Europ.J.Hort.Sci., **78** (3). S. 97–105, 2013, ISSN 1611-4426. © Verlag Eugen Ulmer KG, Stuttgart

Fungi Associated with Blossom-End Rot of Apples in Germany

R. W. S. Weber[1)] and N. Dralle[2)]
([1)]Obstbauversuchsanstalt Jork, Landwirtschaftskammer Niedersachsen, Jork, Germany and [2)]Fakultät Agrarwissenschaften und Landschaftsarchitektur, Hochschule Osnabrück, Germany)

Summary

In a five-year survey of blossom-end rot of apples in Northern Germany, *Botrytis cinerea* and *Neonectria galligena* were the most important pathogens, being present in 41.6 and 33.9 % (respectively) of a total of 1085 infected fruits. Two unidentified *Alternaria* spp. (together 10.2 %), *Diplodia seriata* (6.5 %), *Fusarium avenaceum* and *F. lateritium* (together 4.2 %), *Phoma exigua* and an unidentified *Phoma* sp. (together 1.8 %), and *Stemphylium vesicarium* (1.4 %) were also isolated from disease lesions. In contrast, *Neofabraea alba*, *N. perennans* and *Sclerotinia sclerotiorum* were rare, totalling 5 infected fruits throughout the entire survey. Characteristic symptoms of blossom-end rot were associated with different taxa and are described. Successive years showed substantial variations in the relative contribution of different pathogens to blossom-end rot in Northern Germany, *B. cinerea* being dominant in 2008 and 2011, and *N. galligena* in 2010. In samples collected in 2012 from the Palatinate and Lake Constance regions of Southern Germany, the range of pathogens was as above, and *B. cinerea* was the dominant species in both areas. Implications of these findings for regional fruit production are discussed.

Key words. *Alternaria* – *Botrytis cinerea* – calyx-end rot – *Diplodia seriata* – dry-eye rot – *Fusarium avenaceum* – *Fusarium lateritium* – *Malus domestica* – *Neonectria galligena* – *Phoma exigua* – *Stemphylium vesicarium*

Introduction

Even though infections of apple fruits by necrotrophic fungi may occur throughout the vegetation period, the outbreak of aggressive fruit rots is often delayed until fruit ripening or storage (Noble and Drysdale 1983; Friedrich and Rode 1996; Xu and Robinson 2010). Somewhat exceptionally, large necrotic areas at the blossom ends of immature fruits may be caused by infections which have their origin during flowering. The collective term 'blossom-end rot' is applied to such lesions in the context of the present work, although the terms 'calyx-end rot' or 'dry-eye rot' are also used in the literature if particular pathogens are associated with the disease (Tronsmo and Raa 1977; Hickey 1990; Rosenberger 1990). In most areas and seasons this disease is not an economic problem, fruit losses of 5 % or more being comparatively rare (Grimm 1977; Weber 2008). However, because infected fruits may fall to the ground before harvest or may be removed in the course of manual fruit thinning, crop losses due to blossom-end rot are prone to be underestimated.

There is uncertainty among fruit farmers and their consultants concerning the identity of fungi causing blossom-end rot. Whereas the grey mould fungus *Botrytis cinerea* is widely regarded as an important pathogen in Europe, North America and the Far East (Rosenberger 1990; Weber 2008; Wani et al. 2011), the apple canker pathogen *Neonectria galligena* and a range of other fungi have also been implicated in this disease (Rich 1970; Covey 1985; Kennel 1992). Much of the uncertainty may be due to the erratic appearance of blossom-end rot in successive vegetation periods, and the lack of long-term surveys of its causes. On the time-scale of years or decades, the population dynamics of apple fruit pathogens may fluctuate greatly, as demonstrated in Northern Germany for post-harvest rots which were dominated by *Neofabraea perennans* and *N. alba* up to 1968 and since the mid-1980s, whereas *N. galligena* was the principal storage-rot pathogen in the interim period (Bömeke and Blank 1959; Palm 1986; Palm and Kruse 2012). Further, a recently discovered pathogen, *Phacidiopycnis washingtonensis*, seems to be playing an increasing role as a post-harvest rot in Northern German pome fruit production since at least 2006 (Weber 2012).

The present article summarises results of annual surveys of blossom-end rot carried out in Germany's largest fruit production area, the Lower Elbe region, for five years starting with the last notable outbreak of the disease in 2008 (Weber 2008). Additional information was obtained from the Palatinate and Lake Constance

regions of Southern Germany for the 2012 season, thereby permitting a documentation of this disease in space and time.

Materials and Methods

Collection of samples

Northern German samples were collected from commercial orchards located south of the Lower Elbe river between the western fringe of the city of Hamburg (9.9° E) and the tributary river Oste (9.0° E), at latitudes of 53.4–53.8° N. In the five growing seasons 2008, 2009, 2010, 2011 and 2012, fruits from 6, 3, 1, 4 and 12 (respectively) orchards under integrated pest management and from 2, 5, 9, 4 and 5 (respectively) orchards under organic management were included in the survey. Orchards were chosen according to the annual availability of infected apples, whereby two orchards were sampled in all five years of the survey, and three others in 2–4 years.

In 2012, samples from the Palatinate region were collected from eight orchards under integrated pest management, located between the rivers Saar and Rhine at latitudes of 49.1–49.6° N and longitudes of 6.5–8.2° E. In the same year, apples with blossom-end rot were sampled from four organic and two integrated orchards situated north of Lake Constance at 47.7–47.9° N and 9.3–9.6° E.

For monitoring purposes, 10–20 symptomatic fruits per orchard were collected. In the case of two orchards in the Lower Elbe region in 2012, 6 aliquots of 200 fruits along each of two adjacent rows of the varieties 'Kanzi' and 'Braeburn' were scored for blossom-end rot, and all symptomatic fruits were analysed.

Photographic documentation

Entire fruits with blossom-end rot were photographed with a Sony DSC-W350 digital camera, and close-up photographs were obtained with a Minolta AF macro zoom fitted to 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 in the differential interference contrast (DIC) optical mode. Photomicrographs were taken with the Carl Zeiss digital camera ICc 3, and spore measurements were made with the complementary AxioVision software (version 4.8).

Isolation and identification of fungi

After photographic documentation, sepals were cut off from diseased apples in order to reduce the risk of contamination by saprotrophic fungi. Blossom ends of apples were first wiped with a tissue paper soaked in 70 % (v/v) ethanol and then immersed for 30 s in 70 % ethanol. Following drying in a sterile laminar flow cabinet, two sections (each approx. 3 × 1 mm side length, 2–3 mm deep) were cut across the border between healthy and diseased regions with a flame-sterilised scalpel blade, and placed on potato dextrose agar (PDA) augmented with 200 mg penicillin G and streptomycin sulphate l^{-1} (all media from Carl Roth, Karlsruhe, Germany). Growing colonies of fungi were observed repeatedly, and pure cultures were obtained by sub-cultivation of growing mycelial margins on PDA plates. In order to stimulate sporulation, PDA plates of pure cultures were exposed to a daily 10-min burst of near-UV light (BLB-20W; λ_{max} = 365 nm). Representative isolates were incorporated into the Culture Collection, Obstbauversuchsanstalt Jork (for accession numbers, see Table 1).

Identification of fungi was attempted by microscopy of spores produced on diseased fruits, on isolation plates or in pure culture, using keys to the genera *Fusarium* (Booth 1971; Yli-Mattila et al. 2002; Vitale et al. 2011), *Alternaria* (Ellis 1971), *Stemphylium* (Ellis 1971) and *Phoma* (Boerema et al. 2004), and a description of *Diplodia seriata* (Phillips et al. 2007). Additionally, the sequence of the internal transcribed spacer region (ITS1 – 5.8S – ITS2) of the ribosomal RNA gene cluster was obtained from selected isolates using primers ITS5 and ITS4 (White et al. 1990), and GenBank searches were performed as described in detail by Weber and Zabel (2011).

Pathogenicity tests

Representative isolates of fungi associated with blossom-end rot were screened for their pathogenicity using wound inoculations of apples. Both freshly harvested apples (variety 'Galmac') and fruits approx. 6 weeks ahead of harvest (variety 'Kanzi') were inoculated on 27 August 2012. Fruits were surface-sterilised by being immersed in 70 % ethanol for 30 s, followed by drying in a sterile laminar flow cabinet. Cuts (approx. 4 mm side length, 2 mm deep) were made with a flame-sterilised scalpel on two sides of a triangle, and spore suspensions or pieces of 7 d old agar culture were inserted into the wound. The tissue triangle was then folded back onto the body of the fruit. For each strain examined (see Table 1), two mature and two immature apples were used, each carrying three inoculation sites. Apples were evaluated for the development of fruit rot within and beyond the boundaries of the inoculation wound after 14 d incubation at 20 °C.

Results

Blossom-end rot caused by Botrytis cinerea

Identification of *B. cinerea* was unequivocal on the basis of macroconidiophores developing on infected apples or in pure culture. In a few cases, non-sporulating sclerotial colonies were obtained on the initial isolation plates. In all but one of these strains, formation of macroconidia

Table 1. Identity and characteristics of representative fungal isolates associated with blossom-end rot.

Species	Origin[a]	Accession number[b]	Length of ITS seq.[c]	GenBank reference sequence	Sequence identity[d]	Patho-genicity[e]
Botrytis cinerea	LE 2012	OVB12-077	563 nt	EF207413-415	563/563	++/++
Botrytis cinerea	Pal 2012	OVB12-078	563 nt	EF207413-415	563/563	++/++
Sclerotinia sclerotiorum	LE 2009	OVB09-009	564 nt	EF091808, -809	564/564	++/++
Neonectria galligena	LE 2009	OVB09-003	569 nt	JF499884, -885	569/569	++/++
Fusarium avenaceum	LE 2009	OVB09-005	585 nt	AY147285	571/571	n. d.
Fusarium avenaceum	Pal 2012	OVB12-064	585 nt	AY147285	571/571	++/++
Fusarium avenaceum	LC 2012	OVB12-097	585 nt	AY147285	571/571	++/++
Fusarium lateritium	LE 2012	OVB12-061	585 nt	AF310980	585/585	+/+
Alternaria strain 1	LE 2012	OVB12-072	593 nt	GU584946, -947, -951	593/593	+/+
Alternaria strain 1	LC 2012	OVB12-086	593 nt	GU584946, -947, -951	593/593	n. d.
Alternaria strain 2	LE 2009	OVB09-011	594 nt	HQ630970	594/594	n. d.
Alternaria strain 2	Pal 2012	OVB12-071	594 nt	HQ630970	594/594	+/+
Stemphylium vesicarium	LE 2012	OVB12-070	604 nt	GU065719	548/548	+/++
Stemphylium vesicarium	LE 2009	OVB09-012	604 nt	GU065719	548/548	+/++
Phoma exigua	LE 2012	OVB12-073	565 nt	EU343168	565/565	+/++
Phoma sp.	LE 2012	OVB12-079	564 nt	JX160059	564/564	−/−
Phoma sp.	LC 2012	OVB12-096	564 nt	JX160059	564/564	n. d.
Diplodia seriata	LE 2009	OVB09-007	604 nt	EU650671	580/580	n. d.
Diplodia seriata	LE 2012	OVB12-084	604 nt	EU650671	580/580	++/++
Diplodia seriata	LC 2012	OVB12-089	604 nt	EU650671	580/580	++/++

[a] Abbreviations; LC, Lake Constance; LE, Lower Elbe Region; Pal, Palatinate

[b] Culture collection, Obstbauversuchsanstalt Jork, Germany

[c] including primers ITS5 and ITS4

[d] indicating the number of identical nucleotide positions/total alignment length

[e] denoting limited rot within the margins of the inoculation lesion (+) or spreading fruit rot beyond the inoculation point (++) on unripe/ripe fruits after 14 d incubation at 20 °C; n. d., not determined.

was stimulated by exposure of pure cultures to near-UV light, affording an unequivocal identification as *B. cinerea*. This was confirmed by an analysis of ITS sequences of two poorly sporulating strains (Table 1). Only one culture remained free from macroconidia regardless of cultivation conditions. This was identified as *Sclerotinia sclerotiorum* by ITS sequence analysis (Table 1). Both *S. sclerotiorum* and *B. cinerea* were capable of causing necrotic lesions on immature and mature apples following wound inoculation (Table 1).

Symptoms of blossom-end rot caused by *B. cinerea* were highly characteristic. Infected tissue had a dry, corky appearance and was sharply demarcated against the adjacent healthy tissue which was often additionally highlighted by a red halo (Fig. 1A). Whereas most lesions were of medium size (< 10 mm diam.) and mid-brown to dark grey in colour, some larger lesions were pale to walnut brown (Fig. 1B). Sporulation on or near the sepals was almost always visible in material freshly collected from the field or after incubation in a damp chamber for 3–5 d (Fig. 1C). Whilst many lesions remained encapsulated until harvest time, some infections broke out to give rise to a rapidly progressing fruit rot with alternating paler and darker brown bands and darkened spots around lenticels (Fig. 1D). *Botrytis cinerea* was isolated from the growing margins of such infections.

Blossom-end rot caused by Neonectria galligena

Colonies of *N. galligena* produced a whitish aerial mycelium on PDA which soon developed a bright yellow reverse. Characteristic macroconidia were produced within 7–10 d cultivation at 20 °C. ITS sequence analysis of a representative isolate unambiguously confirmed identification as *N. galligena* (Table 1). This species was capable of causing a spreading fruit rot on immature as well as mature apples that had been artificially inoculated (Table 1).

Blossom-end rot lesions caused by *N. galligena* differed from those of *B. cinerea* in being dark brown in colour,

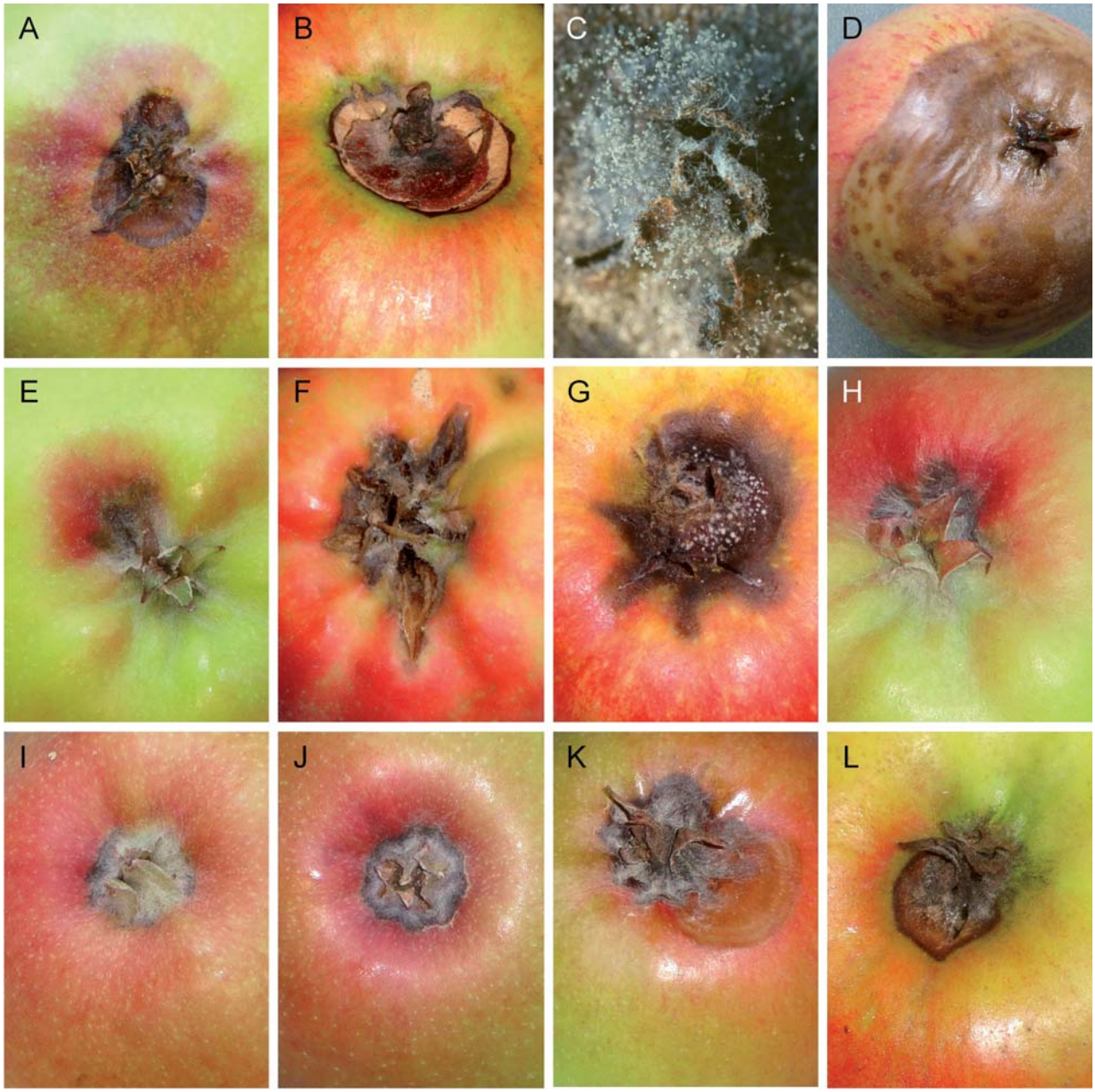


Fig. 1. Symptoms of blossom-end rot caused by different fungi in Northern Germany. (A) Early infection by *Botrytis cinerea* on 'Jonagored', 15 July 2008. (B) Later, more clearly demarcated lesion of *B. cinerea* on 'Jonagored', 17 Oct. 2008. (C) Sporulation of *B. cinerea* on the sepals of an infected blossom end. (D) Outbreak of an aggressive rot from a *B. cinerea* blossom-end infection on 'Topaz', 15 July 2008. (E) Early symptoms of infection by *N. galligena* on 'Kanzi', 17 Aug. 2012. (F) Advanced lesion of *N. galligena* on 'Rubinstar', 9 Sept. 2010. (G) Formation of the first sporodochia of *N. galligena* on an abortive 'Elstar' apple, 24 Aug. 2012. (H) Infection by *Fusarium lateritium* on 'Kanzi', 25 July 2012. (I) Infection by *Stemphylium vesicarium* on 'Braeburn', 28 Aug. 2012. (J) Infection by *Phoma exigua* on 'Braeburn', 28. Aug. 2012. (K) Incipient development of an aggressive infection of *Diplodia seriata* from a blossom-end rot lesion on 'Rubens', 30 July 2009. (L) Infection by *Neofabraea alba* with incipient sporulation on 'Topaz', 1 Oct. 2010.

moist and firm rather than corky in texture, stellate rather than circular in outline, and continuously progressing into the surrounding healthy tissue rather than being demarcated from it (Fig. 1E). Larger lesions developed deep radial cracks (Fig. 1F). Red haloes were also frequently present. Cream-coloured sporodochia were observed mainly on younger fruits in the process of abortion, or on maturing fruits < 6 weeks before harvest (Fig. 1G). Although these closely resembled fructifications of *Neofabraea* spp. when viewed with a hand lens or a stereo microscope, they contained the highly characteristic macroconidia of *N. galligena*.

Fusarium *spp. associated with blossom-end rot*

Two *Fusarium* spp. were isolated from apples with blossom-end rot. The first species produced a relatively slowly growing colony with a pale yellowish-pink reverse which later developed a greenish-black secondary pigment associated with the formation of incipient perithecial initials, suggesting the presence of a *Gibberella* state. Microconidia and chlamydospores were absent. The only ITS sequences with a 100 % match were those of *F. lateritium*. ITS sequences of three isolates of the second species were identical to one another and matched GenBank submissions of *F. avenaceum* as well as *F. tricinctum*. These isolates produced a fast-growing colony with a strong aerial mycelium which was initially white, then turning to shades of yellow and red with a dark pink reverse. Microconidia and chlamydospores were absent, although inflated hyphal cells were produced within the aerial mycelium. These features permitted an identification of *F. avenaceum* in preference to *F. tricinctum*. Whereas *F. lateritium* produced slow lesions on artificially inoculated immature and mature apples, those of *F. avenaceum* were aggressively spreading.

Blossom-end rot lesions yielding *Fusarium* spp. in isolation experiments were inconspicuous. They were dark brown to black in colour, sharply demarcated against the healthy tissue, and confined to the immediate vicinity of the sepal region with occasional short tongue-like protrusions (Fig. 1H).

Alternaria *and* Stemphylium *associated with blossom-end rot*

Isolates of *Alternaria* yielded greyish-green colonies with strong aerial growth. The production of conidia was often abundant after 5–7 d at r.t., but sometimes delayed and sparse. The ITS sequence analysis of four isolates revealed a near-complete identity, the sole difference being a single-nucleotide insertion at the same position in two isolates (Table 1). An identification to species level was impossible on the basis of ITS sequences and spore morphology.

Isolates of *Stemphylium* strongly resembled each other in their production of very large, elongated ellipsoidal conidia with irregular outlines and a distinctly roughened cell wall. Tentative identification of such microscopic features as *S. vesicarium* was confirmed by ITS sequence analysis of two representative isolates (Table 1).

Whereas fruit rot caused by *Alternaria* showed only a limited spread on immature as well as mature apples, *Stemphylium* rot was aggressive on mature fruits (Table 1). In the field, both genera were associated with an inconspicuous phenotype of blossom-end rot, comprising a faint greyish-brown ring which surrounded the entire calyx of the fruit (Fig. 1I). Such lesions resembled sunscald injuries. Weak tongue-like extensions were only rarely seen.

Fungi sporadically associated with blossom-end rot

Phoma exigua was unequivocally identified by ITS sequence analyses, confirmed by microscopy (Table 1). A further *Phoma* sp. was also found. This differed from *P. exigua* at 13 positions of its ITS sequence, and in being unable to cause a fruit rot upon artificial wound inoculation (Table 1). *Phoma* spp. were associated with blossom-end rot lesions that strongly resembled those of *Alternaria* and *Stemphylium*, except that the ring enclosing the calyx was slightly wider (Fig. 1J).

Isolates of *Diplodia seriata* showed a rapid growth on PDA. Colonies were initially hyaline from above and milky-green in reverse, gradually acquiring a deep black pigmentation. The production of highly characteristic conidia from black pycnidia was delayed by several weeks. Identification was confirmed by ITS sequence analysis (Table 1). Aggressively spreading infections by this fungus were observed during a period of 4–6 weeks preceding harvest. Whereas in some cases such infections were traced back to typical blossom-end rot lesions (Fig. 1K), in other cases the rot seemed to originate directly from the calyx cavity. A similarly rapid brown rot developed upon artificial inoculation of immature and mature apples.

Infections by the important post-harvest pathogens *Neofabraea alba* and *N. perennans* were rare. Where present, the lesions were delimited from healthy tissue in a manner resembling *N. galligena* but without radial cracks. Both *Neofabraea* spp. could be identified especially towards harvest time by the production of conidiomata with the highly characteristic conidia on an infected fruit (Fig. 1L), and by growth characteristics in pure PDA culture. Therefore, no ITS sequence analysis was performed.

Temporal and spatial occurrence of blossom-end rot fungi

Taking together all 1085 infected fruits examined in the Lower Elbe region in the five years of this survey, *B. cinerea* was the most important blossom-end rot pathogen, contributing 41.6 % of all infections, followed by *N. galligena* (33.9 %). Further fungi associated with blossom-end rot in order of descending frequencies were *Alternaria* spp. (10.2 %), *D. seriata* (6.5 %), *Fusarium* spp. (4.2 %), *Phoma* spp. (1.8 %), and *Stemphylium vesicarium* (1.4 %). *Neofabraea* spp. were found in only four symptomatic apples, *S. sclerotiorum* in only one fruit.

Variations were observed in the composition of blossom-end rot fungi during the five seasons of this survey in Northern Germany (Fig. 2). Whereas *B. cinerea* dominated in 2008 and 2011, *N. galligena* did so in 2010. Both fungi were about equally prominent in 2012, whereby about one-quarter of the *N. galligena* records were contributed by detailed examinations of two orchards in which trees of the variety 'Kanzi' showed a higher incidence of blossom-end rot as compared to immediately adjacent 'Braeburn' trees. In both orchards, this difference was attributed

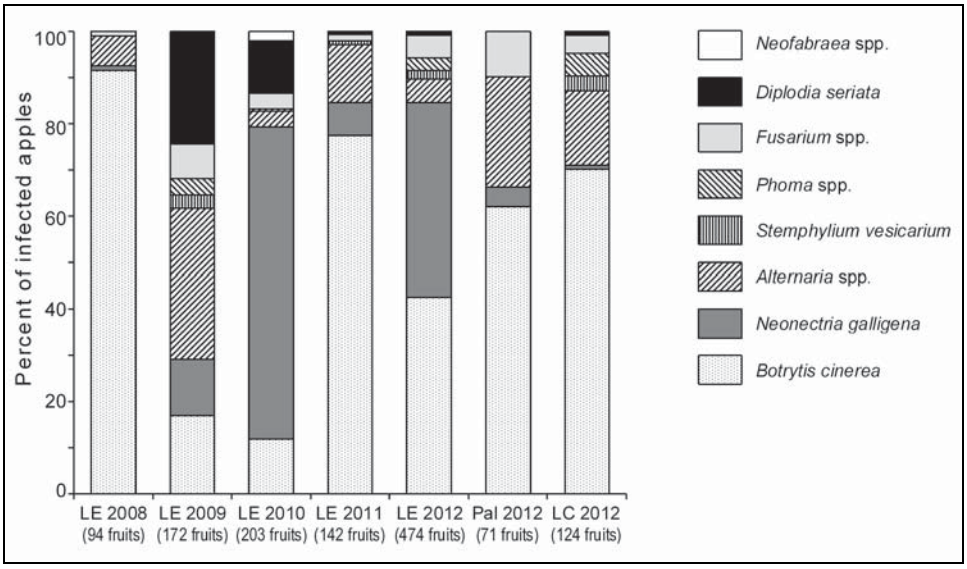


Fig. 2. Association of different pathogenic fungi with blossom-end rot (percent share of infected fruits) in the Lower Elbe region (LE) in 2008–2012, and the Palatinate (Pal) and Lake Constance (LC) regions in 2012.

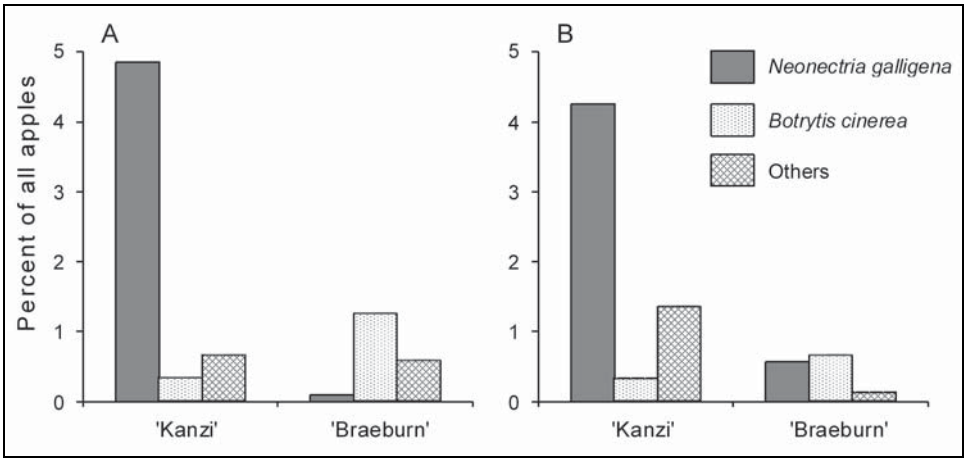


Fig. 3. Incidence of blossom-end rot (percent of total crop) in one orchard under integrated (A) and one under organic (B) management. In both orchards, batches of 1200 apples were sampled from a 'Kanzi' and an adjacent 'Braeburn' row.

to elevated levels of *N. galligena* infections on 'Kanzi' (Fig. 3). An abundance of canker lesions due to *N. galligena* infections was noted on the trunks and twigs of 'Kanzi' but not on the adjacent 'Braeburn' trees.

In 2012, blossom-end rot was observed to be more common than usual in the important apple growing regions of Palatinate and Lake Constance, prompting regional consultants to submit samples for analysis. The results revealed the same diversity of pathogens as in the Lower Elbe region, although a larger share of blossom-end rot was associated with *B. cinerea* and *Alternaria* spp., and a smaller share with *N. galligena* (Fig. 2).

Discussion

In four of the five years of this survey of blossom-end rot of apples in Northern Germany, as well as in other parts of Germany in 2012, *B. cinerea* and/or *N. galligena* were the major pathogens, thereby broadly confirming earlier accounts from Western Europe (Grimm 1977; Kennel 1992). In Northern Germany, substantial variations in the relative importance of these two species in successive seasons were observed. To our knowledge, this phenomenon has not been described previously. Yet longer time series of observations are necessary before any correlation may be attempted between the incidence of specific blossom-end rot fungi and meteorological events during the time between bloom and petal fall, which is believed to be the main infection period (Tronsmo and Raa 1977; Friedrich and Rode 1996). For the present time, we may note that the two years with the highest share of *B. cinerea* in Northern Germany – 2008 and 2011 – were those with unusually dry weather during petal fall (meteorological archive, Obstbauversuchsanstalt Jork).

An exceptionally wide range of fungi associated with blossom-end rot was documented in our survey. In declining order of frequency, *Alternaria* spp. were the most important group after *B. cinerea* and *N. galligena*. The very limited lesions from which *Alternaria* spp. were iso-

lated matched the description given by Rich (1970), and our inoculation experiments confirmed that the Northern German blossom-end rot isolates of *Alternaria* were capable of causing slowly growing lesions in immature apples. The period around petal fall has been identified by Marschall et al. (2006) as the main infection period for *Alternaria* causing lenticel spots which are often located near the blossom end of apples. Infections leading to core rot of apples are initiated at a similarly early developmental stage (Ellis and Barrat 1983). Taken together, there is reasonable evidence to support the conclusions of Rich (1970) against doubts raised by other workers (Kennel 1992) that *Alternaria* spp. should be considered weak pathogens of blossom-end rot.

A similar conclusion concerns *S. vesicarium*, which is better known as the cause of a serious pre- and post-harvest fruit rot of pears with a high capacity to infect very young fruits under experimental conditions (Montesinos et al. 1995) and in the orchard (de Jong and Wenneker 2008). This disease has shown a strong and ongoing northerly spread on pears within Europe during the past two decades (Heijne and van Mourik 2001; Llorente and Montesinos 2006). *Stemphylium vesicarium* had a capacity to cause fruit rots in immature and mature apples in our wound inoculation experiments, and it therefore cannot be ruled out as a cause of blossom-end rot in the field.

Diplodia seriata, cause of black rot of apples, is another fungus with a declining gradient from Southern to Northern Europe (Giraud 2009; Weber et al. 2013). There is good evidence that the recent increase of black rot in northern latitudes in Europe is mediated by global warming (Giraud 2009). To the best of our knowledge, *D. seriata* has not previously been associated with blossom-end rot, although its occurrence as a core-rot pathogen in South Tyrol (Lindner 2010) and as the cause of premature fruit drop in France (Giraud 2009) indicates that it must be capable of infecting apple fruits at an early developmental stage. This contrasts with the infection route leading to black rot in Northern Germany, which is initiated by heavy rainfalls during mid-summer (Quast and Weber 2008).

Fusarium avenaceum and *F. lateritium* are major causes of core rot of apples in Europe (Kennel 1992; Friedrich and Rode 1996; Sørensen et al. 2009 and R.W.S. Weber, unpublished data), giving a plausible explanation for their occurrence in blossom-end rot lesions. The association of *Phoma exigua* with apple fruits is more tentative, although *Phoma* spp., too, have been described as weak agents of core rot (Lindner 1992, 2010).

Of particular interest to us was the near-absence of *Neofabraea* spp. from our survey, even though both *N. alba* and *N. perennans* would have been readily recognised by their production of characteristic conidia on blossom-end rot lesions (this report) and also by their colony morphology on PDA (see Weber 2009). This finding was unexpected in the light of the general acceptance of these fungi as major blossom-end rot pathogens among consultants in Northern Germany (Klopp 2012) and elsewhere (Friedrich and Rode 1996). Similarly, *Sclerotinia sclerotiorum*, a well-described cause of blossom-end rot from North America (Rich 1970; Hickey 1990), was isolated only once in our entire survey. None of these three species therefore seems to be of relevance to this disease in Germany at present.

In general terms, the occurrence of blossom-end rot was too sporadic to permit critical comparisons of the susceptibility of different varieties under otherwise identical conditions. We noted that *B. cinerea* was associated with a wide range of apple varieties throughout the Lower Elbe region, including 'Elstar', 'Jonagold', 'Gloster', 'Kanzi', 'Topaz' and 'Braeburn', the latter two appearing to be especially susceptible in the sense that they contributed a disproportionately large share of all samples examined. In marked contrast, *N. galligena* was associated with elevated levels of blossom-end rot in two 'Kanzi' orchards. Furthermore, in these orchards there were high levels of conidial canker lesions on the main trunks and on 1- to 2-year-old twigs of 'Kanzi' trees. The very high susceptibility of this variety to canker caused by *N. galligena* in the Lower Elbe region has been documented (Palm et al. 2011). The absence of elevated levels of *N. galligena* in neighbouring trees of 'Braeburn', another variety susceptible to blossom-end rot, was notable. In view of ascospore inoculum of *N. galligena* being windborne over large distances whereas conidial inoculum is splash-dispersed mainly within the same tree (Swinburne 1975; Graf 1976), our findings argue for the latter as the source of inoculum for blossom-end rot caused by *N. galligena*, at least under Northern German conditions.

The pruning of canker lesions in winter and early spring should therefore give a measure of control of *N. galligena* as a blossom-end rot pathogen in most situations, leaving *B. cinerea* as the main target for fungicide applications. In view of widespread resistance of this pathogen to several fungicide groups in Northern German soft-fruit production (Weber 2011), a survey of the fungicide sensitivity of blossom-end rot isolates of *B. cinerea* has been initiated. Artificial blossom-time inoculation of orchard trees with spores obtained from pure cultures might provide a basis for spray trials to quantify the efficacies of different compounds. Along similar lines, differences in susceptibility of apple varieties to blossom-end rot may be quantified for different pathogens.

Acknowledgements

We are grateful to pome fruit consultants from the Lower Elbe (Obstbauversuchsring des Alten Landes), Palatinate (DLR Rheinpfalz) and Lake Constance regions (KOB Bavendorf) for collecting samples of infected fruits, and to S. Loss and A. Lutze for technical assistance. Research on the impact of climate change on fruit production in the

Lower Elbe Region (Obstbauversuchsanstalt Jork) is being supported by the German Ministry of Science and Education (KLIMZUG-NORD, grant number 01LR0805 M). N. Dralle was supported by a Landwirtschaftskammer Niedersachsen summer scholarship, which is gratefully acknowledged.

References

Boerema, G.H., J. de Gruyter, M.E. Noordeloos and M.E.C. Hamers 2004: *Phoma* identification manual. CABI Publishing, Wallingford, UK.

Bömeke, R. and H. Blank 1959: Untersuchungen zur Bekämpfung der Lagerfäulen an Finkenwerder. Mitt. d. OVR d. Alten Landes **14**, 202–206 (In German).

Booth, C. 1971: The genus *Fusarium*. Commonwealth Mycological Institute, Kew, UK.

Covey, R.P. 1985: Calyx end rot (*Sclerotinia sclerotiorum*) of apples in Washington. Plant Dis. **69**, 726.

de Jong, P.F. and M. Wenneker 2008: Twee schimmels in één klapp. Fruitteelt **98** (23), 12–13 (In Dutch).

Ellis, M.B. 1971: Dematiaceous hyphomycetes. Commonwealth Mycological Institute, Kew, UK.

Ellis, M.A. and J.G. Barrat 1983: Colonization of 'Delicious' apple fruits by *Alternaria* spp. and effect of fungicide sprays on moldy-core. Plant Dis. **67**, 150–152.

Friedrich, G. and H. Rode 1996: Pflanzenschutz im integrierten Obstbau (third edition). Verlag Eugen Ulmer, Stuttgart (In German).

Giraud, M. 2009: Le Black Rot du pommier. Infos-Ctifl **257**, 36–41 (In French).

Graf, H. 1976: Die Biologie des Obstbaumkrebses (*Nectria galligena* Bres.) als Grundlage seiner gezielten Bekämpfung. Mitt. d. OVR d. Alten Landes **31**, 68–78 (In German).

Grimm, R. 1977: Die Apfel-Kelchfäule und ihre Bekämpfung. Schweizer. Zeitschr. Obstb. Weinb. **113**, 33–37 (In German).

Heijne, B. and B. van Mourik 2001: Zwartfruchtrot op peer neemt toe. Fruitteelt **91** (8), 18–19 (In Dutch).

Hickey, K.D. 1990: Calyx-end rot. In: Jones, A.L. and H.S. Aldwinckle (eds): Compendium of apple and pear diseases. APS Press, St. Paul, USA, 29.

Kennel, W. 1992: Kelchfäule beim Apfel – Ursache und Bekämpfung. Obstbau **17**, 194–197 (In German).

Klopp, K. 2012: Arbeitstagebuch für das Obstjahr 2012. Esteburg-Obstbauzentrum, Jork, Germany (In German).

Lindner, L. 1992: Kernhausfäule beim Apfel: ein ernsthaftes Problem in der Lagerung. Obstb. Weinb. **29**, 191–193 (In German).

Lindner, L. 2010: Kernhausfäulen bei der Apfelsorte "Fuji". Obstb. Weinb. **47**, 20–22 (In German).

Llorente, I. and E. Montesinos 2006: Brown spot of pear: an emerging disease of economic importance in Europe. Plant Dis. **90**, 1368–1375.

Marschall, K., M. Bretagnoll, W. Rizzoli and A. Acler 2006: *Alternaria*. Erkenntnisse zur Biologie. Obstb. Weinb. **43**, 52–53 (In German).

Montesinos, E., C. Moragrega, I. Llorente and P. Vilardell 1995: Susceptibility of selected European pear cultivars to infection by *Stemphylium vesicarium* and influence of leaf and fruit age. Plant Dis. **79**, 471–473.

Noble, J.P. and R.B. Drysdale 1983: The role of benzoic acid and phenolic compounds in latency of fruits of two apple cultivars infected with *Pezicula malicorticis* or *Nectria galligena*. Physiol. Plant Pathol. **23**, 207–216.

Palm, G. 1986: Grundlegende neue Betrachtungsweise in der Bekämpfung bedeutender pilzlicher Schaderreger im Kernobst. Mitt. d. OVR d. Alten Landes **41**, 138–152 (In German).

Palm, G., F. Harms and I. Vollmer 2011: Mehrjährige Befallsentwicklung des Obstbaumkrebses an verschiedenen Apfelsorten an drei Standorten. Mitt. d. OVR d. Alten Landes **66**, 360–363 (In German).

Palm, G. and P. Kruse 2012: Wie ist in Zukunft Lagerfäulnis zu verhindern? Mitt. d. OVR d. Alten Landes **67**, 306–311 (In German).

Phillips, A.J.L., P.W. Crous and A. Alves 2007: *Diplodia seriata*, the anamorph of "*Botryosphaeria*" *obtusa*. Fungal Div. **25**, 141–155.

Quast, G. and R.W.S. Weber 2008: Aktuelles zur Infektionsbiologie von *Diplodia seriata* an Äpfeln im Niederelbegebiet. Mitt. d. OVR d. Alten Landes **63**, 376–383 (In German).

Rich, A.E. 1970: Calyx-end rot of apples. Phytopathology **60**, 1152.

Rosenberger, D.A. 1990: Dry eye rot. In: Jones, A.L. and H.S. Aldwinckle (eds): Compendium of apple and pear diseases. APS Press, St. Paul, USA, 28–29.

Sørensen, J.L., R.K. Phipps, K.F. Nielsen, H.-J. Schroers, J. Frank and U. Thrane 2009: Analysis of *Fusarium avenaceum* metabolites produced during wet apple core rot. J. Agric. Food Chem. **57**, 1632–1639.

Swinburne, T.R. 1975: European canker of apple (*Nectria galligena*). Rev. Plant Pathol. **54**, 787–799.

Tronsmo, A. and J. Raa 1977: Life cycle of the dry eye rot pathogen *Botrytis cinerea* Pers. on apple. Phytopathol. Zeitschr. **89**, 203–207.

Vitale, S., A. Santori, E. Wajnberg, P. Castagnone-Sereno, L. Luongo and A. Belisario 2011: Morphological and molecular analysis of *Fusarium lateritium*, the cause of gray necrosis of hazelnut fruit in Italy. Phytopathology **101**, 679–686.

Wani, A.H., T.A. Wani and T. Un-Nisa 2011: Calyx-end rot disease of apples in Kashmir Valley. J. Mycol. Plant Pathol. **41**, 29–31.

Weber, R.W.S. 2008: *Botrytis cinerea*, Erreger der Kelchgrubenfäule 2008 an der Niederelbe. Mitt. d. OVR d. Alten Landes **63**, 432–434 (In German).

Weber, R.W.S. 2009: Resistenz des Bitterfäule-Erregers *Neofabraea perennans* gegen Thiophanate-methyl: Ko-

nidienkeimung vs. Hyphenwachstum. Erw.-Obstb. **51**, 145–149 (In German).

Weber, R.W.S. 2011: Resistance of *Botrytis cinerea* to multiple fungicides in Northern German small-fruit production. Plant Dis. **95**, 1263–1269.

Weber, R.W.S. and D. Zabel 2011: White haze and scarf skin, two little-known cosmetic defects of apples in Northern Germany. Europ. J. Hort. Sci. **76**, 45–50.

Weber, R.W.S. 2012: Die Gummifäule, eine neue pilzliche Lagerkrankheit des Apfels in Europa. Mitt. d. OVR d. Alten Landes **67**, 86–91 (In German).

Weber, R.W.S., S. Sørensen and P. Maxin 2013: Klimaændringer kan give sommersortråd. Frugt Grønt **12**, in press (In Danish).

White, T.J., T. Bruns, S. Lee and J. Taylor 1990: Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., D.H. Gelfand, J.J. Sninsky and T.J. White (eds): PCR protocols: A guide to methods and applications. Academic Press, San Diego, 315–322.

Xu, X.-M. and J.D. Robinson 2010: Effects of fruit maturity and wetness on the infection of apple fruit by *Neonectria galligena*. Plant Pathol. **59**, 542–547.

Yli-Mattila, T., S. Paavanen-Huhtala, S.A. Bulat, I.A. Alekhina and H.I. Nirenberg 2002: Molecular, morphological and phylogenetic analysis of the *Fusarium avenaceum/F. arthrosporioides/F. tricinctum* species complex – a polyphasic approach. Mycol. Res. **106**, 655–669.

Received 12/17/2012 / Accepted 03/07/2013

Addresses of authors: Roland W. S. Weber (corresponding author), Obstbauversuchsanstalt Jork, Landwirtschaftskammer Niedersachsen, Moorende 53, 21635 Jork, Germany, and Nane Dralle, Fakultät Agrarwissenschaften und Landschaftsarchitektur, Hochschule Osnabrück, Am Krümpel 31, 49090 Osnabrück, Germany, e-mail (corresponding author): roland.weber@lwk-niedersachsen.de.