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The Influence of Genotype and Morphological Characteristics of Rowan (*Sorbus aucuparia*) Leaves on the Development of *Gymnosporangium cornutum*

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ABSTRACT

Rust caused by *Gymnosporangium cornutum* Arthur ex F. Kern is one of the most common diseases of rowan (*Sorbus aucuparia* L.). Currently, there is little information on the development of this disease in forestry literature. In order to eliminate this deficiency, the examination of the influence of *Sorbus aucuparia* genotype and leaf dimensions on the occurrence and abundance of aecia of *Gymnosporangium cornutum* was carried out. The genotype had a statistically significant influence on aecia occurrence, while leaf length, width, circumference, and area had a statistically significant influence on aecia abundance. About 10% of *Sorbus aucuparia* genotypes showed greater tolerance to *Gymnosporangium cornutum*, with a nearly 4-fold lower probability of aecia occurring on leaves. Larger leaves of *Sorbus aucuparia* sensitive genotype had a greater *Gymnosporangium cornutum* aecia abundance compared to smaller leaves. The results obtained for the first time indicate the possibility of selecting and producing reproductive material of *Sorbus aucuparia* tolerant to *Gymnosporangium cornutum*.

Keywords: aecial stage; plant breeding; gene pool; protection measures

INTRODUCTION

Rowan or mountain ash (*Sorbus aucuparia* L.) is one of the most widespread tree species in Europe, except for the southernmost parts and large islands (Räty et al. 2016). This tree species has significant ecological importance in forest ecosystems (Cvjetićanin et al. 2016). Moreover, it represents a source of antioxidants, tocopherols, and pigments (Šavikin et al. 2017).

So far, the variability of characteristics of *Sorbus aucuparia* has been determined. First of all, there is a difference in the morphological parameters of different *Sorbus aucuparia* half-sib families (Sæbø and Johnsen 2000), while the size of anatomical elements differed between certain genotypes (Umarov et al. 2024). There was a difference in time and degree of fruit bearing between different Sorbus aucuparia genotypes (Munilla and Guitián 2014), and seeds from different *Sorbus aucuparia* populations showed differences in germination (Raeisi et al. 2021). In addition, the fruits of *Sorbus aucuparia* had different colours, pH values, antioxidant activities, and contents of vitamin C, organic acids, sugars, and phenolic components depending on the genotype of the trees (Bozhuyuk 2021).

Genus *Gymnosporangium* consists of significant fungal parasites of trees and shrubs, whereby there is a small number of scientific publications related to this genus and its species (Lâce 2017). Rust caused by *Gymnosporangium cornutum* Arthur ex F. Kern causes great damage to Sorbus aucuparia (Karadžić and Milijašević 2003). The pathogen is a heteroecious rust fungus with an incomplete life cycle: spermagonia and aecia are formed on *Sorbus aucuparia*, whereas the primary host is juniper (*Juniperus* spp.), on which telia and basidia are produced (Ellis and Ellis 1985, Karadžić and Milijašević 2003). The characteristic of rust is pronounced yellow-orange aecia of relatively large dimensions on *Sorbus aucuparia* leaves (Karadžić and Milijašević 2003).

When controlling rust on forest species, there are many challenges, including insufficient efficacy in removing the alternative host and the consequences of applying chemical measures (Hamelin 2013). The genetic control of rust pathogens was recognized more than a hundred years ago, and recently this method of protection has been primarily strived for (Oliver 2024).

In accordance with the above, the objective of this research was to examine whether genotypic and morphological variability of *Sorbus aucuparia* leaves influences the development of rust caused by *Gymnosporangium cornutum*. The tested null hypotheses were: I) *Sorbus aucuparia* genotype does not influence the occurrence of *Gymnosporangium cornutum* aecia; II) *Sorbus aucuparia* leaf dimensions do not affect the occurrence of *Gymnosporangium cornutum* aecia; III) In the case of the sensitive genotype of *Sorbus aucuparia*, there is no difference in abundance of *Gymnosporangium cornutum* aecia on leaves of different dimensions.

MATERIAL AND METHOD

Plant Material

Ten rowan (*Sorbus aucuparia*) trees, 7-8 years old, were sampled on the site Bukovica, Montenegro. The distance between the trees was 20 m. The presence of rust caused by *Gymnosporangium cornutum* was confirmed by the presence of aecia. The aeciospores were 20-30 × 18-25 μm, and the aecia were 4-5 mm, within standard dimensions (Figure 1, Figure 2), matching the description of Ellis and Ellis (1985).

For the analysis of the occurrence of aecia, from each tree, 35 leaves were collected at breast height from the middle of the crown by completely random selection. From the tree with a visually pronounced occurrence of aecia, an additional 100 leaves were collected for the analysis of the abundance of aecia. The analysis of aecia abundance was conducted on leaves collected from a single selected tree with pronounced infection in order to assess within-genotype relationships between leaf morphology and the number of aecia. Moreover, leaves were collected from a single tree to avoid potential genotype effects on leaf abundance. Due to a large number of variables, the genotype was excluded from the analysis of aecia abundance.

The leaves were packed in plastic bags and transported to the Institute of Forestry's laboratory in Belgrade. Until the moment of measurement, they were stored in the refrigerator for 24 hours.

Aecia were counted on the underside of leaves. Immediately afterwards, the leaves were scanned, and

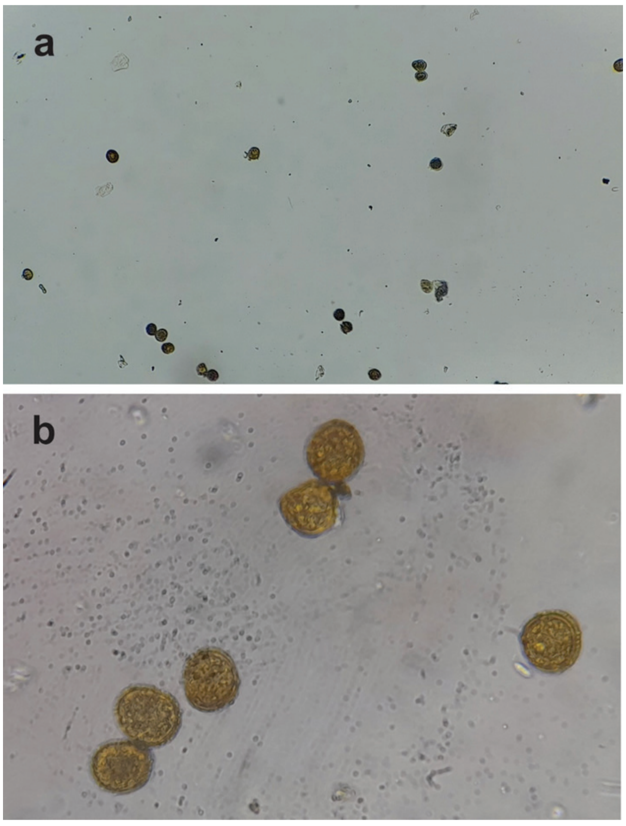

Figure 1. Aeciospores of *Gymnosporangium cornutum*: **(a)** remote representation, 100×; **(b)** close representation, 400×.

leaf blade length, width, leaf circumference, and area were calculated using LAMINA software (Umea University) (Table 1).

Statistical Methods

Binary logistic regression was used for testing the influence of genotype, leaf blade length, leaf blade width, leaf circumference, and leaf area of *Sorbus aucuparia* on the occurrence of *Gymnosporangium cornutum* aecia. The exponential parameter estimates were used to calculate the probability of aecia occurrence.

A general linear model (GLM) was used to test differences in the average *Gymnosporangium cornutum* aecia values across *Sorbus aucuparia* leaf dimensions. Pearson's correlation was used for the analysis of a link between the *Sorbus aucuparia* leaf dimension and the number of *Gymnosporangium cornutum* aecia.

RESULTS

Occurrence of *Gymnosporangium cornutum* Aecia

Binary logistic regression showed statistically significant influence of genotype (Table 2, Figure 2, Figure 3) on the occurrence of *Gymnosporangium cornutum* aecia on *Sorbus aucuparia* leaves. Genotype 1 had the highest probability of occurrence of *Gymnosporangium cornutum* aecia (Exp(B) = 20.503, Figure 2). Genotype 7 had the highest probability of absence of *Gymnosporangium cornutum* aecia (Exp(B) = 3.713, Figure 3). The remaining 80% of genotypes were at the transition between the lowest and highest tolerance to *Gymnosporangium cornutum* (Figure 2).

Abundance of *Gymnosporangium cornutum* Aecia

The general linear model showed that, within the analysed genotype, the number of *Gymnosporangium cornutum* aecia on leaves was influenced by leaf length, width, circumference, and area (Table 2, Figure 2). Pearson's correlation showed statistically significant and positive correlation between leaf length and number of aecia ($r = 0.229$, $p = 0.022$), leaf width and number of aecia ($r = 0.478$, $p < 0.001$), leaf circumference and number of aecia ($r = 0.313$, $p = 0.002$), and leaf area and number of aecia (0.402, $p < 0.001$) within the analyzed *Sorbus aucuparia* genotype. The number of aecia ranged between 1 and 21 per leaf.

Table 1. Dimensions of leaves of tested rowan (*Sorbus aucuparia*) genotypes.

Genotype	Leaflet length (mm)	Leaflet width (mm)	Circumference (mm)	Area (mm^2)
1	53.41 ± 9.61	18.54 ± 2.14	122.39 ± 19.66	786.20 ± 196.97
2	48.94 ± 9.37	16.68 ± 2.14	114.34 ± 19.60	648.10 ± 191.74
3	43.57 ± 8.87	17.52 ± 2.75	99.46 ± 21.18	576.31 ± 223.80
4	40.11 ± 7.62	16.68 ± 2.84	90.79 ± 18.61	504.67 ± 182.10
5	42.88 ± 7.42	15.52 ± 1.59	95.43 ± 16.37	508.67 ± 130.44
6	44.42 ± 8.02	16.47 ± 2.49	99.57 ± 18.14	569.90 ± 180.08
7	37.11 ± 8.62	14.45 ± 2.64	83.78 ± 19.97	417.59 ± 169.99
8	44.49 ± 5.66	18.24 ± 2.31	99.93 ± 13.01	620.42 ± 146.37
9	36.73 ± 6.10	14.94 ± 2.02	84.50 ± 12.55	427.45 ± 108.54
10	36.54 ± 8.58	14.71 ± 2.03	84.08 ± 20.60	418.06 ± 143.21

Table 2. The influence of genotype and morphology of rowan (*Sorbus aucuparia*) leaves on the occurrence of *Gymnosporangium cornutum*.

Source	Wald-Chi Square	df	Significance
Genotype	30.752	9	<0.001
Leaflet length	0.216	1	0.642
Leaflet width	1.048	1	0.306
Circumference	2.071	1	0.150
Area	2.641	1	0.104

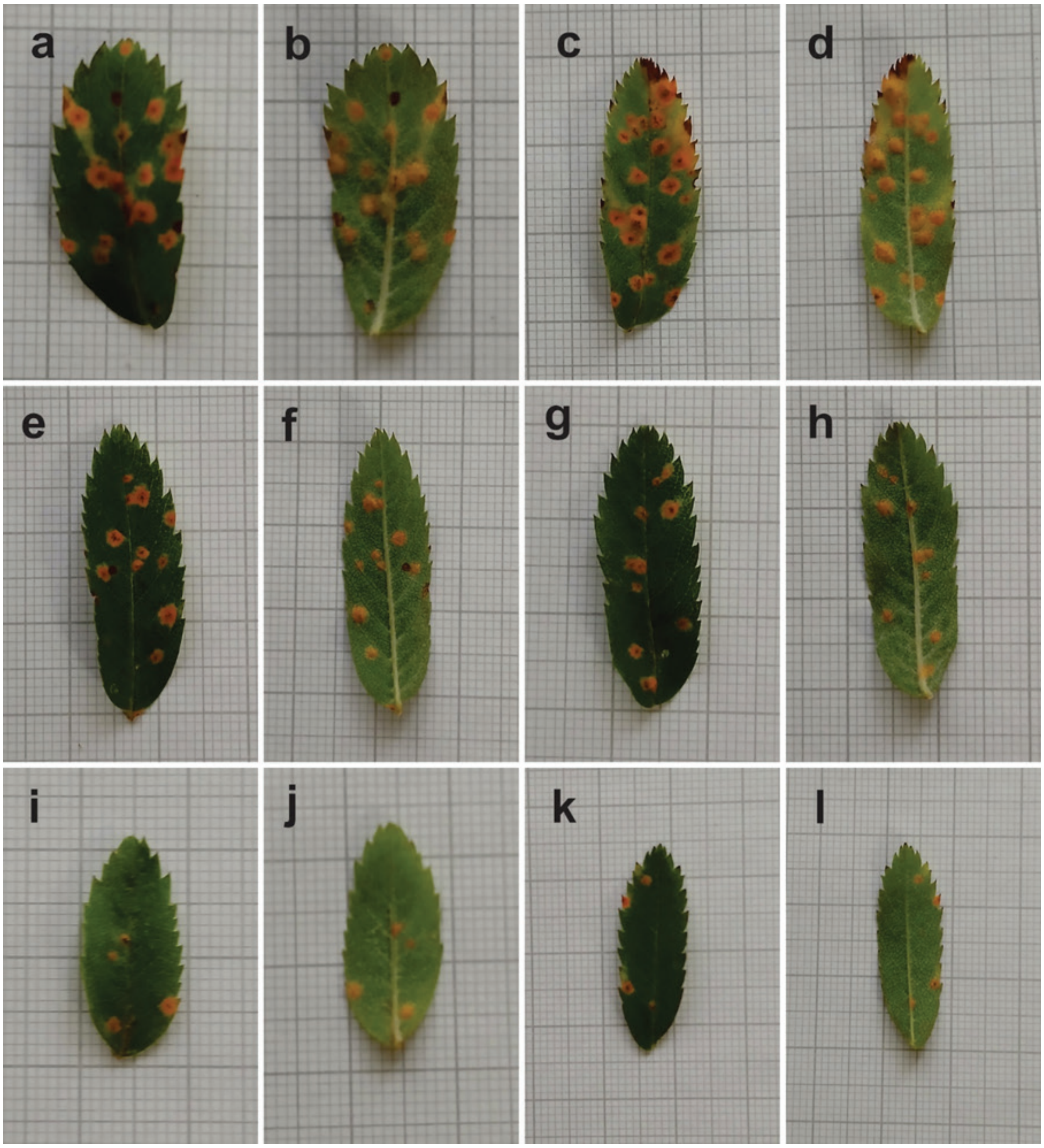

Figure 2. The abundance of *Gymnosporangium cornutum* aecia on rowan (*Sorbus aucuparia*) leaves within the analyzed genotype: **(a, c)** symptoms on the front (adaxial) side of sensitive leaves; **(b, d)** aecia on the back (abaxial) side of sensitive leaves; **(e, g)** symptoms on the front (adaxial) side of moderately tolerant leaves; **(f, h)** aecia on the back (abaxial) side of moderately tolerant leaves; **(i, k)** symptoms on the front (adaxial) side of tolerant leaves; **(j, l)** aecia on the back (abaxial) side of tolerant leaves.

Table 3. The influence of the morphology of rowan (*Sorbus aucuparia*) leaves on the abundance of *Gymnosporangium cornutum* aecia within the analyzed genotype.

Source	Type III Sum of Squares	df	Mean Square	F	Significance
Leaflet length	131.636	1	131.636	5.438	0.022
Leaflet width	571.983	1	571.983	29.013	<0.001
Circumference	245.373	1	245.373	10.646	0.002
Area	403.979	1	403.979	18.852	<0.001

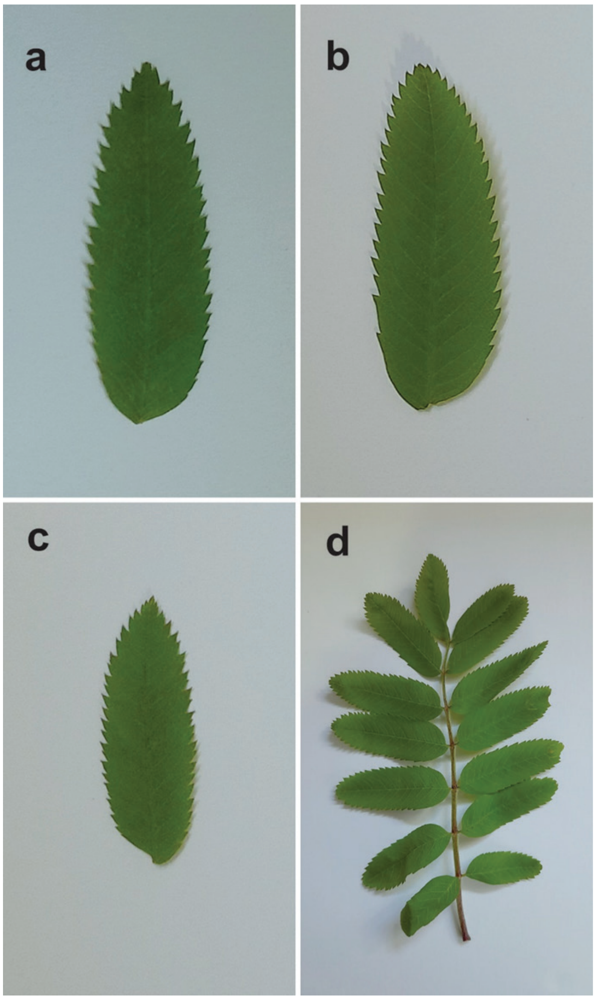

Figure 3. Leaves of *Sorbus aucuparia* tolerant on the occurrence of *Gymnosporangium cornutum* aecia.

DISCUSSION

The genotype of *Sorbus aucuparia* showed great significance, while the dimensions of leaves did not show an influence on the occurrence of *Gymnosporangium cornutum* symptoms. The first null hypothesis is rejected. The alternative hypothesis is accepted that the *Sorbus aucuparia* genotype influences the occurrence of *Gymnosporangium cornutum* aecia. On the other hand, the second null hypothesis that *Sorbus aucuparia* leaf dimensions do not affect the occurrence of *Gymnosporangium cornutum* aecia was accepted. A limitation of this study is that the analysis of aecia abundance in relation to leaf morphology was conducted on a single *Sorbus aucuparia* genotype. Consequently, the observed relationships describe within-genotype variation and should not be generalised to the species level without further multi-genotype validation. Monitoring the health of trees is necessary to ensure long-term resistance to rust, prevent or limit pathogen spread, and reduce the risk of pathogen evolution and the breakdown of host resistance (Hamelin 2022). Genotypes were not genetically verified. In this sense, it would be interesting in the future to monitor, over several years, the influence of micro-locality conditions and ontogenetic factors on the health status of *Sorbus aucuparia* seedlings that exhibited less pronounced symptoms caused by *Gymnosporangium cornutum*. Moreover, the proximity of both hosts required for the spread of *Gymnosporangium cornutum*, primarily common juniper (*Juniperus communis* L.), may interact strongly with the aforementioned factors under increased infection pressure. Therefore, these phenomena should be studied jointly. In this sense, we consider that increasing *Sorbus aucuparia's tolerance* (i.e., mitigating the harmful effects of infection) based on the results of this study provides a stable approach to controlling *Gymnosporangium cornutum*.

For the increase in the representation of *Sorbus aucuparia-tolerant* trees, it is necessary to adjust the methods of regeneration of this species. Examples of the introduction of wild service tree (*Sorbus torminalis* (L.) Crantz), a species belonging to the same genus, showed a positive effect on biodiversity, resistance, and ecosystem functions (Višnjić et al. 2025). Fruit bearing of *Sorbus aucuparia* trees is stimulated by tree diameter and light availability, while competition has a negative effect (Kondrat et al. 2024). The mentioned factors must be considered during natural regeneration. The largest production of

Sorbus aucuparia seeds happens just before the dieback of trees (Pesendorfer et al. 2019). Therefore, in the case of artificial regeneration, it is recommended to wait for an abundant yield of trees with weak rust symptoms to increase the amount of collected high-quality seed. To determine which part of the *Sorbus aucuparia* crown is most susceptible to *Gymnosporangium cornutum*, we recommend the application and analysis of mixed-effects models. Furthermore, to improve the use of tolerant seedlings across different sites, it is necessary to consider the microclimatic characteristics of habitats, which may be more favorable to this type of reproductive material.

Within the sensitive genotype of *Sorbus aucuparia*, a larger number of *Gymnosporangium cornutum* aecia was recorded on larger leaves. Thus, the third null hypothesis was rejected. The alternative hypothesis is accepted: in the case of a sensitive genotype of *Sorbus aucuparia*, there is a difference in the abundance of *Gymnosporangium cornutum* aecia on leaves of different sizes. However, we consider that it is not useful to favour the development of the smaller leaves of *Sorbus aucuparia*. Especially because the effect of leaf size was examined within the genotype susceptible to *Gymnosporangium cornutum*. Larger leaves have several advantages, among which better survival in the shade, greater photosynthesis, more efficient use of water and nutrients, faster acclimatisation, and larger ecosystem productivity stand out (Murphu et al. 2012, Milla and Matesanz 2017, Wright et al. 2017, Song et al. 2018, Lusk et al. 2019, Li et al. 2020). Therefore, the obtained results should primarily be used as a recommendation that *Sorbus aucuparia* trees with small leaves, which are usually a consequence of phenotype or unfavourable habitat conditions, should not be removed from stands affected by *Gymnosporangium cornutum.*

To preserve *Sorbus aucuparia* populations at the edges of the distribution area, it is necessary to connect trees through habitat restoration or the introduction of genetic material (Yousefzadeh et al. 2021). We consider that both approaches can be used for the protection of *Sorbus aucuparia* trees on the edges of the distribution area from *Gymnosporangium cornutum*. During habitat restoration, the focus should be on encouraging the development of trees with less pronounced symptoms, while during the introduction of genetic material, preference should be given to reproductive material obtained from such trees.

Individual mother trees are more important than the population for progeny quality (Sæbø and Johnsen 2000). Therefore, *Sorbus aucuparia's* high tolerance to *Gymnosporangium cornutum*, determined at the individual level, enables an easier initial material selection process with greater flexibility in selecting trees.

CONCLUSIONS

This study demonstrated the significance of genotype and leaf morphology of *Sorbus aucuparia* for the aecial stage of the development of rust caused by *Gymnosporangium cornutum* fungus. The tree genotype influenced the occurrence of *Gymnosporangium cornutum* aecia on *Sorbus aucuparia* leaves, whereas leaf dimensions influenced aecia abundance. For the first time, this study provides a basis for the selection of *Sorbus aucuparia* mother trees with reduced susceptibility to *Gymnosporangium cornutum*, which may contribute to slowing pathogen spread. Future research should focus on progeny tests to obtain the most tolerant reproductive material, which will also be suitable for other forestry needs.

Author Contributions

AV and VP conceived the idea and designed the study, conducted fieldwork, and performed statistical analyses. SL, AL, and LJR performed laboratory analyses. VP, MV, and MĆ performed data curation and visualisation of results. AV, AL, and VP wrote the manuscript.

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Conflict of Interest

The authors declare no conflict of interest.

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