

Control of sooty blotch and black rot of apple through removal of fruit mummies

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ABSTRACT

Several popular apple cultivars retain their aborted fruits as mummies on the tree. In laboratory conditions, overwintered fruit mummies collected from a Northern German apple orchard under organic management released inoculum, which caused black rot due to *Diplodia seriata* and sooty blotch due to *Peltaster cerophilus* on ripe apples. In a field trial conducted over four years in another organic orchard, the manual removal of fruit mummies in winter and again in late June of each year significantly reduced the incidence of both these diseases. However, fruit mummy removal did not significantly affect the development of storage rots due to *Neofabraea alba* and *N. perennans*. The potential, limitations and costs of this phytosanitary measure are discussed in the context of organic apple production.

Key words: *Diplodia seriata*, *Neofabraea alba*, *Neofabraea perennans*, organic production, *Peltaster cerophilus*, storage rot

Abbreviations:

IPM – integrated pest management, ITS – internal transcribed spacer, PDA – potato dextrose agar

INTRODUCTION

Apple fruitlets that become arrested in their development may remain attached to the twig as dead tissue for the remainder of the season or beyond. Such fruit mummies are formed at various time points, notably shortly after blossom, during the ‘June drops’ and in lesser numbers at any subsequent stage until harvest. The physiological basis of the retention of fruit mummies by the tree is an incomplete formation of the abscission

layer at the base of the fruit stalk (Knoche et al. 2000). The tendency to retain fruit mummies is specific to certain apple cultivars; ‘Elstar’, ‘Ingrid Marie’, ‘Gerlinde’, ‘Fresco’ (marketed as Wellant®) and ‘Civni’ (marketed as Rubens®) are among the popular or new cultivars in Northern Germany which retain fruit mummies for up to three seasons (Quast and Weber 2008). By offering dead tissue in close proximity to developing fruits, mummies are an attractive habitat for pathogenic fungi. Several fruit pathogens have been observed as colonisers

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of fruit mummies in Northern Germany and elsewhere. These include *Diplodia seriata* De Not. (teleomorph: *Botryosphaeria obtusa* (Schwein.) Shoemaker) which causes a pre-harvest disease known as black rot (Holmes and Rich 1970, Sutton 1981, Weber 2012), as well as fungi associated with postharvest rots such as *Phacidiopycnis washingtonensis* Xiao & J.D. Rogers which causes rubbery rot (Weber 2011, 2012), and *Neofabraea* spp. which are agents of bitter rot or bull's eye rot (Kennel 1970, Weber 2012). Further, Gleason et al. (2011) have provided observational evidence of the association of *Peltaster* sp., the cause of sooty blotch, with fruit mummies in Northern Germany. Several additional fungi of uncertain pathogenic potential may also be associated with fruit mummies (Weber 2012).

With the exception of *Neofabraea* rots and rubbery rot, diseases caused by the above-mentioned pathogens do not occur to any commercially relevant extent in orchards under integrated pest management (IPM) in Northern Germany, presumably because regional spray schemes against apple scab have sufficient side effects against them. Captan is routinely applied between flowering and three weeks before harvest, and quinone outside inhibitors (QoI) such as trifloxystrobin are also used once or several times per season. Both these compounds are effective against sooty blotch (Sutton and Williamson 2002) and black rot (Smith and Hendrix 1984, Giraud 2009).

Due to a more restricted availability of fungicides, diseases such as black rot and sooty blotch can cause significant crop losses in Northern German organic apple production. Pre-harvest losses may amount up to 10% for black rot (Quast and Weber 2008), whereas >50% of the fruits at harvest may be affected by sooty blotch (Fuchs et al. 2002, Buchleither and Späth 2007). Because markets continue to demand organically produced apples of mummy-bearing cultivars, the labour-intensive manual removal of mummies in winter is occasionally practised by organic farmers in Northern Europe. However, to our knowledge this approach has not been rigorously tested under commercial conditions. Therefore, we conducted an on-farm field trial of the effects of fruit mummy removal on the incidence of relevant fungal diseases over several years. In addition, we examined whether overwintered fruit mummies can act as a source of inoculum for sooty blotch disease and for black rot.

MATERIAL AND METHODS

Location of orchards

Studies were conducted in two orchards located within the Northern German 'Altes Land' region on the southern shore of the Lower Elbe river southwest of the city of Hamburg. An organic apple orchard planted with the cultivar 'Dalinbel' on rootstock M9 located at the Esteburg research station (53.50°N, 9.76°E) was chosen as the source of fruit mummies for this experiment because of a high incidence of sooty blotch and black rot in previous years (Quast and Weber 2008, Gleason et al. 2011). A field trial to examine the effects of the removal of fruit mummies on the incidence of various diseases was conducted in a commercial orchard (53.53°N, 9.74°E) of cultivar 'Elstar' on rootstock M9 under organic management, which was 15 years old at the beginning of our work in 2008. Trees were planted at a distance of 1.2 m within rows and 3.5 m between rows. Rows were in a SW-NE orientation. The tree height was approx. 3 m. The trees possessed dense and intergrading canopies because of restrained pruning by the farmer. No pollinator trees were planted in this orchard because trees of different cultivars bordered the orchard on three sides. There were no windbreaks, although some shelter was provided on the NW border of the orchard by a ridge (4 m tall).

Reagents

Suppliers of reagents and equipment used for molecular biological work were as described by Weber and Zabel (2011). All other reagents were obtained from Carl Roth (Karlsruhe, Germany).

Identification of fungi

Diplodia seriata was identified on the basis of its characteristic conidia (Phillips et al. 2007, Trapman et al. 2008), and on features of its fruit rot which began as a limited black primary lesion in June or July and developed into a spreading brown rot on ripening fruits within 2-3 weeks before harvest (Quast and Weber 2008). Using the primers ITS5 and ITS4 (White et al. 1990) and the methods and equipment described by Weber and Zabel (2011), the ITS1-5.8S-ITS2 sequence of the ribosomal DNA gene cluster was analysed for representative isolates from each of the two orchards examined in this study. These isolates have been deposited in the Esteburg culture collection under the accession numbers OVB07-018 and OVB14-015.

Peltaster cerophilus Jana Frank & Schroers was also isolated from both orchards indicated

above. Mycelial fragments from sooty-blotch colonies on apples were streaked out onto tap-water agar acidified by the addition of 500 µl 90% (v/v) lactic acid 1⁻¹ agar after autoclaving (Batzer et al. 2005). Following repeated subcultivation by mycelial transfer onto the same medium and onto potato-dextrose agar (PDA) augmented with 200 mg penicillin G and streptomycin sulphate 1⁻¹, the isolates were identified by comparing their colony morphology on apple, their growth in pure culture and their ITS sequences with sooty-blotch reference isolates from the Esteburg culture collection.

Neofabraea alba (E.J. Guthrie) Verkley and *N. perennans* Kienholz were identified on the basis of their characteristic conidia formed on infected apples during prolonged storage (Verkley 1999, Weber 2012). Identification of typical isolates had previously been confirmed by ITS sequence analysis (Maxin et al. 2012). Other storage-rot fungi were identified in a similar manner as described previously (Maxin et al. 2012 and references therein).

Artificial inoculation experiments

On 20 May 2009 overwintered fruit mummies were removed from eight ‘Dalinbel’ trees that had shown sooty-blotch symptoms during the two preceding years. In addition, four of the eight trees had shown black rot in 2008. From each tree, three mummies were chosen and suspended on a ‘Golden Delicious’ apple. These apples were incubated in moist chambers where they were sprayed with water once daily to continuous surface wetness. Incubation was for four weeks at room temperature. Eight apples without attached mummies were wetted and incubated in the same way.

Sooty-blotch fungi were isolated as described above. For isolating fruit-rot pathogens, developing lesions were first surface-sterilised by wiping with a paper tissue soaked in 70% (v/v) ethanol. From the advancing edge of the lesions, small segments (2 mm side length) were excised with a sterile scalpel and placed on PDA augmented by penicillin G and streptomycin sulphate. Pure cultures were identified by comparison with the reference strains described above.

Removal of fruit mummies

Fruit mummies (Fig. 1) were removed manually from ‘Elstar’ trees by snapping them off at the point of attachment of the stalk base to the fruit spur. This was conducted twice each year, i.e. in late winter before bud break and again between late June and mid-July following the ‘June drops’. Depending

on the experimental design, mummies were either dropped to the orchard floor or collected and disposed of outside the orchard. All experimental variants were carried out in triplicate, each repeat comprising 25 trees in each of three adjacent rows. Throughout all four years of our experiments, trees were dedicated to the same variants with respect to fruit mummy removal. All trees within the orchard were subjected to uniform fungicide measures, which were conducted according to regional organic practice, mainly comprising the use of copper fungicides and lime-sulphur until the end of flowering and wettable sulphur as well as kaolin-based products between the end of flowering and harvest.

Assessment of black rot

For each experimental repeat, 50 fruits on each of 15 trees were chosen at random and scored for the presence of black rot on 3 Sept. 2008, 2 Sept. 2009 and 1 Sept. 2011, i.e. <2 weeks before the beginning of commercial harvest. The percentage of affected fruits was calculated for each repeat.

Assessment of sooty blotch

For each experimental repeat, sooty blotch symptoms were scored on freshly harvested fruits from the second picking, which took place on 30 Sept. 2010 and 16 Sept. 2011. Individual fruits were removed from each harvest box until a total of 200 fruits were obtained for each experimental repeat. The incidence of sooty blotch was scored as categories zero (no sooty blotch), 1 (small spots), 2 (<10% of fruit surface covered by SBFS), 3 (10–25% covered), 4 (up to 50% covered) and 5 (>50% covered). The disease severity index was calculated according to Mayr and Späth (2008) as follows:

$$\text{Disease severity [\%]} = \frac{\sum (n \times v)}{5 \times N} \times 100$$

whereby v was the sooty-blotch category (i.e. 0, 1, 2, 3, 4 or 5), n was the number of fruits in that category, and N was the total number of fruits scored (i.e. 200 per repeat). The disease severity index was used to indicate the percentage of the fruit surface covered by sooty blotch.

Trial of storage rots

For an enhanced development of storage rots, fruits were taken from the second picking conducted on 30 Sept. 2010 and 16 Sept. 2011. Individual fruits were removed from each harvest box throughout the repeat until a total of 150 fruits had been compiled. These fruits were stored in perforated plastic boxes



Figure 1. Mummies formed as the result of fruit abortion at different developmental stages on an ‘Elstar’ tree in the experimental orchard, photographed in early spring

in a cold room at 2°C and >95% relative humidity. At each of three time points in early December, late January and mid-March, fruits showing incipient rot were removed from the storage boxes and further incubated singly in display trays until sporulation permitted the identification of fungi. The percentage of rotted fruit was recorded at each time point, and computed as the cumulative percentage at the end of storage.

Statistical analysis

Statistical analyses were performed with the software package WinSTAT for Microsoft Excel. All data were analysed using one-way ANOVA, and compared by Tukey’s test ($\alpha = 0.05$).

RESULTS

Identification of fungi

Identification of *N. alba*, *N. perennans* and other storage rots listed in Table 1 was unequivocal on the basis of spore morphology, as well as detailed previous analyses (Maxin et al. 2012). Likewise, the ITS sequences of representative isolates of black fruit rot, OVB07-018 and OVB14-015, were identical to those of other strains previously

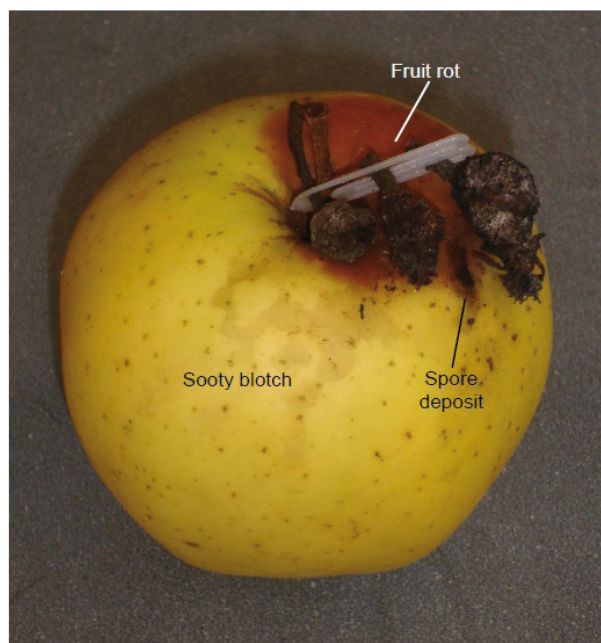


Figure 2. ‘Golden Delicious’ apple after four weeks’ incubation in a moist chamber with daily wetting. Three fruit mummies suspended over the apple at the same position throughout the incubation period have been shifted for the photograph to reveal sooty-blotch colonies due to *Peltaster cerophilus* as well as a spore deposit and fruit rot due to *Diplodia seriata*

Table 1. Percentage of fruits affected by different storage-rot fungi in the controls (no fruit mummies removed) in the 2010 and 2011 harvests. In each year, the control comprised 450 fruits

Species	2010 harvest	2011 harvest
<i>Botrytis cinerea</i>	0.0	1.6
<i>Colletotrichum acutatum</i>	7.1	11.6
<i>Monilinia fructigena</i>	2.8	4.7
<i>Neofabraea alba</i>	17.6	11.6
<i>Neofabraea perennans</i>	24.8	28.0
<i>Neonectria ditissima</i>	7.2	7.4
<i>Penicillium expansum</i>	0.2	2.4
Others	3.1	0.5
Total fruit rot	62.8	67.7

analysed in Northern Germany (Weber and Dralle 2013) as well as to numerous sequences of *D. seriata* in GenBank (e.g. GU121876, KF766161, NR111151). Identification of the two isolates of *P. cerophilus* was based on a 100% identity of their ITS sequences to one another, to sequences of six other Northern German isolates (R.W.S. Weber unpublished) and to published sequences available in GenBank (e.g. JN573679, KF646817; Medjedović et al. 2014). In contrast, the closest match for *Peltaster fruticola* E.M. Johnson et al. gave only 86% identity (HQ386247; Mirzwa-Mróz and Wińska-Krysiak 2011).

Fruit mummies as a source of inoculum

After four weeks of incubation in a damp chamber with daily wetting, thin sooty-blotch colonies were

visible on five of the eight apples to which fruit mummies had been attached, but on none of the eight uninoculated control apples. In addition, a brown fruit rot as well as black conidial deposits were observed on all four apples with mummies collected from trees affected by black rot but on none with mummies from the four symptomless trees, and on none of the uninoculated control fruits. On each fruit the rot originated at or close to the point of attachment of the stalk to the fruit, whereas the sooty blotch colonies were close to the point of contact between the mummies and the fruit surface, spreading downwards onto the side of the fruit (Fig. 2). An analysis of pure-culture isolates as well as spore deposits obtained from these fruits revealed the fruit-rot fungus to be *D. seriata*, whereas cultures of the sooty-blotch fungus were identified as *P. cerophilus*.

Black summer rot trial

A low incidence of black rot was observed in the 2008 season, whereas in 2009 and 2011 the share of infected fruits in the control was 5% or more. In all three years a significant reduction of black rot on maturing apples was observed where fruit mummies had been removed and dropped to the ground (Fig. 3). The percentage by which black rot was reduced rose from 64% in 2008 to 96% in 2009 and 84% in 2011, suggesting an increasing efficacy associated with the repeated removal of fruit mummies from the same trees in successive years. In 2011, no additional effect was obtained

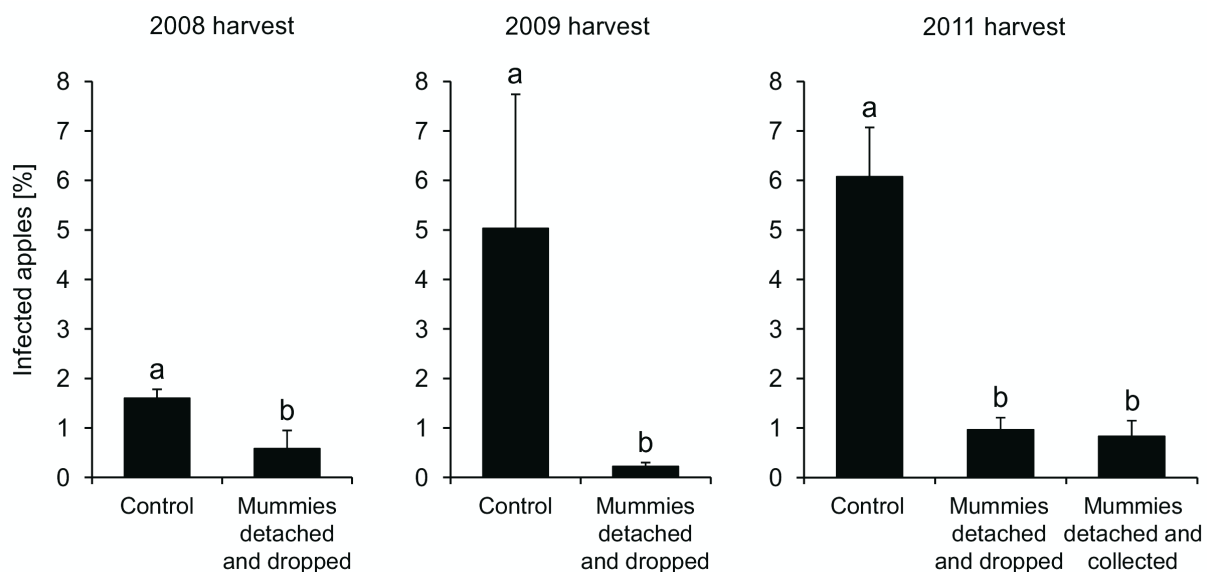


Figure 3. Incidence of black rot (mean of 750 fruits in each of three repeats) due to *Diplodia seriata* in the 2008 (left), 2009 (centre) and 2011 (right) harvests of an organic ‘Elstar’ orchard with or without removal of fruit mummies from the trees. Different letters indicate significant differences using Tukey’s test ($\alpha = 0.05$)

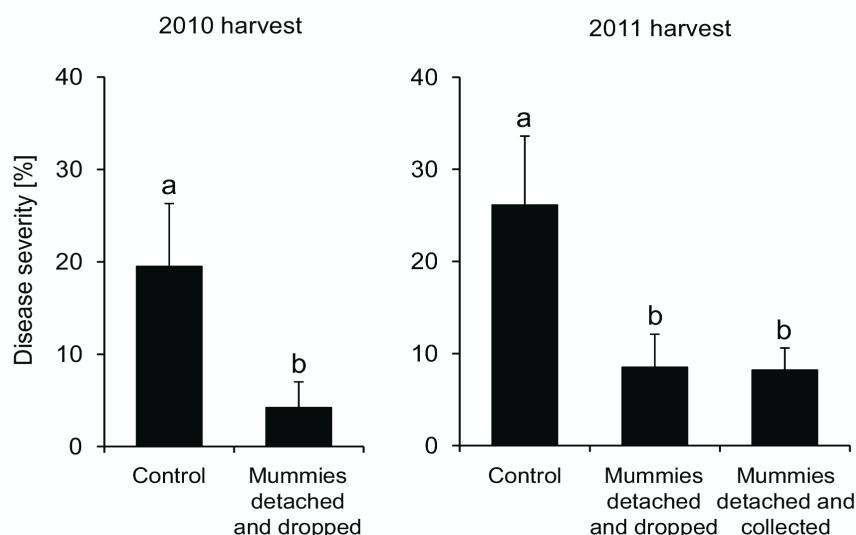


Figure 4. Severity of sooty blotch due to *Peltaster cerophilus* (mean of 200 fruits in each of three repeats) in the 2010 (left) and 2011 (right) harvests of an organic ‘Elstar’ orchard with or without removal of fruit mummies from the trees. Different letters indicate significant differences using Tukey’s test ($\alpha = 0.05$)

when removed fruit mummies were collected and taken out of the orchard, as compared to the standard protocol of dropping them to the ground. Although fruit mummy removal was conducted in 2010, the experiment was not evaluated for black rot in that year.

Sooty-blotch trial

The incidence of sooty blotch was assessed in the 2010 and 2011 seasons. In both years, a significant reduction of sooty blotch was observed in those

variants where fruit mummies had been removed from the trees and dropped to the ground. No additional effect was observed in 2011 when the removed fruit mummies were collected and taken out of the orchard (Fig. 4).

Storage rot trial

After storage for six months in a cold room at an ambient atmosphere, >60% of fruits collected from the control trees not subjected to fruit mummy removal were spoiled by storage rots in both the

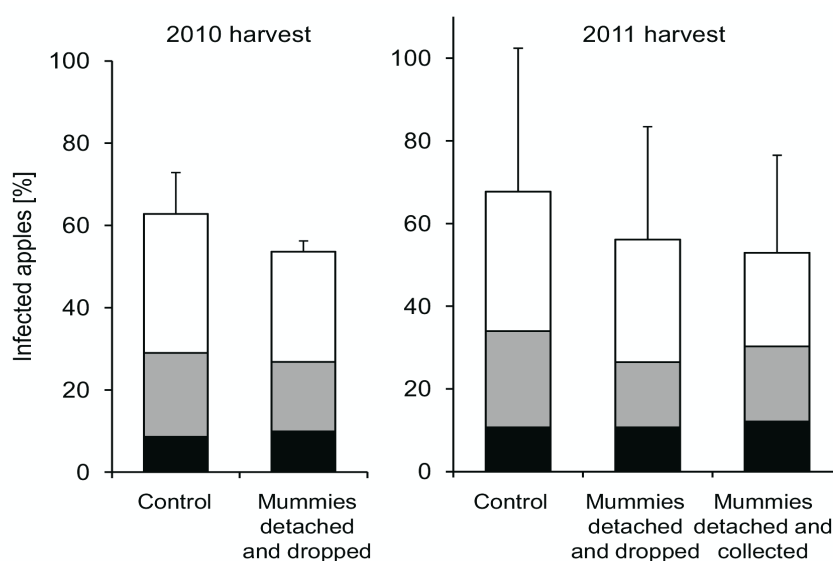


Figure 5. Incidence of fruit rots (mean of 150 fruits in each of three repeats) in the 2010 (left) and 2011 (right) harvests of an organic ‘Elstar’ orchard with or without removal of fruit mummies from the trees. Cumulative data of three evaluations of fruit rot in December (black areas), January (grey areas) and March (white areas) are given. Error bars indicate the SE of cumulative fruit rot after the last evaluation in March

2010 and 2011 harvests. No significant effect of the removal of fruit mummies on the development of storage rot was found, irrespective of whether the detached mummies had been dropped to the ground or collected and taken out of the orchard (Fig. 5). The contribution of fungal species to the total fruit rot was similar in both years of the trial (Tab. 1), the two *Neofabraea* spp. being associated with about two-thirds of all infections. The removal of fruit mummies had no significant effect on the incidence of individual pathogen species such as *N. alba*, *N. perennans*, *Neonectria ditissima* (Tul. & C. Tul.) Samuels & Rossman or *Colletotrichum acutatum* J.H. Simmonds in either of the two years (data not shown).

DISCUSSION

The present study provides direct evidence of the high efficacy of fruit mummy removal against black rot and sooty blotch in an organically managed orchard of ‘Elstar’, one of the most important Northern German apple cultivars, and additionally proves that the fungi causing these two diseases are directly associated with overwintered fruit mummies. In a further trial initiated subsequently but published previously to the present work, Brockamp and Weber (2014) used a different orchard planted with ‘Gerlinde’, another mummy-bearing cultivar, to show that fruit mummy removal was more effective against black rot than repeated sprays with fungicides registered for organic production, such as lime sulphur and clay soil. To our knowledge, these are the first statistically confirmed results of the efficacy of fruit mummy removal against any fungal pathogen in Northern European apple production.

Our findings for black rot tie in with the infection biology of *D. seriata* in Northern Germany, where it is common on fruit mummies but rare on twigs, bark cankers and other possible sources of inoculum (Quast and Weber 2008). In contrast, twig infections are reported as being common in the southeastern United States, where they are assumed to play a more important role as sources of inoculum than fruit mummies (Sutton 1981). Both air- or water-borne ascospores (*Botryosphaeria* state) and the purely splash-dispersed conidia (*Diplodia* state) are capable of infecting growing apples, conidia of *D. seriata* being regarded as more relevant to the short-distance spread within trees (Sutton 1981). Observations of abundant conidia on fruit mummies (Giraud 2009, Weber 2012) coupled with the absence of the ascospore state on

such mummies point to rain splash of conidia as the main route of infection in Northern Germany. A localised spread of infections is further supported by repeated observations of a high incidence of fruit infections on trees of non-mummy bearing cultivars only where these grow in direct contact to heavily infected mummy-bearing trees (Quast and Weber 2008). Temperatures of 20–24°C with at least 9 h of leaf wetness are required for fruit infections to occur (Arauz and Sutton 1989). In Northern Germany, these conditions are most readily met by rainfall at elevated temperatures in June, July and August (Brockamp and Weber 2014). This is also the period in which new fruit mummies become available for colonisation.

The lack of any effect of fruit mummy removal on the incidence of storage rots – notably *Neofabraea alba* and *N. perennans* – was disappointing, given that *N. alba* has been found on about 25% of ‘Elstar’ fruit mummies in Northern Germany (Weber 2012). However, both *Neofabraea* spp. also frequently colonise pruning cuts and other wounds (Blumer 1960, Grove 1990), and it is possible that the resulting bark necroses are more relevant as sources of inoculum in the orchard (Bompeix 1988). Similarly, although fruit mummies may occasionally be colonised by the apple canker fungus *Neonectria ditissima* (syn. *N. galligena*), there is evidence of a strong association of fruit rots caused by this species with canker lesions on the tree (Weber and Dralle 2013).

The recently described species *Peltaster cerophilus* (Medjedović et al. 2014) has been demonstrated to be the most important sooty-blotch fungus affecting organic orchards within the two largest German production areas of the Lower Elbe region in the north and the Lake Constance area in the south (R.W.S. Weber, unpublished). The inoculation experiment reported here has provided the first direct evidence of an association of *P. cerophilus* with overwintered fruit mummies. The strong reduction of sooty-blotch disease associated with the removal of fruit mummies indicates that these play an important role in the infection biology of *P. cerophilus*. The ability to overwinter within the orchard is all the more important because *P. cerophilus* has not been observed to produce ascospores that could be transported over long distances by air currents. This is in contrast to other sooty-blotch fungi and especially the flyspeck fungus *Schizothyrium pomi* (Mont. & Fr.) Arx, which may invade orchards from surrounding vegetation afresh each season

(Cooley et al. 2011, Gleason et al. 2011). Because the infection biology of *Peltaster* spp. is likely to be polycyclic and based on the repeated rain splash and washout of inoculum from conidial fructifications (Williamson et al. 2004), fruit mummy colonisation is a plausible explanation of the dominance of *P. cerophilus* in Northern German organic apple production. However, the fungus must be able to colonise other niches as well because sooty blotch due to *P. cerophilus* is also found on apple cultivars that do not retain their fruit mummies, such as 'Topaz'. This is especially the case in Southern Germany, where the disease pressure is generally higher than in the North (Mayr and Späth 2008).

The work reported here has therefore shown the potential of removing fruit mummies as a phytosanitary control method of black rot and sooty blotch, and its limitations against storage rots. Fruit mummy removal is labour-intensive, one passage through the orchard requiring about 60-100 working hours ha⁻¹ (Brockamp and Weber 2014). Taking a worker's wage to be € 10 h⁻¹ and assuming that mummies are removed twice *per annum*, the costs of this phytosanitary measure would amount to € 1200-2000 ha⁻¹ year⁻¹. In comparison, a 7.5% fruit spoilage due to black rot in an average harvest of 25 t 'Elstar' ha⁻¹ at an average price of € 650 t⁻¹ amounts to a loss of € 1219 ha⁻¹. Considering other benefits such as sooty-blotch control, our results indicate that fruit mummy removal is a realistic control option for organic growers of mummy-bearing cultivars.

Clearly, a single removal of fruit mummies in winter or early spring would be a more pragmatic and labour-saving approach. Although fruit mummies formed and infected in June can become a source of *D. seriata* fruit rot inoculum in July or August of the same season (Giraud 2009, R.W.S. Weber unpublished), this is unlikely to be relevant if the infection cycle has been broken beforehand by removing overwintered colonised mummies. Crop protection measures conducted against storage rots in July and August can show an additional effect of reducing the incidence of black rot (Brockamp and Weber 2014). Therefore, there is a high probability that a single annual fruit mummy removal in winter is sufficient to control *D. seriata* under Northern German conditions. Nonetheless, further work should directly compare the effects of a single removal of fruit mummies in early spring with a removal twice *per annum*, using easily assayed diseases such as black rot.

Irrespective of the above considerations, the low efficacy of fruit mummy removal against storage rots means that this method cannot fully replace the application of pre-harvest fungicides (Brockamp and Weber 2014). Further studies should therefore explore a strategy of reduced fungicide input from mid-season onwards in combination with fruit mummy removal and a hot-water treatment after harvest, which is known to possess a high efficacy against storage rots (Maxin et al. 2012).

CONCLUSIONS

1. Inoculum of *Diplodia seriata* and *Peltaster cerophilus* released from colonised overwintered fruit mummies can infect new apple fruits, causing black rot and sooty blotch, respectively.
2. Removing fruit mummies from organically managed orchards can lead to a significant reduction of the incidence of black rot and sooty blotch before harvest.
3. No such effects were observed on storage rot caused by *Neofabraea alba* and *N. perennans*.

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