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Biometrical assessment of ragweed (*Ambrosia artemisiifolia* L.)

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Abstract

In Lithuania, ragweed is attributed to late-emerging weeds. The highest number of this species' representatives was identified during the 1985–2000 period, while currently only sporadic individuals are being found. The present paper assesses biometrical indicators (number and length of branches, length of male inflorescences and number of flower heads in them, pollen productivity, number of clusters of female flower heads, seed number and weight) of ragweed grown in a cultivated soil in Lithuania and to establish the interrelationships among the indicators tested. At all growth stages, the plants were cultivated in uncontrolled environmental conditions. Common ragweed formed shrubby plants exhibiting high seed and pollen productivity. A strong relationship between inflorescence length and number of male flower heads ($r > 0.85$, $p < 0.01$) was established. The longest male inflorescences (14.46 ± 8.28 cm) were formed in the tops of branches of the first rank and the number of male flower heads was the highest (140 ± 50) in them. A plant grown in a cultivated soil forms approximately 36000 flower heads. It was determined that 3408 ± 2127 pollen grains are produced per anther. The plants grown in the conditions favourable for their development produce about 7.4 E + 09 pollen grains per plant. In the upper part of plant, there was established a strong relationship between the number of clusters of female flower heads and number of seeds ($r > 0.75$, $p < 0.01$). One branch of ragweed has on average 5–6 clusters of female flower heads in which from 1.6 ± 0.3 to 2.3 ± 0.7 seeds are formed, depending on the branch position on the plant. One plant was found to produce 6906 ± 1692 seeds with an average weight of 2.98 mg per seed (SD = 0.36).)

Key words: invasive plant, agricultural weed, flower heads, pollen production, seeds.

Introduction

The common ragweed (*Ambrosia artemisiifolia* L.) is an annual plant belonging to *Asteraceae* family and is distributed as an invasive plant all over Europe. It started to spread in Europe in the second half of the 19th century and currently the dispersal of its populations raises concerns for farmers, conservationists and health protection authorities. Ragweed is a pioneer and opportunistic species, firstly invading roadside territories (Fumanal et al., 2007; Weryszko-Chmielewska, Piotrowska, 2008; Šaulienė et al., 2011 a). At the same time, ragweed is infesting agricultural land, where its presence reduces crop yield and deteriorates its quality. None the less important, economic aspect of ragweed effect is allergy caused by its pollen, and in discrete cases, even asthma fits. A concentration of 6–10 pollen grains m^{-3} is sufficient to trigger allergic reaction, and the level exceeding 10 pollen grains m^{-3} is referred to as high (Bohren et al., 2006). In Lithuania, 5–8 cases are recorded annually when ragweed pollen concentration in the air exceeds this level (Šaulienė, Veriankaitė, 2009).

Ragweed is attributed to late-emerging weeds in Lithuania. The highest counts of the specimens of this species were identified during the 1985–2000 period, while currently only sporadic plants are encountered (Šaulienė et al., 2011 b). Ragweed is rich in micro-elements: the contents of copper, manganese and zinc in leaves and stems are 2.6–3.0 times higher than those in barley grain. The plants flower in August, therefore they do not mature seeds in barley crops as well as maize crops grown for silage, whereas in sugar beet crops and maize crops grown for grain ragweed seeds mature in October (Špokienė, Gudžinskas, 2001).

The factors promoting ragweed invasiveness are climate change and increasing human population, which results in higher travel and transport activities. Restrictions on the use of environmentally unfriendly plant protection products create favourable conditions for the spread of weeds (Bogužas et al., 2010), including ragweed. Advocates of sustainable agriculture in Lithuania witness increased weed activity (Feiza et al., 2010; Marcinkevičienė et al., 2010); therefore a good knowledge of ragweed biological characteristics is one of the most essential aspects in the fight against its intervention. Furthermore, only a few years ago the Panel on Plant Health recommended that the ragweed risk assessment should be revised and updated (European Food Safety Authority, 2007).

The current study was aimed to assess the biometrical characteristics of ragweed grown in a cultivated soil in Lithuania and to establish the interrelations between these attributes.

Materials and methods

The study object. Most *Ambrosia* species are native to East and Central North America. In Lithuania, common ragweed is generally found in the vicinity of railways, where its seeds are spilled while transporting grain from Southern and Central Europe (Šaulienė et al., 2011 a). The ragweed plants grown in Lithuania from the seeds matured in a natural habitat (Canada, Ontario province) were assessed for biometrical characteristics. Such seeds were chosen to prevent genetic adaptations caused by the effects of naturalization processes. Ragweed is a summer monoecious plant 0.2–2.5 m tall (DAISIE, www.europe-aliens.org), an erect, branched, leafy annual with a grooved, often reddish, hairy stem. Leaves compound, divided in oblong, toothed segments, green and hairy above, and with dense, white and pressed hairs beneath. Male flower heads 4–5 mm, involucre cup-like (composed of 5–12 flowers) in slender, bractless, terminal spikes. Female flower heads, usually with one flower, sessile, inconspicuous in small clusters or single in the axils of the upper leaves. Achenes, 4–5 mm, hairy, with 5–6 spines (Wittenberg, 2005).

Cultivation of the test plants. In April 2011, ragweed seeds were sown under laboratory conditions in order to ensure earlier emergence of seedlings and to protect them from frosts. After the seedlings had produced true leaves, the plants were transferred to the greenhouse. At the end of June, ragweed seedlings were transplanted in a cultivated soil in a permanent cultivation site where environmental conditions were not controlled at any of the growth stages. The plants were cultivated in three 10 m^2 experimental plots with 10 common ragweed plants per plot. The soil of the experimental field was neutrally acid light loam rich in nutrients. Three plants from each field were taken for analysis by using random sampling method. All parts of the research were carried out at Šiauliai University.

Determination of biometrical and productivity indicators. At the end of October 2011, the plants were transferred to the laboratory for assessment. Two methods were used. The first assessment method is based on the rank of branches. For this purpose, all the branches of a plant were divided into four ranks. The branch that directly ramifies from the main stem is considered the first rank. The second rank is the branch that ramifies from the branch of the first range, the third – from the second, the fourth – from the branch of the third range. The second assessment method is based on the analysis of biometrical indicators according to plant part. The plant was divided into three parts and one plant part corresponded to 1/3 of plant height. As a result, the data are presented according to the three plant parts.

The plants were assessed for the number and length of branches, length of male inflorescences and flower heads in them, number of clusters of female flower heads, seed number and weight. The branches of the fourth rank were characterised using the number of branches, male flower heads and seeds.

Plant anthers were collected at the beginning of September. Pollen production was estimated using the published methodologies (Prieto-Baena et al., 2003). The average number of pollen grains per stamen was established having assessed stamens collected at different plant heights. Ragweed pollen productivity was calculated based on literature data on the number of male flowers per flower head (Payne, 1963) and on biometrical data collected during the study.

Statistical analysis. The data of measured biometrical indicators were systemised and the results were presented using the main numeric characteristics of position and scatter/dispersion data. The determined mean values of the indicators and standard deviations were analysed by estimating the relationships of biometrical indicators in relation to branch rank and plant part. For data scatter visualisation we used Box-Whisker method, which integrates data width, quartile width, dispersion and standard deviation. Linear regression was used to establish the relationship between the two variables, correlation coefficients (Pearson method) were calculated. Statistical relationship is represented graphically using scatter/dispersion diagram of pair data, coefficient of shift direction was estimated, as well as the ratio of regression and standard deviation.

Results and discussion

Biometrical indicators of ragweed are important both from the scientific and practical viewpoints. The characteristics of this plant have been discussed in the scientific press for more than a decade. Based on researchers' opinion, European Food Safety Authority (2007) has declared that there is a lack of scientific knowledge on ragweed in Lithuania. Such knowledge gaps may have arisen due to the specific spread of ragweed. In 2000, there were identified ragweed overgrowths consisting of more than 1500 plants (Špokienė, Gudžinskas, 2001), and during the expeditions in 2010 there were found only single plants (Šaulienė et al., 2011 a; b).

Having estimated biometrical indicators of ragweed grown in a cultivated soil in 2011 it was found that an average plant height is 134.25 cm (SD = 15.41). Analysis of plant structure indicated that ragweed is characterised by a four-rank branch system. A plant has nearly 7 times fewer first rank branches than second-rank branches (Fig. 1), that are situated on the first rank – on average 8.23 (SD = 3.46). A logical link is observed suggesting that with increasing branch rank number, the branches get shorter. Also, a reduction occurs in the number of lateral branches on each of them, e.g. on average 6.04 (SD = 2.11) on the second-rank branch and 1.86 (SD = 0.85) on the third-rank branch. If plants are cut, branchiness increases and branches of more than six ranges are formed (Simard, Benoit, 2011).

Having estimated average number of ragweed branches in different plant parts it was found that the number of first-rank branches in the 1st plant part was higher than in the other two (Table 1).

Male flowers of common ragweed are concentrated in flower heads and are situated in the inflorescences at the top of branches. Inflorescence length varies depending on branch rank (Fig. 2, on the left). The longest inflorescences are formed at the tops of first-rank branches. In the second-rank branches, the flower heads are clustered in shorter inflorescences. It is noteworthy, that comparison of the second-rank inflorescences revealed the highest variation of the sample. It is obvious that the smallest inflores-

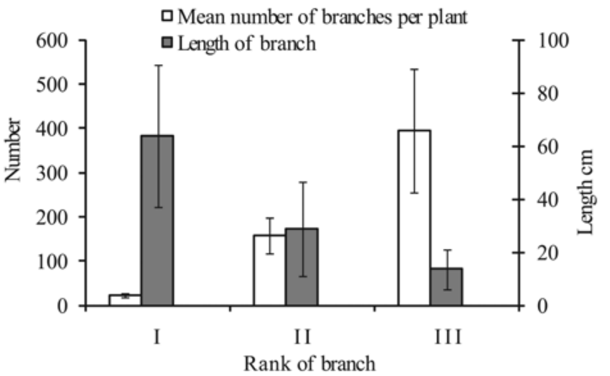


Figure 1. Average number and length of ragweed branches according to branch rank

cences are formed on the shortest (third-rank) branches. However, in terms of plant parts, the average inflorescence length varies inappreciably: 1st – 5.62 (SD = 4.91), 2nd – 5.28 (SD = 4.53), 3rd – 5.23 (SD = 4.62).

Like the highest inflorescence length, the largest number of flower heads (140 ± 50) was recorded in the first-rank branches (Fig. 2, on the right). Three times fewer flower heads are formed on the third-rank branches compared with the first-rank branches. Assessment of ragweed biometrical indicators evidenced that in separate cases even the third-rank branches form up to 250 flower heads. The most inconsistent number of flower heads (23.27 ± 18.32) was established in the inflorescences of the fourth-rank branches. Having estimated average number of flower heads per inflorescence, there were obtained 57 (SD = 42) male flower heads regardless of the plant part. Polish researchers studied common ragweed for ecological characteristics and found that there can be 10–90 male flower heads per inflorescence (Weryszko-Chmielewska, Piotrowska, 2008). Having assessed the number of branches and the number of flower heads in branches it was calculated that common ragweed grown in Lithuania produced on average 36000 male flower heads per plant.

Table 1. Average number and length of ragweed branches according to position on a plant

Plant part	Rank of branch						Length cm	SD
	I		II		III			
	Number	SD	Number	SD	Number	SD		
1st	11.50	4.36	58.50	27.25	147.00	42.92	21.92	18.12
2nd	5.75	2.75	47.75	18.34	131.75	41.95	18.39	14.98
3rd	5.50	3.11	52.25	28.28	117.00	69.60	18.24	16.03

Notes. There was no significant difference between the number of the second- and third-rank branches in different plant parts. There were no differences in terms of biomass between different plant parts. Such results imply that the branches (according to branch-rank) distribute evenly in a plant.

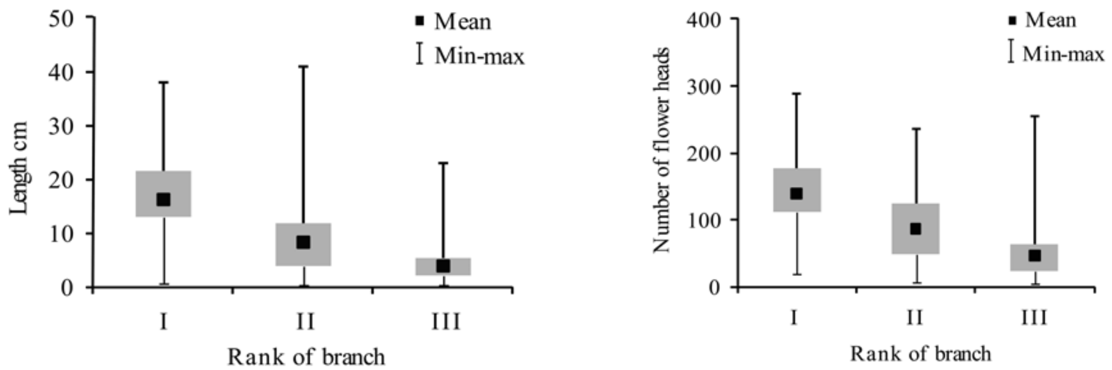


Figure 2. The length of ragweed male inflorescence (on the left) and the number of flower heads (on the right) in the branches of different ranks

The high number of flower heads compels researchers to direct their attention to ragweed effects on human health, since its pollen is attributed to the group of most allergenic ones (Gadermaier et al., 2004; Bohren et al., 2006; Ziska et al., 2011). Our research evidenced that ragweed produces 3408 pollen grains per anther, SD = 2127. Similar findings were published by French and Polish researchers (Fumanal et al., 2007; Weryszko-Chmielewska, Piotrowska, 2008). Having assessed the biometrical indicators of ragweed grown in Lithuania it was ascertained that one plant generates approximately 7.4 billion pollen grains, while the above mentioned researchers reported a smaller number of pollen per plant. Such discrepancies might have been determined by different factors, especially ecological environment of plant growth.

Female flower heads on Lithuania-grown ragweed are arranged in clusters. During plant biometrical data measuring the cluster contained flowers of different ontogenesis stage: from undeveloped to those that matured seeds. Literature findings suggest that the number of flower heads per cluster varies from 7 (Basset, Crompton, 1975) to 3 (Weryszko-Chmielevska, Piotrowska, 2008). Analysis of the obtained data revealed that female flower heads are formed on all ranks of branches (Fig. 3). The same trend is observed in terms of plant parts (Table 1). Average number of clusters per branch is similar (I – 4.06, SD = 4.43; II – 6.25, SD = 4.04; III – 6.21, SD = 1.80). However, their number on the second- and third-rank branches is more consistent, compared with the first-rank branches. The obtained results evidenced that there were cases when the number of clusters in the second-rank branches was up to four times higher compared with the average value.

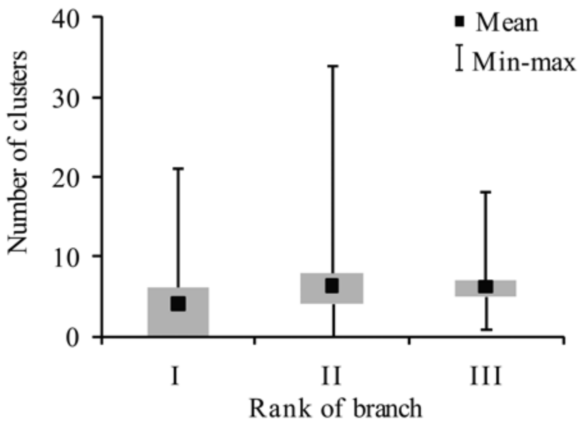


Figure 3. The number of female flower head clusters in the branches of different ranks

Seed production is undoubtedly a major factor of ragweed's, as invasive plant, penetration into agricultural land or wild nature territories. From the sparse information about common ragweed in Lithuania, one can judge that the country's climatic conditions are not suitable for ragweed to mature viable seeds. One of the latest studies on uncontrolled spread of ragweed (Špokienė, Gudžinskas, 2001) indicated that of more than 1500 plants' overgrowth only as few as 250 specimens were recorded in a year's time. It is not clear whether this marked reduction in the population was caused by the frosts that are dangerous for ragweed (Deen et al., 1998; Ziska et al., 2011) or whether it occurred due to insufficient seed maturity/viability.

Assessment of the test plants grown in 2011 showed that one ragweed plant can contain 6906 seeds (SD = 1692). In North America, common ragweed produces on average 210 seeds per plant (SD = 113); however, there are plants that produce nearly 700 seeds (McKone, Tonkyn, 1986). Other tests indicated the seed production to vary from 346 to 6.114 seeds per plant (Fumanal et al., 2007), or even up to 14000 in exceptionally big plants (Basset, Crompton, 1975). Analysis of ragweed grown in a cultivated soil in Lithuania revealed that nearly the same number of seeds is formed in different plant parts (Table 2). This type of information is of special relevance for crop and roadside management, therefore we also estimated the number of seeds in the cluster of female flower heads. Comparison of the clusters showed that the least number of seeds was formed by the flowers present in the 1st part of the plant. Consequently, the existing experience suggesting that to reduce the load of plant effect on the environment one should use timely plant mowing (Simard, Benoit, 2011) is applicable in Lithuania.

Table 2. The indicators of the number of female flower head clusters and seeds in different plant parts

Plant part	Number of female flower head clusters	SD	Number of seeds	SD	Number of seeds per cluster	SD	1000 seed weight g	SD
1st	6.43	3.08	44.76	59.14	1.58	0.33	2.93	0.28
2nd	6.06	2.51	45.45	54.05	1.87	0.58	3.04	0.26
3rd	5.77	2.59	47.64	64.01	2.27	0.73	2.98	0.54

Seed weight is an important indicator when estimating this organ's germoplasmic potential. The average weight of a developed seed is 2.98 mg (SD = 0.36) and it remains similar in all plant parts. Like for other biometrical indicators analysed in the present paper, the scientific information concerning ragweed seed weight is highly controversial (McKone, Tonkyn, 1986; Chikoye et al., 1995; Paquin, Aarssen, 2004; Fumanal et al., 2007). It is known that if ragweed plants growing on the roadsides are cut, the number of seeds declines by 2.89 times (Simard, Benoit, 2011). Although no ragweed overgrowths have been found over the last decade, one should bear in mind the facts indicating that ragweed seeds remain viable for up to 40 years (Gadermaier et al., 2004). The large number of seeds formed in the test plants and sufficient seed weight imply that one of the major factors preventing ragweed spread in Lithuania is climatic peculiarities, especially frosts. Given the fact that the IPCC (2007) report predicts air temperature rises, there exists a real threat of ragweed expansion into Lithuania. Naturalization of ragweed plants has been already documented in the neighbouring countries (Bohren et al., 2006; Fumanal et al., 2007; Weryszko-Chmielewska, Piotrowska, 2008).

Our test plants were grown in a cultivated soil, therefore biometrical indicators presented in the current paper could be considered as a maximally possible result rather than a typical case. The correlation coefficients of results (Table 3) were calculated seeking to highlight the main trends and regularities of ragweed organogenesis.

Analysis of ragweed biometrical data showed that the number of branches per plant depends on the length of branches; however, statistically significant results were obtained only with regard to the third-rank branches. Similarly, the number of male inflorescences and flower heads undoubtedly depends on the length of branch forming the organs, but does not depend either on branch rank or place on a plant.

In the structure of ragweed, the third-rank branches (Fig. 1) account for the largest number and they contain the largest number of female flower head clusters (70% from the total number of clusters in a plant). The calculated correlation coefficients showed (Table 3) that in the third-rank branches the number of female flower head clusters depends not only on the number of branches but also on the length of these branches. Comparison of the number of female flower head clusters and seeds in different plant parts indicated a strong relationship between the variables in the 2nd and 3rd plant parts. Fumanal with colleagues (2007) reported a significant relationship between plant biomass and seed production ($R^2 = 0.86$). Thus, the production of female generative organs as well as seeds can be markedly reduced by the control of development of the third-rank branches.

Table 3. Pearson correlation coefficients among major morphometric indicators of ragweed according to the branch rank and place on a plant

Branch rank / plant part	Branch length and				Number of clusters of female flower heads and	
	number of branches	male inflorescence length	number of male flower heads	number of clusters of female flowers heads	number of branches	number of seeds
I/1st	0.86/–	0.63*/0.77*	0.67*/0.75*	0.05/0.03	0.08/–	–/0.66*
II/2nd	0.92/–	0.79*/0.88*	0.80*/0.83*	−0.04/−0.01	0.64/–	–/0.79*
III/3rd	0.96**/–	0.72*/0.86*	0.70*/0.83*	0.54*/0.08**	0.98*/–	–/0.77*

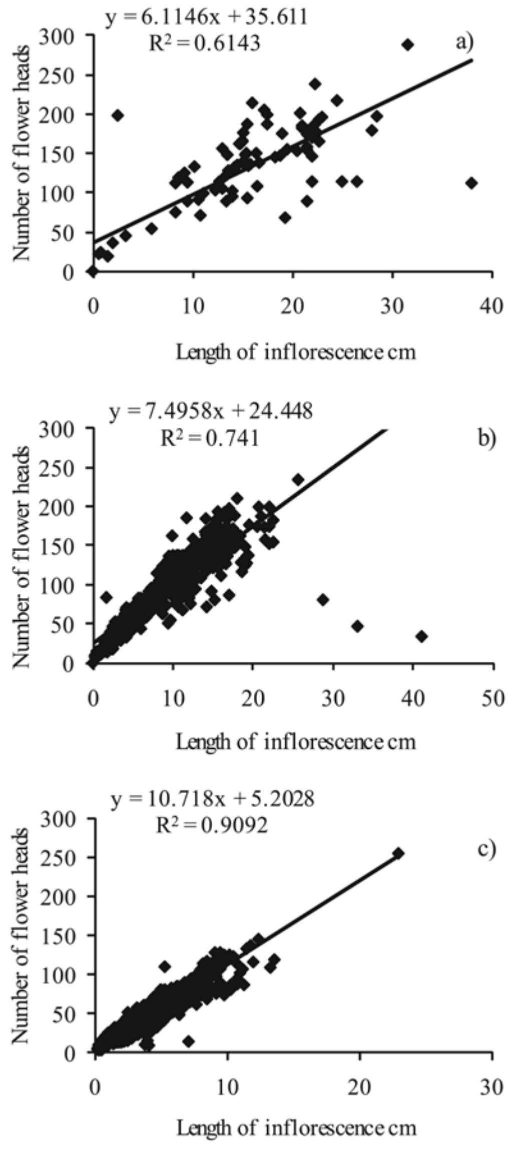
* – $p < 0.01$, ** – $p < 0.05$

Formation of male inflorescences and flowers in them is relevant not only regarding the yield but also ragweed effect on human health. According to clinical experience (Gadermaier et al., 2004), ragweed pollen appears to induce asthma approximately twice as often as what is usual for other pollen allergies (i.e. in 40–50% of ragweed allergic patients). One ragweed plant produces from 26298 to 52037 male flower heads that release billions of pollen grains in the atmosphere that can travel thousands of kilometres (Smith et al., 2008; Šaulienė, Veriankaitė, 2009). A strong relationship was established between the number of male flower heads per plant and inflorescence length. The relationship is nearly the same with respect to plant branch and plant height (Figs 4 and 5).

Analysis of figures shows that the strongest relationship is with the third-rank branches; however, they have the smallest number of male flower heads (Fig. 2), and the largest number of flowers is formed on the branches of the first rank. Consequently, the productivity can be reduced by shortening branches at the 10 cm level twice per vegetation season. This practice gives a 70% reduction of male inflorescences and up to 88.7% of pollen production (Simard, Benoit, 2011). In terms of allergenicity, it is important that ragweed may also induce contact dermatitis, due to the presence of sesquiterpene lactones drained to the plant surface in resin canals (Asero, 2002).

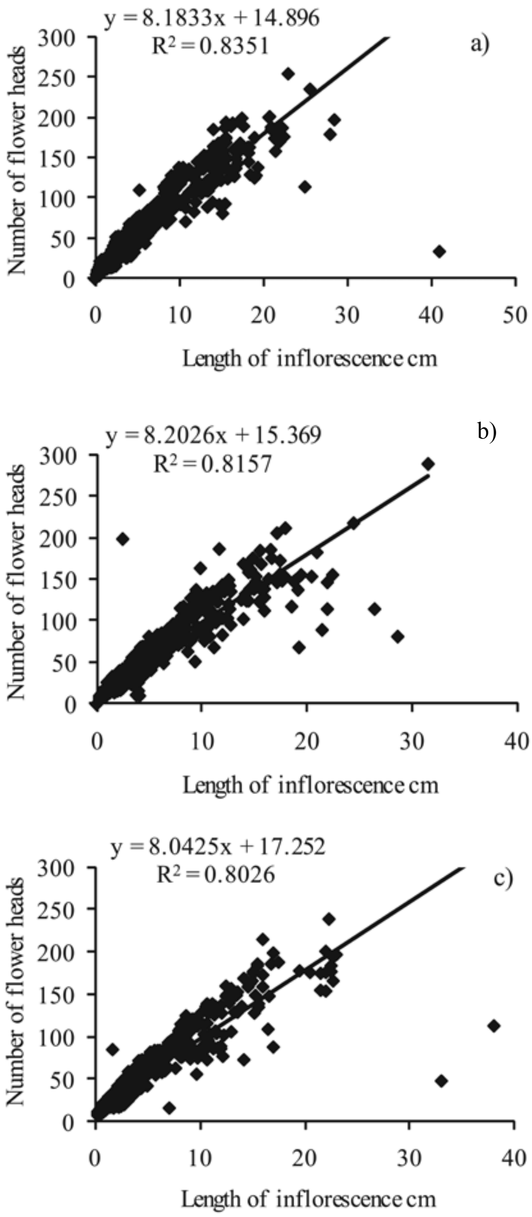
Climate change will inevitably have a positive impact on ragweed invasion in Lithuania, but firstly we will increasingly observe high pollen concentrations in the air as a result of the long distance pollen transport. Latitudinal effects on increasing season length were associated primarily with a delay in first frost of the fall season and lengthening of the frost free period. Overall, these data indicate a significant increase in the length of the ragweed pollen season by as many as 13–27 days at latitudes above ~44°N since 1995 (Ziska et al., 2011).

The fact that currently only a few ragweed plants are found in Lithuania (Šaulienė et al., 2011 b), does not allow us to discontinue monitoring of the situation. It is necessary to carry out comprehensive research into maize plantations. This grain crop (especially grown from the seed bought abroad) harvested in October is an



Note. Linear regression lines, determination coefficient (R^2) are shown on the diagrams: a) first-rank branch, b) second-rank branch, c) third-rank branch

Figure 4. The relationship between the number of ragweed male flower heads and inflorescence length in the branches of different ranks



Note. Linear regression lines, determination coefficient (R^2) are shown on the diagrams: a) 1st plant part, b) 2nd plant part, c) 3rd plant part.

Figure 5. The relationship between the number of ragweed male flower heads and inflorescence length in different plant parts

ecological niche, where ragweed can grow and mature seeds. From the findings presented in this paper it is obvious that a large ragweed seed bank can be accumulated in the soil (the seeds can stay viable for up to 40 years). In ragweed growing places, the soil generally contains from 200 to 2800 seeds m^{-3} at the 5 cm depth, while at the 20 cm soil depth there are found from 250 to 5000 seeds m^{-3} (Tokarska-Guzik et al., 2011). All this suggests that it is vital to carry out monitoring in the sites where ragweed has been recorded and especially ragweed overgrowths (Špokienė, Gudžinskas 2001; Šaulienė et al., 2011 a). Timely application of measures would serve as barriers limiting uncontrolled spread of ragweed.

Conclusions

1. A strong relationship was obtained between ragweed inflorescence length and number of male flower heads (r > 0.85, p < 0.01). The longest male inflorescences (14.46 ± 8.28 cm) are formed on the tops of branches of the first-rank and the number of male flower heads is the highest (140 ± 50) in them. A ragweed plant grown in a cultivated soil produces approximately 36000 flower heads.

2. One anther was found to generate 3408 ± 2127 pollen grains. In the conditions favourable for ragweed development the plant produces about 7.4 E + 09 pollen grains.

3. In the upper part of plant, there was established a strong correlation (r > 0.75, p < 0.01) between the number of clusters of female flowers and number of seeds. One ragweed branch has on average 5–6 clusters of female flower heads, in which from 1.6 ± 0.3 to 2.3 ± 0.7 seeds are formed depending on the position of branch on the plant. It was found that one plant can produce 6906 ± 1692 seeds with an average weight of 2.98 mg per seed (SD = 0.36).

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Kietinės ambrozijos (*Ambrosia artemisiifolia* L.) biometrinis įvertinimas

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Santrauka

Lietuvoje kietinė ambrozija yra priskiriama prie vėlyvųjų piktžolių. Šios rūšies atstovų daugiausia buvo aptinkama 1985–2000 m., o pastaruoju metu randami tik pavieniai individai. Straipsnyje įvertinti Lietuvoje sukultūrintame dirvožemyje išaugintų kietinių ambrozijų biometriniai rodikliai (šakų skaičius ir ilgis, vyriškų žiedynų ilgis ir graižų skaičius, žiedadulkių produktyvumas, moteriškų žiedų graižų klasterių skaičius, sėklų skaičius bei svoris) ir nustatyti jų tarpusavio ryšiai. Visais auginimo tarpsniais augalai buvo kultivuojami nekontroliuojant aplinkos sąlygų. Sukultūrintame dirvožemyje kultivuotos kietinės ambrozijos formavo krūmiškus ir dideliu produktyvumu pasižyminčius augalus. Įvertinus tyrimų rezultatus ir atlikus duomenų statistinę analizę, gautas stiprus ryšys tarp žiedyno ilgio ir vyriškų graižų skaičiaus ($r > 0,85$, $p < 0,01$). Ilgiausi (14,46 ± 8,28 cm) vyriškieji žiedynai formuojasi kietinės ambrozijos pirmos eilės šakų viršūnėse, o jų vyriškųjų graižų skaičius yra didžiausias (140 ± 50 vnt.). Sukultūrintame dirvožemyje išaugintas augalas suformuoja apytiksliai 36000 graižų. Nustatyta, kad vienoje dulkinėje susidaro 3408 ± 2127 žiedadulkių. Vystymuisi tinkamomis sąlygomis išaugę augalai produkuoja apie 7,4 E + 09 žiedadulkių. Augalo viršutinėje dalyje nustatytas stiprus ryšys tarp moteriškų žiedų graižų klasterių ir sėklų skaičiaus ($r > 0,75$, $p < 0,01$). Viena kietinės ambrozijos šaka turi vidutiniškai 5–6 moteriškų žiedų graižų klasterius, kuriuose, priklausomai nuo šakos vietos augale, susiformuoja nuo 1,6 ± 0,3 iki 2,3 ± 0,7 sėklų. Nustatyta, kad viename augale gali būti 6906 ± 1692 sėklų, kurių viena sveria 2,98 mg (SD = 0,36).

Reikšminiai žodžiai: invazinis augalas, piktžolė, graižai, žiedadulkės, sėklos.