Interaction between *Colletotrichum orbiculare* and *Alternaria zinniae* or a *Phomopsis* sp. on *Xanthium spinosum*

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Summary

***Colletotrichum orbiculare* is a potential mycoherbicide for the weed, *Xanthium spinosum*. The possibility of increasing the efficacy of *C. orbiculare* as a mycoherbicide by the addition of either a *Phomopsis* sp. or *Alternaria zinniae* under optimal and sub-optimal conditions of dew was assessed in controlled environment experiments. The addition of *Phomopsis* sp. had no influence on disease caused by *C. orbiculare*. *A. zinniae* did not influence the effect of *C. orbiculare* in the presence of 24 h dew. In the absence of dew, at a concentration of 10^5 spores mL^{-1}, *A. zinniae* significantly reduced ($P<0.05$) anthracnose disease development due to *C. orbiculare*. The addition of either *Phomopsis* sp. or *A. zinniae* to *C. orbiculare* would not increase its efficacy as a mycoherbicide on *X. spinosum*.**

Introduction

Colletotrichum orbiculare (Berk. et Mont.) v. Arx, is a common pathogen of the weed *Xanthium spinosum*, Bathurst burr or spiny cockleburr, in Australia. Controlled environment studies (Auld *et al.* 1988, McRae and Auld 1988) and field experiments (Auld *et al.* 1990) have demonstrated that *C. orbiculare* has potential as a mycoherbicide.

Another pathogen of *X. spinosum*, a *Phomopsis* sp. while not as common or virulent as *C. orbiculare* (Nikandrow *et al.* 1990) also frequently occurs on the weed. In addition isolates of *Alternaria zinniae* M.B. Ellis, collected from other *Xanthium* species in Australia, have shown pathogenicity to *X. spinosum* in glasshouse inoculation studies (Auld *et al.* 1992).

C. orbiculare requires a period of dew or free water for maximum disease development on *X. spinosum* (McRae and Auld 1988). This requirement is a limiting factor in the development of this (Auld *et al.* 1990) and many other potential mycoherbicides (TeBeest *et al.* 1992). The addition of another pathogen to a formulation containing *C. orbiculare* may provide a more effective mycoherbicide, particularly where environmental conditions, such as dew period were sub-optimal.

The aim of the work reported here was to test combinations of *C. orbiculare* and *Phomopsis* sp. or *C. orbiculare* and *A. zinniae* on *X. spinosum* under conditions of adequate dew for *C. orbiculare* and no dew.

Materials and methods

Individual seedlings of *X. spinosum* were raised in a controlled temperature glasshouse (24–26°C) fitted with an automatic watering system in 10 cm diameter plastic pots containing a peat and sand mixture with complete slow-release fertilizer added. For each treatment, 10 plants were inoculated at 5–6 week (4th leaf pair) and placed either in controlled environment chamber (Conviron) (25 ± 1°C, 65% rh, 12 h photoperiod 500 µE m^{-2} s^{-1}) or in a dew chamber (Percival) 25 ± 1°C, for 24 h in the dark and then moved to the controlled environment chamber.

Inoculum of an isolate of *C. orbiculare* (DAR48982) was prepared by sporulation in submerged liquid culture and harvesting spores (see Auld *et al.* 1989) and dried with kaolin (hydrated aluminium silicate) (Mortensen, 1988) (1 spores: 2 kaolin w/w) producing a powder containing 1×10^9 spores g^{-1}.

Fresh inoculum of *Phomopsis* sp. (DAR67505) was prepared by growing new cultures on γ-irradiated carnation leaf agar in near ultra violet light (12 h): 12 h dark for 14 days. Spores were washed off plates with sterile water.

Fresh inoculum of *Altenaria zinniae* (IMI 352085) was prepared from cultures grown on potato dextrose agar (PDA) in petri dishes at 25°C in the dark before cutting into the mycelium and agar in a checkerboard fashion with a sterile scalpel and transferring to 20°C in near ultra violet light (12 h) to promote sporulation. After seven days, spores were collected by washing the plates with sterile water.

Concentrations of suspensions of inocula of *Phomopsis* sp. and *A. zinniae* were assessed with a haemocytometer and adjusted with sterile water. Each fungus was applied separately with a N_2-propelled spray atomizer to completely cover the plants. The range of concentration combinations and dew period treatments in four repeated experiments are shown in Tables 1–4. Plants were examined daily for disease development.

On first appearance of symptoms of anthracnose caused by *C. orbiculare* (i.e. leaf spots and sunken black stem lesions), a rating system of symptoms was used which reflected time to death (1 = no symptoms, 6 = death); the cumulative arithmetic means of treatments were compared when half the plants in the most effective treatment were dead (McRae *et al.* 1988). Untreated control plants (10) in each experiment showed no symptoms.

Results and discussion

C. orbiculare – Phomopsis sp.

In the field disease due to *Phomopsis* is usually only observed on mature plants, although the fungus was often isolated from symptomless young plants. Under conditions of 24 h dew, the addition of *Phomopsis* sp. at 10^5 or 10^6 spores per mL to six week old plants had no effect on the course of anthracnose disease development due to *C. orbiculare* (Table 1).

In the absence of dew, the addition of *Phomopsis* sp. did not increase disease development above that for *C. orbiculare* alone without dew. Increasing concentrations of *Phomopsis* sp. had no significant effect on anthracnose development (Table 2).

C. orbiculare – A. zinniae

Alternaria leaf spot symptoms typically developed two days after inoculation followed by anthracnose symptoms due to *C. orbiculare* occurring on leaves and stems from five days after inoculation. However under conditions of 24 h dew the addition of *A. zinniae* in concentrations of 10^4 or 10^5 spores mL^{-1} had no significant effect on anthracnose development due to *C. orbiculare* whether the latter was applied at 10^5 or 10^6 spores mL^{-1} (Table 3).

Morin *et al.* (1993) found no interaction on the related *X. occidentale* when plants infected with *A. zinniae* were inoculated with *C. orbiculare* and subjected to dew.

Under no dew conditions, at a concentration of 10^5 spores mL^{-1} *A. zinniae* actually reduced anthracnose disease development due to *C. orbiculare* (Table 4). No symptoms of *C. orbiculare* anthracnose appeared on any of the replicate plants. The mechanism of this suppression may have been via competition for infection sites. Such antagonism has been noted for several other pathogen combinations (Reinecke 1981, da Luz and Bergstrom 1987).

It is clear that the addition of either *Phomopsis* sp. or *A. zinniae* to *C. orbiculare* would not increase its effectiveness as a mycoherbicide for *X. spinosum* either under optimal or sub-optimal conditions.

Development of the Bathurst burr mycoherbicide with formulations to reduce dew dependency using other techniques is warranted.

Table 1. Interaction of *Phomopsis* sp. and *C. orbiculare* in the presence of 24 h dew.

Phomopsis sp. spores mL^{-1}	*C. orbiculare* spores mL^{-1}	Mean disease rating (MR_{50})
10^5	10^5	4.59 a
10^6	10^5	4.47 a
10^5	10^6	4.54 a
10^6	10^6	4.63 a
0	10^6	4.75 a
0 no dew	10^6	1.70 b

1 = no symptons, 6 = death; MR_{50} = cumulative mean disease rating when half plants in most successful treatment are dead. Means followed by the same letter are not significantly different (P>0.05) (Tukey's HSD test).

Table 2. Interaction of *Phomopsis* sp. and *C. orbiculare* on *X. spinosum* in the absence of dew.

Phomopsis sp. spores mL^{-1}	*C. orbiculare* spores mL^{-1}	Mean disease rating (MR_{50})
10^4	10^6	1.99 a
10^5	10^6	1.87 a
10^6	10^6	1.87 a
0	10^6	1.49 a
0 24 h dew	10^6	4.40 b

1 = no symptons, 6 = death; MR_{50} = cumulative mean disease rating when half plants in most successful treatment are dead. Means followed by the same letter are not significantly different (P>0.05) (Tukey's HSD test).

Table 3. Interaction of *A. zinniae* and *C. orbiculare* on *X. spinosum* in the presence of 24 h dew.

A zinniae sp. spores mL^{-1}	*C. orbiculare* spores mL^{-1}	Mean disease rating (MR_{50})
10^4	10^5	3.34 a
10^5	10^5	3.41 a
10^4	10^6	3.51 a
10^5	10^6	3.60 a
0	10^6	3.56 a
0 no dew	10^6	1.40 b

1 = no symptons, 6 = death; MR_{50} = cumulative mean disease rating when half plants in most successful treatment are dead. Means followed by the same letter are not significantly different (P>0.05) (Tukey's HSD test).

Table 4. Interaction of *A. zinniae* and *C. orbiculare* on *X. spinosum* in the absence of dew.

A zinniae sp. spores mL^{-1}	*C. orbiculare* spores mL^{-1}	Mean disease rating (MR_{50})
10^3	10^6	1.61 a
10^4	10^6	1.68 a
10^5	10^6	1.00 b
0	10^6	1.51 a
0 24 h dew	10^6	4.61 c

1 = no symptons, 6 = death; MR_{50} = cumulative mean disease rating when half plants in most successful treatment are dead. Means followed by the same letter are not significantly different (P>0.05) (Tukey's HSD test).

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