Proof-of-concept trials for control of DBM by autodissemination

R.A. Vickers1, J.K. Pell2, A. White1 and M.J. Furlong3

1 CSIRO Entomology, 120 Meiers Rd Indooroopilly, Queensland 4068, Australia
2 Plant and Invertebrate Ecology Division, Rothamsted Research, Harpenden, Hertfordshire, UK
3 University of Queensland, St Lucia, Queensland 4067, Australia
Corresponding author: Richard.Vickers@csiro.au

Abstract
On two occasions over a nine-day period, a total of 1100 diamondback moths (DBM), infected with the fungus Zoophthora radicans were released within a 16.4 m x 16.4 m field cage containing 542 DBM-infested potted broccoli plants. Larval and pupal populations on a sub-sample of 25 plants were examined on five occasions over the following 48 days for evidence of Z. radicans infection. Ten DBM-infested plants were placed individually in poly-organza covered cages so that they could not be contacted by infected adults and larvae within the larger field cage. DBM on these plants served as controls.

Infected larvae were first detected on treated plants five days after the initial release of infected adults. After 14 days, 20% of all larvae and pupae sampled were infected and at the final survey, 48 days after initial release, the infection rate was 79% (93% amongst III and IV instar larvae). The detection of infected larvae and pupae on the control plants 35 days after initial release, together with the presence of large numbers of Z. radicans conidia (spores) on microscope slides exposed within the field cage suggest that aerially-borne conidia were a major factor in transmission of the fungus.

There was no evidence to suggest that losses of infected adults and larvae due to predation were significantly higher than those suffered by uninfected adults and larvae. The proportion of uninfected males recaptured at pheromone traps was twice that of infected males, although the difference in terms of cumulative catch only became significant three days after release of the males. The significance of these findings for the development of autodissemination as a practical control technique is discussed.

Keywords
Zoophthora radicans, epizootic

Introduction
Diamondback moth, Plutella xylostella L. (Lepidoptera: Plutellidae), is the most significant and widespread insect pest of crucifers, with an estimated annual management cost of US$1 billion (Shelton et al. 1997). Its pest status is related to the high reproductive potential of the moth, the disruption or lack of natural enemies caused by excessive insecticide use against co-occurring pests and its ability to develop resistance to all currently available insecticides, including toxins of the microbial agent, Bacillus thuringiensis. More imaginative integrated pest management (IPM) strategies are urgently required (Talekar & Shelton 1993, Shelton et al. 1997, Lim et al. 1997). In this regard, the combined use of fungal natural enemies and synthetic female sex pheromones (autodissemination) has the potential to contribute to control of diamondback moth.

The entomopathogenic fungus, Zoophthora radicans Brefeld (Zygomycetes: Entomophthorales) is an important member of the natural enemy complex attacking the diamondback moth. Epizootics commonly develop in larval populations and can eradicate them at a local scale (e.g. Ooi 1981, Yamamoto & Aoki 1983, Riethmacher et al. 1992). However, epizootics are unpredictable and often only occur in large host populations too late in the crop season to maintain damage below the economic threshold. To maximise the potential of Z. radicans for diamondback moth management, epizootics must be initiated early in the season and in low density pest populations. It may be possible to achieve this by manipulating moth behaviour to facilitate autodissemination of fungus to susceptible conspecifics in the crop.

Autodissemination involves the attraction of male moths into specially designed inoculation traps in response to synthetic female sex pheromone. Once inside the trap they become contaminated with infective conidia from a sporulating source of Z. radicans and, on leaving the trap, return to the crop, disseminating disease amongst their own populations (= autodissemination). The benefits of this system over the
The management of diamondback moth and other crucifer pests

conventional use of mycoinsecticides are threefold. Use of a specific sex pheromone targets the inoculum to the diamondback moth as this is the only species entering the trap. Only small quantities of fungal inoculum and pheromone are required, thereby limiting production costs. Whilst inside the trap the fungus can be protected from the damaging effects of UV radiation and an abiotic environment that favours sporulation and infection can be provided.

Laboratory and preliminary field studies have been carried out to test this hypothesis (Pell et al. 1993, Furlong et al. 1995, Vega et al. 2000), but until now, no large scale 'proof of concept trials' have been made. Here we describe trials in Australia designed to quantify the potential to establish early season epizootics of Z. radicans in P. xylostella populations using autodissemination in which dispersal, transmission, epizootic establishment and susceptibility of Z. radicans infected cadavers to predation were measured.

Materials and methods

Cultures

A laboratory culture of P. xylostella was established in April 1998 from larvae collected in an infested crop of broccoli in the Lockyer Valley of south-eastern Queensland. The insects were reared on potted broccoli seedlings under a 14:10 L:D cycle at 18-25°C. The Z. radicans culture was derived from infected P. xylostella larvae collected in a commercial broccoli crop at Gatton in April 1999 and was maintained on a medium of Sabouraud dextrose agar supplemented with egg yolk and milk (SEMA) (Wilding & Brobyn 1980). Isolate virulence was maintained by sub-culturing a maximum of three times at ca. monthly intervals before it was again passaged through the host and re-isolated.

Experiment 1: Initiating an epizootic

The trials were conducted within a field cage measuring 16.4 x 16.4 x 2.3 m (l x w x h) covered with knitted shadecloth that allowed 50% light transmission. Over a period of ca. 5 days during March 2000, 552 broccoli seedlings were planted individually in 200 mm plastic pots and set out 0.6 m apart within and between rows in a grid pattern. Twenty-five plants evenly distributed throughout the cage were selected for regular examination to monitor development of the fungus. A further ten plants, also evenly distributed throughout the cage, were selected as controls. They were placed within individual poly-organza covered cages (450 x 470 x 920 mm (l x b x h) to prevent direct contact with infected adults and larvae from the treated plants. An overhead sprinkler irrigation system provided water as needed.

After ca. 3 weeks the plants were deliberately infested with DBM, both by introducing laboratory-reared larvae, pupae and adults to the cage and by temporarily removing some plants and exposing them overnight to ovipositing females in the laboratory. These procedures ensured the presence of all DBM stages when attempts to initiate an epizootic of Z. radicans commenced. Temperature and humidity were recorded every 30 min within a Stevenson screen in the centre of the cage. Daily temperature and humidity levels at 9:00 am outside the cage were derived from a meteorological station situated 300 m from the trial site.

Release of infected adults

Two hundred and twenty 2-4 day old male moths were released on 3rd May 2000 (day 0) and a second release of 740 males and females was made on 12th May (day 9). A sub-sample of five moths (first release) was retained in individual containers in the field cage to allow determination of mortality due to Z. radicans infection.

Monitoring of sentinel plants

Comprehensive surveys of healthy and infected DBM were made on days 14, 19, 23, 35 and 48. The trial was terminated on day 51. All leaves and stems on the selected plants were examined and larvae and prepupae recorded as infected if there was evidence of rhizoids or sporulation. On day 15, a survey was conducted of ten plants selected at 6 m intervals along a 60 m transect in a 1,800 m² (60 m x 30m) open field of broccoli whose closest boundary was 10 m from the field cage. The purpose of this survey was to determine whether or not Z. radicans was present naturally in the vicinity of the field cage.

Detecting aerially-borne Z. radicans conidia

Pairs of glass microscope slides measuring 75 mm x 25 mm were attached horizontally ca. 50 cm apart with bulldog clips to a bar so that their broad surfaces were parallel with and ca. 30 cm above the ground. The
pairs of slides were evenly distributed throughout the large field cage and were exposed on day 36 for 12 days, after which they were stained with lactophenol cotton blue and examined under a compound microscope for evidence of Z. radicans conidia. Counts were made of the number of conidia appearing in the field of view during a single transect of each slide from one end to the other. It is estimated that 1% of the surface was examined using this technique.

Experiment 2: Loss of DBM by predation

Predation upon infected DBM may adversely influence efforts to initiate an epizootic if a significant proportion of infected individuals is removed before sporulation occurs. Trials were conducted to determine whether there was any difference in levels of predation amongst healthy and infected larvae and adults.

Fourth instar larvae and adults recently killed by the fungus were produced under laboratory conditions. A second group of uninfected larvae and adults, killed by freezing them for 15 minutes at -20° C, served as controls. All cadavers were individually secured with double-sided adhesive tape to 50 mm-square pieces of clear acetate sheet. Protection from crawling predators was provided by encircling half the available larvae and adults with a narrow band of Tanglefoot®. These were designated ‘protected’ and the remainder ‘exposed’.

The acetate sheets were attached with a paper clip to the underside of a leaf within an unsprayed 60 m x 30 m plot of broccoli. Treatments were examined daily for the duration of the trials and scored according to whether or not the larva or adult was present. Trials were conducted on three occasions over a 12 month period. Analyses of variance were performed on the number of adults and larvae lost, weighted by number put out and on proportion lost, with adjustments to allow for the high proportion of zeros.

Experiment 3: Effect of Z. radicans infection on male dispersal

Three hundred and ninety Z. radicans-infected (i.e. inoculated with conidia and maintained under humid conditions for 24 hours to ensure infection) and 361 healthy 2-3 d old laboratory-reared males were released from a central point within a 30 m x 60 m field of broccoli about 1 h before dusk. The treated and untreated males were differentiated by dusting them with yellow and blue Dayglo® powder respectively. Over each of the following three days, pheromone traps, evenly spaced around the circumferences of concentric circles with radii of 5 m (4 traps), 10 m (8 traps) and 15 m (16 traps) centred on the release point, were examined for the presence of marked moths. Contingency tables were constructed to show cumulative numbers of trapped and untrapped untreated and treated moths on the first, second and third days after their release. Chi-squared analyses were used to determine if there were any significant differences between treatments in terms of cumulative catch, irrespective of trap position.

Results

Experiment 1: Initiating an epizootic

Infected larvae were first detected on treated plants four days after the initial release of infected adults. By day 14, 20% of all larvae and pupae sampled were infected and at the final survey on day 48 the overall infection rate had reached 79%. Amongst III and IV instar larvae it was 93%. A single infected I - II instar larva was recorded on day 19 in the controls, representing an infection rate of 0.5%. None were recorded during the subsequent survey conducted on day 23, but over the following 12 days, infections developed very rapidly, reaching 40% by day 35 and 49% by day 48. Figure 1 depicts progress of the epizootic on the treated and control plants over the survey period. No infected larvae were detected during the survey conducted in the open field of broccoli on day 15. Of the five laboratory-infected adults retained to provide a measure of mortality in those released within the field cage, two were dead as a result of Z. radicans infection by the time of the first inspection 72 hours after release. The remaining three died, again as a result of Z. radicans infection, over the following 24 hours.
Detection of aerially-borne conidia
Counts were very variable. In some instances there was more than a three-fold difference between counts on slides within a pair. The mean number of conidia/slide was 1085 ± 710 (s.d.). There was no evidence of any pattern in their distribution throughout the cage.

Meteorological conditions
Over the period of the trial, temperature and humidity within the cage averaged 14.4°C (range 0.7–30.1°C) and 78% (range 26.3–100%) respectively. Diurnal fluctuations in temperature and humidity within the cage for the period 3rd May – 20th June (days 0–48) are depicted in Figure 2. Over the same period, but at 9:00 am, mean temperature and humidity within the cage was 13.0°C and 88.1%, compared with 14.9°C and 80.2% outside the cage.

Experiment 2: Loss of DBM by predation
There was no evidence to suggest that Z. radicans-infected DBM were more susceptible to predation than healthy individuals. Of 339 DBM (166 adults and 173 larvae) placed in the field, only 45 (13.3%) were missing, presumed lost to predation, after three days. They comprised 16 infected and 16 healthy adults and
five infected and eight healthy larvae. After allowing for the high proportion of zeros (i.e. no loss) and for trial and block differences, an analysis of variance revealed no significant difference between losses of infected and healthy individuals ($P<0.05$).

**Experiment 3: Dispersal of infected males**

Sixteen (4.1%) and 29 (8.1%) of the treated and control moths respectively were recaptured and although more control than treated moths were caught on each of the three days following release, differences in terms of cumulative catch irrespective of trap position were not significant until the third day (day 1: $\chi^2=0.16$, n.s.; day 2: $\chi^2=2.99$, n.s.; day 3: $\chi^2=5.14$, $P<0.05$). Total catches over the three days following release were, at 5 m from release point: 13 infected and 18 uninfected moths; 10 m: 1 infected and 3 uninfected and at 15 m: 2 infected and 8 uninfected.

**Discussion**

The results clearly demonstrate that it is possible to initiate an epizootic of *Z. radicans* by releasing inoculated/infected adults into a healthy population of DBM, albeit under conditions favourable to fungal development (reduced incidence of ultra-violet light and an average humidity of almost 78%). However in less humid conditions it may be possible to manipulate the microclimate with strategic irrigations to enhance development of epizootics. Our observation after one particularly dry period, that the majority of *Z. radicans*-infected adults placed in the field during the scavenging trials sporulated immediately after the crop was irrigated, suggests that this may be possible.

The influence of DBM population density on the rate of development of the epizootic is not known, although intuitively one would expect the relationship to be direct because of the increased probability of conidia produced by infected individuals contacting healthy ones with increasing pest density. *Z. radicans* may be a suitable choice of pathogen in this regard, given the substantial contribution made by aerial transmission of the conidia. Under these circumstances physical contact between infected and healthy individuals is less critical for development of epizootics than is the case for *Beauveria* spp., for example, thus facilitating transmission in low population densities (Furlong & Pell 2001).

The next step in evaluating autodissemination as a means of controlling DBM will be to demonstrate that an epizootic can be initiated via inoculation traps and when DBM population densities are at or below the economic threshold. This will require the development of inexpensive traps that provide an environment in which the fungus can continue to sporulate over several days and that provide suitable access to males attracted to them by pheromone. Research is also needed to determine appropriate trap densities and for how long it will be necessary to maintain them in order to generate an epizootic. Given that the strategy would be to initiate epizootics whilst the crop is young and when DBM population densities are low, epizootics may take some time to develop and it may be necessary to maintain traps for several weeks initially. However, where crops are planted sequentially, as is often the case in Australia, it may only be necessary to initiate the epizootic in the first crop of the season. Conidia generated from within the initial crop and distributed aerially may be sufficient to prevent DBM from developing into a threat to subsequent crops.

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