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The impact of diseases on the quality of hops for use in brewing beer

In conventional cultivation, hops must be treated against diseases with pesticides. Since ecological issues complicate approvals and the effectiveness of new products decreases, it is to be expected that not only green cones will be harvested to an increasing extent, but also lots with a visible disease infestation and brown discolored cones. In extensive series of experiments, the effects of a massive infestation with *Peronospora*, powdery mildew, hop aphid and red spider mite in brown hops that are still marketable were investigated for quality characteristics. Healthy green hops were used for comparison. No significant differences could be found between green and brown hops or hop lots in bitter substances, aroma components and polyphenols. 17 comparative brewing trials showed no differences in the analytical characteristics of the green and brown beers, analogous to the hops. Only in rare cases in very late hopped or dry hopped beers a moderate preference for green hops resulted. Contrary to the general expectations of brewers and the doctrines, no significant defects could be found in beers brewed with massively infested hops.

Descriptors: hops, hop diseases, hop pests, hop quality, beer quality

1 Introduction

Hops are exposed to a number of different pests and diseases that can be grouped into discrete categories. The first category comprises viral diseases, such as wilt (*Verticillium*), while the second category consists of harmful fungi, including *Botrytis*, powdery mildew and *Peronospora*. The third category is devoted to insect pests, such as spider mites (red spider mites) or hop aphids. These can be counteracted with synthetic chemical pesticides in conventional agriculture.

The use of pesticides is meanwhile under growing criticism in political discussions, the media and the society generally. It is not merely the consequences for human health that make approval more difficult. The protection of bodies of water and the numerous species which inhabit them has begun to play a crucial role in the approval process for pesticides as well. Plant protection agents of the future will not be highly effective chemical "cudgels". They will be more attuned to and more compatible with the environment; however, this will be offset by their limited efficacy. For this reason, preventing the diseases and controlling the pests affecting hops will become more challenging. As a consequence, hop farmers and brewers will have to learn to tolerate a moderate level of disease and infestation.

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This raises the following questions:

- Can certain varieties no longer be grown with the "green quality" to which brewers are accustomed?
- Will varieties that have to be frequently sprayed with pesticides be eliminated?
- Is it still generally acceptable to produce hops consisting exclusively of green cones, i.e., with no sign of disease or infestation, using any means at one's disposal?
- What effect does disease and/or infestation have on beer quality at all?

Traditionally, brewers reject even slightly discolored lots of hops, as brewing orthodoxy states that using diseased hops results in beer of a lesser quality [1]. The reasoning behind this justification, for example, is that a lot of hops heavily contaminated with powdery mildew or *Peronospora* (downy mildew) exhibits a slightly dull, musty odor. Literature on this subject is rare. In 2005, Weihrauch reported that compared to "healthy" hops in terms of yield and α-acid content, hops with spider mite infestations led to a poorer result in only one of 36 cases [2]. In 2012, he also found that only in isolated cases, did aphid infestation reduce yield and α-acid content [3]. Likewise, Gahr was not able to establish any differences in diseased and healthy hops in analytically tested attributes. Nevertheless, from a sensory standpoint, aberrations have been detected in beer [4]. Pistelli [5] investigated the influence of viral diseases in five Polish hop varieties. On average, infested hops exhibited a reduction in yield by 7 % and a lower α-acid content by 11 %. Fritschova and Krofta investigated the influence of powdery and downy mildew infestation on the hop quality and the flavor and taste of beers made thereof [6]. They observed significant losses of alpha acids and hop oil in diseased hop cones without any effect on beer quality.

Agricultural research within the field of botany on other crops has focused on how photosynthesis is impacted by fungal infestation. Examples include studies by Gordon on sugar beets [7] and by Mandal [8] on a member of the family Plantaginaceae (plantains). In both studies, photosynthesis was clearly impaired by powdery and downy mildew. A review of the literature on this subject is provided by Soumyojit in "Effects of Pathogens on Photosynthesis" [9].

Hops contain significant amounts of secondary metabolites (SM), namely bitter and aroma compounds as well as polyphenols. These SM are largely contained in the lupulin glands of the hop cones. This raises the question: Are the effects of disease and infestation equal with regard to the green material of the cone, i.e., the bracts, and the major location of the SM, namely the lupulin glands? The bracts provide nourishment to pests, but the lupulin does not. It can be assumed that an infestation late in the season may not even affect the lupulin and its constituents.

The current trials were designed to clarify the effects of an infestation on hops. The trials were also devised to determine whether this negatively influences beer quality.

2 Sample Collection

From this point forward, for the sake of clarity, the terms "green" and "uninfested" will be considered synonymous. The same is true for "brown" and "infested".

2.1 Lot Samples Divided into Green and Brown Cones from Crop Years 2018 and 2019

Samples were collected from lots of hops heavily infested with spider mites and powdery mildew. They were manually separated into groups containing green hop cones and obviously discolored brown cones. The 2018 crop comprised 28 lots, while the 2019 crop included 18 lots consisting of a total of 11 varieties.

2.2 Hop samples provided by the Bayerische Landesanstalt für Landwirtschaft (LfL) in Huell

The Bavarian State Research Center for Agriculture, the LfL, regularly carries out analyses on plant protection agents [10]. In these trials, a portion of the hop bines on a single plot is treated against a certain disease, while another portion remains untreated. This results in treated, uninfested/green samples as well as untreated and infested/brown hops. The respective plot is treated against other diseases as needed, thus allowing the disease to be attributed to a defined cause. The following comparisons were made:

- hop aphids on Herkules 2019
- spider mites on Perle (2019) and Herkules (2020)
- powdery mildew on Herkules in 2019 and 2020 (twice)
- *Peronospora* on Hersbrucker (2019), combined with powdery mildew on Herkules (2020)

2.3 A Series of Trials with Bitter Hops from Crop Year 2020

2020 was a challenging year regarding powdery mildew, especially for the bitter varieties Nugget, Taurus and Herkules. For each of these varieties, samples were taken from three uninfested lots (predominantly green cones) and from three lots heavily infested with mildew. Characteristics, such as growing location and the time of the harvest, did not play a role in selection of the lots.

In addition, large-scale production of pellets from lots of Herkules was carried out separately (uninfested as well as heavily infested with powdery mildew) with subsequent processing into CO_2 hop extract.

2.3 Lot Samples from Crop Year 2021

The 2021 growing season was characterized by a *Peronospora* infestation. Hersbrucker, Saphir and Herkules with a high degree of infestation served as representative samples for the trials.

3 Methods

3.1 Visual Inspection

The samples were photographed to demonstrate the difference between uninfested = green and infested = brown. Figure 1 provides a comparison of the uninfested and infested cones. The samples were further analyzed for the degree of infestation (weight of separated brown cones ÷ sample size × 100 %). Figure 2 compares a sample of a lot heavily infested with *Peronospora* with an uninfested lot.

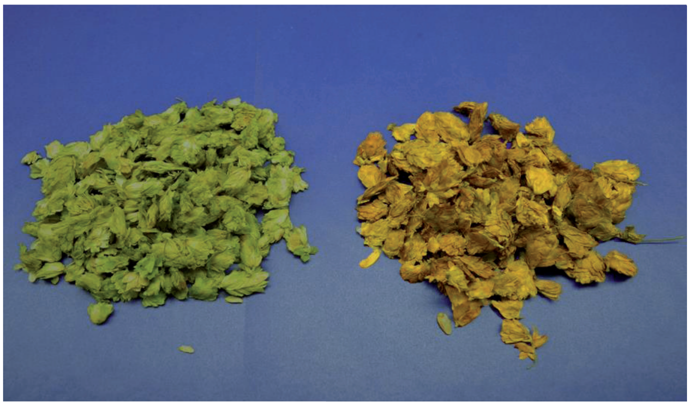


Fig. 1 Comparison of uninfested and infested cones from sorted samples

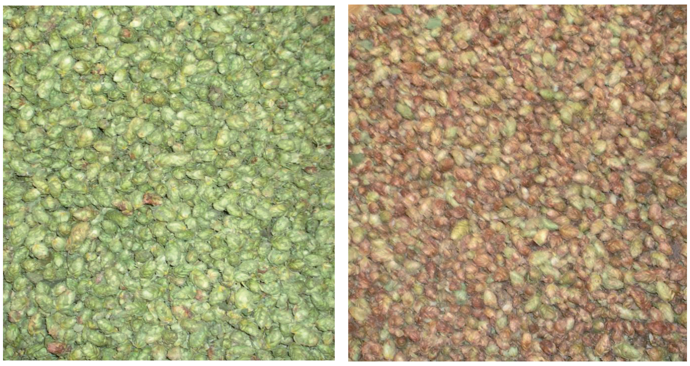


Fig. 2 Sample of an uninfested lot compared to a lot heavily infested with Peronospora

3.2 Sensory Evaluation of the Hop Cones

In the sensory evaluation, a general difference exists in the odor of the raw (unrubbed) hops and the aroma of the lupulin in the crushed cones. Only the raw hop odor revealed a dull or musty note in the infested samples.

3.3 Hop Analysis

In order to minimize systematic deviations, infested and uninfested samples were analyzed in a direct comparison by the same person on the same day using a two-fold or three-fold determination.

3.3.1 Analysis of the Bitter Substances

All lots in the series were analyzed using the ASBC methods Hops-6 α-and β-Acids in Hops and Hop Pellets and Hops-12 Hop Storage Index (HSI) [11, 12]. The HSI was of interest because it was suspected that the infested hops underwent a kind of aging. Parallel to the ASBC method, the conductometric methods EBC 7.4 and 7.5 as well as the HPLC method EBC 7.7 were utilized for the specific determination of the α- and β-acids [13, 14, 15].

3.3.2 Methods for Determining the Hop Oils and Aroma Compounds.

Hydro-distillation according to EBC 7.10 was used to determine the volumetric quantity of hop oils [16]. The relatively high repeatability error of r_{95} = 0.12 m must be taken into account.

Individual hop aroma compounds can be determined using gas chromatography. In this case, MEBAK R-300.07.151 [2016-03] was employed [17]. Of the 40 substances identified and quantified, the most important were categorized into individual groups, such as the monoterpenes and sesquiterpenes, the oxygen fraction (oxygen is present in these molecules causing them to be more soluble in wort and beer), the sum of the esters, the monoterpene and sesquiterpene alcohols, the ketones and the epoxides.

3.3.3 Determination of the Polyphenol Content

The samples in some series were analyzed for their non-specific total polyphenol content according to EBC 7.14 [18]. In addition, an HPLC method was employed for determining the individual low molecular weight polyphenols [19]. The HPLC method EBC 7.7 facilitates the quantification of xanthohumol.

3.3.4 Determination of the Chlorophyll Content

Chlorophyll A and B can be extracted with Ethanol and quantified spectrophotometrically [20, 21].

3.3.5 Mycotoxin Determination

The corresponding analysis was performed externally [22] with results on the following substances:

- Ochratoxin A and aflatoxin B1, B2, G1, G2 are harmful metabolic products of storage fungi [23].
- Substances from fungi of the genus *Alternaria*, especially tenuazonic acid, and the HT-2 toxin formed by *Fusarium* species.

Tenuazonic acid is also found in barley, malt and beer [24]; a maximum permissible concentration in food has not yet been established.

3.4 Beer Production, Laboratory and Sensory Analysis of the Beers

In the following, beers brewed with uninfested hops will be referred to as green beers for the sake of simplicity and, analogously, beers brewed with infested hops as brown beers.

A total of 17 comparisons of green and brown beers were produced at the 2 hl pilot brewery in St. Johann as bottom-fermented, strongly hopped pale lagers. In order to detect possible sensory anomalies due to foreign odors in the brown beers, late hop additions at the end of the boil and in the whirlpool were the rule. Most of the beers were dry hopped between primary fermentation and maturation [25] additionally. The coarsely filtered beers were analyzed for bitterness (IBU) according to EBC 9.8 [26] and the HPLC bitter substances (EBC 9.47) alpha acids, iso-alpha acids, humulinones, and hulupones as well as the xanthohumol and iso-xanthohumol [27]. In some of the trials, the evaluation of foam according to NIBEM [28] or Steinfurt [29] and the analysis of total polyphenols [30] and linalool [31] were also conducted.

The sensory testing was based upon a set of three categories with special consideration given to direct comparisons of the green and brown beers. The triangle test [32] was employed for this purpose in which one of the beers is presented in two glasses while the other is in only one glass. First, the taster must select the sample that is different from the other two. For the tasters who match the samples correctly, their choice and the rationale behind their decision are recorded.

Additionally, the beers were evaluated according to a modified DLG scheme (points from 1 to 5) [33]. A mean was calculated for each beer from the seven sensory attributes in points from 1 to 5. Furthermore, the taster's preference for a series of beers was conducted according to Kramer [34]. A third test was used to assess hop-forward beers based upon the CMA scheme [35]. Here, the five aroma characteristics fruity, floral, citrus, green-grassy and hop-spicy as well as the intensity and quality of the hop aroma are evaluated using a point system from 1 to 10.

In every tasting, additional queries were made of the participants regarding any unusual, strange or unpleasant sensory impressions in the aroma or flavor of the beers.

4 Results

For a comparison of two samples green/brown the confidence intervals (CI) of the respective analyses have to be considered. Those were obtained in this case from the results of collaborative trials of the Hop Analysis Working Group (AHA). Table 1 shows the average CI from the ring analyses in the years 2017 – 2021.

Table 1 Confidence Intervals (CI) for the hop and beer analyses (calculated from the AHA collaborative trials 2017 – 2020)

	Confidence Interval	
Analysis	**low**	**high**
ASBC 6 alpha	0.1	0.2
ASBC 6 beta	0.1	0.2
EBC 7.4	0.1	0.2
EBC 7.5	0.1	0.2
EBC 7.7	0.1	0.4
EBC 7.10	0.05	0.1
EBC 7.13	0.010	0.020
EBC 9.8	0.3	0.5
EBC 9.47		0.4

Table 2 Mean values from the analysis of the bitter substances 2018 and 2019

		Average 2018		Average 2019	
		green	**brown**	**green**	**brown**
α-acids (EBC 7.4)	% w/w	10.16	10.29	12.71	12.66
α-acids (ASBC)	% w/w	10.71	10.95	12.87	12.93
β-acids (ASBC)	% w/w	4.16	4.06	5.09	5.10
HSI		0.332	0.338	0.354	0.358

It is commonly accepted that a single lot of hops will not be homogeneous [36]. For this reason, cones are not generally taken from a set of samples for analysis, but the entire sample is milled first, and then the sample is divided, e.g. according to MEBAK [37]. In this manner, a representative sample of the hop lot can be obtained. This has to be considered when comparing green and brown cones separated from a mixed sample of a single lot or comparing infested and uninfested lots. Even if the differences between two samples or lots are greater than the CI, it has not been proven whether this level of heterogeneity is normal or whether there is a systematic difference between two samples. This is the reason that only through performing a substantial number of comparisons a conclusion can be reached.

4.1 Separating the Green and Brown Cones (crop 2018 and 2019)

The mean for all heavily infested samples exhibited a degree of infestation (brown cones in % relative to the amount sampled) of 21 %, ranging from 8 to 45 %. The differences in color were clear and corresponded to those in figure 1. No visual differences were observed in the lupulin.

In the raw aroma, 21 from 46 brown samples exhibited dull or somewhat musty notes compared to the green cones. In the crushed lupulin, the hop aromas typical of the variety could be perceived, but there were no differences between the odor of the green and brown cones.

Table 2 shows the measured (absolute) values of a selection of the bitter substance analyses averaged over all 46 comparisons. The differences turned out to be remarkably small and lie within the CI (Table 1). The HSI remained unaffected; an increase in the characteristics attributable to aging were not evident.

The individual comparisons of the conductometric values according to EBC 7.4 can be distinguished as follows for the green/brown divided samples. In 20 comparisons, the results for the green and brown hops were identical within the CI. In eight cases, the values for the green hops were higher than those for the brown ones. 18 times the brown cones exhibited higher values than the green ones.

Comparing the analytical values (EBC 7.4, EBC 7.7 and HSI) for each of the seven hop varieties no differences between the varieties could be found.

Table 3 shows the absolute values for the groups of aroma substances in four comparisons of the green and brown cones.

Considering the internal confidence intervals, no differences were able to be determined between the green and brown cones in any of the four comparisons. When the chromatograms were examined, no extra peaks were present in the brown cones that would indicate the additional formation of aroma substances due to an infestation.

Brewing trials were carried out to clarify whether stale, musty smelling cones would impart this off-flavor from the hops to the beer.

Table 3 Four comparisons of green and brown cones; main groups of aroma substances analyzed by GC-FID; values in mg/100 g

	Perle		Herkules A		Magnum		Herkules B		Average		
	green	**brown**	**green**	**brown**	**green**	**brown**	**green**	**brown**	**green**	**brown**	**Average CI**
Monoterpenes	486	478	567	587	928	975	475	544	614	646	100
Sesquiterpenes	562	598	395	382	639	682	375	417	493	520	60
Oxygen fraction	132	115	226	222	209	211	195	251	191	200	40
Esters	67	57	185	173	137	124	148	194	134	137	30
Monoterpene alcohols	12	12	14	19	17	17	16	23	15	18	5
Sesquiterpene alcohols	18	14	9	13	25	33	13	17	16	19	5
Ketones	19	19	23	23	32	33	22	27	24	26	5
Epoxides	22	20	7	8	12	15	7	6	12	12	3

Table 4 Analyses of the two Herkules, the four Saphir and the four Hallertauer beers (XN = xanthohumol; IX = isoxanthohumol; late = whirlpool addition; dry = additional dry hopping)

		Herkules beers		Saphir beers				Hallertauer beers			
		green	brown	green/ late	brown/ late	green/ dry	brown/ dry	green/ late	brown/ late	green/ dry	brown/ dry
Total Polyphenols	mg/l	183	189	187	184	202	197	197	191	218	212
Bitterness	IBU	30	27	19	20	22	23	8	9	11	12
Iso-α-acids	mg/l	31.1	27.2	20.2	19.8	21.1	21.0	6.3	6.6	6.6	6.8
α-acids	mg/l	3.7	3.6	2.6	2.4	3.4	3.8	1.7	1.8	4.0	4.1
Humulinones	mg/l	0.9	1.0	0.8	0.9	1.3	1.6	0.3	1.0	1.0	2.2
XN + IX	mg/l	1.11	1.17	0.84	0.77	0.98	0.98	0.50	0.60	0.57	0.60
Linalool	µg/l	< 2	< 2	43	44	129	132	27	23	126	105

The brewing process was also conceived to allow for the detection of possible off-flavors caused by late hop additions and dry hopping.

The all-malt beers were bottom-fermented and contained the following hops:

- Two Herkules green/brown batches were hopped at the beginning of the boil at 100 g/hl; the duration of the boil was curtailed from the usual 70 to 50 min to reduce the effects of evaporation.
- Four Saphir green/brown batches with a neutral bittering addition at the beginning of the boil (Polaris pellets) with 50 g/hl Saphir at the end of the boil; one portion of the batch was dry hopped with 100 g/hl.
- Two single-variety batches (Hallertauer Mfr.) each were brewed with additions as follows: Beginning of the boil and whirlpool 50 g/hl each; the split batch was dry hopped at 150 g/hl.

For the general beer analyses, such as original gravity, degree of attenuation, pH and color, the beers brewed with the green and brown hops were so similar that listing the results is superfluous. Table 4 shows the constituents derived from hops in the beers. The differences are negligible and reflect the marginal variation in bitter values in the samples. No systematic differences between the beers brewed with green and brown cones could be determined for any of these attributes.

The sensory evaluation of the beers included a triangle test, an evaluation of the harmony of the bitterness and the rank sum test according to Kramer as part of the DLG tasting. A total of 28 to 30 tasters participated. In the triangle test, the green beer was always compared to the brown beer. The statistical evaluation showed that the correct selection of the Herkules and Hallertau beers was statistically significant (Table 5). However, enquiries regarding preference for a particular sample did not reveal any significant difference between the beers brewed with green and brown cones.

Table 5 Results of triangular tests, harmony of bitterness (CI = 0.5) and Kramer tests green versus brown

	Triangle test	Harmony of bitterness	Kramer test
Herkules	*	5.9 : 5.5	near *
Hallertau late	**	6.1 : 5.5	not significant
Hallertau dry	*	6.9 : 6.7	not significant
Saphir late	not significant	6.1 : 5.9	not significant
Saphir dry	not significant	6.3 : 5.9	near *

In general, the DLG tasting did not result in any higher average scores either for the green nor for brown beers. However, the quality of the bitterness in the green beers was rated slightly higher. An additionally conducted rank sum test according to Kramer showed a not significant preferences for the beers brewed with green Herkules and for the beers dry-hopped with green Saphir. Results are also displayed in table 5.

The tasters were unable to detect any unusual nuances in the odor or flavor of the beers brewed with either green or brown hops.

4.2 Samples from the LfL

4.2.1 Crop Year 2019

LfL can provide treated (= green) and untreated (= brown) samples

Table 6 Measured values for bitter substances in treated (= green) and untreated (= brown) lot samples (LfL), crop year 2019; CI see table 1

		Hersbrucker		Perle		Herkules 1		Herkules 2	
		downy mildew		red spidermite		powdery mildew		hop aphid	
		green	brown	green	brown	green	brown	green	brown
α-acids (EBC 7.4)	% w/w	2.0	2.3	7.3	7.7	14.6	15.3	17.8	16.0
α-acids (ASBC)	% w/w	2.0	2.2	6.1	6.2	15.2	15.4	18.3	16.2
β-acids (ASBC)	% w/w	4.3	4.5	3.3	3.6	4.1	4.2	4.7	4.2
HSI		0.287	0.282	0.301	0.291	0.272	0.275	0.272	0.280

Table 7 Comparisons of treated (= green) and untreated (= brown) lots (LfL, crop year 2019); concentrations of aroma substance groups in mg/100g; CI see table 3

	Hersbrucker		Perle		Herkules 1		Herkules 2			
	downy mildew		red spidermite		powdery mildew		hop aphid		Average	
	green	brown	green	brown	green	brown	green	brown	green	brown
Monoterpenes	149	225	548	404	1068	1267	1585	1376	838	818
Sesquiterpenes	283	271	429	563	394	403	538	462	411	425
Oxygen fraction	82	95	135	112	338	365	397	342	238	229
Esters	21	28	96	72	289	313	321	291	182	176
Monoterpene alcohols	7	8	7	5	12	13	17	13	11	10
Sesquiterpene alcohols	38	40	14	13	10	11	14	11	19	19
Ketones	10	10	17	21	33	35	38	33	25	25
Epoxides	7	9	4	5	2	3	3	3	4	5

with a defined degree of infestation as part of pesticide testing. These were *Peronospora* for Hersbrucker, spider mites for Perle, powdery mildew for Herkules 1 and aphids for Herkules 2. The pattern of damage of all four diseases is more or less the same and manifests in a significant browning of the cones similar to figure 2.

Table 6 shows the absolute conductometric values (EBC 7.4) and the values for the ASBC analyses. The infested batches of Hersbrucker (*Peronospora*), Perle (spider mites), and Herkules 1 (powdery mildew) tended to exhibit slightly higher values than the uninfested ones. The reverse was true for Herkules 2 (aphids). The differences between the infested and uninfested samples were negligible.

Table 7 lists the content of the groups of aroma substances determined using GC-FID. Analogous to the bitter substances, the brown powdery mildew and *Peronospora* samples exhibited slightly higher values than the green ones. The opposite was true for the infestations with aphids and spider mites. A clear indication of decline in the aroma substances due to an infestation could not be determined. No signs of any new peaks caused by the infestations were seen in the chromatograms.

Table 8 shows the mycotoxin analyses. Aflatoxins and ochratoxin A were not detectable in any of the samples. The sample infested with powdery mildew contained small amounts of tenuazonic acid and the HT-2 toxin. The EU is currently discussing indicative levels in foods ingested directly by consumers which are significantly higher than the levels detected in hops [38].

All four types of infestation were tested in eight brews. The division into late-hopped beers and those which were also dry-hopped pro-

Table 8 Analysis of the mycotoxins in uninfested and infested samples; data in µg/kg; LOQ = limit of quantification

Muster	Hersbrucker		Perle		Herkules 1		Herkules 2		
Befall	–	downy mildew	–	red spidermite	–	powdery mildew	–	hop aphid	LOQ
Ochratoxin A (OTA)	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.5
Aflatoxin B1	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.1
Aflatoxin B2	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.1
Aflatoxin G1	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.1
Aflatoxin G2	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	0.1
Aflatoxines	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	< LOQ	–
Altenuene (ALT)	–	–	–	–	< LOQ	< LOQ	< LOQ	< LOQ	10
Alternariol (AOH)	–	–	–	–	< LOQ	< LOQ	< LOQ	< LOQ	10
Alternariol monomethyl ether (AME)	–	–	–	–	< LOQ	< LOQ	< LOQ	< LOQ	10
Tentoxin (TEN)	–	–	–	–	< LOQ	< LOQ	< LOQ	< LOQ	10
Tenuazonic acid (TEA)	–	–	–	–	< LOQ	100 ± 40	< LOQ	< LOQ	10
Deoxynivalenol (DON)	< LOQ	< LOQ	–	–	< LOQ	< LOQ	–	–	20
Diacetoscirpenol (DAS)	< LOQ	< LOQ	-	-	< LOQ	< LOQ	-	-	10
Fusarenon X (FX)	< LOQ	< LOQ	-	-	< LOQ	< LOQ	-	-	20
HT-2 Toxin	< LOQ	< LOQ	-	-	< LOQ	37 ± 15	-	-	10
Nivalenol (NIV)	< LOQ	< LOQ	-	-	< LOQ	< LOQ	-	-	20

vided 16 beers. Late hop additions were divided into those hopped at the midpoint of the boil, at the end of the boil, in the whirlpool and those that were dry hopped. These additions were as follows: 400 g/hl with Hersbrucker, 200 g/hl with Perle and 80 g/hl with the two Herkules-hopped beers.

The standard beer analyses exhibited a good reproducibility. The analytical values for the bitter substances in the beers brewed with green and brown hops showed no differences, and therefore listing the analysis results is not necessary. Table 9 contains the results for linalool and the foam index according to Steinfurt. The values for foam were not affected by the infestations (2 x identical, 3 x green higher that brown, 3 x brown higher than green). Linalool values were identical in the late hopped beers. For downy mildew and aphids, the linalool values differed significantly but did not show a clear tendency.

The results of the triangular tests are presented in table 10 (see page 140). None of the allocations turned out to be significant. When considering only the right allocations the bitterness of the dry-hopped green Hersbrucker beer has been rated significantly higher. The Perle beers displayed no difference at all. The powdery mildew beer (Herkules 1 green late) had a better bitterness than the brown one. Looking at the aphid beers (Herkules 2) the intensity of the aroma of the dry-hopped uninfested beer was esteemed higher.

The DLG tasting did not reveal any definite trends. Therefore, in figure 3, the mean scores for the five sensory attributes and foam were averaged for the uninfested and infested beers. The average aroma scores for the five attributes according to the CMA are shown in figure 4. Only

Table 9 **Values for linalool (µg/l) and the foam index according to Steinfurt (SKZ) for the 16 beers brewed from the 2019 samples**

		Linalool		Foam SFT	
		green	brown	green	brown
Downy mildew/ HEB	late	68	66	99	102
	dry	115	132	102	96
Red spidermite/ PER	late	27	22	94	94
	dry	38	31	99	98
Powdery mildew/ HKS 1	late	3	3	87	99
	dry	21	23	95	99
Hop aphid/ HKS 2	late	3	3	94	91
	dry	40	29	99	96

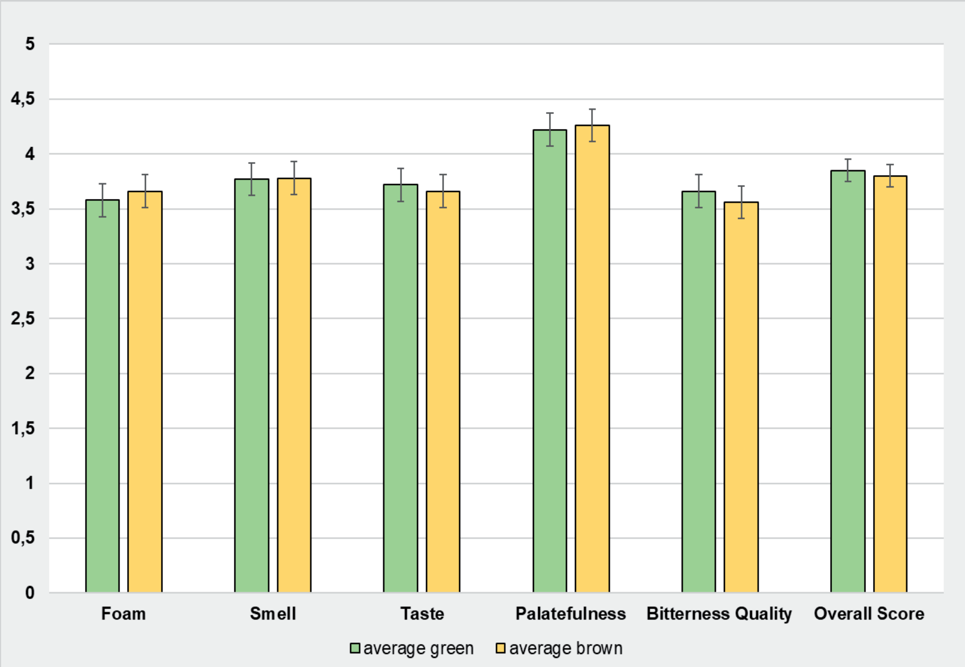
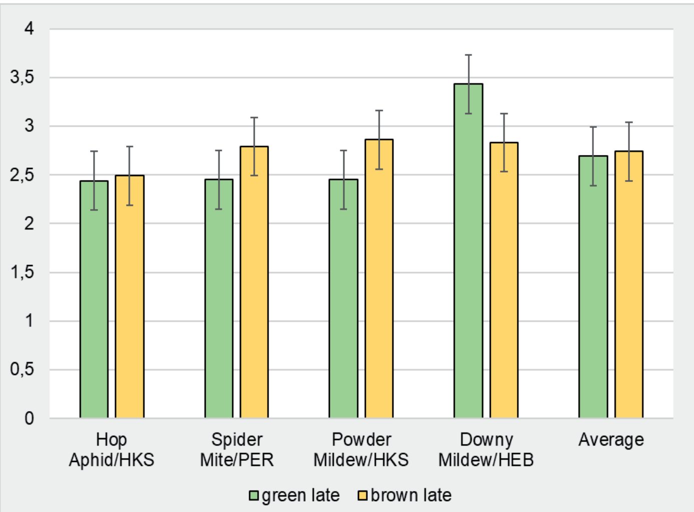


Fig. 3 **Mean scores (DLG) for the five sensory attributes and foam for the uninfested and infested beers**



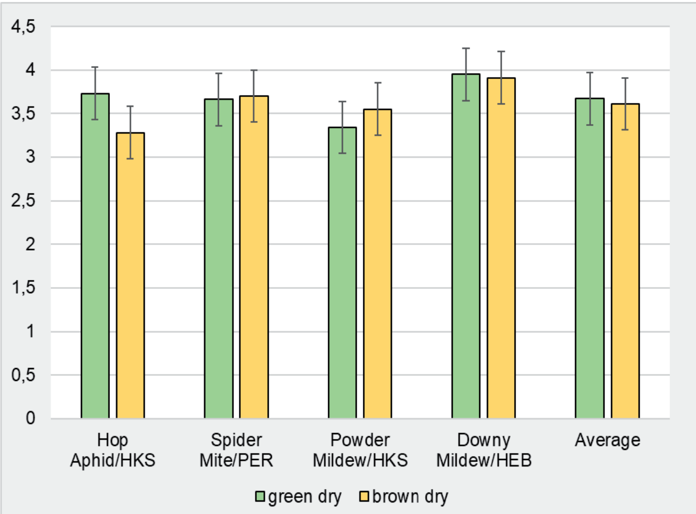


Fig. 4 **Mean aroma scores of the CMA tasting results for the beers brewed from uninfested (green) and infested hops (brown); late hopped only (left), also dry hopped (right).**

Table 10 Results of the triangular tests with 15 to 17 tasters; * significant, ** very significant

			Preference		Bitterness		Aroma	
		right	green	brown	green	brown	green	brown
Downy mildew / HEB	late	10	7	3	6	4	5	5
	dry	7	5	2	6*	1	3	4
Red spidermite / PER	late	8	5	3	4	4	4	4
	dry	7	4	3	4	3	4	3
Powdery mildew / HKS 1	late	9	4	6	2	8*	4	6
	dry	1	1	0	1	0	1	0
Hop aphid / HKS 2	late	7	3	4	5	2	5	2
	dry	11	7	4	8	3	10**	1

the late hopped Hersbrucker showed a tendential difference with favor of the uninfected hops.

In summary, no differences could be established in the series of tastings between the infested and uninfested hops. None of the infested beers exhibited any off-flavors (musty, stale, dull, sweet, etc.).

4.2.2 Samples from Crop Year 2020

Sample collection was performed as it was in 2019 by the LfL but was limited to the variety Herkules. The following comparisons of treated and untreated samples were carried out: 2 × powdery mildew (numbers 1 and 2), *Peronospora* and spider mites. The spider mite trial resulted in three samples: chemically treated, treated with predatory mites [39], and untreated. The treated samples (incl. predatory mites) were green, the untreated ones brown.

Table 11 shows the results for bitterness and hop oils.

- In the hops infested with powdery mildew, the α- and β-acids of the green samples were slightly higher, the values for hop oils and the HSI almost identical.
- In the three samples with spider mite infestations, the α- and β-acids of the green samples had the lowest values, followed by the hops treated with predatory mites. The infested hops exhibited the highest values for the α- and β-acids. The HSI and hop oils did not differ.
- A comparison of the *Peronospora* trials showed lower levels of α- and β-acids and the hop oils in the green samples compared to the infested ones. The HSI remained unaffected.

The mean values for the four comparisons of the green and brown hops demonstrate that there was no obvious damage to the bitter acids and hop oils.

Calculating an average for the conductometric values of the powdery mildew hops of crop 2019 and 2020 there is no significant difference between green samples (15.93 %) and the brown ones (15.70 %).

The *Peronospora* samples were analyzed for chlorophyll A and B. In green hops, the levels (in mg/100 g) were 37.9 (A) and 29.5 (B) compared to 12.4 (A) and 10.2 (B) in the brown hops, a loss of 66 %.

4.3 Powdery Mildew in Bitter Hops from Crop Year 2020

From each of the three bitter varieties Herkules, Taurus and Nugget, three green/uninfested farmers lots and three heavily infested lots with powdery mildew that were on the edge of marketability were selected. The three samples were combined to form a mixed sample. The color differences were striking; regarding the aroma impressions of the raw hops, the brown samples exhibited slightly mustier notes. In the powdery mildew samples, however, the crushed lupulin revealed no abnormalities.

The difference in color was demonstrated by measuring the chlorophyll content. On average, 45 % of the chlorophyll was lost due to infestation.

The results from the analysis of the typical bitter compounds are provided in table 12. Furthermore, the mean values for the three comparisons are given. For Herkules, higher values were meas-

Table 11 Results of the LfL samples (crop year 2020); CI see table 1

		powdery mildew 1		powdery mildew 2		red spidermite			downy mildew		Average	
		green	brown	green	brown	green	brown	preda-tory mites	green	brown	green	brown
α-acids (EBC 7.5)	% w/w	17.5	16.5	15.7	15.3	18.1	18.0	18.8	14.9	17.0	16.55	16.70
α-acids (EBC 7.7)	% w/w	17.0	15.7	13.9	13.6	16.4	17.3	17.1	14.3	16.0	15.40	15.70
β-acids (EBC 7.7)	% w/w	4.2	3.9	3.7	3.8	4.0	4.3	4.2	4.2	4.3	4.03	4.08
HSI		0.286	0.287	0.298	0.288	0.292	0.302	0.29	0.282	0.287	0.290	0.291
Hop oil	ml/100 g	1.8	1.8	1.6	1.4	2.4	2.3	2.3	1.9	2.3	1.93	1.95

Table 12 **Analysis results for bitter substances in the mixed samples combined from 3 hop lots each: conductometric value (CV) according to EBC 7.5, HPLC of the α- and β-acids (EBC 7.7) and HSI analysis (ASBC); CI see table 1**

		Herkules		Taurus		Nugget		Ø	
		green	brown	green	brown	green	brown	green	brown
α-acids (EBC 7.5)	% w/w	16.8	15.2	14.7	16.1	12.9	13.3	14.80	14.87
α-acids (EBC 7.7)	% w/w	15.4	14.1	13.7	15.0	11.8	12.4	13.63	13.83
β-acids (EBC 7.7)	% w/w	4.1	3.8	3.6	4.1	4.0	4.1	3.90	4.00
HSI		0.301	0.331	0.336	0.307	0.299	0.293	0.312	0.310

Table 13 **Analysis of polyphenols (PP) in the HKS hops: total PP; low molecular weight HPLC PP and xanthohumol as well as the ratio of the sum of all low molecular weight PP (lmwPP = HPLC PP + XN) : total PP**

		Pellets green	Pellets brown	CI
Total polyphenols = TPP	mg/100g	3950	3750	160
lm HPLC-Polyphenols = HPLC-PP	mg/100g	525	491	30
Xanthohumol = XN	mg/100g	740	690	50
Sum of low molecular polyphenols = lmPP	mg/100g	1265	1181	
lmPP : TPP	% rel.	32.0	31.5	

Table 14 **Aroma substance groups in mixed samples of three uninfested = green and infested = brown farmers lots each; values in mg/ 100 g; CI see table 3**

	Herkules		Taurus		Nugget		Ø	
	green	brown	green	brown	green	brown	green	brown
Monoterpenes	880	753	1104	1318	920	964	968	1012
Sesquiterpenes	420	312	493	530	549	567	487	470
Oxygen fraction	294	258	185	197	356	325	278	260
Esters	238	197	99	97	258	237	198	177
Monoterpene alcohols	17	14	27	31	20	19	21	21
Sesquiterpene alcohols	12	12	25	28	30	30	22	23
Ketones	20	24	27	33	36	36	28	31
Epoxides	8	8	14	11	19	18	14	12

ured in the green hop samples, while the brown hop samples were higher for Taurus and Nugget. On average, no difference could be established between infested and uninfested lots.

Table 13 shows the major polyphenol analyses for the Herkules hops. The values measured for total polyphenols, low molecular weight HPLC polyphenols and xanthohumol in the brown hops are only slightly lower than the values for those in the green hops.

Table 14 lists the concentrations of the aroma substance groups. The green Herkules lot exhibited slightly higher values than the brown lot, and the reverse was true for Taurus. The Nugget samples were almost identical. The same was true for the mean values from all three comparisons. No abnormalities were observed in the gas chromatograms.

The samples were also analyzed for mycotoxins. Ochratoxin A and aflatoxins were not detected in any of the samples, but tenuazonic acid was at levels ranging from 160 to 1800 µg/kg in the infested hops and 120 µg/kg once in an uninfested sample.

The six hop mixtures were added at a rate of 200 g/hl at the end of the boil and at a rate of 20 and 35 g/hl at the start of the boil, depending upon the α-acid content of the samples. The trial batches were divided after main fermentation; the late-hopped beer was maturated in one tank and the dry-hopped beer (300 g/hl) in a separate tank. It is unusual to use such a large amount of bitter hops at the end of the boil and for dry hopping, but this was purposely carried out to facilitate the detection of any sensory perception which might arise as a result of a particularly heavy infestation.

No significant differences in the bitter components between the green and the brown beers have been observed.

In the triangle test four of six comparisons were correctly assigned conducted with 20 to 26 tasters. However, it was somewhat incongruous that the pairing of the beers hopped late with Nugget could be distinguished in a statistically significant manner while the pairing of the beers dry hopped with Nugget could not. In terms of preference, the green and brown beers were tied. No clear findings emerged for any of the individual attributes either. In assessing the quality of the bitterness, slight preferences were recorded for three of the beers brewed with green hops and three brewed with brown hops.

Table 15 Average DLG scores, quality of the bitterness (DLG) and intensity and quality of the hop aroma (CMA) from the six comparisons with individual CI

	Herkules				Taurus				Nugget			
	late		dry		late		dry		late		dry	
	green	brown	green	brown	green	brown	green	brown	green	brown	green	brown
Average DLG score	4.51 ± 0.07	4.22 ± 0.13	4.31 ± 0.28	4.29 ± 0.40	4.49 ± 0.13	4.34 ± 0.18	4.29 ± 0.18	4.23 ± 0.23	4.30 ± 0.19	4.00 ± 0.33	4.36 ± 0.17	3.86 ± 0.20
Quality of bitterness DLG	4.44 ± 0.33	3.94 ± 0.30	4.25 ± 0.42	4.06 ± 0.64	4.44 ± 0.21	4.19 ± 0.25	4.09 ± 0.32	4.00 ± 0.34	4.14 ± 0.26	3.66 ± 0.45	4.07 ± 0.35	3.41 ± 0.39
Intensity of hop aroma CMA	5.15 ± 0.84	5.24 ± 0.95	7.16 ± 0.93	6.75 ± 0.82	5.38 ± 0.84	5.65 ± 0.88	6.94 ± 0.86	7.25 ± 0.89	5.24 ± 1.04	5.15 ± 1.05	7.07 ± 0.82	6.56 ± 1.18
Quality of hop aroma CMA	7.25 ± 0.81	6.97 ± 1.02	6.88 ± 0.73	6.66 ± 0.75	7.77 ± 0.80	7.59 ± 0.86	7.81 ± 0.80	7.39 ± 0.81	7.09 ± 0.72	6.62 ± 1.03	7.65 ± 0.69	6.31 ± 1.17

The DLG test showed no differences with regard to foam. The mean scores for the size of the bubbles and lacing were 4.09 (green) and 4.12 (brown) for the late-hopped beers and 4.21 (green) and 4.19 (brown) for the dry-hopped beers. An average score was determined for each beer based on the seven attributes evaluated as part of the DLG tasting scheme (Table 15).

The following not statistically proven trends were observed:

- For Herkules, the average DLG score and quality of bitterness are higher for the beers hopped late with green hops compared to those hopped late with brown hops. This difference became less obvious in the dry-hopped beers.
- A comparable but weaker trend was also observed with the beers brewed with Taurus.
- For Nugget, the two beers produced with green hops scored better in both the overall points and the quality of bitterness.

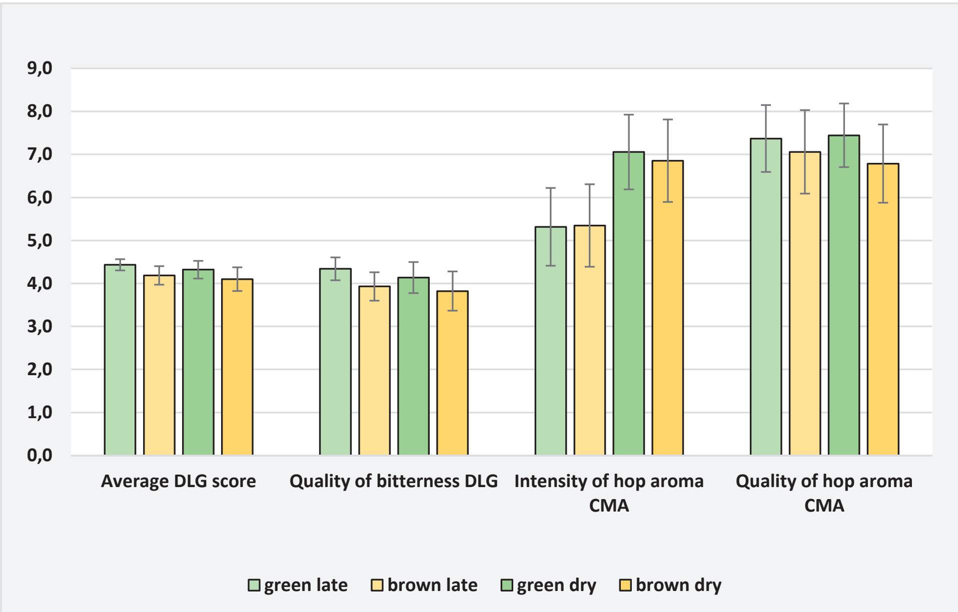


Fig. 5 Mean sensory results from the DLG and CMA tastings (Table 15)

The average results from the three varieties are shown in figure 5 together with their average CI. The green beers receive a better rating for the average DLG score by 0.24 points (late-hopped) and 0.22 points (dry-hopped). As for the quality of bitterness, the differences amount to 0.41 and 0.32 points, respectively. Dry hopping resulted in lower scores for both the overall points and the quality of bitterness, which was an indication that the tasters did not like the use of bitter varieties for dry hopping.

The beers were also evaluated using the CMA scheme for hop-forward beers. Table 15 shows also the points awarded for the intensity and quality of the hop aroma. While the intensity of the hop aroma showed no tendency, the green

Fig. 6 Pellets and extracts produced from uninfested (left) and heavily infested hops (right)

Table 16 Analysis of the six samples from hop lots from crop year 2021; bitter substances according to EBC 7.5 and 7.7; HSI according to the ASBC; hop oil by volume according to EBC 7.10 (CI see Table 1) and sum of chlorophyll A + B (CI = 1 – 2 mg/100 g)

		Hersbrucker		Saphir		Herkules	
		green	brown	green	brown	green	brown
α-acids (EBC 7.5)	% w/w	4.3	5.0	4.3	4.2	20.1	16.5
α-acids (EBC 7.7)	% w/w	3.9	4.7	3.9	3.7	18.8	15.2
β-acids (EBC 7.7)	% w/w	5.7	5.0	5.7	5.2	5.3	4.4
HSI		0.282	0.298	0.340	0.277	0.269	0.281
Hop oil (EBC 7.10)	ml/100g	1.20	0.90	0.75	0.70	2.80	2.00
Chlorophyll A + B	mg/100g	29.9	26.0	34.2	16.9	12.6	7.6

Table 17 Three comparisons of uninfested (= green) and infested (= brown) lots; aroma substance groups; values in mg/100 g; CI see table 3

	Hersbrucker		Saphir		Herkules		Average	
	green	brown	green	brown	green	brown	green	brown
Monoterpenes	378	241	219	314	1335	901	644	485
Sesquiterpenes	283	271	189	181	433	403	302	285
Oxygen fraction	82	78	107	97	284	185	158	120
Esters	20	28	51	41	225	137	99	69
Monoterpene alcohols	9	7	9	12	22	12	13	10
Sesquiterpene alcohols	40	16	15	32	11	13	22	20
Ketones	11	30	35	10	39	29	28	23
Epoxides	4	3	4	4	3	3	4	3

beers always had a non-significant better score in the quality of the hop aroma which becomes also evident when looking to figure 5.

The experimental setup shows that hops very heavily infested with powdery mildew can have an effect on the flavor of the beers, which did not exhibit unpleasant dull or musty flavour notes. The trends indicating a preference for green beers cannot be confirmed with statistical significance. Moreover, the utilization of bitter hop varieties in late hop additions as well as in high amounts for dry hopping are unusual brewing practices with bitter hops.

Hop lots heavily infested with powdery mildew and uninfested lots were processed using commercial equipment separately into pellets and then extracted with CO_2. This provided four products for analysis and for brewing trials. Figure 6 shows the two pellets and the extracts prepared from them. The chlorophyll-influenced color of the pellets is also found in the hop extract; a darker extract with a more intense green color was produced from the green pellets, while the brown pellets yielded hop extract which was lighter in color.

Neither the hop product analyses nor the analytical and sensory results of the brewing trials showed any new or remarkable insights differing from the raw hop findings.

4.4 Downy Mildew in Hop Samples from Crop Year 2021

Sufficient rainfall in the summer of 2021 favored the spread of *Peronospora*, which made it possible to analyze three particularly heavily infested lots of the varieties Hersbrucker, Saphir and Herkules. Lots almost completely free of *Peronospora* served as a comparison.

The data for bitter substances, hop oils and chlorophyll are provided in table 16. When comparing the individual lots, one must take into consideration that the lots were selected exclusively on the basis of their apparent disease. For the Hersbrucker variety, the α-acid content of the uninfested lot was slightly lower than that of the infested lot, and the hop oil content of the green lot was slightly higher. The infested lot had 13 % less chlorophyll.

With nearly identical α-acid values, the green Saphir contained slightly more β-acids and hop oil. The HSI of the green lot is a bit higher compared to the brown lot. The infestation significantly affected the chlorophyll at a loss of approximately 50 %.

The brown Herkules lot was about 20 % lower in α- and β-acids than the green lot with an identical HSI and contained up to 30 % less hop oil. The chlorophyll was 40 % lower.

The six samples were analyzed for mycotoxins. No dangerous aflatoxins, ochratoxin A or *Fusarium* toxins were found.

The major groups of hop aroma compounds analyzed by GC-FID are shown in table 17.

No additional peaks were present in the chromatograms of the infested lot samples. The values for the green Hersbrucker were slightly higher for almost all attributes; no significant differences were observed for Saphir. The infested Herkules exhibited lower

Table 18 Results of the triangle tests including the significance and preference for correctly assigned beers, preference for quality of bitterness and intensity of aroma as a ratio of green to brown beers

Variety	Hopping	Significance	Preference	Bitterness	Aroma
HEB	late	-			
	late + dry	*	11 : 7	12 : 6	10 : 7
SIR	late	**	8 : 11	8 : 11	6 : 12
	late + dry	***	13 : 8	10 : 10	15 : 6
HKS	late	**	11 : 8	11 : 8	12 : 6
	late + dry	***	8 : 8	10 : 14	15 : 9

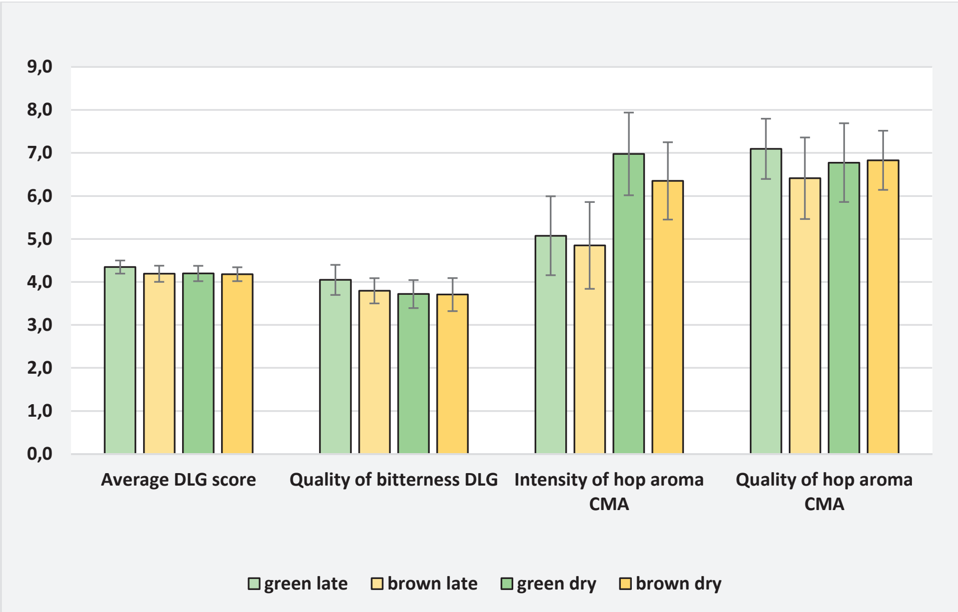


Fig. 7 Average results from the DLG and CMA sensory evaluations

values than the uninfested hops, both in terms of bittering capacity and aroma.

The beers were late hopped and dry hopped at a rate of 250 g/hl with Hersbrucker and Saphir, however, with Herkules at 150 g/hl. The analyses of colour, foam, bitter units, iso-alpha acids, linalool and polyphenols displayed no significant differences between green and brown beers and therefore are not shown.

Table 18 lists the results of the triangular tests. Of the six comparisons, five were statistically significant. The beers brewed with green hops were assigned a preference three times, the beers produced with brown hops once, and none of the samples in one instance. The results with regard to quality of the bitterness were evenly heterogenous. A higher intensity of aroma was attributed four times to the beer brewed with the green hops (one time was statistically significant) and one time for the beers brewed with brown hops.

The Saphir beers proved that the results were also able to impart an ambivalent impression: the late-hopped brown beers were preferred more often and were assigned a better bitterness quality and especially a higher aroma intensity, while the reverse was true for the dry-hopped beers.

The results from the sensory evaluation according to the DLG (average score and quality of bitterness) and CMA (intensity and quality of hop aroma) are not presented as individual values for each variety as the differences were neglectable. The figure 7 shows analogous to figure 5 the average results of the three varieties. The late hopped beers received higher values in the green beers in all descriptors. These not statistically significant differences disappear in the dry hopped beers besides in the intensity of the hop aroma. None of the beers exhibited an abnormal or strange aroma.

5 Summary

Hops are exposed to pests and diseases that are combated in conventional agricultural practices with synthetic chemical pesticides. Plant protection agents of the future, however, will not be highly effective “cudgels”. Their improved compatibility with the environment will be offset by their limited efficacy. Hop cultivation will therefore have to tolerate moderate disease and infestation in the future. As part of beer brewing orthodoxy, diseased and infested hops are thought to negatively impact the quality of beer. This rationale is anchored in the fact that a slightly musty or dull raw odor is evident in hops heavily infested with powdery or downy mildew.

The focus of this research was to determine the effects of massive disease infestations on the compounds in hops and on the resultant beer quality.

Different approaches were taken to collecting samples in crop years 2018 to 2021:

- A total of 46 strongly infested but still certified lots were selected from the crop years 2018 and 2019. The hops were separated manually into only green, uninfested cones and brown, infested cones.
- The LfL regularly conducts testing on plant protection agents: a portion of the hop bines in a plot are specifically treated against a disease, while another remains untreated. This ensures that the infestation is attributable to a defined pest. Four comparisons for the crop years 2019 and 2020 were available.
- Three uninfested lots and three lots of bitter varieties heavily infested with powdery mildew were sampled from crop year 2020.
- Four hop lots from crop year 2021 exhibited an extensive *Peronospora* infestation.

All samples were analyzed for bitter substances, while aroma substances, polyphenols, mycotoxins and chlorophyll were measured in selected samples. In addition, 17 comparisons of uninfested and infested hops were carried out in trials through the production of late-hopped and dry-hopped beers.

The results from the research trials can be summarized as follows:

- Separation into green and brown cones revealed no differences in α- and β-acids or the HSI values. Four comparisons of the hop aroma compounds also yielded identical results. No peaks occurred in infested samples which might indicate the formation of aroma compounds brought about by infestation. Six brewing trials with uninfested and infested hop cones generated comparable beer analyses with no significant findings in sensory analysis.
- The samples collected by LfL for the 2019 and 2020 crop years were grouped by the type of infestation: hop aphids, spider mites, powdery and downy mildew. Variations in the concentrations of bitter and aroma compounds among the infested and uninfested samples were negligible. Additional "infestation peaks" could not be detected in the chromatograms. The particularly hazardous aflatoxins and ochratoxin A were not present in any of the samples. Tenuazonic acid was detectable at harmless concentrations in the powdery mildew infested hops. However, the chlorophyll content is lower as a result of infestation. Beer analyses conducted on the four beers exhibited no abnormalities between infested and uninfested beers. The tastings also revealed no statistically significant differences.
- Heavily mildew-infested and uninfested bitter varieties (2020 crop year) from commercially available lots showed a 45 % degradation of chlorophyll, on average. Ochratoxin A and aflatoxins were not detectable in any of the samples. Furthermore, there were no significant differences in bitter or aroma compounds. Taste tests of late-hopped and dry-hopped beers dosed at high rates revealed a tendency (no statistical significance confirmed) to assign a lower preference to the beers brewed with the mildew-infested hops.
- Three comparisons of heavily *Peronospora*-infested batches with uninfested ones exhibited a chlorophyll loss of one-third. Mycotoxins were not detectable in any of the samples. No noteworthy differences were observed in either the bitter or aroma compounds. The six hops were used to brew late and dry-hopped beers. No statistically significant difference between the uninfested and infested hops was able to be detected for any of the sensory attributes evaluated. However, the beers brewed with uninfested hops tended to receive higher scores.
- Generally: Chlorophyll is affected by diseases. Infested hops show 15 to 66 % lower contents. Even in totally brown cones chlorophyll is still existing. Up to now chlorophyll losses cannot be correlated with a certain disease. Furthermore, the biogenesis of secondary metabolites seems to be independent from the chlorophyll content of the cones.

6 Conclusion

Hops heavily impacted by disease and infestation with pests exhibited no measurable damage to the relevant hop compounds compared to uninfested hops. Similarly, beers brewed with heavily infested hops showed no clear defects compared to uninfested hops, despite an extreme emphasis on high dosing rates in late and dry hopping. Nevertheless, some comparisons showed only a tendency to favor beer brewed with green hops in sensory tests. In conclusion, given that severe disease infestation does not cause clear deficiencies in hop compounds and beer quality, it follows that moderate infestation will not exert negative effects on beer. However, at this time, these results do not reflect the doctrines generally accepted by those in the industry.

The current objective in combating disease in hops with pesticides is to produce green hops with no visible signs of infestation or disease. The future goal of disease control could be maintaining the quality of substances in the lupulin glands and not retaining a green color.

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