Effect of plant age, temperature and dew period on the severity of anthracnose of cowpea caused by *Colletotrichum dematium*

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Three factors that influence anthracnose of cowpea caused by *Colletotrichum dematium* were studied in the greenhouse, namely age of the plant at inoculation, incubation period of the pathogen and temperature. Cowpea seedlings were inoculated with a 5×10^5 ml^{-1} conidial suspension of *C. dematium* at three, six and nine weeks after sowing. The inoculated seedlings were maintained in humidity chambers (RH 95 %) for 12, 24 or 36 hours and then transferred to greenhouses at 20, 25 or 30 °C, respectively. Disease severity was rated on a 0–5 scale. Plants inoculated three weeks after sowing were more resistant to infection than those inoculated at six and nine weeks. There were no significant differences in disease severity between plants maintained in humidity chambers for 12 or 24 hours. Cowpea plants were more susceptible to *C. dematium* at the age of nine weeks and maintained at temperatures of 25 and 30 °C for 24–36 hours at high humidity.

Key words: anthracnose, *Colletotrichum dematium*, cowpea, humidity, temperature, *Vigna unguiculata*.

Cowpea (*Vigna unguiculata* (L.) Walp.) is grown in the tropics and subtropics of Africa, India and Asia where leaves, green pods and grain are consumed, especially by resource-poor farmers (Rachie 1985). Anthracnose of cowpea, caused by *Colletotrichum dematium* (Pers.) Grove, was first recorded in India and Malaysia in 1977 (International Mycological Institute 1977), and has recently been reported from the subtropical regions of southern Africa (Smith & Aveling 1997) where mean temperatures during the cowpea growing season range from 6–27 °C. Anthracnose diseases of legumes generally cause severe economic losses in wet, humid, tropical areas (Emechebe & Florini 1997) and are less common in drier savanna regions (Lenne 1992). However, *Colletotrichum truncatum* (Schwein.) and *Colletotrichum capsici* (H. Syd.) F. Butt. & Bisley, causing brown blotch of cowpea, are more prevalent in the latter climates (Emechebe 1981).

Surveys conducted in Botswana, Mozambique and Zimbabwe in 1998 and 1999 during the cowpea-growing season (unpubl. data) indicated that *C. dematium* was more prevalent in monocultured fields than in inter-cropped fields and also in areas of high humidity, especially in Mozambique and Zimbabwe. *C. dematium* was not present in either monocultured or inter-cropped fields in Botswana where temperatures ranged from 28–40 °C during the cowpea growing season. Preliminary greenhouse studies furthermore indicated that inoculation with *C. dematium* of cowpea plants younger than three weeks, incubated in a humidity chamber for less than 12 hours after inoculation and maintained at less than 20 °C, resulted in very low disease incidence and severity.

Studies under controlled conditions are essential to provide more specific information on the effects of the environment on disease development. The aim of this study was to investigate the effect of plant age at inoculation, temperature and incubation period (dew period) on infection of cowpea by *C. dematium* in the greenhouse.

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Materials and methods

Inoculum preparation

A culture of *C. dematium* (PPRI 6121) originally isolated from cowpea in KwaZulu-Natal (Smith & Aveling 1997), was obtained from the National Collection of Fungi, ARC-Plant Protection Research Institute, Pretoria. Stock cultures maintained at –70 °C in 30 % glycerol were subcultured on potato-dextrose agar (PDA). A conidial suspension was prepared by flooding the surface of a 7-day-old culture with 10 ml sterile distilled water and the concentration was adjusted to 5×10^5 conidia ml^{-1}.

Plant material and experimental design

Cowpea (cv. Rhino) seedlings were reared in 15 cm diameter pots (2 l capacity) in pasteurised

potting soil. Six seeds were planted in each of 216 pots and thinned to four after emergence. Each pot was watered every second day with 200 ml tap water and also received 100 ml Hoagland's solution once a week. The experiment was conducted in three greenhouses with temperatures maintained at 20, 25 and 30 °C, respectively. Each greenhouse was divided into two blocks with 36 pots per block. At 3, 6 and 9 weeks after planting, respectively, the main stems of the plants in nine randomly-selected pots in each block in each greenhouse were inoculated with 2 ml *C. dematium* inoculum per plant using a thistle brush. Plants in the remaining pots served as control and were brushed with sterile water. Three of the nine pots with inoculated plants, and one with uninoculated plants, from each inoculation time within each block at each temperature were placed in a humidity chamber (RH 95 %) for 12, 24 or 36 hours. Thereafter, the plants were returned to the respective greenhouses in which the relative humidity ranged between 55 and 80 %.

Disease assessment

Inoculated plants were assessed for symptom expression seven days after inoculation. The disease scale of Stovold & Smith (1991) was used, where 0 = no symptoms; 1 = few small lesions, 10–20 % stem area infected; 2 = slight, 21–40 % stem area infected; 3 = moderate, 41–60 % stem area infected; 4 = severe, 61–80 % stem area infected with lesions on the leaves; 5 = very severe, >81 % stem area infected with total defoliation and plant collapse being evident or inevitable.

Statistical analysis

A combined analysis of variance (ANOVA) (GenStat 2000) was conducted on disease severity rating over all treatments and means were separated using Fisher's protected least significant difference ($P \leq 0.05$)

Results and discussion

Combined ANOVA indicated that plant age ($P < 0.001$), dew period ($P = 0.041$) and interaction of age and temperature ($P = 0.001$), significantly affected disease severity. The disease was expressed as early as 12 hours after inoculation. There were no significant differences in disease severity between dew periods of 12 and 24 hours and of 24 and 36 hours. However, mean disease rating increased significantly from 1.28 after 12 hours of inoculation to 1.67 after 36 hours.

Studies by Tu (1983) on the effect of precipitation on anthracnose of bean (*Phaseolus vulgaris* L.) caused by *Colletotrichum lindemuthianum* (Sacc. & Magnus) Scribn., indicated that a wet period of 10 hours was necessary for the fungus to establish infection. In the above experiments, patterns of disease spread were closely associated with heavy rains, with new infections appearing 3–7 days after each rain shower, depending on the temperature. Anthracnose caused by *Colletotrichum coccodes* (Wallr.) S. Hughes on tomato (*Lycopersicon esculentum* Mill.) was more severe when fruit was exposed to continuous wetness (more than 95 % humidity) for more than 10 hours (Dillard 1992). The present greenhouse results showed that a dew period of 12 hours of high humidity was required by *C. dematium* to initiate disease and that extended periods of high humidity promoted infection.

No significant differences in disease severity were evident at three weeks (Fig. 1). At six weeks, disease severities at 20 and 30 °C were similar (1.61) and slightly lower, albeit not significantly (1.28), at 25 °C. At nine weeks, disease severity increased significantly from 20 to 25 °C, and slightly from 25 to 30 °C. There was no significant difference in disease severity between nine-week-old plants maintained at 20 °C (1.25) and six-week-old plants maintained at 25 °C (1.28). Highest disease severity therefore occurred at 25 and 30 °C when plants were nine weeks old.

The cowpea crop is widely grown throughout the tropics and subtropics (Williams 1975) where temperatures range from 15–35 °C. Germination base temperature of cowpea seeds is 8.5 °C, but temperatures above 21 °C are required for vegetative growth and above 30 °C for flowering (Coetzee 1995). Results of the present study indicate that *C. dematium* is able to infect stems of cowpea at 20 °C, but the disease becomes significantly more severe at 25–30 °C. Disease severity tests with other species of *Colletotrichum* indicated that *C. lindemuthianum* (Prasanna 1985), *Colletotrichum truncatum* (Schwein.) Andrus & W D Moore (Emechebe & McDonald 1979; O'Connell et al. 1993), *Colletotrichum acutatum* J H Simmonds and *Colletotrichum gloeosporioides* (Penz.) Penz. & Sacc. (Zulfiqar et al. 1996), *C. capsici* (Pring et al. 1995) and *Colletotrichum destructivum* O'Gara (Latunde-Dada et al. 1996), are all more severe at temperatures ranging from

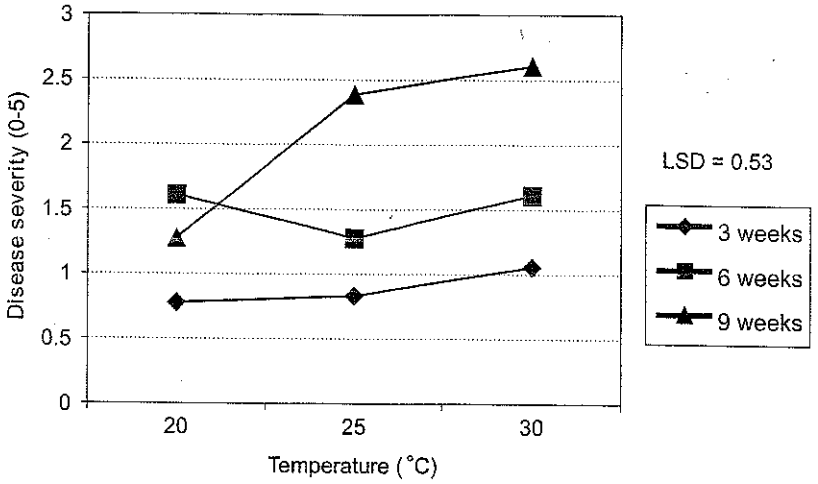

Fig. 1. Effect of age of cowpea plants and temperature on disease severity of *Colletotrichum dematium* in the greenhouse.

22 to 33 °C on their respective hosts.

In conclusion, results obtained in this study confirm those of Gourly (1966), Sutton (1962) and Lenne & Sonoda (1978) implicating *C. dematium* as a pathogen of mature tissue. The study further indicated that *C. dematium* can initiate infection on cowpea at a temperature of 20 °C but was more severe at 30 °C. An incubation period of 12 hours at a high humidity was necessary for infection and disease development and prolonged periods of high humidity resulted in severe infection. These factors are important for predicating disease incidence or severity and should be useful in determining infection by *C. dematium* in the field and in developing disease models and disease forecasting.

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References

Coetzee J 1995. *A traditional crop in Africa.* Africa Crops Info '95 Leaflet. Vegetables and Ornamentals Plant Institute and the Grain Crops Institute, Agricultural Research Council, Pretoria.

Dillard H R 1992. *Colletotrichum coccodes*: the pathogen and its hosts. In: Colletotrichum: *biology, pathology and control,* 225–236 (Eds J A Bailey & M J Jeger). CAB International, Wallingford.

Emechebe A M 1981. Brown blotch of cowpea in northern Nigeria. *Samaru Journal of Agricultural Research* **1**: 20–26.

Emechebe A M & Florini D A 1997. Shoot and pod diseases of cowpea induced by fungi and bacteria. In: *Advances in cowpea research,* 176–192. (Eds B B Singh, D R Mohan Raj, K E Dashiell & L E N Jackai). Co-publication of the International Institute of Tropical Agriculture (IITA) and Japan International Research Centre for Agriculture (JIRCAS). IITA, Ibadan, Nigeria.

Emechebe A M & MacDonald D 1979. Seed-borne pathogenic fungi and bacteria of cowpea in northern Nigeria. *PANS* **25**: 401–404.

GenStat 2000. GenStat for Windows. Release 4.2, 5th edition. VSN International, Oxford.

Gourley C O 1966. The pathogenicity of *Colletotrichum dematium* to table beets and other hosts. *Canadian Journal of Plant Science* **46**: 531–536.

International Mycological Institute 1977. *Herbarium records and additional records of* Colletotrichum *species on legumes.* International Germplasm Associates, Milnthorpe.

Latunde-Dada A O, O'Connell R J, Nash C, Pring R J, Lucas J A & Bailey J A 1996. Infection and identity of the hemibiotrophic anthracnose fungus (*Colletotrichum destructivum*) from cowpea (*Vigna unguiculata*). *Mycological Research* **100**: 1133–1142.

Lenne J M 1992. Infection strategies of *Colletotrichum* species. In: Colletotrichum: *biology, pathology and control,* 134–166 (Eds J A Bailey & M J Jeger). CAB International, Wallingford.

Lenne J M & Sonoda R M 1978. *Colletotrichum* spp. on tropical forage legumes. *Plant Disease Reporter* **62**: 813–817.

O'Connell R J, Uronu A B, Waksman G, Nash C, Keon J P & Bailey J A 1993. Hemibiotic infection of *Pisum sativum* by *Colletotrichum truncatum. Plant Pathology* **42**: 774–783.

Prasanna K P R 1985. Seed health testing of cowpea with special reference to anthracnose caused by *C. lindemuthianum*. *Seed Science and Technology* **13**: 821–827.

Pring R J, Nash C, Zakaria M & Bailey J A 1995. Infection process and host range of *Colletotrichum capsici*. *Physiological and Molecular Plant Pathology* **46**: 137–152.

Rachie K O 1985. Introduction, In: *Cowpea: research, production and utilisation*, xxi (Eds S R Singh & K O Rachie). John Wiley, Chichester.

Smith J E & Aveling T A S 1997. *Colletotrichum dematium*: causal agent of a new cowpea stem disease in South Africa. *Plant Disease* **81**: 832.

Stovold G E & Smith H J P 1991. The prevalence of and severity of diseases in the coastal soybean crop of New South Wales. *Australian Journal of Experimental Agriculture* **31**: 545–550.

Sutton B C 1962. *Colletotrichum dematium* (Pers. ex Fr.) Grove and *C. trichellum* (Fr. ex Fr.) Duke. *Transactions of the British Mycological Society* **45**: 222–232.

Tu J C 1983. Epidemiology of anthracnose caused by *Colletotrichum lindemuthianum* on white bean (*Phaseolus vulgaris*) in southern Ontario: survival of the pathogen. *Plant Disease* **67**: 402–404.

Williams R J 1975. Diseases of cowpea (*Vigna unguiculata* (L.) Walp.) in Nigeria. *PANS* **21**: 253–267.

Zulfiqar M, Blansky R H & Timmer L W 1996. Infection of flower and vegetative tissues of citrus by *Colletotrichum acutatum* and *C. gloeosporioides*. *Mycologia* **88**: 121–128.

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