

Unmasking the black spot mystery in organic apple cultivars and the path to effective control strategies

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Abstract

*In recent years, organic apple orchards have been affected by mysterious black, silver-centered spots, primarily on the sun-exposed side of the fruits. Research confirmed *Elsinoë pyri* as the causal pathogen using molecular identification. To investigate infection timing, window trials were conducted in 2024 and 2025, applying treatments during specific periods. Results of 2025 indicated that treatments in April-May-June are most effective, with May being critical, and that three-month treatment blocks outperform single-month applications. Symptoms were more frequent on sun-exposed sides and on upper leaves of the shoots. These findings provide a basis for effective control strategies, while further research will explore the influence of weather events and the possible presence of the pathogen in flower- and or leaf buds.*

Keywords: *Elsinoë pyri*, genetic identification, window trials, infection period

Introduction

In recent years, organic apple orchards have been suffering from mysterious black spots, predominantly appearing on the sun-exposed side of the fruits. Initially, this phenomenon was observed in the recent scab-resistant variety Natyra. Different possible causes were investigated, including lenticel spot and physiological issues related to nutrient deficiencies. Various isolation attempts, however, did not yield any conclusive results.

To address this problem, a research project ('Raadselspot') was initiated aiming to uncover the underlying cause of these symptoms and to better understand the damage development. The first objective focused on the identification of a possible causal organism through molecular analyses. A breakthrough occurred in summer 2023, when the black spots had further developed into lesions containing a silver-colored center (figure 1). This damage pattern had strong resemblance to the known Topaz spot-pathogen and thus initiated a more detailed search for *Elsinoë pyri*. Similar symptoms have since been observed in other organic varieties, such as Rewena, Jonagored, Rusticana and Wurtwinning. Interestingly, this pathogen primarily causes severe infections in organic orchards, while infections in conventional orchards remain limited.



Figure 1: Black silver-centered spots on Natyra leaves and apples.

Due to the limited information available on the life- and disease cycle of *E. pyri*, the second objective of this project aims to determine the period of infection. To achieve this, a field trial was conducted in which different plots received treatments during a specific month to identify the most critical infection periods. The goal of these trials is to identify the key moments of infection and to determine the optimal timing for treatment applications.

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Material and Methods

Identification of pathogen

Apples and leaves showing black silvery-centered spots were collected for isolation. The lesions were cut out and sterilized by surface sterilization. These sterilized lesions were placed on potato dextrose agar (PDA) medium and got incubated (in the dark at 20°C for approximately 4 weeks). To obtain pure mycelial cultures of the target fungi, a mycelial plug was transferred to fresh PDA medium and incubated under the same conditions. (Chandelier, et. al., 2021)

Fungal identification was performed based on morphological characteristics and molecular analysis. For genetic identification, DNA was extracted from pure mycelium and the extracted DNA was thereafter sequenced using universal ITS primers. Additionally, direct DNA extraction from sterilized lesion were used for qPCR analysis (Chandelier, et. al., 2021).

Window trials

Two years in a row, field trials were carried out to gain more insight into the infection moment of *E. pyri*. In these trials, each object received treatment only during a specific period in time. These are called window trials, as they aim to determine the best time window to treat the orchard in order to reduce symptoms.

The first year (2024), the trial was conducted in a Rewena orchard. Each treatment month consisted of four applications of Kumulus (sulfur) at 2 kg/ha leaf wall area. For each treatment month, three replicates were included, with six trees per replicate. The trial ran from April to August. The orchard was monitored regularly and evaluations were performed when the first symptoms became visible. At two dates, both, leaves and fruits were evaluated.

Table 1: Overview of treatment schedule of second year window trial. Each colored block consists of 3 applications of Hydro Super 25WG at 0.4 kg/ha leaf wall area (0.1 kg active ingredient copper per hectare leaf wall area).

	March	April	May	June	July	August
Object 1 (untreated)						
Object 2						
Object 3						
Object 4						
Object 5						
Object 6						
Object 7						
Object 8						
Object 9						
Object 10						
Object 11						

In the second year (2025), the window trial was adapted based on the results from the first year. This trial started one month earlier and treatment blocks covering three months were added to the protocol (Table 1). For each treatment month or block, three replicates were included, with ten trees per replicate. The Trial was conducted in the variety Natyra with the product Hydro Super 25WG (copper hydroxide). In each month, the trial plots received three

treatments at 0.4 kg/ha leaf wall area (0.1 kg pure copper per hectare leaf wall area). Evaluations on leaves and fruits were again carried out from the moment symptoms became visible.

Results

Identification of the pathogen

The isolation of mycelium from infected tissue showed low success rates. Due to the slow growth of the target fungus, fast-growing fungi often contaminated the PDA plates. In addition, even when isolation was successful, DNA extraction often failed. However, after optimizing the extraction protocol, successful detections of *E. pyri* from mycelium were obtained. Nevertheless, direct DNA extraction from infected tissue in combination with qPCR analysis, as described by Chandelier, et al. (2021), showed very good success rates.

Window trials

The first window trial (2024) was evaluated in May and July, whereas a 100% infection rate was already observed in the untreated control in May. There was almost no reduction in infection following Kumulus application, regardless of the month of application. However, the efficacy based on symptom severity was 57,4% and 24,4% for the April and May application periods, respectively. By the second evaluation, these values had decreased to 38.1% for the April application period and 12.3% for the May application period (data not shown). Unfortunately, the apple yield was too low to have reliable results. Due to extremely high infection rates in this orchard, the results should be carefully interpreted. In the past, there have been no fungicidal treatments in this orchard, which explains the high infestation in combination with a very wet season. One key takeaway used for the following window trial was the indication that earlier applications can help reduce symptom severity.

The second window trial (2025) was evaluated in September, as symptom development occurred much later than during the first window trial. Both, fruits and leaves were assessed. However, due to the low infection levels on the apples, a second evaluation will be conducted after storage in March. The results of the leaf assessments are presented in Figure 2. They indicate that the three applications during May 2025 were crucial for effectively suppressing symptom development. With an efficacy of 63.3%, May clearly stood out compared to the lower efficacies observed for the other individual months. Moreover, treatments applied during the April–May–June period resulted in an efficacy of 86.4%.

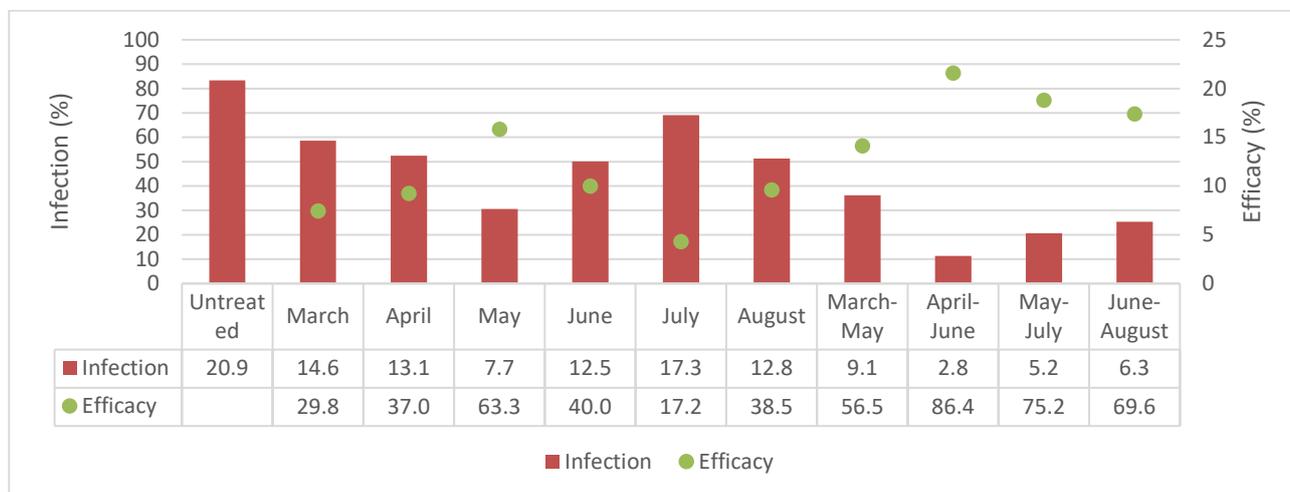


Figure 2: Results of the 2025 window trial, evaluated in September. Infection percentages (blue dots) and treatment efficacies (red bars) are presented.

In general, three-month block treatments performed better than single-month applications, with the April–May–June block showing the highest efficacy.

The results also showed 7.4% higher infection on the South side of the trees, confirming that symptoms occur more frequently on the sun-exposed side. In addition, for each shoot, the oldest five leaves and leaves from position six to the shoot tip were evaluated separately to be able to draw initial conclusions about the infection periods based on leaf growth on the long shoots. The results showed an 8% higher infestation on the leaves from position 6 onwards.

Discussion

With confirmation that *E. pyri* was indeed responsible for the mysterious black silver-centered spots on organic apples, the first steps toward management could be initiated. From the literature, it is known that the fungus requires rain and temperatures between 10°C and 26°C for infection, with an optimum around 18–22°C (Scheper et al., 2013). The fungus is presumed to overwinter on infected leaves and remaining fruits. There is also some indication that *E. pyri* may overwinter in leaf or flower buds (Chandelier et al., 2021).

It is therefore important to know when infection happens in the orchards, which was the goal of the window trials. Results of both window trials give us first indications that optimal timing of treatment may fall in the months April, May and June, with May being the most crucial month of treatment in 2025. Moreover, three-month block treatments showed more efficacy than single-month treatments.

The next step in this project is to compare the results with weather events to identify possible links with symptom development and infection periods, since 2025 was a dry year with less rain compared to 2024. In addition, buds collected from the orchard used for the window trial will be evaluated for the presence of *E. pyri*, providing better insight into the potential latent presence of the fungus.

Although the window trials provide first indications of possible infection periods, the available data remain limited and are restricted to a single growing season. Therefore, these results should be interpreted with caution and mainly serve as a basis for further experiments. Additional trials under different conditions and across multiple years are required to confirm these initial findings before practical management strategies can be formulated.

Acknowledgements

We would like to thank our local fruit growers for the good cooperation and their will to set out these trials in field and also thank CCBT (Coördinatiecentrum praktijkgericht onderzoek en voorlichting voor de biologische teelt) for financing this project

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