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Evaluation of resistance to *Pseudoperonospora humuli* and of the content of alpha acids and hop oils in hops of selected genetic resources of hop *Humulus lupulus* L.

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Abstract: Twenty hop genotypes were selected for the evaluation of resistance to primary and secondary *Pseudoperonospora humuli* infection and of alpha acid and hop oil content in the hops. From the wild hop genotypes, two from Canada and one from Belgium showed resistance. Among the registered hop varieties, the Czech varieties Kazbek and Boomerang were the most resistant. Both wild hop genotypes from Canada showed the highest content of alpha acids among the wild hop entries, namely 4% w/w. The lowest variability of the alpha acid content in the wild hop category was found in two wild hop varieties from the Caucasus, one from Austria and one from Lithuania. The highest content of hop oils was determined in two hop genotypes from Canada and two from Belgium. Wild hop genotypes from the Caucasus have the lowest variability of hop oils among the wild hop entries. Two hop genotypes from Canada and one from Belgium were selected for breeding aimed at drought resistance.

Keywords: hop downy mildew; hop genotypes; *Humulus lupulus* L.; primary and secondary infection; resistance

In the Czech Republic, genetic resources of hops are part of the "National Programme on Conservation and Utilization of Plant Genetic Resources and Agrobiodiversity". The Czech genetic resources of hops are kept in an *ex-situ* field collection (Charvátová et al. 2017). Wild hops have enormous importance for the development of new hop varieties (de Witte & Stocklin 2010). Wild hops are selected by natural selection. As a result, they gain important characteristics which are further used for breeding aimed at resistance to fungal diseases, pests, and drought. Wild hops are part of breeding programmes. In the Czech Republic, the first variety with Russian wild hops in its origin, Kazbek, was registered in 2008 (Nesvadba et al. 2013). Now, genotype 5495 is undergoing registration tests. It originated from wild hops from Canada (Straková et al. 2020). Depending on their origin, wild hops show different chemical compositions. Wild hops from North America have a different composition of hop resins (Hampton et al. 2002) and are part of a separate genetic group (Patzak et al. 2010). During expeditions, wild hops from dry areas are collected (Nesvadba et al. 2009). Subsequently, they can be used for breeding aimed at drought resistance. At the same time, these genotypes must show resistance to *Pseudoperonospora humuli.*

Hop downy mildew is currently the most serious fungal disease threatening hop production in all

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hop-growing areas in the northern hemisphere and in Argentina (Ojijambo et al. 2015). The extent of the losses depends on the susceptibility of the variety, the onset of infections and weather conditions. Late infection during the period of flowering and the creation of hop cones results in stopping the development. However, it also brings about a reduction in the content of bitter acids, which eventually has an impact on the market value of the hops (Gent 2015).

Pseudoperonospora humuli reproduces both sexually and asexually. These changes in the life cycle allow the pathogen to survive in unfavourable conditions in the environment and to spread rapidly under optimal conditions. A typical symptom of invasion by the pathogen *Pseudoperonospora humuli* is spike-like shoots. Affected shoots grow from an infected hop rhizome, which is a primary infection. The development of spike-like shoots is closely connected with the growth of hop plants after dormancy in the spring season and can be predicted based on a short-term prognosis of hop downy mildew (Mitchell 2010). The first signs can appear on young shoots early in the spring (a more humid spring) or at the beginning of summer (Chee et al. 2006). A secondary infection spreads during the vegetation period. Buds, apical meristems, leaves, inflorescence, and cones become infected. The affected cones can barely close. Their aroma is unimpressive and their value for brewing beer is lower due to a reduction in the content of bitter substances (by up to 25%) and polyphenols (Royle & Kremheller 1981).

Appropriate spraying against a primary infection is key for the correct functioning and treatment against a secondary infection from *Pseudoperonospora humuli*. It is a prerequisite for success in combating secondary infections. Growing hop varieties resistant to hop downy mildew can significantly reduce the intensity of the protection. However, the need for fungicidal interventions will not be fully eliminated.

The content of alpha acids is very important when evaluated in commercial and beer-brewing contexts. Hop varieties are purchased at prices per kilogram of alpha acids according to hop categories – aroma hops or bittering hop varieties. Alpha acids give beer the necessary bitterness (Mikyška & Krofta 2012). The content of hop oils is very important for the new category of "flavour hops" with specific aromas. These hops are used for dry hopping (Nesvadba et al. 2016). Good input materials are the basis for breeding research (Čerenak et al. 2015).

MATERIAL AND METHODS

As to the evaluation methodology of the field collection, the planting of new genotypes is repeated three times and evaluated over a period of 5 years. The evaluation was performed in the years 2016 to 2020 within the Czech Republic's collection of genetic hop resources. The collection is located in Stekník, near Žatec. The hop field is situated in a warm and dry region at an altitude of 215 m a.s.l. The sum of temperatures above 10 °C is 2 600–2 800 °C per year. As far as the pedological characteristics are concerned, the soils in the region are alluvial.

Plant material. Benchmark genotypes: registered Czech varieties Kazbek, Blues, Boomerang, Gaia, and the new genotype N2. English variety Pilgrim.

Wild hops: 14 wild hops from the following countries, characterised as very dry localities without groundwater, arid areas with sparse vegetation (perennial and annual grasses and other herbaceous plants) were selected: Austria, Canada, Belgium, Spain, Russia (Caucasus), Lithuania, Switzerland, the USA. The objective is to gain new genetic material for breeding aimed at drought resistance, which will also show resistance to *Pseudoperonospora humuli.*

Evaluation of the resistance to *Pseudoperonospora humuli* was based on the Hop Classifier (Rígr & Fáberová 2000) according to a point scale and is divided into two categories:

(1) Primary infection (occurrence of spike-like shoots)
- 3 – resistant (no occurrence of spike-like shoots)
- 5 – medium resistance (1 to 5 spike-like shoots were found)
- 7 – susceptible (6 and more spike-like shoots were found)

(2) Secondary infection (damage to hop cones – 500 randomly selected hop cones)
- 3 – resistant (no damage)
- 5 – medium resistance (damage below 10% of hop cones)
- 7 – susceptible (damage above 10% of hop cones)

Each year, eight individual hop plants in four repetitions were evaluated. In total, two evaluations were conducted, one in the spring season – measurements of the primary infection. The primary infection was evaluated before training, depending on the timing of the pruning. The second evaluation was accomplished during hop harvest – measurements of the secondary infection. Both evaluations were performed according to the Methodology of hop collection (Nesvadba et al. 2018). The native infection in field conditions,

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with the accompanying weather components of the temperature, precipitation and relative humidity data, was evaluated. It should be noted; the hops were not chemically treated during the monitored growing seasons. Therefore, the weather conditions were favourable for spreading the disease in each year.

Prior to the analyses, the obtained hop cones were dried at a constant temperature of 55 °C. The chemical analyses to determine the content and composition of the hop resins in the hop cones were performed using the High-performance liquid chromatography (HPLC) method (EBC 7.7 1998) and the content of hop oils was analysed based on gas chromatography (Verlang 1998). The values determined in the analyses are based on a 100% dry substance.

Basic statistical methods were used for the evaluation: the average, standard deviation and variability are expressed in % (100 times the coefficient of variation). The *t*-test was used to determine the difference between the hop varieties. The difference between the sets is established based on a significance level α, which determines the probability of the difference between the tested sets (Meloun & Mitický 1994).

RESULTS AND DISCUSSION

Genotypes with an average value of 3.00 (no spike – like shoots in this genotype during the time of evaluation) show significantly different resistance to a primary infection by *Pseudoperonospora humuli* than the other genotypes (Table 1). This group includes eight wild hops and the only two registered Czech varieties (Kazbek and Boomerang) and one English variety (Pilgrim). Five other genotypes with an average value of 4.20 have a significantly different resistance only compared to a group of genotypes with an average resistance of 5.80. This group includes the Czech variety Gaia and genotype N2. No statistical significance was determined among the other genotypes. The results show that the Czech variety Blues, Ursdon from the Caucasus, Rhona from Switzerland, and Madame from Spain have the highest susceptibility. The wild hops Ursdon from the Caucasus, Rhona from Switzerland and Madame from Spain have the highest susceptibility to a primary infection (7 points), but this susceptibility was not found in the same years. This means that

Table 1. Evaluation of the resistance to the primary infection caused by *Pseudoperonospora humuli* in the selected hop genotypes (Stekník 2016–2020)

Genotype	Origin	Average (% w/w)	SD	*CV* (%)
Francuzy	Lithuania	3.00	0.000	0.00
Kazbek	Czech Republic	3.00	0.000	0.00
Pilgrim	England	3.00	0.000	0.00
Boomerang	Czech Republic	3.00	0.000	0.00
Belt	USA	3.00	0.000	0.00
Sunža	Caucasus	3.00	0.000	0.00
Kabarda	Caucasus	3.00	0.000	0.00
Poperinge	Belgium	3.00	0.000	0.00
Fishing lakes	Canada	3.00	0.000	0.00
Antler	Canada	3.00	0.000	0.00
Toses D'alas	Spain	3.00	0.000	0.00
Kauno	Lithuania	4.20	1.095	26.08
Gaia	Czech Republic	4.20	1.095	26.08
Boekhoute	Belgium	4.20	1.095	26.08
P132	Austria	4.20	1.095	26.08
N2	Czech Republic	4.20	1.095	26.08
Blues	Czech Republic	5.00	0.000	0.00
Ursdon	Caucasus	5.00	1.414	28.28
Rhona	Switzerland	5.80	1.095	18.89
Madame	Spain	5.80	1.095	18.89

SD – standard deviation; *CV* – coefficient of variation

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it is not influenced by a particular year. Instead, it has a genetic basis.

Only four wild hops and the N2 genotype show an average value of resistance to a secondary infection from *Pseudoperonospora humuli* (Table 2). Four other genotypes, three of them of Czech origin (Kazbek, Boomerang and Blues) and the Ursdon wild hops from the Caucasus, have an average resistance value of 3.4. These two groups of genotypes have a significantly different resistance than the genotypes with an average resistance of 4.50 or 5.00 (with 99% probability). The statistical significance was not determined among the groups of genotypes with average values of 3.00, 3.40 and 3.80. It is interesting that the English variety Pilgrim with an average resistance value of 4.50 has a high susceptibility. The highest susceptibility to the secondary infection was found in seven genotypes with an average value of 5.00. At the same time, with 99% probability, these genotypes have a significantly different resistance than the other hop genotypes. The results show that no genotype has the highest susceptibility (7 points). All the genotypes fall into the categories resistant or medium resistance (3 or 5 points).

The resistance class means no occurrence of spike-like shoots in the primary infection and no damage on the hop cones in the secondary infection. These genotypes have an increased durability to infection, or the symptoms are mild. There are no serious losses in production even if favourable weather conditions occur.

Within the evaluation of both the primary and secondary infection, the wild hops Boekhoute from Belgium and Fishing lakes from Canada, as well as the Czech variety Kazbek, have the highest resistance with an average value of 3.00. From the perspective of statistical significance, these three genotypes are complemented by the Czech variety Boomerang and the Antler wild hops from Canada, which both have average resistance values to a primary infection of 3.00 and average resistance values to a secondary infection of 3.40 and 3.80, respectively. The results show that it was possible to gain three new wild hops for hop breeding aimed at drought resistance, which also show resistance to *Pseudoperonospora humuli*. In addition, the Czech varieties Kazbek and Boomerang are suitable also. The Madame wild hops from Spain

Table 2. Evaluation of the resistance to the secondary infection caused by *Pseudoperonospora humuli* in the selected hop genotypes (Stekník 2016–2020)

Genotype	Origin	Average (% w/w)	SD	*CV* (%)
Rhona	Switzerland	3.00	0.000	0.00
Boekhoute	Belgium	3.00	0.000	0.00
Poperinge	Belgium	3.00	0.000	0.00
Fishing lakes	Canada	3.00	0.000	0.00
N2	Czech Republic	3.00	0.000	0.00
Kazbek	Czech Republic	3.40	0.894	26.31
Boomerang	Czech Republic	3.40	0.894	26.31
Ursdon	Caucasus	3.40	0.894	26.31
Blues	Czech Republic	3.40	0.894	26.31
Gaia	Czech Republic	3.80	1.095	28.83
P132	Austria	3.80	1.095	28.83
Antler	Canada	3.80	1.095	28.83
Pilgrim	England	4.50	1.000	22.22
Francuzy	Lithuania	5.00	0.000	0.00
Kauno	Lithuania	5.00	0.000	0.00
Belt	USA	5.00	0.000	0.00
Sunža	Caucasus	5.00	0.000	0.00
Kabardina	Caucasus	5.00	0.000	0.00
Madame	Spain	5.00	0.000	0.00
Toses D'alas	Spain	5.00	0.000	0.00

SD – standard deviation; *CV* – coefficient of variation

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with the highest susceptibility to both the primary and secondary infection are wholly unsuitable.

The results (Tables 3 and 4) show that all Czech varieties and the English variety have a higher content of alpha acids than the tested wild hops. However, a statistical significance, when compared to wild hops, was determined in all the registered hop varieties except for Kazbek, which showed a significant difference from the wild hops with an alpha acid content below 3% w/w. The English variety Pilgrim does not have any statistical significance with respect to the Antler wild hops from Canada. With a probability of 99%, the Antler resistant hops from Canada show a significant difference in the content of alpha acids compared to the wild hops with a content below 3% w/w. The second Canadian hop variety (Fishing lakes) resistant to *Pseudoperonospora humuli* has a significantly different alpha acid content than the wild hops with a content below 2.50% w/w (95% probability) and the wild hops with an alpha acid content below 2.00% w/w (99% probability). The third wild hops, Poperinge from Belgium, have an average alpha acid content of just 1.99% w/w. All three resistant hops show variability in the alpha acid content of between 20.25% and 33.06%. This range is similar to that of the Czech hop varieties Gaia, Vital, Bor, Harmonie, Sládek, Bohemie Saaz Late and Saaz (Nesvadba et al. 2020). Among the wild hops, variability below 20 % in the alpha acid content was found in four wild hops (Sunža from the Caucasus, 132 from Austria, Francuzy from Lithuania, and Kabardina from the Caucasus). By contrast, Toses D'alas from Spain has the highest variability in the alpha acid content (48.05%). The alpha acid content in the wild hops ranges between 1.35 and 4.24% w/w. This range is lower than that of the entire collection of wild hops, which is between 0.10 and 8.87% w/w (Nesvadba et al. 2011).

All the Czech hop varieties and the N2 genotype have a significantly different hop oil content than all the wild hops. The English variety Pilgrim has a significantly different hop oil content only among wild hops with a content below 0.45% w/w. Among the wild hops, the resistant wild hops show a high hop oil content, which is significantly different from the wild hops with a hop oil content below 3.00% w/w. The Poperinge resistant wild hops from Belgium have a variability in the hop oil content of 24.58 %, but both resistant wild hops from Canada have a high variability in the hop

Table 3. Average content and variability in the alpha acid in the selected hop genotypes (Stekník 2016–2020)

Genotype	Origin	Average (% w/w)	SD	*CV* (%)
Gaia	Czech Republic	13.34	1.654	12.40
Boomerang	Czech Republic	12.06	0.954	7.91
Blues	Czech Republic	7.47	0.842	11.27
Pilgrim	England	6.96	1.646	23.64
Kazbek	Czech Republic	5.42	0.994	18.35
Antler	Canada	4.24	0.859	20.25
N2	Czech Republic	4.13	0.972	23.56
Fishing lakes	Canada	4.09	1.353	33.06
Boekhoute	Belgium	2.86	0.893	31.24
P132	Austria	2.74	0.449	16.39
Belt	USA	2.64	0.623	23.56
Kabardina	Caucasus	2.43	0.454	18.69
Kauno	Lithuania	2.21	0.652	29.45
Francuzy	Lithuania	2.18	0.376	17.30
Sunža	Caucasus	2.17	0.253	11.66
Madame	Spain	2.07	0.760	36.79
Poperinge	Belgium	1.99	0.446	22.41
Toses D'alas	Spain	1.87	0.898	48.05
Ursdon	Caucasus	1.50	0.323	21.53
Rhona	Switzerland	1.35	0.390	28.97

SD – standard deviation; *CV* – coefficient of variation

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Table 4. Average content and variability in the hop oil in the selected hop genotypes (Stekník 2016–2020)

Genotype	Origin	Average (% w/w)	SD	*CV* (%)
Boomerang	Czech Republic	2.35	0.170	7.25
Gaia	Czech Republic	1.96	0.486	24.83
Kazbek	Czech Republic	1.16	0.170	14.68
N2	Czech Republic	1.15	0.300	26.10
Blues	Czech Republic	1.07	0.141	13.15
Pilgrim	England	0.73	0.274	37.27
Boekhoute	Belgium	0.53	0.148	27.86
Antler	Canada	0.51	0.214	42.09
Poperinge	Belgium	0.49	0.121	24.58
Fishing lakes	Canada	0.48	0.202	41.94
Kauno	Lithuania	0.47	0.119	25.35
Belt	USA	0.42	0.184	43.64
Francuzy	Lithuania	0.33	0.091	27.24
Kabardina	Caucasus	0.29	0.145	49.44
Toses D'alas	Spain	0.29	0.141	49.18
P132	Austria	0.27	0.103	38.04
Madame	Spain	0.17	0.073	43.98
Rhona	Switzerland	0.16	0.043	27.37
Sunža	Caucasus	0.15	0.023	14.95
Ursdon	Caucasus	0.09	0.018	20.33

SD – standard deviation; *CV* – coefficient of variation

oil content of 41.94% and 42.09%, respectively. The lowest variability among the wild hops was found in Sunža from the Caucasus (14.95%). In contrast, the highest variability in the hop oil content was determined in the Kabardina wild hops from the Caucasus and Toses D'alas from Spain (below 49% w/w). The hop oil content in the wild hops ranges between 0.09 and 0.53% w/w, which is a smaller range than that of the entire collection of wild hops, i.e., between 0.04 and 1.03% w/w (Nesvadba et al. 2010).

The registered hop varieties have high alpha acid and hop oil contents, as was assumed. However, the Antler and Fishing lakes wild hops from Canada have an alpha acid content at the same level as some of the registered Czech hop varieties (Saaz, Saaz Late, Saaz Shine and Saaz Brilliant). The Poperinge wild hops from Belgium have a similar alpha acid content as the Czech flavour hop variety Mimosa. The Czech variety Boomerang is very interesting, showing very high alpha acid and hop oil contents as well as a lower variability in the contents, which was always below 10%. Among the three identified wild hops resistant to *Pseudoperonospora humuli*, both wild hops from Canada have a higher alpha acid content than the Poperinge wild hops from Belgium. Their hop oil content is at the same level. The Boekhoute wild hops from Belgium are very interesting as they have the highest hop oil content (0.53% w/w) among the wild hops and their alpha acid content amounts to 2.86% w/w. Unfortunately, they show a higher susceptibility to the primary infection.

CONCLUSION

Among the fourteen wild hops tested, three wild hops resistant to both the primary and secondary infection from *Pseudoperonospora humuli* were identified, namely Fishing lakes from Canada, Antler from Canada, and Poperinge from Belgium. Wild hops from Canada have a significantly higher alpha acid content than the Poperinge wild hops from Belgium and are more suitable for breeding aroma hops with a required alpha acid content above 3% w/w. In the context of the hops trade, the hop oil content is not as important as the alpha acid content. However, it is important when it comes to the use for different types of beer. A higher hop oil content is desirable for special beers and dry hopping. From this perspective, the resistant Poperinge wild hops from Belgium are more suitable for breeding special flavour hops because they show a more stable hop oil content

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than wild hops from Canada. All three wild hops will be included in the breeding programme aimed at drought resistance. It can be assumed that the gained ascendants will have the required resistance to *Pseudoperonospora humuli*. Among the registered varieties, the Czech hop varieties Boomerang and Kazbek show the highest resistance to both the primary and secondary infection. Both hop varieties are also suitable for hop breeding because they have low variability in the hop oil and resin contents. The Boomerang hop variety is suitable for breeding bittering hops and Kazbek is suitable for breeding aroma hops and flavour hops. The results achieved are very important for breeding aimed at drought resistance.

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