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White Haze and Scarf Skin, Two Little-Known Cosmetic Defects of Apples in Northern Germany

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Summary

A milky discolouration of red-pigmented apple varieties such as 'Redprince' and 'Elstar' from Northern Germany was due to the abnormal development of air spaces in hypodermal tissues. This physiological condition was identified as scarf skin, which is well-known in the United States but is reported here for the first time from Europe. White haze, caused by a superficial colonisation of the apple cuticle by mycelial yeasts, was also observed in Northern Germany, and two *Tilletiopsis* spp. were isolated and characterised. The occurrence of scarf skin and white haze in Northern Germany is discussed with reference to other fruit producing regions.

Key words. apple – epidermis – russeting – scarf skin – *Tilletiopsis* – white haze

Introduction

Cosmetic defects of the apple surface are particularly annoying to producers because they reduce the marketability of physiologically healthy fruits. Russeting and sooty blotch are two skin conditions widely recognised in most fruit producing areas. Russeting is a physiological condition caused by the formation of corky periderm tissue due to meristematic activities of the hypodermal layer beneath the epidermis (Lindner 2008). In contrast, sooty blotch is the result of a colonisation of the fruit surface by epiphytic fungi with melanised hyphae and/or fructifications (Gleason et al. 2011). In the Lower Elbe Valley, Germany's largest coherent apple production area, both of these conditions are common, russeting being a major problem with a long history (Roemer 1961) whereas sooty blotch is of a more recent occurrence and is confined to organic farming (R.W.S. Weber, in preparation).

In recent years we have observed two further types of skin defect in Northern Germany, identified here as white haze and scarf skin. Symptoms of white haze appear if the colonisation of fruit surfaces by epiphytic yeasts of the genus *Tilletiopsis* becomes visible as a greyish-white plaque either before harvest or after long-term storage (Boekhout et al. 2006; Baric et al. 2010), whereas apple fruits affected by scarf skin acquire a whitish or opalescent sheen due to the formation of subepidermal air spaces (Byers 1977; Ferree et al. 1984a). Although both conditions are well-known elsewhere, the present report appears to be the first critical account of white haze for Germany, and of scarf skin for the whole of Europe.

Materials and Methods

Plant material

Apple fruits showing unusual skin conditions were collected from commercial farms at harvest or after long-term storage (for details see Table 1 and 2). From each collection, five symptomatic fruits were chosen for microscopic examination and for the isolation of fungi as outlined below.

Microscopy

The surface of fruits was examined with an Olympus SZ60 stereo microscope fitted with a Sony α350 digital camera. For light microscopy, material was viewed with a Carl Zeiss Axio Scope A1 using x10, x40 and x100 Plan-Neofluar objectives with differential interference contrast (DIC) optics. Photomicrographs were taken with a Carl Zeiss digital camera ICc 3, and measurements were made with the AxioVision software (version 4.8).

Isolation of fungi

For isolating microorganisms from subepidermal tissue, symptomatic fruits were thoroughly washed in tap water and swabbed with 70 % (v/v) ethanol. Five small cubes of tissue (1.5 mm side length) were cut from affected regions of each fruit and placed on 1 % malt extract agar (MEA) augmented with penicillin G and streptomycin sulphate (each at 200 mg l^{-1}), or on King's B medium (media supplied by Carl Roth, Karlsruhe, Germany). In order to isolate epiphytic microorganisms, a

Table 1. Documented cases of scarf skin in Northern Germany in 2009 and 2010.

Variety	Date	Farm No.	Locality	Management	Severity[1)]
'Elstar'	Oct. 2009	1	Hollern	integrated	20 %
'Jonagold'	5 Oct. 2009	2	Neuenfelde	integrated	25 %
'Redprince'	24 Feb. 2010	3	Neuenfelde	integrated	>50 %
'Elstar'	24 Feb. 2010	3	Neuenfelde	integrated	25 %
'Redprince'	24 Feb. 2010	4	Drochtersen	integrated	25 %
'Gala'	Oct. 2010	5	Bassenfleth	integrated	25 %
'Elstar'	Oct. 2010	5	Bassenfleth	integrated	>30 %
'Gloster'	Oct. 2010	5	Bassenfleth	integrated	20 %
'Redprince'	Oct. 2010	5	Bassenfleth	integrated	10 %

[1)] Percent of fruits affected by scarf skin

Table 2. Occurrence of white haze in Northern Germany in 2010.

Variety	Date	Farm No.	Locality	Management	*Tilletiopsis* sp.
'Elstar'	15 Oct. 2010	6	Langförden	integrated	B
'Elstar'	16 Oct. 2010	7	Gross Hove	organic	B
'Elstar'	16 Oct. 2010	8	Drochtersen	organic	B
'Elstar'	16 Oct. 2010	9	Jork	unmanaged	G

B = *Tilletiopsis* isolate OVB10-013; G = *Tilletiopsis* isolate OVB10-015

piece (1.5 cm diam.) of the surface of each affected fruit was sliced off, fixed to the underside of a Petri dish lid with a streak of vaseline, and suspended above MEA or King's B agar plates (modified from Boekhout et al. 2006). Single-spore isolates of fungi were transferred to potato dextrose agar (PDA; Carl Roth), grown at 20 °C and stored at 4 °C.

In order to obtain actively liberated spores (ballistospores), a 7 d old PDA culture of each isolate was inverted for 10 min onto thinly poured tap-water agar (TWA) plates, mounting squares of agar with freshly discharged spores for microscopy. Dimensions of 25 spores were measured for each isolate. The germination of liberated spores was examined after 24 h incubation on TWA, TWA augmented with 1 % (v/v) apple juice, and PDA. In addition, material was collected from the surface of 7 d old cultures for microscopic examination.

Determination of ITS sequences

Mycelium was scraped from the surface of PDA cultures incubated for 14 d at 20 °C using a sterile spatula. DNA was extracted from 100 mg mycelium using the FastDNA Spin Kit and the FastPrep 24 Cell Homogenizer (MP Biomedicals, Solon, Ohio, USA). For amplifying the internal transcribed spacer (ITS) region of the nucleus-encoded ribosomal gene cluster, primers ITS4 (5'-TCCTCCGCTTATTGATATGC) and ITS5 (5'-GGAAGTAAAAGTCGTAACAAGG) were used (White et al. 1990). The PCR reaction mix (total vol. 50 µl) contained approx. 1 ng DNA, 0.5 µM of each primer, and 25 µl of 2x Dream Taq PCR Master Mix (Applied Biosystems, Foster City, California, USA). Cycling conditions included one period of 2 min at 94 °C, 35 cycles of 30 s at 94 °C, 30 s at 50 °C and 60 s at 72 °C, and one period of 10 min at 72 °C.

Subsamples (1 µl) of PCR products were separated at 2 V cm^{-1} in 1 % (w/v) agarose in TBE buffer (pH 7.8) containing 89 mM Tris, 89 mM boric acid and 2 mM EDTA. The gel was then stained by incubation in SYBR Green I dye (Lonza, Rockland, Maine, USA) and viewed with an excitation wavelength of 420–500 nm. Purification of the amplicon from the PCR mixture was achieved using the QIAquick PCR purification kit (Qiagen, Hilden, Germany). Sequencing was performed externally by Eurofins MWG Operon (Ebersberg, Germany) with ITS4 and ITS5 as primers. Chromatograms were viewed, and sequences edited, using Chromas Lite 2.01 (Technelysium, Brisbane, Australia). Sequence searches were made in GenBank using the BLASTN function (Zhang et al. 2000).

Results

Scarf skin

Affected fruits were characterised by a milky appearance of the epidermis which was most obvious over red-pigmented regions except for the immediate vicinity of lenticels (Fig. 1A). To the naked eye, the fault seemed to be due to a corrosion of the epicuticular wax layer. However, when this layer was removed by scraping with a scalpel blade, air spaces were observed between the highly pigmented hypodermal cells underlying the epidermis

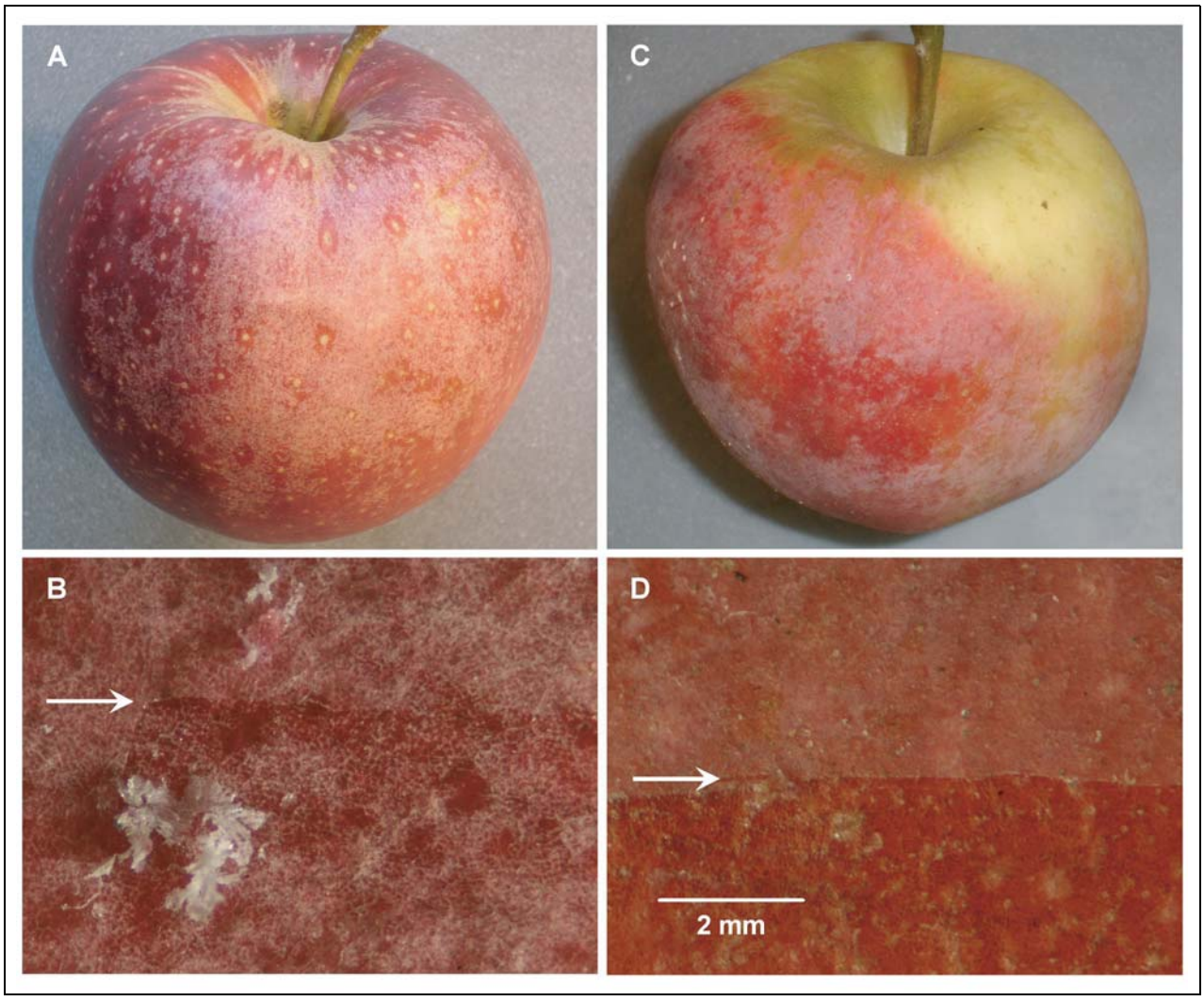


Fig. 1. Appearance of scarf skin on 'Redprince' (A, B), and of white haze on 'Elstar' (C, D) at harvest time (October 2010). In the magnified images B and D the surface was scraped off horizontally below a line indicated by arrows. B and D to same scale.

(Fig. 1B). When the surface scrapings were examined by light microscopy, no microorganisms were apparent, and none were consistently isolated from apples affected by this condition which was therefore assumed to be due to physiological factors. The symptoms as observed here were identical in every way with detailed descriptions of 'scarf skin' from North America (Byers 1977; Ferree et al. 1984a).

In the past, scarf skin has been occasionally noticed in Northern Germany as a skin condition without commercial relevance, especially on fruits from younger, vigorously growing trees (<10 yr old; G. Palm, pers. comm.). During the past two years, incidence of scarf skin has been sufficiently severe in a few cases for concerned fruit farmers to bring the condition to our attention (Table 1).

White haze

In contrast to scarf skin, the whitish discolouration of fruits affected by white haze (Fig. 1C) was easily removed by scraping, revealing the full fruit pigmentation underneath (Fig. 1D). The white plaque consisted of fungal hyphae and elongated spores when viewed with the light microscope. This feature is typical of 'white haze', i.e. the colonisation of fruit surfaces by anamorphic smut fungi (Lindner 2006). Spores were discharged in great quantity from colonised surfaces onto agar media, permitting the isolation of fungi. Two different species were obtained from a total of four samples collected during harvest in October 2010 (Table 2). Both were referable to the genus *Tilletiopsis* (Ingold 1984, 1991; Boekhout 2010). All three isolates of the first species possessed an identical ITS sequence which showed a 100 % identity also to several GenBank accessions (AY259053, -056, -058, -060, -063, -065, -077, -078, and -079). These had been identified as *Tilletiopsis* sp. B belonging to the *T. pallescens* clade by Boekhout et al. (2006). Because the matching GenBank accessions covered only 72 % of the ITS sequence obtained in the current work, the latter has been deposited in GenBank (accession number JF828314). The fourth isolate (GenBank accession JF828315) was identical to GenBank accessions AY259076, -077 and -080, termed *Tilletiopsis* sp. G by Boekhout et al. (2006).

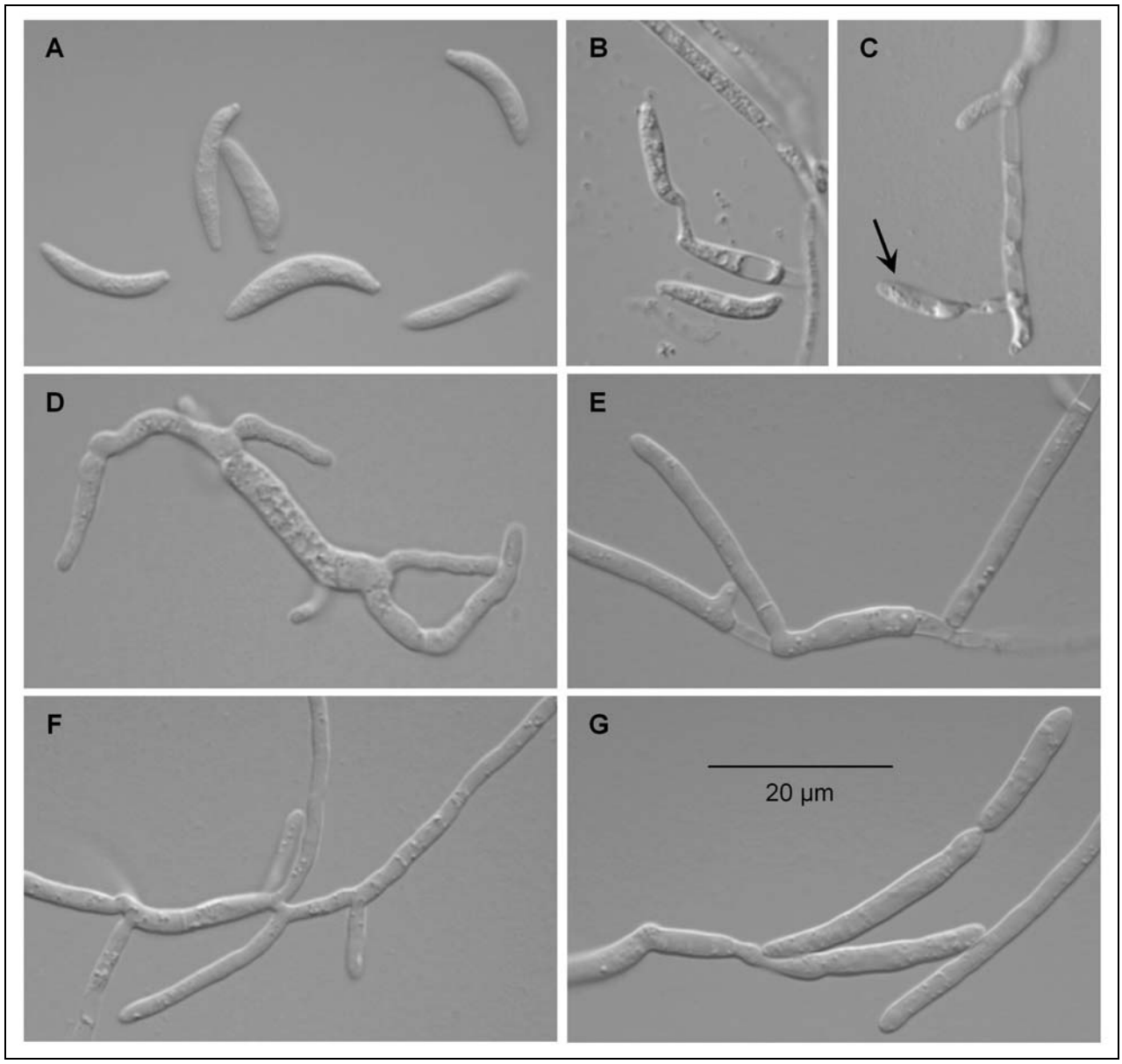


Fig. 2. Microscopic features of *Tilletiopsis* isolates OVB10-013 (species B) and OVB10-015 (species G). A: Freshly liberated ballistoconidia of sp. B. B: Germination of a ballistoconidium by repetition on the surface of a 7 d old colony of species G. C: Production of a ballistoconidium (arrow) on a sterigma in species G grown on PDA. D and E: Ballistospore germination of sp. B after 24 h incubation on TWA (D) and PDA (E). F: Ballistospore germination of sp. G after 24 h incubation on PDA. G: Production of elongated yeast-like conidia in sp. B after 24 h incubation on PDA with a coverslip. All images to same scale.

Critical microscopic examination of the two different *Tilletiopsis* spp. isolated in the present work revealed no differences between freshly discharged ballistoconidia, which were curved towards the hilar end (Fig. 2A) and measured 12.8–19.1 × 2.3–3.4 µm (average 15.8 × 2.8 µm) in *Tilletiopsis* sp. B (isolate OVB10-013), and 11.3–20.7 × 2.3–3.2 µm (average 15.7 × 2.8 µm) in *Tilletiopsis* sp. G (isolate OVB10-015). When the surfaces of 7 d old colonies of both species were scraped with a scalpel blade, some conidia showed germination by repetition to produce a new ballistoconidium (Fig. 2B). Ballistoconidia of both species were produced on sterigmata arising laterally from hyphae (Fig. 2C), and liberated actively. They showed mycelial germination on all agar media, although details of germination differed. Thus, after 24 h incubation species B germinated by short, strongly curved hyphae on TWA with or without added apple juice (Fig. 2D), whereas on PDA long straight hyphae with empty intercalary compartments delimited by retraction septa were produced (Fig. 2E). In contrast, species G produced long and straight hyphae without empty compartments on all media (Fig. 2F). Elongated yeast-like conidia were produced from short lateral branches by both species when growing PDA cultures were covered with a microscope coverslip for >8 h or when a coverslip was applied onto fresh ballistospores on PDA (Fig. 2G).

Discussion

To the best of our knowledge, the present article contains the first published accounts of white haze from Germany, and of scarf skin from Europe. There is a striking discrepancy between the lack of previous information from Germany, and the importance ascribed to both conditions elsewhere. White haze has been identified as a serious problem in apple producing regions close to Germany such as the Netherlands (Boekhout et al. 2006) and South Tyrol (Lindner 2006; Baric et al. 2010), whereas scarf skin can be a criterion for the downgrading of dessert apples in North America (Byers 1977). One reason for the lack of data from Germany might be that both skin conditions have been present but overlooked or gone unreported for some years. This is plausible considering the lack of diagnostic facilities in German horticulture.

Two seminal publications examining the phylogenetics of white haze fungi are those of Boekhout et al. (2006) and Baric et al. (2010). Although the taxonomy of *Tilletiopsis* is still in a state of flux, it can be safely concluded that both Northern German *Tilletiopsis* spp. isolated in the present work are taxonomically distant from each other, but very similar or identical to species also found in the Netherlands. There, *Tilletiopsis* sp. B was the dominant fungus whereas sp. G was isolated sporadically only from asymptomatic fruits (Boekhout et al. 2006). In the Netherlands, white haze was observed chiefly as a post-harvest condition that developed during ultra-low oxygen (ULO) storage whereas symptoms were prominent at or before harvest time in Northern Germany (this report) as well as in South Tyrol (Baric et al. 2010). However, in South Tyrol an altogether different species composition was found, featuring neither sp. B nor sp. G (Baric et al. 2010). Clearly, more detailed surveys of *Tilletiopsis* spp. associated with white haze are necessary with respect to region, time of appearance, and crop management system.

Three of the four Northern German cases of white haze in October 2010 were from organically managed or derelict orchards, indicating that the fungicides employed in integrated production may collaterally control white haze. Boekhout et al. (2006) have obtained preliminary evidence that dithianon, dodine and captan treatments were effective against white haze. For the control of severe infections of *Venturia inaequalis* (apple scab) in Northern Germany in 2010, these fungicides were widely deployed from blossom time until the end of June (dithianon, dodine) or until 21 d before harvest (captan). It is unknown whether any of the crop protection measures available to organic farmers are effective against white haze.

Whilst *Tilletiopsis* spp. may be common on apple surfaces, the development of symptoms visible to the unaided eye is favoured by cool, moist conditions, notably a rainy period just before harvest (Boekhout et al. 2006). These conditions prevailed in Northern Germany in 2010, but not in previous years. Other favourable conditions include the use of hail nets which raise the humidity around the trees, and the application of nitrogen-containing foliar fertilisers (Christanell 2009; Baric et al. 2010). At present, neither of these additional factors applies to Northern German apple production to any large extent. Taken together, the available facts indicate that Northern Germany is currently only at low risk to white haze. A more detailed knowledge of the factors influencing white haze symptom development will be required before predictions of the possible impact of climate change on the occurrence of white haze can be made.

The concept of scarf skin has changed over time. The term was first used by Beach et al. (1905) as a criterion for describing whitish epidermal discolourations as a variable but natural trait of certain apple varieties. Later Byers (1977) understood scarf skin as a physiological disorder associated with particular environmental conditions. Subsequent research in the United States has revealed that scarf skin is initiated during a 30- to 50-day period following petal fall (Ferree et al. 1984a). This is the sensitive period also for fruit russeting. Whilst most of the factors causing scarf skin are as yet unknown (Ferree et al. 1984a, b), russeting is triggered by one or several factors including surface wetness, epiphytic fungi and a variety of chemical agents (Faust and Shear 1972; Creasy 1980; Lindner 2008; Christanell 2009). Treatments with the fungicide dodine may encourage both russeting and scarf skin (Ferree et al. 1984a; Christanell 2009). In contrast, both conditions may be reduced by post-blossom applications of gibberellins alone or in combination with prohexadione-calcium (Ferree et al. 1984a; McArtney et al. 2006; McArtney 2010). There is therefore good evidence that scarf skin and russeting are physiologically related. There is also an anatomical connection in the sense that both conditions have their origin in the hypodermis which produces a periderm tissue in the case of russeting (Lindner 2008) but develops air spaces in scarf skin (Ferree et al. 1984b).

With hindsight, it is safe to say that scarf skin has occurred sporadically during the past 10 years in Northern Germany, but in contrast to the United States it has not led to the downgrading of fruit quality in the marketing chain. The more frequent observation of scarf skin in the 2009 and 2010 seasons may be related to the current trend towards cultivating red-pigmented apple varieties such as 'Redprince', which show this condition more clearly. Dodine has been registered since 2007 for the control of apple scab (*Venturia inaequalis*) in Northern Germany, and is being used repeatedly during the post-blossom period. However, no effect of dodine on fruit russeting has been observed in critical experiments with one of the most sensitive varieties, 'Golden Delicious' (Palm and Kruse 2010). Dodine is therefore unlikely to stimulate scarf skin in varieties of current commercial relevance.

Scarf skin and white haze are easily identified using the methods and illustrations provided here. It is hoped that this article will raise an awareness of both these cosmetic defects so that more information of their causes and importance may be generated.

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