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RURAL INDUSTRY RESEARCH FUNDS
FINANCIAL YEAR 1990/91

# FINAL REPORT

Authorised Body: COTTON RESEARCH COUNCIL

Project Number: DAN 27L

Project Title: *MYCOHERBICIDES FOR BIOLOGICAL CONTROL OF NOOGOORA AND BATHURST BURRS*

Field of Research: Biological Control of Weeds Field Code: 7 Weed Control

Organisation: NSW Agriculture & Fisheries

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Anticipated Commencement Date: 1 July 1986. Anticipated Completion Date: 30 June 1989

Funds Provided/Sought from Other Sources: NSW Agriculture & Fisheries provided the salaries of research staff and operating costs for laboratory and glasshouse facilities. The Rural Credits Development Fund granted $60,000 over 3 years beginning 1985 for capital items and maintenance. The Australian Wool Corporation granted approximately $100,000 over three years for a technical officer and additional maintenance requirements. Sandoz Ltd (Switzerland) provided financial assistance.

Aims: The aim of the project was to develop naturally occurring plant pathogens as mycoherbicide biological control agents for *Xanthium* spp., Noogoora and Bathurst burrs.

Industry Significance: Noogoora and Bathurst burrs are important weeds of irrigated and dryland cotton. In some areas hand chipping is still used to control them because of difficulties in controlling them with conventional herbicides.

FINAL REPORT: TECHNICAL SUMMARY. PROJECT NUMBER DAN 27L.
MYCOHERBICIDES FOR BIOLOGICAL CONTROL OF NOOGOORA AND BATHURST BURRS.

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Organisation: NSW AGRICULTURE & FISHERIES

The project began with a survey of pathogens of *Xanthium* spp. Subsequently two research streams developed. One investigating the use of *Phomopsis* spp. on Noogoora and Hunter burrs and the other investigating *Colletotrichum orbiculare* for control of Bathurst burr.

*Phomopsis* was shown to be a latent pathogen and to have a wide host range. Young plants were not susceptible to rapid disease development and leaf senescence was required for aggressive disease development in older plants.

The research on the development of the fungus *Colletotrichum orbiculare* as an innundative biocontrol agent (mycoherbicide) for Bathurst burr, *Xanthium spinosum* led to a commercial agreement between the international Swiss based company, Sandoz, and NSW Agriculture & Fisheries to produce a product based on NSW Agriculture & Fisheries fungal isolates and technology.

A series of controlled environment experiments examined the influence of dew period, humidity, temperature and light, as well as their interaction, on infection and disease development. The results indicated that applications should be made in the late afternoon or evening for greatest efficacy. An overriding requirement for leaf wetness is provided when the aqueous spore suspensions are applied but there is further scope for formulation development. In addition the fungus was shown to perform poorly on moisture stressed plants. However in this, it is no different to many conventional herbicides.

The fungus was initially produced on a range of solid media but for eventual commercial application mass production in liquid culture is a far more convenient technique. Using a laboratory fermentor, sporulation of the fungus was achieved in submerged liquid culture and in further work improvements in the media were made. This allowed production of up to $10^{6.8}$ spores $ml^{-1}$ in 10 days.

Spores in liquid were only viable for 12-24 hours, hence it was important to develop a process which could give the spores "shelf-life". This was achieved using a drying down process with kaolin.

Preliminary field tests in spring/summer 1987/88 and 1988/89 were promising with percentage kills on natural populations varying from 58-100%.

This research was made the subject of a patent invented by Dr Bruce Auld and owned by NSW Agriculture & Fisheries.

NSW Agriculture & Fisheries advertised for expressions of interest in the development of a product based on this research. Responses were received from five companies, including four internationals. The chosen company, Sandoz, has offered to undertake product development and to pay royalties on sales of the product. In addition the company is assisting with funding further research. Prototype material produced by Sandoz is being field tested on *X. spinosum*.

FINAL REPORT

TITLE: MYCOHERBICIDES FOR BIOLOGICAL CONTROL OF NOOGOORA AND BATHURST BURRS

RESEARCHERS INVOLVED:

Principal Researcher:
Dr Bruce Auld, Principal Research Scientist
Associates:
Dr Cheryl McRae, formerly Technical Officer (Scientific)
(subsequently WRDF Ph.D. Scholar, now WRDF Post-doctoral Scholar)
Ms Madeleine Say, Technical Officer (Scientific)
Mr Geoff Millar, Technical Officer (Scientific)
Mrs Karen Radburn, Assistant
Dr Alex Nikandrow, Senior Plant Pathologist
Mrs Helen Ridings, Senior Biometrician
Taxonomic Consultant: Mr John Walker, Principal Research Scientist, BCRI, Rydalmere
Overseas Associates:
Professor G.E. Templeton and Dr Greg Weidemann, University of Arkansas, Fayetteville ARK. USA.
Overseas Students:
Ms Karin Jongerius and Ms Marie-Antionette Smit, Wageningen Agricultural University, The Netherlands.

BACKGROUND:

*Xanthium* species are important weeds in Australia in summer crops and as wool contaminants. *X. occidentale*, Noogoora burr and *X. italicum*, Hunter burr are weeds of sheep grazing land as well as high value crops, cotton and soybeans on which the cheap herbicide 2,4-D cannot be used. *X. spinosum*, Bathurst burr, is one of the most widespread and important weeds in Australia. It occurs in coastal tablelands and plains areas of eastern Australia and is a problem in grazing and cropping areas. In 1983-84 a survey of fungal pathogens of *Xanthium* species was made.

*PHOMOPSIS*

The survey of diseases on *Xanthium* species in N.S.W. showed an unidentified species of *Phomopsis* to be associated with a die-back disease of *X. spinosum*, *X. occidentale*, *X. italicum* and *X. orientale*.

A technique used to incorporate macerated mycelium of the fungus into a matrix of sodium alginate with 2% oatmeal allowed the production of large numbers of spores with a consistently high proportion of alpha (infective) spores.

Production of alpha spores was favoured at temperatures between 20 and 25$^{o}$C under a light regime of 12 hr UV or 12 hr fluorescent with a 12 hr dark period in a humid atmosphere for 3-4 weeks. No alpha or beta spores were produced in the dark. Spore production remained optimal at storage temperatures of 5 and 10$^{o}$C for up to 4 weeks, but decreased more quickly with storage at higher

temperatures. In general, factors which favoured alpha spore production, did not favour the production of beta spores, although storage temperatures in excess of 15°C decreased production of both spore types.

In host range testing *Phomopsis* was pathogenic to 15 of the 21 species tested.

Studies of the factors affecting disease development showed that infection of Noogoora burr can occur in any green leaf or stem tissue, however the aggressive form of the disease, which develops at nodes, requires the presence of dead petioles for at least 2 weeks after infection. The aggressive disease develops at temperatures between 15 and 25°C, developing quickest at 25°C. Water stress prevents disease development in 5 week-old plants, but not in 10 week-old plants, while nutrient stress prevented disease development in plants of both ages. Plants less than 6 weeks old did not develop aggressive disease.

Development of the non-aggressive form of disease requires floral initiation and plant senescence. Generally, plants with this form of disease died at a similar rate to senescing, uninoculated plants.

*COLLETOTRICHUM*

Taxonomic Collections and Investigations of *Colletotrichum* spp.

Some 80 isolates of the anthracnose fungus of *X. spinosum* were collected. The species of *Colletotrichum* occurring on *Xanthium spinosum* in N.S.W. is not *C. xanthii*, as was first thought. Examination of the type collection of *C. xanthii* showed the type to be different from N.S.W. collections and identical to *C. acutatum*, a common fruit rotting fungus in northern N.S.W. N.S.W. collections of *Colletotrichum* on *X. spinosum* most closely resemble *C. orbiculare*, however, cultural characteristics and spore morphology do not completely agree with any of the three currently accepted concepts of *C. orbiculare*. This species most commonly occurs as a latent fruit rotting pathogen of cucurbits, but has also been recorded on celery (Apiaceae) and on saffron thistle (Asteraceae).

Pathogenicity testing and screening of isolates of *C. orbiculare*

Isolates have been screened for spore production on solid substrates and in submerged culture and screened for pathogenicity to *X. spinosum* and other *Xanthium* spp. and host range tested. The work identified the ten best isolates from the original 80 in terms of rate of death caused to target plants (see McRae, Ridings and Auld, 1988). Some isolates were also pathogenic to other *Xanthium* species under certain circumstances. However as the disease is spread by rain splash it does not appear to be a serious threat to non-target plants.

Disease development and optimal conditions for development

This work has been completed. Increasing inoculum concentration readily caused death of *X. spinosum* in controlled environment studies reaching a plateau at $10^7$ spores $ml^{-1}$. Concentrations $\geq 10^6$ spores $ml^{-1}$ killed plants in $\leq$ 3 weeks (see Auld, McRae and Say, 1988). Increasing dew periods increased disease reaching a plateau at 24 hours, however some plants died even with no applied dew period. The optimum dew period (24 hr) temperature is between 20-25°C. However there is an interaction with length of dew period

so that a short dew period at high temperature (e.g. 8 hours at 35°C) also favours disease development (see McRae and Auld, 1988).

Disease development is significantly affected if there is a delay in onset of dew or more than 8 hours of light before the onset of dew; this is due to the need for a dark period for functional (melanized) appressoria to form. These findings indicate the need for late afternoon or evening applications of the fungus for maximum disease development in the field.

**Testing potential stress factors on disease development**

The influence of host moisture stress, simulated rainfall after inoculation, cold temperatures and host age/flowering status on *C. orbiculare* development on *X. spinosum* were investigated in controlled environment chambers.

Moisture stress to 50% relative leaf water content before inoculation significantly decreased disease development compared with unstressed controls. Disease development on plants given the same stress up to three times after inoculation was not significantly different from controls (Table 1).

Table 1. Effect of water stressed *X. spinosum* on disease development

| Stress | Mean disease rating* Days after inoculation 7 | 14 | 21 | 28 |
|---|---|---|---|---|
| Pre inoculation | 3.08 a | 3.08 a | 3.08 a | 3.08 a |
| Post inoculation x 1 | 3.92 b | 4.25 b | 4.58 b | 5.58 b |
| Post inoculation x 2 | 3.75 b | 4.33 b | 4.67 b | 5.50 b |
| Post inoculation x 3 | 3.67 b | 3.92 b | 4.25 b | 5.25 b |
| Control | 3.08 a | 4.33 b | 4.58 b | 5.00 b |

(Means in columns followed by same letter not significantly different ($p > 0.05$))
*See McRae, Ridings and Auld, 1988: 1 = no symptoms, 6 = death

Simulated rainfall (15 mm) as soon as 1 h after inoculation did not affect disease development (Table 2). Application of the fungus in the field before or soon after rain should enhance its effect as a mycoherbicide.

Table 2. Effect of simulated rain (15 mm) after inoculation on disease development

| Treatment<br>Delay before rain (hrs) | Mean disease rating |
|---|---|
| 1 | 4.58 a |
| 2 | 4.75 a |
| 4 | 4.73 a |
| 8 | 4.22 a |
| 16 | 4.65 a |
| 24 | 4.58 a |
| 48 | 4.60 a |
| Control - inoculated, no rain | 4.48 a |
| Control - no inoculation, rain | 1.00 b |

(Means followed by same letter not significantly different ($p > 0.05$))

Exposure to 10°C for 3 or 7 days after inoculation did not significantly affect disease development after 14 days from inoculation compared with plants at 25°C for 14 days, but delayed the onset of disease development.

Disease developed more quickly in 3 wk old plants than in 5 wk old plants. Flowering in 5 wk old plants (as would occur in late autumn germinating co-hosts) did not affect disease development compared with vegetative plants of the same age (Table 3).

Table 3. Effect of age and flowering in *X. spinosum* on disease development

| Age (wks) | Flowering status | Mean disease rating |
|---|---|---|
| 5 | flowering | 3.80 a |
| 5 | vegetative | 3.68 a |
| 3 | vegetative | 4.14 b |

(Means followed by same letter not significantly different $p > 0.05$)

**Development of Mass Production Techniques**

In initial tests the fungus was induced to sporulate in submerged culture fermentation (see Auld, McRae and Say, 1988). Subsequent modification of the culture medium allowed production concentrations $>6 \times 10^6$ spores $ml^{-1}$.

Table 4

| Medium | Spore production; millions per ml Days after inoculation 3 | 7 | 10 |
|---|---|---|---|
| 8 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 storage**

Under room temperature storage the spores in suspension are viable for <24 hours. We have developed an air drying technique in which the spore slurry is mixed with kaolin. Spores have remained viable for >9 months up to the time of writing this report.

Field applications

*Preliminary Experiments, Summer 1987-88*

At this stage the inoculum production capability for any one application was limited. Consequently small plot (up to 2.5 $m^2$) applications were made on a total of seven different occasions at two sites using suspensions of $10^6$ or $10^7$ spores $ml^{-1}$ to assess the robustness of the fungus as a mycoherbicide in the field. One site, near Wellington was a dryland pasture situation and the other site was in an irrigated soybean field at Leeton. Periods of leaf wetness after inoculation ranged from 0 to 6 hrs. After eight weeks percent plant kill varied from 89-100% in treated plots compared with 3-13% in control plots. There did not appear to be any effect from increasing concentration from $10^6$ to $10^7$ spores $ml^{-1}$.

*Summer 1988-89*

Following these preliminary field tests, experiments with natural populations of *X. spinosum* in a dryland pasture and in an irrigated soybean crop were undertaken in the summer of 1988/89. In addition the effect of providing artificial dew to plants grown in the field was assessed at two other sites, Orange and Forbes.

Although previous work in controlled environment experiments highlighted the importance of dew period, the relationship between hours of leaf wetness after inoculation (Table 5) and success of *C. orbiculare* as a mycoherbicide (Tables 6, 7, 8) was not consistent.

Table 5. Environmental conditions for 14 h following inoculation

| Site | Inoculation date | Max. temp. °C | Min. temp. °C | Leaf wetness h | Relative humidity Min.% | Relative humidity period>84% h |
|---|---|---|---|---|---|---|
| Leeton | 22 December 1988 | 30 | 16 | 0 | 79 | 13 |
| | 3 January 1989 | 31 | 17 | 2.0 | 60 | 8 |
| | 16 January 1989 | 30 | 21 | 0 | 64 | 4 |
| Forbes | 10 January 1989 | 25 | 10 | 0.3 | 39 | 5 |
| Orange | 17 January 1989 | 25 | 11 | 13.0 | 69 | 13 |
| Cowra | 19 January 1989 | 23 | 9 | 12.0 | 76 | 13 |
| | 14 February 1989 | 24 | 15 | 0 | 65 | 9 |

At Cowra, although no dew was recorded at the second application time, inoculation resulted in 100 percent kill at either $10^6$ or $10^7$ spores $ml^{-1}$ (Table 6). However relative humidity was above 84% for nine hours after that application. (This level of humidity is the lowest at which there is significant germination of spores of *C. orbiculare*.) In addition periods of heavy rain after inoculation may have increased subsequent disease development. The unusually high death rate in the adjacent untreated control plots between 8 and 12 wk after the second application (Table 6) was due to the fact that the co-operating farmer let sheep into the field on two occasions. Stem pieces of *X. spinosum* become caught in wool and if badly diseased readily break off. At a further distance from the treated areas (> 20 m) only 9 of 100 *X. spinosum* plants counted at random were dead.

Table 6. Effect of *C. orbiculare* applied at *X. spinosum*, Cowra

| Application date | Treatment spores $ml^{-1}$ | Time (wk): | % Plants killed 2 | 8 | 12 |
|---|---|---|---|---|---|
| 19 January 1989 | $10^6$ | | 8 a | 93 a | 98 a |
| | Untreated control | | 0 b | 15 b | 15 b |
| 14 February 1989 | $10^7$ | | 42 a | 77 a | 100 a |
| | $10^6$ | | 27 a | 59 b | 100 a |
| | Untreated control | | 0 b | 16 c | 94 b |

For each application time means followed by the same letter in the same column are not significantly different ($p > 0.05$)

The variation in efficacy of *C. orbiculare* at Leeton was likewise not related to actual dew period but to humidity. The better result from the first application (Table 7) followed 13 h of high humidity after inoculation.

Although 2 h of leaf wetness and 6 additional hours of high humidity were recorded at the second application time at Leeton, higher temperatures may have been partly responsible for the lower percentage kill compared with plants experiencing nine hours of high humidity at Cowra. Older *X. spinosum* plants were more difficult to kill from the first soybean sowing time but not the second, suggesting that environmental factors may override age effects noted in controlled environment studies.

Table 7. Percentage *X. spinosum* plants killed compared with untreated controls, soybean crops, Leeton, 1989

| Soybean sowing time | Percent plants killed<br>Application date *C. orbiculare*<br>22 Dec 88 | 3 Jan 89 | 16 Jan 89 |
|---|---|---|---|
| 20 October 89 | 100 a | 58 b | - |
| 2 December 89 | - | 72 a | 78 a |

For the same soybean sowing time, means followed by the same letter are not significantly different ($p > 0.05$) based on square root transformation.

The provision of an artificial dew period to plants at Forbes (Table 8) produced a dramatic increase in the rate and final number of plants killed compared with those left in the field under conditions of low humidity at application (Table 5) and high temperatures. In contrast at Orange, dew period in the field (Table 5) and subsequent conditions (Fig. 8) were very suitable for disease development.

Table 8. Effect of artificial dew on death of field grown inoculated plants 1989

| Site | Dew Treatment /subsequent location | % plants killed<br>Time (wk): 4 | 8 | 12 |
|---|---|---|---|---|
| Orange | 18 hr art. dew/field | 95 a | 100 a | 100 a |
| | 13 hr dew field/glasshouse | 75 ab | 90 a | 90 a |
| | 13 hr dew field/field | 60 b | 100 a | 100 a |
| | Untreated | 5 c | 40 b | 40 b |
| Forbes | 18 hr art. dew/field | 100 a | 100 a | 100 a |
| | 0.3 hr dew field/glasshouse | 60 b | 80 a | 100 a |
| | 0.3 hr dew field/field | 25 c | 50 b | 50 b |
| | Untreated | 25 c | 25 c | 25 c |

For each site, means followed by the same letter in the same column are not significantly different ($p > 0.05$)

In the eastern part of its range, including areas represented by Cowra, Orange and Wellington, *X. spinosum* control by *C. orbiculare* appears promising using simple aqueous based suspensions. In drier areas the formulation of the spore

suspension into a product with a low rate of evaporation could play a major role in achieving a consistent effect with the fungus in the field.

**Commercial Development**

In May 1988 NSW Agriculture & Fisheries advertised in the press for expressions of interest in the development of a mycoherbicide for Bathurst burr based on this research.

Responses were received from five companies including four internationals. The chosen commercial partner, Sandoz, is based in Basle, Switzerland but has an Australian branch. Under the terms of an agreement between the parties Sandoz will, with further cooperation from NSW Agriculture & Fisheries, proceed to product development and pay royalties to NSW Agriculture & Fisheries on product sales and partially fund further research led by Dr Auld.

Field testing of prototype material produced by Sandoz has begun in summer 1989/90.

**PUBLICATIONS**

Patent Specification:
Auld, B.A. (Inventor). (1988). Title: Mycoherbicide: 14 pp. with 6 figures. Patent 18454/88.

Auld, B.A., McRae, C.F. and Say, M.M. (1988). Control of *Xanthium spinosum* by a fungus. *Agriculture, Ecosystems and Environment* 21: 219-223.

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

McRae, C.F., Ridings, H.I. and Auld, B.A. (1988). Anthracnose of *Xanthium spinosum* quantitative disease assessment and analysis. *Australasian Plant Pathology* 17: 11-13.

Nikandrow, A., Millar, G.D. and Auld, B.A. (1987). *Phomopsis* spp., fungi with a potential for biological control of *Xanthium* weeds. *Proc. 6th conf. Aust. Plant Path. Soc.*, Adelaide. Abstract. p.92.