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*Short communication*

# Involvement of a phytotoxic peptide in the development of the Northern leaf blight of corn

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## Abstract

Non pathogenic isolates of *Exserohilum turcicum* successfully infect corn plants in the presence of synthetic *E. turcicum* toxin during inoculation. The toxin significantly increased the number of appressoria and the ramification of germinating conidia both on host leaves and on artificial media. These findings indicate that this toxin plays an important role in infection of Northern leaf blight.


Northern leaf blight of corn incited by the fungus *Exserohilum turcicum* pass Leonard and Suggs is one of the major diseases of corn in most growing areas, and causes substantial damages (Perkins and Pedersen, 1987; Raymundo and Hooker, 1981; Simone, 1978). Previous studies have shown that *E. turcicum* produces a phytotoxin which inhibits chlorophyll formation on etiolated corn leaves and root elongation of corn seedlings (Bashan and Levy, 1992; Bashan et al., 1995). The degree of chlorophyll biosynthesis inhibition was correlated with the pathogenicity of the fungus and the susceptibility of the host (Bashan and Levy, 1992). Recently, the phytotoxic compound was isolated and its structure was determined. This compound (the so called E.t. toxin) is a small peptide composed of three amino acids (glycine – serine – glutamine) and was synthesized in the laboratory. Both the synthetic and the natural peptides inhibited chlorophyll synthesis and root elongation of corn seedlings. We have no evidence that the toxin alone (natural and synthetic) may produce necrosis on leaves (Bashan et al., 1995). In the present study, we investigate the effect of the synthetic peptide on conidial germination and appressorial formation of *E. turcicum* on susceptible corn plants and Johnson grass.

† This article is dedicated to the memory of Yehouda Levy who passed away recently.

The susceptible corn line A619 and the susceptible sweet corn cultivar Jubilee were used in all experiments. Plants were grown in the greenhouse in 1l plastic pots containing a mixture of soil, peat and vermiculite (1:1:1 v/v). After 4 to 5 weeks, when plants reached the fifth leaf stage they were used for inoculation. *Exserohilum turcicum* was isolated from an infected corn field and from Johnson grass (*Sorghum halepense*) as described previously (Abadi et al., 1989) and propagated on lactose casein hydrolysate agar (Tuite, 1969). Conidia were harvested from two-week-old cultures and suspended in distilled water. Suspensions were adjusted to 30,000 conidia per ml and mixed with a toxin solution to a final concentration of 250 ppm.

The synthetic peptide was prepared in the laboratory of E. Meir at the Hebrew University in Jerusalem and the natural peptide was detected by HPLC (for more details, see Bashan et al., 1995).

For germination studies, 10 $\mu$l droplets of conidial suspensions with or without toxin were placed on Petri dishes containing 2% agar. After incubating at 25 °C for 24 h in the dark, germinating spores and ramifications of germ tubes were counted microscopically. On plants, 10 $\mu$l droplets of the conidial suspensions with or without the toxin were placed on the adaxial surface of the fourth and fifth leaves of plants grown as described above. Plants were incubated in a mist chamber in the dark at 25 °C for 24 h. Then, inocu-

*Table 1.* Germination and appressorial formation of *Exserohilum turcicum* conidia on leaves as affected by E.t. toxin

| Isolate source | Infected plant | Toxin concentration, ppm | Germination, % | Appressorial[a] formation, % | Lesion size, mm$^2$ |
|---|---|---|---|---|---|
| Corn | Corn (A619) | 0 | 97.5 | 87.5a | 11.33$^a$ |
| Corn | Corn (Jubilee) | | | | 7.166$^a$ |
| Johnson grass | Johnson grass | 0 | 97.0a | 82.5a | 6.56$^a$ |
| Corn | Johnson grass | 0 | 35.5b | 39.1b | 0 |
| Johnson grass | Corn* | 0 | 36.2b | 50b | 0 |
| Corn | Johnson grass | 250 | 92.5a | 76.6a | 420 |
| Johnson grass | Corn | 250 | 96.6a | 77.5a | 500 |

Different letters indicate significant differences between means in columns ($n = 20, p = 0.05$) according to Duncan's Multiple Range test.
* – on both corn lines A619 and Jubilee.
[a] – percentage of germinating tubes producing appresoria.

*Table 2.* Germination of *Exserohilum turcicum* conidia on agar as affected by E.t. toxin

| Isolate source | Toxin ppm | Germination, % | Conidia with one germtube % | Ramification[a] % |
|---|---|---|---|---|
| Johnson grass | 0 | 70.5 | 38.9 | 30.6 |
| Johnson grass | 250 | 79.2 | 23.7 | 65.5 |
| Corn | 0 | 59.6 | 36.2 | 23.4 |
| Corn | 250 | 92.8 | 11.6 | 81.2 |

[a] Ramification means two or more germination tubes for each conidia.

lated leaves were excised and stained with calcoflower (Cohen et al., 1987). Germination percentage and germ tube ramification were analyzed microscopically.

*Exserohilum turcicum* isolated from corn plants was pathogenic to corn but did not infect Johnson grass. Vice versa, the isolate collected from Johnson grass was non-pathogenic on corn (Table 1). However, the Johnson grass isolate caused much bigger necrotic lesions than the corn isolate on corn plants when the toxin was added to the inoculum. Vice versa, in the presence of toxin, fungal corn isolates caused bigger necrotic lesions than the Johnson grass isolate on Johnson grass leaves. These significant increases in lesion size in plants inoculated in the presence of toxin may be due to the effect of the toxin on germination, appressorial formation and germ tube ramification. No significant effect of the toxin was observed at toxin concentrations below 250 ppm.

Conidial germination of the *E. turcicum* corn isolate was 97.5% on corn leaves, but only 35.5% on Johnson grass leaves. A similar phenomenon was observed with Johnson grass isolate. Germination of conidia was 97.0% on Johnson grass leaves and decreases to 36.2% on corn leaves. However, in the presence of the toxin, conidial germination of the corn isolate increased to 92.5% on Johnson grass leaves, and that of the Johnson grass isolates was 96.6% on corn. A similar effect of the toxin was observed for appressorial formation (Table 1). The endogenous E.t. toxin content of the Johnson grass isolate as detected by HPLC analysis is 1/6 compared with the E.t. toxin content of the corn isolates.

Table 1 shows that the percentage of germinating spores that develop appressoria on the non-host plant was significantly increased in the presence of toxin. Likewise, ramification of germinating conidia increased drastically for both isolates (Table 2). These results indicate that besides its toxic effects on the host plants this peptide may also be a regulator of fungal development as it increased conidial germination and germ tube branching.

Toxins play an important role in the initial infection of plants caused by various fungi (Scheffer and Livingston, 1984; Yoder and Scheffer, 1969). In many cases, phytotoxins produced by parasitic fungi are able to produce at least a part of the symptoms caused by living pathogens in either host or non-host plants (Comstock and Scheffer, 1973). In some cases, breakdown products of a toxin may also account for symptom production (Walton and Panaccione, 1993). Many pathogenic fungi taxonomically related to *E. turcicum* such as *Bipolaris maydis, H. sacchari, Cochliobolus carbonum* and *Cochliobolus victorae*, are known to produce phytotoxins.

The loss of the ability of *H. victorae* and *C. carbonum* to produce toxin was associated with a decrease in aggressiveness (Xiao et al, 1992). Corn plants resistant to *C. carbonum* were found to contain a specific enzyme which neutralizes toxin activity (Meeley et al, 1992). Cuq et al. (1993) showed that *E. turcicum* produces a lipophilic phytotoxin that has been structurally characterized as monocerin. They showed that this molecule causes brown necrotic lesions on punctured leaves and inhibits the root growth of pre-germinated seeds, but no correlation between pathogenicity of the fungus and toxin production was indicated. When grown in culture, *E. turcicum* produces toxins which are associated with fungal aggressiveness and host susceptibility (Bashan and Levy, 1992). In this study, we showed that the synthetic peptide which is presumed to be identical with a peptide produced by *E. turcicum* (Bashan et al., 1995) increases lesion size, germination and appressorial formation of *E. turcicum* and this may increase the aggressiveness of non-pathogenic isolates. This indicates that *Exrserohilum turcicum* toxin is an important virulence factor of the fungus.

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