

Epidemiology of *Elsinoë piri*, the causal agent of Lenticel Spot in organic apple production

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Abstract

*The fungal pathogen *Elsinoë piri* has been increasingly linked to lenticel spot disease on apples (*Malus domestica*) in the Netherlands, particularly affecting late-season cultivars such as 'Natyra' ('SQ159'; integrated grown as 'Magic Star'). This disease poses a significant challenge to organically managed orchards, which appear especially susceptible. Infected fruits develop small, dark lesions localized around lenticels, primarily on the sun-exposed surfaces, resulting in a marked reduction in external fruit quality and marketability. Despite its growing economic impact, the epidemiology and life cycle of *E. piri* remain poorly understood. It is hypothesized that the pathogen overwinters on fallen leaves and mummified fruit, with spore release triggered by warm and wet conditions. To elucidate the infection biology and timing of *E. piri*, field and laboratory trials were conducted focusing on 'Natyra' apples. Additionally, the presence and incidence of the pathogen were assessed in 'SQ159' apples from both organic and integrated production systems. A deeper understanding of *E. piri*'s infection cycle is essential to develop targeted and sustainable management strategies.*

Keywords: *Elsinoë piri*, Lenticel spot, *Malus domestica*, life cycle, fungal pathogen

Introduction

Apple (*Malus domestica*) is a major fruit crop in the Netherlands, with cultivars such as 'Elstar' occupying a central position in both integrated and organic production systems. Although widely appreciated by growers and consumers, 'Elstar' is highly susceptible to apple scab caused by *Venturia inaequalis*. To reduce the need for fungicides and enhance orchard resilience, breeding programmes have introduced scab-resistant cultivars, including 'SQ159', marketed as 'Magic Star'/'Sprank' in integrated production and as 'Natyra' in organic systems. This cultivar is a crossbreed of 'Elise' and a scab resistant WUR-selection (Fresh Forward, 2025).

From 2022 onwards, symptoms of black lenticel spotting have been increasingly observed in 'Natyra' apples as well as leaves. These dark, localized lesions are often characterized by a white centre and compromise external fruit quality which leads to considerable economic losses, as affected apples are frequently downgraded to juice-grade. The symptoms are present mostly on the sun-side of the apples and resemble Topaz-spot, a blemish associated with the fungal pathogen *Elsinoë piri* (Korsgaard et al., 2014). Little is known about the precise life cycle of this pathogenic fungus. It is believed to overwinter in orchards on fallen, infected leaves and unharvested fruit, and to release its spores in spring (Scheper et al., 2013). The pathogen favours warm and humid conditions for successful infection. However, this particular fungal strain is difficult to cultivate in pure culture (Chandelier et al., 2022; Fan et al., 2017). Consequently, its presence on 'Natyra' may need to be assessed using DNA-based detection methods.

The study aims to investigate the causal agent associated with black lenticel spots in emerging apple cultivars, with a specific focus on 'Natyra'. To this end, we conducted fungal isolations in both organic and integrated orchards, evaluated pathogenicity through Koch's

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postulates, analysed fungal communities present in rinse-water samples, and assessed the efficacy of sulphur-based treatments. Together, these efforts contribute to a better understanding of the disease and provide a basis for developing strategies to reduce the incidence of black lenticel spots in apple cultivars like the 'SQ159'.

Material and Methods

Fungal Isolation and Identification

Symptomatic and asymptomatic 'SQ159' apples were collected from 17 organic and 18 integrated orchards. To isolate the causal agent, symptomatic fruits were rinsed with sterile water, lesions/black spots were sprayed with 70% ethanol until droplet runoff, the skin was removed aseptically with a scalpel, and tissue under the lesion was isolated and placed onto potato dextrose agar (PDA; Oxoid, U.K.) after which the isolations were incubated at 20 °C in the dark. Fungal morphotypes were sub-cultured, and DNA was extracted (LGC Mag Plant Kit). Isolates were identified through ITS sequencing (ITS1/ITS4), and when necessary additional genetic markers TEF1, LSU, and RPB2 were amplified. Sequencing was performed by Baseclear. Koch's postulates were fulfilled by inducing lenticel spot symptoms through drop inoculation of clean 'Natyra' apples with a 2×10^4 conidia/mL spore suspension of isolated *E. piri* strains. Following symptom development, the isolates were re-isolated, and the identity of *E. piri* was confirmed through sequencing.

Apple Rinse Water (Filter) Analysis

Symptomatic and asymptomatic 'SQ159' apples were rinsed in 1.5 L of water containing Tween for 30 minutes. The rinse water was then filtered using a vacuum pump, after which the filters were frozen and subjected to DNA extraction. The extracted DNA was subsequently analysed by qPCR targeting *E. piri*.

Sulphur Field Trial

Orchard Sites

In 2024, the efficacy of sulphur against lenticel spot development on 'Natyra' was tested in three organic orchards with severe *E. piri* infections in 2023. The orchards were located in the provinces Zeeland and Noord-Brabant and all consisted of 'Natyra' trees on M9 rootstock with planting years 2015 and 2021 in Zeeland and 2016 in Noord-Brabant. Each orchard contained eight plots arranged in a randomized complete block design with four treated and four control plots. In each plot one central row was monitored. Five trees per row were selected, and on each tree ten fruits and ten leaves were labelled for repeated assessment.

Spray Programme

All orchards received wettable sulphur during the primary scab season. From 15 May onward, sprays were stopped in control plots, while treated plots continued with 5 kg ha⁻¹ ThiovitJet every ten days between 1 June and 15 July. Fruits and leaves were assessed eight times between June and October, with 400 fruits and 400 leaves assessed each time. Monitoring data was used to calculate infection degree and sulphur efficacy.

Results

Fungal Isolation and Identification

Isolations from organic apples yielded *E. piri* as the most dominant species, whereas in integrated apples *E. piri* was not detected. In these integrated samples, no single fungal species was clearly dominant, although taxa such as *Stemphylium vesicarium* and *Alternaria alternata* were frequently isolated. Identification of *E. piri* required LSU, PBR2, TEF and ITS3/4 markers, as ITS1/4 failed to amplify these isolates. The identity of the causal agent, *E. piri*, responsible for lenticel spots on 'Natyra', was confirmed through Koch's postulates as described in the Materials and Methods.

Apple Rinse Water (Filter) Analysis

The qPCR of the rinse water resulted in the detection of *E. piri* in only the organic apples, *E. piri* was not found in the rinse water of integrated grown 'SQ159'. This confirms consistent differences between production systems.

Sulphur Field Trial

The effect of continued wettable sulphur application after the primary scab infection period was assessed by comparing treated and untreated 'Natyra' plots across three orchards for the presence of *E. piri* symptoms. In all control plots, the percentage of symptomatic apples and leaves increased over the growing season, with the highest infection in apples reaching 36%. Sulphur applications significantly reduced the total number of apples with lenticel spots in orchards 1 and 2. After treatment, the percentage of apples with lenticel spots was reduced to 7% in orchard 1 and 10.5% in orchard 2, corresponding to an overall efficacy of 62–64% in preventing lenticel spot formation. Leaf-spot efficacy was lower and less consistent, with only orchard 2 showing a statistically significant reduction. In contrast, orchard 3 showed no significant treatment effect on either apples or leaves, with an efficacy of only 11% in preventing lenticel spot formation on apples, indicating a possible different disease dynamic at that site.

Discussion

This study shows that *E. piri* is the causal agent of black lenticel spots on 'Natyra', which was consistently detected in organic apples. This causal role of *E. piri* was confirmed through Koch's postulates. Integrated 'Magic Star'/'Sprank' fruits also exhibited black spots, however these spots were less frequent and differed in appearance. Rinse-water analyses likewise showed that *E. piri* was present only in organic samples and absent in integrated ones. System-specific differences in fungal communities suggest that lower fungicide use in organic orchards may favour *E. piri*, while integrated management suppresses it or promotes other fungi such as *S. vesicarium* and *A. alternata*.

Observations indicate that the disease is not restricted to 'Natyra' but also occurs in several other organically grown cultivars, such as 'Topaz', 'WUR28' and 'Santana', suggesting a wider relevance for organic apple production.

Sulphur treatments reduced symptom spread in two orchards but were ineffective in a third orchard, reflecting differences in local spraying regimes and disease pressure. Where infection pressure was moderate, sulphur substantially suppressed lenticel spot development, whereas in orchard 3 low infection pressure overrode the protective effect. Overall, lenticel spots caused by *E. piri* in 'Natyra' and other cultivars appears to be shaped by pathogen presence, orchard system, and site-specific factors, highlighting the need for tailored management, continued monitoring, and further study of *E. piri* epidemiology.

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