Australasian Plant Pathology (1996) 25: 50-54

Root and stalk rot of maize caused by *Phaeocytostroma ambiguum* recorded for the first time in New South Wales

G.E. Stovold, A. Newfield and M.J. Priest

NSW Agriculture, Biological and Chemical Research Institute, PMB 10, Rydalmere, New South Wales 2116 Australia

Abstract

Phaeocytostroma ambiguum is reported for the first time in New South Wales associated with root and stalk rot of maize plants near Darlington Point in the Riverina district. Symptoms observed on field-infected plants were reproduced on the roots of seven maize hybrids, inoculated with pure cultures of *P. ambiguum* and grown under glasshouse conditions. The hybrid 108/7222 showed significantly less root infection than the hybrids 766/6213, 797/7529 and 925/7323. Typical symptoms of stalk rot were also reproduced on the artificially inoculated hybrids except for 108/7222 and 797/7529, which appeared to be resistant to stalk infection.

Introduction

Stalk and root rots are common in maize (*Zea mays* L.) production areas throughout the world, often causing yield losses of 10 to 20% in susceptible hybrids (Shurtleff 1980). A range of fungi is associated with stalk and root rots, the most common belonging to the genus *Fusarium*. Other important incitants of this disease include *Diplodia* spp., *Pythium* spp., and *Macrophomina phaseolina* (Tassi) Goid. Many other fungi have also been associated with stalk and root rot (Shurtleff 1980).

In Australia, *Fusarium graminearum* Schwabe Group 2 (Francis and Burgess 1977) and *F. moniliforme* Sheldon, have been reported as the major causes of stalk and root rot of maize grown in New South Wales (NSW) and southern Queensland (Wenholz and Darragh 1927; Edwards 1933; 1935; 1936; Pont 1963; Purss 1963; Anon 1971; Francis and Burgess 1975). Additionally, *Marasmius sacchari* Wakk. var. *hawaiiensis* Cobb and *M. graminum* (Lib.) Berk. var *brevispora* Dennis have been reported from the Atherton Tableland, Queensland during dry seasons (Pont 1973). Ramsey (1990) tested the pathogenicity of a range of fungi isolated from maize in north Queensland and reported *Pyrenochaeta indica* Viswanathan as a cause of root rot.

The fungus *Phaeocytostroma ambiguum* (Mont.) Petr. (syn. *Phaeocytosporella zeae* Stout) has been associated with stalk rot of maize in North America (Stout 1930; Koehler and Boewe 1957) and its ability to cause a dark brown root rot demonstrated by Craig and Koehler (1958). This fungus has also been reported from France (Messiaen 1955) and Serbia (Smiljakovi *et al.* 1979). The only record in Australia is on *Sorghum bicolor* (L.) Moench in Queensland (Sutton 1980). This paper reports the first occurrence of *P. ambiguum* on maize in NSW and describes its pathogenicity in glasshouse experiments.

Methods

Identification of *Phaeocytostroma ambiguum* In May 1990, maize plants showing symptoms of root and stalk rot were forwarded to the Plant Disease Diagnostic Laboratory at Rydalmere from the Darlington Point area of the Riverina district of New South Wales. Scattered lodging and premature senescence were reported in several fields in the area, particularly those cropped successively to maize, but widespread damage was not evident. Affected plants showed bleached, straw coloured lesions with a dark margin at the base of the stalks and extensive rotting of the roots where lesions similar to those present on the stalks were evident (Figure 1). Black fungal fruiting bodies were present on the diseased stalk and roots and these were excised for microscopical examination. Small pieces of infected stem and root tissue were removed

and plated onto potato dextrose agar (PDA) and PDA acidified with lactic acid. Spores from the fruiting bodies were also suspended in sterile water and streaked onto PDA and acidified PDA.

Pathogenicity tests An isolate of *P. ambiguum* (DAR 66077) was grown on sterile corn meal sand medium (3 g corn meal, 100 g washed sand, 14 mL tapwater, autoclaved 30 min at 121°C) in 500 mL flasks for 28 days at room temperature (18–22°C). This inoculum was used to inoculate pasteurised (60°C for 30 min) potting mix soil (loam 50%, sand 25%, peat moss 25%) with balanced fertiliser added. Pots (15 cm diameter) were two thirds filled with soil and four separate lots of 10 g of inoculum placed in each pot. Additional soil was added and four maize seeds surface-sterilised in 70% ethanol for 30 sec followed by 2 min in 10% sodium hypochlorite were planted directly above the inoculum, and covered with soil.

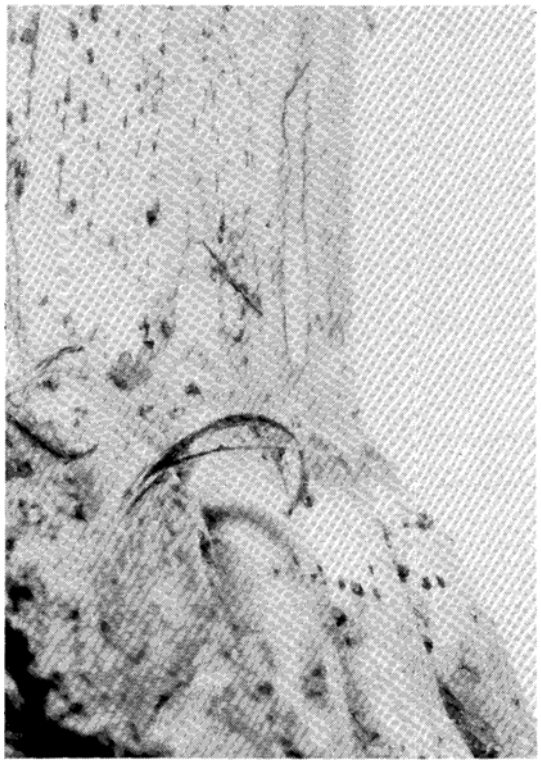

Figure 1 Typical symptoms of root and stalk rot caused by *Phaeocytostroma ambiguum* on maize plants sampled from Darlington Point, New South Wales.

Seven varieties of hybrid maize (Table 1) were inoculated. There were three inoculated pots and one uninoculated pot of each hybrid. Plants were maintained under normal summer/autumn glasshouse conditions (15–40°C) throughout the test. Additional slow-release fertiliser (Nutricote) was added after 5 weeks at a rate of 10 g per pot to maintain growth.

After 94 days, when plants were at the early grain fill stage two pots of each hybrid and the uninoculated pot were harvested by washing all loose soil from the root system. The extent of root infection was determined by cutting ten roots (approx. 25 cm) from each plant and measuring the length of rotted tissue. Five pieces of diseased root tissue (1 cm long) from each plant were plated to acidified PDA to check for the presence of the inoculated fungus. Root systems and lower stem tissue were examined microscopically to determine if fruiting bodies were present. Top weights and root weights of each plant were recorded after drying overnight at 80°C.

One pot of each inoculated hybrid was left for additional symptom development during maturation and senescence. These plants were harvested and symptoms on roots and stalks noted as above, 143 days after inoculation.

Results

Identification of *Phaeocytostroma ambiguum* Microscopic examination of prepared slides, made from the fruiting structures on the diseased stalks and roots enabled the identification of the fungus as *P. ambiguum*, according to the description given by Sutton (1964; 1980). The fungus was readily isolated onto PDA, and acidified PDA directly from diseased root and stem tissue. Spores from the fruiting bodies germinated readily on both media and yielded pure cultures. Pycnidia were formed on artificial media within 4 weeks and enabled confirmation of the association of *P. ambiguum* with the symptoms of stalk rot and root rot.

Description Cultures on PDA moderately fast growing with white to pale grey-brown aerial mycelium, reverse of culture pale brown. Numerous black, irregular, thin stroma present throughout the plate. Conidiomata mostly multilocular and convoluted with irregular ostioles. Conidia formed enteroblastically on long, thin, hyaline phialides

with a minute collarette, pale brown, ellipsoidal to pyriform, 12–16 x 5–6 μm. Paraphyses, scattered amongst the phialides, hyaline, septate and occasionally branched, usually much longer than the phialides. Dried specimens and living cultures have been lodged in Herb. DAR.

Pathogenicity test During the 94 day growth period of the plants in the glasshouse there were no visual symptoms of stalk rot or reduced growth of inoculated plants compared with the uninoculated controls for any of the hybrids tested. Dry weight of tops and roots were not significantly different for inoculated and uninoculated plants and these data are not presented. However, when soil was washed from the roots, extensive discoloration of the root system of inoculated plants was evident. The degree of damage, as measured by percentage healthy root length, is given in Table 1. *P. ambiguum* was recovered from all hybrids and on some, pycnidia containing mature spores were observed (Table 1).

All hybrids were infected but the degree of root infection varied. Analysis of variance on square root transformation of percentage healthy root length indicated hybrid 108/7222 was significantly less infected than hybrids 797/7529, 766/6213 and 925/7323 (Table 1). Although recovery of *P. ambiguum* from diseased roots was variable, 108/7222 yielded 55% recovery which was less than all other hybrids except 507/7457 and 925/7323. Hybrid 108/7222 also had a low level (12% of plants) with mature pycnidia compared with the other hybrids.

When the remaining plants were harvested after 143 days, 100% of roots of all hybrids were diseased except 797/7529 and 108/7222 (88% and 90%, respectively). Unlike the other hybrids, 797/7529 and 108/7222 failed to develop symptoms of stalk rot, further evidence that these two hybrids have a degree of tolerance to the disease.

Representative samples of plants artificially infected with *P. ambiguum* have been lodged in the Rydalmere Herbarium (DAR 67452-67458).

Discussion

P. ambiguum was consistently associated with diseased roots and stalk tissues of maize plants showing scattered lodging in the Riverina district of New South Wales in 1990. This is the first record of this disease in New South Wales. Although *P. ambiguum* was first reported on maize in Illinois, United States of America (Stout 1930) and subsequently reported from France (Messiaen 1955) and Serbia (Smiljakovi *et al.* 1979), it is generally regarded as a weak pathogen present on mature stalks and roots. In artificial inoculation tests, Craig and Koehler (1958) reported that *P. ambiguum* caused extensive infection of seedling roots, but they detected no differences in dry weight of inoculated and uninoculated plants. In Illinois the incidence of *P. ambiguum* on rotted stalks was low compared with the incidence of *Diplodia zeae* (Schw.) Lev. and *Fusarium* spp. (Koehler and Boewe 1957).

Table 1 Pathogenicity of *Phaemocytostroma ambiguum* to maize hybrids in glasshouse inoculations

Maize hybrid	% root length healthy		% recovery of *P. ambiguum* from root pieces		% plants with mature pycnidia present on root system	
	I[A]	U[A]	I	U	I	U
108/7222	58.4 (7.40)[B]a	100	55	0	12.5	0
986/7300	36.97 (5.73)ab	100	91.7	0	57.0	0
507/7457	37.51 (5.62)ab	100	45	0	100	0
551/8422	33.45 (5.31)ab	100	90	0	83.3	0
766/6213	23.10 (4.30)b	100	60	0	62.5	0
797/7529	28.41 (4.82)b	100	60	0	42.8	0
925/7323	18.39 (3.88)b	100	48.5	0	71.4	0

[A]I = Inoculated, U = Uninoculated

[B]Numbers in brackets are square root transformations. Numbers followed by the same letter are not significantly different ($P>0.05$).

We have not conducted surveys of maize fields to determine the incidence of *P. ambiguum* in New South Wales, but there has been no evidence in previously published work that it is a common cause of stalk rot of maize. The outbreak observed in the Riverina district in 1990 may have been predisposed by unusual seasonal conditions or management factors favouring infection. There is no reason to believe the fungus is a recent introduction and it is more likely that it has been present for some time as a low level pathogen, producing symptoms only on mature plants. The lack of visible differences between inoculated and uninoculated plants in our glasshouse pathogenicity tests and the fact that stalk infections appear only on mature plants, when other fungi are also present, could explain why this disease has not previously been observed.

Although glasshouse inoculations indicated that all the hybrids tested were susceptible, this represents only a small percentage of the genetic material available and more tolerant genotypes may exist. Evidence of some tolerance was observed in the hybrid 108/7222, but further testing would be needed to confirm if this apparent tolerance is sustainable under field conditions.

Our findings on the incidence and virulence of *P. ambiguum* support previous reports, which indicate that this fungus occurs at a low incidence on mature stalks and roots of maize, is able to cause extensive infection of root systems, but under optimal conditions of water and nutrition has no measurable effect on plant dry weights. There has been no research reported on the host range or other aspects of the biology of the fungus. It may well be a weak pathogen of a range of grasses and cereals surviving on crop debris in a similar way to other fungi, which attack maize roots and stalks. Based on our findings, and reports in the literature, control would be effected through maintenance of optimal irrigation and nutrition, crop rotation to avoid build up of the fungus in maize trash and careful selection of hybrids to avoid those that exhibit susceptibility under commercial cultivation.

Acknowledgements

Dr. I. Barchia provided statistical analysis of the data.

References

Anon. (1971) – Maize diseases in Queensland. *Queensland Agricultural Journal* **97**: 519-522.

Craig, J. and Koehler, B. (1958) – *Pyrenochaeta terrestris* and *Phaeocytosporella zeae* on corn roots. *Plant Disease Reporter* **42**: 622-623.

Edwards, E.T. (1933) – A new Fusarium disease of maize. *Agricultural Gazette of N.S.W.* **44**: 895-897.

Edwards E.T. (1935) – Studies on *Gibberella fujikuroi* var. *subglutinans* the hitherto undescribed ascigerous stage of *Fusarium moniliforme* var. *subglutinans* and its pathogenicity on maize in New South Wales. *New South Wales Department of Agriculture, Science Bulletin* **49**: 1-68.

Edwards, E.T. (1936) – Root and basal stalk rot of maize. Burning of stalks essential for control. *Agricultural Gazette of N.S.W.* **47**: 259-261.

Francis, R.G. and Burgess, L.W. (1975) – Surveys of Fusaria and other fungi associated with stalk rot of maize in Eastern Australia. *Australian Journal of Agricultural Research* **26**: 801-807.

Francis, R.G. and Burgess, L.W. (1977) – Characteristics of two populations of *Fusarium roseum* 'Graminearum' in Eastern Australia. *Transactions of the British Mycological Society* **68**: 421-427.

Koehler, B. and Boewe, G.H. (1957) – Causes of stalk rot in Illinois. *Plant Disease Reporter* **41**: 501-504.

Messiaen, C.M. (1955) – Les principales maladies du mais en France et leurs caractères distinctifs. Station de Pathologie végetale du Sud-ouest, France, B.T.I. 105: 1-4.

Pont, W. (1963) – Maize diseases are common in North Queensland. *Queensland Agricultural Journal* **89**: 357-365.

Pont, W. (1973) – Studies on root rot and stalk rot of maize in North Queensland caused by *Marasmius sacchari* Wakker var. *hawaiiensis* Cobb and *Marasmius graminum* (Lib). Berk. var. *brevispora* Dennis. *Queensland Journal of Agricultural and Animal Sciences* **30**: 225-237.

Purss, G.S. (1963) – Maize diseases in Southern Queensland. *Queensland Agricultural Journal* **89**: 680-687.

Ramsey, M.G. (1990) – Etiology of root and stalk rots of maize in North Queensland: 2. Pathogenicity of fungi, including *Pyrenochaeta indica*, a new record. *Australasian Plant Pathology* **19**: 2-12.

Shurtleff, M.C. (1980) – *Compendium of Corn Diseases.* American Phytopathological Society, St Paul, Minnesota, USA.

Smiljakovi , H., Dragani , M. and Vidakovi , J. (1979) – *Phaeocytosporella zeae* Stout, Malo poznat provzrokova trule i stabla kukuruza *Zaštita Bilja XXX* (147): 47-52.

Stout, G.L. (1930) – New fungi found on the Indian corn plant in Illinois. *Mycologia* **22**: 271-287.

Sutton, B.C. (1964) – Coelomycetes III. *Mycological Papers*, No. 97. Commonwealth Mycological Institute.

Sutton, B.C (1980) – *The Coelomycetes*. Commonwealth Mycological Institute.

Wenholz, H. and Darragh, W.T. (1927) – The root, stalk and ear rot diseases of maize. *Agricultural Gazette of N.S.W.* **38**: 39-49.

Manuscript received 9 June 1995, accepted 22 September 1995.