The Influence of Environmental Factors on Anthracnose of *Xanthium spinosum*

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This research was funded by the Australian Wool Corporation, the Rural Credits Development Fund, and the Cotton Research Committee.
Accepted for publication 7 March 1988.

ABSTRACT

McRae, C. F., and Auld, B. A. 1988. The influence of environmental factors on anthracnose of *Xanthium spinosum*. Phytopathology 78:1182-1186.

Anthracnose on *Xanthium spinosum* (Bathurst burr, spiny clotburr, or spiny cockleburr) is caused by the fungus *Colletotrichum orbiculare*. The optimum dew period temperature for anthracnose development was between 20 and 25 C. The optimum post dew period temperature for disease development was 30 C. Anthracnose development increased with the duration of dew period. Maximum disease development resulted from a 48-hr dew period at 25 C. Long dew periods (48 hr) at low temperatures increased disease development compared with short dew periods, while significant anthracnose development resulted from an 8-hr dew period at a high temperature (35 C). Disease development was increased by two consecutive dew periods of at least 12-hr duration. Exposure to light for up to 8 hr at the beginning of a 24-hr dew periods did not reduce disease development. A dark period of at least 10 hr during the dew period significantly increased disease development. All experiments were undertaken in controlled environments. The results are discussed in terms of possible use of *C. orbiculare* as a mycoherbicide against *X. spinosum*.

Additional key word: biological weed control.

Xanthium spinosum L. (Bathurst burr, spiny cockleburr, spiny clot burr) is among the world's worst weeds. It has been recorded in 39 countries as a weed of some 13 crops (8). It is proclaimed a noxious weed in all states of Australia. *X. spinosum* is considered a serious weed in eastern Australia due to contamination of wool by its woody spiny fruits ("burrs") and competition with pasture and crops (16). *Colletotrichum orbiculare* (Berk. et Mont) v. Arx, (Walker & Nikandrow, *unpublished data*) which causes an anthracnose disease, is presently being considered as a potential mycoherbicide for *X. spinosum* (2). Symptoms of this disease have been described previously (17). The objectives of this study were to determine the effect of environmental factors on anthracnose development of Bathurst burr.

MATERIALS AND METHODS

General. Seedlings of Bathurst burr were grown from seed collected from plants harvested west of Coonamble, New South Wales. Burrs were each cut at the distal end to expose the tips of the seed before being sown. This technique produces uniform and rapid germination (1). Seedlings were grown in germination trays and individually transplanted at about 10 days into 10-cm diameter plastic pots containing a steam sterilized soil-sand-peat potting mixture (1:3:1). Plants were kept in a temperature-controlled glasshouse at 24–26 C fitted with automatic watering. For each treatment, 10 plants were inoculated at 5–6 wk, when the fourth leaf pair had fully expanded, with an isolate of *C. orbiculare* obtained from an infected Bathurst burr plant from Coolah, New South Wales (Number 001; Agricultural Research and Veterinary Centre collection, Orange, N.S.W., Australia). The experimental design was completely random and each experiment was repeated. The inoculum was prepared from 3–4-wk-old cultures of the fungus grown on water agar sprinkled with irradiated carnation leaf pieces kept in the dark at 25 ± 1 C. Spores were collected from the media by washing the carnation pieces in water and filtering the spores through cheesecloth.

Inoculum suspensions of 10^6 spores per milliliter (2) were prepared using a hemacytometer and sprayed on to seedlings with a hand operated applicator until incipient runoff. Eighty microliters per liter of surfactant (Plus 50, Ciba-Geigy) was added to all spore suspensions.

After inoculation, plants were covered with plastic bags, which were secured around the plastic pot with elastic bands to create an environment of high humidity. This practice simulated a dew period. Control plants were sprayed only with water and surfactant in all experiments. A standard set of conditions for growth chambers was used unless otherwise specified: 25 ± 1 C, 65% RH 12-hr photoperiod (500 $\mu E\ m^{-2}\ s^{-1}$).

The severity of disease development on each plant was assessed daily using a 1–6 disease rating scale; 1 = no disease symptoms, 6 = plant death (17).

All data were statistically analyzed by comparing the arithmetic mean of daily disease ratings of each treatment until the day that 50% of the plants in the most effective treatment were dead. This analysis technique reflected the time to plant death in each treatment (17). Analysis of variance was performed on the data and orthogonal polynomials fitted where appropriate.

Effect of dew period temperature on disease development. Seedlings were inoculated and given dark dew periods of 24 hr at 5, 10, 15, 20, 25, 30, or 35 C. After the dew period, seedlings were transferred to standard conditions. Disease development was rated from 5 to 14 days after inoculation.

Effect of temperature after dew period on disease development. Seedlings were inoculated and given a dark dew period at 25 ± 1 C for 24 hr. After the dew period seedlings were transferred to the same set of temperatures (above) in lighted growth chambers (100 $\mu E\ m^{-2}\ s^{-1}$, 12-hr photoperiod). Disease development was rated from 4 to 14 days after inoculation.

Effect of dew period duration on disease development. Seedlings were inoculated and given dark dew periods (25 ± 1 C) of 4, 8, 12, 18, 24, and 48 hr. All seedlings remained in the darkened growth chamber until 24 hr after inoculation, except the 48-hr dew period treatment, which was exposed to 48 hr of dark. Relative humidity in the chamber was maintained at about 30% to inhibit post dew period germination of the fungus. One treatment was not covered with plastic bags and acted as a 0-hr dew period treatment. The inoculum on these plants was dry within 1 hr of inoculation. Disease development was rated from 5 to 22 days after inoculation.

Disease development and the interaction of temperature and dew period. Seedlings were inoculated and placed into darkened growth chambers set at 5, 10, 15, 20, 25, 30, and 35 C. Plants that were exposed to 5, 10, and 15 C were given a 48-hr dew period. Those maintained at 20, 25, and 30 C were subjected to an 8, 18, or

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48-hr dew period, while those maintained at 35 C received only an 8-hr dew period. A 24-hr dew period was also imposed on each temperature treatment for comparative purposes. The plants remained in the growth chambers until 24 hr after inoculation (except the 48-hr dew period treatments) and were then transferred to standard conditons. Disease development was rated 8–23 days after inoculation.

Effect of multiple dew periods on disease development. Plants were inoculated and given the following dark dew periods (25 ± 1 C): 6-hr dew period/18-hr dry; 6-hr dew period/18-hr dry on 2 consecutive days; 6-hr dew period/18-hr dry, on 4 consecutive days; 12-hr dew period/12-hr dry; 12-hr dew period/12-hr dry, on 2 consecutive days; 24-hr dew period; and a control treatment sprayed with distilled water only and covered for 24 hr. The dry portion of the treatment was created by removing the plastic bags and turning the lights on (500 $\mu E\ m^{-2}\ s^{-1}$ for 12 hr at 25 ± 1 C (except the 6-hr dew period treatments, which were exposed to an additional 6 hr of darkness at the beginning of the dry period). Humidity was controlled at about 30% in the dry period to ensure that there was no spore germination during this time. Disease expression was rated between 5–16 days after inoculation.

Effect of light during dew period on disease development. Plants were inoculated and given the following dew periods (25 ± 1 C) in the light (500 $\mu E\ m^{-2}\ s^{-1}$) or in the dark: 0-hr light/24-hr dark; 8-hr light/16-hr dark; 12-hr light/12-hr dark; 14-hr light/10-hr dark; 18-hr light/6-hr dark; 20-hr light/4-hr dark; 24-hr light/0-hr dark. After the dew period, all treatments were transferred to standard conditions. Disease expression was recorded from 5 until 14 days after inoculation.

In a second experiment, seedlings were inoculated as described previously, exposed to 8 hr of light (500 $\mu E\ m^{-2}\ s^{-1}$) at the beginning of a 24-hr dew period (25 ± 1 C) and then transferred to a second growth chamber (25 ± 1 C) for dark periods of 16, 12, 10, 8, or 6 hr. All except those in the 8-hr light/16-hr dark treatment were then returned to the light for the remainder of the 24-hr dew period. A control treatment with a 24-hr dark dew period was included. After the dew period plants were placed in the standard growth conditions mentioned previously. Disease development was assessed from 4 until 13 days after inoculation.

RESULTS

Effect of dew period temperature on disease development. The optimum dew period temperature for disease development was between 20 and 25 C. There was no significant difference between these temperature treatments. (Fig. 1). Disease development at 15 and 30 C was significantly lower ($P < 0.05$) than between 20 and 25 C. Disease expression was significantly 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. 2).

Effect of dew period duration on disease development. Disease development increased with duration of dew period (Fig. 3). A 48-hr dew period resulted in significantly more ($P < 0.05$) disease than 24 hr. Small basal lesions eventually occurred on seven plants (disease rating 2) in the 0-hr dew period treatment, which accounts for the small amount of disease; 1.2 on the disease rating scale (Fig. 3). These plants were very slow to die compared with those at longer dew periods (Table 1).

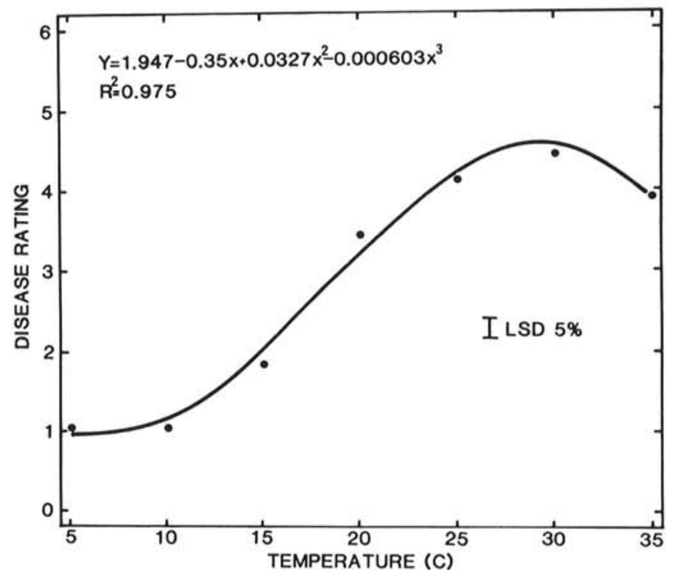

Fig. 2. Effect of post dew period temperature on anthracnose development on *Xanthium spinosum*. (Disease ratings are presented as the mean daily disease rating up to the day when 50% of the plants in the most effective treatment were dead.)

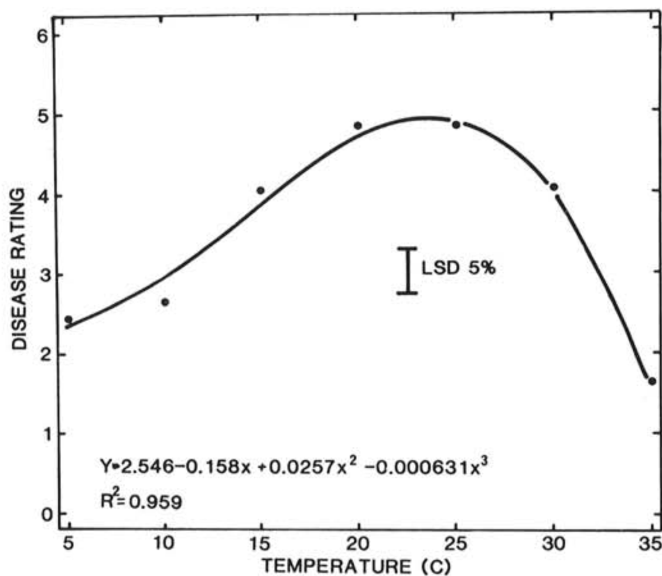

Fig. 1. Effect of 24-hr dew period temperatures on anthracnose development on *Xanthium spinosum*. (Disease ratings are presented as the mean daily disease rating up to the day when 50% of the plants in the most effective treatment were dead.)

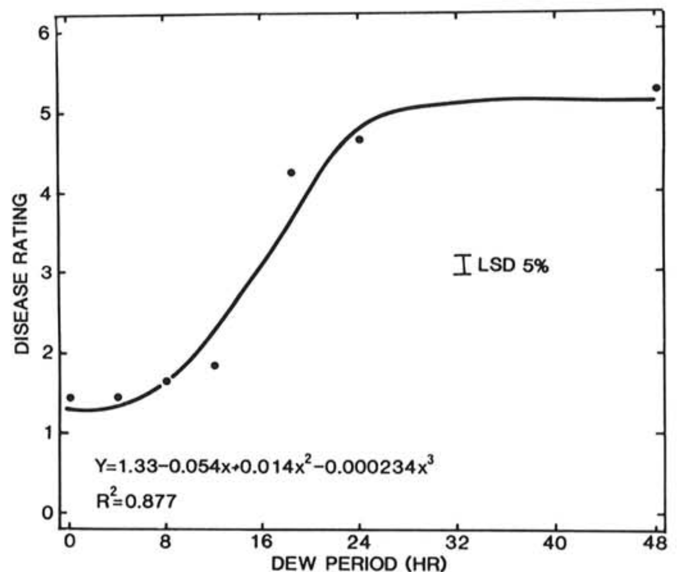

Fig. 3. Effect of duration of dew period at 25 C on anthracnose development on *Xanthium spinosum*. (Disease ratings are presented as the mean daily disease rating up to the day when 50% of the plants in the most effective treatment were dead.)

Effect of temperature/dew period interaction on disease expression. A 48-hr dew period at 15, 20, or 25 C resulted in the highest level of disease development (Fig. 4). However, there was not significant difference between disease levels at these three treatments. At a dew period of 18 hr, 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 an 8-hr dew period at 35 C and the disease from 8-hr dew at 20 or 25 C; 18-hr dew at 20, 25 or 30 C, or 48-hr dew at 10 or 30 C.

Effect of multiple dew periods on disease development. An uninterrupted 24-hr dew period resulted in significantly more disease ($P < 0.05$) than any of the multiples of shorter dew periods (Table 2). There was no significant difference ($P < 0.05$) between the disease resulting from 6-hr dew or multiples of 6-hr dew. However, 12-hr dew on consecutive nights did significantly ($P < 0.05$) increase disease expression compared with a 12-hr period but was still significantly less than one 24-hr period.

Effect of light during dew period on disease development. There was no significant difference ($P > 0.05$) between disease development resulting from one 24-hr dew period in darkness or disease development resulting from 8 hr of 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 a 24-hr dark dew period or dew periods consisting of 8 hr of light followed by 16, 12, or 10 hr of darkness during the dew period (Fig. 6). Dark intervals of less than 10 hr significantly reduced ($P < 0.05$) disease development.

TABLE 1. The effect of duration of dew period on the rate of Bathurst burr mortality caused by the anthracnose fungus *Colletotrichum orbiculare*

Dew period (hr)	Time to death of 50% of plants (days after inoculation)
0	>16
4	16
8	14
12	15
18	13
24	10
48	7

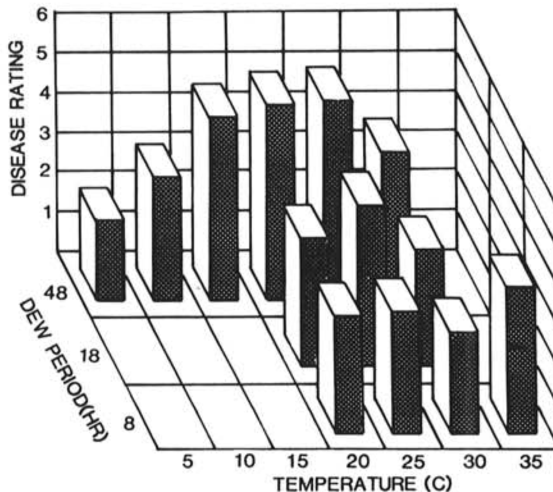

Fig. 4. The interaction between temperature and duration of the dew period on anthracnose development on *Xanthium spinosum*. (Disease ratings are presented as the mean daily disease rating up to the day when 50% of the plants in the most effective treatment were dead.)

TABLE 2. Effect of daily dew periods on anthracnose severity on Bathurst burrs (dew periods were created by covering plants with plastic bags in the dark at 25 ± 1 C)

Dew periods (hr/day)	Disease rating (scale 1–6)
6	1.6 ab[z]
6; 6	1.81 ab
6; 6; 6; 6	1.49 a
12	2.25 b
12; 12	3.99 c
24	4.86 d

[z]Values followed by the same letter do not differ significantly ($P > 0.05$).

DISCUSSION

The results indicate that the optimum temperature range for fungal infection and Bathurst burr anthracnose development is

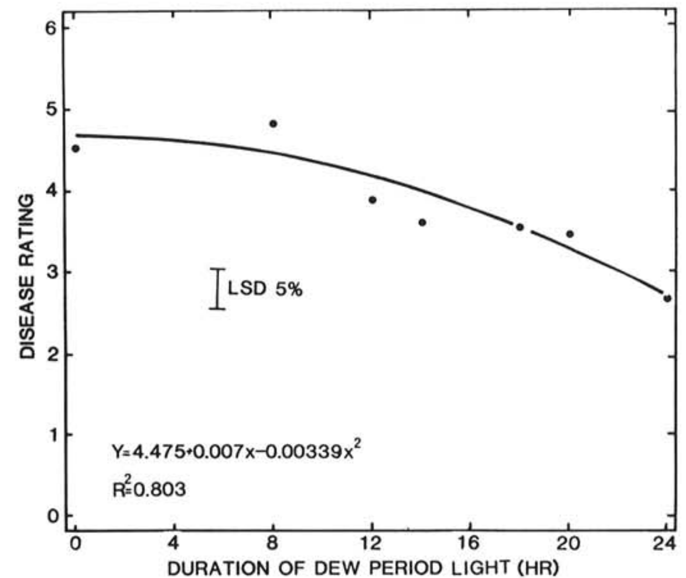

Fig. 5. Effect of light duration starting at the commencement of a 24-hr dew period on anthracnose development on *Xanthium spinosum*. (Disease ratings are presented as the mean daily disease rating up to the day when 50% of the plants in the most effective treatment were dead.)

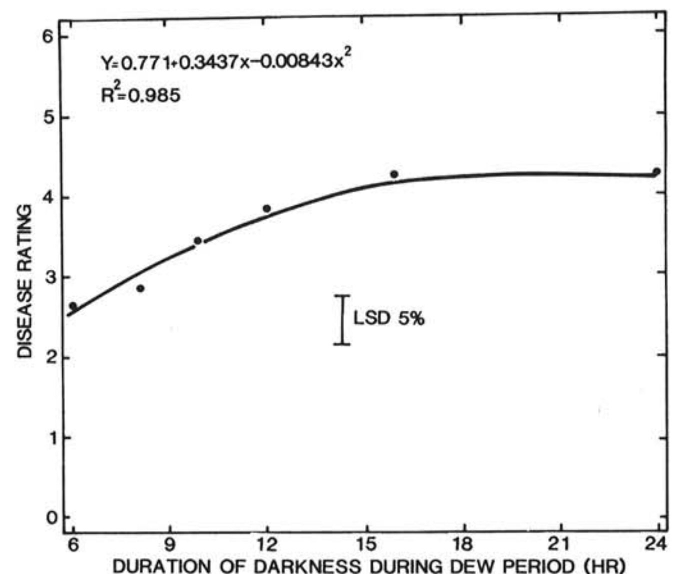

Fig. 6. Effect of darkness during the dew period after an 8-hr light period on anthracnose development on *Xanthium spinosum*. (Disease ratings are presented as the mean daily disease rating up to the day when 50% of the plants in the most effective treatment were dead.)

similar to other anthracnose diseases caused by *C. orbiculare* and other *Colletotrichum* species (6,9,15,23,25,28,29,31). More importantly, the results show that the temperature range at which maximum disease development occurs is within the temperature range that occurs during the host's growth season. *X. spinosum* occurs in areas where average summer temperatures are in the order of 20–35 C (7) and temperature is therefore not likely to be a limiting factor for *C. orbiculare* as a mycoherbicide.

Maximum disease expression occurred after a 48-hr dew period. It is of course unrealistic to expect dew periods of such length in areas where *X. spinosum* grows; however, some disease symptoms resulted from dew periods as low as 4 hr; 50% of those diseased plants were dead 16 days after inoculation. This is comparable to the time to death of Bathurst burr in the field as a result of some herbicide applications (McRae, *unpublished data*).

Appressorial dormancy (21) may account for the increased disease development observed after 12 hr of dew on two consecutive nights compared with that resulting from one 12-hr dew period. Preliminary histological studies indicate that appressorial formation is greatest at about 12 hr (under optimum conditions), so that some of the appressoria formed during the first dew period may have experienced a period of dormancy enforced by the subsequent adverse conditions (12 hr of light at low humidity) and resumed the infection process during the second dew period. Other workers have implicated appressorial dormancy in the latency of anthracnose of barley and bananas (19,27). Anthracnose latency on Bathurst burr would enhance the effectiveness of a mycoherbicide preparation of the fungus.

Our results indicate that germination of *C. orbiculare* spores was not affected by light. This was evident from the fact that light up to 8 hr into the dew period did not significantly ($P > 0.05$) reduce disease development compared with a 24-hr dark dew period. Histological studies show that maximum germination occurs at about 6 hr after inoculation (McRae, *unpublished data*). There are reports in the literature regarding other *Colletotrichum* species that are consistent with this finding (24,26) and some that are conflicting (22). The requirement for at least 10 hr of darkness after the germination period (8 hr of light) indicates that appressorial formation of *C. orbiculare* requires darkness. Kubo et al (10–14) demonstrated that melanization of *C. orbiculare* appressoria is essential for the subsequent expression of the penetration ability of the appressoria, that melanin pigmentation begins 6 hr after inoculation, and that most appressoria are darkly pigmented by 12 hr after inoculation. It follows, then, that the requirement for darkness is related to melanin biosynthesis in appressoria. If this requirement is not met, appressoria penetration would be reduced, which would be reflected by reduced disease expression.

Most anthracnose fungi require high humidity conditions for germination, penetration, and sporulation in and on their host (3–6,18,20,34,35). Preliminary studies by the authors suggest that this is also true for *C. orbiculare* on *X. spinosum*. The most likely restricting factors to disease development in terms of mycoherbicide application therefore appear to be humidity and duration of dew period. Effective control of northern jointvetch in soybean by *C. gloeosporioides* (Penz.) Sacc. f. sp. *aeschynomene* is also related to high relative humidity (28). The implication of this is that a mycoherbicide preparation of *C. orbiculare* should be applied to Bathurst burr late in the afternoon, to utilize high prevailing relative humidity and also to allow appressorial formation to occur in darkness.

However, these restricting factors can to some extent be overcome by artificial inoculation techniques. The humidity level at inoculation and to some extent during the dew period may be increased by using more efficient wetting agents or invert emulsions, or applying the fungus as a gel (33) or granular preparation (32). Such inoculum preparations would also overcome problems associated with a time delay between inoculum challenge and onset of dew conditions (2). Mycoherbicide application could also be timed to occur when environmental conditions most favor infection. Such conditions may occur at or near sunset and after summer storms. Our results indicate that the latter example is potentially a very effective application time because the disease resulting from 8 hr of dew at 35 C (conditions that may be expected after summer storms) was not significantly different from disease resulting from 18 hr of dew at 20, 25 or 30 C. Seventy percent of these inoculated plants died from anthracnose 23 days after inoculation.

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