”Topaz-spot” in apple identified as “Elsinoe leaf and fruit spot” (*Elsinoe pyri* (Wor.) Jenkins)

M. Korsgaard[1], S. E. Glazowska[2], M. Schiller[2], P.R. Johnston[3] and O. S. Lund[2]

Abstract

*A fungal-like apple disease, known as “Topaz-spot” in Europe, appeared in Denmark in the year 2000. It has now spread throughout Denmark as a problem in untreated, organic production of apple (*Malus domestica*). The causing organism of the symptom “Topaz spot” has been identified as the ascomycete* Elsinoe pyri *(Wor.) Jenkins. The fungus was identified by pyrosequencing analysis of DNA and by isolation of* E. pyri *from infected apple skin (Glazowskaet al., 2013).*

Symptoms of E. pyri, called “Elsinoe leaf and fruit spot”, appeared as numerous brown spots on leaves and black spots on fruits. Leaf spots were typically 0.5-4 mm in diameter and fruit spots 2-5 mm, both with a pale centre surrounded by a dark halo.

To our knowledge this is the first time Elsinoe pyri has been associated with “Topaz spot”.

The apple cultivar ‘Topaz’ is susceptible to E. pyri, but other cultivars are even more susceptible. A survey of 85 apple cultivars in 11 unsprayed Danish organic orchards was carried out in 2013. The disease was present in all orchards throughout the country and in all but two cultivars. Infections of the cultivars of “Elsinoe leaf and fruit spot” on leaves ranged from 0 to100 %.

Keywords: *Elsinoe pyri,* Topaz spot, organic apple production, apple cultivars, apple disease

Introduction

Black spots on apples appeared in Denmark around the year 2000. At first, the symptom appeared in the cultivar ‘Prima’ in two unsprayed organic orchards on Zealand and Funen. It was believed at that time, that the spots were a kind of resistance reaction to Apple Scab, caused by *Venturia inaequalis*. In 2003, it was found again in the cultivar ‘Topaz’ in an organic, unsprayed orchard in Southern Jutland. In 2008 also the cultivar ‘Rubinola’ showed the symptoms in an unsprayed organic orchard on Zealand. The symptoms seemed to appear only in cultivars with Vf-scab resistance.

The spots were known in Europe as “Topaz-spot” (Anon. 2011), as symptoms mainly were found in the Vf-resistant cultivar ‘Topaz’ (Trapman & Jansonius 2008).

In Denmark, since 2009 the Topaz-spots also appeared in apple cultivars with no Vf-resistance like the cultivar ‘Red Aroma’, which is extensively produced throughout Scandinavia. In 2012, more than 95 % of ‘Red Aroma’ apples were ruined by Topaz-spots in an unsprayed Danish organic orchard (Korsgaard, 2012). The potential losses in private – usually unsprayed – gardens are also large. Topaz-spot may cause great economic losses in unsprayed orchards, as the spotted apples can only be used by the industry for processing into juice and other products. Topaz-spot has so far not been a problem in orchards using fungicides, and it was thus expected that the causal organism is a fungal

[1] mak@ecoadvice.dk, Gefion, Fulbyvej 15, DK-4180 Soroe, Denmark
[2] Schiller@plen.ku.dk; glaz@plen.ku.dk; osl@plen.ku.dk, University of Copenhagen, Faculty of Science, Department of Plant and Environmental Sciences, Hoejbakkegaard Allé 13, DK-2630 Taastrup, Denmark
[3] JohnstonP@landcareresearch.co.nz,Landcare Research, Private Bag, 92170 Auckland, New Zealand

species. However, this fungal species has only recently been identified, and its prevalence on different apple cultivars in Denmark has not previously been surveyed.

Materials and methods

In 2013 we identified the fungal species causing Topaz-spots in apple and started evaluating the susceptibility of a large number of apple cultivars to Topaz-spot.

The identification was initially performed by purification of total DNA from apple fruit skin of two cultivars, 'Pigeon fra Juellinge' and 'Rifbjerg Skarlagen Pearmain' grown in Denmark near Copenhagen, with severe symptoms of Topaz-spot. Fungal DNA was analysed by barcode pyrosequencing (MWG-Biotech, Germany) using polymerase chain reaction (PCR) primers targeting the ribosomal *ITS2* region of ascomycetes (Louarn *et al.,* 2012). Fungal isolates, from Topaz-spot infected epidermal fruit tissue of the cultivars 'Ahrista' and 'Rifbjerg Skarlagen Pearmain', were recovered on potato dextrose agar and subjected to DNA extraction for PCR amplification and sequencing. The PCR primers ITS1F and ITS4 were used to target amplification of the two internal transcribed spacers (*ITS1 and ITS2*) including the 5.8S unit, located between the genes encoding the small and large ribosomal subunits (18S and 28S rDNA).

A survey of 85 apple cultivars was carried out in 2013. The evaluation of the susceptibility to Topaz-spot in apple cultivars took place in 11 unsprayed orchards, located in different regions throughout Denmark. All cultivars in each orchard were evaluated by including 15 trees per cultivar, evenly distributed in the orchard. The proportion of leaves attacked by Topaz spot was scored on a scale from 0-100 %. Two of the orchards were museum collections comprising only one or two trees of each cultivar. In these orchards the data collection was reduced accordingly.

Results

Identification and morphology of the causal species:

The fungal species causing Topaz-spot was initially identified by pyrosequencing analysis of DNA and then verified by isolation of the fungus from infected apple skin.

More than 15 fungal species were identified by pyrosequencing analysis, but only a single DNA sequence of 182 bp was found to show both consistent and specific accumulation in all symptomatic skin samples (n=6). A BLAST search in the GenBank non-redundant nucleotide database revealed 100 % identity to the *ITS2*rDNA part of the *ITS1-5.8S-ITS2* sequence of two *Elsinoe pyri* isolates, ICMP 18257 and ICMP 18437, from New Zealand (GenBank Accession Nos. KC626006, KC626007).

Two independent fungal isolates were recovered from Topaz-spot infected epidermal fruit tissue of the cultivars 'Ahrista' and 'Rifbjerg Skarlagen Pearmain'. After 7-14 days, light brown, wrinkled, stromatic colonies were formed, later becoming red to dark brown, with a growth rate of approximately 4 mm per week. Chain-like hyphae were observed within the medium at the edges and underneath the stromatic tissue. Conidia were hyaline, oblong to clavate, measuring 2-3.6 x 4.5-9 µm (n=50) (Figure 1). Sequencing of the *ITS1-5.8S-ITS2*rDNA of these isolates resulted in two identical 628 bp sequences (GenBank Accession Nos. KC928079, KC928080) with 99 % identity to the *ITS1-5.8S-ITS2* sequence of the *E. pyri* isolates from New Zealand.

The fungal species responsible for the "Topaz-spot" symptoms was identified as the ascomycete *Elsinoe pyri* (Wor.) Jenkins (Glazowska *et al.*, 2013).

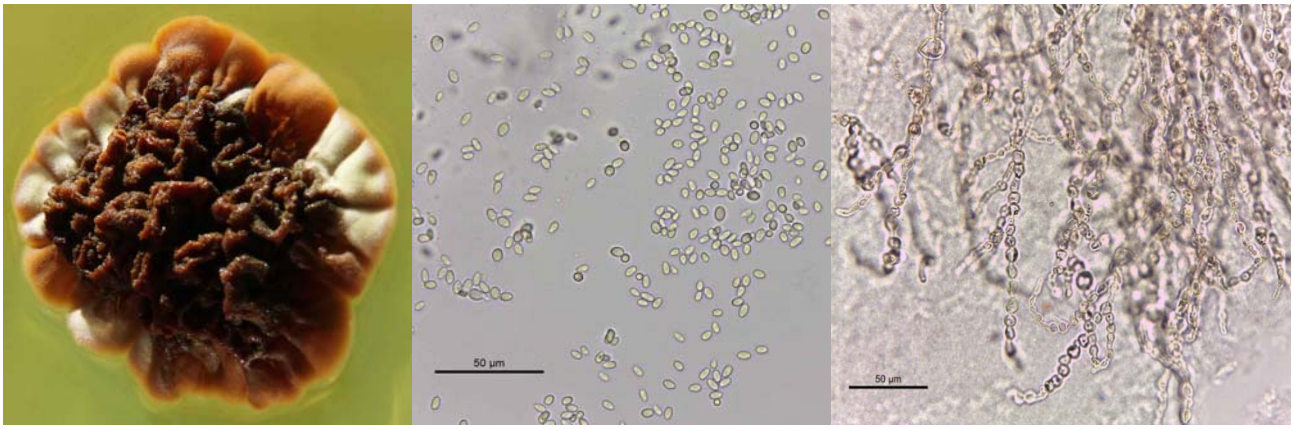

Figure 1: Colony, conidia spores and hyphaes of *Elsinoe pyri.* Photos by Korsgaard and Glazowska.

Disease symptoms

The symptom of *E. pyri* is known as “Elsinoe leaf and fruit spot”. Elsinoe leaf and fruit spot appeared as numerous brown spots on leaves and black spots on fruits (Figure 2). Their typical sizes were 0.5-4 mm in diameter on leaves and 2-5 mm on fruits, both with a pale, silvery centre surrounded by a dark halo. Symptoms occurred mainly on fruits and leaves exposed to sunlight, suggesting that this fungus has a low requirement for humidity and/or a large need of light.

Prevalence of the disease

Apple cultivars with Vf-scab-resistance like ‘Topaz’, ‘Prima’ and ‘Rubinola’ were susceptible to *E. pyri*, but other cultivars were even more susceptible to *E. pyri*. The disease was present in all orchards throughout the country and in all cultivars, except for the two old Danish cultivars: ‘Annasæble’ and ‘Bodilsæble’. Both cultivars are grown in a very small scale, resulting in very few observations. The Elsinoe leaf and fruit spot infection level in leaves of different cultivars varied between 0 and 100 %. The infection level of the 24 most frequently produced cultivars in the11 organic orchards investigated in details are shown in Figure 3. In the cultivars ‘Cox Orange’, ‘Jonagored’, ‘Otava’, ‘Ingrid Marie’ and ‘Gravenstein’ more than 70 % of the leaves were infected by Elsinoe leaf and fruit spot. In the cultivars ‘Ritt Bjerregaard’, ‘Bodil Neergaard’, ‘Resista’, ‘Alkmene’, ‘Holsteiner Cox’, ‘Pigeon’, ‘Discovery’ and ‘Fredrik’ less than 20 % of the leaves were infected by Elsinoe leaf and fruit spot.

Figure 2: Typical symptoms of *Elsinoe pyri* on fruit and leaves of apple. Photo on left was taken in year 2000 of the disease on 'Prima'. Photo on the right is from 2012 in the cultivar 'Red Aroma' showing Elsinoe spots on both fruit and leaves.

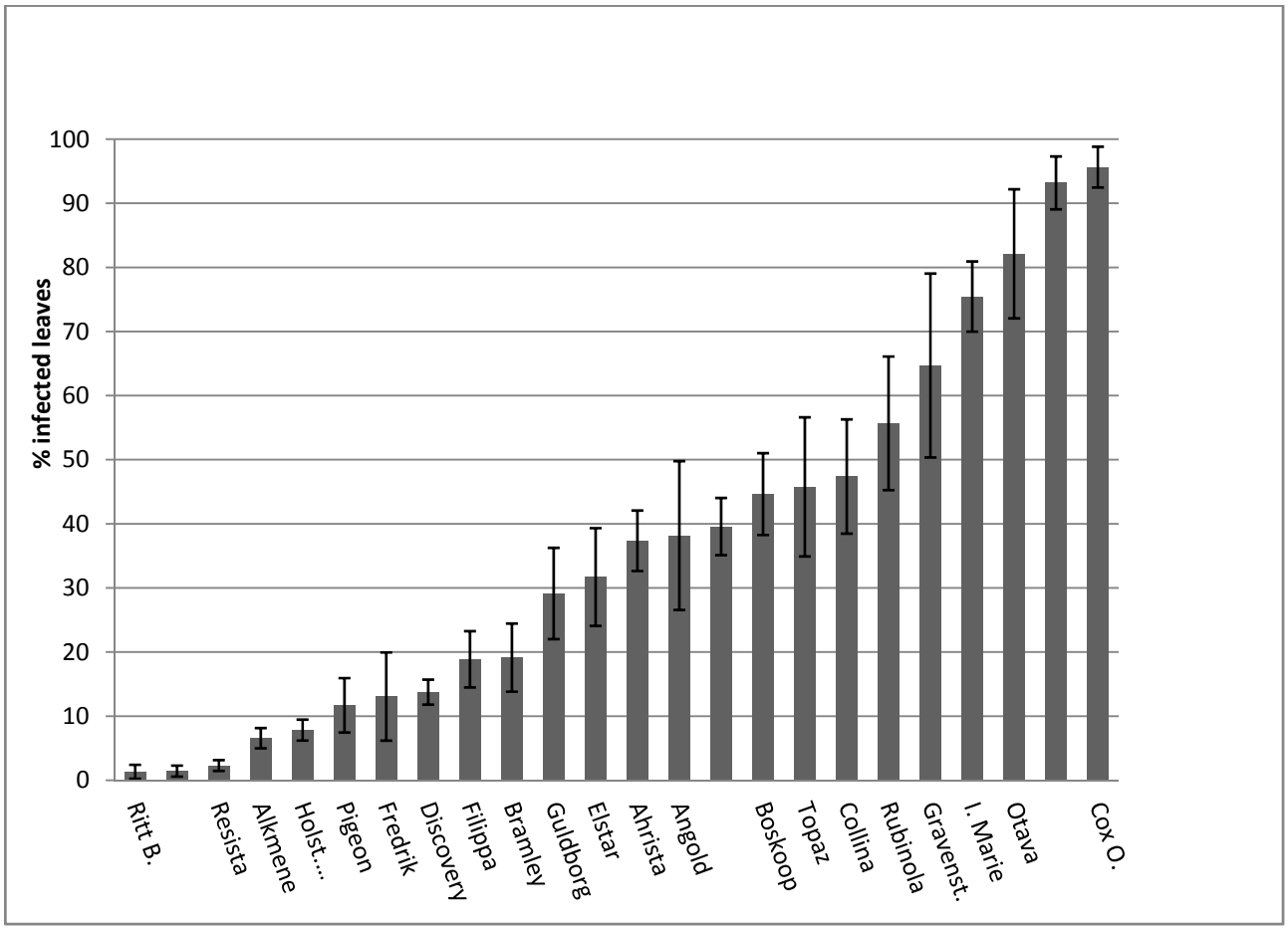

Figure 3: Susceptibility to "Elsinoe Leaf and Fruit spot" in 24 apple cultivars. Data collected in 2013 from 11 Danish organic orchards, where no fungicides were used. (Bars showing 95 % confidence)

The relation between rainfall and temperature in May, June and July and the level of leaf-infection by Elsinoe leaf and fruit spot was investigated. The rainfall varied between 70 mm at the driest location Fejø to 213 mm at the wettest location Broager. The cultivar 'Red Aroma' was present in 10 orchards, and 'Holsteiner Cox' and 'Discovery' in 8 orchards.

The infection level in the cultivar 'Red Aroma' and 'Discovery' did not correspond to the level of rainfall or temperature. In 'Holsteiner Cox' there was a tendency that the level of infection of Elsinoe leaf and fruit spot was higher at locations with more rainfall (Figure 4).

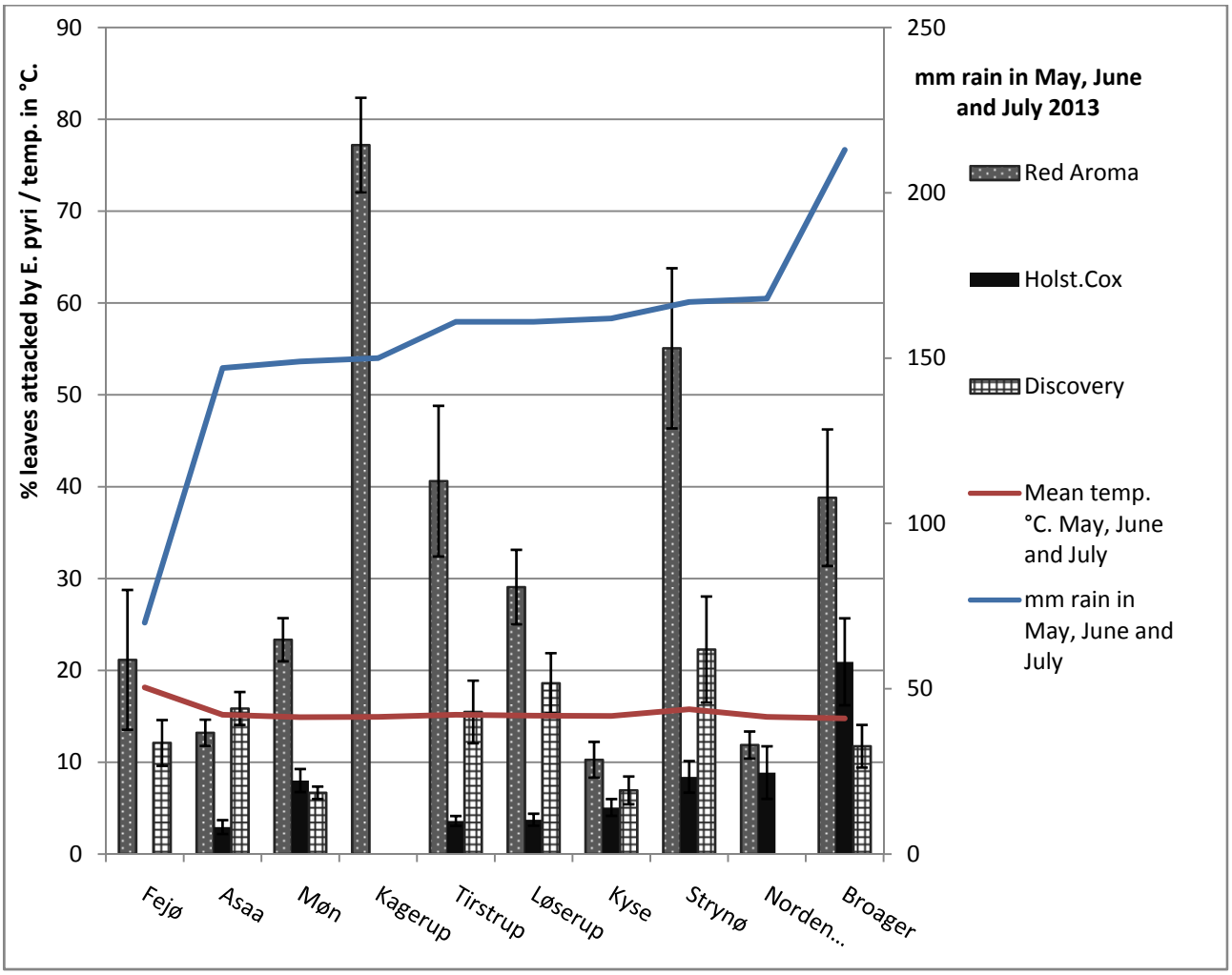

Figure 4: The percentage of leaves showing "Elsinoe leaf and Fruit spots" in the apple cultivars 'Red Aroma', 'Holsteiner Cox' and 'Discovery'. Data collected from 10 unsprayed, orchards located in different regions across Denmark. The precipitation and mean temperature in May-July 2013 are shown for each location.

Discussion

The ascomycete *Elsinoe pyri* (Wor.) Jenkins was identified as the fungal species responsible for the Topaz-spot symptoms. The symptom of *E. pyri* has historically been known as "Elsinoe leaf and fruit spot", and to our knowledge, this is the first time *E. Pyri* has been associated with the Topaz-spot disease. It is important, that we now know these two diseases to be due to the same pathogen.

E. pyri was first described in Russia by Nikolai Nikolaevich Woronichin in 1914 as a species of *Plectodiscella* (Woronichin, 1914).In 1932, the American mycologist Anna E. Jenkins transferred it to the genus *Elsinoë*, changing the name from *Plectodiscella piri* to

Elsinoë piri (Wor.) Jenkins, and the anamorph stage: *Sphaceloma pirinum* (Pegl.) Jenkins (Jenkins, 1932; Jenkins & Horsfall, 1929). In this paper we have used the New Zealand modern way of spelling: *Elsinoe pyri*.

Jenkins (1932) reported the fungus to be present on apples imported from Italy, Switzerland, Ireland, Hungary and Portugal to America. Later Jenkins (1943) reported *E. pyri* in apples in the states of Oregon and Washington and in 1946 she reported *E. Pyri* in apples imported from France and Spain (Jenkins, 1946). Thus, *E. pyri* was apparently common in Europe in the 1930-1940's and in New Zealand the disease was first reported in 1954 (Atkinson, 1971). Nevertheless, this disease has been very sparsely investigated. Because *E. pyri* is not a problem in fungicide treated orchards, this might explain the low interest in the disease.

Recently, *E. pyri* has been studied in New Zealand. Scheper *et al.* (2013) found that conidia of *E. pyri* germinated at all temperatures between 10°C and 26°C, with an optimum greater than 20°C. They also found that the first spots in inoculated leaves became visible 20 days after inoculation, and that these spots developed into typical Elsinoe spots six weeks after inoculation.

However, we still need more information about this fungus and its life cycle. Jenkins presumed that it infects not only the leaves and fruits but also the twigs (Jenkins, 1932). We still lack proof of that, and we need to know where it over winters. How does it develop? What are the climatic and environmental needs for distribution, infection and development of the fungus? Does it produce mycotoxins? What are the interactions between *E. pyri* and other microorganisms on the apple skin?

Answers to these questions are important in order to develop methods to control this disease, with or without the use of biological or organically approved fungicides.

Although the disease has been reported to infect both apple and pear (Woronichin, 1914; Jenkins, 1932), we have not observed any symptoms of *E. pyri* in pears in the investigated orchards. It would be interesting to know more about the incidence of the Elsinoe leaf and fruit spot disease in the cultivars of pears grown today.

The evaluation of the 85 cultivars of apple showed large differences in their infection level. The frequent occurrence of *E. pyri* in Vf-resistant cultivars is more likely due to the reduced use of fungicides in these cultivars, masking the higher susceptibility of other cultivars. These data were obtained in one year only, and they do not yet show the entire picture of the susceptibility of the cultivars to *E. pyri*. Differences in levels of infestation, climate and management between the orchards may also affect the level of infection. In 2013 it was noticed that vigorous growth induced by strong pruning seemed to reduce the infection level of *E. pyri* in several cultivars.

The survey of the cultivars will be repeated in 2014, and further data will give more valid information of the susceptibility of each cultivar and the interactions between level of infection and the climate, together with the management of the orchard.

Acknowledgements

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