On the alkaloid content of ergot (*Claviceps purpurea*)

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Abstract

In the last decades, data on the content of toxic alkaloids in ergot and the relationship of the ergot contamination in rye and the alkaloid content in ergot has been scarce. Therefore, the level of ergot contamination (g/kg rye) and the alkaloid contents (mg/kg ergot) of ergot grown on 7, 21 and 24 artificially infected rye varieties at three German locations in 2002, 2003 and 2004 were determined. In addition, three different ergot size fractions and rye kernels of ergot infected ears were analysed on their alkaloid contents to investigate if small sclerotia show higher alkaloid contents than large ones and if the ergot alkaloids are able to penetrate the grain.

In general, the factor *rye variety* had only minor influence on the ergot alkaloid content, but the extent of ergot contamination in rye was highly affected. The results concerning the effect of the *location* on the ergot alkaloid content were inconsistent, but proved to be significant for the ergot contamination. However, an influence of the *year* on the ergot alkaloid content can be derived from the data observed although the ergot contamination remained unaffected. Both parameters, ergot contamination and ergot alkaloid content, varied to a large extent and showed no relationship in the majority of cases. For this reason the analysis of the toxic alkaloids should be preferred to evaluate potential risks at feeding instead of the ergot contamination in grain, which is current practice in the European Union until now. Concerning the different ergot size fractions, the alkaloid contents differed not significantly from each other. Since no ergot alkaloids were detected in rye kernels of ergot infected ears, a transfer from sclerotia into grain seems not to be relevant.

Keywords: Ergot, alkaloid content, sclerotia size, rye

Zusammenfassung

Zum Alkaloidgehalt von Mutterkorn (*Claviceps purpurea*)

Über den Gehalt von Mutterkorn an toxischen Alkaloiden sowie der Beziehung zwischen der Mutterkornbelastung des Roggens und dem Alkaloidgehalt des Mutterkorns liegen nur wenige aktuelle Informationen vor. Aus diesem Grund wurden die Mutterkornkontamination (g/kg Roggen) sowie der Alkaloidgehalt des Mutterkorns (mg/kg Mutterkorn) erfasst, welches von 7, 21 und 24 künstlich infizierten Roggensorten aus den Jahren 2002, 2003 und 2004 von drei Standorten in Deutschland stammte. Außerdem wurden drei verschiedene Sklerotiengrößen sowie Roggenkörner von mutterkorninfizierten Ähren auf deren Alkaloidgehalte analysiert, um festzustellen, ob kleine Sklerotien höhere Alkaloidgehalte aufweisen als große, und ob Alkaloide in das Korn übergehen können.

Insgesamt hatte der Faktor *Roggensorte* kaum einen Einfluss auf den Alkaloidgehalt des Mutterkorns, jedoch war die Höhe der Mutterkornbelastung des Roggens stark von der Roggensorte abhängig. Die Ergebnisse bezüglich des Effektes des Standortes auf den Alkaloidgehalt waren uneinheitlich, zeigten jedoch einen signifikanten Einfluss auf die Höhe der Mutterkornbelastung. Eine jahresbedingte Beeinflussung des Alkaloidgehaltes kann aus dem Datenmaterial abgeleitet werden, wobei die Mutterkornkontamination nicht durch den Faktor *Jahr* beeinflusst war. Die Parameter Mutterkornkontamination im Roggen und Alkaloidgehalt im Mutterkorn variierten in großem Umfang und zeigten in den meisten Fällen keine Beziehung zueinander. Aus diesem Grund sollte der Alkaloidgehalt herangezogen werden, um mögliche Risiken bei der Verfütterung von mutterkornkontaminiertem Futter zu bewerten anstatt der Feststellung des gewichtsbezogenen Mutterkorngehaltes im Getreide, was die heutige gängige Praxis in Europa darstellt. Bezüglich der verschiedenen Mutterkorngrößenfraktionen waren keine signifikanten Unterschiede des Alkaloidgehaltes festzustellen. Da keine Ergotalkaloide in den Roggenkörnern von mutterkornkontaminierten Ähren festgestellt wurden, scheint ein Übergang von den Sklerotien in das Korn nicht von Relevanz zu sein.

Schlüsselworte: Mutterkorn, Alkaloidgehalt, Sklerotiengröße, Roggen

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1 Introduction

A maximum amount of 1000 mg ergot/kg grain is permitted in the European Union [Council Directive 2002/32/EC of 7 May 2002] in order to avoid intoxications of animals caused by ergot contaminations of feeding stuffs. The factors known to have an influence on the ergot (*Claviceps purpurea*) contamination of rye are well established (Mielke, 2000; Engelke, 2002). Investigations on the factors affecting the content and pattern of alkaloids, which are known to be the main toxic components of ergot (Rotter et al., 1985; Wirth and Gloxhuber, 1994), in ergot grown on rye or related grass species were mostly conducted for pharmaceutical purposes before the 1960's, e.g., by Mothes and Silber (1952), Silber and Bischoff (1954) or Meinicke (1956). Due to technological progress, it was later possible to cultivate alkaloids producing *C. purpurea* strains in submerged cultures for the industrial production of alkaloids in larger quantities (Minghetti and Crespi-Perellino, 1999). Hence, besides some data, e.g., from Canada (Young, 1981a, b; Young et al., 1982) or European regions (Wolff, 1989; Richter, 2003), scarce recent information on the alkaloid contents and patterns of ergot grown on cereals can be found. For that reason, it was the objective of the present study to extend the existing data by investigating the alkaloid contents of ergot.

The size of ergot sclerotia varies from few mm to more than 4 cm. As the sclerotia size is an important factor for different sieving strategies, sclerotia from the same size as rye kernels may remain in cleaned rye. Hence it was of interest, if sclerotia of different sizes vary concerning their content of alkaloids.

The parasitic fungus *C. purpurea* obtains all nutrients from the host plant. Thus, by analysing kernels of ergot infected rye ears it was studied, if a transfer of alkaloids over the fungus-plant junction and an accumulation in grains might be possible.

2 Materials and methods

2.1 Origin and sampling of ergot

In the years 2002, 2003 and 2004, different rye varieties (n = 7, n = 21 and n = 24), mainly experimental hybrids and two conventional varieties (LPEP 3 and LPEP 4), were cultivated at three locations (environments E1-3) in Northern Germany (E1 = Petkus near Berlin, E2 = Klausheide near Nordhorn, E3 = Bergen near Celle). The artificial infection (inoculation) of the rye with *C. purpurea* was done according to procedures developed by the plant breeding company *Lochow Petkus GmbH*. For the primarily infection ergot sclerotia were brought on the ground before and after soil cultivation prior to rye sowing. The test varieties were then randomised allocated in arrays with three replicates at each location in 2003 and 2004; in 2002 only one replicate was used. In order to increase the infection stress for the test varieties pollen sterile rye was cultivated between each test variety and each array (Figure 1). Male infertile rye is highly susceptible to *C. purpurea* contaminations and can therefore act as an efficient vector for secondary infection over honey dew. To delay the moment of flowering of the test varieties, the respective parcels were treated with a growth regulator (Moddus®). In EC 49 the pollen ste rile rye was then inoculated with a spore suspension over a needle apparatus. The test varieties were additionally inoculated in EC 61 with a spore suspension over a pesticide spray. In the years 2002 and 2003, the same conidia suspension was used for inoculation. After rye harvest, the sclerotia were sorted out and the level of ergot contamination of the rye varieties was determined by weighing. The ergot samples were ground to 0.5 mm and dry matter- and alkaloid contents were analysed.

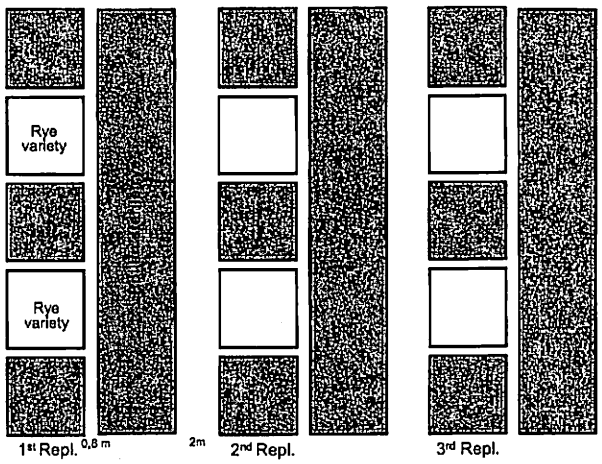

Figure 1:
Arrangement of the *Claviceps purpurea* inoculation experiments with different rye varieties (3 replicates)

At location E2, just before harvesting in 2003, 10 intact ergot contaminated ears were randomly chosen from each rye variety and cut from the rye plants. The ergot sclerotia space (n = 2392), as well as the rye kernels, were manually sorted out from the ears. The sclerotia length was measured and the ergot was fractionated according to size (fraction small: < 1 cm; fraction medium: 1 – 2 cm; fraction large: > 2 cm) and rye variety. The dry matter- and alkaloid contents were analysed after grinding to 0.5 mm.

2.2 Analyses

The alkaloid contents [ergometrine (EM); ergotamine (ET); ergocornine (ECOR); α-ergocryptine (ECRY);

ergocristine (ECRIS); ergosine (ESIN) and their *–inine* (-ine) isomers] of ergot and diets were analysed with a slightly modified HPLC-method developed by Wolff *et al.* (1988) as described by Mainka et al. (2005). The detection limits were 10 µg/kg for EM and EM-ine and 5 µg/kg for the other alkaloids and their isomers at a sample weight of 5 g. The mean recovery rate of the alkaloids in the diets was 79 %. The results of the analyses were not corrected for the recovery. The sum of all identified alkaloids (*-ine* and *–inine* isomers) is termed as total alkaloids. The contents of EM, ET, ECOR, ECRY and ECRIS are summed to the key alkaloid content.

2.3 Calculations and statistics

A one-factorial design of analysis of variance (ANOVA) was applied to analyse differences between rye varieties, locations, years and size fractions of ergot. Because of the high number of rye varieties, only the probability values (p) are shown. Variety LPEP 4 was used as the reference variety since it was one of the two "non-hybrids" and was cultivated in all years and at each location to determine significant variety differences (t-test, $p < 0.05$) for ergot contamination and ergot alkaloid content. To analyse any interactions [rye variety x location] and [rye variety x year], complete two by two factorial designs of ANOVA were used by considering only the same rye varieties for the respective years and locations. Significant differences between means were evaluated by the t-test or the Student-Newman-Keuls-test ($p < 0.05$).

All statistics were carried out using the Statistica for Windows ™ operating system (StatSoft Inc., 1994).

3 Results

Unfortunately, the sample quantities were not as high as intended. Because of adverse environmental conditions for ergot growth, sclerotia were produced at only two (E1 and E2), one (E2) and two (E2 and E3) locations in the years 2002, 2003 and 2004, respectively (Table 1).

Table 1:

Scheme of the successfully inoculated rye varieties in the different locations (E1 – E3) in the respective year

	E1	E2	E3
2002	n = 7	n = 7	
2003		n = 21	
2004		n = 24	n = 24

3.1 Ergot contamination and alkaloid content in 2002

The alkaloid analyses of the ergot from seven different rye varieties grown in two environments in the year 2002 demonstrated a significantly higher mean total alkaloid content of the ergot grown in E1 (1054 mg/kg DM) as compared to E2 (534 mg/kg DM) ($p = 0.014$) (Figure 2 and Table 2).

The key and *–inine* alkaloid portions of total alkaloids amounted to 83 % (69 – 89 %) and 15 % (11 – 22 %) in E1 and 71 % (60 – 76 %) and 22 % (18 – 31 %) in E2. The average alkaloid patterns are given in Table 3. In contrast to the alkaloid contents, the mean level of ergot contamination was significantly lower ($p = 0.046$) in E1 (8 g/kg rye) as in E2 (24 g/kg rye) (Figure 2 and Table 2). The correlation coefficient between the ergot contamination of rye and the total alkaloid content of ergot was r = - 0.383 ($p > 0.05$; n = 14).

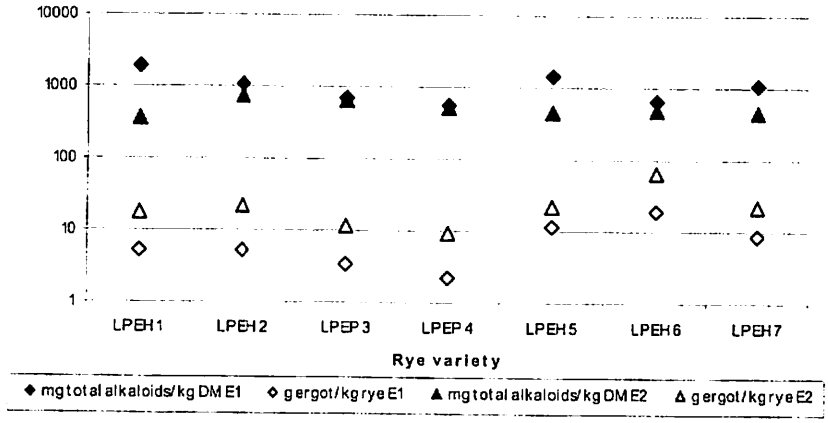

Figure 2:

Influence of different environments (E 1 or E 2) on the ergot contamination of diverse rye varieties and on the total alkaloid content of the ergot in 2002 (n = 1); semi-logarithmic illustration

LPEH = "*Lochow Petkus Hybrid*" (Hybrid rye variety)

LPEP = "*Lochow Petkus Population*" (Conventional rye variety)

Table 2:

Probability values of ergot contamination and total alkaloid content arranged according to the influence factors rye variety, location and year and the respective interactions

	Ergot	Total alkaloids
Rye variety	E2 / 2003: < 0.001	E2 / 2003: 0.010
	E2 / 2004: < 0.001	E2 / 2004: 0.418
	E3 / 2004: < 0.001	E3 / 2004: 0.501
	E2 / 2002/2003/2004: 0.005	E2 / 2002/2003/2004: 0.074
Location	E1/E2 / 2002: 0.046	E1/E2 / 2002: 0.014
	E2/E3 / 2004: < 0.001	E2/E3 / 2004: 0.693
Year	E2 / 2002/2003/2004: 0.949	E2 / 2002/2003/2004: < 0.001
Rye variety x location	E2/E3 / 2004: < 0.001	E2/E3 / 2004: 0.053
Rye variety x year	E2 / 2002/2003/2004: 0.124	E2 / 2002/2003/2004: 0.884

Table 3:

Mean alkaloid patterns (%) of the ergot in E1 and E2 in 2002 (n = 1), in E2 in 2003 (n = 3) and in E2 and E3 in 2004 (n = 3)

	E1 / 2002	E2 / 2002	E2 / 2003	E2 / 2004	E3 / 2004
Ergometrine	10.6	6.8	5.1	5.3	8.9
Ergometrinine	1.9	1.2	1.6	1.2	2.1
Ergotamine	4.1	13.5	14.8	21.3	3.6
Ergotaminine	1.0	5.1	4.0	9.0	1.0
Ergocornine	0.5	2.0	7.0	3.3	1.1
Ergocorninine	0.1	1.2	2.4	1.4	0.4
α-Ergocryptine	0.2	5.9	14.4	3.3	1.5
α-Ergocryptinine	0.2	2.7	5.5	1.9	1.3
Ergocristine	67.3	42.8	23.8	11.1	4.7
Ergocristinine	12.0	10.1	3.1	3.6	0.9
Ergosine	2.2	7.1	15.8	28.8	56.7
Ergosinine	0.1	1.6	2.4	9.9	17.9

Table 4:

Level of ergot contamination of different rye varieties and total alkaloid contents of the ergot in E2 in 2003 (n = 3)

Rye variety	*Ergot [g/kg rye]*	*Min - Max*	*Total alkaloids [mg/kg ergot DM]*	*Min - Max*
Year 2003				
LPEH 1	22	10 - 30	200	176 - 230
LPEH 1 (100)	3 *	2 - 3	343	17 - 589
LPEH 1 (50)	6	4 - 7	243	79 - 450
LPEH 2	45 *	21 - 70	246	129 - 325
LPEH 2 (100)	5	5 - 7	317	41 - 683
LPEH 5	14	5 - 19	96	33 - 211
LPEH 6	36 *	2 - 49	66	55 - 84
LPEH 7	36 *	14 - 47	48	35 - 66
LPEH 8	84 *	47 - 120	67	52 - 94
LPEH 9	110 *	78 - 151	61	27 - 98
LPEH 10	33 *	12 - 45	179	44 - 364
LPEH 11	32 *	19 - 38	194	131 - 270
LPEH 12	74 *	30 - 113	115	33 - 237
LPEH 13	38 *	29 - 42	51	43 - 60
LPEH 14	5	3 - 8	68	7 - 147
LPEH 14 (100)	7	5 - 8	119	85 - 159
LPEH 15	23 *	14 - 42	42	18 - 81
LPEH 16	18	10 - 25	54	26 - 84
LPEH 17	13	2 - 31	270	130 - 453
LPEP 3	13	7 - 19	151	52 - 239
LPEP 4	8	5 - 11	133	39 - 190
Mean	*30*	*3 -110*	*146*	*42 - 343*

* Significantly different as compared to LPEP 4 (t-test, $p < 0.05$).

LPEH = "*Lochow Petkus Hybrid*" (Hybrid rye variety); (50) (100) = Frequency of restorer genes

LPEP = "*Lochow Petkus Population*" (Conventional rye variety)

3.2 Ergot contamination and alkaloid content in 2003

The ergot contamination as well as total alkaloid contents of the harvest from different rye varieties at E2 in 2003 demonstrated high variations (3 – 110 g ergot/kg rye and 42 – 343 mg total alkaloids/kg ergot DM) (Table 4). Significant differences between some rye varieties and the reference variety (LPEP 4) were found only for ergot contamination (Table 4). However, significant differences between several varieties analysed by a one- factorial ANOVA were detected for the ergot contamination ($p < 0.001$) and the total ergot alkaloid content ($p = 0.010$) either (Table 2). The average portions of key alkaloids and *–inine* isomers of total alkaloids amounted to 65 % (59 – 74 %) and 19 % (6 – 36 %), respectively. The mean alkaloid pattern is shown in Table 3. The correlation coefficient between the ergot contamination of rye and the total alkaloid content of ergot was r = - 0.311 ($p < 0.05$; n = 75).

3.3 Ergot contamination and alkaloid content in 2004

The mean total alkaloid contents of the ergot grown on 24 rye varieties in 2004 amounted to 1223 mg/kg DM (863 – 1620 mg/kg DM) in E2 and 1243 mg/kg DM (818 – 1635 mg/kg DM) in E3 (Table 5).

The key and *–inine* alkaloid portions of total alkaloids

Table 5:

Level of ergot contamination of different rye varieties and total alkaloid contents of the ergot in E2 and E3 in 2004 (n = 3)

	E2				E3			
Rye variety	Ergot [g/kg rye]	Min - Max	Total alkaloids [mg/kg ergot DM]	Min - Max	Ergot [g/kg rye]	Min - Max	Total alkaloids [mg/kg ergot DM]	Min - Max
Year 2004								
LPEH 1	30 *	26 - 36	1233	1187 - 1259	90	33 - 128	1334	1190 - 1464
LPEH 1 (50)	6	4 - 8	1130	991 - 1355	9	6 - 11	988	742 - 1206
LPEH 2	60 *	30 - 77	1384	1087 - 1688	90 *	66 - 121	1497	1269 - 1633
LPEH 2 (100)	4	0.3 - 9	1620	852 - 2457	10	9 - 13	971	857 - 1102
LPEH 5	12	8 - 19	1319	1200 - 1524	41	17 - 64	1025	648 - 1309
LPEH 6	19	6 - 36	1251	1146 - 1354	65 *	47 - 90	1134	718 - 1428
LPEH 7	17	12 - 22	1144	1081 - 1239	64	28 - 113	818	707 - 878
LPEH 8	59 *	39 - 71	1217	1124 - 1388	79	46 - 126	1140	898 - 1594
LPEH 9	35 *	25 - 43	1384	1179 - 1668	80 *	56 - 94	1207	829 - 1557
LPEH 10	18	15 - 21	1239	978 - 1694	77 *	54 - 91	1303	1083 - 1496
LPEH 12	57	20 - 83	1251	1145 - 1436	223 *	178 - 279	1635	1276 - 1924
LPEH 13	32 *	28 - 40	1138	987 - 1421	72	48 - 107	1539	1224 - 1867
LPEH 14	11	2 - 23	1457	1030 - 2043	78	20 - 156	1061	779 - 1674
LPEH 17	11	8 - 12	1126	1042 - 1197	37	27 - 44	1049	805 - 1248
LPEH 18	39 *	32 - 44	1201	1027 - 1543	163 *	140 - 177	1316	1000 - 1563
LPEH 19	31 *	21 - 37	1345	987 - 1663	147 *	122 - 166	1207	555 - 1653
LPEH 20	63	40 - 103	1354	1240 - 1450	130 *	108 - 159	1272	940 - 1475
LPEH 21	27	19 - 33	1227	901 - 1494	96 *	83 - 103	1547	1488 - 1627
LPEH 22	85 *	63 - 120	1196	1067 - 1291	149 *	111 - 169	1575	1481 - 1641
LPEH 23	49 *	46 - 56	863	301 - 1253	191 *	99 - 261	897	478 - 1598
LPEP 24	9	9 - 10	1078	914 - 1197	20	11 - 26	1294	1168 - 1469
LPEH 25	38	22 - 47	1021	886 - 1125	170	89 - 294	1468	1008 - 1762
LPEH 26	23	15 - 32	944	929 - 967	104	23 - 149	1606	1002 - 2007
LPEP 4	12	6 - 21	1222	1120 - 1342	21	13 - 33	948	626 - 1135
Mean	*31* [a]	*4 - 85*	*1223*	*863 - 1620*	*92* [b]	*9 - 223*	*1243*	*818 - 1635*

* Significantly different as compared to LPEP 4 (t-test, $p < 0.05$).

[a, b] Different letters in one line indicate significant differences between means of each location at $p < 0.05$.

LPEH = "*Lochow Petkus Hybrid*" (Hybrid rye variety); (50) (100) = Frequency of restorer genes
LPEP = "*Lochow Petkus Population*" (Conventional rye variety)

were 44 % (25 – 60 %) and 27 % (15 – 35 %) in E2 and 20 % (13 – 37 %) and 24 % (20 – 30 %) in E3, respectively. The corresponding alkaloid patterns are presented in Table 3. Significant differences regarding the total alkaloid contents were found neither between rye varieties at both locations ($p = 0.418$ for E2 and $p = 0.501$ for E3), nor between the two locations ($p = 0.693$), nor was an interaction found between rye variety and location ($p = 0.053$). In contrast, in terms of ergot contamination, several rye varieties differed significantly when compared to LPEP 4 as well as when analysed by one-factorial ANOVA ($p < 0.001$) at E2 and E3 (Tables 4 and 5) and showed nearly the same ranking in their susceptibility for ergot contamination at E2 and E3. [E.g., rye varieties LPEH 1 (50) and LPEH 2 (100) demonstrated the slightest ergot contamination at both locations when compared with the others (Table 5)]. Furthermore, the ergot contamination was significantly higher at E3 ($p < 0.001$). This context is confirmed by the interaction, which was found between rye variety and location ($p < 0.001$) (Table 2). Such relationship concerning the total alkaloid contents was not detected. The correlation between ergot contamination in rye and total alkaloid content of ergot was found to be quite low in E2 ($r = -0.133$; $p > 0.05$) and E3 ($r = 0.224$; $p > 0.05$) either ($n = 75$).

3.4 Comprehensive ergot contamination and alkaloid content (2002 – 2004)

The one-factorial ANOVA demonstrated significant differences of the mean levels of ergot contamination of the six investigated rye varieties over 3 years at $p = 0.005$ as well as the comparison with LPEP 4 did (Table 6), but no influence of the year ($p = 0.949$) or interactions between rye variety and year ($p = 0.124$) were detected (Table 2). In contrast, the mean total alkaloid content was significantly influenced by the year at $p < 0.001$. However, the differences due to the rye variety were not significant ($p = 0.074$), nor were the interactions between both fractions ($p = 0.884$). Except for LPEH 2, which shows the highest alkaloid content in every year, no variety dependence for high or low alkaloid contents was detected.

3.5 Alkaloid contents of different ergot size fractions and rye kernels

Concerning the alkaloid contents of different ergot sizes and rye kernels, the majority of the sclerotia (82 %) were present in the medium size fraction, and only 15 % and 3 % in the small and large one, respectively. The total alkaloid contents (mg/kg DM) of the small, medium and large ergot fraction amounted to 116 (0.1 – 516), 125 (21 – 327) and 139 (1 – 679) and did not differ significantly from each other. The mean key and *–inine* alkaloid portions of total alkaloids were 58 % (41 – 69 %), 61 % (39 – 71 %) and 55 % (32 – 74 %) and 26 % (12 – 52 %), 23 % (18 – 29 %) and 24 % (16 – 33 %), respectively. Neither the ergot fraction ($p = 0.449$) nor the rye variety ($p = 0.081$) had a significant influence on the alkaloid content. No interaction between both was found either ($p = 0.199$). Additionally, ergot alkaloids were not detected (< detection limit) in rye kernels of ergot infected ears.

Table 6:

Level of ergot contamination of six rye varieties and total alkaloid contents of the ergot in 2002, 2003 and 2004 in E2 (2002: n = 1; 2003 and 2004: n = 3)

	2002		2003		2004		Overall means rye variety	
Rye variety	Ergot [g/kg rye]	Total alkaloids [mg/kg ergot DM]	Ergot [g/kg rye]	Total alkaloids [mg/kg ergot DM]	Ergot [g/kg rye]	Total alkaloids [mg/kg ergot DM]	Ergot [g/kg rye]	Total alkaloids [mg/kg ergot DM]
E 2								
LPEH 1	18	378	22	200	30	1233	23 *	604
LPEH 2	22	739	45	246	60	1384	42 *	790
LPEH 5	22	465	13	96	12	1319	16	627
LPEH 6	64	488	36	66	19	1251	40 *	602
LPEH 7	21	468	36	48	17	1144	25 *	553
LPEP 4	9	536	8	133	12	1222	10	630
Overall means year	*26*	*512* [a]	*27*	*132* [b]	*25*	*1259* [c]		

* Significantly different as compared to LPEP 4 (t-test, $p < 0.05$)

[a, b] Different letters in one line indicate significant differences between means of each year at $p < 0.05$.

LPEH = "*Lochow Petkus Hybrid*" (Hybrid rye variety); (50) (100) = Frequency of restorer genes

LPEP = "*Lochow Petkus Population*" (Conventional rye variety)

4 Discussion

In general, the alkaloid content of ergot varied in a wide range but in the reported range of variation (Young, 1981a; Young 1981b; Young and Chen, 1982; Wolff, 1989; Wolff and Richter et al., 1989; Komarova and Tolkachev, 2001; Richter, 2003). However, diverse methods of alkaloid analysis and considerations of different alkaloids summed to the so called total alkaloid content make an exact comparison with the literature quite difficult.

4.1 Influence factors on ergot contamination and alkaloid content

Factors known to affect the ergot contamination of rye are the location and year, or rather the climate, the virulence of the *C. purpurea* strain, the rye variety, esp. its extent of pollen production, sowing time, seed density and number of ears per m², length of flowering, crop rotation, soil cultivation, nutrient supply and infection stress, e.g., by weed (Freudenberg-Rosendahl, 1951; Hecht, 1951; Mothes and Silber, 1952; Richter et al., 1989; Jungehülsing, 1995; Mielke and Betz, 1995; Mielke, 2000; Doleschel and Pichlmaier, 2002; Engelke, 2002; Hartl et al., 2003; Hartl 2004).

The results of the present study correspond to the literature findings since influences of the variety and location on the ergot contamination of rye were found as well (Table 2). The ergot contamination of the conventional rye varieties and the hybrids which contained restorer genes was mostly lower as compared with the hybrids (Tables 4, 5 and 6 and Figure 2). This finding is in accordance to literature and due to the higher capacity of pollen production by conventional rye varieties and restorer genes containing hybrids (Mielke and Betz, 1995; Engelke, 2002; Wilde, 2005). An effect of the year on ergot contamination was not detected (Table 6), which is quite atypical due to varying annual climatic conditions, but might be explained by the limited number of observations (n = 6) and their high variations. In addition, the loss of some locations in each year, where no ergot sclerotia were produced (Table 1), is presumably mainly explainable by adverse environmental conditions in the respective year and at the respective location.

Alterations of the alkaloid content and pattern in ergot are mainly the cause of the *C. purpurea* strain, or rather the strain population (the unity of the different strains), but the environmental conditions (e.g., the climate, the soil, the nutrient supply and the host plant) seem to play an important role as well (Bekesy, 1940; Schulze, 1953; Silber and Bischoff, 1954; Meinicke, 1956; Kolsek et al., 1957; Hofmann, 1964; Kybal et al., 1976; Young, 1981b). Additionally, the numerous *C. purpurea* strains show special geographical extensions, which mostly explain the different alkaloid contents and patterns often found at diverse locations (Christensen, 1980; Komarova and Tolkachev, 2001). Furthermore, the total alkaloid contents may vary in dependence on the maturity stage of the sclerotia since large sclerotia showed higher alkaloid contents in some investigations (Bekesy, 1940; Schulze, 1953; Silber and Bischoff, 1954). However, the alkaloid contents of single sclerotia differ in wide ranges (Bekesy, 1940; Schulze, 1953; Silber and Bischoff, 1954; Young, 1981a;b), but the average content of a particular ergot source is highly constant (Silber and Bischoff, 1954).

Although the same inoculation suspension was used in E2 and E3 in 2004, different alkaloid patterns were found (Table 3). Noticeably, the mean total alkaloid contents varied only moderately (Table 5), which is in contrast to the results of the year 2002 (Figure 2). Probably due to adverse or favourable environmental conditions, some strains of the *C. purpurea* mix could have been promoted more than others (Bekesy, 1940; Schulze, 1953; Meinicke, 1956; Jungehülsing, 1995), which could have resulted in the expression of different alkaloid patterns. The varying total alkaloid contents and dissimilar alkaloid patterns in the years 2002, 2003 and 2004 at location E2 (Tables 3 and 6) are possibly also the result of different environmental conditions in the respective year and of different conidia suspensions, containing different *C. purpurea* strains, used in 2002/2003 and 2004. The host plant was apparently no environmental influence factor since a significant effect of the rye variety was not detected. Hence, the interaction between the *C. purpurea* strains and the environmental conditions seem to be essential.

In general, an influence of the rye variety on the alkaloid content cannot be derived from the data observed. As compared with the reference variety (LPEP 4) no differences were found (Tables 4, 5 and 6) and the one-factorial ANOVA did not show significant effects in the majority of cases as well (Table 2). The use of a conventional variety as a reference for hybrid rye seems not to be helpful to compare ergot alkaloid contents. Meinicke (1956), Wolff and Richter et al. (1989), Bush et al. (1997) as well as Young (1981b) found different alkaloid contents of ergot as dependent on the host plant (different wild grass and grain species). In the current experiments, only high bred rye varieties were used. The larger botanical distance of cultivated grain and wild grass species might presumably be an explanation, but also the special virulence of the huge number of *C. purpurea* strains for infecting different host plants is of importance (Jungehülsing, 1995). In the present study, the same conidia suspensions were used for the inoculation of different locations in one year (Figure 2 and Table 5). However, the diversity of the fungus strain populations at the locations due to varying environmental conditions might be an explanation for the altered alkaloid

contents in E1 and E2 in 2002. Since no significant location effect was found in 2004 (Tables 2 and 5), the results are inconsistent. However, an influence of the year on the alkaloid content can be concluded (Tables 2 and 6), and confirms the findings in literature (Mothes and Silber, 1952; Hecht, 1951; Richter 2003). The alterations are the result of different environmental conditions, but the different inoculation suspensions in 2002/2003 and 2004 have to be considered as well.

Deduced from the present analyses, the total alkaloid contents per kg rye (DM) may vary markedly between 0.002 mg and 2.457 mg per 1000 mg ergot/kg rye. Due to the low and non-significant correlations between the ergot contamination of rye and the alkaloid content of ergot in the majority of cases, no close relationship is concluded. For that reason, the analysis of the toxic alkaloids in rye instead of a survey of the ergot contamination seems to be more convincing to evaluate the feeding hazards.

4.2 Alkaloid content of different ergot size fractions and rye kernels

Bekesy (1940) as well as Silber and Bischoff (1954) found higher alkaloid contents in large than in small sclerotia, which was attributed to a better nutritional status. In the present study, only a trend towards higher alkaloid contents in large sclerotia was observed. Hence, a substantial higher alkaloid contamination of the medium sclerotia fraction (1 – 2 cm length), which size is similar to rye kernels, is not concluded. However, ergot fragments of broken sclerotia have to be considered as well.

Wolff and Richter (1989) detected ergot alkaloids, principally fat soluble ones, in different grain kernels and wheat germ oil and considered the transfer of ergot alkaloids from the sclerotia into the kernels during plant growth, which is not confirmed by the current analyses since no alkaloids were detected in rye kernels of ergot infected ears. A transfer of alkaloids from the fungus into grain kernels cannot be concluded from the present data.

6 Conclusions

Since the ergot contamination in rye and the alkaloid content of the ergot are highly variable and not closely related to each other, the analysis of the toxic alkaloids should be preferred to evaluate potential risks at feeding instead of the ergot contamination in grain. Small sclerotia seem not to be more toxic than larger ones as the alkaloid contents of different ergot size fractions differed not significantly from each other. A transfer of alkaloids from ergot sclerotia into grain cannot be concluded because ergot alkaloids were not detected in rye kernels of ergot infected ears.

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