Black rot (*Diplodia seriata*) in organic apple production – infection biology and disease control strategies

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Abstract

*During a three-year trial (2011 - 2013) at an organic fruit farm in the Lower Elbe region (Northern Germany), the manual removal of fruit mummies and preventive applications of crop protection compounds (Myco-Sin and lime sulphur) were tested for their influence on reducing black rot (*Diplodia seriata*) and fungal storage rots. Aspects of the infection biology of* D. seriata *were examined by monitoring the spore release (conidia) in response to rainfall and by scoring overwintered fruit mummies for colonisation by* D. seriata*. The removal of fruit mummies, either as a stand-alone treatment or in combination with Myco-Sin or lime sulphur applications, was able significantly to reduce pre-harvest fruit infections caused by* D. seriata*. A correlation between spore potential, the amount and intensity of rainfall and high temperatures was observed during the monitoring of primary infections. Storage rots, chiefly caused by* Neofabraea *spp., were controlled by a combination of fruit mummy removal and spray applications, the highest efficacies being observed with Myco-Sin.*

Keywords: apples, black rot, *Diplodia seriata*, storage rots, fruit mummy removal, preventive spraying

Introduction

In the Lower Elbe region (Northern Germany), black rot of apples was first observed as a pre-harvest disease in August 2007 and has been recorded in every season since. In affected orchards, optimal infection conditions such as temperatures around 20°C combined with high rainfall have resulted in annual crop losses over 5 % at harvest. The causal fungus *Diplodia seriata* overwinters in bark lesions, dead twigs and mummified apples on the tree. During high rainfall conidia washed from fruit mummies onto the surface of fruits cause primary infections through lenticels (Quast & Weber, 2008). In North America, ideal conditions have been characterised as a 9 h period of leaf wetness at 20-24°C for fruit infections and 4.5 h wetness at 26.6°C for leaf infections (Arauz & Sutton, 1989). First fruit symptoms are small black spots of limited size which enter a stage of latency. These lesions enlarge rapidly during fruit maturation prior to harvest and lead to an outbreak of brown fruit rot (Weber et al., 2008). The most strongly affected apple varieties are those retaining their aborted fruits as fruit mummies, like 'Elstar', 'Gerlinde' and 'Dalinbel'. Leaf infections appear as brown necrotic spots with a purple halo (frogeye spots).

In 2011 a three-year project was initiated at the ESTEBURG Fruit Research and Advisory Centre. The aim was to develop disease control strategies against black rot (*D. seriata*) and fungal storage rots by testing different preventive spraying programs and the manual removal of fruit mummies as a possible sanitation measure. Aspects of the infection biology of *D. seriata* were also examined, notably a characterisation of meteorological events leading to spore release and fruit infections.

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Material and Methods

An on-farm trial was established in an organic apple orchard near Hamburg-Neuenfelde within 2 x 2 rows of the variety ‘Gerlinde’ (planted in 2000 at a distance of 1.0 x 3.5 m; 2.5 m canopy height). The treatments were replicated three times along the length of the row with twelve trees per replicate (block design). Evaluations were made using the central trees in the inner rows, giving 10 trees per replicate (60 trees per variant). Six different treatments were tested (Table 1), each block being dedicated to the same treatment in each of the three years of the trial.

Table 1: Details of treatments throughout the trial (2011-2013)

No.	Treatment	Application rate	Date		
			2011	2012	2013
1	Untreated control	-	-	-	-
2	Fruit mummy removal	(i) before bud burst (ii) after June drop	29.03. 29.06.	29.02. 02.07.	06.03. 08.07.
3	Fruit mummy removal + Myco-Sin	4 kg ha^{-1} m^{-1} can. ht.	as in No.2 and 6	as in No.2; and 6	as in No.2 and 6
4	Fruit mummy removal + Lime sulphur	7.5 l ha^{-1} m^{-1} can. ht. (2011) or 6 l ha^{-1} m^{-1} can. ht. (2012, 2013)	as in No.2 and 5	as in No.2 and 5	as in No.2
5	Lime sulphur	7.5 l ha^{-1} m^{-1} can. ht. (2011) or 6 l ha^{-1} m^{-1} can. ht. (2012, 2013)	08.07. 15.07. 25.07.	12.07. 20.07.	-
6	Myco-Sin	4 kg ha^{-1} m^{-1} can. ht.	08.07. 18.07. 25.07. 03.08. 12.08. 29.08.	13.07. 20.07. 31.07. 09.08. 21.08. 06.09.	19.07. 26.07. 05.08. 15.08. 29.08. 13.09.

m can. ht. = metre canopy height = height of the tree - height of the trunk

In treatments 2, 3 and 4, fruit mummies were removed manually twice each year, *i.e.* before bud burst and again at the end of the June drop period; fruit mummies were always disposed of outside the orchard. Spray applications commenced in early July, using lime sulphur and the plant strengthener Myco-Sin (based on acidic clay soil, aluminium sulphate and plant extracts). Myco-Sin (4 kg ha^{-1} m^{-1} canopy height) was applied regularly at 7-10 d intervals or just before rainfall on altogether six dates every year, the last application being between the first and second fruit pickings. Lime sulphur sprays were timed to cover impending infection periods of *D. seriata* (high temperature + rainfall). In 2011 and 2012, three and two applications (respectively) were made until end of July, as determined by registration details for apple scab and recommended pre-harvest intervals. Due to a lack of rainfall in July 2013, lime sulphur was not applied in that year. All crop protection compounds were applied with a hand-held spray device, permitting a water flow rate of 500 l ha^{-1} m^{-1} canopy height.

Fruit infestation caused by *D. seriata* was evaluated on 60 trees per variant with 100 fruits per tree at harvest. For examination of storage rots, fruits of the second picking (900 apples per variant) were placed in a cold store (>90 % r.h., 2°C) from September until April, and evaluated on three dates during this period. Infected fruits were isolated and further incubated until the production of spores permitted a microscopic identification of causal fungi.

The release of spores (conidia) from fruit mummies was quantified in response to rainfall. Between June and September, five small containers were placed on the trees before rainfall. Each container was covered with a coarse (1 mm) mesh to which five fruit mummies were attached. Spores released from fruit mummies during rain were quantified by microscopic examination of the collected fluid. Likewise, the presence of *D. seriata* and other pathogens on fruit mummies removed from the trial orchard was determined (Weber, 2012).

Results

In all three years of the trial, the release of conidia was associated with rainfall exceeding 5 mm, higher spore releases being associated with higher rainfall. If these conditions coincided with a mean daily temperature above 15°C, fruit infections were observed as typical minute black spots about 5-7 d after the event. An example of these observations is given in Fig. 1 for the 2012 season.

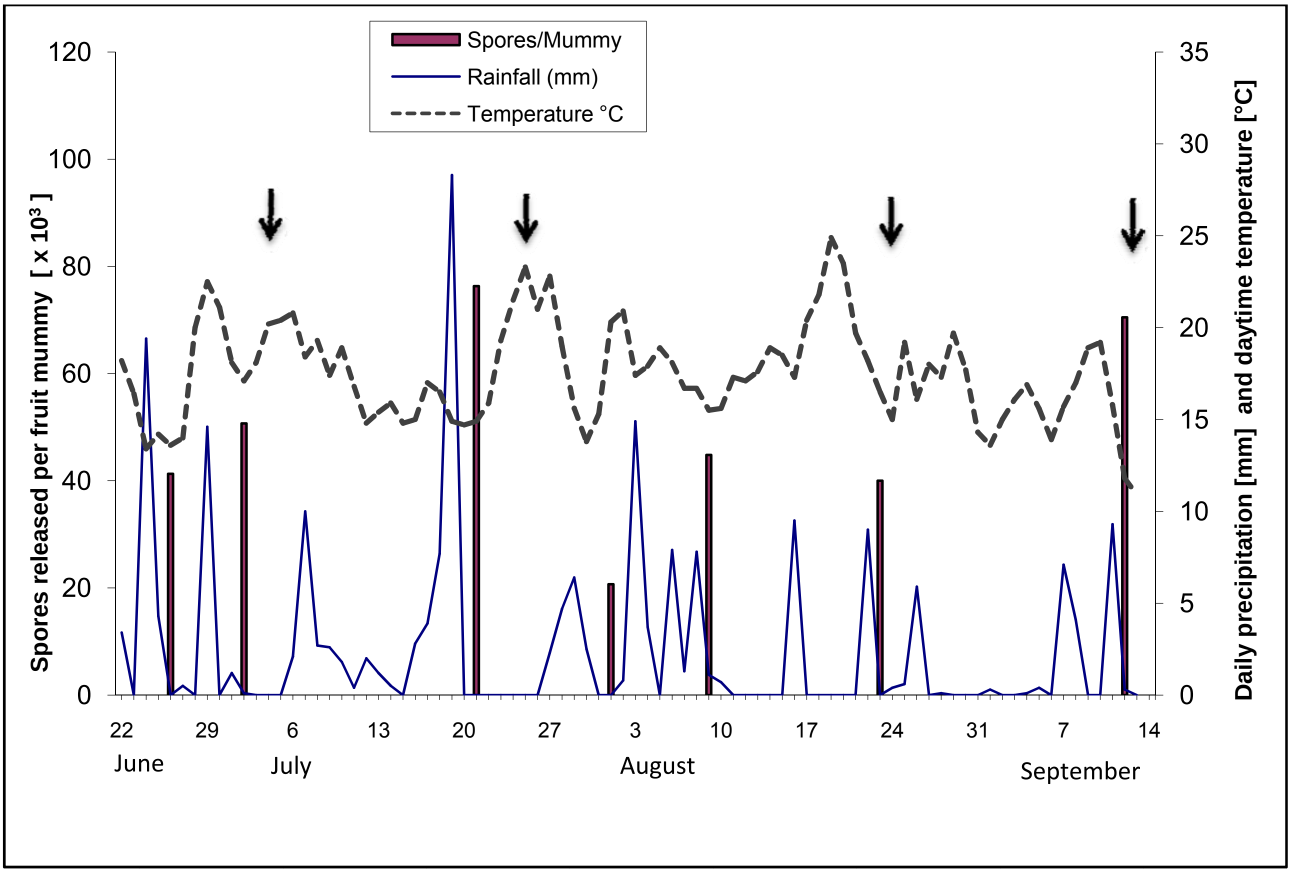

Figure 1: Daily rainfall, average daily temperature, release of conidia and reconstructed infection dates (arrows) in the field in 2012.

In 2012, rainfall events exceeding 10 mm at the end of June and in August caused a moderate spore elution of up to 50.000 conidia per fruit mummy. Optimal infection conditions were recorded on 29 June and 22 Aug., and new infections were clearly associated with these dates. Yet higher rainfall (30 mm) on 19 July led to a spore release of 76.000 conidia per mummy.

Of the fruit mummies removed from the trial in early spring 2012 and 2013, 53.2 % and 59.0 % (respectively) were colonised by *D. seriata*, whereas 38.9 % and 18.3 % (respectively) were colonised by *Neofabraea alba*.

In the three years of this trial, fruit infestation caused by *D. seriata* on untreated control trees were 4.2 %, 2.6 % and 7.5 % (Table 2). In 2012, infection levels were too low to permit any statistical analyses of variance. All plant protection treatments reduced the incidence of black rot. The removal of fruit mummies, either as a stand-alone treatment or in combination with Myco-Sin or lime sulphur applications, consistently gave the highest control levels. In 2011 and 2013, fruit infestation was significantly reduced by removal of fruit mummies alone, giving efficacies of 76.2 % and 80.0 %, respectively. Lime sulphur failed to yield any significant effect either on its own or as an addition to fruit mummy removal, whereas Myco-Sin produced a significant effect on its own in 2013 (Table 2).

Table 2: Incidence of pre-harvest fruit infections by *D. seriata* and efficacies of different treatments (calculated according to Abbott, 1925) in the three years of the trial. Statistically significant differences were calculated using the Tukey test (α = 0.05).

Treatment	2011		2012		2013	
	Fruit infestation [%]	Efficacy [%]	Fruit infestation [%]	Efficacy [%]	Fruit infestation [%]	Efficacy [%]
Untreated control	4.2 a	-	2.6 a	-	7.5 a	-
Fruit mummy removal	1.0 b	76.2	0.9 a	64.5	1.5 b	80.0
Fruit mummy removal + Myco-Sin	1.5 ab	64.3	0.6 a	76.9	1.7 b	77.3
Fruit mummy removal + Lime sulphur	1.4 ab	66.7	0.6 a	76.9	2.0 b (no lime sulphur)	73.3
Lime sulphur	4.0 a	4.8	1.8 a	30.8	-	-
Myco-Sin	1.9 ab	54.8	0.6 a	76.9	1.7 b	77.3

For statistical analysis, data expressed in percentages were arcsin(radq(x/100))-transformed.

An evaluation of the 2011 and 2012 harvests for storage rots is presented in Table 3. In 2011 and 2012, a total of 20.4 % and 13.3 % (respectively) of fruits from untreated control trees were spoiled by storage rots. Myco-Sin alone and/or in combination with fruit mummy removal was able significantly to reduce the incidence of postharvest fruit rot. The effects of lime sulphur applications or of fruit mummy removal were not significant.

Table 3: Occurrence of spoilage due to storage rots in the 2011 and 2012 harvests. Statistically significant differences were calculated using the Tukey test (α = 0.05).

Treatment	**Infestation [%]**	
	2011	**2012**
Untreated control	20.4 a	13.3 a
Fruit mummy removal	15.6 ab	14.3 a
Fruit mummy removal + Myco-Sin	7.9 b	6.4 b
Fruit mummy removal + Lime sulphur	11.3 ab	12.6 ab
Lime sulphur	12.2 ab	10.6 ab
Myco-Sin	7.8 b	8.4 ab

For statistical analysis, data expressed in percentages were arcsin(radq(x/100))-transformed.

Most storage rots (50-60 %) were caused by *Neofabraea* spp., *N. alba* being the dominant species. Other pathogens were identified as *Neonectria galligena*, *Penicillium expansum*, *Botrytis cinerea* and *Monilinia fructigena*. However, only *N. alba* was significantly reduced by the combination of fruit mummy removal and Myco-Sin applications or by Myco-Sin alone. The share of fruits infected by *N. alba*was 11.5 % in 2011 and 7.9 % in 2012, and this was reduced to 3.5 % and 3.1 % (respectively) by Myco-Sin applications and fruit mummy removal (Table 4). The infrequent occurrence of the other pathogens prevented any statistical evaluation of their suppression by any of the treatments included in this trial.

Table 4: Occurrence of different fungi on stored fruits of the 2011 and 2012 harvests. Statistically significant differences were calculated using the Tukey test (α = 0.05).

Treatment	Occurrence of fungal pathogens [% of harvested fruits]											
	Neofabraea alba		***Neofabraea perennans***		***Neonectria galligena***		***Botrytis cinerea***		***Penicillium expansum***		***Monilinia fructigena***	
	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012	2011	2012
Untreated control	11.5a	7.9a	0.0	0.4	3.2	1.4	2.2	0.9	1.5	1.2	1.7	1.7
Fruit mummy removal	7.9ab	7.7ab	0.4	0.6	3.5	3.0	1.7	0.8	0.8	0.1	0.8	2.0
Fruit mummy removal + Myco-Sin	3.5b	3.1b	0.2	0.0	0.7	0.3	1.5	0.6	0.4	0.9	1.6	1.4
Fruit mummy removal + Lime sulphur	6.4ab	7.7ab	0.9	0.3	2.3	1.1	0.4	1.2	0.5	0.2	0.5	1.7
Lime sulphur	8.7ab	7.2a	0.8	0.1	0.8	1.1	0.3	0.3	0.7	0.3	0.7	0.9
Myco-Sin	5.2ab	4.5b	0.0	0.4	0.4	0.3	0.1	1.3	1.0	0.6	0.4	1.3

For statistical analysis, data expressed in percentages were arcsin(radq(x/100))-transformed.

Discussion and conclusions

In 2011-2013, black rot of apples (*D. seriata*) caused fruit losses up to 7.5 % in untreated control trees. These results corroborate field observations of a strong coincidence between the retention of fruit mummies by different apple varieties and their tendency to develop black rot (Holmes & Rich, 1970; Quast & Weber, 2008). The manual removal of fruit mummies, either as a stand-alone treatment or in combination with Myco-Sin or lime sulphur applications, was able significantly to reduce these infections. Effects comparable to fruit mummy removal were achieved by spraying with Myco-Sin from July until harvest, while applications of lime sulphur were not effective. The combination of fruit mummy removal and Myco-Sin was also the most effective strategy against storage rots, especially those caused by *N. alba*. Efficacies of approx. 50 % for Myco-Sin were comparable with previous work (Palm & Kruse, 2012).

A correlation between high temperatures, rainfall and existing spore potential was consistently observed in our trial, the extent of spore release corresponding well with the intensity and amount of preceding rainfall. If suitable temperatures were present, periods of rainfall gave rise to new infections on the developing fruits. In July and August 2011, several weeks of daily rainfall combined with single days of elevated temperatures caused fruit losses of 4.2 % at harvest, whereas in summer 2013 long periods of high temperature with single days of intensive rainfall led to a fruit infestation of 7.5 % in untreated control trees.

Especially in the first year, the manual removal of fruit mummies was associated with a high expenditure of manual labour amounting to 60-100 h ha^{-1} (L. Brockamp, unpublished). In our trial, the manual removal was conducted twice, i.e. once in early spring before bud burst, and again after the June drop period. In order to achieve a more favourable balance between input of work and efficacy, a thorough single fruit mummy removal in February/March may be sufficient for practical purposes. Our results to date indicate that this must be carried out by hand because current mechanical devices are unable to remove much more than 50 % of fruit mummies present on trees.

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