The composition and taxonomic significance of fatty acid patterns in three white rust species: *Albugo amaranthi, A. candida and A. tragopogonis* (Peronosporales, Albuginaceae)

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The fatty acid composition of the biotrophic white rust species *A. amaranthi, A. candida* and *A. tragopogonis* was identified by means of GC and GC/MS analysis. Besides the common saturated fatty acids of C14 to C22 chain length, several unusual monounsaturated and polyunsaturated fatty acids were present in significant amounts in the lipid profiles of the three species. Each taxon revealed its own characteristic pattern which was uniform among populations of different geographic origin and independent from the host species. Fatty acids were obtained from minute amounts of sporangia collected from the surface of desiccated host plants. Comparison of herbarium specimens revealed a decrease of polyunsaturated compounds after storage for 30 months at room temperature, but still allowed unquestionable species differentiation.

Keywords: chemotaxonomy, polyunsaturated fatty acids, GC-MS analysis, oomycetes

Fatty acids (FAs) play an outstanding role as the major energy storage in oomycetes. Some of them may as well be an important factor for normal growth and development of these organisms (HERMAN & HERMAN 1985). Many of the biotrophic species of this group accumulate large amounts of lipids in their sporangia (DICK 1995) and recent studies on the sunflower downy mildew pathogen *Plasmopara halstedii* (SPRING & HAAS 2002; SPRING et al. 2003) revealed the presence of unusual high amounts of polyunsaturated fatty acids (PUFAs). The occurrence of C20 + PUFAs, like arachidonic acid (20:4n-6) and eicosapentaenoic acid (20:5n-3), in marine organisms, some fungi and bacteria has recently gained particular interest due to biotechnological reasons (reviewed by SAYANOVA & NAPIER 2004). In addition, the usefulness of FA pattern evaluation for phylogenetic and taxonomic studies was shown many times (e.g. VILJOEN, KOCK & THOUPOU 1989; ROMANO et al. 2000; SANINA, GONCHAROVA & KOSTETSKY 2004). Their exploration in Peronosporomycetidae has long been restricted to taxa of the saprophytic Saprolegniales and the few pathogenic species of the Peronosporales which could be cultivated on synthetic media (reviewed by LÖSEL 1988). More recent studies have shown that minute amounts of sporangia occurring on the host's surface are sufficient to establish the FA composition of biotrophic downy mildews as well (SPRING & HAAS 2002; SPRING et al. 2003). In continuation of our chemical survey of Peronosporales we now present the FA analysis of three white rust species of the genus *Albugo* representing ca. two thirds of the taxa common to Central Europe. The work is aiming to test the usefulness of FA profiling in fresh and herbarium preserved samples of different geographic origin and host relationship for the taxonomy of this difficult to classify group of organisms.

Material and methods

Sample material

Samples of *A. amaranthi* (SCHWEIN.) KUNTZE (1891)*, A. candida* (PERS.) KUNTZE (1891) and *A. tragopogonis* (DC.) GRAY (1821) were collected from infected host plants in the field and classified according to BRANDENBURGER (1985). Plant tissue with whitish blisters of nearly mature subepidermal sporangia was harvested and dried between paper in order to make vouchers for deposition in the herbarium Hohenheim (HUH). All samples except one originated from South Germany (Tab. 1).

Lipid extraction, preparation of methyl esters and GC analysis

Preparation of samples for GC was carried out essentially as described earlier (SPRING & HAAS 2002). However, lipid ex-

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Tab. 1: Specimens, origin and hosts of *Albugo* used in this study.

taxon	host	origin	collect. date	sample ID
A. amaranthi	*Amaranthus patulus*	Bad.-Württ., Krotzingen	07.07.2002	Aa468
	Amaranthus patulus	Bad.-Württ., Leinfelden-Echterdingen	30.07.2002	Aa488
	Amaranthus patulus	Rheinl.Pfalz, Mainz, Oppenheim	11.08.2002	Aa494
	Amaranthus retroflexus	Bad.-Württ., Esslingen, Scharnhausen	18.09.2002	Aa504
	Amaranthus sp.	Bad.-Württ., Stuttgart, Plieningen	07.08.2002	Aa568
A. candida	*Capsella bursa-pastoris*	Bad.-Württ., Esslingen, Scharnhausen	20.11.2001	AcH14
	Capsella bursa-pastoris	Bad.-Württ., Esslingen, Scharnhausen	15.09.2001	AcH15
	Capsella bursa-pastoris	Bad.-Württ., Tübingen, Hirschau	24.04.2002	Ac448
	Capsella bursa-pastoris	Bad.-Württ., Stetten	09.06.2002	Ac460
	Sinapis arvensis	Bad.-Württ., Stuttgart, Obertürkheim	09.10.2002	Ac500
	Capsella bursa-pastoris	Bad.-Württ., Esslingen, Scharnhausen	18.09.2002	Ac505
A. tragopogonis	*Helianthus annuus*	South Africa, Free State, Bethlehem	15.02.2003	At528
	Helianthus annuus	Bad.-Württ., Stuttgart, Plieningen	07.08.2003	At567

traction and transmethylation of FAs were combined to one single preparation step modified according to LARSON and GRAHAM (2001). Air-dried samples of zoosporangia (usually 1–5 mg) were harvested from the surface of infected host plants by means of a micro suction device. In cases of very sparse sporulation several hundreds of sporangia were collected with a fine spatula and rinsed off by dipping the tool in n-hexane.

The sample material was then subjected to an extraction / transmethylation procedure with 250 µl BF_3 in MeOH (14 %, w:w), 60 µl *tert*.-butyl methyl ether and heating at 90 °C for 30 min. After cooling, fatty acid methyl esters (FAME) were recovered by adding 0.5 ml petroleum ether and 0.5 ml H_2O. Routine FID-GC of FAME was carried out with a Shimadzu 17 A gas chromatograph equipped with a DB-23 capillary column (30 m × 0.25 mm i. d., J&W), on-column injector and FID. Operating conditions of the chromatograph were: detector temperature 260 °C, linear velocity of helium carrier gas 30 cm/s. The column temperature was initially set at 140 °C for 2 min, subsequently increased by 5 °C/min to 240 °C and maintained at this temperature for 8 min. For resolution of peak overlaps of the FAs C 20:5 and C 22:0, analyses were repeated on a CP-Select CB for FAME capillary column (50 m × 0.25 mm i.d., Varian-Chrompack) with the following operating conditions: detector temperature 260 °C, linear velocity of helium carrier gas 25 cm/s. The column temperature was initially set at 120 °C and increased by 4 °C/min to 240 °C.

Identification of FAME was usually achieved by comparison of retention times with standards.

Gas chromatography in combination with electron ionization mass spectrometry (GC/EI-MS)

GC/EI-MS analyses (electron energy 70 eV) were carried out with a Hewlett-Packard 5890 series II gas chromatograph interfaced to a Hewlett Packard 5971A mass spectrometer (Waldbronn, Germany). A 50 m x 0.25 mm i.d. fused-silica capillary column coated with 0.2 µm df of 100 % cyanopropyl polysiloxane (CP-Sil 88, Chrompack, Middelburg, The Netherlands) was installed in the GC oven. He 5.0 was used as the carrier gas at a constant flow rate of 1.0 ml/min. Injections were performed in split mode (split ratio 1:10). The injector and transfer line temperatures were set at 250 °C and 280 °C. The GC oven program started at 60 °C (hold time 1 min), then was raised at 7 °C/min to 180 °C and finally raised at 10 °C/min to 240 °C (hold time 5min). The ion source temperature was ~175 °C.

In the full scan mode, *m/z* 59 – *m/z* 400 were recorded at a sampling rate of 3 scans/s. Additional analyses in the selected ion monitoring (SIM) mode were performed using *m/z* 74, *m/z* 87, and *m/z* 316 ($[M]^+$) at 2.13 cycles/s for verification of 20:5 n-3 in *A. tragopogonis* (At567). FAMEs in the samples were identified by both identical retention times and mass spectra in comparison with individual standard compounds.

Results

Fatty acid patterns of three *Albugo* species

Lipid extracts of sporangium samples belonging to the taxa *A. amaranthi* (sample Aa568), *A. candida* (sample Ac505) and *A. tragopogonis* (sample At567) were analysed by means of GC/MS for the identification of fatty acids. All three samples shared the presence of the common saturated FAs (14:0, 16:0, 18:0, 20:0, 22:0), but differed significantly in their relative amount (Tab. 2). C 24:0 was found only in minute amounts in two of the samples. Amongst the unsaturated FAs the samples shared the presence of palmitoleic acid (16:1n-7), oleic acid (18:1n-9), α-linoleic acid (18:2n-6), α- and γ-linolenic acid (18:3n-3, 18:3n-6), as well as the less common stearidonic acid (18:4n-3). *Albugo amaranthi* differed from the two

Tab. 2: The fatty acid composition (identified by GC/MS analyses; ratio to total peak area) of *A. amarathi* (Aa568), *A. candida* (Ac505) and *A. tragopogonis* (At567)

Fatty Acid	*A. amaranthi*	*A. candida*	*A. tragopogonis*
14:0	0.4	5.3	2.6
16:0	21.5	9.1	41.7
16:1n-9c*	–	3.0	–
16:1n-7	1.3	1.2	7.4
16:2**	0.4	0.1	0.6
18:0	21.3	26.3	11.5
18:1n-9	4.5	16.8	3.3
18:2n-6	26.6	10.3	13.9
18:3n-6	7.3	6.3	2.0
18:3n-3	4.3	1.7	3.6
18:4n-3	0.9	1.7	1.7
20:0	4.3	2.7	2.6
20:3n-3	–	0.7	0.7
20:4n-6	–	3.3	7.0
20:5n-3	–	1.3	0.5
22:0	1.6	2.1	1.1
24:0	–	tr	0.3

* no standard available; position of the double bond tentatively assigned; ** position of the double bond not determined; tr = trace amounts (< 0.1); – = not detected

other species in the lack of PUFAs with a carbon chain length higher than 18. *Albugo candida* and *A. tragopogonis* contained significant amounts of arachidonic acid (20:4n-6), whereas eicosapentaenoic acid (20:5n-3) and eicosatrienoic acid (20:3n-3) were less abundant. *Albugo candida* was the only species to contain an isomer of 16:1, for which no commercially standard was available. According to GC-retention data and literature reports on FAs in other organisms, the structure was tentatively identified as 16:1n-9c.

Intraspecific variation of the fatty acid profiles

Comparison of the FA profiles of independent and geographically separated populations of *A. amaranthi* (5 samples), *A. candida* (6 samples) and *A. tragopogonis* (4 samples) revealed a high degree of taxon specific homogeneity (Fig. 1). The qualitative differences found in the model isolates described above (Tab. 2) were constantly present in all populations of a taxon. Quantitative differences were low to moderate, except for one sample of *A. candida* (AcH15) which showed a significant higher content of 16:1n-9c than the other 5 populations.

No host specific influence on the FA patterns was observed in *A. amaranthi* collected from *Amaranthus patulus* and *Amaranthus retroflexus*. Similarly, the pattern of a *A. candida* sample from *Sinapis arvensis* could not be distinguished from the samples isolated from *Capsella bursa-pastoris*.

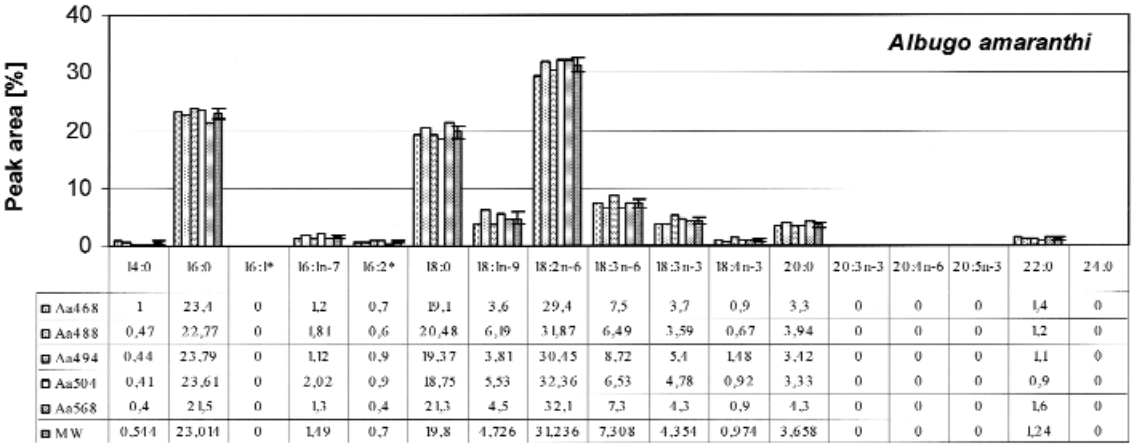
Samples of *A. amaranthi* collected in Baden, Württemberg, and Rheinland-Pfalz did not show any typical differences that were indicative for the existence of geographical races within the area screened. Interestingly, the four populations of *A. tragopogonis* from cultivated sunflower showed nearly identical FA patterns (Fig.1), although one sample derived from South Africa, while the others were collected near Stuttgart, Germany.

Stability of FAs in herbarium specimens

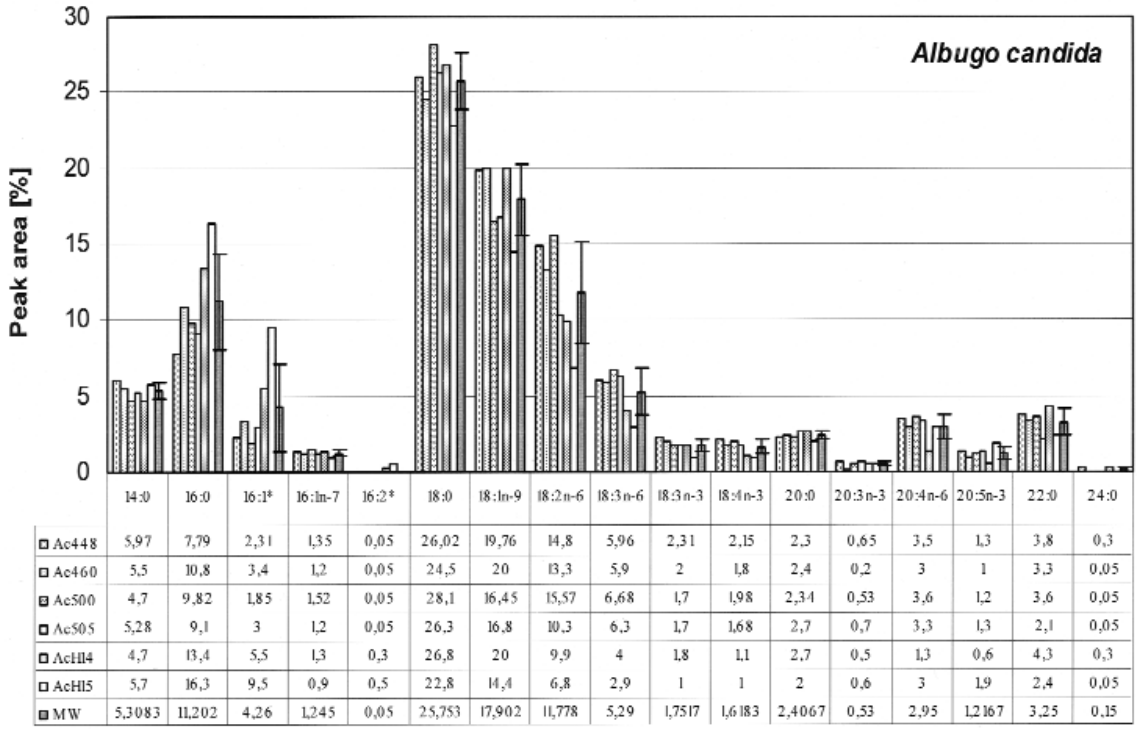
Three samples of *A. candida* from *Capsella bursa-pastoris* were extracted shortly after collection (Ac448, Ac460; Ac505) while two (AcH14, AcH15) were gained from host plants after 30 months of storage (room temperature) on air-dried herbarium specimens. Comparison of the FA patterns gave very similar results for both types of samples (Tab. 3). However, although samples stored for more than 2 years at room temperature showed all FAs characteristic for *A. candida*, the relative amount of unsaturated fatty acids was significantly lower. This was most evident for α-linoleic acid (18:2n-6) and α-linolenic acid (18:3n-6), while the n-3 PUFAs were less affected and the monounsaturated fatty acids (MUFAs) remained nearly constant when compared with samples from freshly harvested sporangia.

Discussion

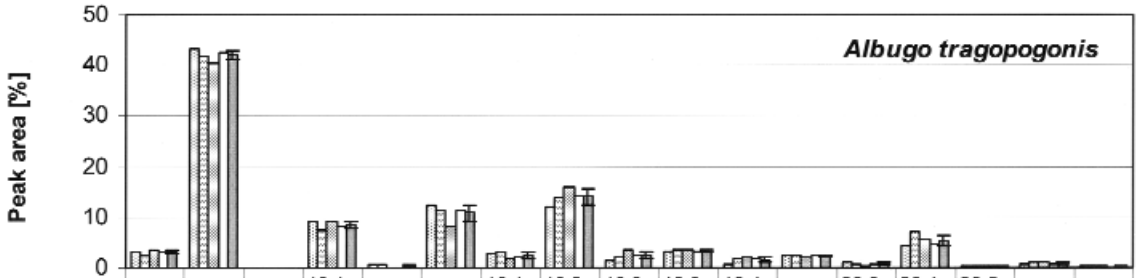
Within the Peronosporomycetidae, the white rusts (Albuginaceae) with the only genus *Albugo* (Pers.) Roussel 1806 form a well distinct group based on their unique mode of sporangium formation and other characters. Recent molecular genetic analysis suggests a basal phylogenetic position of the Albugina-



	14:0	16:0	16:1*	16:1n-7	16:2*	18:0	18:1n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:0	20:3n-3	20:4n-6	20:5n-3	22:0	24:0
Aa468	1	23,4	0	1,2	0,7	19,1	3,6	29,4	7,5	3,7	0,9	3,3	0	0	0	1,4	0
Aa488	0,47	22,77	0	1,81	0,6	20,48	6,89	31,87	6,49	3,59	0,67	3,94	0	0	0	1,2	0
Aa494	0,44	23,79	0	1,12	0,9	19,37	3,81	30,45	8,72	5,4	1,48	3,42	0	0	0	1,1	0
Aa504	0,41	23,61	0	2,02	0,9	18,75	5,53	32,36	6,53	4,78	0,92	3,33	0	0	0	0,9	0
Aa568	0,4	21,5	0	1,3	0,4	21,3	4,5	32,1	7,3	4,3	0,9	4,3	0	0	0	1,6	0
MW	0,544	23,014	0	1,49	0,7	19,8	4,726	31,236	7,308	4,354	0,974	3,658	0	0	0	1,24	0



	14:0	16:0	16:1*	16:1n-7	16:2*	18:0	18:1n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:0	20:3n-3	20:4n-6	20:5n-3	22:0	24:0
Ac448	5,97	7,79	2,31	1,35	0,05	26,02	19,76	14,8	5,96	2,31	2,15	2,3	0,65	3,5	1,3	3,8	0,3
Ac460	5,5	10,8	3,4	1,2	0,05	24,5	20	13,3	5,9	2	1,8	2,4	0,2	3	1	3,3	0,05
Ac500	4,7	9,82	1,85	1,52	0,05	28,1	16,45	15,57	6,68	1,7	1,98	2,34	0,53	3,6	1,2	3,6	0,05
Ac505	5,28	9,1	3	1,2	0,05	26,3	16,8	10,3	6,3	1,7	1,68	2,7	0,7	3,3	1,3	2,1	0,05
AcHl4	4,7	13,4	5,5	1,3	0,3	26,8	20	9,9	4	1,8	1,1	2,7	0,5	1,3	0,6	4,3	0,3
AcHl5	5,7	16,3	9,5	0,9	0,5	22,8	14,4	6,8	2,9	1	1	2	0,6	3	1,9	2,4	0,05
MW	5,3083	11,202	4,26	1,245	0,05	25,753	17,902	11,778	5,29	1,7517	1,6183	2,4067	0,53	2,95	1,2167	3,25	0,15



	14:0	16:0	16:1*	16:1n-7	16:2*	18:0	18:1n-9	18:2n-6	18:3n-6	18:3n-3	18:4n-3	20:0	20:3n-3	20:4n-6	20:5n-3	22:0	24:0
At528	3,3	43,4	0	9,3	0,6	12,3	2,7	11,9	1,4	3,3	0,8	2,6	1	4,3	0,4	0,6	0,4
At567	2,6	41,7	0	7,4	0,6	11,5	3,3	13,9	2	3,59	1,7	2,6	0,7	7	0,5	1,1	0,3
At642	3,4	40,6	0	9,2	0	8,2	1,6	16,1	3,5	3,5	2,1	2,1	0,5	5,6	0,3	1	0,2
At643	3,2	42,4	0	8,1	0,1	11,5	2	14,3	2,6	3,3	1,7	2,5	0,8	4,7	0,3	0,8	0,1
MW	3,125	42,03	0	8,5	0,433	10,88	2,4	14,05	2,375	3,423	1,575	2,45	0,75	5,4	0,375	0,875	0,25

Fig. 1: Comparative FA profiles in populations of *A. amaranthi*, *A. candida* and *A. tragopogonis*.

Values are given as peak area relative to the total area of FAs. Mean values and standard deviation for each FA are shown in the very right column. * position of the double bond tentatively assigned for 16:1n-9c, not determined for 16:2.

Tab. 3: Relative fatty acid composition (% of total content) of *A. candida* sporangia extracted shortly after the harvest of infected host plants and after 30 months of storage at room temperature, respectively

	SFA	MUFA	PUFA	n-3 FA	n-6 FA
Fresh samples					
Ac448	46.2	23.5	30.8	6.5	24.3
Ac460	46.0	24.6	27.9	5.6	22.2
Ac505	45.6	21.0	25.4	5.4	19.9
Mean value	**45.9**	**23.0**	**28.0**	**5.8**	**22.1**
SD +/-	0.25	1.50	2.21	0.48	1.79
Stored samples					
AcH14	52.2	26.8	19.5	4.0	15.2
AcH15	49.3	24.8	17.7	4.5	12.7
Mean value	**50.8**	**25.8**	**18.6**	**4.3**	**14.0**
SD +/-	1.45	1.00	0.90	0.25	1.25

SFA, MUFA, PUFA, saturated, monounsaturated, polyunsaturated fatty acids, respectively

ceae within the Peronosporomycetidae (HUDSPETH, STENGER & HUDSPETH 2003). Among the ca. 40 species (BIGA 1955; CHOI & PRIEST 1995), some are of economic importance due their ability to infect crop plants. Thus *A. candida* has its host preference in Brassicaceae species (PETRIE 1988), while *A. tragopogonis* is a major pathogen of cultivated sunflower with economic impact particularly in some regions of the southern hemisphere (reviewed by GULYA, RASHID & MASIREVIC 1997). Classification in some taxa (e.g. variations of *A. tragopogonis*) is equivocal and traditional phenotypic characters based on morphology (particularly the oospore wall) or virulence behaviour appear insufficient to solve the problem (CHOI & PRIEST 1995). While molecular genetic markers are now rapidly developed to reinvestigate the systematics and phylogeny of oomycetes (for review see SPRING 2004), chemical characters of these organisms have long been understudied due to the difficult accessibility of sufficient sample amounts. Recent investigations on some biotrophic taxa of Peronoporales species have shown that µg to mg amounts of sporangia are appropriate to analyse the FA composition and to use the data for diagnostic and taxonomic work (SPRING & HAAS 2002; 2004; SPRING et al. 2003). For the white rusts only a single sample of *A. candida* had been investigated so far (SPRING & HAAS 2002), thus prohibiting further taxonomic conclusions.

The current investigation unraveled a high diversity in the FA composition of the three *Albugo* species most common to Central Europe. Besides the saturated FAs of C14 to C24 chain length, these species contained some monounsaturated and polyunsaturated compounds which are less common in Eumycota and higher plants, but are abundant in marine algae and Straminipila (LÖSEL 1988), hence supporting the modern phylogenetic treatment of the Peronosporomycetidae (DICK 2001). In contrast to *Plasmopara halstedii*, a much higher amount and diversity of unsaturated C18 FAs was found in *Albugo*, while C20 PUFAs were less abundant. This could be an interesting feature for future investigations linking chemical with molecular genetic characters by tracing the phylogeny of desaturases and elongases as key enzymes of the metabolic pathway of FAs (for reviews see SPERLING & HEINZ 2001; SAYANOVA & NAPIER 2003).

Employing multiple independent populations of the three *Albugo* species supported the taxon specificity of FA patterns similarly to the situation previously found in *Plasmopara halstedii*. All three white rust species could be classified unambiguously by means of qualitative (16:1 isomer in *A. candida,* no C20 PUFAs in *A. amaranthi*) or quantitative differences in FAs, thus coinciding their phenotypic differentiation. The intraspecific variability of the patterns was similarly low as previously found for *Plasmopara halstedii* (SPRING & HAAS 2002). Nevertheless, it was unexpected that the four samples of *A. tragopogonis* were nearly identical in all aspects, although one of them had been grown more than 10 000 km from the others. Unfortunately, and unlike in the other two species, it was not yet possible to compare host specific influences on the FA pattern of *A. tragopogonis* which is reported to infect a relatively wide range of Asteraceae thus leading to the description of at least 5 independent varieties (see DICK 2002).

For a broader screening of FA profiles in biotrophic oomycetes, sampling from fresh material is a major obstacle, since accession of infected plant material is difficult and extremely time consuming. For that reason, we have tested the stability of FAs in desiccated plant samples stored at room temperature as a model for the use of herbarium collections. Although the results must be regarded as preliminary and need careful repetition with a broader range of samples over a longer period, it appears possible to use herbarium specimens up to an age of two to three years for comparative chemotaxonomic studies. Only the PUFAs decomposed to a certain degree, but still allowed to unequivocally classify the two *A. candida* samples.

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