European Journal of Plant Pathology **110:** 929–937, 2004.

Can phyllosphere yeasts explain the effect of scab fungicides on russeting of Elstar apples?

P.R. Gildemacher[1], B. Heijne[1,*], J. Houbraken[2], T. Vromans[2], E.S. Hoekstra[2] and T. Boekhout[2]
[1]*Applied Plant Research PPO, Lingewal 1, P.O. box 200, 6670 AE Zetten, The Netherlands;* [2]*Centraalbureau voor Schimmelcultures, Uppsalalaan 8, P.O. box 85167, 3508 AD Utrecht, The Netherlands; *Author for correspondence (Phone: +31-488-473700; Fax +31-488-473717; E-mail: bart.heijne@wur.nl)*

Accepted 19 April 2004

Key words: apple, enzyme activity, *in vitro* susceptibility, russet, yeast

Abstract

In 1999 and 2000, the effects of scab fungicides on yeast composition and russeting of Elstar apples were assessed. Yeast composition of fungicide-treated and untreated young apple fruit with or without russet symptoms was investigated and enzyme activity of the yeasts was studied. *Cryptococcus albidus*, *C. laurentii*, *Rhodotorula glutinis*, *Sporobolomyces roseus* and *Metschnikowia pulcherrima* dominated the apple fruit surface. Russeted apple fruitlets had a higher red yeast density than non-russeted fruitlets. *In vitro* fungicide susceptibility of the dominant yeast species varied. Dithianon and dodine were active against all tested species, captan and tolylfluanid showed specificity for certain species, whereas pyrimethanil and bupirimate were largely ineffective. Fungicide treatment in the field had a clear effect on the yeast composition. *Metschnikowia etschnikowia pulcherrima* was eliminated from the phyllosphere and cryptococcoïd species diminished by both captan and dithianon. The red yeast population was not significantly changed by either fungicide. Captan, dithianon, tolylfluanid and pyrimethanil reduced russet in the field in both years, dodine and kresoxim-methyl only in 2000. All yeast species had cutinolytic activity, all but *M. pulcherrima* and *Debaryomyces hansenii* were lipolytic, and some of the isolates showed proteolytic activity.

Introduction

Russeting of the apple cultivar 'Elstar', one of the most important cultivars in The Netherlands, results in substantial economic losses due to reduced fresh market value (Gildemacher, 2000) and storability (Walter, 1967; Tromp et al., 1976). Russeting is the formation of cork on the apple skin by epidermal cells as a reaction to the death of epidermal and hypodermal tissue (Meador and Taylor, 1987). The death of a limited number of epidermal cells can result in a considerable russeted surface (Walter, 1967). The characteristics of the cuticle and epidermis determine the susceptibility of apple varieties to russet (Babin et al., 1977; Eccher, 1978).

The most vulnerable period for russet formation ranges from 1 to 4 weeks after flowering (Creasy, 1980; Creasy and Swartz, 1981). The diminishing susceptibility after this period is probably related to the development of a thicker cuticle during the first 8 weeks after full bloom, as Miller (1982) showed. Russet formation of the apple skin is the result of complex biotic and abiotic interactions, which explains the irregular occurrence of russet over the years (Vogl et al., 1985; Gildemacher, 2000).

Important weather conditions favouring russet formation are high relative humidity (Babin et al., 1977; Creasy 1980; Creasy and Swartz, 1981; Bonany and Carbo, 1995), dew (Vogl et al., 1985), exposure to sunlight (Jackson et al., 1977; Eccher

and Noe, 1993) and large fluctuations between day and night temperatures (Walter, 1967). Nitrogen superfluity (Faust and Shear, 1972; Hatch, 1975) and boron deficiency (Porreye, 1980; Smith et al., 1987) can augment russeting. The use of gibberellins can diminish russeting (Eccher, 1978; Taylor, 1978; Wertheim, 1982).

It has been reported that the fungicides captan (Walter, 1967; Vogl et al., 1985; Jones et al., 1994), polyram (Bremer and Bünemann, 1982; Vogl et al., 1985) and sulphur (Wundermann, 1981; Eccher and Maffi, 1986) reduce russet. It has been suggested that microbes occurring on young apples may induce russet. Inoculation of apples with the yeast-like fungus *Aureobasidium pullulans* and the red yeast *Rhodotorula glutinis* increased russet formation (Matteson Heidenreich et al., 1997). Yeasts are a normal part of the microflora occurring on apples (Davenport, 1976; Bizeau et al., 1989; Chand-Goyal and Spotts, 1996). These studies suggest a rather limited number of yeast species occurring on the apple surface. Different numbers of yeasts were observed in different developmental stages of the apple (Davenport, 1976; Pennycook and Newhook, 1981). Among the factors influencing the yeast population are weather conditions (Davenport, 1976; Andrews and Kennerly, 1978; Pennycook and Newhook, 1981), animal vectors (Davenport, 1976) and fungicide regime (Hislop and Cox, 1969; Andrews and Kennerly, 1978; Calvente et al., 1999).

To test the hypothesis that apple russeting is influenced by phylloplane yeast species we investigated the influence of scab fungicides on the apple phylloplane yeast population and russet of Elstar apples, and the enzymatic activities of different phylloplane yeast species.

Materials and methods

Experimental practices in the orchard

Apple trees of cultivar Elstar were used as experimental trees at Randwijk experimental station. The trees had an average height of 2.5 m and were planted in 1996 in a 1.25×3.00 m configuration with Delcorf and Everest as pollinators within the row. The experimental field comprised five double rows of Elstar, alternated with double rows of Jonagold. A randomised complete block design with 10 replicates was used. On every single Elstar row one replicate was placed with six experimental trees per treatment, flanked by a buffer tree on both sides.

Fungicide treatments are summarised in Table 1. No fungicides other than the indicated treatments were used during the entire season. The fungicide treatments were applied with an Empass experimental sprayer with a hand-held spray gun and a 1.2 mm nozzle, under 10 bar with a resulting discharge of 1.4 l/min. Trees were sprayed until run off, with 1000 l/ha, approximately 0.4 l per tree. Timing of sprayings was based on weather data using the Welte scab warning system (Batzer et al., 2000). Spraying dates, sampling dates and phenological data are presented in Figure 1.

Table 1. Doses of tested fungicides in 1999

Fungicide	Conc.a.i.[b]	Recommended dose	
		ha^{-1}	a.i. (mg/l)[c]
Water			
Captan[a]	83%	1.5 kg	1.245
Dithianon	75%	0.5 l	0.375
Tolylfluanid	50%	1.5 kg	0.750
Dodine	45%	1.3 l	0.585
Bupirimate	25%	0.5 l	0.125
Kresoxim-methyl	50%	0.2 l	0.100
Pyrimethanil	40%	0.75 l	0.300

[a] First two sprayings 1999 were with 1.2 kg ha^{1}, thereafter always 1.5 kg ha^{1}.
[b] Concentration active ingredient.
[c] Active ingredient in mg l^{-1}, based on 1000 l/ha spraying volume.

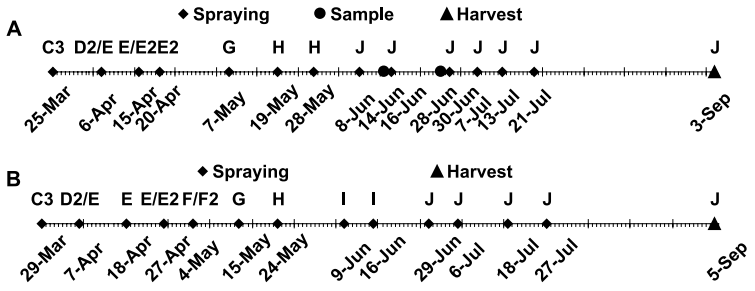

Figure 1. Spraying, sampling and harvest dates and corresponding phenological data indicated by letters according to Tromp et al. (1976). A, 1999; B, 2000.

Within each of the captan, dithianon and untreated plots a random sample of ten fruitlets was taken on June 14, 1999, 6 weeks after full bloom. On June 28, 8 weeks after full bloom, the first signs of russeting became apparent and a second sample was taken of ten russeted and ten smooth fruitlets per plot. All samples were kept in plastic bags and stored at −18 °C till transport to the laboratory for analysis of yeast composition.

All experimental trees were harvested normally and the fruit was stored at 3 °C until assessed for russet. Russet was assessed separately for the stalk and calyx half of the apples. Five russet grades were distinguished; 1 = smooth, 2 = slight, 3 = moderate, 4 = strong and 5 = very strong. A russet index for both sides of the apple was calculated comparable to a method used by Wertheim (1982):

$$(\#\text{grade } 1\times 1+\#\text{grade } 2\times 3+\#\text{grade } 3\times 5+\#\text{grade } 4\times 7+\#\text{grade } 5\times 9)/\text{total}\#$$

where # is the number of apples.

Statistical analysis of the russet figures was done by analysis of variance followed by identification of significantly different treatment pairs through *t*-tests.

Isolation of yeasts from the apple skin

Prior to isolation of the yeasts from the sample apples, the stalk and the calyx were removed. Subsequently, all 10 apples per treatment were transferred to a sterile Stomacher bag, containing 100 ml sterile water and placed overnight on a shaker to obtain a maximum release of yeast cells present on the apples. Ten-fold dilutions (till 10^{-3}) were made and 0.1 ml was plated in duplicate onto 1% yeast extract, 0.5% bactopeptone, 4% glucose, 2% agar (YPGA) with 50 mg/l penicillin-G and 30 mg/l streptomycin (PS).

Ballistospore-producing yeasts were isolated by the spore-fall method. Five pieces (approx. 1 cm^2) of apple skin were attached with double sided adhesive tape to the lid of the Petri-dish containing YPGA/PS. After 24 h at 20 °C, the lids were replaced by new sterile ones. After incubation for 4 – 5 days at 20 °C in darkness, all the macroscopically different yeast species were isolated and identified.

Yeast identification

All isolates obtained were grouped according to phenotypic characteristics, such as colony colour and morphology. From these groups a representative selection was made and identified, using the API 32C Yeast ID Kit-system (Biomérieux, 's-Hertogenbosch, the Netherlands). The ability to utilise different nitrogen sources, peptone (blank), nitrate, ethylamine, L-lysine and cadaverine, and the ability to grow at 30, 35, 37 and 40 °C was also investigated, as was the macroscopic appearance of the colony and the micromorphology of the yeast cells. The ability of the red yeasts to discharge ballistospores was investigated. Identifications were made by the Yeast Identification Program, version 4.0 (Barnett et al., 2000).

Screening of enzyme activity

Cutinase was detected as described by Dantzig et al. (1986), with *p*-nitrophenyl butyrate (Sigma) as the substrate. The yeast isolates were grown

onto acetate medium containing 0.5% yeast extract, 0.5% peptone, 0.5% ammonium acetate and 2.0% agar. After incubation for 10 days at 24 °C, a loop of cells was transferred onto a glass slide and overlaid with freshly prepared 0.026% *p*-nitrophenyl butyrate containing 0.011% Triton X-100. Distinct yellow staining was considered a positive reading. Lipase was detected according to the method described by Sierra (1957), using Tween 40, Tween 60 and Tween 80 as lipid substrates. Protease was detected as described by Braga et al. (1998), using agar plates containing 0.75% casein.

Fungicide susceptibility tests

Fungicide susceptibility of the most frequently found microorganisms on the apple samples, *Metschnikowia pulcherrima, Cryptococcus laurentii, C. albidus, Sporobolomyces roseus* and *R. glutinis* and of the yeast-like fungus *Aureobasidium pullulans*, was tested using a poison food technique (Dhingra and Sinclair, 1985; Smolka, 1993). Growth of the selected isolates on YPGA containing different concentrations of captan, dithianon, dodine, tolylfluanid, pyrimethanil and bupirimate was tested. The fungicides were mixed with YPGA in a concentration range of 0, 0.01, 0.1, 0.5, 1, 2, and 7 times the dose recommended by the supplier, based on a spraying volume of 1000 l/ha (Table 1).

Yeast suspensions of 4 to 5×10^7 cells per ml were prepared in sterile distilled water. Ten point-inoculations were made per dish with a preparation needle dipped in the yeast suspension. After seven days incubation in the dark at 20 °C the number of visible colonies per dish was counted and multiplied with the average rate of growth of all inoculation points in one dish. Good growth, moderate growth, bad growth or no growth scored 7, 5, 3 or 0 points, respectively. Consequently, the score in the test ranged between 350 points for unhampered growth of all inoculation points in all five dishes until zero for total suppression.

Results

Effect of fungicide treatment on russeting

There was a significant interaction between year and treatment effect. In both 1999 and 2000 fungicide treatment had a significant effect on the severity of russet. In 1999 tolylfluanid, captan and dithianon significantly reduced russet for the calyx side when compared to the control, the dodine and kresoxim-methyl treatments. Pyrimethanil reduced russet significantly compared to the control, but not compared to the dodine and kresoxim-methyl treatments (Table 2). The russet index of the stalk cavity side of the apple was not significantly influenced by fungicide treatment.

In 2000, russeting was more severe, resulting in a higher index for the calyx side of the apples. In 2000, all fungicide treatments reduced the russet index compared to the control for both sides of the apple, except the stalk side of the apples in the pyrimethanil treatment (Table 2). Regarding the calyx side, captan, dithianon, and tolylfluanid reduced russet significantly compared to kresoxim-

Table 2. Effect of different scab control fungicides on the russeting index of Elstar in 1999 and 2000

	1999		2000	
	Calyx end	Stalk end	Calyx end	Stalk end
Tolylfluanid	1.98 a	4.08	3.34 a	3.62 bc
Dithianon	2.05 ab	4.08	3.35 a	3.66 a
Captan	2.15 abc	4.08	3.46 a	3.71 a
Pyrimethanil	2.29 cd	3.87	3.40 ab	3.88 cd
Dodine	2.4 de	3.98	3.71 ab	3.86 ab
Kresoxim-methyl	2.41 de	4.05	3.53 b	3.93 bc
Control	2.5 e	3.88	4.22 c	4.10 c
F.prob.	<0.001	ns	<0.001	< 0.001
LSD 0.05	0.19		0.26	0.19

Data within the same column followed by a same letter are not significantly different ($P = 0.05$).

methyl. Stalk side russet was reduced most by captan and dithianon, but was not significantly different from treatment with dodine. Kresoxim-methyl and tolylfluanid reduced russet significantly less than the other treatments.

Qualitative and quantitative yeast analyses

The number of isolates obtained per species and their corresponding percentage of the total population is given in Table 3. In total 208 and 385 isolates were obtained from the apple fruit 6 and 8 weeks after full bloom in 1999, respectively. Most isolates belonged to the basidiomycetes. *C. albidus* and *C. laurentii* were the most common yeast species. Also red yeasts belonging to the genera *Rhodotorula*, *Sporidiobolus*/*Sporobolomyces* were frequently detected. *M. pulcherrima* was the most common ascomycetous yeast. Some differences occurred between the yeast species isolated 6 and 8 weeks after full bloom. In particular, the filamentous fungus *Phoma macrostoma* was found abundantly 6 weeks after full bloom, but was almost absent after 8 weeks. *C. albidus* and *R. glutinis* were relatively more dominant 8 weeks after full bloom compared to two weeks earlier.

The quantitative yeast analysis 8 weeks after full bloom showed significant differences between the russeted and non-russeted samples regarding the amount of red yeasts. The difference for cryptococcoïd yeasts and *M. pulcherrima* was not significant (Figure 2). Both captan and dithianon

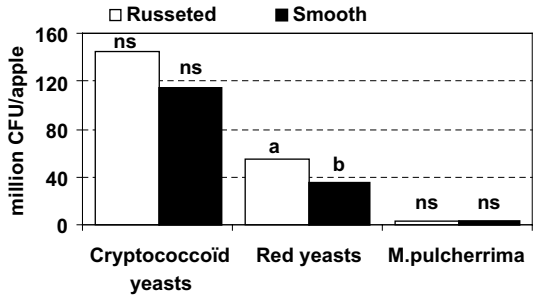

Figure 2. Yeast population of russeted and smooth apple fruitlets eight weeks after full bloom. Different letters behind treatment values indicate a significant difference. Cryptococcoid yeasts and *M. pulcherrima*: not significant; red yeasts: F $P = 0{,}02$; $LSD_{0,05} = 16$.

practically eliminated *M. pulcherrima* from the apple surface. Also the Cryptococcoïd yeast population was significantly reduced by captan, and even more by dithianon (Figure 3).

In vitro *enzyme activity*

A summary of the results of the enzyme assays is presented in Table 4. All the tested isolates were cutinolytic. With the exception of *M. pulcherrima* and *D. hansenii*, all of the tested yeast isolates were also lipolytic. All the tested *C. laurentii*, *R. aurantiaca* and *R. minuta* isolates were protease negative, but all the tested *D. hansenii*, *M. pulcherrima*, *R. mucilaginosa* and *S. roseus* isolates were proteolytic. *A. pullulans*, *C. albidus*, *P. macrostoma* and

Table 3. Yeast species isolated from apple fruits 6 and 8 weeks after full bloom in 1999

Species	6 weeks		8 weeks	
	No. of isolates	%[a]	No. of isolates	%[a]
Aureobasidium pullulans	1	0.5	1	0.3
Cryptococcus albidus	24	11.5	70	18.2
Cryptococcus laurentii	66	32.0	133	34.6
Debaryomyces hansenii	2	1.0	0	0
Metschnikowia pulcherrima	25	12.0	55	14.3
Phoma macrostoma	29	13.9	2	0.5
Rhodotorula aurantiaca	1	0.5	1	0.3
Rhodotorula glutinis	18	8.7	70	18.2
Rhodotorula minuta	2	1.0	2	0.5
Rhodotorula mucilaginosa	1	0.5	0	0
Sporobolomyces johnsonii	1	0.5	1	0.3
Sporobolomyces roseus	38	18.3	50	13.0
Total	208		385	

[a] Relative percentage, the number of isolates per species relative to the total number of isolates.

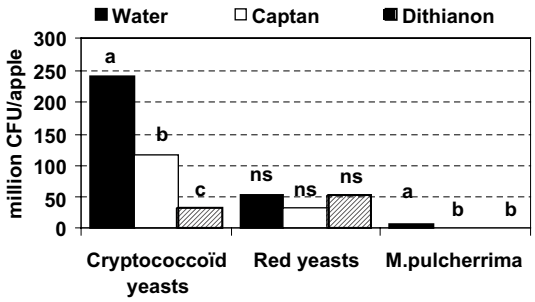

Figure 3. Yeast population of apple fruitlets eight weeks after full bloom subject to different fungicide treatments. Different letters behind treatment values within the same yeast group indicate a significant difference. Cryptococcoid yeasts: F $P < 0{,}001$, $LSD_{0,05} = 51$; red yeasts: not significant; *M. pulcherrima*: F $P < 0{,}002$; $LSD_{0,05} = 4{,}0$.

R. glutinis showed a variable proteolytic activity. Different isolates of the same species showed differences in the level of enzyme activity (data not shown).

Yeast susceptibility to fungicides in vitro

The results of the *in vitro* tests of yeast susceptibility to fungicides are presented in Figure 4. Dodine suppressed growth of all species from 10% of the recommended fungicide dose onwards. Dithianon suppressed growth of all species at half the recommended dose, with the exception of *C. albidus,* which was not totally suppressed until inoculated on the full dose. Captan suppressed growth of *A. pullulans*, *C. albidus* and *M. pulcherrima* above 10% of the recommended dose. The growth of *C. laurentii* was clearly reduced from the same dose onwards, but was not completely inhibited until seven times the recommended dose. The red yeasts *S. roseus* and *R. glutinis* seemed completely unaffected by the captan in the growing medium. Tolylfluanid stopped *M. pulcherrima* at 10% of the dose. *A. pullulans* and *C. albidus* were suppressed considerably in growth from 10% of the dose onwards, but still visible at 7 and 2 times the dose respectively. *R. glutinis* was completely unaffected, while *S. roseus* and *C. laurentii* were only slightly affected at seven times the advised tolylfluanid dosage. Bupirimate showed only slight action at 1 and 2 times the advised dose against a selection of the fungi. Pyrimethanil showed partial inhibition of several species from 0.5 times the recommended concentration onwards.

Discussion

All tested fungicides reduced russet, especially in 2000, in contrast to results of other authors who were not able to detect a significant russet reducing effect of captan (Schwabe and van der Merwe, 1991) or dithianon (Jones et al., 1994). Captan, dithianon, tolylfluanid and dodine had a higher russet reducing effect than pyrimethanil and kresoxim-methyl, while all suppressed apple scab (*Venturia inaequalis*) effectively, suggesting the involvement of another fungal agent in russeting. Matteson Heidenreich et al. (1997) identified phyllosphere yeasts as a russet inducing factor.

The yeast population on the apple fruit was clearly decreased by the spraying of both captan and dithianon. Hislop and Cox (1969), Andrews

Table 4. Summary of enzyme activities of different yeasts species and the filamentous fungi *Aureobasidium pullulans* and *Phoma macrostoma* isolated from apple skin

Species	No. of isolates	Cutinase positive (%)	Lipase positive (%)	Protease positive (%)
Aureobasidium pullulans	2	100	100	50
Cryptococcus albidus	36	100	100	83
Cryptococcus laurentii	46	100	100	0
Debaryomyces hansenii	2	100	50	100
Metschnikowia pulcherrima	30	100	0	100
Phoma macrostoma	6	100	100	67
Rhodotorula aurantiaca	2	100	100	0
Rhodotorula glutinis	35	100	100	91
Rhodotorula minuta	2	100	100	0
Rhodotorula mucilaginosa	1	100	100	100
Sporobolomyces roseus	36	100	100	100

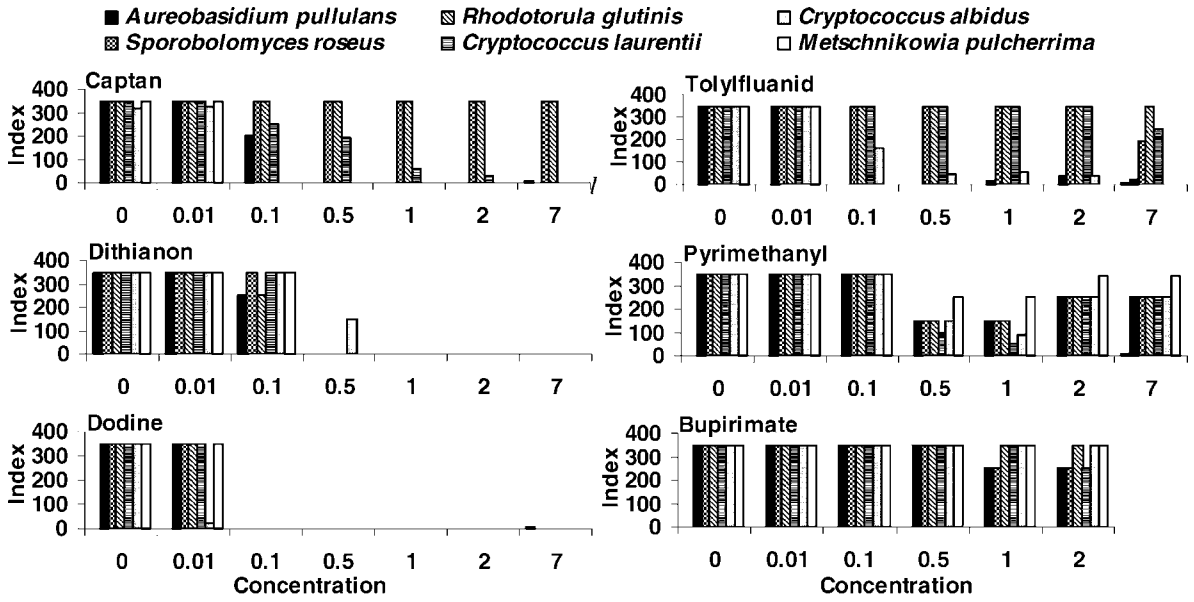

Figure 4. Effects of different fungicides at different concentrations on the in-vitro growth of selected yeast species. 350 means no growth inhibition of all inoculated points, 0 means complete inhibition. Concentration 1 represents the dose on the label, based on a spraying volume of 1000 litre per hectare, 0.01 is 1% of the dose, 7 is seven times the dose.

and Kenerly (1978) and Calvente et al. (1999) also demonstrated the influence of pesticide regimes on phylloplane microflora under field circumstances. *M. pulcherrima* and the cryptococcoïd yeasts proved vulnerable, while the red yeasts were relatively insensitive. Fungicides thus have distinct effects on individual component yeast species and can influence fungal species composition on apple fruits.

The vulnerability of *M. pulcherrima* found in the field was confirmed by the *in vitro* experiment. The moderate *in vitro* action of captan against *C. laurentii* was in line with the field observations. In contrast to the field observations, all yeast growth, including the red yeasts, was inhibited in the Petri dishes at low concentrations of dithianon.

Picco and Mangiarotti (1989) found no *in vitro* effect of dithianon on *A. pullulans, Rhodothorula* sp., *S. roseus* and *M. pulcherrima* and concluded that yeasts and yeast-like species in general were not very sensitive to fungicides. Smolka (1993) found an effect of tolylfluanid on two different strains of *S. roseus* where we found none. An explanation for the differences between these studies could be the existence of less sensitive or resistant strains. Smolka (1993) found one *S. roseus* strain susceptible to carbendazim while another strain was not affected. The inconsistencies between these *in vitro* experiments and between our field observations and *in vitro* experiment emphasize that caution should be taken in extrapolating laboratory results to the orchard practice.

The russet reducing effects of the fungicides were higher in the year 2000 when russet was more pronounced than in 1999. Andrews and Kenerly (1978) found a marked effect of season on fungicide efficacy on microorganisms in an apple orchard. They explained these variations by assuming the effect of different rainfall patterns close to pesticide applications.

The skin of young apple fruits was dominated by a limited number of fungal microorganisms, as was earlier reported for apple leaves by Andrews and Kenerly (1978). More than 98% of the population of fungal microorganisms on the apple fruit comprised only six species, the yeasts *C. albidus, C. laurentii, R. glutinis, S. roseus* and *M. pulcherrima* and the filamentous fungus *P. macrostoma.* All these species are frequently reported to occur in apple orchards all over the world (Hislop and Cox, 1969; Davenport, 1976; Andrews and Kenerly, 1978; Andews and Kenerly, 1980; Pennycook and Newhook, 1981; Picco and Mangriotti, 1989).

The slight changes in the composition of microorganisms between the two sampling dates suggest a dynamic population development in

which different species occupy different niches on the apple fruit skin and where changes in circumstances can cause changes in the population composition. Larger changes in species composition over longer time interfalls were previously observed by Hislop and Cox (1969), Davenport (1976), Pennycook and Newhook (1981) and Picco and Mangiarotti (1989).

All the yeast species isolated produced enzymes with the potential to degrade cutin. This may induce or aggravate russet as Matteson Heidenreich et al. (1997) suggested. Dickman and Patil (1988) showed that the fungus *Colletotrichum gloeosporioides* can infect papaya fruit directly by producing cutinase, while *Mycosphaerella* sp., a wound pathogen, could only infect intact fruit when cutinase was added. Similar mechanisms may play a role when russet of apple and the role of enzyme-producing yeasts are concerned. The yeasts may induce russet directly, but could also stimulate russet by exposing the apple surface to environmental factors through enzymatic degradation of the apple cuticle. Dickman and Patil (1988) showed that certain fungicides and insecticides protected papaya fruit in the field from *C. gloeosporioides* by cutinase inhibition. It would be interesting to test the efficacy of cutinase inhibitors as anti-russet agents.

The results of our study support the hypothesis that phyllosphere yeasts play a role in the development of russet. More yeasts were found on the young apples showing the first signs of russet, and both the severity of russet as well as the yeast population on the apple surface was reduced by the use of fungicides. All isolated yeasts produced enzymes that are potentially harmful for the apple skin.

Matteson Heidenreich et al. (1997) identified the red yeast *R. glutinis*, which was rather insensitive to both fungicides used in the field in this research, as one of the yeasts that can induce russet. The russet-reducing effect of dithianon and captan we observed is thus more likely to be due to their action against other yeast species. This suggests that species other than those previously identified also induce russet. It also implies that the russet-reducing effect of fungicides might be further improved by better targeting of the yeast species responsible for russet induction.

However, one has to be cautious when searching for a chemical solution to russet. Yeasts on the apple skin contribute to preventing storage diseases caused by *Botrytis cinerea* (Filonow et al., 1996) and *Penicilium expansum* (Janisiewicz, 1987). Moreover, in spite of the ample use of scab fungicides in apple growing today, russet is a major concern of commercial apple growers. This indicates that, although yeasts may well play an important role in the development of russet, it is not likely that a satisfying solution will be found by adjusting fungicide regimes alone. It is unlikely that a complicated physical defect such as apple fruit russet is the result of only one factor.

References

Andrews JH and Kenerly CM (1978) The effects of a pesticide program on the non-target epiphytic microbial populations of apple leaves. Canadian Journal of Microbiology 24: 1058–1072

Andrews JH and Kenerly CM (1980) Microbial populations associated with buds and young leaves of apple. Canadian Journal of Botany 58: 847–855

Babin J, Bidabé B, Bondoux P, Bouchier de l'Ecluse R, Brian C, Huet J, Le Lezec M and Trillot M (1977) La Rugosité des Pommes et des Poires. Institut national de vulgarisation pour les fruits, légumes et champignons, Paris

Barnett JA, Payne RW and Yarrow D (2000) Yeasts, characteristics and identification, 3rd ed., Cambridge, pp 1–1139

Batzer U, Berling R and Helm HU (2000) Experiences with the scab simulation programme Schorfexpert. Erwerbobstbau 42: 1–7

Bizeau C, Ponchant D and Moreau M (1989) Evolution of yeast flora on cider apples in Brittany. Yeast, Spec. issue 7th Int. Symp. Yeasts, pp S491–S494

Bonany J and Carbó J (1995) Relación entre los parámetros climáticos y la incidencia de 'russeting' en manzanas 'Golden Delicious'. In: Programa y Resúmenes. VI Congreso de la Sociedad Española de Ciencias Hortícolas. Barcelona, 25 al 28 Abril 1995 (p 221)

Braga AA, Benevides de Morais P and Linardi VR (1998) Screening of yeasts from Brazilian amazon rain forest for extracellular proteinases production. Systematic and Applied Microbiology 21: 353–359

Bremer H and Bünemann G (1982) Nebenwirkungen organischer Schorffungizide. II. Ertrag und Fruchtqualität bei 'Golden Delicious'. Gartenbauwissenschaft 47: 204–211

Calvente V, Benuzzi D, Obuchowitz N, Hough G and De Tosseti MIS (1999) Changes in surface microflora of apple and pear fruits by application of pesticides and their relation with biocontrol of post-harvest diseases. Agro-Food Industry Hi-Tech 10: 30–33

Chand-Goyal T and Spotts RA (1996) Enumeration of bacterial and yeast colonists of apple fruits and identification of epiphytic yeasts on pear fruits in the pacific Northwest united States. Microbiological Research 151: 427–432

Creasy LL (1980) The correlation of weather parameters with russet of Golden Delicious apples under orchard conditions.

Journal of the American Society for Horticultural Science 105: 735–738

Creasy LL and Swartz HJ (1981) Agents influencing russet on Golden Delicious apple fruits. Journal of the American Society for Horticultural Science 106: 203–206

Dantzig AH, Zuckermann SH and Andonov-Roland MM (1986) Isolation of a *Fusarium solani* mutant reduced in cutinase activity and virulence. Journal of Bacteriology 168: 911–916

Davenport RR (1976) Distribution of yeasts and yeast-like organisms from aerial surface of developing apples and grapes. In: Dickinson CH and Preece TF (eds) Microbiology of aerial plant surfaces (pp 325–359) Academic Press, London, UK

Dickman MB and Patil SS (1988) The role of cutinase from *Colletrichum gloeosporioides* in the penetration of papaya. In: Singh RS (ed) Experimental and conceptual plant pathology, Gordon and Brach Science Publishers, New York, USA

Dhingra OD and Sinclair JB (1985) Basic plant pathology methods. CRC Press, Bota Raton, Florida, USA

Eccher T (1978) Russeting of Golden Delicious apples as related to endogenous and exogenous gibberellins. Acta Horticulture 80: 381–385

Eccher T and Maffi A (1986) Treatments for prevention of Golden Delicious russeting. Acta Horticulture 179: 821–822

Eccher T and Noe N (1993) Influence of light on shape and quality of Golden Delicious apples. Acta Horticulture 329: 156–158

Faust M and Shear CB (1972) Russeting of apples, an interpretive review. HortScience 7: 233–235

Filonow AB, Vishniac HS, Anderson JA and Jansiewicz WJ (1996) Biological control of *Botrytis cinerea* in apple by yeasts from various habitats and their putative mechanisms of antagonism. Biological Control 7: 212–220

Gildemacher PR (2000) Duizend-en-één factoren van invloed op verruwing. Fruitteelt 90, 19: 18–19

Hatch AH (1975) The influence of mineral nutrition and fungicides on russeting of 'Goldspur' apple fruits. Journal of the American Society for Horticultural Science 100: 52–55

Hislop EC and Cox TW (1969) Effects of Captan on the non-parasitic microflora of apple leaves. Transactions of the British Mycological Society 52: 223–235

Jackson JE, Palmer JW, Perring MA and Sharples RO (1977) Effects of shade on the growth and cropping of apple trees. III. Effects on fruit growth, chemical composition and quality at harvest and after storage. Journal of Horticultural Science 52: 267–282

Janisiewicz WJ (1987) Postharvest biological control of blue mold on apples. Phytopathology 77: 481–485

Jones KM, Bound SA, Oakford MJ and Wilson D (1994) A strategy for reducing russet in Red Fuji apples while maintaining control of black spot (*Venturia inaequalis*). Australian Journal of Experimental Agriculture 34: 127–130

Matteson Heidenreich MC, Corral-Garcia MR, Momol EA and Burr TJ (1997) Russet of apple fruit caused by *Aureobasidium pullulans* and *Rhodotorula glutinis*. Plant Disease 81: 337–342

Meador DB and Taylor BH (1987) Effect of early season foliar sprays of GA4 + 7 on russeting and return bloom of Golden Delicious apple. HortScience 22: 412–417

Miller RH (1982) Apple fruit cuticles and the occurrence of pores and transcuticular canals. Annals of Botany 50: 355–371

Pennycook SR and Newhook FJ (1981) Seasonal changes in apple phylloplane microflora. New Zealand Journal of Botany 19: 273–283

Picco AM and Mangiarotti AM (1989) La micoflora saprofita epifitica del melo I trattamenti anticritticogamici. Rivista di Patologia Vegetale S. IV 25: 1–7

Porreye W (1980) Utilisation du bore dans la culture de Golden Delicious. Revue de l'Agriculture 33: 813–834

Schwabe WFS and van der Merwe J (1991) Influence of captan and mancozeb sprays on russeting of Golden Delicious apples. Deciduous Fruit Grower 41: 54–57

Sierra J (1957) A simple method for the detection of lipolytic activity of micro-organisms. Antonie van Leeuwenhoek 23: 15–22

Smith CB, Morrow CT and Greene GM (1987) Corking of 'Delicious' apples (*Malus domestica* Borkh) on four root-stocks as affected by calcium and boron supplied through trickele irrigation. Journal of Plant Nutrition 10: 1917–1924

Smolka SE (1993) Assesment of pesticide side effects on beneficial microorganisms from the phylloplane. IOBC WPRS Bulletin 16: 155–160

Taylor BL (1978) Effects of gibberellin sprays on fruit russet and tree performance of Golden Delicious apple. Journal of Horticultural Science 53: 167–169

Tromp J, Jonkers H and Wertheim SJ (eds) (1976) Grondslagen van de fruitteelt. Staatsuitgeverij,'s Gravenhage, 356 pp

Vogl M, Streitberg H and Hanke V (1985) Fruchtberostung bei der Apfelsorte 'Gelber Kostlicher'. Archiv für Gartenbau 33: 371–386

Walter TE (1967) Russeting and cracking in apple, a review of world literature. Annual Report of East Malling Research Station 1966: 83–95

Wertheim SJ (1982) Fruit russeting as affected by various gibberellins. Journal of Horticultural Science 57: 283–288

Wundermann H (1981) Pflanzenschutzmittel und Fruchtberostung bei 'Golden Delicious'. Hinweise für die Nachblüte-Behandlungen. Obstbau 6: 188–192