**Temperature and leaf wetness affect the severity of leaf spot on lettuce and wild rocket incited by *Fusarium equiseti***

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**Abstract** Eight experimental trials have been carried out under controlled conditions in order to have a better understanding of the effect of temperature and leaf wetness duration on the incidence and severity of leaf spot caused by *Fusarium equiseti* on lettuce cv. Elisa and wild rocket cv. Grazia. Lettuce resulted very susceptible to *F. equiseti*, particularly at temperatures of 25-30°C, with a higher disease index than 50% and a higher disease severity than 25%. At such temperatures, 1-3 hours of leaf wetness were sufficient to cause a high disease incidence and severity, while at least 12 hours of leaf wetness were necessary to cause high losses at lower temperatures. Disease incidence and severity were higher on the wild rocket at the highest temperatures (30-35°C). Only one hour of leaf wetness was sufficient to cause significant levels of disease incidence and severity at the highest temperatures, while longer periods (6-12 hours) were necessary to cause significant losses at lower temperatures. The possible causes of the recent spread of *F. equiseti* in northern Italy on a number of crops as well as the threat represented by such a pathogen are discussed hereafter.

**Keywords** *Lactuca saviva*; *Diplotaxis tenuifolia*; epidemiology; foliar pathogen

**Introduction**

*Fusarium equiseti* is a soil inhabitant that is generally considered a weak pathogen, which can infect the seeds, roots, tubers and fruit of several crops. This species, which commonly occurs in tropical and subtropical regions (Booth 1978; Bosch and Mirocha 1992), has also been observed in temperate regions, and has been reported as being a causal agent of disease on cereals (Bottalico, 1988; Kosiack et al. 2005) as well as on many plants, including cotton, lentils, sugar beet, cumin, potatoes, cowpeas, pines, ginseng, asparagus, on which it causes a variety of symptoms (Farr and Rossman 2016). *F. equiseti* is also well known for its mycotoxins production, and in particular nivalenol, diacetoxyscirpenol and zearalenone (Jimenez et al. 1997; Bosch and Mirocha 1992; Bottalico and Perrone 2002; Goswami et al. 2008). Three leafy vegetables that are grown intensively in Italy for ready-to-eat mix salads and fresh consumption have recently been found to be new hosts of this species, with a new type of leaf spot on lettuce (*Lactuca sativa*) (Garibaldi et al. 2016), cultivated rocket (*Eruca sativa*) (Garibaldi et al. 2016) and wild rocket (*Diplotaxis tenuifolia*) (Garibaldi et al. 2015).

Since very little is known about the epidemiology of this pathogen, this study was undertaken in order to understand the effect of temperature and leaf wetness duration on the incidence and severity of leaf spot caused by *F. equiseti* on lettuce and wild rocket under controlled conditions.

**Materials and methods**

Plant and pathogen material

 Eight experimental trials (4 on lettuce, 4 on wild rocket) were carried out in growth chambers located at the Centre of Competence for innovation in the Agro-environmental sector of the University of Torino in Grugliasco (Torino) from March 2016 to June 2016, in which the environmental conditions reported in Table 1 were tested.

 Cv. Elisa lettuce plants (T&T, Sant’Anna di Chioggia, Italy) and cv. Grazia wild rocket (Enza Zaden, The Netherlands) were used in the present test. The plants were grown in 2L-pots filled with a peat: pomix: pine bark compost: clay (40:10:30:20 v/v) mix (TS 4, Turco Silvestro, Albenga, SV), with a pH 6-6.5and2 kgm-3 of NPK. The substrate were disinfested at 90°C for 30 min. Before the inoculation, the plants were kept for 20 to 25 days at temperatures ranging from 22 to 25°C.

Growth chamber trials

Trials were carried out in growth chambers with a 12 h/d fluorescent light regime, at temperatures of 10, 15, 20, 25, 30 and 35 °C. After inoculation, the plants were kept at each temperature for 0, 1, 3, 6, 12,24 and 48 hours, and were enclosed in clear polyethylene moist chambers (100 ×100 ×50 cm), covered with a transparent polyethylene film (50 m thick), in order to create a dew chamber with 100% RH.

A randomised block design was used in all the trials, with 4 replicates. Each replicate was represented by 1 pot with 20- 25 plants. The trials were repeated four times for each pathosystem, under completely controlled environmental conditions.

The strain coded Feq 7 of *Fusarium equiseti* was used for the inoculation of the lettuce, while isolate Feq 5/14 was used for the wild rocket. Both strains were grown on potato dextrose agar (PDA, Merck, Darmstadt, Germany), amended with streptomycin sulphate, for 7-10 days at 20-23 °C, with a 12 hour photoperiod. A suspension containing a concentration of 1x10 6conidia/ml of the pathogen was used for inoculation of twenty to twenty-five-day-old plants.The suspension was applied to all the surfaces, 24-72 hours after transfer of the plants to the growth chambers. Five ml of inoculum was sprayed onto 20-25 plants.

Disease evaluation and statistical analysis

Symptoms were recorded on 50 to 100 leaves/treatment 4-8 days after inoculation. Disease incidence (expressed as percent of infected leaves) and disease severity (expressed as percent of infected leaf area) were evaluated. Disease severity was recorded by adopting a scale from 0 to 5 (0 = no symptom; 1 = up to 5 % of infected leaf area; 2 = 6 to 10% of infected leaf area; 3 = 11 to 25% of infected leaf area; 4 = 26 to 50% of infected leaf area; 5= 51 to 100 % of infected leaf area). Disease severity was calculated using the formula: DS= [∑(n° leaves × x 0-5) / (total of recorded leaves )] with x 0-5 = (x0=0; x1= 5%; x2=10%; x3=25%; x4=50%; x5=75%) .

The data were arc-sin-transformed and analysed by means of univariate ANOVA, with Tukey’s HSD test (*P*<0.05), using SPSS software 22.0. One-way Anova was used to investigate the effect of increasing the temperatures and hours of incubation at high relative humidity (RH)on disease incidence (DI, expressed as % of infected leaves) and disease severity (DS, expressed as % of affected leaf area). The results obtained in trials 1-4 on the lettuce and and wild rocket are reported in Table 2, on the basis of the results obtained from ANOVA. The standard error is reported.

**Results**

The used inoculation method led to a notable disease incidence and severity on both of the crops tested in all the trials. Lettuce in general resulted to be more severely affected by leaf spot caused by *F. equiseti* than wild rocket (Figures 1-6). One-way analysis of variance confirmed that the temperature (*P*<0.0001), leaf wetness duration (*P*<0.001) and their interactions were significant factors (*P*<0.0001) of influence on disease incidence and severity caused by *F. equiseti* on lettuce and wild rocket (Table 2). The differences between experiments 1-2 and 3-4 can be explaned because sometimes fungi and bacteria that are grown and transferred several times on artificial media are interested by a decrease of virulence (Table 2).

Lettuce resulted to be very susceptible to *F. equiseti*, in particular at temperatures of 25 and 30°C, with a higher disease index than 50% and a higher disease severity than 25%(Figure 1). At such temperatures, 1 and 3 hours of leaf wetness were sufficient to cause high disease incidence and severity. At least 12 hours of leaf wetness were necessary to cause high losses at lower temperatures (Figures 2 and 3).

 The disease incidence and severity values on the wild rocket were lower than those reached on the lettuce, even for the conditions more favourable for disease development (Figures 4 and 5). The same trend was also observed for the wild rocket. Disease incidence and severity was higher at the highest temperatures (30 and 35°C). Although only one hour of leaf wetness was sufficient to cause significant levels of disease incidence and severity at the highest temperatures, longer periods (6 and 12 hours) were necessary to cause significant losses at the lower temperatures (Figure 6).

**Discussion**

*F. equiseti* has often been found on cereals, but it is not considered to be a component of the Fusarium head blight complex (Bottalico 1988). This fungus has been reported on a number of other crops, including muskmelon (Adams et al. 1987), pumpkin (Elmer 1996), cotton (Chimbekujwo 2000), cumin (Reuveni 1982), potato (Rai 1979), cowpea (Aighe and Favole 1999), asparagus (Vujanovic et al. 2006), pineapple (Stepien et al.2013) and ginseng (Punja 1997).

There is little information on the environmental factors that favour this pathogen. Punja et al. (1997), studying populations of *F. equiseti* taken from ginseng, observed the best *in vitro* growth of the pathogen on potato dextrose agar at temperatures of 22-30 °C. Such a temperature requirement explains why this species has been reported more frequently in warm regions.

The sudden appearance of the pathogen in northern Italy and its spread to different hosts could be related, at least partially, to the increase in temperature observed in northern Italy as a consequence of climate changes. *F. equiseti* is spreading quickly in northern Italy on different leafy vegetables, such as lettuce, and wild and cultivated rocket, which are grown intensively in this region as monocultures or in succession. This fact can easily favour the survival of the pathogen, which is a good saprotroph, in soil or crop debris. Such a feature makes it a pathogen that can easily adapt to many different cropping systems, and in particularly to those of a very intensive nature.

*F. equisenti* could represent a serious threat for many Mediterranean crops. The risk posed by its appearance and spread is further increased by the capability of this species to produce mycotoxins, already described not only for cereals (Bottalico 1988), but also for ginseng (Goswami et al. 2008) and pineapple (Stepien et al. 2013).

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**Table 1** Experimental layout of the trials carried out on lettuce cv. Elisa and wild rocket cv. Grazia

|  |  |
| --- | --- |
|   |  **Trial**  |
|  | 1 | **2** | **3** | **4** |
| **Lettuce**  |  |  |  |  |
| Temperature °C | 15,20,25,30 | 15, 20,25,30 | 10,15, 20, 25,30 | 10, 25 |
| Hours at high RH | 0,1,3,6,12,24,48 | 0,1,3,6,12, 24, 48 | 0, 1, 3,6,12,24,48 | 0, 1, 3,6,12,24,48 |
| Start of trial | 7/03/2016 | 21/03/2016 | 6/05/2016 | 13/06/2016 |
| Artificial inoculation  | 8/03/2016 | 22/03/2016 | 9/05/2016 | 14/06/2016 |
| End of trial | 17/03/2016 | 6/04/2016 | 17/05/2016 | 20/06/2016 |
| **Wild rocket** |  |  |  |  |
| Temperature °C | 15, 20, 25, 30 | 15,20,25,30 | 10,15, 20,25,30,35 | 10,15, 20, 25 |
| Hours at high RH | 0,3,6,12,24,48 | 0,1,3,6,12,24,48 | 0,1,3,6,12, 24, 48 | 0, 1, 3,6,12,24, 48 |
| Start of trial | 7/03/2016 | 21/03/2016 | 6/05/2016 | 30/05/2016 |
| Artificial inoculation  | 8/3/2016 | 22/3/2016 | 9/5/2016 | 31/05/2015 |
| End of trial | 17/3/2016 | 06/04/2016 | 17/5/201 | 10/6/2016 |
|  |  |  |  |  |

**Table 2** Effect of the ‘trial’ factor considering the disease incidence (DI, expressed as % of infected leaves) and severity (DS, expressed as % of affected leaf area) during the trials carried out to investigate the effect of temperatures and hours of incubation at high relative humidity (RH) on *Fusarium equiseti/*lettuce and *Fusarium equiseti/* wild rocket pathosystems

|  |  |  |  |
| --- | --- | --- | --- |
| Trial N. | *Fusarium equiseti*- lettuce | Trial N. | *Fusarium equiseti-*wild rocket |
| DI |  | DS |  | DI |  | DS |  |
| 1 | 43.4 | ±2.8 ba | 20.6 | ± 1.6 b | 1 | 31.3 | ± 1.9 b | 16.7 | ± 1.2 b |
| 2 | 44.7 | ± 2.6 b | 19.6 | ± 1.4 b | 2 | 39.6 | ± 2.2 c | 15.3 | ± 1.1 b |
| 3 | 30.8 | ± 3.2 a | 12.2 | ± 1.6 a | 3 | 15.6 | ±1.4 a | 6.8 | ± 0.7 a |
| 4 | 31.0 | ± 2.5 a | 10.5 | ± 0.9 a | 4 | 15.0 | ± 1.3 a | 6.5 | ± 0.7 a |

a Values with the same letter in the same column are not significantly different, according to Tukey’s Test (P < 0.05). Standard error is reported.

**Figure 1** Effect of temperature on leaf spot disease incidence (DI, expressed as % of infected leaves) and severity (DS, expressed as % of infected leaf area), caused by *Fusarium equiseti*, on lettuce cv. Elisa. The reported data are the average values of trials 1 and 2. Columns superscripted with the same letter are not significantly different at *P*≤0.05 (Tukey’s Test)

**Figure 2** Effect of hours of incubation at high relative humidity (RH) on leaf spot disease incidence (DI, expressed as % of infected leaves) and severity (DS, expressed as % of affected leaf area), caused by *Fusarium equiseti*, on lettuce cv. Elisa. The reported data are the average values of trials 1 and 2. Columns superscripted with the same letter are not significantly different at *P*≤0.05 (Tukey’s Test)

**Figure 3** Disease incidence (DI, expressed as % of infected leaves) and severity (DS, expressed as % of infected leaf area) caused by *Fusarium equiseti*, on lettuce cv. Elisa for a temperature range of 15,20,25 and 30°C for 0,1,3,6,12,24 and 48 hours of incubation at high relative humidity (RH)

**Figure 4** Effect of temperature on leaf spot disease incidence (DI, expressed as % of infected leaves) and severity (DS, expressed as % of infected leaf area), caused by *Fusarium equiseti*, on rocket cv. Grazia. The reported data are the average values of trials 3 and 4. Columns superscripted with the same letter are not significantly different at *P*≤0.05 (Tukey’s Test)

**Figure 5** Effect of hours of incubation at high relative humidity (RH) on leaf spot disease incidence (DI, expressed as % of infected leaves) and severity (DS, expressed as % of affected leaf area), caused by *Fusarium equiseti*, on rocket cv. Grazia. The reported data are the average values of trials 3 and 4. Columns superscripted with the same letter are not significantly different at *P*≤0.05 (Tukey’s Test)

**Figure 6** Disease incidence (DI) and severity (DS) of *Fusarium equiseti*, on rocket cv. Grazia for a temperature range of 10, 15, 20, 25, 30 and 35°C for 0, 1, 3, 6, 12, 24 and 48 hours of incubation at high relative humidity (RH)