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Effects of Rust Fungus Infection and Aphid Infestation on Plant Height and Seed Production of Impatiens Parviflora DC. Stands

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Abstract

The effects of the rust fungus *Puccinia komarovii* Tranzschel and the aphid *Impatientinum asiaticum* Nevsky 1929 on plant height, seed number per capsule, and seed mass were investigated in eight small balsam (Impatiens parviflora DC.) stands in Hungary and one in Poland. Two stands were infected by rust, five by aphids, and two were healthy. The lowest average plant height was 37.5 cm, the highest 94.7 cm, both measured in aphid-infested stands. Examining the stands separately, no relationship appeared with the type of damage. For data pooled across stands, differences were significant: rust-infected plants were the tallest, healthy shoots were the shortest, while aphid-infected plants fell in between. Mean seed number per capsule was the lowest (1.53) in the pest-free Nagybörzsöny stand, and the highest (2.33) in the aphid-infested Bielsko-Biała stand. In pairwise comparison of stands, average seed number did not differ in most cases. Significant positive correlation was found between average plant height and average seed number per capsule. Seeds were the heaviest in the healthy stand, whereas they were lighter in rust-infected and aphid-damaged stands. The pests tested had no detrimental effect on *Impatiens parviflora*, but both pest types somewhat reduced the seed mass. The applicability of the studied pests as biological control agents against host plant invasion is also discussed.

Keywords: *Impatientinum asiaticum*; invasive neophyte; *Puccinia komarovii*; shoot height; seed mass; seed number; small balsam

1. Introduction

The distribution area of some alien species that have previously introduced to the Hungarian flora is still showing continuous expansion today. [1–4]. In the case of such increasingly invasive species – which include the small balsam (*Impatiens parviflora* DC.) – it is particularly important to gain detailed knowledge about their life history and ecological relationships, as this may be useful in controlling these species.

I. parviflora is native to Central Asia, and its first subspontaneous occurrence in Europe was recorded in 1831 near the Geneva Botanical Garden [5]. Subsequently, in the initial stages of its invasion, it was observed primarily as a garden escapee in various cities [6]. It now occurs in almost all European countries [7–12] and has also been introduced to North America [13]. Its distribution mainly occurs in the understory of closed forests [8], and its detection therefore requires intensive field work. Remote sensing methods, which have been successfully used in the monitoring of other invasive species [14–16], are practically not applicable to *I. parviflora.*

The success of invasive plant species in new geographic regions is often attributed to the absence of natural enemies that keep these species under control in their original habitats [17,18]. Small balsam is now quite common along the riverbanks and in the humid or mesophytic forests of Hungary, and its appearance in these places is usually abundant [19,20] (Figures 1 and 2).

Figure 1. Dense stand of *Impatiens parviflora* at a riverine woodland in Hungary (photo by P. Csontos).

The natural pests of *I. parviflora*, the rust fungus *Puccinia komarovii* Tranzschel and the aphid *Impatientinum asiaticum* Nevsky 1929, have also become known from its Hungarian populations [21]. Host-specific pests of invasive species are often used in biological control [22], although these interventions are not always successful [23]. However, a variety of the aforementioned rust fungus species (*Puccinia komarovii* var. *glandulifera*) is already being used in biological control of *Impatiens glandulifera* Royle in Great Britain [24,25].

In this paper, we seek to answer the question of whether host-specific pests, namely infection by *Puccinia komarovii* and damage by the aphid species *Impatientinum asiaticum*, cause a decrease in

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vitality or a decline in the development and reproductive success of the host plants in Impatiens parviflora stands?

Figure 2. Zygomorphic flower (upper picture) and one-seeded capsules (lower picture) of *Impatiens parviflora* (photos by P. Csontos).

2. Results

The tallest plant height, averaging 94.7 cm, was found in the stand surveyed near Bielsko-Biała (Poland), while the individuals of the stand at Kis-Sváb-hegy (Budapest, Hungary) grew to the lowest, where an average height of 35.7 cm was measured. Interestingly, both extreme average values were found among the stands damaged by aphids (Figure 3), and similarly, the smallest and largest specimens measured among all examined individuals were also found in this group (25 cm and 126 cm, respectively). The stands infected with rust fungus were slightly higher than the healthy stands, but a significant difference was found only in one of the stands. Overall, it can be said that the stands with three different health status (rust or aphid infected and pest-free), if all nine are examined

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independently, did not form clear groups based on the height of their individuals. However, there was a tendency that the stands with rust fungus infection were somewhat taller than the healthy stands.

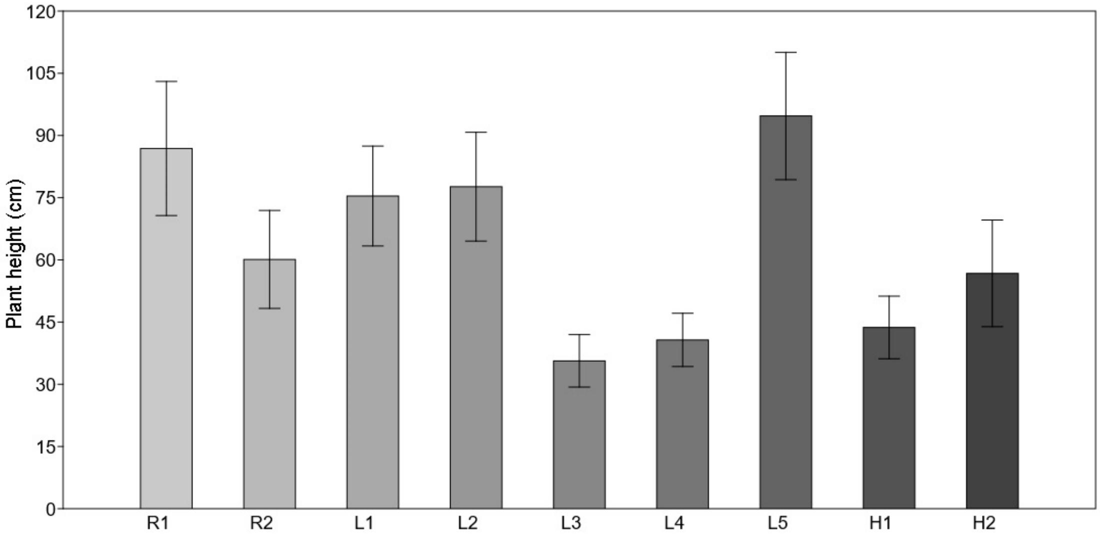

Figure 3. The average height of individuals of *Impatiens parviflora* in pest-exposed and healthy populations. R1–2: infected by rust fungus *Puccinia komarovii*, L1–5: damaged by aphid *Impatientinum asiaticum*, H1–2: healthy stands. See materials and methods section for more information about stands R1–H2.

When data from stands with the same health status were combined, all three types were significantly different in terms of average plant height of *I. parviflora* (Table 1). The rust-infected specimens grew the tallest, followed by the aphid-infected ones, while the healthy plants were the shortest.

Table 1. Statistical comparison of plant height data of healthy, rust-infected and aphid-damaged *Impatiens parviflora* stands. Results of the Kruskal-Wallis test (a) and Dunn's test (b).

(a) Kruskal-Wallis test				
Health category	Number of individuals	Median of plant height (cm)	Sum of ranks	Mean of ranks
Rust infected	40	72,5	4617.0	115.43
Aphid-damaged	100	65,0	9208.0	92.080
Healthy	40	49,5	2465.0	61.625
(b) Dunn's test				
Group 1	Group 2	Difference in rank means	P value	Conventional notation
Rust infected	Aphid-damaged	23.345	P<0.05	*
Rust infected	Healthy	53.800	P<0.001	***
Aphid-damaged	Healthy	30.455	P<0.01	**

When we examined the number of seeds per capsules, we did not find any five-seeded capsules – which would have been the maximum possible [26], and in some stands no four-seeded capsules were found either. Overall, however, capsules with one to four seeds were present in all three stand types (Figure 4). The greatest variability in seed number per capsule (similarly to the case of body height) was observed in aphid-damaged populations.

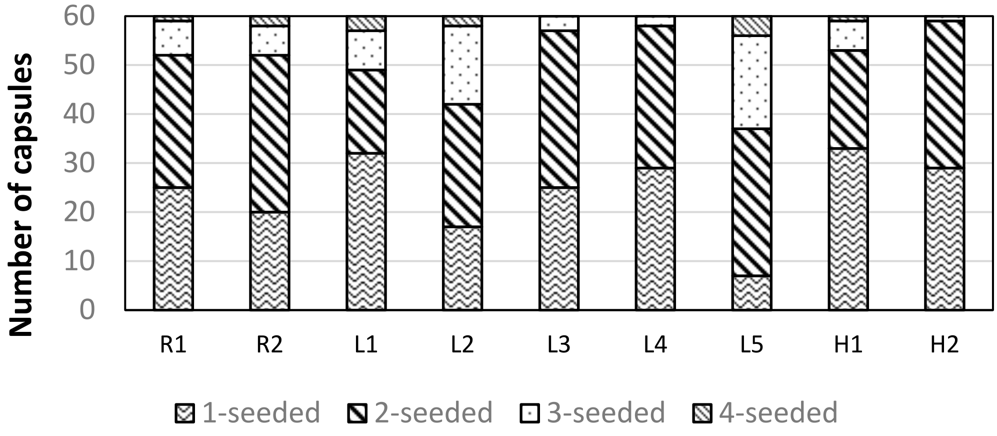

Figure 4. Number of capsules according to their seed content in nine stands of *Impatiens parviflora*. See Figure 3 for explanations of stand labels, and Materials and Methods section for further information about stands.

The results of the statistical analysis performed on the seed number per capsules data are summarized in Table 3. The lowest average seed number was found in the pest-free Nagybörzsöny (H2) stand (1.53 seeds/capsule), and the highest was observed in the aphid-infested (L5) stand in Bielsko-Biała (2.33 seeds/capsule). The number of seeds per capsule of the L5 stand was significantly higher than that of all other stands except the L2 stand. (It should be noted that the height of the individuals was also the tallest in the L5 stand.) Furthermore, the aphid-damaged stand at Normafa (L2) showed significant differences compared to the three populations with very low average number of seeds per capsules – which were the stands surveyed near Nagybörzsöny (H2; 1.53 seeds/capsule), Budakeszi (L4; 1.55 seeds/capsule) and Vámosszabadi (H1; 1.58 seeds/capsule) (Table 3). In the other possible pairings of the stands, their number of seeds per capsule data did not differ significantly.

Table 2. Results of pairwise comparisons of nine *Impatiens parviflora* stands based on the number of seeds per capsule data examined in 60 capsules per stand. The statistical evaluation was based on the Kruskal-Wallis test followed by Dunn's test. (See Table 1 for more information about stands R1–H2; ns= not significant.

Stand pairs compared	Rank-mean difference	Level of significance	P-value
R1 vs. R2	-19,800	ns	P>0,05
R1 vs. L1	17,675	ns	P>0,05
R1 vs. L2	-57,108	ns	P>0,05
R1 vs. L3	13,592	ns	P>0,05
R1 vs. L4	31,475	ns	P>0,05
R1 vs. L5	-109,630	***	P<0,001
R1 vs. H1	33,183	ns	P>0,05
R1 vs. H2	34,058	ns	P>0,05
R2 vs. L1	37,475	ns	P>0,05
R2 vs. L2	-37,308	ns	P>0,05
R2 vs. L3	33,392	ns	P>0,05
R2 vs. L4	51,275	ns	P>0,05
R2 vs. L5	-89,825	*	P<0,05
R2 vs. H1	52,983	ns	P>0,05
R2 vs. H2	53,858	ns	P>0,05
L1 vs. L2	-74,783	ns	P>0,05
L1 vs. L3	-4,083	ns	P>0,05
L1 vs. L4	13,800	ns	P>0,05
L1 vs. L5	-127,300	***	P<0,001
L1 vs. H1	15,508	ns	P>0,05

L1 vs. H2	16,383	ns	P>0,05
L2 vs. L3	70,700	ns	P>0,05
L2 vs. L4	88,583	*	P<0,05
L2 vs. L5	-52,517	ns	P>0,05
L2 vs. H1	90,292	*	P<0,05
L2 vs. H2	91,167	*	P<0,05
L3 vs. L4	17,883	ns	P>0,05
L3 vs. L5	-123,220	***	P<0,001
L3 vs. H1	19,592	ns	P>0,05
L3 vs. H2	20,467	ns	P>0,05
L4 vs. L5	-141,100	***	P<0,001
L4 vs. H1	1,708	ns	P>0,05
L4 vs. H2	2,583	ns	P>0,05
L5 vs. H1	142,810	***	P<0,001
L5 vs. H2	143,680	***	P<0,001
H1 vs. H2	0,875	ns	P>0,05

The relationship between the average heights of the stands and the average number of seeds per capsule of the stands is shown in Figure 7. The correlation coefficient (r) of the line fitted by linear regression was 0.7485, and the slope of the line deviated significantly from zero (P= 0.0203).

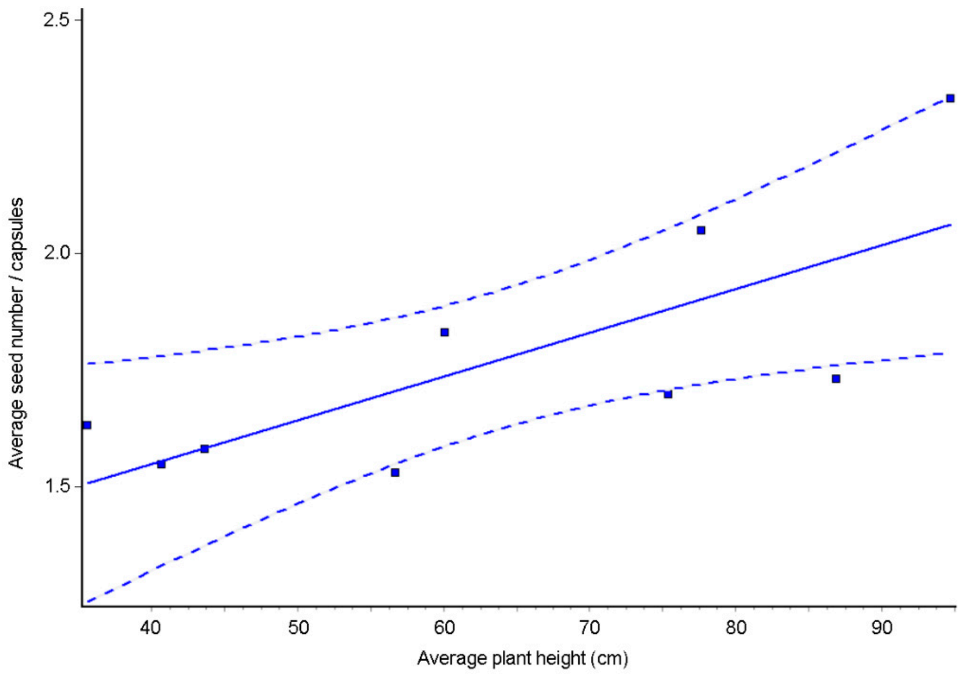

Figure 5. Linear regression between average plant height (cm) and average seed number per capsule (with 95% confidence interval indicated) based on nine stands of *Impatiens parviflora* (r = 0,7485).

The results of the statistical comparison of the seed mass data among stands using the Kruskal-Wallis test and the subsequent Dunn's test are shown in Figure 8. The healthy stand (H1) was characterized by the heaviest seed mass, and it differed significantly from seed masses of all pest-exposed stands. Seed masses of the two rust infected stands fell into the same statistical group, however within aphid damaged stands wider variability of seed mass was detected.

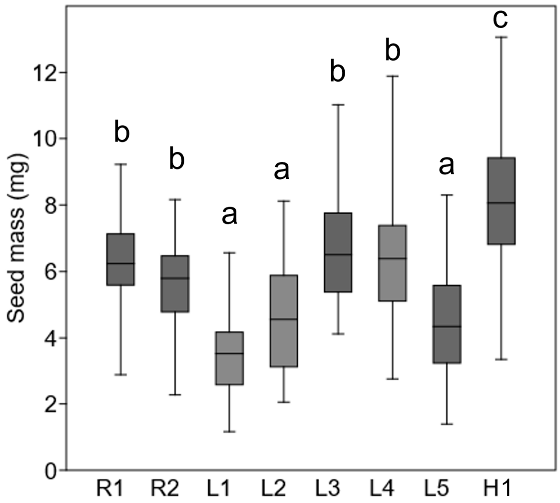

Figure 6. Box and whisker plots of seed masses of *Impatiens parviflora* in healthy (H1) and pest-exposed (R1─2: infected by rust fungus *Puccinia komarovii*, L1─5: damaged by aphid *Impatientinum asiaticum*) stands. Different letters above the boxes indicate significant differences among stands.

3. Discussion

The height of adult individuals of I. parviflora is given by Coombe [26] as 20 cm to 150 cm, by Chmura [27] as 4 cm to 152 cm, and by Csiszár and Bartha [20] as 10 cm to 150 cm. The individual height data observed in the present study are consistent with these data. There is also literature reporting that the average height of individuals of I. parviflora populations in different habitats can differ significantly [6,28,29]. Based on these, it can be reasonably assumed that the site effects in the stands we examined were stronger than the differences that could have been caused by the presence of pests. However, when samples of stands belonging to the same category were treated together, the differences that had previously remained hidden, became detectable due to the increased sample size.

The greatest individual height observed in rust-infected stands may be related to the fact that *P. komarovii* infection can cause shoot and hypocotyl elongation, as has been previously shown for I. parviflora individuals [30,31] and regarding the hypocotyl stem only, for a related species, *I. glandulifera*, too [32]. One explanation for the development of this phenomenon is that the effect of rust fungus on shoot elongation may result in a better dispersal of the spores of such fungi. This is because the spores of the fungus can be carried by the wind from the higher leaves of elongated host plants, where they develop, more easily and over greater distances, so that rust variants with this trait can spread more widely. This selection may ultimately have led to the increasingly frequent presence and then fixation of the trait responsible for shoot elongation in rust fungi.

However, severe rust infection caused stunting and death of individuals of stands in Slovakia [30,33]. All this may be related to the higher humidity of the habitats in Slovakia, which may have had a positive effect on the prevalence of rust fungus infection there, while in the slightly drier conditions of Hungary, rust fungus infection remained more limited.

As for the higher average height of aphid-damaged stands than healthy ones, it is supposed that specimens from stands living in more humid habitats generally grew taller and under such conditions their leaves also have a softer tissue structure than those of specimens growing in drier conditions. Consequently, this plant trait attracts aphids to such *I. parviflora* stands, because they can feed more easily on the softer leaves.

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The average values of the number of seeds per capsule varied between 1.53 and 2.33 in the studied stands. In two stands (L1 and H1) single-seeded capsules and in six other stands (R1, R2, L2, L3, L5 and H2) two-seeded capsules were the most frequent, while in stand L4, single- and double-seeded capsules were equally common. This is a slightly higher fecundity than that observed in *I. parviflora* populations in five Polish forests, where in all cases single-seeded capsules dominated [6]. We did not examine the total seed yield per individual, because determining it would have required an extraordinary amount of field work due to the species' prolonged seed dispersal and the dynamochor capsule type. Based on literature data, in good habitats, in the case of 4-5-seeded capsules, the seed yield can reach 10,000 seeds per individual [26], however, in the stands we examined, the seed yield was certainly significantly lower than this. None of the rust-infected stands showed significant differences from healthy stands in terms of seed number per capsule. Of the five aphid-infested stands, one (L5) showed a higher average seed count compared to both rust-infected and healthy stands, in another case (L2) we measured a higher seed count only compared to healthy stands, but the other three aphid-infested stands did not show significant differences. Therefore, it cannot be stated that the number of seeds per capsule in aphid-infested stands is consistently higher than in other stands. Furthermore, the multiple significant differences detected between individual stands within the group of aphid-infested stands in terms of seed number per capsules may actually indicate that the presence of aphids does not necessarily have a significant effect on this plant trait.

Based on our studies, it seems that the number of seeds per capsule may be primarily affected by the characteristics of the habitat. Trepl [34] also refers to the effect of habitat when he discusses that the seed yield per individual ranges from a few single-seeded capsules to 2000 seeds per individual. In our case, the existence of a site effect is also suggested by the correlation shown by linear regression, in which taller plants were associated with a higher seed number, since taller plant size is generally an indicator of a more favorable habitat. However, the picture is complicated by the fact that rust infection can also cause taller growth, and the quality of the habitat (e.g. its humidity) may also play a role in the development and spread of the infection. Nevertheless, to establish more precise relationships, i.e. to gain a detailed understanding of the relationships between growing site, fungal infection, plant height and seed number, further studies are necessary, involving more *I. parviflora* stands and supplemented by instrumental measurements of habitat variables.

Regarding seed mass, our results show that both rust and aphids significantly reduced the average seed mass, and thus the largest seeds were found in the healthy stand. This means that pest-affected *I. parviflora* plants are generally less able to provide their seedlings with reserve nutrients, which ultimately results in a reduced chance of survival. The average seed mass of the five examined stands damaged by aphids was not the same within the group, as in three stands we measured a significantly lower seed mass was found compared to the other two stands. This suggests that the seed quality of the stands can be influenced not only by the presence of pests, but also by the characteristics of the growing site.

As for the applicability of the pests examined in plant protection, the rust fungus in principle could be a promising candidate due to its high host specificity. In this regard, its negative impact on the seed mass of the host plant deserves attention. Beside to small balsam, *P. komarovii* has so far only been detected on the Himalayan balsam (*I. glandulifera*), but the latter balsam species is affected by a separate variant, *P. komarovii* var. *glanduliferae*, described in 2015 [24]. The aphid, *Impatientinum asiaticum* did not show as close host specificity as rust fungus. It attacks *I. parviflora* and *I. glandulifera* in Hungary [21,35], but it was reported in addition to the above, even on *I. noli-tangere* [38]. However, we consider the use of aphids to be possible in the future, given that they can act as vector organisms and be able to introduce other pathogens, especially plant viruses, into the host plant [39]. Finally, we mention that *I. parviflora* has several other pests besides rust fungus and aphids [35,38], although, the usefulness of these pests in biological control against the invasive host plant has not yet been clearly demonstrated.

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4. Materials and Methods

To investigate the impact of pests, we surveyed nine *I. parviflora* stands. Eight stands were located in Hungary and one in southern Poland (Table 3). Two of the surveyed stands were damaged by the rust fungus *P. komarovii* and five by the aphid *I. asiaticum*. We did not find a stand where both pests occurred simultaneously.

The rust fungus was widespread in the infected stands and was usually observed on the majority of the leaves of individual host plants (Figure 7). In the stands attacked by aphids, the pests also occurred in large numbers, practically on all host plant specimens, with usually 30–60 individuals damaging each plant. Two additional stands were free from pests (Figure 8).

Figure 7. Small balsam specimens infected by *Puccinia komarovii* under old beech stand at Normafa, Buda Mts., Hungary (photo by P. Csontos).

In the inner area of each stand free from edge effects, 20-20 individuals were randomly selected, which were removed from the soil, and their shoot height above the ground was measured with centimetre accuracy. Subsequently, 60-60 mature capsules were selected from additional specimens from the inner area of each stand and the number of mature seeds in them was determined, the maximum value of which, typical for the species, can be 5 seeds per capsule [26]. The seeds found in the capsules were collected for seed weight measurement. The number of seeds from 60 capsules per population varied between 93 and 140. Since some seeds were found to be damaged or diseased, the weight of 70-70 completely healthy seeds per population were measured on a digital analytical balance (Sartorius R180D) with a readability of 0.01 mg.

Table 3. Site characteristics of the sampled *Impatiens parviflora* stands. (R = infected by rust fungus *Puccinia komarovii*, L = damaged by aphid *Impatientinum asiaticum*, H = healthy stand.

Code	Geographical location; vegetation type	GPS coordinates	Altitude above sea level	Health status	Date of sampling (yyyy.mm.dd)
R1	Normafa, Budai Mts.; old beech stand	47° 29' 57.7"N 18° 58' 10.1"E	460 m	R	2013.07.20.
R2	Hárs Mt..Budai Mts.; oak stand	47° 31'44"N 18° 57'22.6"E	390 m	R	2013.07.23.
L1	Denevér street, Budapest; ash grove at valley bottom	47° 29' 35.8"N 18° 59' 34.9"E	327 m	L	2013.07.26.

L2	Normafa, Budai Mts.; gap in a mixed stand of beech and sessile oak	47° 30' 8.4''N 18° 57' 54.6''E	490 m	L	2013.07.29.
L3	Kis-Sváb Hill, Budai Mts.; mixed forest of lime and ash on slopes	47° 30' 17.5''N 19° 00' 40.9''E	197 m	L	2013.07.26.
L4	near Budakeszi, Budai Mts.; sessile oak- hornbeam stand	47° 30' 25.4''N 18° 56' 38.4''E	320 m	L	2013.07.27.
L5	near Bielsko-Biała, Poland; mixed deciduous forest	49° 47' 43''N 19° 06' 48.8''E	450 m	L	2013.09.09.
H1	Vidra farm, Vámosszabadi; riverine willow-poplar woodlands	47° 47' 5.4''N 17° 37' 22.6''E	115 m	H	2013.08.26.
H2	Nagybörzsöny, Börzsöny Mts.; sessile oak-hornbeam forest	47° 55' 42.6''N 18° 50' 36.3''E	270 m	H	2016.07.21.

Figure 8. Healthy specimens of small balsam collected in a sessile oak-hornbeam forest at Nagybörzsöny, Börzsöny Mts., Hungary (photo by P. Csontos).

The height data of the individuals were analyzed in two ways: (i) first, the nine stands were considered separately and compared with each other, (ii) then the individuals of the same type of stands were combined, and the resulting three groups (i.e. rust-infected, aphid-infected and healthy) were analyzed using statistical methods. The Gaussian distribution was not met in any case for all groups to be compared based on the Kolmogorov-Smirnov test, therefore the non-parametric Kruskal-Wallis test was used to evaluate the data. The post-hoc test was the Bonferroni procedure in the first case and the Dunn's test in the second.

The graphical interpretation of the seed number per capsule data of the stands is presented in three groups (1-seeded, 2-seeded, and 3 or more seeded), however, for the statistical comparison of the individual stands, the basic data were used, which were analyzed with the Kruskal-Wallis test and the subsequent Dunn's test. The relationship between plant height and seed number per capsule was examined using linear regression, and linearity was checked using the Wald-Wolfowitz Runs test.

In the case of the seed mass data analysis, we could only consider data from eight stands, as seed mass measurement data were not available for the H2 stand. The comparison of the stands was again carried out using the previously applied Kruskal-Wallis test and the subsequent Dunn's test.

Significant differences were accepted in all cases at $p<0.05$. The InStat [36] and PAST [37] software packages were used to perform the calculations.

5. Conclusions

The impact of pests on the studied plant traits was not uniform. Rust infection increased the height of the host plant. The individuals of the aphid-damaged stands were also taller than those of the healthy stands, although to a lesser extent. In the latter case, the large differences among the studied stands indicate that the effect of the growing location also played an important role. Neither of the two pests tested had a clearly detectable effect on the number of seeds per capsule, but instead a positive correlation was found between the height of the individuals and the number of seeds per capsule. As for the seed weight, both pests significantly reduced the average seed mass in the affected stands, which may be important for biological control against the host plant known as an invasive species. Especially the rust fungus, *P. komarovii* can be considered promising to use in biological plant protection because this fungus shows very high host specificity. In this regard, its reducing effect on the seed mass of the host plant can be important.

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