

***Alternaria zinniae*: a Candidate for the Biocontrol of *Xanthium* Weeds**

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Summary

The effects of some environmental factors on disease caused by *Alternaria zinniae* on two widespread weeds of arable land, *Xanthium occidentale* and *Xanthium italicum*, were investigated. Plants were inoculated quantitatively in an inoculation tower with suspensions of conidia in water, incubated in a dew chamber and transferred to controlled environment cabinets. Maximum disease development on *X. occidentale* and *X. italicum* occurred after dew periods of 18 and 36 hours respectively. The optimum temperatures after the dew period for development of disease on *X. italicum* and *X. occidentale* were 25°C and 25 and 30°C, respectively. Water stress in the host, after the dew period, increased the proportion of necrosis on leaves by 2.5 times in *X. occidentale* and by 25 times in *X. italicum*. *A. zinniae* is a potential candidate for biocontrol of at least two *Xanthium* weeds.

Introduction

Several species of *Alternaria* have recently been investigated as potential mycoherbicides of annual weeds (4). Quimby (8) proposed the investigation of *Alternaria helianthi* (Hansf.) Tubaki & Nishihara as a biological control agent for Noogoora burr *Xanthium occidentale* Bertol. (Asteraceae).

Species in the Noogoora burr complex are serious weeds of summer crops and pastures in arable land (6). In Australia Noogoora burr and Hunter burr, *Xanthium italicum* Mor., are the two most widespread species from the complex (6).

In 1989 *Alternaria zinniae* M.B. Ellis (IMI No. 336377) was isolated from necrotic lesions on roadside plants of *X. occidentale* west of Armidale N.S.W. This paper reports the results of a study on the effects of some environmental factors on development of disease caused by *A. zinniae* on *X. occidentale* and *X. italicum*.

Materials and Methods

Cultures of *A. zinniae* were grown in Petri dishes on V8 juice agar inoculated with a suspension of conidia and then covered with sterile filter paper. Cultures were incubated at room temperature (20-25°C) beneath two white and two blacklight (Phillips TL40w/08) fluorescent tubes (12h on/off cycle). Conidia were dislodged into distilled water by scraping the colony surface and washing conidia onto a 38µm sieve.

Plants were grown in a heated glasshouse (22-28°C) in 10cm plastic pots containing a sand-peat-soil potting mix (1:1:1 v/v) until four to six weeks old. Plants were then inoculated in a spore inoculation tower (2) with suspensions of conidia in distilled water, transferred to a darkened dew chamber (3) set at 22±3°C for 48 hours and then to a controlled environment cabinet (25±1°C; 60-75% RH; 14 hour daily photoperiod of 391±11 µE.m⁻² s⁻¹). The density of inoculum settling in the inoculation tower was determined by

counting the number of conidia that settled on four horizontal 22x22mm glass coverslips placed a few centimetres above the plants during inoculation.

Percentage necrosis was assessed using the key described by Allen (1) and a key made by the senior author. Necrosis and lesion numbers were expressed as the mean per leaf per plant. Cotyledons, leaves less than one centimetre long at the time of inoculation and leaves that grew after inoculation were not assessed.

The environmental factors tested were: (i) dew period duration - at four, eight, 12, 18, 24, 36 and 48 hours after inoculation with 402 conidia cm^{-2} plants were transferred from the dew chamber to the controlled environment chamber in which the photoperiod was not operated until all dew treatments were finished.

(ii) post dew period temperature - plants of *X. occidentale* and *X. italicum* were inoculated with 83 and 236 conidia cm^{-2} respectively. Following the dew period plants were transferred to controlled environment chambers set at 20 ± 1 , 25 ± 1 and $30 \pm 1^\circ\text{C}$ and a 14 hour daily photoperiod (375, 391 and 357 $\mu\text{E m}^{-2} \text{s}^{-1}$ respectively).

(iii) water stress in the host - prior to inoculation with 222 conidia cm^{-2} and following the dew period, water was either supplied to plants or withheld from plants for three days in a two x two factorial experiment. Plants were watered to saturation after removal from the dew chamber.

Results

Development of necrosis on leaves increased as dew period duration increased to 18 hours for *X. occidentale* and to 36 hours for *X. italicum* (43.5% and 50.1% necrosis respectively at one week after inoculation). Longer dew periods gave no further significant increase in necrosis on leaves. Dew periods of eight hours or less gave less than 3% necrosis in both species. The minimum dew periods required to give maximum numbers of lesions on stems were 24 and 36 hours for *X. occidentale* and *X. italicum* respectively.

Necrosis on leaves of *X. occidentale* was significantly greater ($P < 0.01$) at post dew period temperatures of 25 and 30°C (26%) than at 20°C (6%). Necrosis on leaves of *X. italicum* was greater at 25°C (9.7%) than at 20 and 30°C (2.7 and 3.5% respectively). Both species of *Xanthium* developed significantly more lesions ($P < 0.05$) on stems at 20°C than at 25 or 30°C .

Plants exposed to water stress were noticeably wilted at the end of each period of water stress. Necrosis on leaves of both *X. occidentale* and *X. italicum* was increased by 2.5 and 25 times when the post dew period water stress was not preceded by pre-inoculation water stress (Table 1). Water stress before inoculation did not have a significant effect on necrosis except that it negated the effect of water stress after the dew period (Table 1).

Table 1. The effect of water stress on disease development on *Xanthium occidentale* and *Xanthium italicum* inoculated with *Alternaria zinniae* at 14 days after inoculation.

treatment before inoculation		water stress		no water stress	
		water stress	no water stress	water stress	no water stress
treatment after dew period					
*%necrosis	<i>X. occidentale</i>	14.4b	13.36b	49.7a	18.4b
leaf ⁻¹ plant ⁻¹	<i>X. italicum</i>	1.0c	0.2c	25.5b	0.9c

*Values followed by the same letter do not differ significantly ($P < 0.05$)

Discussion

The dew periods required for maximum infection of *X. occidentale* and *X. italicum* were longer than those that would occur in the field during fine weather. If the same dew periods are required under field conditions then the use of *A. zinniae* as a mycoherbicide may need to coincide with rainy periods. Research is needed to see if the same dew period requirements found in the laboratory also apply to disease development in the field.

When infection on leaves and stems were both considered the optimum post dew period temperature ranges for disease development were 20-30°C and 20-25°C for *X. occidentale* and *X. italicum* respectively. Species in the Noogoora burr complex occur in temperate, sub-tropical and mediterranean regions and seeds germinate through spring to early summer (6). Temperatures suitable for the use of *A. zinniae* as a mycoherbicide are thus likely to coincide with the early stages of burr growth.

Water stress prior to inoculation did not decrease the susceptibility of *X. occidentale* and *X. italicum* to *A. zinniae* and therefore may not limit the use of *A. zinniae* as a mycoherbicide. The stimulation of necrosis by water stress after inoculation could potentially be exploited in irrigated cropping.

A. zinniae is a potential candidate in the control of at least two *Xanthium* weeds. All species in the Noogoora burr complex should be tested for their susceptibility to disease caused by *A. zinniae* so that the occupation, by a more resistant species, of the niche vacated by a less resistant species can be foreseen. The efficacy of mycoherbicides can be improved by the use of surfactants and nutrients in the inoculum formulation (5). Research in the area of formulations may widen the limits set by environmental factors on the use of *A. zinniae* as a mycoherbicide.

References

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