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Microbial biodiversity associated with the walnut *Juglans regia* L. in South Tyrol (Italy)

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The endophytic and epiphytic microflora from walnut trees in South Tyrol (Northern Italy) was analyzed. A total of 16 trees from 8 sites were sampled at three dates during the warm season (May, July and September) in 2005, using plant material from three different tissue types (leaves, fruit and lignified twigs). Samples were either transferred directly onto nutrient medium or surface sterilized prior to plating to distinguish between epiphytic and endophytic growth. A total of 3,880 culturable isolates were obtained, the vast majority of the isolates (3,742) belonging to fungi (96.4 %); only 138 (3.6 %) were bacteria. Fungal isolates were classified into 30 genera, Alternaria, Penicillium, Phoma, Botryosphaeria, Fusarium, Cladosporium, Phyllosticta *and* Epicoccum *being the most common taxa. Although epiphytic growth predominated (73.9 % of the identified isolates), a significant number of endophytes (26.1 %) were isolated as well. More isolates were obtained from leaves (45.3 %) than from twigs (31.8 %) and fruit (23.0 %). Seasonal effects were not very distinct, but a tendency towards higher isolation success in September was observed.*
Keywords: *Juglans regia*, endophytes, phyllosphere, fungi, bacteria

***Die mikrobielle Biodiversität des Walnussbaumes* Juglans regia *L. in Südtirol (Italien)*.** *Die endophytische und epiphytische Mikroflora von Walnussbäumen in Südtirol (Norditalien) wurde analysiert. Insgesamt wurden Proben von 16 Bäumen an acht Standorten zu drei Zeitpunkten während der warmen Jahreszeit (Mai, Juli und September) im Jahr 2005 gesammelt, wobei Pflanzenmaterial von drei verschiedenen Gewebetypen verwendet wurde (Blätter, Nüsse und verholzte Zweige). Die Pflanzenproben wurden entweder direkt auf ein Nährmedium gegeben oder zuerst oberflächensterilisiert, um epiphytisches von endophytischem Wachstum zu unterscheiden. Insgesamt wurden 3880 Stämme isoliert, davon waren ein Großteil Pilze (94,4 %) und nur 138 (3,6 %) Bakterien. Die Pilzisolate wurden 30 Gattungen zugeordnet, von denen* Alternaria, Penicillium, Phoma, Botryosphaeria, Fusarium, Cladosporium, Phyllosticta *und* Epicoccum *die häufigsten waren. Obwohl epiphytisches Wachstum vorherrschte (73,9 % der identifizierten Isolate), konnte auch eine signifikante Zahl von Endophyten isoliert werden (26,1 %). Die Isolierungsrate aus Blättern (45,3 %) war höher als jene aus Zweigen (31,8 %) und Nüssen (23,0 %). Jahreszeitliche Unterschiede waren nicht stark ausgeprägt, aber ein Trend zu höheren Isolierungsraten im September wurde beobachtet.*
Schlagwörter: *Juglans regia*, Endophyten, Phyllosphäre, Pilze, Bakterien

***La biodiversité microbienne du noyer* Juglans regia *L. au Tyrol du Sud (Italie)*.** *La microflore endophytique et épiphytique des noyers au Tyrol du Sud a été analysée. Des échantillons de 16 arbres dans huit habitats au total ont été prélevés à trois moments pendant la saison chaude (mai, juillet et septembre) de l'année 2005, tout en utilisant des matériaux végétaux de trois types de tissus différents (feuilles, noix et branches lignifiées). Soit les échantillons des plantes étaient appliqués directement sur le substrat nutritif, soit leur surface était préalablement traitée, afin de pouvoir distinguer la croissance épiphytique de la croissance endophytique. 3880 souches au total ont été isolées, dont la plupart étaient des champignons (94,4 %) et 138 (3,6 %) seulement des bactéries. Les isolats des champignons ont été attribués à 30 genres, dont* Alternaria, Penicillium, Phoma, Botryosphaeria, Fusarium, Cladosporium, Phyllosticta *et* Epicoccum *étaient les plus fréquents. Alors que la croissance épiphytique était prépondérante (73,9 % des isolats*

identifiés), un nombre significatif d'endophytes a également pu être isolé (26,1 %). Le taux d'isolement des feuilles (45,3 %) était plus élevé que celui des branches (31,8 %) et des noix (23,0 %). Les différences saisonnières n'étaient pas très fortes, mais une tendance vers des taux d'isolement plus élevés en septembre a été observée.
Mots clés: *Juglans regia*, endophytes, phyllosphère, champignons, bactéries

The walnut tree *Juglans regia* L. (Juglandaceae) is native to lower mountainous areas from the eastern Mediterranean to Central Asia and is widely planted in temperate regions worldwide as an ornamental tree as well as for fruit and timber production. The centers of commercial walnut plantation are China and the USA, especially California, with approximately 80,000 ha and a yearly production of 240,000 tons. In South Tyrol walnut trees are found mainly as single trees around farm houses at elevations between 220 and 1200 m asl, and the fruits are used for desserts and liquor production. Walnut trees prefer deep, fertile soils and a warm, temperate climate with moderate to higher yearly precipitation (> 700 mm).

Only a limited number of microbial and insect species is known to attack walnut trees and cause significant damage (Blaschke and Bussler, 2008; Teviotdale et al., 2002), which might be explained by the production of different chinons (e.g. juglon and plumbagin) and polyphenols with strong anti-microbial activity in leaves and other plant tissues of walnut trees.

The most common walnut diseases are fungal anthracnose, caused by *Gnomonia leptostyla* (anamorph *Marssonina juglandis*); bacterial walnut blight, caused by *Xanthomonas campestris* pv. *juglandis*; and the viral blackline, caused by the cherry leafroll virus strain CLRV-W (Teviotdale et al., 2002). Whereas a number of studies have been published on the mycoflora of the edible seeds and the occurrence of macro fungi on the stems, only a few reports are available on the biodiversity of micro fungi on walnuts in Central Europe (Juhásová et al., 2005). Therefore, this study was conducted with the following aims:

- Investigation of microbial biodiversity associated with walnut trees in South Tyrol
- Distinction between endophytic and epiphytic microbial growth
- Observation of potential differences in the biodiversity based on type of plant tissue, geographic origin and sampling date

Material and methods

Plant samples were collected from a total of 16 walnut trees from eight sites in South Tyrol (Fig. 1): Mals (3 trees; 1050 m asl) and Laas (5 trees; 840 to 930 m asl) in Vinschgau Valley; Dorf Tirol (1 tree; 300 m asl) and Marling (1 tree; 300 m asl) in the Burggrafenamt area; Bozen (1 tree; 310 m asl); Girlan (1 tree; 430 m asl) and Eppan (1 tree; 550 m asl) in the Überetsch area; and Laimburg (3 trees; 230 m asl) in the Unterland area. The trees grow next to farm houses or in open meadows and were not treated with pesticides (with the exception of one tree at Laimburg treated with an insecticide). Age and cultivar of the trees were not determined, all trees, however, were mature and tall, produced abundant fruit and did not show severe symptoms of diseases.

Five samples with a surface of approx. 5 x 5 mm were collected from each plant tissue and transferred onto Potato Dextrose Agar- (PDA; Merck) and Nutrient Broth Agar- (NDA; Sigma-Aldrich) plates, respectively. Plant surface was sterilized by dipping the samples into 70 % ethanol for one minute before plating. Growing cultures were transferred onto new plates for further purification and identified microscopically to genus level. Two common morphotypes, which were difficult to distinguish based on morphological characters, were identified at the Austrian Center of Biological Resources and Applied Mycology (ACBR), Vienna, by sequencing the ITS 1 and 2 region of the ribosomal gene cluster and a blast search of the GenBank of the National Center for Biotechnology Information (NCBI), 8600 Rockville Pike, Bethesda, MD 20894, USA (Altschul et al., 1997).

Bacterial colonies were analyzed based on the following tests: Gram-staining, potassium hydroxide (KOH) string test, catalase and oxidase activity, production of endospores, morphology and motility.

Results and discussion

The microbial flora from walnut trees (*Juglans regia*) in South Tyrol (Northern Italy) was examined during the 2005 vegetation period. In order to obtain a representative overview of the bacterial and fungal biodiversity, sampling was carried out at regular intervals from July until September from three different plant tissues (leaves, fruit and wooden twigs) from trees growing at eight sites in the province. A total of 3,880 micro-

Fig. 1: Origin of plant samples
(1 = Mals, 2 = Laas, 3 = Dorf Tirol, 4 = Marling, 5 = Bozen, 6 = Girlan, 7 = Eppan, 8 = Laimburg)

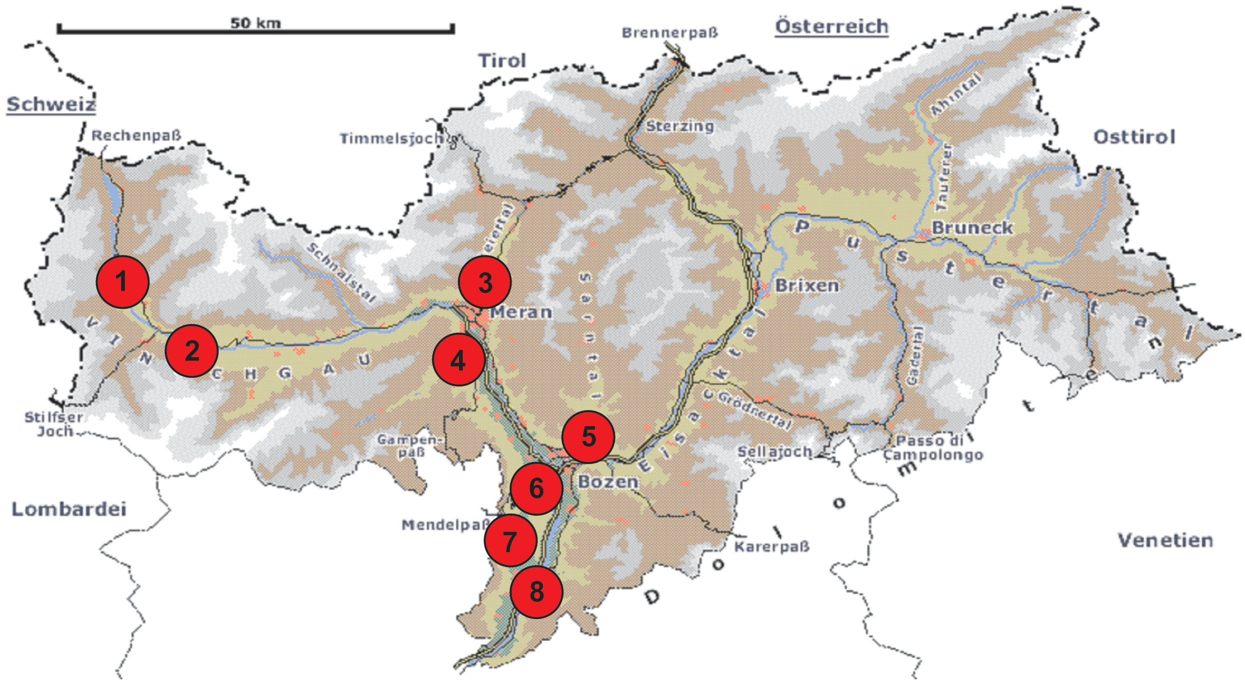

Fig. 1. Origin of plant samples: 1) Mals, 2) Laas, 3) Dorf Tirol, 4) Marling, 5) Bozen, 6) Girlan, 7) Eppan and 8) Laimburg

bial strains were isolated on artificial media, 96.4 % of them fungi and 3.6 % bacteria. Because identification on species level solely based on morphological characters is difficult and might lead to erroneous results especially with endophytic taxons (Sieber, 2007), microbial strains were only identified to genus level (Table 1).

Fungal isolates

Out of 3,742 fungal isolates, 3,233 isolates (86.4 %) were classified into 30 genera. Most of the unidentified fungal isolates did not sporulate on PDA-medium (mycelia sterilia). In a previous study on endophytic fungi from the grapevine *Vitis vinifera,* we found a similar number of genera (Schweigkofler and Prillinger, 1997), whereas other authors isolated up to 100 and more fungal species from wooden and herbaceous plants (Carroll and Carroll, 1978; Petrini, 1986).
Taxonomically, the fungal isolates from walnuts belong to one order of the Zygomycota, nine orders of the Ascomycota and one order of the Basidiomycota (Table 2). The predominant role of ascomycetous fungi on aerial surfaces and as endophytes of wooden plants is well established (Petrini, 1986; Schweigkofler and Prillinger, 1997 and 1999; Sieber, 2007). Based on Sieber (2007), most endophytes from angiosperms belong to Diaphortales, whereas members of the Heliotiales predominate the endophytic flora of the gymnosperms. In this study, we isolated only two taxons belonging to Diaporthales (*Melanconium* and *Phomopsis*) and three genera of Helotiales (*Botrytis, Monilinia* and *Cylindrosporium*), all five of them at low frequencies. The three most common genera were *Alternaria*, *Phoma* (both belonging to Pleosporales) and *Penicillium* (Eurotiales) with a cumulative frequency of nearly 60 % of all isolates. Eight additional genera were isolated at frequencies of above 1 %, among them one basidiomycetous (*Rhizoctonia*), and seven ascomycetous (*Fusarium, Botryosphaeria, Cladosporium, Phyllosticta, Epicoccum, Aureobasidium* and *Colletotrichum*). White yeasts (Saccharomycetales) were isolated only at low frequencies, whereas red basidiomycetous yeasts (e.g. *Rhodotorula*, *Sporobolomyces*) were not present at all. The two zygomycetous genera *Mucor* and *Rhizopus* added up to 1 % isolation frequency. Two morphotypes (called LB-1 and LB-2) isolated in high numbers were identified using molecular analysis of the ITS-gene region. The ITS-gene region

Table 1: Isolation of microbial strains from *Juglans regia* of different geographic origin in South Tyrol; strains were identified to genus level. Percentage levels refer to the total number of identified isolates from a certain origin

	SUM	%	Mals	%	Laas	%	Marling	%	Dorf Tirol	%	Eppan	%	Girlan	%	Bozen	%	Laimburg	%
Fungi total	3742	100	476	12.7	1044	27.9	316	8.4	276	7.4	260	6.9	249	6.7	382	10.2	739	19.7
Fungi identified	3233	86.4	393	82.5	886	84.4	296	93.6	245	88.7	217	83.4	214	85.9	334	87.4	648	87.6
Alternaria	557	17.2	21	5.3	181	20.4	64	21.6	58	23.7	41	18.9	28	13.1	56	16.8	108	16.7
Penicillium	515	15.9	39	9.9	89	10.0	69	23.3	66	26.9	39	18.0	79	36.9	27	8.1	107	16.5
Phoma 2	476	14.7	69	17.6	138	15.6	28	9.5	32	13.1	36	16.6	27	12.6	49	14.7	97	15.0
Phoma 1	367	11.4	34	8.7	118	13.3	35	11.8	15	6.1	20	9.2	14	6.5	49	14.7	82	12.7
Fusarium	285	8.8	63	16.0	118	13.3	3	1.0	8	3.3	5	2.3	4	1.9	41	12.3	30	4.6
Botryosphaeria	266	8.2	38	9.7	84	9.5	19	6.4	17	6.9	15	6.9	21	9.8	26	7.8	46	7.1
Cladosporium	204	6.3	49	12.5	39	4.4	27	9.1	14	5.7	14	6.5	5	2.3	26	7.8	30	4.6
Rhizoctonia	133	4.1					9	3.0	8	3.3	8	3.7	11	5.1	17	5.1	27	4.2
Phyllosticta	106	3.3	10	2.5	32	3.6	3	1.0	5	2.0	6	2.8	5	2.3	11	3.3	34	5.2
Epicoccum	102	3.2	10	2.5	19	2.1	11	3.7	10	4.1	8	3.7	2	0.9	3	0.9	39	6.0
Aureobasidium	84	2.6	17	4.3	7	0.8	8	2.7	5	2.0	10	4.6	7	3.3	22	6.6	9	1.4
Colletotrichum	41	1.3					16	5.4	4	1.6	1	0.5					20	3.1
Yeast	14	0.4	2	0.5	3	0.3	2	0.7			1	0.5			2	0.6	5	0.8
Rhizopus	21	0.6	1	0.3							8	3.7	9	4.2			3	0.5
Mucor	14	0.4	4	1.0	2	0.2	1	0.3	1	0.4	1	0.5	1	0.5			4	0.6
Drechslera	10	0.3	1	0.3	7	0.8			2	0.8								
Diplodia	8	0.2	2	0.5	4	0.5					1	0.5	1	0.5				
Aspergillus	4	0.1	3	0.8											1	0.3		
Melanconium	4	0.1	1	0.3	1	0.1	1	0.3			1	0.5						
Sphaeropsis	4	0.1															2	0.3
Phomopsis	3	0.09									1	0.5			1	0.3		
Botrytis	2	0.06			1	0.1									1	0.3		
Cylindrocarpon	2	0.06			1	0.1											1	0.2
Cylindrosporium	2	0.06													2	0.6	1	0.2
Dendryphion	2	0.06									1	0.5						
Monilinia	2	0.06			1	0.1											1	0.2
Nectria	2	0.06			2	0.2												
Acroconidiella	1	0.03			1	0.1												
Geotrichum	1	0.03			1	0.1												
Ovularia	1	0.03			1	0.1												
Verticillium	1	0.03			1	0.1												
Bacteria total	138	100	25	18.1	34	24.6									12	8.7	34	24.6
Bacillus	30	21.7	9	36.0	7	20.6									3	25.0	4	11.7
Xanthomonas	35	25.4	4	16.0	12	35.3									4	33.3	6	17.6
other bacteria	73	52.9	12	48.0	15	44.1									5	41.7	24	70.6

of isolate LB-1 had a length of 502 bp, those of isolate LB-2 566 bp. The sequences can be obtained from the GenBank (accession no.: DQ640319 and DQ640318). The blast-search for LB-1 showed high homology to *Botryosphaeria* species, esp. *B. sarmentorum* and *B. iberica* (max. identity: 98 %), whereas isolate LB-2 was most closely related to *Phoma* species, especially *P. sojicola* (max. identity: 98 %).

Based on our results, the overwhelming proportion of microbes associated with the walnut tree is not host specific. Species belonging to the genera mentioned above are widespread saprophytes and/or plant pathogens associated with a wide range of higher plants; most of them produce abundant airborne pigmented spores (mainly conidia, less common ascospores), which are well adapted to UV- and drought stress.

Alternaria spp. is a widespread pathogen of apple trees in South Tyrol, causing symptoms on leaves and fruit at warm and moist weather conditions (Marschall et al., 2006), but is also common as an endophyte in grapevines (Schweigkofler and Prillinger, 1997). In Northern Italy an Alternaria leaf spot has been described on walnuts with symptoms being circular, necrotic spots bordered with concentric zones of darker tissue (Belisario et al., 1999). The genus *Phoma* contains a large number of plant pathogenic species that produce asexual spores in pycnidia, among them *P. juglandis*, a minor walnut pathogen. *Penicillium* (the most common fungal isolate on the fruit) is well-known to infect walnut kernels and produce mycotoxins. *Fusarium* species are widespread pathogens especially of herbaceous plants but can also attack forest trees (e.g. *Pinus* spp.). Dispersal of *Fusarium* conidia can be airborne and very abundant at moist weather conditions (Garbelotto et al., 2008) and might be enhanced by the activity of beetles (Gordon et al., 2001). On walnut fruit *Fusarium* spp. were found to be associated to BAN (Brown Apical Necrosis), a complex disease which can lead to severe fruit drop (Belisario et al., 2002). *Botryosphaeria* is a pathogen of wooden plants (e.g.

Table 2: Taxonomic position of fungi isolated from walnut trees based on classification system used by the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov)

Phylum	Subphylum	Class	Subclass	Order	Family	Genus
Zygomycota	Mucoromycotina			Mucorales	Mucoraceae	*Mucor*
						Rhizopus
Ascomycota	Pezizomycotina	Dothideomycetes	Dothideomycetidae	Capnodiales	Mycosphaerellaceae	*Acroconidiella*
						Ovularia
					Davidiellaceae	*Cladosporium*
					Dothioraceae	*Aureobasidium*
				Botryosphaeriales	Botryosphaeriaceae	*Botryospaehria*
						Diplodia
						Phyllosticta
						Sphaeropsis
			Pleosporomycetidae	Pleosporales	Pleosporaceae	*Alternaria*
						Dendryphion
						Drechslera
						Phoma
					Leptosphaeriaceae	*Epicoccum*
		Eurotiomycetes	Eurotiomycetidae	Eurotiales	Trichocomaceae	*Aspergillus*
						Penicillium
		Leotiomycetes		Helotiales	Sclerotiniaceae	*Botrytis*
						Monilinia
					Dermateaceae	*Cylindrosporium*
		Sordariomycetes		Phyllachorales	Phyllachoraceae	*Colletotrichum*
						Verticillium
			Hypocreomycetidae	Hypocreales		*Fusarium*
					Nectriaceae	*Cylindrocarpon*
						Nectria
			Sordariomycetidae	Diaporthales	Melanconidaceae	*Melanconium*
					Valsaceae	*Phomopsis*
	Saccharomycotina	Saccharomycetes		Saccharomycetales	Dipodascaceae	*Geotrichum*
						Yeast species
Basidiomycota	Agaricomycotina	Agaricomycetes	Agaricomycetidae	Polyporales	Corticiaceae	*Rhizoctonia*

the grapevine *V. vinifera*) and might cause a trunk disease. Wood-staining fungal species with dissemination primarily by insect vectors, like *Ophiostoma*, *Leptographium* and *Ceratocystis*, were not found in this study. Unexpectedly, we did not find isolates of *Marssonina juglandi,* causal agent of fungal anthracnose of the walnut. We found perithecia of the teleomorph stage *Gnomonia leptostyla* (Diaporthales) on leaf debris under a walnut tree in fall. Ascospores produced on the perithecia start the infection cycle of *M. juglandi* in spring. The dry weather in 2005 might have been unfavorable for the growth of *M. juglandi,* which prefers wet conditions. Black spots were observed on some walnut trees, but not so premature defoliation, a typical symptom for heavy infestation by this pathogen. The absence of *M. juglandi* might be explained by sampling only plant tissue showing no symptoms of disease. In addition, leaf debris (where ascospores are produced) was removed from under some of the walnut trees by the owners.

The majority of the fungal isolates was obtained from non-sterile plant tissues (73.9 %), but 841 (26.1 %) were isolated after surface sterilization and, therefore, are considered to be endophytes (Table 3). Members of most genera were isolated both as endophytes and epiphytes with the exception of *Verticillium* and *Phomopsis*, which were isolated only after surface sterilization and *Drechslera* and *Nectria*, for which only epiphytic growth was observed.

Climatic conditions

Microbial growth depends on a variety of environmental parameters. Among them temperature and relative humidity play a predominant role. The climate in South Tyrol's lower valleys is sub-Mediterranean with warm summers and mild winters with occasional snowfall. The year 2005 was particularly dry and sunny: at the Research Centre Laimburg an annual precipitation of only 619 mm was measured, compared to the 818 mm yearly mean. Whereas the mean yearly temperature of 2005 was very similar to long-time mean (11.4 °C vs. 11.5 °C), the summer was warmer

Table 3: Amount of identified fungal isolates differing in sampling date, tissue type and surface sterilization

	Total	%	Epi-phytes	%	Endo-phytes	%
All samples:	3233	100	2392	73.9	841	26.1
Sampling date:						
May	1064	32.9	723	68.0	341	32.0
July	783	24.2	691	88.3	92	11.7
September	1386	42.9	978	71.5	408	28.5
Tissue type:						
Leaves	1465	45.3	1076	73.4	389	26.6
Fruit	743	23.0	526	70.8	217	29.2
Twigs	1029	31.8	794	77.2	235	22.8

with a total of 108 "summer days" (temp. max. 25 °C), compared to the mean 99 days. The same general pattern was observed in other areas from which walnut samples were obtained. The sample sites in Vinschgau Valley (Mals and Laas), however, are located at significantly higher elevations compared to the other sites and characterized by both lower temperatures and less precipitation. The average number of fungal isolates per tree is slightly lower in Vinschgau Valley compared to the lower and warmer areas: 476 fungal isolates from three trees in Mals (average: 158 isolates/tree) and 1,044 fungal isolates from five trees in Laas (average: 209 isolates/tree), compared to an average of 278 fungal isolates from each one of the eight trees located in the lower and warmer areas. The sampling date had a certain influence on the isolation frequency with most strains isolated in September, followed by May and July (Table 3). The climatic parameters (temperature, relative humidity, precipitation, and UV irradiation), the composition of the leaf surface (senescence) and the availability of nutrients all change over the vegetation period and could play a role for the microbial colonization. Whereas so-called resident species inhabit the phylloplane over a prolonged period in different geographic regions, the colonization pattern of transient species is more irregular and can oscillate during the season (Davenport, 1976). Among the yeasts, typical resident species on apples and grapevines are *Aureobasidium* and certain red yeasts (e.g. *Rhodotorula*), whereas most white ascomycetous yeasts have a more transient appearance (Davenport, 1976). The darker pigmentation of the resident species might function as UV protection. We isolated a relevant number of *Aureobasidium* strains, but surprisingly no red yeast species.

A slight effect of the tissue type on the isolation success was observed (Table 3): most isolates were found on leaves (1,465; belonging to 28 genera), followed by twigs (1,029; 27 genera) and nuts (743; 22 genera). The same genera (*Phoma, Alternaria, Penicillium, Fusarium, Botryosphaeria*) were predominant in all tissues, *Phoma* being the most common on both leaves (26.5 % of the leaf isolates) and twigs (25.9 %), and *Penicillium* (29.1 %) the most common on nuts. *Alternaria*, among the three most common genera on leaves (21.6 %) and twigs (18.6 %), was less common on nuts (6.6 %). The relative proportion of endophytic versus epiphytic growth was similar for all tissue types.

Bacterial isolates

A total of 138 bacterial strains was isolated in this study. Based on morphological and physiological tests, 30 isolates (22 %) belonged to the genus *Xanthomonas*, and 35 (25 %) to the genus *Bacillus*. The remaining 73 isolates (53 %) were not identified, but could be distinguished into four distinct groups with matching characteristics (three of them Gram-negative and one Gram-positive). The *Xanthomonas*-isolates were not characterized further but most likely belonged to *X. campestris* pv. *juglandis*, causal agent of bacterial walnut blight. Surface lesions on immature fruit, typical for that disease, were observed regularly on the walnut trees sampled. Symptoms of bacterial canker of walnuts (caused by *Brenneria nigrifluens,* syn. *Erwinia nigrifluens*), have not been observed in this study.

Similar to the fungal isolates, the average number of bacterial isolates per tree is slightly lower in Vinschgau Valley (av. 7.4) compared to South Tyrol's lower and warmer areas (av. 9.8). A slow but steady increase of bacterial growth was observed from July (26.1 % of total isolates) to August (31.2 %) and September (42.8 %). In general, more bacteria were isolated from leaves (52.2 %) than from nuts (29.0 %) and twigs (18.8 %), but differences were found based on bacterial taxonomy: most *Xanthomonas* strains (73.3 %) were isolated from leaves, 10 % from nuts and 16.7 % from twigs, whereas only 34.8 % of the *Bacillus* strains were isolated from leaves, 45.7 % from nuts and 20 % from twigs. The differences in bacterial growth patterns might be explained by the presence of antibacterial substances and phenolic compounds in walnut tissue (Pereira et al., 2007), extracts of walnut leaves selectively inhibited the growth of Gram-positive bacteria, whereas Gram-negative bacteria and fungi were resistant to the extracts.

Conclusion

Walnut trees are a habitat for a wide variety of fungal and (to a lesser extent) bacterial taxons, some of which can pose a serious threat to plant health. The only sporadic cultivation of walnuts in South Tyrol might prevent the establishment of severe epidemics typical for monocultures. Measures should be taken to further prevent the accumulation of microbial inoculum, e.g. removal of leaf debris.

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