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# Studies of host–pathogen interaction between maize and *Acremonium strictum* from Cameroon

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**Abstract**

Different effects of *Acremonium strictum* from Cameroon on maize cultivars Ndock 8701, CMS 8704 and CMS 8501 were investigated. Observations of symptoms and re-isolation of the pathogen showed that the disease causes chlorosis, leaf necrosis, stem necrosis, barren plants and wilting symptoms. Reduction in growth and yield is demonstrated. In the cultivar Ndock 8701 the pathogen showed systemic development in the host tissues with inter- and intracellular colonization of the vascular bundle and adjacent tissues including the protoxylem lacuna, xylem vessels, metaxylem, sieve tubes, protophloem and metaphloem. Gels and gums were observed in the maize xylem vessels after fungal invasion and are part of the host defence response. Coloration corresponding to acidic carbohydrates and phenolic compounds was recorded. This is the first demonstration of the pathogenic nature of *A. strictum* in maize from Cameroon as well as the observation of gels and gums. This pathogen must be regarded as important considering its interaction in maize.

## Introduction

Maize is one of the leading cereal crops of the world (FAO, 1997). Its cultivation is limited by diseases which cause grain losses of about 11% of the total production (Oerke et al., 1994). In Cameroon, where about 650,000 tons of maize are produced yearly (Anonymous, 1987; NCRE, 1993), diseases are important constraints. Diseases such as blight, stalk and ear rot and smut have been reported with localized yield losses of about 11–50% (Nankam, 1991; Ngoko, 1994; Cardwell et al., 1997).

During testing of maize seed samples from Cameroon for seed-borne fungi at the Danish Government Institute of Seed Pathology for Developing Countries (DGISP) between 1993 and 1995, thirteen fungi belonging to nine genera were identified including *Acremonium strictum*, *Bipolaris maydis*, *Botryodiplodia theobromae*, *Colletotrichum graminicola*, *Fusarium moniliforme*, *Fusarium oxysporum* and *Macrophomina phaseolina* (Tagne, 1995). Among these fungi, *A. strictum* was one of the most frequent (Tagne, 1995). This research studied the interaction between this fungus and maize, and describes the major pathogenic effects of *A. strictum* in infected maize tissues.

## Materials and methods

### *Maize and* A. strictum *isolates*

Plants of the maize cultivar Ndock 8701 (DGISP number 35817) from Cameroon were grown in a greenhouse from non-infected seeds identified during seed-health testing. The seeds were sown one per pot in 20 cm

diameter plastic pots filled with soil. The greenhouse temperature was about 26 °C and fertilizer 20 : 10 : 10 NPK was applied at about 20 g per plant 3 weeks after sowing. For field experiments in Cameroon, healthy seeds of cultivars CMS 8704, CMS 8501 and Ndock 8701, commonly used by farmers, were planted.

*A. strictum* Gams isolated from maize seed of cultivar CMS 8501 was cultured on Potato Dextrose Agar (PDA) and used for inoculation. This isolate was deposited at the Mycological Laboratory of DGISP, Denmark, and later at the Plant Pathology Laboratory of IRAD, Cameroon. It was also deposited in the type culture collection of CAB International Mycological Institute, Kew, England, under the reference number IMI 363644. The identification of the fungus was based on cultural characteristics on different media and microscopic observations. The characters fit the description of *A. strictum* made by Gams (1971) and Domsch et al. (1980). The identification, the conclusion made on the pathogenic nature of the fungus and the knowledge from the literature (Harris, 1936; Sabet et al., 1970; Raju and Sangam, 1976) that *A. strictum* is the only known species of *Acremonium* causing disease in maize, confirmed that IMI 363644 was *A. strictum* W. Gams (*Cephalosporium acremonium* auct. mult.) (Gams, 1971).

*Preparation of the inoculum and inoculation of plants in the greenhouse*

*A. strictum* (IMI 363644) was grown on PDA. Sterile toothpicks were placed on top of the agar and incubated at 20 °C under a NUV cycle produced by Philips tube of 12-h light and darkness for 10 days to allow the fungus to colonize the toothpicks. The plants were inoculated with *A. strictum* 3 and 7 weeks after sowing. Twenty-four plants were inoculated at each stage, using a modified toothpick method developed by Young (1943). In each of the 24 plants, the toothpick collected aseptically from the medium with the fungal growth was inserted at the second internode. Six plants were used as control, three with sterile toothpicks and three without toothpicks. The inoculated plants and the control plants in the greenhouse were arranged in a completely randomized design.

*Disease evaluation in the greenhouse*

The inoculated plants were evaluated once a week until 110 days after sowing by visual observation of symptoms, measurement of plant height and stem circumference. Maize stems were split about 90 and 60 days after inoculation, for the two inoculation times, respectively, and evaluated for necrotic vascular bundle and brown discoloration of infected tissues. The pathogen was re-isolated from surface-disinfected plant parts sampled from roots, root crowns, nodes, internodes, leaf sheaths and leaves, plated on wetted blotter paper, and incubated under the conditions described above.

*Collection of maize tissues from the greenhouse for histological studies*

Plant parts were collected from different nodes and internodes of 110-day-old maize plants about 60 days after inoculation with *A. strictum*. From each part, pieces of about 4 mm width and 5 mm length were cut. Each piece was divided into 2: one was fixed in formalin–ethanol–acetic acid (FAA), the other one was plated on PDA and incubated under NUV light as described above. After incubation, the single specimens were recorded for the presence of *A. strictum*, and if found, the corresponding fixed specimen was processed for microscopy.

*Preparation of tissues for microscopy*

The tissues were fixed in 4% FAA, dehydrated sequentially in 70% and 96% ethanol, and finally embedded in cold polymerizing resin (Technovit 7100, Heraeus Kulzer, Germany). Sections of 7 μm thickness were made using a Leitz rotation microtome (Neergaard, 1997). Staining was done with Toluidine Blue O, 0.05% at pH 4.4 (Siegel, 1967; Neergaard, 1997). The stained sections were mounted in DPX (FLUKA) mounting media and observed under light microscopy. Description of stained tissues follows Siegel (1967) and Neergaard (1997).

*Field experiment*

A field experiment was carried out at the Institute of Agricultural Research for Development (IRAD) Nkolbisson, Cameroon, in 1997 using the three maize cultivars. Each experimental plot had four rows of 5 m length with three replications. Two seeds were sown per hill at 0.5 m gaps in the rows with 0.75 m between the rows. The inoculation was performed when the maize plants were 4 weeks old by inserting toothpicks infested with the pathogen in the second internode of each plant. The same isolate as in the greenhouse

trials (IMI 363644) was used and the inoculum was prepared in the same way as described above. In each plot, the two middle rows were inoculated and the two outside rows served as controls. Weeding and fertilizer application (NPK) were carried out appropriately. Height of plants and stem circumference were measured: plant health and symptoms on leaves were recorded. At maturity, the stems were split and evaluated for necrotic vascular bundle. The harvested ears were weighed and ear weight reduction for each cultivar was determined as the mean difference from the three replicates between the inoculated plants and the controls. The percentage weight reduction from the non-inoculated control was calculated. This value indicates the percentage reduction of the yield.

*Data analysis*

Quantitative data were subjected to statistical analysis, and the separation of means was done using the Least Significant Difference (LSD) at the 97.5% level of significance.

## Results

*Symptoms on leaves, effect on plant growth and recovery of the pathogen in greenhouse experiments*

With the plants inoculated in the greenhouse when 3 weeks old, progressive discoloration and veinal necrosis were observed on the leaf blade elongating either upward or downward. No symptoms were observed on the non-inoculated controls. Chlorotic areas were found in the blade developing from the area adjacent to the point of inoculation, whereas the leaves from the plant inoculated with sterile toothpicks showed no symptoms (Figure 1). Recovery of the pathogen was possible at a distance of about 25 cm upward (blade) and 10 cm downward (leaf sheath) from the point of inoculation 3 weeks after inoculation. The leaves became yellow and finally died from the upper to the lower part (Figure 1C).

On the plants inoculated in the greenhouse 7 weeks after sowing, all the leaves infected through their sheath were either dead or showing necrotic yellowing after 1 week. This necrotic yellowing was observed on one side of the leaf blade (Figure 1D). This symptom developed in an acropetal manner and faster compared to the plants incoculated when 3 weeks old. Dead leaves showed reddish lines following the development of necrotic veins. The same type of observations and dead leaves were recorded on plants in the field for all 3 maize cultivars 5–10 days after inoculation. No similar symptoms were found in the non-inoculated control plants.

Growth of plants inoculated when 3 weeks old was poor compared to the non-inoculated plants. A mean reduction of 12% was found in stem circumference (Table 1). This reduction was statistically significant at the 97.5% level of confidence (LSD). A mean reduction of 21% was also found in the height of inoculated plants (Table 1). This reduction was statistically significant at the 97.5% level of confidence (LSD). None of the plants inoculated at this stage tasseled or produced ears, thus failing to reach stage 7 of the maize growth stages described by Chiarapa (1971). The inoculated plants became stunted and barren. Early wilting was recorded with some plants, while others wilted just before the completion of the test.

The symptoms in plants inoculated when 7 weeks old and observed from 1 week to 60 days after inoculation, were very similar to those recorded from plants inoculated when 3 weeks old. Brown disrupted tissues were noted in the stem at the point of inoculation in all plants, while no such effect was found on the control plants inoculated with sterile toothpicks. This necrosis was clearly observed after longitudinal splitting of the stem (Figure 2).

Heavy growth of the pathogen was recorded in samples taken at random from inoculated plants. Its distribution in the different plant parts was as follows: roots (96%), root crown (88%), nodes (88%), internodes (83%), leaf sheaths (29%) and leaves (25%) (Table 2).

*Effect on plants in the field*

In the field, reduction in stem diameter and height of plants was found in inoculated plants of each cultivar as a result of pathogen presence, compared to the non-inoculated control plants (Table 3). Twenty-four percent mean reduction in stem diameter was recorded for maize plants of cultivar Ndock 8701, 15% for CMS 8501 and 5% for CMS 8704 (Table 3). This reduction in stem diameter was statistically significant at a 97.5% LSD for the cultivars Ndock 8701 and CMS 8501. A 20% reduction in height was recorded for maize plants of the cultivars Ndock 8701, 0.5% for CMS 8501 and 12% for CMS 8704 (Table 3). This reduction in plant height was statistically significant at a 97.5% LSD for the cultivars Ndock 8701 and

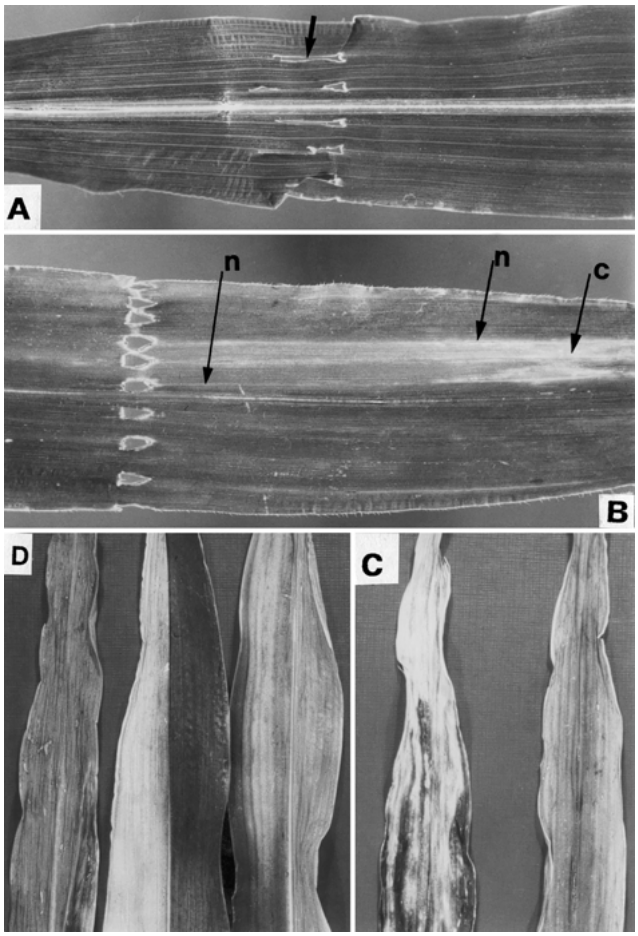


*Figure 1.* Leaf symptoms of black bundle disease caused by *A. strictum* on maize. (A) Leaf inoculated only by the toothpick (arrow). (B) Necrotic veins (n), chlorosis (c). (C) From left to right, progress of vein necrosis and systemic yellowing 2–3 weeks after toothpick inoculation. (D) From left to right, wilted leaf and lateral leaf yellowing observed in plants inoculated when 7 weeks old.

*Table 1.* Reduction in height and stem circumference of maize plants grown in the greenhouse about 90 days after inoculation with *A. strictum*

| Treatment | Number of plants | Plant height (cm) | Reduction in height (cm) | Percentage of reduction | Stem circumference (mm) | Reduction in stem circumference (mm) | Percentage of reduction |
|---|---|---|---|---|---|---|---|
| Control | 6 | $238 \pm 20$[1] | — | — | $50 \pm 2$ | — | — |
| Inoculated | 24 | $188 \pm 43$[2] | 50 | 21 | $44 \pm 3$[1] | 6 | 12 |

[1]The data in the table are means of measurements made on individual plants.
[2]Statistically significant differences ($P < 0.05$, $\alpha = 0.03$).

CMS 8704. The inoculated plants grew to maturity, but showed disrupted tissues at the point of inoculation, stunted growth and ear weight reduction. A 26% reduction in ear weight was obtained for the cultivar CMS 8501, 12% for Ndock 8701 and 1% for CMS 8704 (Table 3). This reduction was statistically significant at a 97.5% LSD for the cultivars Ndock 8701 and CMS 8501.

*Symptoms in split stems*

In split stems of inoculated plants taken from the greenhouse after 90 and 60 days, respectively, for the first and second sets of plants, discoloration of vascular bundle tissues was observed developing from the root crown area upwards in the stem. The intensity of the discoloration changed from dark to light brown in the lower and upper internodes of the stem, respectively. Eleven plants (46%) of the 24 inoculated when 3 weeks old, and 24 plants (100%) inoculated when 7 weeks old were found with necrotic and discolored tissues. In the stalk of a number of plants, discolored bundle tissues and brown rotten-like tissues were recorded at the same time. No such observation was found in any of the control plants with or without toothpicks (Figure 2). Large areas of black dead tissues were also observed in each of stem as a result of pathogen infection of the stems. A small area of dead tissues was found in the stem of plants inoculated with sterile toothpicks (Figure 2). Necrosis of the vascular bundles extended upwards in the stalks of the inoculated plants. No color change or necrosis was found in control plants (Figure 2). The same necrotic and discolored tissues were found in plants of the three maize cultivars inoculated in the field with their length varying from 11–58, 8–103 and 8–20 cm for the cultivars Ndock 8701, CMS 8501 and CMS 8704, respectively (Table 3).

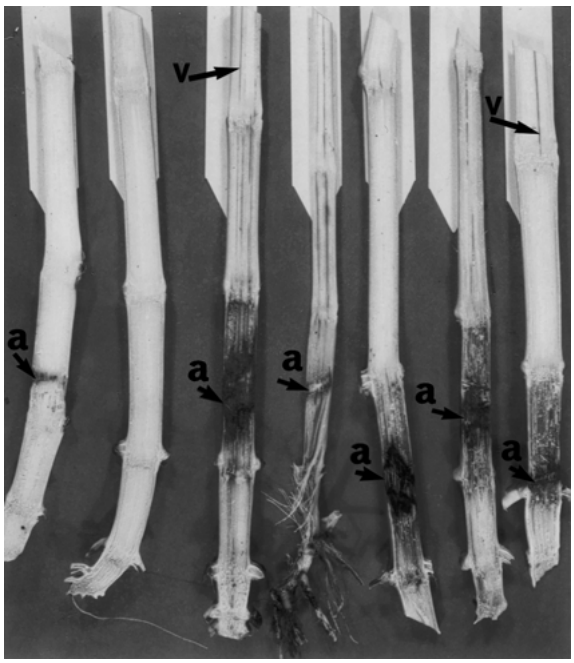


*Figure 2*. Split stems of plants inoculated with *A. strictum* when 7 weeks old; from left to right, control plants inoculated with sterile toothpick and without inoculated plants with area of inoculation with toothpick (arrow a) and necrotic vascular bundle (arrow v).

*Microscopical studies of infected tissues*

During the observation of stem tissue sections stained with Toluidine Blue O, cells of the infected vascular bundle tissues were dark brown to non-infected and healthy cells (Figure 3A,B). This discoloration progressed to adjacent tissues of the vascular bundles. Red, red-violet and blue-green colored substances were also observed in intact adjacent tissues (Figure 3C). In some cases cell walls had dissolved around the protoxylem lacuna (Figure 4B), while a compressed appearance of cells was noticed in the middle of intact and well-differentiated cells of the vascular system.

Yellow substances (non-stained) filled the metaxylem, protoxylem lacuna and xylem vessels of the vascular bundles. The color of these substances varied from light to deep yellow, from the area showing advanced symptoms to adjacent areas (Figure 4C,D). Hyphae extended into the protoxylem, xylem vessels, metaphloem and in some cases appeared in the protoxylem lacuna, intra- and intercellularly with a deep to light-violet appearance (Figure 4A–C).

*Table 2*. Recovery of *A. strictum* from different parts of maize plants 60–90 days after inoculation of the second internodes

| Treatment | Number of plants | Roots | Crown | Internodes 3–10 | Nodes 1–10 | Leaf sheaths (8–14)[1] | Leaves (8–14) |
|---|---|---|---|---|---|---|---|
| Control | 48[2] | 0 | 0 | 0 | 0 | 0 | 0 |
| Inoculated | 12 | 96 | 88 | 88 | 83 | 29 | 25 |

[1]Brackets indicate order of leaf sheaths or leaf development on the plant.

[2]The numbers in the table are the mean of the percent recovery found in the designated part of the plant out of the inoculated and non-inoculated.

*Table 3*. Necrosis in split stems, reduction in height, diameter and yield of maize in the field after inoculation with *A. strictum* at the second internode

| Maize cultivar | Height of inoculated plants (cm) | Height of control plants (cm) | Reduction in height (cm) | Diameter of inoculated plants (mm) | Diameter of control plants (mm) | Reduction in stem diameter (mm) | Ear weight reduction | Length of necrosis in inoculated plants (cm) | Necrosis in control plants (cm) |
|---|---|---|---|---|---|---|---|---|---|
| Ndock 8701 | 123 ± 12[2] | 153 ± 2.35 | 30[1] ≈ 20% | 16 ± 0.2 | 21 ± 1.2 | 5[1] ≈ 24% | 12%[1] | 11–58 | 0 |
| CMS 8501 | 214 ± 1.4 | 215 ± 0.06 | 1 ≈ 0.5% | 16.6 ± 0.7 | 19.6 ± 2 | 3[1] ≈ 15% | 26%[1] | 8–103 | 0 |
| CMS 8704 | 190 ± 28 | 216 ± 4.7 | 26[1] ≈ 12% | 17.7 ± 1.6 | 18.6 ± 0.4 | 0.9 ≈ 5% | 1% | 8–20 | 0 |
| Mean | 176 ± 42 | 195 ± 29 | 19 ≈ 10% | 16.7 ± 0.7 | 19.7 ± 0.9 | 3 ≈ 15% | 13% | 9–61 | 0 |

[1]Denotes significant statistical difference compared to the control at 97.5% LSD.
[2]The measurements of the height and circumference are means from many individual plants and also from the three replicates.

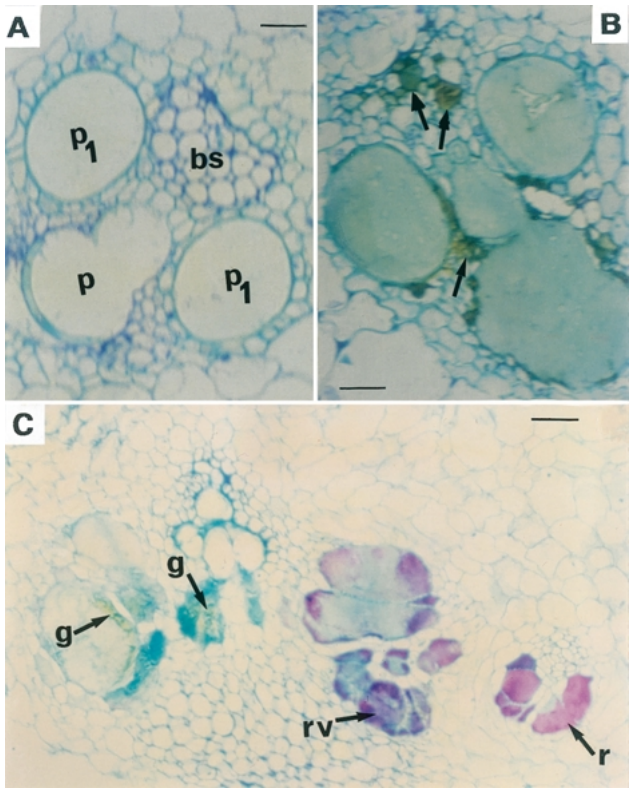


*Figure 3*. Maize stem sections from the axis of 110-day-old plants after inoculation with *A. strictum*, stained with Toluidine Blue followed by light microscopy (A and B bar = 30 μm). (A) Tissue of a healthy plant: Anatomical description of maize vascular bundle, P = protoxylem lacuna; P1 = xylem vessels; bs = sieves tubes and companion cells. (B) Section of infected plant similar to (A) with brown to black symptoms (arrows) in the vascular bundle, caused by *A. strictum*. (C) (r) = red = acidic carbohydrates, (rv) = red violet = neutral-acidic carbohydrates, (g) = blue green = polyphenolic substances (bar = 50 μm).

## Discussion

*Disease and symptoms in plants*

All the plants inoculated in the greenhouse and the field showed symptoms in leaves including vein necrosis, chlorosis, yellowing and wilt developing in an acropetal manner. These observations proved the pathogenic nature of *A. strictum* on maize of Cameroon. The symptoms on leaves are similar to those observed by Reddy and Holbert (1924) and Raju and Sangam (1976). The chlorosis observed may have been due to the activity of chlorophyllase enzyme. Pegg (1981) stated that chlorosis can be a response to water stress, and since there was no water stress, we concluded that chlorophyllase was responsible. Other symptoms recorded in all the inoculated plants were barren stalks and plants, stunted plants and nubbin ears. Similar symptoms were described by Green (1981) as a general wilt symptom and a characteristic of fungi infecting the vascular system.

These results indicate that *A. strictum* is not only the vascular disease pathogen described earlier, but also a wilt pathogen of maize, and this is in accordance with the conclusions of Harris (1936) and Sangam et al. (1976). The distribution of the pathogen in different parts of the maize plant, i.e., most roots, root crown, nodes, internodes, leaf sheaths and leaves, indicated that *A. strictum* develops systemically in the host maize. Such systemic development of *A. strictum* has been demonstrated earlier in sorghum (Bandyopadhyay et al., 1987), but not in maize.

Split stems showed a necrosis of vascular bundle tissues developing through internodes and a brown discoloration of tissues. Similar observations were made of symptoms of vascular bundle disease of maize caused

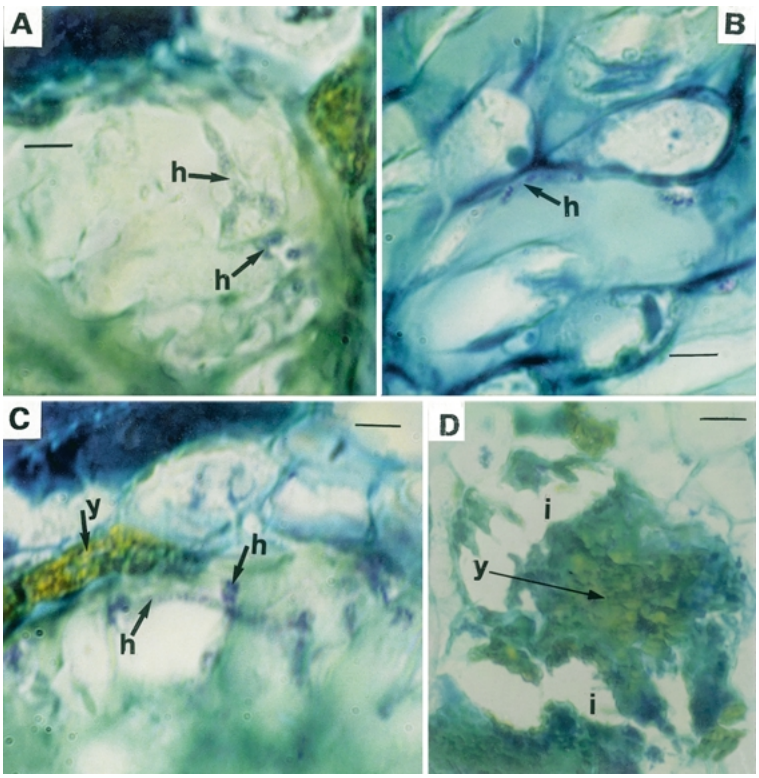


*Figure 4.* Maize stem sections from the axis of 110-day-old plants after inoculation with *A. strictum*, stained with Toluidine Blue followed by light microscopy (A, B and C bar = 2.8 μm). (A) (h) = hyphae in the protoxylem lacuna; (B) (h) = hyphae in the cells adjacent to the vascular bundle. (C) (h) = hyphae, (y) = yellow substances in the cells. (D) disintegrated tissues (i); yellow substances in the tissues (y) (bar = 5 μm).

by *A. strictum* (Reddy and Holbert, 1924; Gupta and Renfro, 1977; Harris, 1936; Sabet et al., 1970; Raju and Sangam, 1976). Green (1981) attributed similar symptoms to vascular wilt and stated that it is a disease syndrome due to either the actual presence of the pathogen or to its propagules. Browning of vascular elements and adjacent tissues may indicate oxidized phenolic compounds induced by the mycelium in the vessels (Pegg, 1981) due to the deposition of dark pigments in vessel, tracheid walls and surrounding parenchyma cells.

Reductions in ear weight (12% and 26%) and stem diameter (24% and 15%) recorded for the cultivars Ndock 8701 and CMS 8501, respectively, were significantly higher than for the cultivar CMS 8704. These observations agree with the results of Reddy and Holbert (1924) relating growth reduction caused by *A. strictum* and loss of production per plant. Differences between the reaction of the cultivar CMS 8704 and cultivars Ndock 8701/CMS 8501 may reflect genetic differences. This agrees with The (1992), who described Ndock 8701 and CMS 8501 in one genetic pool and CMS 8704 in another genetic pool. It is therefore concluded here that the maize cultivar CMS 8704 is more resistant to the isolate of *A. strictum* tested than CMS 8501 and Ndock 8701.

### *Histological studies of infected tissues*

Fungal hyphae and host plant reactions were observed in the protoxylem lacuna, xylem vessels, metaxylem, sieve tubes, protophloem and metaphloem. These hyphae occurred inter- and intracellularly in stem tissues of 110-day-old maize plants. Sabet et al. (1970) made similar observations in maize plants. Similar patterns of tissue colonization were described by Green (1981) and Beckman et al. (1953) for other wilt fungi including *Fusarium oxysporum* f. sp. *cubense* in banana and *Ceratocystis fagacearum* in oak.

Early wilt and brown discoloration of vascular bundle elements were recorded. Similar discolorations were discussed by Green (1981) and Pegg (1981),

who suggested that wilt is the result of toxins produced by the pathogen and involves the deposition of melanin-like compounds on the walls of xylem vessels and neighboring parenchyma cells. After inoculation of maize plants with *A. strictum*, yellow substances filling the metaxylem, protoxylem lacuna and xylem vessel of the vascular bundles were observed, similar to substances and reactions found and discussed by Pegg (1981) and Green (1981) for other wilts (*F. oxysporum* f. sp *cubense* in banana and *C. fagacearum* in oak).

The color recorded in the cells after staining with Toluidine Blue O, indicating acidic carbohydrates (red), acidic-neutral carbohydrates (red violet), polyphenolic substances (blue green) and unstained substances (yellow) are in accordance with Siegel (1967) and Neergaard (1999). This describes the encounter of *A. strictum* with maize. Similar observations were made by Beckman and Zaroogian (1967) after studies with banana and different pathogens including *F. oxysporum* f. sp. *cubense*.

The substances recorded in the maize xylem vessels in this study are not membrane-bound invagination into vessels and therefore are not tyloses. This is the first time that they have been reported for *A. strictum* and maize. Beckman and Zaroogian (1967) found similar gels or gum substances in diseased banana vascular tissues infected by *Fusarium* sp., stained violet with Methylene Blue and orange with Safranin O. This indicated an acidic polymer probably pectic in nature. In other host–pathogen interactions pH of the vessel gums were 3.5–4 (E. Neergaard, pers. observ.) and this indicates acidic carbohydrates.

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