Technical Review TR-TF 003

Tree Fruit

A review of the literature of the *Neofabraea* species complex, causative agents of *Gloeosporium* rot in stored apple

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Apples are stored for up to 12 months following harvest and are subject to attack from numerous post-harvest pathogens. *Neofabraea* species are considered an important post-harvest disease worldwide and losses of up to 35% have been reported for this disease (Sutton *et al.*, 2014). An apple rot survey of UK pac houses undertaken from 2008 - 2013 as part of Horticultural Development Company (HDC) projects TF193 and CP90, identified an increasing incidence in the occurrence of fruit rot caused by *Neofabraea* species in stored apples over the duration of the survey. On this basis the HDC commissioned this literature review of the fungus responsible and the current control options available, to inform future research into this disease.

Figure 1. Left: Apple rot; Top right: Neofabraea alba colony morphology; Middle right: Neofabraea alba spores; Bottom right: N.perennans/N.malicorticis symptoms.

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Importance of the fungus

Losses in store

Neofabraea species can induce wood lesions on trees in the orchard but it is the post-harvest rot that develops in the store where the majority of losses due to this fungus are experienced. The fungus characteristically gains entry to developing fruit in the orchard through the lenticels and can remain latent for some time after harvest. In the UK, fruit rot caused by *Neofabraea* species in store has changed in significance over the years, the level and frequency depending on seasonal weather conditions and cultural factors in the orchard and/or changes in store practice.

Apple rot surveys carried out in the UK have revealed a rise and fall in the prevalence of this disease in store over the last century. In the 1920's, *Neofabraea* (then referred to as *Gloeosporium*) was recorded in a comprehensive survey of rots developing in store on Bramley's seedling among other cultivars (Kidd and Beaumont, 1924). Although quantitative data was not reported on the incidence of the rot, the authors state that *Neofabraea* (*alba*) was 'one of the less frequent fungi' recorded in the survey. Conversely, a survey in the 1930's, sampling numerous apple cultivars from the main fruit growing areas of England (Wilkinson, 1954), recorded *Neofabraea* as the predominant rot developing in store in 1937/8 and 1938/9 (Figure 1). In the 1960's, just prior to the introduction of post-harvest fungicide treatments and the large scale adoption of controlled atmosphere storage across the industry, losses of up to 50% were recorded in some consignments of Cox, mainly due to rotting caused by *Neofabraea* species (Preece, 1967).

TABLE 1. *Type and frequency of fungal rots recorded in cold stored apples during* 1937–9

Fungus	Percentage rots 1937–8	Percentage rots 1938–9
Gloeosporium spp.	15·41	8·16
Botrytis cinerea	6·56	8·08
Penicillium expansum	3·31	7·42
Sclerotinia fructigena	0·54	1·51
Alternaria spp.	0·23	0·04
Cylindrocarpon mali	0·20	0·79
Rhizopus nigricans	0·04	0·15
Diaporthe perniciosa	0·02	0·04
Fusarium spp.	0·01	0·07
	Season 1937–8	Season 1938–9
Total number apples stored	21,600	12,822
Number of rotted fruits	5,691	3,370
Percentage rotted fruits	26·32	26·26

Figure 2. An excerpt from Wilkinson (1954) which showed that *Neofabraea* (*Gloeosporium* species as it was then known) was the predominant rot developing on cold stored apples in the 1930's.

Apple rot surveys undertaken in the 1980's show that fruit rot caused by *Neofabraea* species was reduced to trace levels in most of the years surveyed and this rot was only a problem when fruit nutrition was sub-optimal (Berrie, 1989). Berrie (1993) attributed the decline in the significance of *Neofabraea* rot to 'the introduction of post-harvest fungicide treatments, combined with improved storage of fruit and a better understanding of the importance of fruit mineral composition in preserving fruit quality'. With the phasing out of the practice of post-harvest fungicide treatment, Berrie (1993) demonstrated alternative control strategies to mitigate losses due to storage rots in an era without post-harvest fungicide treatments. The efficacy of pre-harvest fungicide treatments were tested against post-harvest fungicide treatments and initial data was presented on the rot risk assessment concept which determines treatment according to need (Berrie, 1993).

Following a decline in the incidence of fruit rot caused by *Neofabraea* species in the 1990s to negligible levels in the UK, a recent increase has been observed. During the most recent survey, spanning 2008-2013 growing seasons, undertaken as part of HDC project TF193 (*Sustainable control of storage rots of apple, 2008-2010*) and the HDC fellowship project CP90 (*Succession planning to sustain the UKs expertise in field and laboratory plant pathology research and development, 2011-present*), fruit rot caused by *Neofabraea* species has been increasing in incidence in all apple cultivars surveyed. In 2010 and 2011 the percentage of apple samples surveyed which contained fruit rot caused by *Neofabraea*, was on average between 80 and 100% on susceptible cultivars (Figure 2b). Against this trend, the incidence in the 2013 rot survey dropped significantly (average incidence for susceptible cultivars = 56%) and further still (16%) in the 2014 survey. A historic resume of the rot surveys discussed in this section is presented in Figure 2a.

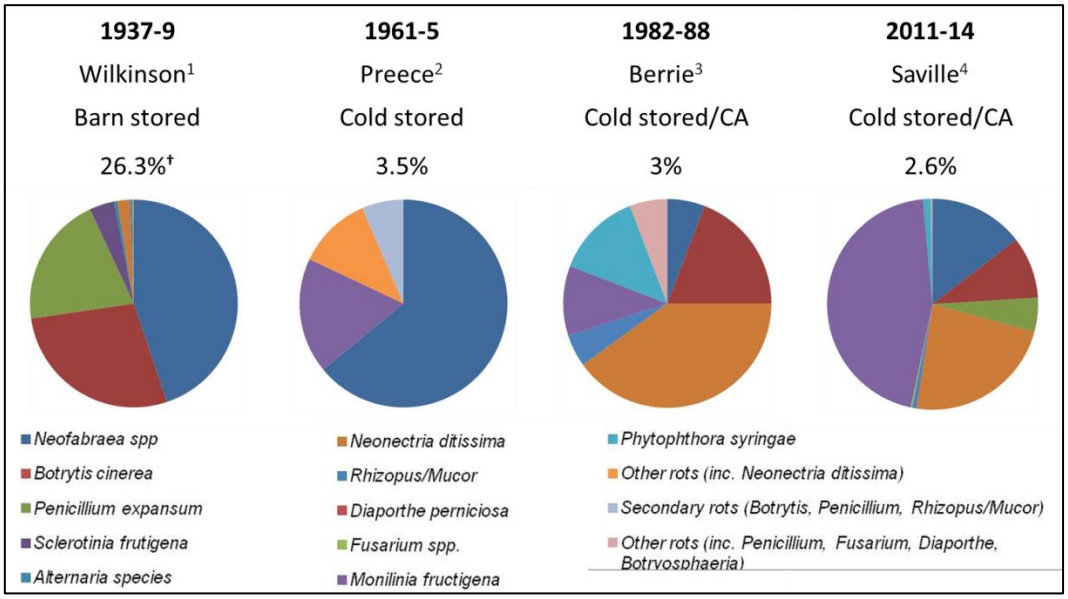

Figure 2. The rise and fall of *Neofabraea* rot incidence in the UK. **(a)** Data compiled from four rot surveys spanning the last 75 years. The data set is for Cox, as this is the common cultivar recorded across all surveys ([1]Wilkinson, 1984, [2]Preece, 1967, [3]Berrie, 1989, [4]Saville, 2013, † Average total losses due to rots during survey period). The categorisation of taxa in the legend are described as recorded in the literature so some inconsistencies between data sets are present i.e. *Sclerotinia frutigena* is the same as *Monillinia fructigina* and rots have been grouped in certain surveys (e.g. 'other rots'). As far as possible common colour coding has been used to represent these inconsistencies.

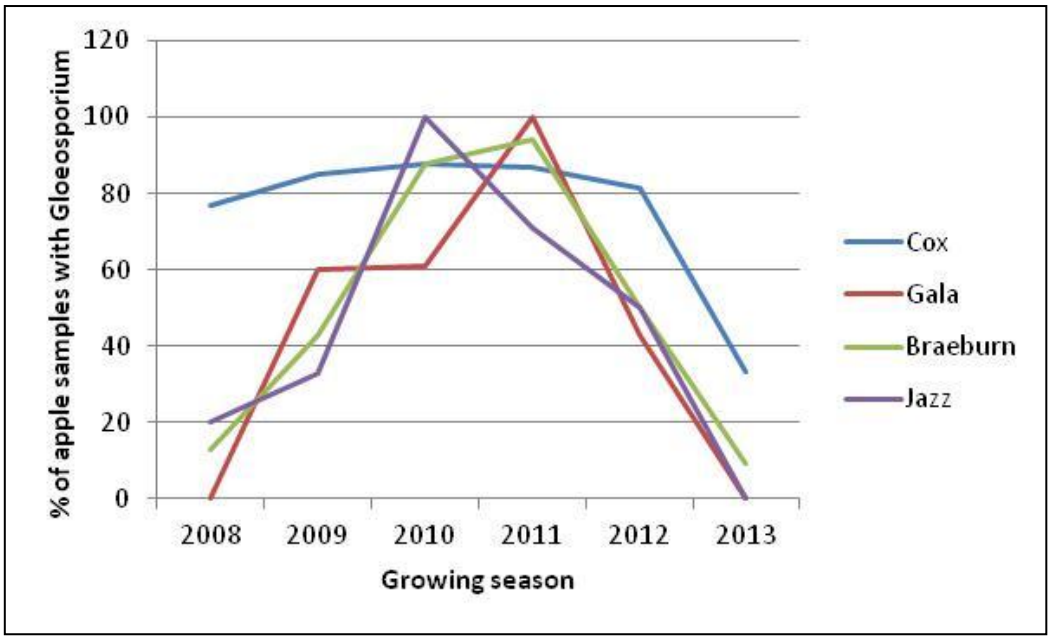

Figure 2 (b) *Neofabraea* incidence of four susceptible cultivars in rot surveys spanning 2008-2013 growing seasons. The graph shows the percentage of apple samples with *Neofabraea* (*Gloeosporium*) - Data from Berrie, 2010 and Saville, 2013.

The recent rise and fall of fruit rots caused by *Neofabraea* species could have many explanations including;

i. Trends in fungicide use – post-harvest treatments were replaced by pre-harvest treatments (Bellis/Switch/Geoxe) which have been increasingly used in recent years due to conditions favourable to storage rots.
ii. Changes in orchard practice leading to an increase/decrease in inoculum availability.
iii. Variability in climatic conditions across growing seasons affecting inoculum availability, infection and nutrient uptake.
iv. Late harvests leading to reduced time in store for symptom expression from harvest to assessment (the survey period is fixed, spanning from January – March).
v. Changes in management practice for other targets ('primary' disease) influencing the significance of *Neofabraea* species ('secondary' disease).

All of the above factors will be further explored in the subsequent sections of this review.

Fruit rot caused by *Neofabraea* species is the principal cause of decay of stored apple in Europe as reported in France (Bompeix *et al.,* 2000), Norway (Landfald, 1983) and Germany (Maxin *et al.,* 2005). The disease is also significant in North America. For example in British Columbia, a survey of packhouses revealed that bull's-eye rot accounted for 40% of diseased Golden Delicious apples and 9% of diseased McIntosh apples (Sholberg and Haag, 1996). The wood lesions caused by this disease can also be a serious problem in climates which favour the disease. For example in the Fraser valley, British Columbia, *Neofabraea* canker incidence on a per tree basis was reported to be between 50 and 80% (Rahe, 1997). Reports of losses of pear in USA between 5 and 50% (Lennox *et al.,* 2004) highlight the potentially significant economic losses that can be caused by *Neofabraea* species.

The fungus

Nomenclature

Historically the *Neofabraea* species as we know them today have been ascribed to various taxonomic classifications. Verkley (1999) provides a comprehensive overview of *Pezicula*, the genus to which *Neofabraea* species used to belong and provides evidence based on molecular taxonomy, that *Neofabraea* is a separate evolutionary lineage.

Neofabraea is a genus containing seven species; *Neofabraea malicorticis, Neofabraea corticola, Neofabraea perennans, Neofabraea alba, Neofabraea krawtzewii, Neofabraea populi* and *Neofabraea eucalypti*. An eighth species, *Cryptosporiopsis kienholzii,* previously referred to in the literature as *Neofabraea* sp. nov. (De Jong *et al*., 2001) until the naming of the anamorphic state (Spotts, 2009), is a recent addition to this genus. All are pathogens of woody hosts and *Neofabraea malicorticis, Neofabraea perennans, Neofabraea alba* and *Cryptosporiopsis kienholzii* are pathogenic on *Malus* and *Pyrus* species.
The numerous synonyms and previous taxonomy classifications of the telomorphs and anamorphs of these fungi are described in Table 1. *Colletotrichum acutatum* is also included in this table because this species has historically been grouped within *Gloeosporium* (*Gloeosporium fuctigenum*).

Symptoms caused on the host

The wood lesions caused by *Neofabraea* spp. are variously referred to dependent on the causative casual organism. **Anthracnose canker**, caused by *Neofabraea malicorticis* and to a lesser extent by *N. alba* (which require wounding to infect), first appears as small circular red-purple spots. As they enlarge they become elliptical, sunken and orange-brown in colour. As the cankers mature a crack delineates the healthy and diseased tissue and erumpent, cream coloured acervuli become evident. The bark eventually

sloughs off exposing string-like fibres of the inner bark. **Perennial canker,** caused by *Neofabraea perennans*, appears as elliptical, sunken and orange, purple or brown lesions in their first year. In contrast to anthracnose canker which rarely enlarge after the first year, perennial canker continue to expand year on year marked by host produced layers of callus tissue. The raised callus tissue provides a court for the woolly apple aphid (*Eriosoma lanigerum*), which make small galls in the canker margin which then rupture in cold winters, becoming infection sites for reinfection by conidia of *Cryptosporiopsis perennans* (the anamorph of *N. perennans*) promoting the annual expansion of the cankers.

Table 1. A table describing the numerous synonyms and previous taxonomical classifications of the telomorphs and anamorphs of the *Neofabraea* which are pathogenic on *Malus*.

Telomorph (sexual stage)		**Anamorph (asexual stage)**	
Current nomenclature	**Previous nomenclature**	**Current nomenclature**	**Previous nomenclature**
Neofabraea alba (Guthrie) Verkley	*Pezicula alba*	***Phlyctema vagabunda*** Desm.	*Gloeosporium album* *Trichoseptoria fructigena*
Neofabraea perennans (Kienholz) Dugan, R.G. Roberts & G.G. Grove	*Pezicula malicorticis*	***Cryptosporiopsis perennans*** (Zeller & Childs) Wollen	*Gloeosporium perennans*
Neofabraea malicorticis H.S. Jacks	*Pezicula malicorticis*	***Cryptosporiopsis curvispora*** (Peck.) Gremmen	*Gloeosporium malicorticis* *Macrophoma curvispora* *Myxosporium malicorticis*
Telomorph never been observed		***Cryptosporiopsis kienholzii*** Seifert, Spotts & Lévesque	*Neofabraea sp. nov*
Glomerella acutata, G. cingulata[1] (Stoneman) Spauld. & H. Schrenk		***Colletotrichum acutatum*** J. H. Simmonds ***Colletotrichum gloeosporioides*** (Penz.) Penz. & Sacc.	*Gloeosporium fuctigenum*

[1] previously classified within *Gloeosporium* genus
Information curated from MycoBank (Robert *et al.*, 2005)

Neofabraea species can also infect fruit causing a rot in storage. The fruit rot, variously referred to as 'Bull's eye rot', 'lenticel rot', 'bitter rot', 'ripe spot' and '***Gloeosporium* rot'**, is the most economically important symptom caused by these pathogens (the cankers themselves rarely kill trees but rather infect small branches and twigs which act as inoculum sources for disease spread). In this review hereafter, the symptom on stored apple will be referred to as *Gloeosporium* rot, the common name for this rot in the UK. *Gloeosporium* rot symptoms are rarely seen in the orchard in the UK which is in contrast to other apple growing regions around the world. Rather, symptoms generally appear after 3-5 months of storage in controlled atmosphere. The rot symptoms, like the canker symptoms, are species specific but generally form circular lesions which are light to dark brown with a lighter brown to tan centre (particularly for *N. perennans* and more uniformly brown for *N. alba*) and may be flat or slightly sunken (more so for *N. perennans* and *N. malicorticis*). The affected tissue is firm (softer for *N. alba*). Rot lesions are usually less than 25 mm in diameter. Older lesions may exhibit cream-colored spore masses. *Neofabraea* rots mostly occur on the cheeks of the fruit (originating from infection of the lenticels), but stem and calyx end *Gloeosporium* rots are also observed. Figure 3 provides a pictorial guide of the rot symptoms caused by *Neofabraea* species and rot caused by other fungi (*Colletotrichum acutatum* and *Cylindrocarpon mali* (Nectria rot)) which exhibit similar rot symptoms.

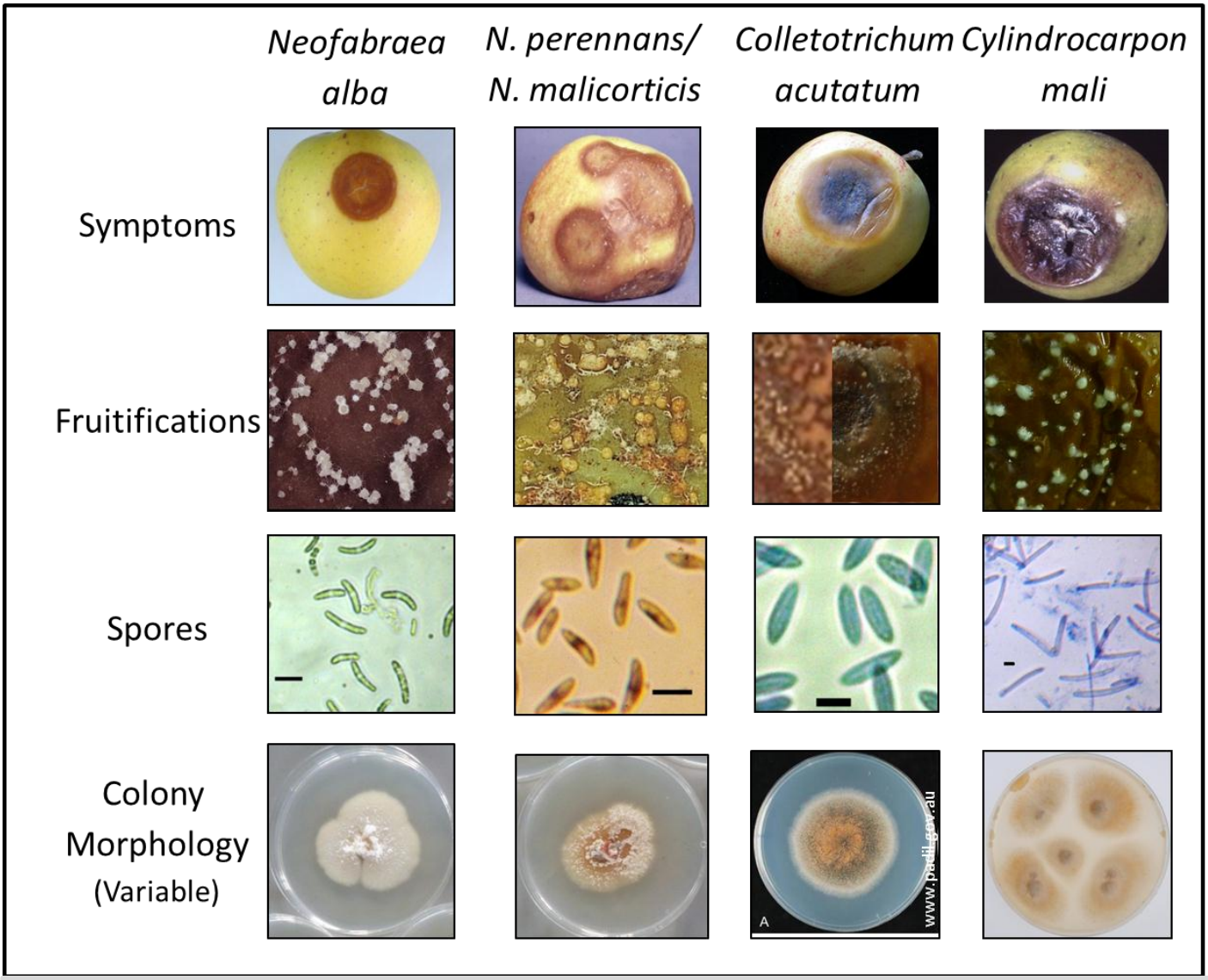

Figure 3. A pictorial guide of the symptoms, fruitifications, spores and colony morphology of the *Neofabraea* species and two other rot fungi (*Colletotrichum acutatum* and *Cylindrocarpon mali* (Nectria rot)). Adapted from le Point Sur, No.5, May 2014 (a Ctifl publication) by kind permission of M. Giraud. For HDC levy payers only.

Morphological identification and culture characteristics

Distinction between the Neofabraea species by apple rot symptoms alone is challenging particularly between N. malicorticis and N. perennans. Spore morphology and morphological features can be used to identify organisms to the species level when in culture. However, as detailed in Table 2, distinguishing features are slight or overlapping, particularly between N. malicorticis and N. perennans. Added to this, conidial characteristics of the Neofabraea spp. are known to vary significantly with environment, thus other methods of differentiation have been sought so accurate species level diagnosis can be determined.

Culture characteristics provide a useful diagnostic tool to distinguish between species of the same genus which are morphologically similar. Much work has been invested in determining methods for the distinction of N. malicorticis and N. perennans in culture. The utilisation of nutrients in media is one such method, however utilization of, for example, potassium chloride and sodium nitrate, did not yield diagnostic differences between the two fungi (Miller, 1932). Miller (1932) also demonstrated that the fungi react similarly to temperature and acidity although did report that the growth of N. malicorticis is retarded to a greater extent than N. perennans in tannic acid media. Overall, Miller (1932) and later Kienholz (1939) found that there was greater intra species physiological variability (i.e between isolates) than between the two species. More recently, Dugan et al. (1993a) reported that they were unable to find a single, practical method to separate the two fungi in culture. Due to the inter species similarities and intra species morphological variability of the Neofabraea genus, robust species identification is now reliant on molecular identification.

Molecular identification

The morphological differentiation between the Neofabraea species is slight and has resulted in incorrect identification of species in past studies. Molecular methods of species identification provide a tool in many cases to definitively classify the species based on species specific differences in DNA sequence.

De Jong et al. (2001) determined the phylogenetic relationships among Neofabraea species based on DNA sequencing of ITS nuclear rDNA, mitochondrial rDNA and the β-tubulin gene. These three genetic loci are highly polymorphic between species and are often used in phylogenetic studies to differentiate species within a genus. This study found that although the ITS region exhibited variation between the three species, variation was insufficient to separate N. malicorticis and N. perennans, whilst the β-tubulin gene had sufficient phylogenetically informative base pairs to differentiate between all of the species within the Neofabraea. All the sequences determined in the study are freely available on Genbank, a web based bioinformatic resource. The study also identified a new species, Neofabraea sp. nov. (later named Cryptosporiopsis kienholzii, Spotts et al., 2009), not formally recognised using the diagnostic characteristics described above (Table 2).

A method has subsequently been described for the determination of species using multiplex DNA amplification (polymerase chain reaction) based on the size of the product amplified (Gariépy et al., 2003). It should be noted that an error in the description of the Neofab- upTub-100 primer (TGA TGA GAC CTT CTG TAT GC) in which the position of the final G and C should be switched before commencing this assay.

Table 2. Morphological diagnostic features of the members of the *Neofabraea* genus which commonly infect apple.

Asexual stage	Acervuli	Macroconidia	Microconidia	Sexual stage	Apothecia	Asci	Ascospores
Plyctema vagabunda	0.4 - 0.8 mm Conidia form a greyish to pale brownish mass	2-4 x 14-30 µm Unicellular, cylindrical Weakly to strongly curved, rounded or pointed ends. (see picture in Fig. 3)		***Neofabraea alba***	0.5 – 1 mm Sessile, circular or irregular, often merged	13-24 x 125-150 µm Clavate and Cylindrical	7-10 x 20 – 30 µm Hyaline, straight or slightly curved, Initially aseptate later develop 3-6 septa
Cryptosporiopsis perennans	0.5 – 1 mm Subepidermial and then erumpent	3-6 x 10.5-17.7 µm Unicellular, hyaline and straight. (see picture in Fig. 3) Borne on simple or branched hyaline conidiosphores	1.5-2.5 x 5-8µm produced in culture	***Neofabraea perennans***	Rare and developed in the stromata of old acervuli	8-14 x 86-170 µm Clavate, inoperculate	4-8.5 x 11.5-22.5 µm Hyaline, unicellular, ellipsodial, coarsely granular.
Cryptosporiopsis curvispora	0.2-1.2 mm Subepidermal then erumpent Simple or branched, hyaline conidiophores emerge	3-6 x 15-35 µm Unicellular, hyaline Sickle to 'U' shaped (occasionally straight) (see picture in Fig. 3)	1.5-2.5 x 5-8µm produced in culture	***Neofabraea malicorticis***	0.1-1 mm Sessile (occasionally short stalked) Colour: Grey to flesh	10-20 x 75-150 µm Clavate, inoperculate Short-pedicellate at base	5-9 x 12.5-26 µm Hyaline, unicellular, ellipsodial, coarsely granular.

Information curated from compendium of apple and pear diseases and pests, second edition, pp51-52 (Sutton et al. 2014)

Neofabraea species identification; a UK case study

A small study was carried out to determine the species level identification of a *Neofabraea* culture collection curated from UK origin as part of the rot survey (CP 90). The collection consisted of 104 isolates collected over two storage seasons mostly from packhouses which store fruit grown in Kent, but also including samples from a packhouse in Herefordshire. Colony morphology, spore size and molecular identification (on a subset of 70 isolates) as described above were all used to determine the species level of the isolates.

Of the 104 isolates, it was not possible to identify 11 on any of the criteria used (data not shown). Species identified based on molecular analysis (Table 3, highlighted in green) are considered the most reliable diagnosis due to the variability and overlap of spore morphology between the *Neofabraea* species. Amplification of the ITS and β-tubulin loci was followed by sequencing to determine species specific polymorphisms. Of the 70 isolates for which molecular identification was undertaken; 30% did not amplify, 46% were identified as *N. alba*, 13% were identified as *N. perennans,* 9% were identified as *N. perennans* or *N. malicorticis* (only ITS sequence data was available for these isolates which is insufficient to distinguish *N. perennans* and *N. malicorticis*). A single isolate, R142/12/5, did not match any of the reference sequences used. Further analysis revealed that this isolate is the putative new *Neofabraea* species initially described by De Jong *et al.* (2001) and subsequently named *Cryptosporiopsis kienholzii* (Spotts *et al.,* 2009). To the best of our knowledge, this is the first reported recording of this species in the UK.

Unfortunately, spores were only generated in a small proportion of isolates (20%). Where spore morphology and molecular identification data was available, both were in agreement. The criteria with the least confidence for accurate species identification is colony morphology. For this reason subsequent conclusions of species distribution are based on molecular identification and spore morphology.

The metadata associated with each of the isolates (i.e. cultivar and location collected) means that a picture of the species distribution can be drawn as presented in Figure 4. In terms of cultivars (Figure 4a), *N. Alba* is dominant in all cultivars apart from Cox in which *N. perennans* is the dominant species. In terms of the geographic distribution of species (Figure 4b), in this small study, isolates were only collected from Kent and Herefordshire, the two major apple growing regions in the UK. Despite the small scale of this study, obvious trends are evident in the species distribution with *N. alba* dominant in Kent whilst *N. perennans* is the dominant species in Herefordshire. The dominance of *N. alba* causing *Gloeosporium* rot in Kent grown apples is consistent with the apparent absence of tree *Neofabraea* cankers usually associated with *N. perennans* and *N. malicorticis*. Additionally, a single isolate of *Cryptosporiopsis kienholzii* was recorded in Herefordshire but was absent in the isolates sampled from Kent.

Studies in other apple growing regions around the world have been carried out and show similar trends. For example in North West USA a similar survey found that *N. alba*, *N. perennans*, and *C. kienholzii* accounted for 6.0, 81.3, and 12.7 % of 150 isolates obtained from apple fruit, respectively, demonstrating that *N. perennans* is the predominant causative agent of *Gloeosporium* rot in this region (Spotts *et al.*, 2009). The study also reported a geographical distribution much like that observed in the UK with *N. alba* being the most common species in Oregon and *N. perennans* was most common in Washington. This exercise is important for monitoring which species are present so that control strategies can be tailored more specifically.

Table 3. Species identification based on molecular, spore or colony morphology of a collection of UK *Neofabraea* isolates.

Isolate code	Origin	Cultivar	Date of collection	Molecular identification	Spore size (LxW) µm	Identification based on spore size	Identification based on colony morphology
R142/12/9	Wyefruit-farm 14	Cox	15/03/2012	*N. perennans*	None found		
R142/12/3	Wyefruit-farm 14	Cox	15/03/2012	*N. perennans* OR *N. malicorticis*	None found		
R141/12/7	Wyefruit-farm 7	Cox	15/03/2012	Did not amplify	None found		*N. perennans* OR *N. malicortios*
R141/12/6	Wyefruit-farm 7	Cox	15/03/2012	Did not amplify	None found		*N. perennans* OR *N. malicortios*
R149/12/6	Armsbury	Erg Russet	20/03/2012	*N. alba*	None found		
R149/12/3	Armsbury	Erg Russet	20/03/2012	*N. alba*	None found		
R149/12/2	Armsbury	Erg Russet	20/03/2012	*N. alba*	None found		
R142/12/2	Wyefruit-farm 14	Cox	15/03/2012	Did not amplify	18.3*3.3	*N. alba*	
R142/12/7	Wyefruit-farm 14	Cox	15/03/2012	*N. alba*	None found		
R141/12/2	Wyefruit-farm 7	Cox	15/03/2012	*N. perennans*	None found		
R141/12/1	Wyefruit-farm 7	Cox	15/03/2012	Did not amplify	None found		*N. alba*
R141/12/3	Wyefruit-farm 7	Cox	15/03/2012	*N. perennans*	8.3-11.6*3.3	*N. perennans* OR *N. malicorticis*	
R141/12/4	Wyefruit-farm 7	Cox	15/03/2012	*N. perennans*	None found		
R141/12/5	Wyefruit-farm 7	Cox	15/03/2012	Did not amplify	None found		*N. perennans* OR *N. malicortios*
R141/12/8	Wyefruit-farm 7	Cox	15/03/2012	Did not amplify	8.3-11.6*3.3	*N. perennans* OR *N. malicorticis*	
R151/12/12	Newmafruit	Cox	22/03/2012	*N. perennans* OR *N. malicorticis*	None found		
R151/12/7	Newmafruit	Cox	22/03/2012	Did not amplify	None found		*N. perennans* OR *N. malicortios*
R151/12/12	Newmafruit	Cox	22/03/2012	*N. alba*	None found		
R149/12/8	Armsbury	Erg Russet	20/03/2012	*N. alba*	None found		
R149/12/10	Armsbury	Erg Russet	20/03/2012	*N. alba*	None found		
R149/12/7	Armsbury	Erg Russet	20/03/2012	*N. alba*	16.6*3.3	*N. alba*	
R151/12/6	Newmafruit	Cox	22/03/2012	Did not amplify	None found		*N. perennans* OR *N. malicortios*
R151/12/5	Newmafruit	Cox	22/03/2012	*N. alba*	16.6*3.3	*N. alba*	
R150/12/2	FWM	Braeburn	22/03/2012	*N. alba*	None found		
R149/12/9	Armsbury	Erg Russet	20/03/2012	*N. alba*	None found		
R151/12/11	Newmafruit	Cox	22/03/2012	*N. alba*	14.9-19.9*3.3-4.9	*N. alba*	
R151/12/3	Newmafruit	Cox	22/03/2012	*N. alba*	16.6*1.66	*N. alba*	
R149/12/10	Armsbury	Erg Russet	20/03/2012	*N. alba*	None found		
R149/12/5	Armsbury	Erg Russet	20/03/2012	*N. alba*	None found		
R149/12/4	Armsbury	Erg Russet	20/03/2012	Did not amplify	None found		*N. alba*
R148/12	Bardsley	Cox	20/03/2012	Did not amplify	None found		*N. perennans* OR *N. malicortios*
R138/12/4	Wye fruit	Cox	15/03/2012	Did not amplify	None found		*N. perennans* OR *N. malicortios*
R139/12/3	Wyefruit-farm 17	Gala	15/03/2012	Did not amplify	None found		*N. alba*
R138/12/6	Wyefruit-farm 7	Cox	15/03/2012	Did not amplify	10*3.3	*N. perennans* OR *N. malicorticis*	
R136/12/1	Wyefruit-farm 72	Gala	15/03/2012	*N. alba*	None found		
R139/12/6	Wyefruit-farm 17	Gala	15/03/2012	*N. perennans*	None found		
R139/12/5	Wyefruit-farm 17	Gala	15/03/2012	Did not amplify	16.6*1.66	*N. alba*	
R139/12/4	Wyefruit-farm 17	Gala	15/03/2012	*N. alba*	None found		
R138/12/7	Wyefruit-farm 7	Cox	15/03/2012	*N. perennans* OR *N. malicorticis*	None found		
R138/12/8	Wyefruit-farm 7	Cox	15/03/2012	*N. perennans*	16.6*3.3	*N. perennans* OR *N. malicorticis*	
R139/12/9	Wyefruit-farm 17	Gala	15/03/2012	Did not amplify	None found		*N. alba*
R131/12	FWM-Waddenhall	Braeburn	08/03/2012	Did not amplify	None found		
R127/12/2	FWM-Umson farm	Bramley	08/03/2012	*N. alba*	14.9-19.9*1.66	*N. alba*	
R122/12/1	Bardsley	Cox	07/03/2012	*N. alba*	None found		
R115/12	Ledford	Cox	02/03/2012	*N. alba*	None found		
R142/12/5	Wyefruit-farm 14	Cox	15/03/2012	***	None found		
R142/12/6	Wyefruit-farm 14	Cox	15/03/2012	*N. alba*	21.6*3.3	*N. alba*	

Table 3 Continued

Isolate code	Origin	Cultivar	Date of collection	Molecular identification	Spore size (LxW) µm	Identification based on spore size	Identification based on colony morphology
R145/12	The Breach	Bramley	20/03/2012	*N. alba*	16.6*3.3	*N. alba*	
R114/12/1	Ledford	Cox	02/03/2012	*N. alba*	None found		
R114/12/2	Ledford	Cox	02/03/2012	*N. alba*	None found		
R138/12/5	Wye fruit	Cox	15/03/2012	*N. perennans* OR *N. malicorticis*	10*5	*N. perennans* OR *N. malicorticis*	
R112/12/2	FWM-Chedley	Gala	02/03/2012	*N. alba*	None found		
R113/12/1	Ledford	Cox	02/03/2012	*N. alba*	None found		
R113/12/3	Ledford	Cox	02/03/2012	*N. alba*	None found		
R112/12/3	FWM-Chedley	Gala	02/03/2012	*N. alba*	None found		
R112/12/4	FWM-Chedley	Gala	02/03/2012	Did not amplify	16.6*3.3	*N. alba*	
R112/12/1	FWM-Chedley	Gala	02/03/2012	*N. alba*	None found		
R138/12/7	Wye fruit	Cox	15/03/2012	*N. perennans*	None found		
R100/12/3	Armsbury	Rosie Red	01/03/2012	Did not amplify	19.9*3.3	*N. alba*	
R111/12/1	FWM-Beacon Hill	Braeburn	02/03/2012	*N. alba*	None found		
R132/12/3	Newmafruit	Braeburn	08/03/2012	*N. perennans* OR *N. malicorticis*	None found		
R138/12/9	Wye fruit	Cox	15/03/2012	*N. perennans*	None found		
R138/12/8	Wye fruit	Cox	15/03/2012	*N. perennans* OR *N. malicorticis*	None found		
R138/12/6	Wye fruit	Cox	15/03/2012	*N. perennans*	None found		
R132/12/4	Newmafruit	Braeburn	08/03/2012	*N. alba*	None found		
R132/12/2	Newmafruit	Braeburn	08/03/2012	*N. alba*	None found		
R138/12/1	Wye fruit-farm 7	Cox	15/03/2012	Did not amplify	10-16.6*1.66-3.3	*N. perennans* OR *N. malicorticis*	
R138/12/5	Wye fruit-farm 7	Cox	15/03/2012	Molecular analysis not carried out	8.3-16.6*1.66-3.3	*N. perennans* OR *N. malicorticis*	
R138/12/2	Wye fruit-farm 7	Cox	15/03/2012		14.9*3.3	*N. perennans* OR *N. malicorticis*	
R123/12/2	Armsbury	Braeburn	07/03/2012		None found		*N. alba*
R134/12/3	Wye fruit	Cox	16/03/2012		10*3.3	*N. perennans* OR *N. malicorticis*	
R122/12/3	Bardsley	Cox	07/03/2012		None found		*N. alba*
R111/12/3	FWM-Beacon Hill	Braeburn	02/03/2012		None found		*N. alba*
R100/12/1	Armsbury	Rosie Red	01/03/2012		None found		*N. alba*
R100/12/2	Armsbury	Rosie Red	01/03/2012		None found		*N. alba*
R90/12/1	The Breach	Cox	22/02/2012		None found		*N. alba*
R90/12/2	The Breach	Cox	22/02/2012		None found		*N. alba*
R90/12/3	The Breach	Cox	22/02/2012		None found		*N. alba*
R90/12/4	The Breach	Cox	22/02/2012		None found		*N. alba*
R90/12/5	The Breach	Cox	22/02/2012		None found		*N. alba*
R90/12/8	The Breach	Cox	22/02/2012		None found		*N. alba*
R90/12/7	The Breach	Cox	22/02/2012		None found		*N. alba*
R90/12/6	The Breach	Cox	22/02/2012		None found		*N. alba*
R93/12/3	Gadkgains	Jazz	17/02/2012		None found		*N. alba*
R93/12/1	Gadkgains	Jazz	17/02/2012		None found		*N. alba*
R93/12/4	Gadkgains	Jazz	17/02/2012		None found		*N. alba*
R93/12/2	Gadkgains	Jazz	17/02/2012		None found		*N. alba*
R93/12/5	Gadkgains	Jazz	17/02/2012		None found		*N. alba*
R91/12/2	FWM-Goldings	Braeburn	17/02/2012		None found		*N. alba*
R82/12	FWM-Chedley	Gala	09/02/2012		None found		*N. alba*
R84/12	Newmafruit	Gala	10/02/2012		None found		*N. alba*
R55/12/1	FWM-Chedley	Gala	02/02/2012		None found		*N. alba*
R40/12/1	Ledford	Cox	26/01/2012		None found		*N. alba*

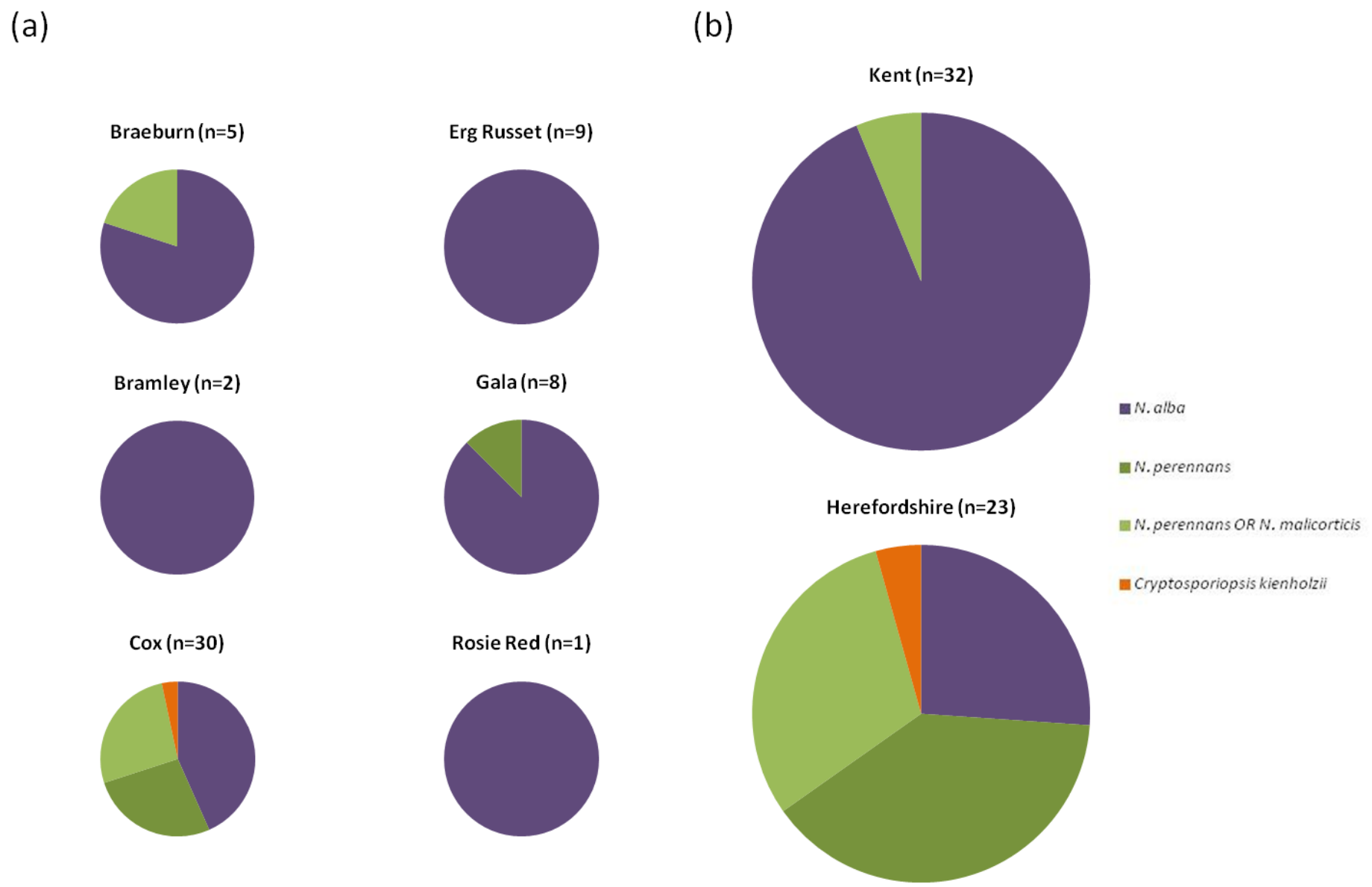

Figure 4. Neofabraea species identification of a UK isolate collection as determined by molecular identification and spore morphology by (a) cultivar and (b) region.

Epidemiology

Inoculum sources and over wintering

Unlike *N. perennans* and *N. malicorticis, N. alba* seldom causes cankers due to the low pathogenicity of this species on woody tissue. *N. alba* is thought to survive on dead bark tissue (caused for example by frost or sunburn), remaining fruit stalks and mummified fruit of apple and pear and as a saprophyte on various woody and herbaceous plants (Verkley, 1999) whilst *N. perennans* and *N. malicorticis* are considered as facultative pathogens on host woody tissue. *N. alba* has been shown to be pathogenic on the woody tissue of olive trees (Zazzerini *et al.*, 1979) and more recently on related *Fraxinus* species causing coin canker (Rossman *et al.*, 2002). Other hosts of *Neofabraea* species, as reported in the compendium of apple and pear diseases and pests (Sutton *et al.*, 2014) include; crab apple, pear, quince, peach, serviceberry, apricot, cherry, flowering quince, hawthorn and mountain ash. Thus wild hedgerow species may harbour a significant source of inoculum for UK orchards. In addition, windbreaks have been hypothesised as acting as a mechanical factor, recirculating airborne/splash dispersed spores (pers. comm. Michel Giraud, ctifl, France). A group in the Netherlands has developed and validated a sensitive real time PCR technique enabling the detection and quantification of *Neofabraea* species (pers. comm. Marcel Wenneker, Wageningen, Netherlands). The use of this tool will greatly increase our knowledge of the epidemiology of these diseases by determining the most significant orchard substrates harbouring inoculum and analysing spore trap data.

Sporulation and timing of infection

Measurements of spore populations of *N. alba* in a Cox orchard in the UK from April 1959 to March 1960 showed that spores are dispersed throughout the year, with a maximum discharge in the Autumn (Burchill and Edney, 1960). Data collected in France suggests sporulation ceases during frost periods before recommencing in spring until May (Bompeix, 1973). Spore release is promoted by rainfall, especially in cool conditions. Asexual spores (conidia), borne from cankers, are splash dispersed to fruit, other branches within or between trees during rains or irrigation. Infection occurs most readily when the weather is cool and damp. Recent preliminary studies in Belgium, in which fruit is covered at different times throughout the season to protect fruit from new spores, has shown that the 8 to 6 weeks prior to harvest is an important infection period for this disease (Pers. comm. Kjell Hauke, PC fruit, Belgium).

Disease spread

According to Sharples (1959) conidia are dispersed by rain splash within the orchard (and capable of initiating canker and fruit rot) whilst ascospores, borne from apothecia, lead to longer distance dispersal of the disease to previously unaffected orchards. Apothecia production is promoted by exhaustion of nutrients of dead host tissue (i.e. old prunings).

Host interactions

Effect of host nutrition

The accumulation of nutrients in developing fruit can vary widely from season to season regardless of nutrient availability. Seasonal factors which may affect nutrient availability include the relative strengths of vegetative and reproductive links (Zavalloni *et al.,* 2001) and the weather conditions, in particular the accessibility of water (Failla *et al.*, 1990) e.g. dry weather during the vegetation season disrupts Ca uptake. Łysiak (2013) demonstrated in an eight year study on cv. Ligol apple that nutrient accumulation varied significantly over the eight seasons assessed; nitrogen content varying by 47%, phosphorus content by

29%, potassium content by 39%, magnesium content by 24% and calcium content by 45%. However, the study found no correlation between concentration of macro elements and incidence of *Gloeosporium* rot (or other storage rots) for this cultivar. Other studies contradict this showing that nutrition is an important factor. Sharples (1980) identified that fruit low in calcium are more prone to *Gloeosporium* rot with a K:Ca ratio of greater than 30 predisposing the fruit to *Gloeosporium* rot. During a study to determine orchard factors which influence storage rots, from the data which informed the rot risk assessment (Webster *et al.*, 2001), *Gloeosporium* rot was at low levels during the period of assessment. None-the-less correlations were evident between *Gloeosporium* rot incidence and nutrition. Crop load and fruit size, which influences calcium dispersal, are therefore included in the risk assessment for this disease.

Effect of harvest time

Edney (1964) demonstrated that the development of *Gloeosporium* rot on apples inoculated with *N. perennans* was promoted by delayed harvest time. Subsequent work has corroborated this finding in the field. In a Norwegian study, a crop of organically grown apples (cv. Aroma) were harvested over five weeks (on optimal harvest date, as determined by starch levels, one or two weeks before and one or two weeks after) over three seasons. Storage losses due to total fruit decay significantly increased with delayed harvest and *Gloeosporium* rot followed this trend. The relationship between harvest date and disease incidence (including *Gloeosporium* rot) has not always been clear (Wilkinson and Sharples, 1967, Valiuskaite *et al.,* 2006, Gualanduzzi *et al.,* 2005 and Neri *et al.,* 2005) and will likely be strongly influenced by cultivar, location, climate and inoculum presence.

Cultivar

Cultivars do differ in susceptibility to *Gloeosporium* rot. Anecdotal evidence suggests that Bramley, Gala, Braeburn, Red Delicious, Granny Smith, Jonagold and Jazz have low susceptibility; Golden Delicious, Fuji and Cameo have medium susceptibility and Cox is highly susceptible (pers. comm. Michel Giraud).

Control

Cultural control

Cultural control in the orchard is an important aspect for the control of this disease but may often be overlooked. Due to the different epidemiology of the causative agents of fruit rot in store, cultural control strategies differ. For instance in the case of *N. perennans* and *N. malicorticis*, which are able to survive on living wood in the form of cankers, the removal of cankers at the pruning stage will be important to remove the innoculm source from the orchard. However in the case of *N. alba*, which is less pathogenic on living wood and more often found on old prunings in the orchard and on pruning snags, it is important to adopt a sanitary approach, removing prunings from the orchard or shredding prunings in the orchard. If a particular orchard has a history of *Gloeosporium* rot developing in stored apple or *Neofabraea* cankers are evident in the orchard, then removal of cankers from the trees and other pruning should be reserved for warmer, drier periods to reduce the spread of the disease. Painting of pruning wounds is another possibility to prevent reinfection of fresh wounds. Amendment of the paint with appropriate insecticides to discourage woolly apple aphid from establishing on cut surfaces is recommended in the USA (Dugan, 1993) for effective control of perennial canker (*N. Perennans*). However, Dugan also reasons that the process is costly and labour intensive.

Timing of harvest has been shown to be a crucial factor in determining the incidence of *Gloeosporium* rot, and other storage rots, subsequently developing in store. Therefore, particular attention to optimum picking date should be made to reduce post-harvest losses.
Recommendations for post-harvest cultural control include rapid cooling of fruit, and storage with low oxygen atmosphere and at low temperature. Indeed, the higher incidence of *Gloeosporium* rot in Cox

(Figure 2) may partly result from the higher temperatures that this cultivar is stored at to mitigate low temperature breakdown, a physiological disorder to which Cox is particularly susceptible.

The apple rot risk assessment (Webster *et al.,* 2001) is a decision support tool for rot control which assists in determining the need for pre-harvest treatments (see below) where a risk has been identified and to predict the storage potential of fruit consignments. Clear factors for rot risk assessment of *Gloeosporium* rot were not identified due to the low incidence of the disease when the correlative studies were conducted. Consequently, decisions on risk are mainly based on orchard rot history, rainfall and crop load which in turn influences mineral composition. These factors and risk thresholds are summarised in Table 4. A marketing plan can be formulated by determining risk prior to harvest based on the rot risk assessment and on physiological traits such as mineral composition and firmness.

Table 4. A summary of the rot risk assessment criteria described in Webster *et al.* (2001) for *Gloeosporium* rot.

Factor	Risk criteria
Orchard rot history	Moderate-high incidence of *Gloeosporium* rot = risk
Rainfall in month pre-harvest	Greater than average = risk
Crop load	Light crop = risk
Mineral composition	Low calcium (i.e. K/Ca ratio >30) = risk

A complementary method to determine the risk of *Gloeosporium* rot developing in store was described by Dugan (1993). The method involves removing a random sample from each consignment of apples to be put in store for medium to long term and subjecting them to temperatures of 18 - 22 °C (64-70 °F) at high humidity for 1 month; fruit which develop a high incidence of *Gloeosporium* rot should then be marketed/exported early. Practically this method may not be suitable for internal markets but rather for fruit export.

Fungicides

Most UK growers do not currently use specific treatments for control of *Neofabraea* but rather achieve incidental control by targeting other diseases. Application of fungicide or copper based products during the dormant season is practiced to reduce infections leading to Nectria canker. Application of the fungicide benomyl in the dormant season reduced the incidence of *Gloeosporium* rot significantly the following season over the three years tested, primarily as a result of the reduction in the sources of inoculum overwintering on branches and shoots (Kennel, 1989). The effect of copper (oxychloride) was also tested in the first year only of this trial. However, commercial control was not achieved with this treatment. Work in the 1950's at East Malling investigated the importance of spray timing for the control of *Gloeosporium* rot during the growing season. Using captan, three spray schedules (July, August and September, August and September and September only) were applied on cv. Cox over two growing seasons (1955 and 1956). The harvested fruit was stored, assessed for *Gloeosporium* rot and compared to an unsprayed control. A single spray of captan in mid-September was effective in reducing *Gloeosporium* rots and additional sprays in July and August improved control further (Moore and Edney, 1958). Captan and dithianon applied as part of a scab control programme are likely to be achieving incidental control of *Gloeosporium* through the growing season. Pre-harvest treatments, such as sprays of Switch, Bellis or Geoxe, starting 4 – 6 weeks prior to harvest are currently used in high risk seasons for general storage rot control. Fungicide resistance of isolates of *Gloeosporium* collected from fruit has been reported in unpublished data from the 1990's. In total 115 isolates were tested for sensitivity to benomyl at 2 parts per million (ppm) and 20 ppm. Over 80% of the isolates collected were resistant to benomyl at 20 ppm with the incidence of resistant isolates strongly influenced by orchard fungicide treatment and post-harvest fungicide application (Berrie and Luton, 1996).

Hot water treatment

Post-harvest heat treatment of fresh produce prior to storage for the prevention of rot development has been demonstrated in numerous temperate, sub-tropical and tropical fruit, vegetables and flowers. As a surface pathogen, *Neofabraea* species, which reside in a quiescent state in the lenticels of the fruit skin, would be amenable to heat treatment. First demonstrated by Burchill (1964) this is indeed the case, with the results subsequently being corroborated and methods refined for incubation in hot air (e.g., 72 hours at 40 ◦C; Tahir *et al.*, 2009; Fallik *et al.*, 2001) and by hot-water dipping (HWD) for up to 3 minutes (Maxin *et al.*, 2005; Amiri and Bompeix, 2011). The practice is now used by a growing number of organic growers to prevent the development of *Gloeosporium* rot in high risk situations. Hot water treatment in combination with a number of antifungal agents, have also been tested, with the potential to produce additive or synergistic effects to reduce post-harvest disease development to acceptable levels. For example, Trapman *et al.* (2010) tested the efficacy of HWT combined with treatment with BoniProtect (*Aureobasidium pullulans*) to control storage diseases, including *Gloeosporium* rot. A trial on cv. Pinova in 2006 showed that a HWT (2 minutes, 51 °C) alone or in combination with BoniProtect gave a 65% and 82% reduction in storage rots respectively compared to the untreated control in which *Gloeosporium* rot was the predominant disease. The reason that this control measure has not been adopted more widely (i.e. beyond the organic growers) is probably because the temperatures required to control the pathogen can also damage the fruit. With fruit quality paramount this control measure is not widely practiced. In addition the practice is not amenable to the current post harvest procedures and has a high energy cost associated with it (i.e. heating up and cooling down the fruit).

Future research needs

- Continued monitoring of *Gloeosporium* rot incidence in store together with species identification using the methods presented in the review.
- Although not present in great numbers, increase our understanding of the new species (epidemiology, fungicide sensitivity, host susceptibility etc) in preparation for the future.
- Determine the source of inoculum of *N. alba* (the dominant species in Kent). A small study using the molecular diagnostic tool described in this review, or better still an RT PCR assay developed in the Netherlands (Wenneker, in press), could identify alternative hosts or substrates which could inform management of this disease in the future.
- Innovations in canker control from the vine industry and from European apple canker (*N. ditissima*) control may be applicable for the control of *Neofabraea* cankers.
- Increasing our understanding of what affects the expression of latent infection in stored apple.
- Keeping informed about developments in Europe where *Gloeosporium* rot is the predominant rot in stored apple.

Main points for growers

- The nomenclature of the causative agents of *Gloeosporium* rot has recently changed to *Neofabraea* species complex which includes *N. alba*, *N. perennans, N. malicorticis* and *Cryptosporiopsis kienholzii* (Table 1).

- Data from the apple rot survey shows that *Gloeosporium* rot is sporadic in rank, incidence and loss from year to year in the UK. *Gloeosporium* rot has become increasingly important by rank in recent years. For example in 2011, *Gloeosporium* rot was the second most recorded rot in cv. Cox (a highly susceptible cultivar). The average incidence (that is the percentage of apple samples surveyed which contained *Gloeosporium* rot) in susceptible cultivars has been as high as 80%. However, this fell to 16% in the 2014 rot survey. Losses due to *Gloeosporium* rot are still relatively low, particularly when compared to those experienced in England in the 1960's and those currently experienced in other parts of Europe (Figure 2).

- *Gloeosporium* rot is most evident in the store although wood lesions will be present in the orchard. Symptoms on fruit and wood can be mistaken with those caused by other fungi (Figure 3 and section 3.2).

- Species identification of isolates collected from two apple growing regions of England suggest that *N. alba* are predominant in Kent and *N. perennans* (and *N. malicorticis*) are predominant in Herefordshire. *N. perennans* (and *N. malicorticis*) are the predominant species on cv. Cox. Species identification revealed that *Cryptosporiopsis kienholzii* is present in the UK which, to the best of our knowledge, is the first reported recording (Figure 4).

- The pathogen is currently not targeted specifically but is probably controlled incidentally through treatments targeted at other wood/fruit rot pathogens such as *Neonectria ditissima*. Further practices which are important for *Gloeosporium* rot control are consideration of nutrition and storage (Section 7).

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