Proc. VII. Int. Symp. Biol. Contr. Weeds, 6-11 March 1988, Rome, Italy.
Delfosse, E.S. (ed.). Ist. Sper. Patol. Veg. (MAF), pp. 435 - 43 (1989).

Potential for *Xanthium spinosum* Control by *Colletotrichum orbiculare* as a Mycoherbicide

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Abstract

***Xanthium spinosum*, Bathurst burr, spiny cockleburr or spiny clotburr is a serious weed in Australia in pastures and summer crops. Several isolates of the fungus *Colletotrichum orbiculare* were collected from anthracnose lesions on *X. spinosum* in eastern Australia. In this paper we report on in vitro production of the fungus and the effect of inoculum concentration and environmental factors on its ability to kill *X. spinosum* in controlled environments. Viable spores were produced in submerged shake culture using a modified Richards solution in concentrations up to 6.8 x 10^6/ml. At concentrations of 10^7 spores/ml the fungus killed 6-wk-old plants within 14 d. Disease increased with increasing dew period, although some plants died after 0 h dew. The optimum dew period temperature for disease development was between 20-25°C. The post dew period temperature optimum was 30°C. A dark period of at least 10 h during the dew period and within 8 h of inoculation significantly increased disease development. A delay in onset of dew period of > 4 h significantly reduced disease development. Field applications would need to be made in the late afternoon for maximum effect.**

Introduction

Xanthium spinosum L. (Asteraceae), Bathurst burr, spiny cockleburr or spiny clotburr, an annual, native to South America, is widespread as a weed throughout the world especially in temperate regions. It has been recorded in 39 countries as a weed in 13 crops (Holm *et al.* 1977). In Australia it has been regarded as a major weed since the early 1850s (Hocking and Liddle 1986). It occurs from humid coastal areas to the arid inland from lat. 15°S to lat. 45°S. *X. spinosum* is particularly troublesome on sheep grazing properties in south eastern Australia (Martin and Carnahan 1982), where its fruits are contaminants of wool, but it is also a weed of horticulture and irrigated summer crops (Hocking and Liddle 1986). Although susceptible to 2, 4-D (2,4-dichlorophenoxy acetic acid), herbicides often cannot be used because of the proximity of susceptible crops, especially cotton.

The fungus *Colletotrichum orbiculare* (Berk et Mont.) v. Arx (Coelomycetes) (Walker, Nikandrow and Millar, unpubl. data) was found causing anthracnose on *X. spinosum* in several locations during a survey in south eastern Australia in 1984 (Nikandrow, Weidemann and Auld, unpubl. data). Although usually causing only leaf and stem lesions, a few plants were found occasionally which had apparently been killed by the fungus. Specific isolates of the fungus were pathogenic to *X. spinosum* in preliminary tests, producing symptoms of seedling blight and anthracnose on older plants.

We investigated the potential of *C. orbiculare* as an inundative biological control agent. The work reported here examines: (a) *in vitro* production of spores; (b) response of *X. spinosum* to increasing inoculum concentration; and (c) environmental effects on disease development.

Materials and Methods

Spore Production

In preliminary experiments spores were produced on a range of media in petri dishes and in submerged liquid culture (Auld *et al.* 1988) which offers the most potentially efficient means of mass production. In this experiment we set out to improve spore production by modifying the media used. Flasks containing 200 ml of culture media were inoculated with 20 ml of a solution of 10^4 spores/ml. Culture media were based on a modified Richards medium containing 15% V8 (Trademark Campbell Soup Co.) juice adjusted to pH 7 with NaOH or 15% V8 juice only (Table 1). Cultures were incubated in a Gallenkamp orbital incubator at 12 h 15°C dark and 12 h 20°C light. Harvests were made by filtering mycelium off and centrifuging spores from liquor for 30 min at 10,000 r.p.m. Counts were made from resuspended spores by measuring two samples using a haemocytometer and viability checked on diluted samples plated on PDA.

Inoculum Concentration

Ten 6-wk-old plants each in separate pots of sterile loam were sprayed for each of 5 concentrations (10^3 to 10^7 spores/ml) (Fig. 1) to complete leaf wetness using an atomizer and covered with plastic bags for 24 h to simulate dew; 10 plants were sprayed with sterile water as controls. Plants were grown in controlled environment chambers (Conviron) at 25°C constant 20-40 % RH with a 12 h photoperiod; watering was by ground level drip emitters at the edge of pots. Time of appearance of first symptoms and time of death were noted.

Environmental Influence on Disease Development

General. Inoculum suspensions of 10^6 spores/ml were prepared using a haemocytometer and sprayed on to seedlings with a hand operated applicator until incipient runoff. Eighty microlitres of surfactant (Plus 50, Ciba Geigy) was added per litre of spore suspensions. After inoculation plants were covered with plastic bags which were secured around the plastic pot with elastic bands to simulate dew. Control plants were sprayed only with water and surfactant in all experiments. There were 10 plants in each treatment.

The severity of disease development on each plant was assessed using a 1 to 6 disease rating scale which reflected time to death (McRae *et al.* 1988): 1 = no disease symptoms, 6 = plant death. Disease development was rated daily from the onset of symptoms until 14 to 28 d after inoculation. Treatments were compared by taking their cumulative mean disease rating up to the time when half the plants in the most severely diseased treatment were dead.

Unless otherwise specified standard conditions in the growth cabinets were 25 ± 1°C; 12 h photoperiod (500 $\mu E/m^2/s$); 65% RH.

Dew period length and delayed onset. In the first experiment dew periods of 0, 4, 8, 12, 18, 24 and 48 h applied immediately after inoculation were compared by noting the time (after inoculation) to death for 50% of replicates. In the second experiment periods of delay (0, 4, 8, 12, 16, 20, 24 h) before onset of dew period were compared at six days after application when half the replicates in the most severely diseased treatment were dead. During the delay period plants were held at 20 to 40% RH.

Effect of dew period temperature on disease development. Seedlings were inoculated and placed into darkened growth chambers for 24 h at 5, 10, 15, 20, 25, 30 or 35°C and then transferred to a growth cabinet at standard conditions.

Effect of temperature after dew period on disease development. Seedlings were inoculated and incubated in a darkened growth chamber for 24 h and then transferred to lighted growth chambers set at 5, 10, 15, 20, 25, 30 and 35°C.

Disease development and the interaction of temperature and dew period. Seedlings were inoculated, placed into darkened growth chambers set at 5, 10, 15, 20, 25, 30 and 35°C. Plants which were exposed to 5, 10, and 15°C were given a 48 h dew period, those maintained at 20, 25 and 30°C were subjected to an 8, 18 or 48 h dew period while those maintained at 35°C received only an 8 h dew period. A 24 h dew period was also imposed on each temperature treatment to check against the dew period temperature experiment above. The plants remained in the growth chambers until 24 h after inoculation (except the 48 h dew period treatments) and were then transferred to standard conditions.

Effect of light during dew period on disease development. Plants were inoculated, placed into either a growth chamber in the light or in the dark for the following periods: 0 h light/24 h dark; 8 h light/16 h dark; 12 h light/12 h dark; 14 h light/10 h dark; 18 h light/6 h dark; 20 h light/4 h dark; 24 h light/0 h dark. After the dew period, all treatments were transferred to standard conditions.

In a second experiment, seedlings were inoculated as described previously, exposed to 8 h light at the beginning of a 24 h dew period and then transferred to a second growth chamber for dark periods of 16, 12, 10, 8 or 6 h. All except those in the 8 h light/16 h dark treatment were then returned to the light for the remainder of the 24 h dew period. A control treatment with a 24 h dark dew period was included. After the dew period plants were placed in the standard growth conditions mentioned previously.

Results

Spore Production

Maximum spore production was obtained with dilute solutions of modified Richards solution, although all media produced in excess of 10^6 spores/ml at ten days after inoculation (Table 1).

Table 1. Culturing *Colletotrichum orbiculare* (Berk et Mont.) v. Arx in various media.

Medium	Spore production (millions/ml) Days after inoculation		
	3	7	10
V8 juice	0.0036	1.4	3.2
Modified Richards (MR)	0.0026	0.7	1.1
MR 50%	0.0013	0.5	1.4
MR 20%	0.0065	1.5	6.8
MR 10%	0.0014	1.8	6.1

Inoculum Concentration

Increasing inoculum concentration had a marked effect on disease development (Fig. 1). So much so that in other experiments spore concentrations of 10^7/ml tended to mask the effect of other imposed treatments and subsequently 10^6 spores/ml was used routinely. Concentrations of $\geq 10^6$ produced leaf and stem lesions; plants eventually being killed by lesions girdling the main axis near the base of plants. All plants inoculated with $\geq 10^6$ spores died within 21 d. Sporulation on stem lesions occurred within four days of their appearance. At concentrations of 10^3 to 10^5 spores/ml lesions were generally restricted to leaves.

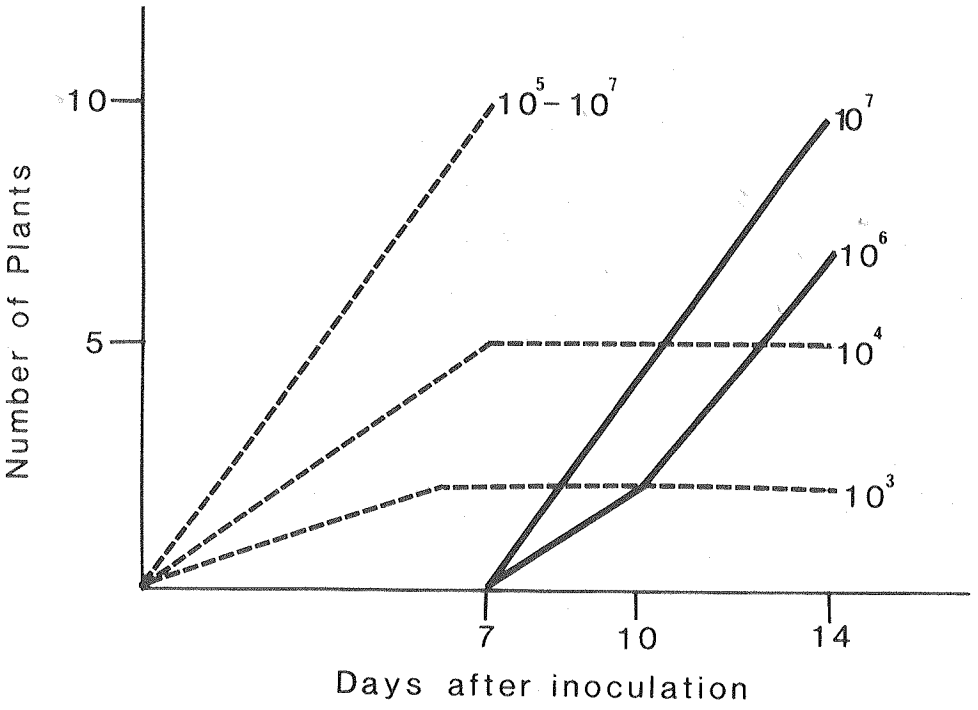

Figure 1. Effect of inoculum concentration of *Colletotrichum orbiculare* (Berk et Mont.) v Arx (spores/ml) on appearance of disease symptoms (broken lines) and death (solid lines) of *Xanthium spinosum* L.

Environmental Influence on Disease Development

Dew period length and delayed onset. Plants were killed after short dew periods following inoculation (Table 2). Even with no dew period, 70% of treated plants eventually died. A delay in onset of dew period after inoculation when plants were maintained at 20 to 40% RH had a major influence if the delay was over 4 h (Table 3).

Effect of dew period temperature on disease development. The optimum dew period temperature for disease development was between 20-25°C. There was no significant difference between these temperature treatments. (Fig. 2). Disease development at 15 and 30°C was significantly lower ($P < 0.05$) than between 20 and 25°C. Disease expression was much lower ($P < 0.001$) at 5, 10 and 35°C.

Effect of post dew period temperature on disease development. The optimum post dew period temperature was 30°C. Disease expression was significantly greater ($P < 0.05$) at this temperature than at any other temperature examined (Fig. 3).

Table 2. Effect of length of dew period on half life of *Xanthium spinosum* L.

Dew Period (h):	0	4	8	12	18	24	48
Time to death of 50% of replicates (d after inoculation):	>16	16	14	15	13	10	7

Table 3. Effect of delay in onset of dew on disease development.

Delay in onset of dew period (h):	0	1	2	3	4	6	24
Mean disease rating: (see text)	4.9	4.1	4.0	3.4	3.2	1.4	1.3

LSD 5% 0.6

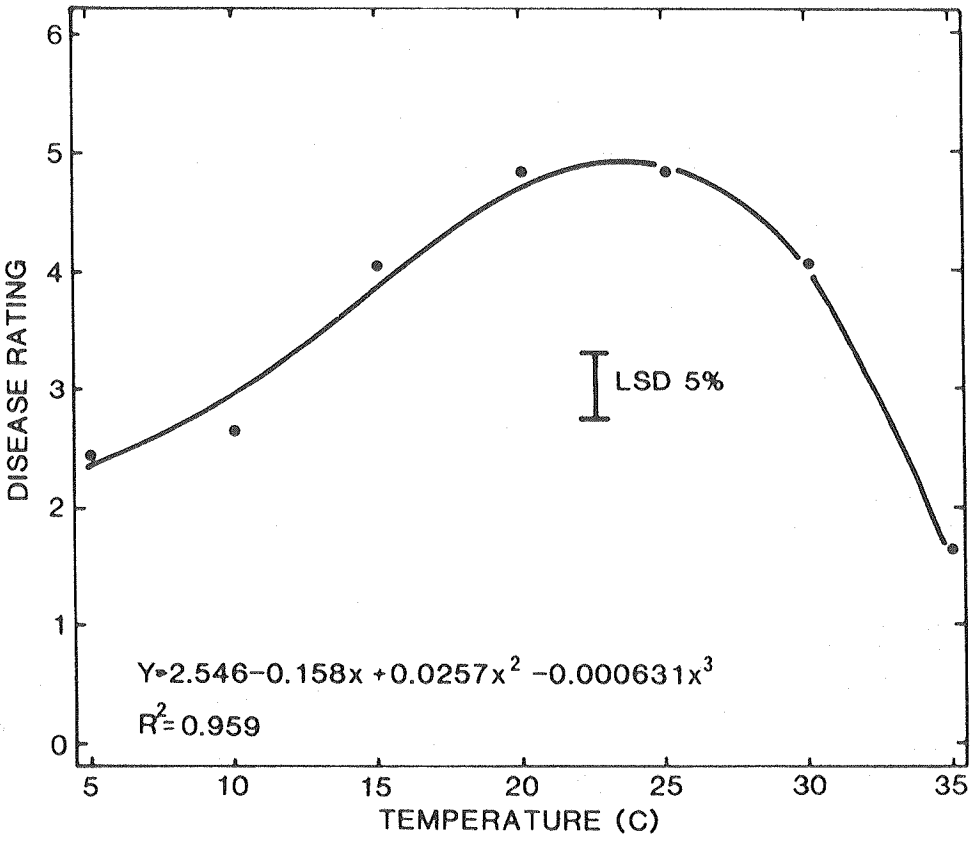

Figure 2. Effect of dew period (24 h) temperatures on disease development on *Xanthium spinosum* L.

Effect of dew period/temperature interaction on disease expression. A 48 h dew period at 15, 20 or 25°C resulted in the highest level of disease development (Fig. 4). However, there was no significant difference between disease levels at these three treatments. At a dew period of 18 h, disease development was significantly higher ($P < 0.05$) at 25°C than at 20 or 30°C. There was no significant difference between the disease resulting from 8 h dew period at 35°C and the disease from 8 h dew at 20 or 25°C; 18 h dew at 20, 25 or 30°C or 48 h dew at 10 or 30°C.

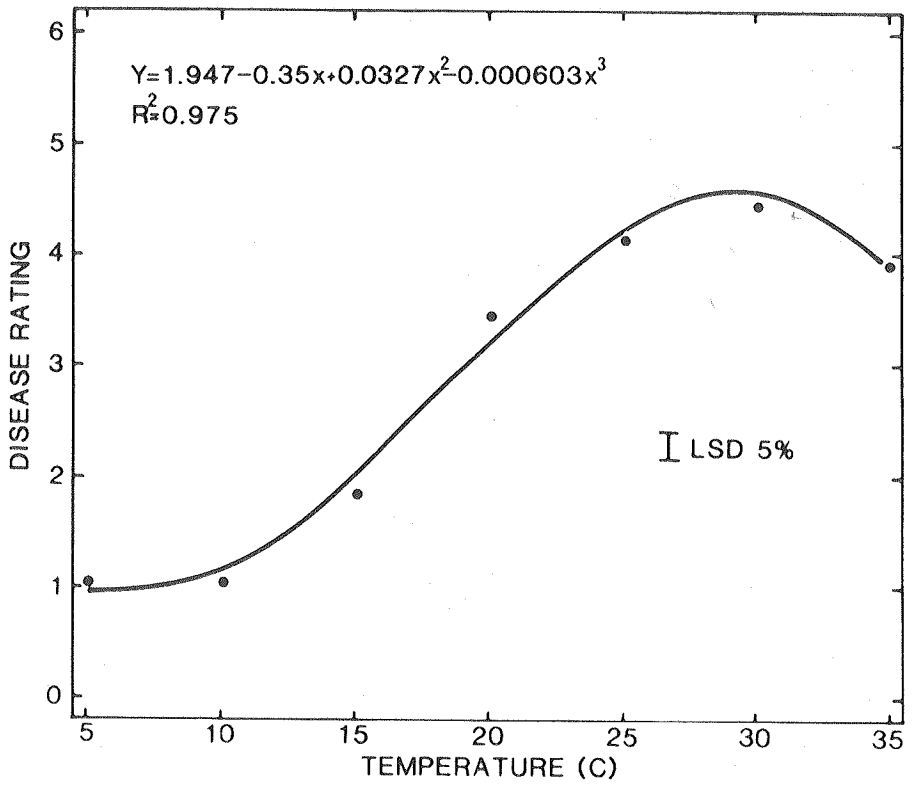

Figure 3. Effect of post dew period temperature on disease development on *Xanthium spinosum* L.

Effect of light during dew period on disease development. There was no significant difference ($P > 0.05$) between disease development resulting from 24 h dew period in darkness or disease development resulting from 8 h light at the commencement of the dew period (Fig. 5). However disease development resulting from all the other treatments was significantly less ($P < 0.05$) than either of these treatments. The general trend was a decrease in disease expression as the period of light at the commencement of the dew period increased.

There was also no significant difference ($P > 0.05$) between the level of disease which resulted from the unnatural 24 h dark dew period or dew periods consisting of 8 h light followed by 16, 12 or 10 h darkness during the dew period (Fig. 6). Dark intervals of < 10 hr significantly reduced ($P < 0.05$) disease development.

Discussion

Any large scale use of the fungus would require mass production of inoculum and submerged culture fermentation is the simplest means to achieve this. Production of viable spores in inexpensive media using shake culture indicates that mass production should be achievable economically. However techniques for ensuring long "shelf life" of spores, which would be desirable in a commercial product (Bowers 1982), still remain to be investigated.

The ability of the fungus to kill *X. spinosum* in 14 d under optimal conditions and to kill plants under sub-optimal conditions of dew which are likely to occur in the environment in which the weed occurs is encouraging. The need for dew within 4 h of inoculation for significant infection and the dark requirement in the dew period implies a requirement for late

afternoon or early night applications in the field for maximum effect. The requirement for a dark period is related to the formation of melanized appressoria (McRae and Auld, unpubl. data).

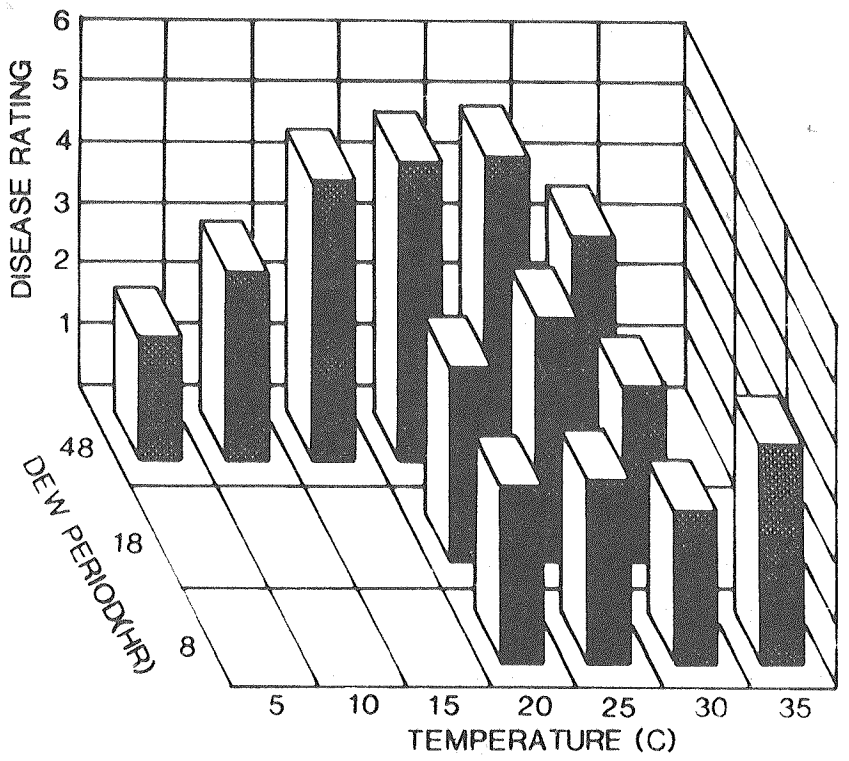

Figure 4. The interaction between temperature and duration of dew period on disease development on *Xanthium spinosum* L.

The temperature range for optimal infection and maximum disease development is within that which occurs during the hosts growing season (McRae and Auld 1988). The fact that the effective dew periods for inoculation were less for high temperatures was also encouraging.

Most anthracnose fungi require high humidity conditions for germination, penetration and sporulation in and on their host. Preliminary studies by the authors suggest that this is also true for *C. orbiculare* on *X. spinosum*. The most likely restricting factor to disease development in terms of mycoherbicide application appears to be occurrence and duration of dew period. However these restricting factors can to some extent be overcome by artificial inoculation techniques.

In its natural occurrence, the fungus appears to be poorly adapted for dispersal being spread by rain splash. Untreated controls within a few centimetres of treated plants in growth chamber and glasshouse experiments did not become infected in these experiments. Thus the fungus would be applied in the same way and with the same equipment as conventional herbicides direct to target plants as a "mycoherbicide" (Templeton *et al.* 1979). *C. orbiculare* is known as a pathogen of the Cucurbitaceae (Sutton, 1980). However, the fact that it is poorly adapted to spread and that cucurbits growing in most of the weed infested areas are also weeds (*Citrullus colocynthis* [L.] Schrader, *C. lanatus* [Thunb.] Mansf. var. *lanatus* and *Cucumis myriocarpus* Naudin [Auld and Medd 1987]) indicate that this is unlikely to be a major set-back to the development of the fungus for widespread use although further host range testing remains to be done.

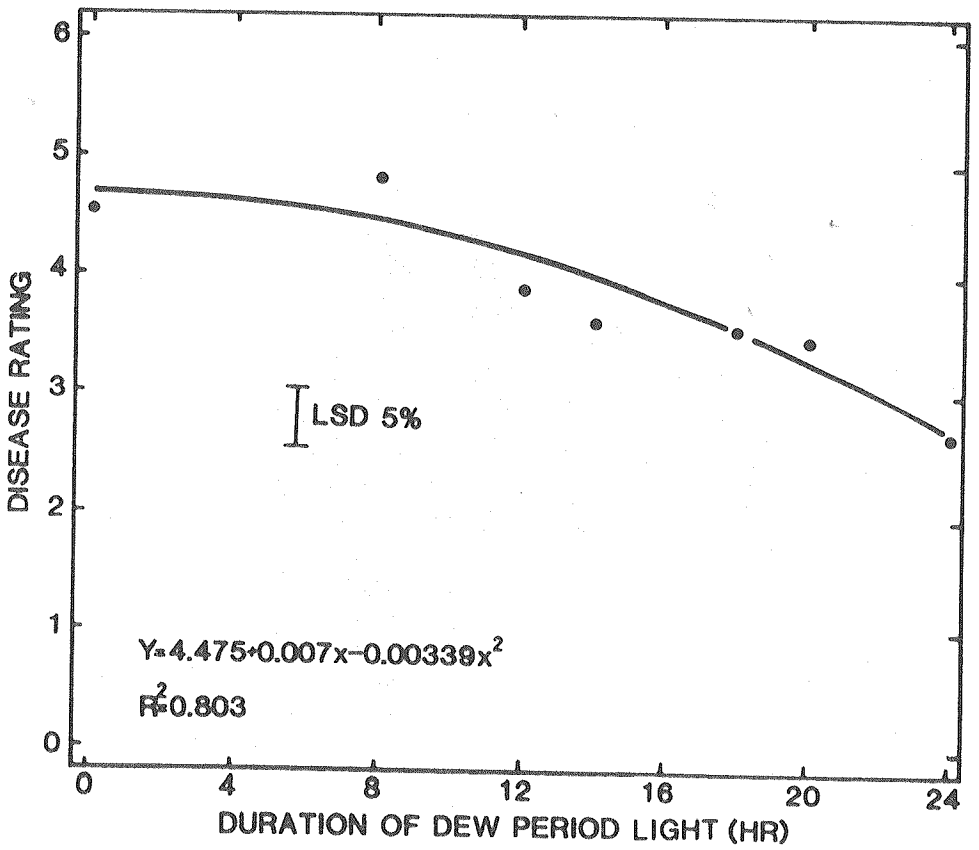

Figure 5. Effect of light during dew period on disease development on *Xanthium spinosum* L.

Acknowledgements

This project is financially supported by the Australian Wool Research Trust Fund, the Rural Credits Development Fund (Australia) and the Cotton Research Council (Australia). The authors thank J.F. Brown and G.E. Templeton for comments on the draft manuscript.

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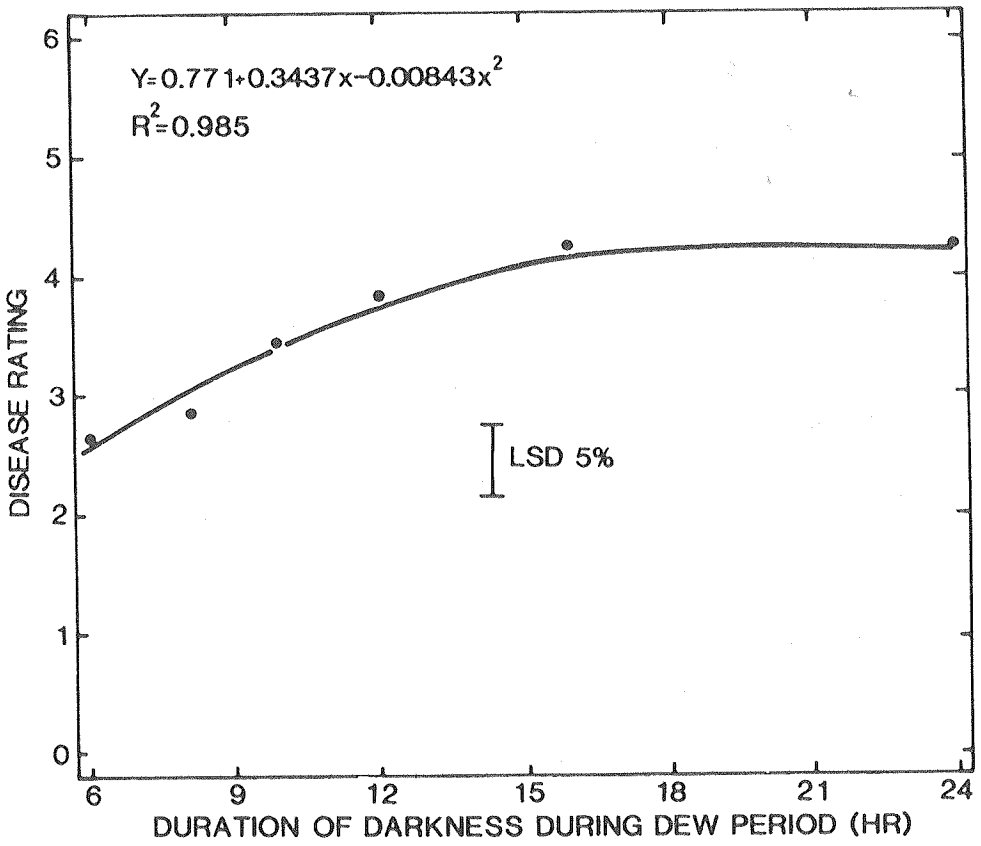

Figure 6. Effect of darkness during dew period, after an 8-hr light period, on disease development on *Xanthium spinosum* L.