Fusarium species of the Liseola section associated with stalk and ear rot of maize in southern Italy, and their ability to produce moniliformin

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Fusarium species of the Liseola section, isolated from infected maize (stalks, seeds), and from soil after a maize crop in southern Italy, were identified as belonging to F. moniliforme and F. proliferatum. F. moniliforme was isolated more frequently from stalks and seeds than from soil, while F. proliferatum was mainly present in soil. Moniliformin (up to 1520 mg kg\(^{-1}\)) was produced by only some isolates of F. proliferatum from seeds and soil.

According to the nomenclature of Booth (1971), both Fusarium moniliforme Sheldon and Fusarium moniliforme var. subglutinans Wollenw. & Reink. have often been associated with stalk and ear rot of maize (Kommedahl & Windels, 1981; Maric, 1981). Attempts to identify the Fusarium isolates of the Liseola section associated with fusariosis in southern Italy have led to the recognition, together with F. moniliforme, of another morphologic group of isolates showing characteristics rather different from those reported for F. moniliforme var. subglutinans (Bottalico, Visconti & Solfrizzo, 1984).

In recent taxonomic investigations of Fusarium species within the Liseola section increasing importance has been placed on phialide morphology and microconidial arrangement, as well as physiological characteristics. Such criteria have necessitated the recognition of other species (Burgess & Liddell, 1983; Nelson, Toussoun & Marasas, 1983) and/or varieties (Gerlach & Nirenberg, 1982; Kuhlman, 1982). This encouraged us to reinvestigate the taxonomic features of the isolates from southern Italy, and to assay their capability to produce moniliformin. The production of this toxin by Fusarium isolates of the Liseola section is not universal (Bottalico et al., 1982; Rabie et al., 1982; Marasas et al., 1986).

**MATERIALS AND METHODS**

Samples were taken in 1984 from three different maize fields in Basilicata (southern Italy) which

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**Table 1. Percentage of maize stalks infected with Fusarium species of the Liseola section**

<table>
<thead>
<tr>
<th>Field</th>
<th>No. of hybrids examined</th>
<th>Percentage of stalks infected*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>13</td>
<td>Fusarium spp.: 83.8 F. moniliforme: 47.6 F. proliferatum: 11.5</td>
</tr>
<tr>
<td>2</td>
<td>11</td>
<td>97.2 F. moniliforme: 78.1 F. proliferatum: 33.6</td>
</tr>
<tr>
<td>3</td>
<td>10</td>
<td>96.0 F. moniliforme: 58.5 F. proliferatum: 3.6</td>
</tr>
</tbody>
</table>

* Ten stalk cores, sampled in August after anthesis, were examined for each hybrid.

**Table 2. Percentage of maize seeds infected with Fusarium species of the Liseola section**

<table>
<thead>
<tr>
<th>FAO maturity</th>
<th>No. of samples examined</th>
<th>Percentage of seeds infected*</th>
</tr>
</thead>
<tbody>
<tr>
<td>200-300</td>
<td>15</td>
<td>Fusarium spp.: 4.1 F. moniliforme: 3.3 F. proliferatum: 0.4</td>
</tr>
<tr>
<td>400-500</td>
<td>19</td>
<td>21.8 F. moniliforme: 20.9 F. proliferatum: 0.9</td>
</tr>
<tr>
<td>600-700</td>
<td>19</td>
<td>50.3 F. moniliforme: 48.1 F. proliferatum: 1.9</td>
</tr>
</tbody>
</table>

* One hundred seeds were examined for each sample.
were planted with ten hybrids belonging to two different classes of FAO maturity (600-700 and 400-500).

In August, after anthesis, ten stalk cores (7 mm diam) were taken for each hybrid per field from the lowest internode of green stalks, using a tool similar to that described by Kommedahl et al. (1979). The cores were brought to the laboratory in small plastic bags, shaken in 1% NaOCl for 30 s, placed on pentachloronitrobenzene (PCNB)-peptone-agar medium (Nash & Snyder, 1962), and incubated for 7 d at 23-25°C.

Fifty-three commercial seed samples of different hybrids, belonging to three FAO maturity classes, were examined (Table 2). One hundred seeds per sample were left for 12 h under running water, then surface treated with 0.5% NaOCl for 1 min, cut in halves, and placed on PCNB medium for 5-7 d.

Three gram soil samples, obtained by combining three randomly selected samples (3-15 cm deep), were collected for four months (April, May, June and July) from three different fields where maize was previously grown. Each soil sample was passed through a 2 mm sieve, then successively diluted with water to give a final dilution of 1:1000. Aliquots of one ml of final soil dilutions, mixed in a shaker for 30 s, were pipetted into Petri dishes (10 dishes per treatment) to which 15 ml of cool PCNB medium for 5-7 d.

Identification

Fusarium colonies on PCNB medium were subsequently transferred to potato-saccharose-agar medium (PSA), and incubated for 7 d at about 24° under fluorescent lamps for 12 h d⁻¹. Single-spore cultures of Fusarium species of the Liseola section were then obtained on PSA plates, and finally they were identified in accordance with the nomenclature of Nelson et al. (1983). Observations were made also using water-agar with sterile soil, or with sterile rice seed, or with sterile carnation leaf (Nelson et al., 1983), or using KCl media prepared by adding 2, 4, 6 or 8 g of KCl to a litre of 1.5% water-agar (Fisher et al., 1983).

Moniliformin analysis

Moniliformin production on autoclaved seeds and analysis of the toxin in dried colonies were carried out using methods described in a previous report (Bottalico et al., 1982). Forty-three isolates of F. moniliforme from stalks (20), seeds (22) and soil (1), and thirty-three isolates of F. proliferatum from stalks (18), seeds (8) and soil (7) were tested for moniliformin production.

RESULTS

On the basis of phialide morphology and microconidial arrangement, the Fusarium isolates of the Liseola section from maize stalks, seeds, and field soil were divided into two groups. One group was characterized by microconidia always borne in chains on monophialides (Figs 1-2), while the other group showed microconidia borne both in false heads and in chains, usually on polyphialides but sometimes also on monophialides (Figs 3-8). According to Nelson et al. (1983) they were identified as F. moniliforme Sheldon and F. proliferatum (Matsushima) Nirenberg, respectively.

Some isolates of F. proliferatum with clavate microconidia also produced some pyriform microconidia, but only on rice seeds and never on water-agar, or on other nutrient-poor media. As reported by Nelson et al. (1983), this characteristic was not constant, and the percentage of pyriform microconidia was usually very low (0.5-1%). On water-agar, the isolates of F. proliferatum generally produced many false heads and short chains, but the abundance and length of the chains was increased by decreasing the water potential of the medium, and longer microconidial chains were formed on medium with 4 g l⁻¹ KCl. Isolates of F. proliferatum also produced thickened hyphae and conidia on different media.

F. moniliforme was isolated more frequently than

Table 3. Presence of Fusarium species of the Liseola section in maize field soil, from three sites, in four months

<table>
<thead>
<tr>
<th>Site</th>
<th>April</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>April</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>April</th>
<th>May</th>
<th>June</th>
<th>July</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusarium spp.</td>
<td>38</td>
<td>42</td>
<td>40</td>
<td>32</td>
<td>70</td>
<td>50</td>
<td>30</td>
<td>4</td>
<td>34</td>
<td>42</td>
<td>16</td>
<td>20</td>
<td>418</td>
</tr>
<tr>
<td>F. moniliforme</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td>12</td>
<td>2</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>16</td>
</tr>
<tr>
<td>F. proliferatum</td>
<td>4</td>
<td>16</td>
<td></td>
<td></td>
<td>16</td>
<td>28</td>
<td>6</td>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td>66</td>
<td>110</td>
</tr>
</tbody>
</table>

* Colonies grown on 10 agar-PCNB plates, each inoculated with 1 ml 1 : 1000 diluted solution from 3 g of soil.
Figs 1–2. *Fusarium moniliforme*, simple phialides bearing microconidia in chains (× 400). Figs 3–10. *Fusarium proliferatum*, phialides and polyphialides bearing microconidia in false heads and chains, × 100, Fig. 3; × 250, Figs 4–5; × 400, Figs 6–8; water-agar medium cultures without KCl (Fig. 9) or with 4 g l⁻¹ KCl (Fig. 10) and longer chains of microconidia (× 100).
Fusarium spp. of the Liseola section

Table 4. Production of moniliformin by Fusarium species of the Liseola section isolated from maize in southern Italy*

<table>
<thead>
<tr>
<th>Fusarium species</th>
<th>Source</th>
<th>No. of isolates tested</th>
<th>No. of toxin producing isolates</th>
<th>Amount of moniliformin produced (mg kg⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>F. moniliforme</em></td>
<td>Stalk</td>
<td>20</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Seed</td>
<td>22</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Soil</td>
<td>1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><em>F. proliferatum</em></td>
<td>Stalk</td>
<td>18</td>
<td>3</td>
<td>221-1520</td>
</tr>
<tr>
<td></td>
<td>Seed</td>
<td>8</td>
<td>3</td>
<td>467-1213</td>
</tr>
<tr>
<td></td>
<td>Soil</td>
<td>7</td>
<td>3</td>
<td>—</td>
</tr>
</tbody>
</table>

* Isolates were grown on autoclaved corn kernels at 25-27 °C for 3 weeks.
† Identified according to Nelson et al. (1983).

F. proliferatum, both from stalks from each of the three fields (Table 1) and from seeds of different FAO maturity classes (Table 2). In contrast, from soils the ratio was reversed, and *F. moniliforme* was not found at all in two fields (Table 3).

None of the forty-three isolates of *F. moniliforme* from soil, seeds or stalks was able to produce moniliformin. This toxin was produced by 6 out of 33 isolates of *F. proliferatum* (up to 1520 mg of toxin per kg of dried colony). All the toxin-producing isolates were obtained from soil or seeds. Isolates from stalks failed to produce moniliformin (Table 4).

**DISCUSSION**

It appears that in southern Italy maize represents an important host for both *F. moniliforme* Sheldon and *F. proliferatum* (Matsushima) Nirenberg. However, *F. moniliforme* is more widely distributed in vegetative parts, while *F. proliferatum* is more frequently present in soil, and the thickened hyphae formed by *F. proliferatum* could represent soil survival structures. Some similar taxonomic characteristic could lead to confusion of *F. proliferatum* with *F. subglutinans* (Wollenw. & Reink.) Nelson, Toussou & Marasas, the latter being characterized by microconidia always borne in false heads on polyphialides. As a result of this investigation, we support Fisher et al. (1983), who recommend the use of KCl (4 g l⁻¹) in a water-agar medium for the improved identification of *Fusarium* species belonging to the section Liseola.

Isolates of *F. proliferatum*, but not of *F. moniliforme*, were able to synthesize moniliformin. The negative results concerning *F. moniliforme* are in accordance with our previous report (Bottalico et al., 1982) and those of others (Kriek et al., 1977; DeLucca et al., 1982; Thiel, Meyer & Marasas, 1982), but there are also reports of positive results (Cole et al., 1973; Burmeister, Cigler & Vesonder, 1979; Allen et al., 1981; Rabie et al., 1982; Marasas et al., 1986). Such conflicting data may be related to the geographical origin of the isolates, as well as to the use of different systems of naming isolates.

The ability of *F. proliferatum* to produce moniliformin appears to be restricted to some isolates, and it is not clear why all isolates from stalks were negative. Marasas et al. (1986) found all strains of *F. proliferatum* were able to produce moniliformin, but they analysed only a few isolates from maize and did not specify their origin. It appears that further studies are necessary to assess the relationship between source and toxigenicity for isolates of this species.

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**REFERENCES**


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