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Epiphytic fungi on apple leaves and their value for control of the postharvest pathogens *Botrytis cinerea, Monilinia fructigena* and *Penicillium expansum*

Epiphytische Pilze auf Apfelblättern und ihre Eignung für die Bekämpfung der Apfelfäuleerreger *Botrytis cinerea, Monilinia fructigena* und *Penicillium expansum*

C. J. Falconi, K. Mendgen*
Universität Konstanz, Fakultät für Biologie, Lehrstuhl für Phytopathologie, D-78464 Konstanz

Summary

Fungal populations were examined each month on apple leaves, cv. 'Golden Delicious', during 1989. Out of 32 different fungal species studied, 21 were present throughout the season in large numbers. Of these, 368 isolates were selected and tested *in vitro* and *in vivo* for antagonistic activity against the postharvest pathogens *Botrytis cinerea, Monilinia fructigena* and *Penicillium expansum.* If applied to wounded apples, isolates of *Aureobasidium pullulans, Epicoccum purpurascens, Sordaria fimicola* and *Trichoderma polysporum* (20 µl containing 10^7 spores/ml) provided good protection against the three postharvest pathogens at a ratio of 100:1 (antagonists to pathogen). Better control with much smaller numbers of antagonists was obtained with mixtures, such as *E. purpurascens* plus *A. pullulans, S. fimicola,* or *T. polysporum.* After the application of such mixtures to freshly wounded apples, ratios between 1:1 until 10:1 (antagonists to pathogen) were sufficient to control postharvest decay over a broad range of concentrations of the micro-organisms.

Key words: Apple; postharvest pathogens; *Botrytis cinerea; Monilinia fructigena; Penicillium expansum;* biological control

Zusammenfassung

Während der Saison 1989 wurden die Pilzpopulationen auf Apfelblättern der Sorte 'Golden Delicious' monatlich untersucht. Von den 32 verschiedenen Arten, die im Laufe des Jahres gefunden wurden, waren 21 im Verlauf der Saison in grossen Mengen anwesend. Aus diesen Arten wurden 368 Isolate hergestellt und *in vitro* und *in vivo* auf antagonistische Aktivität gegenüber den Erregern der Apfelfäule, *Botrytis cinerea, Monilinia fructigena* und *Penicillium expansum* getestet. Isolate von *Aureobasidium pullulans, Epicoccum purpurascens, Sordaria fimicola* und *Trichoderma polysporum* (10^7 Sporen/ml bei 20 µl/Wunde) schützten die verwundeten Früchte sehr gut gegen die Erreger der Apfelfäule, solange das Verhältnis Antagonist zu Pathogen mindestens 100:1 betrug.

Ein deutlich besseres Ergebnis wurde mit Mischungen von Antagonisten erzielt. Wenn *E. purpurascens* mit *A. pullulans, S. fimicola* oder *T. polysporum* gemischt wurden, reichte ein Verhältnis von 1:1 bis 10:1 von Antagonist zu Pathogen in den frisch verwundeten Äpfeln aus, um die Fäule in einem weiten Konzentrationsbereich der Mikroorganismen zu bekämpfen.

* Corresponding author

Stichwörter: Apfel; Nachernte-Fruchtfäule; *Botrytis cinerea; Monilinia fructigena; Penicillium expansum;* biologische Bekämpfung

1 Introduction

Microbial populations on leaves and fruits of apple trees develop and change in typical ways during the season (ANDREWS and KENNERLY 1980; LEBEN 1971; STADELMANN and SCHWINN 1976, 1979). Among these micro-organisms, pathogenic fungi may become prevalent, depending on the climatic conditions and on the capability of the pathogen to infect the different apple cultivars. To prevent this infection, fungicides are applied at regular intervals. Because of unfavourable weather in 1988, and the infection pressure by fungicide-resistant strains of apple scab (HERMANN et al. 1989; SIEBELS 1991) up to 28 fungicide treatments had to be applied in the orchards around Lake Constance (Bodensee) within 3 months (MENDGEN et al. 1991). Also for the postharvest pathogens *Botrytis cinerea, Penicillium expansum* (PALAZON and PALAZON 1987) and *Monilinia fructicola* (ZHANG et al. 1991), a loss of sensitivity to fungicides has been reported.

As an alternative to this extensive reliance on chemical control, antagonistic micro-organisms have been used to prevent the infection of apples by the major pathogens (CULLEN et al. 1984; JANISIEWICZ 1987; PUSEY and WILSON 1984; TRONSMO and YSTASS 1980). Control of the pathogen *B. cinerea* was achieved by a postharvest treatment of apples with *Trichoderma pseudokoningii* (TRONSMO and RAA 1977), *Acremonium breve* (JANISIEWICZ 1988), *Cryptococcus laurentii* (ROBERTS 1990), some yeasts (MCLAUGHLIN et al. 1990), or *Pseudomonas cepacia* (JANISIEWICZ et al. 1991). More recently (SCHIEWE and MENDGEN 1992), antagonistic fungi were found that control the two postharvest pathogens *Pezicula malicorticis* and *Nectria galligena* under laboratory conditions.

The first antagonists used for biological of postharvest fruit diseases were isolated from the soil (PUSEY and WILSON 1984; TRONSMO and RAA 1977). Since then, effective antagonists have also been found among the epiphytic microflora of apples (JANISIEWICZ 1987). The use of the natural microflora of fruit trees for the selection of micro-organisms antagonistic to single pathogens has become a standard practice [e.g., grapes (FERREIRA 1990) citrus (CHALUTZ and WILSON 1990) mangoes (KOOMEN et al. 1990)]. However, since a variety of pathogens may contribute to losses even under modern storage conditions (REYES 1990), a complex system of biological control is needed, where several antagonistic micro-organisms are combined.

Here, we report on the interaction of several isolates of fungi resident on leaves as antagonists with the three postharvest pathogens, *B. cinerea, M. fructigena* and *P. expansum* and their potential use for the control of postharvest decay.

2 Methods

2.1 Sampling of fungal isolates

Leaves of *Malus sylvestris* var. *domestica* cv. 'Golden Delicious' were collected in an orchard in Hegne, 10 km east of Konstanz from June to October 1989. The orchard had not been treated with fungicides since 10 years. A group of 10 trees, centrally located in the apple orchard was sampled monthly. On each sampling date, 40 leaves were collected randomly from each tree, bulked and taken immediately to the laboratory. The phylloplane fungi were detached from the leaves by shaking them for 10 min at 250 rpm in 0.01 mol/l phosphate buffer (pH 7.0) containing 0.001 % Tween 20 at 4° C. The resulting suspension was diluted four times (10^{-1} to 10^{-4}), aliquots (0.1 ml) of each dilution were mixed with 20 ml molten (35° C) potato dextrose agar (PDA) containing streptomycin sulfate 0.5 g/l, and poured into Petri dishes. The cultures were incubated at 20° C for 3 to 8 days in the dark. Single spore isolates were produced from fungal colonies by streaking on PDA. Isolates were identified whenever possible to the species level, according to the specifications of ARX (1981), ELLIS (1971) and SUTTON (1980). In some cases, identification was completed and/or confirmed by the CBS Institute, Baarn, The Netherlands. Total colony counts, and counts of each species were made as colony-forming-units (cfu)/per

gram leaf fresh weight. The varying population levels of the isolated leaf micro-organisms were assessed by the Wilcoxon non-parametric range test, as described by MELGAREJO et al. (1985). Single spore isolates of *Botrytis cinerea, Monilinia fructigena* and *Penicillium expansum* were isolated from infected apples, from the same orchard in Hegne. They were also grown on PDA and tested for pathogenicity at regular intervals.

2.2 Inhibition of spore germination of postharvest pathogens

Fungal conidia were obtained by washing the mycelia growing on PDA with 2 ml of sterile 0.05 % Tween 20. The resulting suspensions were diluted with sterile distilled water to a concentration of 10^6 conidia/ml as determined with a hemarocytometer. Conidial suspensions of the postharvest pathogens (10^6 conidia/ml) were prepared in filter-sterilized apple juice and mixed with the spores from fungal leaf isolates. Of the mixed suspension, 0.1 ml aliquots were plated in wells (20 mm in diameter). After 24 h at 4° C, germination of 200 spores was evaluated. A spore was regarded germinated as soon as the germ tube was longer than the diameter of the spore. The experiments were repeated three times.

2.3 Inhibition of mycelial growth

Inhibition of the pathogens by phylloplane fungi was evaluated in dual cultures by the inhibition of radial growth as described by MELGAREJO et al. (1985): Mycelial plugs of the pathogens and of potential antagonists were cut from actively growing colonies on PDA, and transferred to the assay plates, at a distance of 3 cm. In the controls, a mycelial plug of one of the pathogens and a sterile PDA plug were used. After 10 days at 4° C, inhibition was determined. The experiments were repeated three times.

2.4 *In vivo* test

For *in vivo* assays, apples (cv. 'Golden Delicious') were harvested at commercial maturity from trees of the same orchard where the fungal isolates had been collected and stored in polyethylene bags at 4° C in the dark until use. After surface sterilization by soaking the apples in 70 % ethanol for 5 min, followed by several rinses with sterile water, apples were wounded by removing a plug with a cork borer (3 mm wide, 3 mm deep) and immediately inoculated with the conidia. Generally, two wounds were applied on the equator of each fruit, one wound with both the antagonists and the pathogens, the other with the pathogens only (controls). First, 20 µl of a suspension of a single antagonist (10^7 spores/ml in water) was applied to one of the wounds. The other wound received 20 µl of water only. After 20–30 min, both wounds were inoculated with *B. cinerea, M. fructigena* or *P. expansum* (20 µl containing 10^5 spores/ml).

When two antagonistic fungi were used in mixtures, spores of each antagonistic fungus were suspended in 20 µl 0.05 mol/l phosphate buffer, pH 7.0. Twenty µl containing 10^5 spores/ml of *Epicoccum purpurascens* (KC7574) and 20 µl containing 5×10^5 spores/ml of *Aureobasidium pullulans* (KC1503) were applied. In the other variants, 20 µl containing 10^5 spores/ml of *E. purpurascens* (KC 7574) were mixed either with 20 µl containing 10^5 spores/ml of *Trichoderma polysporum* (KC1014) or with identical amounts of *Sordaria fimicola* (KC8964), *Cladosporium cladosporioides* (KC1107), *Acremonium strictum* (KC1026) or *Chaetomium globosum* (KC1047). To the control wounds, 40 µl of phosphate buffer was added. After 20–30 min, the pathogens (20 µl containing 10^5 spores/ml) were added to both wounds.

Immediately after inoculation, apples were placed into airtight sealed plastic containers at 4° C for 3 weeks. These conditions correspond to those in apple storage containers used by local farmers.

2.5 Statistical analysis

In the *in vivo* test on fruits, each treatment in each experiment consisted of 10 single-fruit replicates, in a randomized complete block design. Each experiment was repeated twice. The data were subjected to analysis of variance using the Waller-Dunkan K-ratio t test ($p \leq 0.05$) and the Statistical Analysis System software SAS Institute Inc., Cary, NC., USA (1989).

3 Results

3.1 Fungal populations on apple leaves

The fungi regularly recovered from apple leaves are listed in Table 1. The number of colony-forming-units from most species varied significantly during the year. Out of the 32 different fungi repeatedly observed, 21 were present in significant numbers throughout the season (Table 1). Fungi generally assumed to have antagonistic activities, such as *A. strictum, Trichoderma* spp. or *S. fimicola,* tended to increase in number during late summer.

Table 1. Changes in fungal populations isolated from apples leaves (cv. 'Golden Delicious') during 1989. (+) Indicate fungi present in low amounts only (< 10 CFU's/g fresh weight) and are considered as transient microorganism. Values followed with equal letters do not differ significantly ($P \leq 0.05$) in the Wilcoxon nonparametric range test. Values are means of $\log_{10}$ CFU's/g fresh weight

Tab. 1. Fluktuation der Pilzpopulation auf Apfelblättern (Sorte 'Golden Delicious') während der Saison 1989. (+) zeigt an, daß diese Pilze nur in niedriger Anzahl vorhanden waren (< 10 CFU/g Frischgewicht). Dies sind transiente Mikroorganismen. Ergebnisse die mit gleichen Buchstaben versehen sind, unterscheiden sich nicht signifikant ($P < 0,05$) im Wilcoxon Test. Die Ergebnisse sind Mittelwerte, ausgedrückt als $\log_{10}$ CFU/g Frischgewicht

	June	July	August	September	October
Acremonium strictum	+	+	2,5 b	4.5 a	4.0 a
Acremonium sp.	3.6 b	4.7 a	3.1 b	3.3 b	5.5 a
Alternaria alternata	4.7 a	3.0 b	4.8 a	5.1 a	5.2 a
A. tenuissima	4.5 a	3.7 b	3.2 b	3.6 b	5.1 a
Aspergillus clavatus	+	+	1.8 b	4.4 a	3.9 a
Aspergillus niger	3.1 b	+	3.3 b	5.0 a	3.1 b
Aspergillus sp.	1.4 b	2.7 b	2.4 b	4.0 a	3.6 b
Aureobasidium pullulans	4.1 b	5.0 a	5.3 a	4.2 b	4.9 a
Botryosphaeria sp.	1.5 b	+	3.0 b	4.4 a	5.1 a
Botrytis cinerea	2.8 b	3.4 b	5.1 a	6.1 a	4.6 a
Cephalosporium sp.	1.4 b	3.6 b	4.8 a	2.3 b	4.6 a
Chaetomium globosum	2.0 b	2.8 b	3.6 b	4.3 a	5.2 a
Cladosporium cladosporioides	4.0 b	4.3 b	5.3 a	6.3 a	5.5 a
C. herbarum	4.1 b	5.0 a	4.8 a	5.5 a	4.7 b
C. sphaerospermum	3.4 b	3.6 b	5.4 a	4.7 a	6.3 a
Coniothyrium sp.	2.2 c	3.3 b	3.8 b	5.2 a	5.1 a
Dendryphiella sp.	+	+	+	4.0 a	+
Epicoccum purpurascens	2.6 c	4.4 b	5.5 a	6.3 a	5.4 a
Fusarium sp.	1.4 b	+	+	3.8 b	4.7 a
Monilinia fructigena	1.8 c	3.0 b	3.6 b	4.2 a	4.5 a
Penicillium frequentans	2.2 c	2.8 c	4.4 b	5.6 a	5.7 a
P. purpurogenum	3.1 b	4.9 a	4.7 a	3.5 b	3.0 b
Phoma macrostomum	3.6 b	4.8 a	4.9 a	5.3 a	4.6 a
Phoma pomorum	3.0 b	3.3 b	6.1 a	5.3 a	5.4 a
Phyllosticta sp.	1.5 c	3.8 b	4.5 a	4.9 a	5.0 a
Sordaria fimicola	+	+	2.0 b	2.5 b	3.7 a
Stemphylium sp.	4.1 b	3.8 b	6.2 a	6.1 a	4.5 b
Spilocaea pomi	4.0 b	5.8 a	4.9 a	5.2 a	4.3 b
Trichoderma viride	+	+	2.4 b	3.8 a	4.0 a
Trichoderma polysporum	+	+	+	3.0 a	+
Trichotecium roseum	+	1.8 b	2.1 b	4.6 a	5.2 a
Ulocladium sp.	+	2.0 b	3.9 b	5.2 a	3.5 b

Table 2. Inhibition by 42 selected fungi of spore germination, mycelial growth and apple lesions from *Botrytis cinerea* (B. c.), *Monilinia fructigena* (M. f.), and *Penicillium expansum* (P. e.)

Tab. 2. Einfluß von 42 ausgewählten Pilzen auf die Sporenkeimung, das Mycelwachsrum und die Faulstellen, die durch *B. cinerea* (B. c.), *M. fructigena* (M. f.) und *P. expansum* (P. e.) auf Äpfeln hervorgerufen wurden

		Inhibition (%)								
		Spore germination			Mycelial growth			Apple lesions		
Isolare		B. c.	M. f.	P. e.	B. c.	M. f.	P. e.	B. c.	M. f.	P. e.
Aureobasidium pullulans	KC1503	60	20	45	8	4	20	96	95	75
	KC1506	52	19	44	4	12	22	70	90	78
	KC1507	56	18	40	3	18	25	87	92	64
	KC1512	58	13	44	5	19	21	80	50	60
Trichoderma polysporum	KC1014	85	90	90	95	98	99	93	80	87
	KC1218	84	89	88	92	97	98	90	75	80
	KC1393	84	83	80	90	94	98	89	60	78
	KC1412	83	86	90	85	92	80	85	72	65
	KC1553	80	90	94	80	90	74	70	68	42
	KC1684	98	90	87	85	90	96	28	12	6
	KC1714	92	98	90	80	75	92	14	38	10
Sordaria fimicola	KC8964	95	80	60	98	99	98	89	86	90
	KC7252	94	77	58	95	98	93	78	82	87
	KC4325	92	80	60	94	96	95	80	78	70
	KC7758	95	80	55	90	95	90	85	80	74
	KC4297	96	75	54	88	93	92	82	50	64
	KC3337	94	72	56	85	90	85	75	62	80
	KC4283	92	73	60	80	86	88	74	70	85
	KC2684	42	30	25	34	20	10	85	80	96
Cladosporium cladosporioides	KC1107	80	60	20	15	56	55	62	42	20
	KC1215	78	54	18	4	12	15	45	30	18
	KC1336	79	60	19	2	15	18	42	26	10
	KC1669	77	58	20	8	0	30	30	20	15
	KC1712	75	52	18	6	2	25	28	40	10
Acremonium strictum	KC1026	35	38	80	4	7	15	30	44	55
	KC5310	34	37	79	2	3	13	25	30	42
	KC5864	31	32	80	1	5	11	22	40	48
	KC5141	30	34	77	0	2	10	20	44	10
	KC5558	30	19	74	2	5	7	15	52	8
	KC5106	28	38	75	10	6	9	10	40	30
	KC5712	26	36	67	3	6	8	12	35	18
	KC5853	20	37	50	4	5	11	8	10	12
Chaetomium globosum	KC1047	80	78	70	78	87	60	59	84	46
	KC1560	76	75	65	42	55	56	42	75	40
Epicoccum purpurascens	KC7574	80	85	80	25	70	67	93	98	95
	KC1348	79	84	70	12	45	50	85	94	90
	KC1352	80	83	77	23	50	48	78	80	87
	KC7561	69	80	72	22	30	38	65	30	85
	KC8530	70	82	70	18	20	15	63	20	70
	KC2213	73	85	74	10	15	19	62	18	45
	KC4326	78	82	76	25	12	60	60	11	40
	KC3367	60	80	78	18	20	54	42	15	8

3.2 Inhibition of spore germination

During the season, 368 fungi were isolated and tested for their antagonistic qualities. A selection of the most effective fungi is shown in Table 2. Some characteristics are typical for the different fungal species. *A. pullulans* has only an intermediate effect on spore germination of *B. cinerea, P. expansum,* and even less influence on *M. fructigena.* Some *S. fimicola* isolates considerably inhibited spore germination of *B. cinerea* and *M. fructigena,* but not so much *P. expansum.* Also *C. cladosporioides* had only a minor effect on *P. expansum.* On the other hand, some *A. strictum* isolates efficiently suppressed spore germination of *P. expansum,* but not so much *B. cinerea* and *M. fructigena.* Many isolates of *C. globosum, T. polysporum* and *E. purpurascens* isolates effectively suppressed the germination of all three postharvest pathogens.

3.3 Inhibition of mycelial growth

A. pullulans, C. cladosporioides and *A. strictum* isolates had only minor effects on mycelial growth of all three pathogens. *C. globosum* and *E. purpurascens* isolates reduced hyphal growth at an intermediate, variable level. In contrast, the majority of *T. polysporum* and *S. fimicola* isolates were quite efficient, with some noticeable exceptions (e.g., *S. fimicola* KC2684).

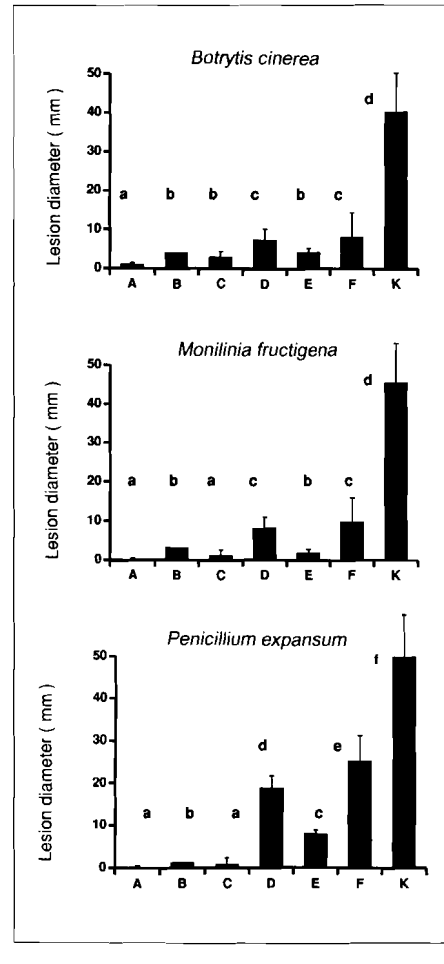

Fig. 1. Influence of mixtures of antagonistic fungi on lesion development on apples (Values followed by the same letter do not differ significantly).
A: *A. pullulans* (KC1503) + *E. purpurascens* (KC7574)
B: *T. polysporum* (KC1014) + *E. purpurascens* (KC7574)
C: *S. fimicola* (KC8964) + *E. purpurascens* (KC7574)
D: *C. cladosporioides* (KC1107) + *E. purpurascens* (KC7574)
E: *A. strictum* (KC1026) + *E. purpurascens* (KC7574)
F: *C. globosum* (KC1047) + *E. purpurascens* (KC7574)
K: Control
Abb. 1. Der Einfluß von Mischungen antagonistischer Pilze auf die Bildung von Faulstellen (Werte mit gleichen Buchstaben unterscheiden sich nicht signifikant).

3.4 *In vivo* test on apples

Attificially wounded apples were inoculated both with a potential antagonist and one of the pathogens and subsequently stored under conditions that are similar to those applied in practice. One isolate of *E. purpurascens* (KC7574) was most efficient at a concentration of 10^7 spores/ml (Table 2). This corresponds to a ratio between pathogen and antagonist of 1:100. Good control was also obtained with *A. pullulans* (KC1503) and *T. polysporum* (KC1014), *S. fimicola* (KC8964 and KC2684) and *E. purpurascens* (KC7574). Often, *in vitro* antagonistic activity of the isolates did not correspond to the activities observed *in vivo* (Table 2). We also applied mixtures of two antagonists, however at much lower concentrations (Fig. 1). Excellent control was obtained with the mixtures of *E. purpurascens* (KC7574) and *A. pullulans* (KC1503). Also, a combination of *E. purpurascens* (KC7574) with *S. fimicola* (KC8964) or with *T. polysporum* (KC1014) gave good results. In these experiments, the ratio pathogen-antagonist was 1:2 or 1:5 in the case of *E. purpurascens* (KC7574), and *A. pullulans* (KC1503).

In Figure 2, we varied the ratio between the pathogen and the mixture of the antagonists *E. purpurascens* (KC7574) and *A. pullulans* (KC1503) by using different spore concentrations of pathogen and antagonists. No lesions were observed as long as the two antagonistic fungi together surpassed the

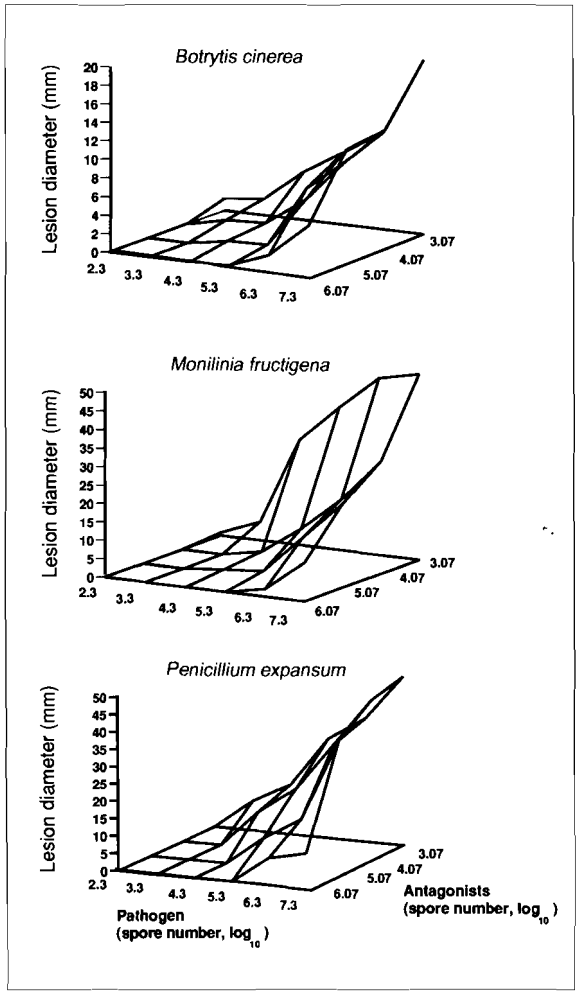

Fig. 2. Concentration-dependent effect of a mixture of *Aureobasidium pullulans* (KC1503) and *Epicoccum purpurascens* (KC7574) on lesion development on wounded apples induced by postharvest pathogens. The ratio of *A. pullulans* and *E. purpurascens* in the mixture was 5:1.
Abb. 2. Konzentrationsabhängigkeit des Einflusses einer Mischung von *A. pullulans* (KC1503) und *E. purpurascens* (KC7574) auf die Fäuleentwicklung in verwundeten Äpfeln. Das Verhältnis von *A. pullulans* zu *E. purpurascens* betrug 5:1 als die Mischung in die Wunde pipettiert wurde.

number of pathogenic fungi by a factor of 10. Some control was already obtained as long as the number of pathogenic spores was matched by at least a similar number of antagonistic spores.

4 Discussion

The method used in this study to quantify the number of propagules of fungi is only a rough means of estimating the presence of resident micro-organisms on leaves (LEBEN 1971; STADELMANN and SCHWINN 1976). It takes into account only those micro-organisms which can be washed away from the leaves and grown on nutrient agar. It does not allow to distinguish between single spores, large fungal colonies or hyphae, which may be difficult to wash away at similar rates. However, in spite of these disadvantages, the method is capable to indicate population trends and shows that many fungal species occur in high numbers on the apple leaf surface, including pathogenic fungi such as *B. cinerea, M. fructigena* and *Spilocaea pomi.* This roughly corresponds to results obtained in experiments with apple and pear trees from other regions (ANDREWS and KENERLY 1980). The higher number of fungal species found in this study (32 different species) may be due to the fact that this orchard was not treated with fungicides during the last 10 years.

This paper highlights the interaction of fungi under different conditions such as artificial media or apple wounds. It is interesting to note the variation of different isolates of fungal species in their ability to inhibit spore germination, mycelial growth or lesion development in apple of three different pathogenic fungi. Isolates may inhibit spore germination or mycelial growth poorly, but suppress apple lesion formation efficiently (*S. fimicola,* KC2684). Some isolates inhibited spore germination and mycelial growth, but did not affect the development of apple lesions considerably (*T. polysporum,* KC1714, KC1648). Other isolates were efficient in all qualities tested (*S. fimicola,* KC8964, KC7252; *T. polysporum,* KC1014, KC1218). These few examples give a general impression of the complexity of interactions which may occur on the surfaces of apple leaves or fruits. Therefore, the selection of a single isolate for the biological control of apple storage disease, as has been done before (JANISIEWICZ 1987; MENDGEN et al. 1991; ROBERTS 1990), is unlikely to include the different possible mechanisms antagonistic fungi have developed against other fungi.

We did not study the mechanisms of antagonistic activity. It may be the result of physical contact, competition for space and nutrients, or may even be an effect of toxins and antibiotics (PUSEY 1989; SY et al. 1991; WILSON et al. 1991). *E. purpurascens,* an effective fungus for the control of pathogens in this study, is known to produce numerous antifungal and antibacterial compounds such as flavipin and epicorazin (MALLEA et al. 1991). This has to be considered before the consumption of such apples. The mechanisms of antagonistic action of the other antagonistic fungi used here must be studied also before they may be safely applied in practice (JEFFRIES and JEGER 1990; WILSON et al. 1991).

Our studies have shown that the antagonists are more or less efficient towards each pathogen. Also, different antagonists may be more adapted to the variable conditions on leaves or fruits (JANISIEWICZ 1988; MENDGEN et al. 1991). Therefore, we applied defined mixtures of fungi antagonistic to *B. cinerea, M. fructigena* and *P. expansum.* Thus, we were able to reduce the concentrations of antagonists needed for a protection against postharvest decay considerably. In addition, it is interesting that the effective ratio antagonist-pathogen was rather constant over a broad range of the concentrations applied. This ratio reached 1:1 under optimal conditions and seems unsurpassed by any other biocontrol system against three pathogens we know.

Experiments were performed at 4° C and conditions of high humidity and low oxygen. Similar conditions for storage are used by many farmers in the lake Constance (Bodensee) area. Additional experiments performed at 20° C showed, that even lower concentrations of the antagonists can be effective (results not shown), but we think that these results are of minor importance in practice.

Currently, we are performing field experiments using mixtures of antagonistic fungi presented in this work and, in addition, two antagonistic micro-organisms effective against *P. malicorticis,* and *N. galligena* (SCHIEWE and MENDGEN 1992). In variants, we add calcium (CONWAY et al. 1991) or film polymers (ELAD et al. 1990) to the sprays. During growth of apples, this combination of different techniques will create an environment on their surface which simultaneously improves fruit quality, favors antagonists and suppresses pathogens.

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Literature

ANDREWS, J. H., C. M. KENERLEY: Microbial populations associated with buds and young leaves on apple. – Can. J. Bot. **58**, 847–855, 1980.

ARX, J. A.: The genera of fungi sporulating in pure culture. – J. Kramer, Vaduz, 1981.

CHALUTZ, E., C. L. WILSON: Postharvest biocontrol of green and blue mold and sour rot of citrus fruit by *Devaryomyces hansenii*. – Pl. Dis. 74, 134–137, 1990.

CONWAY, W. S., C. E. SAMS, J. A. ABOTT, B. D. BRUTTON: Postharvest calcium treatment of apple fruit to provide broad spectrum protection against postharvest pathogens. – Pl. Dis. 75, 620–622, 1991.

CULLEN, D., F. M. BERBEE, J. H. ANDREWS: *Chaetomium globosum* antagonizes the apple scab pathogen *Venturia inaequalis* under field conditions. – Can. J. Bot. **62**, 1814–1818, 1984.

ELAD, Y., N. AYISH, O. ZIV, J. KATAN: Control of gray mold, *Botrytis cinerea*, with film-forming polymers. – Pl. Path. **39**, 249–254, 1990.

ELLIS, M. B.: Dematiaceous Hyphomycetes. – Commonw. Mycol. Inst., Kew, 1971.

FERREIRA, J. H. S.: *In vitro* evaluation of epiphytic bacteria from tables grapes for the suppression of *Botrytis cinerea*. – S. Afr. J. Enol. Vitic. **11**, 38–41, 1990.

HERMANN, M., R. SZITH, V. ZINKERNAGEL: Reduced sensitivity of some scab isolates *(Venturia inaequalis)* from Steiermark (Austria) to EBI-fungicides. – Gartenbauwissenschaft **54**, 160–165, 1989.

JANISIEWICZ, W. J.: Postharvest biological control of blue mold on apples. – Phytopathology 77, 481–485, 1987.

JANISIEWICZ, W. J.: Biocontrol of postharvest diseases of blue mold with antagonistic mixtures. – Phytopathology **78**, 194–198, 1988.

JANISIEWICZ, W. J., L. YOURMAN, J. ROITMAN, N. MAHONEY: Postharvest control of blue mold and gray mold of apples and pears by dip treatment with pyrrolnitrin, a metabolite of *Pseudomonas cepacia*. – Pl. Dis. 75, 490–494, 1991.

JEFFRIES, P., M. J. JEGER: The biological control of postharvest diseases of fruit. – Biocontrol News Inf. **11**, 333–336, 1990.

KOOMEN, I., J. C. DODD, M. J. JEGER, P. JEFFRIES: Postharvest biocontrol of anthracnose disease of mangoes. – J. Sci. Fd Agric. **50**, 137–138, 1990.

LEBEN, C.: The bud in relation to the epiphytic microflora. – In: PREECE, T. F., C. H. DICKINSON (eds.): Ecology of leaf surface microorganisms, pp. 117–127. London, Academic Press, 1971.

MALLEA, M., D. PESANDO, P. BERNARD, B. KHOULALENE: Comparison between antifungal and antibacterial activities of several strains of *Epicoccum purpurascens* from the Mediterranean area. – Mycopathologia **115**, 83–88, 1991.

MCLAUGHLIN, R. J., M. E. WISNIEWSKI, C. L. WILSON, E. CHALUTZ: Effect of inoculum concentration and salt solutions on biological control of postharvest diseases of apple with *Candida* sp. – Phytopathology **80**, 456–461, 1990.

MELGAREJO, P., R. CARRILLO, M. SAGASTA: Mycoflora of peach twigs and flowers and its possible significance in biological control of *Monilinia laxa*. – Trans. Br. mycol. Soc. **85**, 313–317, 1985.

MENDGEN, K., A. SCHIEWE, C. J. FALCONI: Biologische Bekämpfung von Pflanzenkrankheiten. – PflSchutz-Nachr. **45**, 5–20, 1991.

PALAZÓN, I. J., C. F. PALAZÓN: La resistencia de hongos causantes de las podredumbres en post-cosecha. – Inf. téc. econ. Agraria **71**, 18–31, 1987.

PUSEY, P. L.: Use of *Bacillus subtilis* and related organisms as fungicides. – Pestic. Sci. **27**, 133–140, 1989.

PUSEY, P. L., C. L. WILSON: Postharvest biological control of stone fruit brown rot by *Bacillus subtilis*. – Pl. Dis. **68**, 753–756, 1984.

REYES, A. A.: Pathogenicity, growth and sporulation of *Mucor mucedo* and *Botrytis cinerea* in cold or CA storage. – Hort. Sci. **25**, 549–552, 1990.

ROBERTS, R. G.: Postharvest biological control of gray mold of apple by *Cryptococcus laurentii*. – Phytopathology **80**, 526–530, 1990.

SAS Institute Inc.: SAS/STAT user's guide, version 6, Fourth Edition. – Volume **1**, SAS Institute Cary, NC, 1989.

SCHIEWE, A., K. MENDGEN: Identification of antagonists for biological control of the postharvest pathogens *Pezicula malicorticis* (Jacks.) Nannf., and *Nectria galligena* Bres. on apples. – J. Phytopath. **134**, 29–237, 1992.

SIEBELS, C.: Sensitivität von *Venturia inaequalis* Populationen aus dem Bodenseegebiet gegenüber Sterolbiosyntheseinhibitoren. – Diplomarbeit Univ. Konstanz, 1991.

STADELMANN, F., F. J. SCHWINN: Influence of scab infection on total number of phyllosphere microorganisms of apple and pear. – Trans. Br. mycol. Soc. **66**, 163–167, 1976.

STADELMANN, F., F. J. SCHWINN: Der Einfluß von *Venturia inaequalis* und *V. pyrina* auf die saprophytische Mikroflora von Apfel und Birnblättern. – Phytopath. Z. **94**, 139–164, 1979.

SUTTON, B. C.: Coelomycetes. – Commonw. Mycol. Inst., Kew, 1980.

SY, A. A., L. ALBERTINI, M. MOLETTI, C. L. HAMAT: Mécanismes potentiels régissant le contróle biologique des agents phytopathogènes. – Cryptogamie Mycol. **12**, 133–147, 1991.

TRONSMO, A., J. RAA: Antagonistic action of *Trichoderma pseudokoningii* against the apple pathogen *Botrytis cinerea*. – Phytopath. Z. **89**, 216–220, 1977.

TRONSMO, A., J. YSTASS: Biological control of *Botrytis cinerea* on apple. – Pl. Dis. **64**, 1009, 1980.

WILSON, C. L., M. E. WISNIEWSKI, C. L. BILES, R. MCLAUGHLIN, E. CHALUTZ, S. DROBY: Biological control of postharvest diseases of fruits and vegetables: alternatives to synthetic fungicides. – Crop Prot. **10**, 172–177, 1991.

ZHANG, Y., M. BODMER, J. RÜEGG: Variation in sensitivity to sterol biosynthesis inhibitor (SBI) of different isolates of *Monilinia* species. – Z. PflKrankh. PflSchutz **98**, 317–322, 1991.