaf blotch, caused by the fungus *Guignardia aesculi*, accidentally introduced from North America (Jagiello et al. 2017). The first symptoms of tree infection are light brown patches on leaves, and the leaves are getting smaller and smaller every year (Raimondo et al. 2003). After a few years the tree dies (Madej 1971; Gilbert et al. 2004; Baraniak et al. 2005, Snieškienė 2011; Baranowski and Dankowska 2012; Dzięgielewska et al. 2017). This tree species in recent years has been also attacked by the insect pest horse-chestnut leaf miner [HCLM, *Cameraria ohridella* (Deschka & Dimić, 1986), Gracillariidae, Lepidoptera]. Larvae of this moth feed on leaf parenchyma, leading to rusty discoloration and dehydration of leaves (which may be shed already in mid-July), and gradual dieback of branches (Thalmann et al. 2003; Jagiello et al. 2017). HCLM invasion was first observed in Europe in 1984, when it was recorded near Lake Ohrid in Macedonia (Deschka and Dimić 1986). The species has spread to most of European countries, migrating at a rate of about 60 km/year. It was recorded in Spain and France in 2000, in England after crossing the English Channel in 2002, whereas in Denmark, Ukraine, and Sweden in 2003 (Gilbert et al. 2005). In Poland this species was first reported in 1998 near Wrocław (south-western part of the country). The pest quickly

spread in the south of Poland, next it was found in Warsaw, Puławy, and Poznań (central Poland), but now it is widespread in our country (Głowacka *et al.* 2009).

The expansion of HCLM, which threatens the survival of common horse-chestnut in Europe, is associated with a complex of factors resulting mostly from human impact on the environment. The most important factors are: climate change (causing thermal and water stress), soil salinity, and pollution associated e.g. with road traffic, but also dispersal of the moths and leaves with larvae by wind as well as road and sea transport, which is facilitated by e.g. open borders and a lack of detailed control of vehicles and goods. The trees growing along major transportation routes are colonized first and next the pest is dispersed by people, animals, wind, and vehicles onto other trees (Głowacka *et al.* 2009). Container ports in West Europe are currently also a threat, as goods are transported from them in virtually all directions, and trees in those cities are infested by HCLM (Gilbert *et al.* 2005). Simultaneously, to save trees of this species, research was started (e.g. Percival and Holmes 2016) as well as many institutional measures (e.g. spraying with insecticides, injection of insecticides into the trunk or the soil, or placing of bands with specific pheromones on their trunks) and voluntary actions (e.g. raking of leaf litter).

Adult individuals of HCLM are very small moths, with body length reaching 3-4 mm and wing span of only 5-8 mm (Jaworski 2009). During emergence they are abundant on horse-chestnut trunks. Currently their density is so high that in one leaf up to 300 of its larvae can be observed, forming mines (empty corridors, devoid of parenchyma). Feeding on horse-chestnut leaves lasts from May till September, and up to 98% of photosynthetic area can be lost due to this (Percival *et al.* 2011), as the moths reduce the green, photosynthetic area of leaves and cause their earlier drying and shedding (Nardini *et al.* 2004). Moreover, seed weight is lower, and also the affected seedlings are weaker (Takoš *et al.* 2008).

Dendrochronology is one of the methods used for reconstruction of environmental conditions (Schweingruber 1996). Tree-ring width curves reflect primarily the weather conditions (which are the most variable environmental factors), but also changes in groundwater level, the level of pollution, fires, management practices, construction works near the trees, pest outbreaks, etc. (Schweingruber 2007; Koprowski *et al.* 2018). Invasions of pests cause depressions of growth (or even absence of some tree-rings), pale late wood, multiple rings of traumatic resin canals, etc. (Koprowski and Duncker 2012; Lynch 2012; Leland *et al.* 2016). If no direct long-term observations of individual trees or forest stands are available, then dendrochronological methods make it possible to reconstruct with a high precision the timing of extreme events (Koprowski and Duncker 2012; Zhang 2015).

The major aims of this study were: (1) to assess the effect of infestation by HCLM on ring-width dynamics in common horse-chestnut; (2) to determine the date of invasion of the pest; and (3) to compare the growth-climate response in the period before and after the invasion of HCLM. We also assessed tree health in the investigated avenue as well as the activity and damages caused by HCLM. We hypothesized that the invasion of HCLM lowered the dynamics of horse-chestnut tree-ring width and that the growth-climate response differed between the periods before and after the invasion of HCLM.

Materials And Methods

Field research was conducted in 2017 on common horse-chestnut trees (*Aesculus hippocastanum* L.), growing along a 400-m section of a public road in a village of Buk in Dobra Commune in West Pomerania Province. The trees were planted on both sides of the road, forming an avenue (Fig. 1). The study area is located in NW Poland, 12 km from Szczecin, about 400 m from the German border. The climate of Szczecin is predominantly influenced by the polar-sea air masses from the North Atlantic, and also affected by local circulation from the South Baltic and the water bodies surrounding the city. The average annual temperature in 1948–2016 was +8.7°C, but the average temperature in the warmest years reached +10.9°C, compared to +7.1°C in the coolest years. The warmest month is July, with the average temperature of +18.1°C (varying between years: 15.2–22.7°C), and the coolest is January, with the average air temperature of -0.4°C (range: -8.8–5.1°C). Thus the average annual amplitude equals to 18.5°C. The average annual rainfall for the city of Szczecin amounts to 545 mm (range: 347–796 mm). The most humid month is usually July, with 71 mm of average rainfall (but varying between years: 5–185 mm), whereas the driest one is February, with the average rainfall amounting to 31 mm (range: 3–77 mm) (Meteorological Yearbooks). According to Heinz and Schreiber's (1987) division into climatic zones and subzones, the area is in subzone 7a, with mean long-term minimum temperature from -17.7°C to -15.0°C.

The infested leaf area was assessed on the basis of the percentage contribution of the injured part of the leaf to the total leaf area. For this purpose, symptoms of HCLM infestation were recorded throughout the growing season of 2017 (every 2 weeks, from early May to late September), and in mid-August 20 leaves from 30 trees were randomly collected from the lower part of each tree crown and mean values were calculated.

The trees grow about 1 m from the road edge, spaced about 10 m apart. This avenue was selected because of preservation of its design, absence of protective measures, and lack of removal of tree leaves, which could influence the development of trees and HCLM.

Samples from 30 common horse-chestnut trees for the dendrochronological analysis were taken with the help of a Pressler increment borer at breast height (1.3 m above ground) in April 2017. In the laboratory, the cores sampled were glued into wooden mounts, and after drying, their surfaces were cut with a preparation knife to get legible images of growth rings. The measurements of the annual tree-ring widths (TRW), with 0.01 mm accuracy, were made using the Dendrometer program (Mindur 2000), starting from the innermost parts, towards the bark. Altogether 3274 annual growth rings were measured. Then, using the classic method of dendrochronological dating (cross-dating) (Cook and Kairiukstis 1992; Schweingruber 1989), a ring-width chronology was constructed, and its quality was tested with the COFECHA program, from the DPL package (Holmes 1983, 1994; Grissino-Mayer 2001). The constructed RES chronology (de-trended, autocorrelation removed) was subjected to indexation (using a negative exponential curve and autoregressive modelling), to eliminate the age trend and to emphasize the annual variability of the annual growth ring widths, in the ARSTAN program (Cook and Holmes 1999). An

expressed population signal (EPS) of ≥ 0.85 was used to ensure a reliable chronology length (Wigley *et al.* 1984).

Our dendroclimatological analyses encompassed an analysis of the signature years, correlation, and response function. The signature years are the years in which a majority of the trees examined demonstrate the same incremental trends: increase of TRW with respect to the neighbouring years (positive signature year, marked as +) or decrease in increment width (negative year, marked as -) (Meyer 1997–1998). The signature years were calculated from a minimum of 10 trees, assuming the minimum threshold of unanimity of the incremental reactions at 95%. In the analysis of the correlation and response function, the RES tree-ring index chronology was compared with meteorological data from a weather station situated 12 km SE from the plot investigated. The values of monthly average air temperatures (T) and total monthly precipitation (P) were analysed for the period of 69 years (1948–2016), whereas insolation (IN), for the period of 52 years (1965–2016) (Meteorological Yearbooks). The analyses of correlation and response function were made in 2 periods: before the determined date of HCLM invasion (till 1999) and after the invasion (2000–2016). For every year in which an annual growth ring was formed, its width was confronted with meteorological data for the period of 16 months: from June of the year preceding growth (pJUN) till September of the current growth year (SEP). In every case the multiple regression coefficient of determination (r^2) was calculated, determining the strength of the relationship between the features analysed (RESPO program from the DPL package, Holmes 1994).

Relative growth changes in plot chronology were detected using Nowacki and Abrams' (1997) method. Running comparisons of sequential 10-year ring-width means were used to detect periods of decrease and increase of TRW, while discounting short- and long-term climatic change and tree aging trend. Percentage growth change (%GC) was calculated in yearly increments across individual tree-ring chronology by using the formula:

$$\%GC = [(M_2 - M_1)/M_1] \times 100,$$

where M_1 = preceding 10-year mean, and M_2 = subsequent 10-year mean (Nowacki and Abrams 1997; Black and Abrams 2004; Läänelaid *et al.* 2014; Cedro and Sotek 2016).

Results

Tree health

In the study area, the first mines on common horse-chestnut leaves in 2017 were observed from mid-May. Since then, the mean area of mines in leaves regularly and markedly increased. Very strong infestation (up to 70–80% for most of the trees) was recorded already in the second half of August. This resulted in earlier shedding of leaves, started in late August. The decreasing amount of parenchyma and photosynthetic pigments in leaves markedly affected the physiological processes in the infested trees.

Site chronology

The constructed chronology named BU, spanning 116 years (1901–2016), is based on ring-width curves of 22 trees (Fig. 2). The largest number of rings was measured for tree BU13, i.e. 136 rings from the innermost part to the bark, followed by 134 rings for BU3 and 132 for BU1. This indicates that the trees were about 145 years old, and the avenue was planted in the 1880s. Mean TRW was 3.54 mm (for individual trees: 2.22–4.31 mm). The chronology can be regarded as representative, as the value of expressed population signal (EPS) is high: 0.92. The curves of ring widths and their indexed values do not indicate unambiguously the year of invasion of HCLM on the studied trees, so we used the curve of relative growth change to determine the date of invasion (Fig. 3). Relative growth changes show that tree growth rate increased in 1916–1929, 1939–1948, 1955–1974, 1980–1999, and at the end of the chronology, in 2011–2016, whereas it decreased in 1910–1915, 1930–1938, 1949–1954, 1975–1979, and 2000–2010. Considering the direction of the spread of HCLM (from Macedonia), and the first record of this folivore in Poland (Wrocław in 1998, Łabanowski and Soika 1998), we looked for reduction of ring width caused by HCLM after this date. The impact of HCLM infestation on horse-chestnut TRW in the avenue in Buk started in 2000 (Fig. 3). This is not the date of appearance of *Cameraria ohridella* on the studied trees but of noticeable (measurable) influence of HCLM infestation on tree-ring width.

Dendroclimatology

The analysis of signature years indicates 34 years when more than 95% trees showed consistent growth responses in relation to the preceding year. Negative pointer years prevailed (22): 1910, 1915, 1918, 1923, 1925, 1930, 1934, 1938, 1940, 1948, 1954, 1956, 1960, 1964, 1970, 1973, 1975, 1977, 1982, 1992, 1997, and 2010. Only 12 pointer years were positive: 1924, 1939, 1950, 1955, 1957, 1961, 1987, 1993, 2007, 2009, 2011, and 2013. In 2000, as many as 91% of the analysed trees produced narrower growth rings than in the preceding year. Positive pointer years were characterized by higher precipitation than the long-term mean, with sufficient rainfall (or snowfall) in spring and summer (especially in June), whereas the number of sun hours was lower than the long-term mean. Negative pointer years usually were preceded by years with water shortages, and also during the pointer years the recorded precipitation was lower than average. Low precipitation in May, June or July, linked with high air temperature and strong insolation, were the major causes of negative pointer years.

In the year 2000, weather conditions were favourable for horse-chestnuts. Mean annual air temperature in 2000 (9.9°C) was 1.2°C higher than the long-term mean (8.7°C), while annual precipitation (572 mm) was nearly 30 mm higher than the long-term mean (545 mm), and the number of sun hours (1612 h) was slightly higher than the long-term mean (1588 h). Summer months of that year were colder, with higher precipitation and lower insolation than long-term means. Only April and May in 2000 were warmer, more sunny, and drier than long-term means (Fig. 4). In that year, no renovation of the road and the associated infrastructure took place, which could have affected negatively the root systems of trees, and consequently also tree health in general (oral communication in the Commune Office). In 2000–2005, the curve of relative growth change shows that tree-ring width was reduced by 70% and stayed low till 2010. Starting from 2011, however, in spite of the presence of HCLM and early defoliation of the trees every year, relative growth change increased by 50% (Fig. 3). Positive pointer years after the date of HCLM

invasion were recorded in 2007, 2009, 2011, and 2013. In all those years, mean annual precipitation was higher than average (the year 2007 was the wettest during the study period, with annual precipitation of 796 mm) and high rainfall was observed in summer months (June and July), e.g. 185 mm in July 2011 (compared to the long-term mean of 71 mm for this month) and 141 mm in June 2013 (compared to the long-term mean of 60 mm). The negative pointer year 2010 was characterized by low precipitation in June-July.

The analyses of correlation and response function made for the period before the invasion (1948-1999), and after the invasion (2000-2016) of HCLM showed different growth-climate responses (Fig. 5). Before the invasion, tree-ring width was affected predominantly by weather conditions in May and June. Lower than average air temperature, lower number of sun hours, and higher precipitation contributed to formation of broader tree-rings. In the year preceding growth, few relationships were observed (in September, October, and December). The highest multiple regression coefficient of determination was calculated for insolation (45%), lower for precipitation (38%), and the lowest for temperature (26%). After the invasion of HCLM (2000-2016), similar relationships were found for precipitation in June of the current year, whereas in July the regression coefficient was negative. However, the greatest changes can be noticed in the year preceding growth and in winter: values of correlation and regression are usually positive for precipitation and negative for temperature and insolation. A cold, clouded, and moist previous growing season as well as frosty and clouded winter contributed to formation of broader tree-rings. For each of the analysed climatic factors, r^2 increased, which indicates very strong growth-climate relations. For insolation it reached 83%, for air temperature 74%, and for precipitation 40%.

Discussion

The spread of HCLM across Europe has been noticed by its inhabitants. HCLM infestation of horse-chestnut trees – present in most of European countries for several hundred years, planted in parks, gardens, and avenues as ornamentals – has been widely discussed in the mass media. The rapid spread of HCLM is facilitated by the modern lifestyle and technological progress: frequent travels, a lack or limited border control, and heavy road traffic (Łabanowski and Soika 1998; Gilbert *et al.* 2004; Baraniak *et al.* 2005; Kosibowicz 2005; Głowacka *et al.* 2009; Snieškienė 2011).

Another contributing factor is the observed climate change, causing extension of the potential range of distribution of this pest. Over the past three decades, HCLM has spread throughout Europe - even crossing mountain barriers, seas and canals - it has even been observed in England, Denmark and Sweden in the early 2000s, thousands of kilometers from its first sighting in 1984 at Lake Ohrid in Macedonia (Gilbert 2005). Because of the disturbance of the life cycle, the trees do not initiate the normal winter dormancy and are prone to frost damage, especially if winter starts early and is more severe. Repeated infection of trees for a few years leads to their weakening (Percival *et al.* 2011), leading to e.g. lower growth dynamics and formation of narrower tree-rings. The rate of development of individual stages of the HCLM life cycle depends on insolation and temperature. Probably high insolation and high temperature in summer stimulate females to lay eggs faster (Jäckel *et al.* 2006; Bystrowski *et al.* 2008).

That is why a change in the growth-climate response during the HCLM outbreak was observed after the year 2002, i.e. it started to be more dependent on conditions in the year preceding growth and the strength of the dependence increased, especially for temperature (from 26% before the invasion to 74% after the invasion) and insolation (from 45% to 83%). Lower than average air temperature, lower number of sun hours, and higher precipitation at the end of the preceding year negatively affect the last stages of the HCLM life cycle, which results in a smaller number of overwintering parasitoids and weaker infestation in the following year (Samek 2003). After such a pattern of weather conditions, trees in the following growing season are healthier and develop wider growth rings.

Common horse-chestnut is rarely studied by dendrochronologists. In Slovenia its radial growth depends on moisture conditions (precipitation-evapotranspiration index, SPEI) in summer months: June-August, as droughts cause strong reductions of tree-ring width (Simon and Lena 2016). In Poland similar research was conducted by Wilczyński and Podlaski (2007), but effects of HCLM infestation on tree-ring width were studied only by Bednarz and Scheffler (2008). According to Wilczyński and Podlaski (2007), radial growth rate of this species in mountainous regions is affected most significantly by temperatures in winter (December-March) and August as well as precipitation in December in the year preceding growth. High temperatures in those months and high precipitation in December result in formation of broad tree-rings. Horse-chestnuts growing in urban areas (Kraków, southern Poland) are sensitive to weather conditions in the year preceding growth: correlation coefficients were negative for temperatures in July, August, and September, while positive for precipitation in August of the preceding year and June of the current year (Bednarz and Scheffler 2008). The HCLM outbreak in Kraków probably started in 1997, which was followed by strong growth reductions, lasting till 2005. Those results are consistent with our data from north-western Poland (horse-chestnut avenue in the village of Buk). Although the outbreak started here 5 years later (our study area is located about 700 km NW of Kraków), the growth response of trees and growth-climate relations indicate that weather conditions in the preceding year were the dominant factors shaping the growth dynamics. Research conducted by Salleo *et al.* (2003) shows a different response of common horse-chestnuts to HCLM infestation: they reported an increased number of false rings, an increase in tree-ring width, and an increase in hydraulic efficiency of wood for 4 years after the invasion (which probably started in north-eastern Italy in 1997).

Besides, the recently recorded (2011-2016) increase in radial growth dynamics of the studied trees, in spite of HCLM infestation every year, suggests that the infested trees have developed a defence strategy (Łabanowski and Soika 1998; Malinowski 2008; Baranowski and Dankowska 2012). This issue has not been studied yet, but the appearance of defence mechanisms in the studied trees seems to be evidenced by an increase in their vitality: many young boughs with uninfested or less infested leaves, intensive scarring of wounds, intensive flowering and fruiting, broader tree-rings, as compared with previous years. Moreover, natural parasites of HCLM appear (e.g. *Minotetrastrichus frotalis*), which infest this pest, but its infestation rate is lower than those of other species of leaf miners (Bystrowski *et al.* 2008).

Conclusions

At the time of climate change and strong increase in human impact, and the resultant spread of invasive species, it is necessary to study species that are prone to infestation by pests that did not inhabit a given region earlier. Horse-chestnut trees, although they have been intentionally introduced to many parts of Europe, are now familiar and valuable elements of our landscape. The large numbers of dying horse-chestnut trees or the remarkable deterioration of the condition of this species, observed in recent years mostly due to the HCLM invasion, is subject not only of scientific research but also of public discussions. That is why research on the growth-climate relations in the period before the HCLM invasion and during the invasion is an important contribution to current knowledge about species attacked by pathogens and invasive species. Results of this study can also contribute to development of a strategy of conduct during invasion of new alien pests.

Our most interesting finding is the improved tree health in the last few years, in spite of infestation by HCLM. The increased tree-ring width, large number of young branches, or wound healing can indicate that the trees have developed some natural defence mechanisms and/or some parasites have attacked the invaders. Research on these mechanisms as well as mutual relationships (tree – invasive pest – its parasite – climate change) may help us control pest outbreaks and epidemics in the future, as in the case of e.g. the already spreading box tree moth (*Cydalima perspectalis*) or the fungus causing anthracnose on London plane (*Gnomonia platani*).

Declarations

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Data Availability Statement: The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request. The data presented in this study will be available after positive decisions in REpOD (Repository for Open Data, <https://repor.icm.edu.pl/>, with DOI).

Conflicts of Interest: The authors declare no conflicts of interest.

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Figures

Figure 1

Location of the study area. (source of the map: www.geoportal.gov.pl)

Figure 2

Individual sequences of tree-ring width (thin black lines), making up the local horse-chestnut chronology (BU; green line), and number of samples in the chronology (red line).

Figure 3

Running relative growth change of the horse-chestnut chronology (BU), showing the relative growth change between mean tree-ring width of 10 preceding and mean of 10 subsequent years.

Figure 4

Mean monthly air temperature (T), monthly precipitation (P), and monthly insolation (IN) in the year of the outbreak (2000) – dark blue, green and orange colour, compared with mean values for 1948-2016 (T, P) and 1965-2016 (IN) – bright blue, green and orange colour.

Figure 5

Results of correlation (CC) and response function (RF) analyses for the horse-chestnut chronology (BU) during the outbreak of *Cameraria ohridella* (2000-2016) for temperature (T), precipitation (P) and insolation (IN), and before the outbreak (T, P 1948-1999, *IN 1965-1999). Bars denote significant values ($p \leq 0.05$); p, previous year.