Short Communication

OCCURRENCE OF Fusarium equiseti AS A CONTAMINANT OF DIPLOTAXIS TENUIFOLIA SEEDS

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SUMMARY

Fusarium equiseti, the causal agent of leaf spot of both wild [Diploptaxis tenuifolia (L.) DC] and cultivated [Eruca vesicaria (L.) Cav.] rocket, is a new pathogen on these crops, which has increasingly been found on several commercial farms in Italy. In order to investigate the possible transmission of this pathogen through infested seeds, both wild and cultivated rocket seeds, from eight commercially available seed lots, have been collected from four farms suffering from severe field losses. F. equiseti was identified through morphological observations and molecular analysis based on the elongation factor 1 alpha gene (EF-1α). Four out of six seed lot samples of wild rocket were found to be contaminated, while the pathogen was not isolated from the two tested seed lots of E. vesicaria. The highest level of detected infestation was 5 out of 800 non-disinfected Diplotaxis sp. seeds. F. equiseti was not isolated from disinfected seeds. Nine of eleven isolates of F. equiseti obtained from seeds were pathogenic on wild rocket. This work demonstrates that F. equiseti can be transmitted by Diplotaxis tenuifolia seeds. The external nature of seed contamination of Diplotaxis sp. by F. equiseti has been proved. More extensive essays on seed lots of cultivated rocket are needed in order to have a better understanding of the seed transmissibility of the pathogen on such a host.

Keywords: seed-borne pathogens, leaf spot, seed health, leafy vegetables.

Wild [Diploptaxis tenuifolia (L.) DC] and cultivated [Eruca vesicaria (L.) Cav.] rocket are increasingly grown in many countries, because of their extensive use as salad and/or as a garnish. In Italy, these leafy vegetables are cultivated in intensive cropping systems in open fields and under greenhouses over an area of approximately 3,600 hectares, with up to five successive crops per year on the same soil. Moreover, rocket seed production in Italy covers an area of about 500 hectares (Anonymous, 2012, 2014).

In recent years, several diseases caused by soil-borne and foliar pathogens have been observed for the first time on wild and cultivated rocket in Italy (Gilardi et al., 2013; Garibaldi et al., 2015). Many of these diseases have been shown to be seed-borne, and their transmission through infected seeds has been demonstrated (Gullino et al., 2014; Gilardi et al., 2015).

The leaf spot of wild and cultivated rocket, caused by Fusarium equiseti, is an increasingly important disease that affects many production areas in both Southern and Northern Italy. Yield losses of about 10% have been observed at temperatures between 15 and 28°C and under high relative humidity (Garibaldi et al., 2011, 2015). Black-brown and greasy lesions with a well-defined border, surrounded by a violet-brown halo, have been observed on plants at the cotyledon stage and after 20 to 25 days from sowing. The same pathogen is associated with leaf spot, root and crown rot and fruit rot, which result in seed decay and seedling infection on different crops, such as cereals, ginseng, cumin, hibiscus, cyperus, corn, watermelon and aleppo pine (Adams et al., 1987; Rai, 1979; Reuveni, 1982; Correll et al., 1991; Kosiak et al., 2003; Goswami et al., 2008; Punja et al., 2008; Hassan et al., 2014; Gupta et al., 2013; Lazreg et al., 2014; Li et al., 2014, 2015). Such a broad host range increases the risk caused by the recent introduction of F. equiseti into Italy. Its sudden appearance in different cultivation areas could be explained due to its spread through contaminated seeds.

In order to investigate the possible contamination of commercial seed lots of wild and cultivated rocket, a seed test was conducted on two seed samples of E. sativa and six of Diploptaxis spp. collected from commercial farms in...
Veneto, Piedmont, Lombardy and Campania that have suffered severe losses in the field.

Eight seed lots (Table 1) were assayed for the presence of *F. equiseti* on Potato Dextrose Agar (PDA, Merck, Germany), amended with 25 mg l⁻¹ of streptomycin sulphate, as described by Mathur and Kongsdal (2003). Isolations were carried out on seeds that had only been washed in distilled water (not disinfected) or disinfected by soaking for 1 min in 1% sodium hypochloride, rinsed in sterile distilled water and dried on filter paper. Fifteen seeds were placed in each Petri plate, which was then sealed with parafilm and incubated at 25°C for a 12 h photoperiod for 7 days. The Petri plates were checked regularly for the development of fungal orange-brown colonies, with spores produced in chains. Four out of six wild rocket isolates were pathogenic (Table 3), with affected leaf area ranging from 5.1 to 30.6% of affected leaf area. Two out of nine isolates from the seeds were not pathogenic (Table 3).

*F. equiseti* is a member of the FIESC, a genetically highly diverse group, with 30 phylogenetically distinct species (O’Donnell et al., 2009, 2012), divided into two clades designated as *F. incarnatum* and *F. equiseti* (Castella and Cabañes, 2014). Differences in pathogenicity among the tested isolates obtained from wild rocket seeds observed in the present study could be correlated with phylogenetic species known within the FIESC, as reported also in other pathosystem (Castella and Cabañes, 2014; Villani et al., 2016).

The external nature of seed contamination of *D. tenuifolia* sp. by *F. equiseti* has been proved in the present study. This result is in agreement with similar results pertaining to other plant species, such as cowpea (Aigbe et al., 1999), ginseng (Punja et al., 2008) and *Carya illinoinensis* (Lazarotto et al., 2014). The pathogen was not isolated from symptomatic leaves of *E. vesicaria*. Other assessments should be made in which the evaluation should be extended to new seed lots of cultivated rocket. The occurrence of *F. equiseti* on wild rocket seeds represents a potential source of inoculum, and favours the long distance spread of the pathogen. *Alternaria* sp. was also constantly isolated from the rocket seeds, thus confirming that the quick spread of this pathogen in Italy and in

<table>
<thead>
<tr>
<th>Host</th>
<th>Cultivar</th>
<th>Seed lot</th>
<th>Seed company</th>
<th>Harvesting Year</th>
<th>Origin of the sample</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Eruca vesicaria</em></td>
<td>Rucola coltivata</td>
<td>1</td>
<td>La Semiorto, Sarno, (Salerno), Italy</td>
<td>2014</td>
<td>Piedmont</td>
</tr>
<tr>
<td><em>E. vesicaria</em></td>
<td>Rucola coltivata</td>
<td>2</td>
<td>Franchi Sementi, Grassobbio (Bergamo), Italy</td>
<td>2015</td>
<td>Piedmont</td>
</tr>
<tr>
<td><em>D. tenuifolia</em></td>
<td>Extrema</td>
<td>3</td>
<td>Ortis, Cesena (Forlì-Cesena), Italy</td>
<td>2015</td>
<td>Veneto</td>
</tr>
<tr>
<td><em>Diplotaxis tenuifolia</em></td>
<td>Summer</td>
<td>4</td>
<td>Orosem, Azzano, San Paolo (Bergamo), Italy</td>
<td>2014</td>
<td>Lombardy</td>
</tr>
<tr>
<td><em>D. tenuifolia</em></td>
<td>Luna</td>
<td>5</td>
<td>T&amp;T Vegetable seeds, Chioggia (Venezia), Italy</td>
<td>2014</td>
<td>Veneto</td>
</tr>
<tr>
<td><em>D. tenuifolia</em></td>
<td>Giove</td>
<td>6</td>
<td>T&amp;T Vegetable seeds, Chioggia (Venezia), Italy</td>
<td>2014</td>
<td>Veneto</td>
</tr>
<tr>
<td><em>D. muralis</em></td>
<td>Rucola selvatica</td>
<td>7</td>
<td>Suba seed, Longjano (Forlì-Cesena), Italy</td>
<td>2014</td>
<td>Campania</td>
</tr>
<tr>
<td><em>D. muralis</em></td>
<td>Rucola selvatica</td>
<td>8</td>
<td>Suba seed, Longjano (Forlì-Cesena), Italy</td>
<td>2015</td>
<td>Campania</td>
</tr>
</tbody>
</table>
other countries could, at least partially, be explained by the use of contaminated seeds (Tidwell et al., 2014; Gilardi et al., 2015). Previous studies provided evidence that *Plectosphaerella cucumerina*, a pathogen of wild rocket that has frequently been detected on several farms that grow this crop for the ready to eat sector, is also a seed contaminant (Gilardi et al., 2013).

Owing to the extensive host range of *F. equiseti* and the economic value of rocket, this pathogen could also be a threat to this crop in other production areas, and the present results suggest that seeds may be important in disseminating different pathogens. Considering the external nature of the contamination of wild rocket seeds a conventional treatment of the seeds might eliminate the risk of infection in contrast with a more problematic endophytic contamination.

Identifying the primary source of inoculum is of critical importance for an effective disease management. Quick and reliable diagnostic tools and seed dressing methods need to be investigated and made available to seed companies and growers. Among the tested fungicides, benomyl, thiophanate-methyl, propiconazole and fludioxonil have been shown to be able to reduce the mycelial growth of *F. equiseti* from ginseng *in vitro* at 10 mg/l, while fludioxonil is already effective at 1 mg/l (Punja et al., 2008). Information regarding the chemical management of this pathogen is very scarce, especially in relation to the very limited availability of registered fungicides for rocket.

Moreover, disease management should rely above all on preventative methods, such as early diagnosis of the pathogen and seed dressing.

**ACKNOWLEDGEMENTS**

The research leading to these results has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No. 634179 “Effective Management of Pests and Harmful Alien Species - Integrated Solutions” (EMPHASIS). The authors thank Dr. Marguerite Jones for language revision. The technical support of Giuseppe Ortu is kindly acknowledged.

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