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CONTENTS

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Maize seed orientation in the substrate and its influences on germination, seedling structure, and transmission of *Fusarium moniliforme*

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The percentage of seedlings emerging during the first 4 days after sowing and the mesocotyl length after 7 days in sand at 25°C was strongly influenced by the orientation of the seed in the seedbed. Maize seeds emerged faster when oriented vertically with the pedicel end facing down (VD) or horizontally with the embryal side facing up (HU). The mesocotyl portion of the seedling was shorter (11mm) when sown at HU and VD, providing a fast and easy emergence to the seedling. It was much longer (20mm) when the seed was oriented horizontally with the embryal side facing down (HD) and vertically with the pedicel end facing up (VU). We concluded that the length of the mesocotyl portion of the seedling varies with the orientation of the seed in the substrate at the same depth. The seeds sown in the orientation HU and VU disclosed a higher germination of 95 percent and 88 percent respectively, compared to the seeds sown in the orientation HD and VD which had 68 percent and 53 percent germination respectively. Seed to seedling transmission of *Fusarium moniliforme* was recorded at a ratio of 1:1 in the untreated seeds, and infection of the fungus was found in the third leaf lamina and other sections of 10 day old seedlings.

This demonstrates for the first time the systemic development of *F. moniliforme* above the crown portion of 10 day old seedlings. Treatment with Thiram contact fungicide improves the germination of highly infected seeds and also reduces the seed to seedling transmission of *F. moniliforme*. The efficiency of this seed treatment depended on the orientation of the seeds in the seedbed.

Key words: Maize seed, substrate germination transmissions, seedling.

Introduction

Healthy seed of good quality is an important step in the improvement of food production (Mathur, (1989) especially in developing countries where many other constraints limit the boost of food production.

Fusarium moniliforme Sheldom is a pathogen causing root, stalk, ear and kernel rot of maize. This pathogen has a worldwide distribution and has frequently been reported from seed and soil (King, 1981; Kim, *et al.*, 1984). Infection in maize seeds reduces the germination and seedling vigour (Futrell and Kiglore, 1969; Scott and Futrell, 1970; Styer and Cantliffe, 1984). A seed to seedling transmission ratio of 1:1 (100 percent) has been reported by Foley (1962) and Kim, *et al.* (1984). Munkvold, *et al.* (1997) and Munkvold and Carlton (1997) concluded that this fungus moves systemically from the seed to the stalk and into the kernel of maize plants grown in the field. Seed to seedling transmission of *F. moniliforme* results into root rot, seedling blight, stalk rot and important yield loss either because of lodged plants or rotten ears and kernels (Lawrence, *et al.*, 1981; Oerke, *et al.*, 1994; Munkvold and Carlton, 1997).

Preliminary observations on the germination of maize seeds suggested that the contact area between the mesocotyl and the seed surface is influenced by the position of the seed in the substrate. Such variations in the contact area between the surface and the mesocotyl portion of the seedling could influence seed to seedling transmission of *F. moniliforme.* This influence could occur as a result of increased contact between the growth of the fungus outside the kernel and the mesocotyl portion of the seedling.

The present research was carried out in 1994 to investigate the influence of seed orientation in the substrate on seedling emergence, seedling structure, germination and its impact on the transmission of *F. moniliforme.*

Materials and Methods

Seed orientation and seedling development

A sample of maize, cultivar CMS 8807 from Cameroon (DGISP 35822) was used. The growth stages of the plant involved were from 0 to 3 (Chiarappa, 1971). Four orientations of the seed in the substrate were considered for sowing and are described as follows (Figure 1):

Vertical with the pedicel end facing down (VD).
Vertical with the pedicel end facing up (VU).
Horizontal with the embryal side facing down (HD).
Horizontal with the embryal side facing up (HU).

Three replicates of 50 seeds were sown in each of the 4 orientations. The seeds were sown 10 per plastic pot (12x12 cm), filled with sand mixed with water, at about 160 ml water per litre of sand. The seeds were covered with about 1 cm of sand. Each pot was placed in a growth chamber at 25° C under an alternate cycle of 12 hours darkness and light produced by a Phillips fluorescent lamp TLF 40 w/34 (ISTA, (1993). Recording of emerged seedlings was carried out at intervals of 12 hours until emergence of all viable seeds. After completion of germination in 7 days after

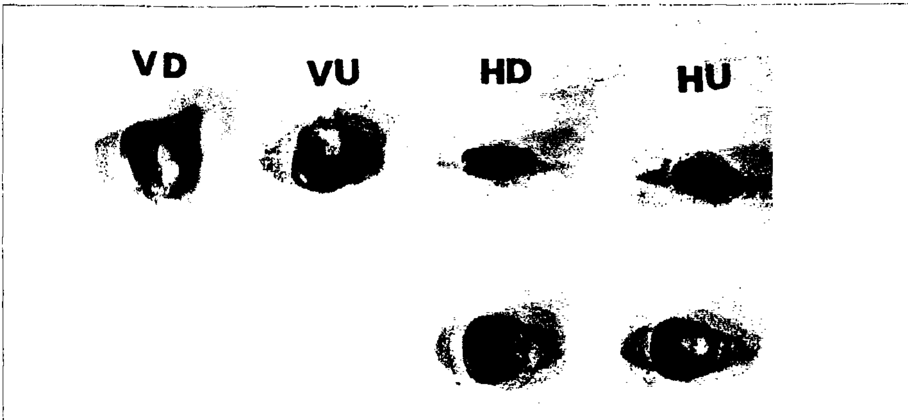

Figure 1: Orientations of the seeds in the seedbed. From left to right first line, vertical with the pedicel end facing down (VD), vertical with the pedicel end facing up (VU), horizontal with the embryal side facing down (HD), horizontal with the embryal side facing up (HU). Second line upper views of the last 2 orientations.

sowing according to ISTA rules (ISTA, (1999), normal seedlings, abnormal seedlings and dead seeds were evaluated. Seedling course was described from each of the 4 orientations and the length of the mesocotyl portion of the seedlings from each of the 4 orientations was recorded. The experiment was repeated three times.

Seed orientation and transmission of F. ***moniliforme***

Two maize seed samples from Egypt (DGISP 34009 and 32675) identified during seed health testing with 100 percent infection of *F. moniliforme* were used. Experiments were conducted with treated and untreated seeds. For seed treatment a systemic fungicide Benlate 50 wp (Dupont) powder formulation was used and a contact Thiram 80 powder formulation. The dosage for the 2 fungicides was 1mg per g of seed (0.1 percent w / w) and 2mg per g of seed (0.2 percent w / w) as recommended by the manufacturer as well as Raju and Sangam (1977). The treatment was carried out by putting the working samples into a glass flask, which was placed on a mechanical shaker at 600 rpm for 20 minutes with a gentle addition of the fungicide. The seeds were sown in 3 replications in some tests and 4 replications in other tests depending on the total number of seeds in the samples. In each replicate 15 seeds were sown in each of the 4 orientations described above. Each seed was sown individually in a plastic pot (9x9 cm) filled with sterile moisturized sand.

After 7 days under the temperature and light conditions described above, seedling emergence was recorded. Ten days after the sowing date abnormal seedlings and dead seeds were recorded and the seedlings were then taken from the pots, gently washed under tap water and evaluated for germination. The remains of the germinated kernel attached to the seedling were separated under aseptic conditions (lamina air flow with a flame and a sterile scalpel). Each seedling was surface disinfected separately in 1 percent sodium hypochlorite for 1 minute, and cut into sections of about 1 to 2 cm. The three sections plated for recovery of *F. moniliforme* (Figure 2) were described as follows:

Section one (SI), portion of the seedling from the seed to the first node including the node.

Section two (SII), about 2 cm portion of the seedling, approximately 1 cm on either side of the rupture coleoptile tip. This portion was separated into coleoptile tip SII-1, first leaf sheath SII-2, second leaf lamina (SII-3) and third leaf lamina (SII-4).

Section three (SIII), approximately 2 cm portion of the seedling 1 cm on either side of the point of separation of the lamina of the first leaf, separated into different parts as done for SII.

The scalpel and forceps were sterilized in 95 percent alcohol for about 1 minute after each operation while cutting the seedlings into parts or separating the components of each part. The components were plated on blotter and incubated at +20° C +/- 2° C under alternating 12 hours light (NUV) and darkness. Plates were first examined for growths of *F. moniliforme* after 7 days, and recording continued until 10 days after the start of incubation. These growth of *F. moniliforme* were identified following the keys characters described by Booth (1971) and Burgess, *et al.*, (1993). After preliminary observations with all the 4 orientations, consistent transmission studies on treated seeds were conducted with the 2 orientations HU and HD identified as giving minimum contact and the maximum contact respectively between the seed and the seedling shoot.

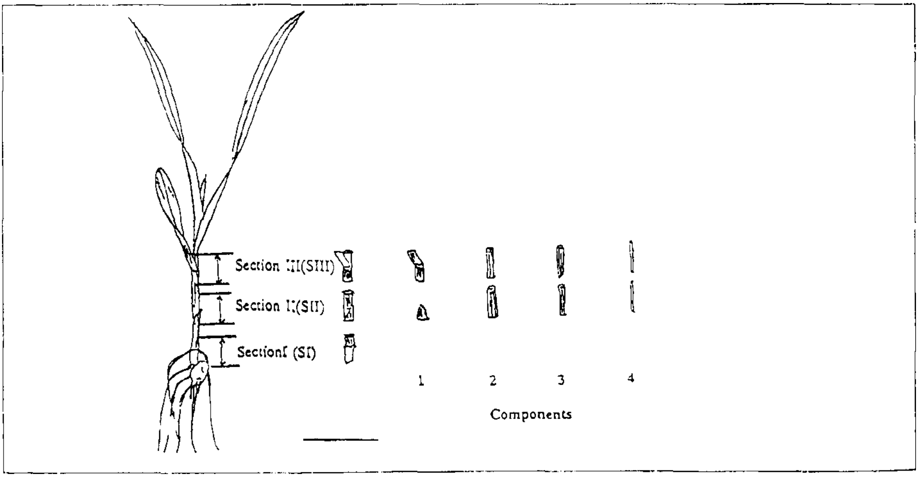

Figure 2: Diagrammatic presentation of sections of 10-day-old seedling and their components. From outside to inside of each section (1-4), SII: coleoptile tip, first leaf sheath, second leaf lamina and third leaf lamina, and SIII. (Bar 5 cm).

Statistical analysis

Data collected for germination and transmission of *F. moniliforme* were analyzed for statistical differences using multiple range analysis of variance and LSD at $\alpha \leq 5$ percent.

Results

Seedling course in the substrate

After initiation of the first development stage the maize seedling shoot grew easily toward the surface of the substrate if the seed was oriented vertically with the pedicel end facing down (VD) or horizontally with the embryal side facing up (HU). When oriented vertically with the pedicel end facing up (VU) a positive geotropism occurred first, before a subsequent elongation toward the surface of the substrate. For the horizontal with the embryal side facing down (HD), the seedling shoot had to mediate the obstacle caused by the seed before elongating toward the surface of the substrate. The seedling shoots from the orientations HU and VD travelled a short way compared to those of the orientations HD and VU (Figure 3). Consequent to this movement of seedling shoots the mesocotyl portion of seedlings from HD and VU has a larger contact area with the seed compared to those from HU and VD. The largest contact area was observed from HD, followed by VU, HU and VD.

Time required for seedling emergence

Figure 4 shows the percentage of emerged seedlings at intervals of 12 hours. Significant difference at 99 percent level of confidence (LSD) was found between the percentage of emerged seedlings from the orientation HU and VD compared to HD and VU, 72 hours, 84 hours and 96 hours after sowing (Figure 4). Faster emergence was recorded in orientations VD and HU.

Length of mesocotyl portion of the seedling

The length of the mesocotyl portion of the seedling ranged from 0.7 cm to 2.9 cm. Significant statistical differences at 99 percent level of confidence (LSD) were observed between the length of mesocotyl portion of the seedling from the orientation HU and VD compared to HD and VU. The mesocotyls of seedlings from HD and VU were significantly longer (20mm) than those of seedlings from HD and VU (11mm) (Figure 5).

Germination of treated and untreated seeds

From the germination of untreated seeds sown in the 4 orientations, differences were observed. The seed sown in the orientation HU and VU emerged better and disclosed a higher germination of 95 percent and 88 percent respectively, compared to the seeds sown in the orientation HD and VD recorded with 68 percent and 53 percent germination respectively (Table 1).

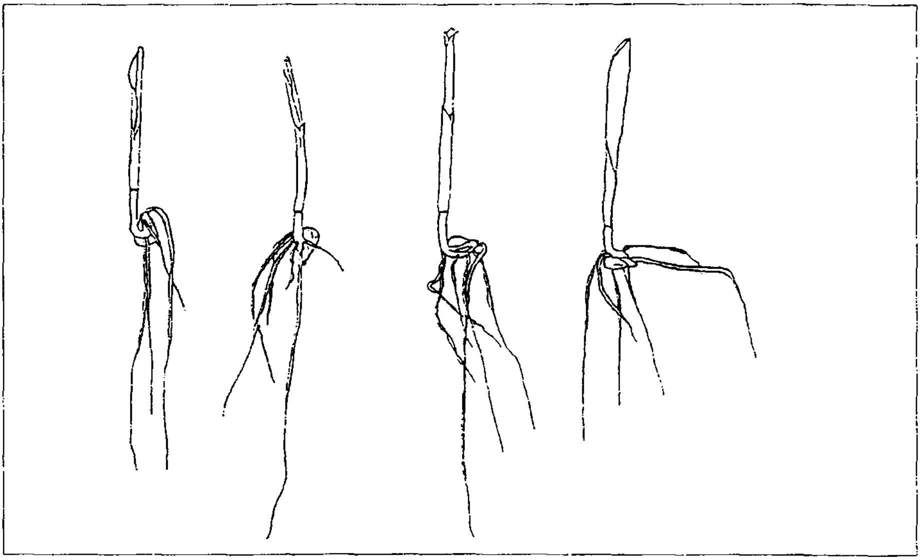

Figure 3: Diagramatic presentation of seedling course during the maize germination according to the orientation, from left to right vertical with the pedicel end facing up (VU), vertical with the pedicel end facing down (VD), horizontal with the embryal side facing down (HD), horizontal with the embryal side facing up (HU) (5 day old seedling) (Bar 3 cm).

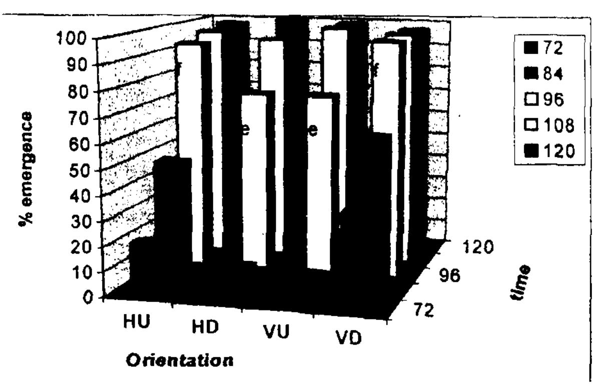

Figure 4: Seedling emergence (*percent*) at intervals of 12 hours from 72 hours to 120 hours after sowing. Significant difference at 99 *percent* (LSD) between (a) and (b), (c) and (d), (e) and (f).The numbers in the legend shows various recording times after sowing.

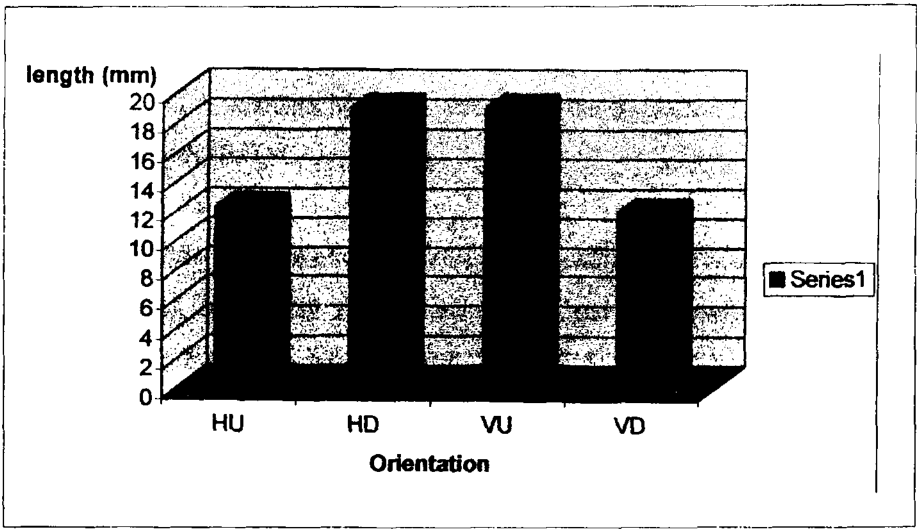

Figure 5: Length of mesocotyl, statistical significant difference at 99 percent (LSD) between a and b.

Table 1: Germination of treated maize seeds with Thiram 80 at 0.2 percent w/w and untreated seeds sown in different orientations.

Orientation and Treatment	% Emergence	% Germination	% Abnormal	% Dead seed
HU	100 *	95*	5	0*
HUT	100	98	2	0
HD	73*	68*	5	27*
HDT	97	95	3	2
VU	93	88	5	7
VUT	98	98	0	2
VD	58*	53*	20	27*
VDT	75	70	28	2

(*) Denotes significant difference at 99 percent (LSD) in the column. The numbers in the table are average from 4 replicates of 50 seeds per treatment or orientation: horizontal with the embryal side facing up untreated (HU) and treated (HUT), horizontal with the embryal side facing down untreated (HD) and treated (HDT), vertical with the pedicel end facing up untreated(VU) and treated (VUT), vertical with the pedicel end facing down untreated (VD) and treated (VDT).

These difference in the germination due to the orientation of the seed in the seedbed were statistically significant at 99 percent LSD. From the orientations HU, VU and VD 5 percent abnormal seedlings were recorded whereas 20 percent was recorded from the VD orientation. Higher levels of dead seed were recorded from the orientation HD (27 percent) and VD (27 percent) compared to HU (0 percent) and VU (7 percent). These differences were statistically significant at 99 percent LSD.

In all the 4 orientations, the germination of the treated seed was higher compared to the untreated. This improvement of the germination due to seed treatment was statistically significant at 99 percent LSD for the orientation HD. The best performance in the germination was obtained from the seed treated and sown in the orientation horizontal with the embryal side facing up (HUT 98, percent) and the vertical with the pedicel end facing up (VUT, 98 percent). The germination of the treated seeds in the orientation HU disclosed no dead seeds and that from VU no abnormal seedlings (Table 1), as a result of the interaction between the orientation of the seed in the seed bed and the treatment. The treated seeds sown in the orienation VD gave the lowest germination of 53 percent with a higher number of abnormal seedlings (20 percent) and dead seeds (27 percent). After the treatment the germination of the seeds in this orientation was improved up to 70 percent but remaind the lowest compared to other orientations with a lot of abnormal seedlings (28 percent) and dead seeds (27 percent).

*Transmission of **F. moniliforme** on untreated and treated seeds*

In the untreated maize seeds, a seed to seedling transmission ratio of 1:1 was recorded (Table 2). There was no difference in the transmission of *F. moniliforme* from untreated seeds as a result of orientation in the substrate. The maximum recovery of *F. moniliforme* was from the crown portion of the seedling and the fungus was extensively distributed in different parts of the seedling above the crown portion including coleoptile, leaf sheat and leaf lamina (Table 2). This shows the systemic development of *F. moniliforme* in 10 day old seedlings.

The growth of *F. moniliforme* was completely eradicated by the treatment with Benlate and the data presented in Table 2 are based only on the seeds treated with Thiram and those untreated.

After treatment with Thiram at 0.1 percent and 0.2 percent, the level of *F. moniliforme* found in the seedlings was reduced compared to the untreated (Table 2). This reduction was higher and statistically significant at 95 percent (LSD) in horizontal with the embryal side facing up (HU) (Table 2). This reduction of *F. moniliforme* in seedlings from treated seeds compared to untreated seeds followed the same trend and remained statistically significant at 95 percent (LSD) in section one (SI) also section two (SII) and its components (Table 2).

Table 2: Transmission of *Fusarium moniliforme* in maize seedlings after treatment with Thiram 80.

Orientation and Treatment Seedling section	HU	HD	HUT 0.1%	HDT 0.1%	HUT 0.2%	HDT 0.2%
SI	100.00*	100.00	63.60*	86.60	77.73*	91.07
SII.1	85.71	66.66	38.22*	30.00	17.77*	53.33
SII.2	47.61	42.22	24.61*	0.00	2.20*	22.20
SII.3	7.14	20.00	5.77	3.33	2.20	6.60
SII.4	4.76	15.55	3.03	3.33	0.00	2.20
SII	88.33*	73.33	41.66*	58.66	20.00*	55.66
SIII.1	2.38	15.55	0.00	7.03	0.00	7.17
SIII.2	7.14	15.55	0.00	10.73	2.33	0.00
SIII	9.33	26.66	0.00	17.66	2.33	9.00

* Denotes significant difference at 05 percent (LSD) in the line; the numbers in the table are the mean of 3 replications in 2 orientations: horizontal with the embryal side facing up untreated (HU), treated at 0.1 percent w/w (HUT 0.1 percent) and treated at 0.2 percent w/w (HDT 0.2 percent), horizontal with the embryal side facing down untreated (HD), treated with 0.1 percent w/w (HDT 0.1 percent) and treated with 0.2 percent w/w (HDT 0.2 percent) SII = cumulative recovery from all components of section II; SIII=cumulative recovery from all components of section III.

Discussion

Seed orientation and vigor testing for maize

The length of the mesocotyl recorded ranged from 0.7 to 2.9 cm during this work. Purseglove (1972) found this portion to be between 0 and 3 cm and also depending on the depth of planting. The present results have identified the orientation of the seed in the substrate as an additional factor that extends or shortens the length of this mesocotyl portion of the maize seedling.

Vigor testing for corn seed lots was previously carried out using total emergence and growth of seedlings within 3 days, first count of germination (4 days after sowing) or measurements of seedlings length (Woodstock, 1969; Grabe, 1976; Burris, 1977; Perry, 1981). According to the ISTA vigor committee 1974 to 1977 seedling growth, soil emergence and percentage of field emergence were components of seed vigor (Perry, 1977; Wilson, *et al.*, 1992). This work has shown very clearly that these indicators of vigor are significantly influenced by the orientation of the seed in substrates. This result provides as an additional proof that the vigor test earlier used is obsolete and this is in accordance with the present rules for seed testing from the International seed testing Association (ISTA). In case poorly equipped laboratories in developing countries should follow this old testing procedure, it is suggested that a uniform orientation be applied.

When evaluating the germination potential of seed lots, variation in conditions including temperature and moisture are used to reveal differences in the performances of seed lots (Isely, 1950; Grabe, 1976; Tekrony, 1995). According to these authors, tests devised to detect the response of seed lots to unfavorable conditions provides valuable information supplementing the tests carried out under favorable conditions and predicts the field performance of the seed lots. These aims are clearly indicated in the objectives of the vigor test. These objectives are defined as: << identification of seed lots which are capable of rapid and uniform seedling emergence, in the field and seed lots with high emergence ability in unfavorable environmental conditions >> (Perry, 1977). The 2 orientations HD and VU which gave delayed emergence of seedlings, could be regarded as an additional type of unfavorable condition, that should be considered during testing of seed lots to predict their field performance.

Maize seed treated with Thiram 80 and sown horizontally with the embryal side facing down (HDT, Table 1) or vertically with the pedicel end facing down (VDT, Table 1) showed a higher and statistically significant increase in germination. This observation probably means that this orientation, which provides the best contact of the seedlingshoot with the seed, gives the contact fungicide a better effect. Beneficial effects of contact fungicides have also been reported by Raju and Sangam (1977).

Seed orientation and transmission of ***F. moniliforme***

Seed to seedling transmission ratio of *F. moniliforme* was found to be 1:1 in the untreated maize seeds. A similar ratio of seed to seedling transmission of *F. moniliforme* was reported by Foley (1962) and Kim, *et al.* (1984). This transmission is not influenced by the seed orientation. Furthermore the orientation does not influence the efficiency of systemic fungicide Benlate in controlling *F. moniliforme* in treated seeds.

F. moniliforme was substantially recorded in all the sections of the seedlings plated and their components (Table 2). This illustrates very clearly that this fungus develops systemically in the 10 days old seedlings studied. Systemic transmission of *F. moniliforme* from maize seed to plant as well as the systemic development in the plants was earlier investigated by Foley (1962), Kedera, *et al.* (1994) and Munkvold, *et al.* (1997). However, the investigations in 10 days old seedlings performed in this study were conducted for the first time. Munkvold, *et al.* (1997) stated that seedling infections of *F. moniliforme* which persist, are seldom detectable above the crown tissue until the late reproductive stage or even after maturity. This work shows very clearly that in 10 day old seedlings the seed-borne infection of *F. moniliforme* is observed after its transmission in the seedling above the crown tissues (coleoptile, leaf sheaths and leaf lamina). Therefore the statement of these authors is not valid in this study.

This statistically significant reduction of *F. moniliforme* infection of seedlings from seeds treated with Thiram and oriented horizontally with the embryal side facing up (HU) means that this orientation provides a fast and easy way to the seedling shoot and thereby offfers to the seedling the opportunity to escape the inoculum of *F. moniliforme* on the surface of the seed. This is in accordance with the observation of Sing and Singh, 1977, Lawrence, *et al.*, 1981 and Kim, *et al.*, 1984, who demonstrated that the infection of *F. moniliforme* in maize is external and internal. According to these authors, the internal infection is in the mycelium in the inner layer of the pericarp, the endosperm and the embryo. The present results also hold true, even in cases where the Thiram seed treatment may have temporarily suppressed the external infection of the fungus. An opposite effect is observed when the seed has the HD orientation and in this case the course is longer and the contact between the seedling shoot and the seed is greater. This HD orientation probably provides a higher exposure of the seedling shoot in terms of contact and duration. The comparison of these two contrasting scenarios supports the novel observations of this investigation.

Adverse effects of a number of factors on seedlings during laboratory testing have been studied by Reddy (1935) and Rice (1944) quoted by Isely (1950). These authors indicated that adverse conditions should be created during evaluation of fungicides; the method of treatment as well as the reactions of treated and untreated seeds. The orientation with the embryal side facing down should be regarded as a method conferring unfavorable conditions useful during fungicides evaluation.

After sectioning and plating the crown portion of the seedling yielded higher records of *F. moniliforme* compared to other portions. This section, therefore, would be the best section to plate in studies dealing with the transmission of this pathogen into maize seedlings. This is in accordance with the conclusion of Anderegg and Gurthrie (1981), who found a positive correlation between the level of seed infection with *F. moniliforme* and the amount of infection in the crowns of subsequently developed plants.

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