Know-how for Horticulture™

Control of diamondback moth in brassica vegetables with fungi

Dr. Richard Vickers
CSIRO Entomology

Project Number: VG03034

VG03034

This report is published by Horticulture Australia Ltd to pass on information concerning horticultural research and development undertaken for the vegetable industry.

The research contained in this report was funded by Horticulture Australia Ltd with the financial support of the Vegetable Industry.

ISBN 0 7341 0902 4

Published and distributed by:
Horticultural Australia Ltd
Level 1
50 Carrington Street
Sydney NSW 2000
Telephone: (02) 8295 2300
Fax: (02) 8295 2399
E-Mail: horticulture@horticulture.com.au

HAL

Know-how for Horticulture™

FINAL REPORT

Project No. VG03034 (Completion date 31.3.04)

Title: Control of diamondback moth in Brassica vegetables with fungi

Author: Dr. Richard Vickers

Research Provider: HAL and CSIRO Entomology

Project No. VG03034

Project Leader: Dr Richard Vickers
CSIRO Entomology
120 Meiers Rd
Indooroopilly
QLD 4068

Ph: (07) 3214 2824
Richard.Vickers@csiro.au

Other personnel: Andrew White
Karryn Waterworth
Shama Khan

Purpose: To provide an account of research that investigated the feasibility of harvesting diamondback moths from an infested crop as a means of providing adults for a novel control technique known as inundative autodissemination.

Funding sources:

Horticulture Australia

CSIRO

Date: 31 March 2004

Disclaimers:

Contents

Media Summary …… 4

Technical Summary …… 5

Report

Introduction and Literature Review ……. 7

Materials and Methods …… 8

Results ……. 10

Discussion ……... 13

Technology transfer …… 14

Recommendations …….. 14

Acknowledgments …….. 14

Bibliography ……... 15

Media Summary

Diamondback moth (DBM) is the most significant and widespread pest of cruciferous crops in the world. It has up to 20 generations a year, is resistant to almost all currently available insecticides and it costs more than US$1 billion a year to manage worldwide.

One potential alternative to insecticidal control involves the use of a naturally occurring fungus that can be introduced to healthy populations by releasing adult DBM that have been artificially infected. The technique is called inundative autodissemination and adults may be sourced from cultures established specifically for the purpose or, alternatively, from infested crops using a vacuum. The latter method was investigated in this research project. Three issues were of particular relevance:

- Does vacuuming adversely affect the longevity of the moths?
- Does vacuuming adversely affect the ability of the moths to fly?
- Can sufficient moths be harvested by vacuum for inoculation and release?

Trials carried out in the laboratory and in the field demonstrated that neither adult longevity nor the ability to fly was adversely affected by vaccuming. However the numbers of adults that could be harvested by vacuum from crops with low-level DBM infestations was very low. At 0.3 larvae/metre of row, when many growers would apply insecticides, it would take almost 81 hours to collect 100 moths.

It was concluded that vacuuming infested crops is not suitable as a means of providing adults to implement autodissemination. However adults sourced from commercial suppliers of biological control agents may prove a viable alternative and should be encouraged if results from a concurrent investigation of autodissemination prove satisfactory.

Technical Summary

Introduction.

High reproductive potential, lack of natural enemies caused by excessive insecticide use and resistance to currently available insecticides make diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Plutellidae) the most significant and widespread pest of crucifers.

For these reasons alternatives to insecticidal control are required and a technique that is currently being investigated involves the propagation of artificially-induced epizootics of the entomopathogenic fungus, *Zoophthora radicans*. In nature such epizootics can reduce populations to zero, but are unpredictable and often occur too late in the season to benefit growers. Strategies to induce epizootics in a predictable and timely fashion are now being investigated, including one that relies on the release of *Z. radicans*-infected adults into DBM infested crops to spread the disease amongst healthy DBM.

The technique will require a reliable supply of adults suitable for infection and release. The objective of the research reported here was to determine whether that could be achieved by using a vacuum sampler to harvest adults from crops already infested with DBM.

Three issues were investigated. First, is the longevity of adults harvested by vacuum adversely affected by the process? If so then the secondary conidia essential to propagation of the epizootic may not be produced by the cadavers and the epizotic will not develop. Second, does vacuuming have an adverse impact on the ability to fly and thus on the distribution of fungal spores throughout the crop? And finally, can harvesting infested crops by vacuum yield sufficient adults for infection and release? In general terms, the more adults/plant, the better the chances of success. However whilst releases of 1–3 adults per plant have been used in previous studies, there is no experimental evidence prescribing minimum effective ratios. For the purposes of this study an arbitrary target of one infected adult for every 100 plants was adopted.

Materials and Methods.

Vacuuming and adult longevity. Within two days of emergence from a laboratory culture, 20 adults (males and females) were captured individually with a glass vial. Ten were transferred to each of two 850 ml plastic container provisioned with 5 % sugar solution. These adults served as the controls. Twenty more were removed from the emergence cage with a Warrior® garden blower/vacuum, subjected to 10 minutes of the airstream created by the vacuum and then transferred to plastic containers as previously described for the control insects. All containers were inspected daily and the number of dead adults noted. Observations ceased when all adults were dead.

Vacuuming and dispersal ability. Within 3 days of emergence, 108 laboratory-reared males were subjected to 10 minutes of the airstream created by a Warrior® garden blower/vacuum and then marked with yellow Dayglo® powder. Eighty nine control moths were collected individually with a tube and dusted with blue Dayglo® powder. Both sets of moths were provisioned with water. The moths were then released from

the central point of a 90 m x 21 m plot of cabbages containing a grid of 20 DBM pheromone traps. After 5 days catches were removed and identified by colour. Mean distance between the central release point and capture point was determined for both groups.

Collecting adult moths from infested crops by vacuum. Five hundred potted brocolli plants within a field cage (l x b x h = 16.4 x 16.4 x ca. 2.4 m) were deliberately infested with DBM sourced from CSIRO cultures. On 16 occasions at approximately weekly intervals, numbers of larvae and pupae on all plants within 5 randomly selected 1 m lengths of row was recorded and a Warrior® garden blower/vacuum was used to collect adult DBM from the crop for 10 minutes, during which time ca 100 plants were sampled. Regressions of larval count versus number of adults captured 7 and 14 days later, of numbers of pupae versus adults 7 and 14 days later and the proportion of plants infested versus numbers of adults captured on the day of the survey and 7 and 14 days later were calculated.

Results.

Vacuuming had no effect on adult longevity. Mean longevity of vacuumed and control adults following imposition of the treatments were, respectively, 4.9 days and 4.3 days. The means were not significantly different. The mean distance travelled by the treated (i.e. vacuumed) and control males was 10.6 m and 8.5 m respectively. There was no significant difference between the means.

There was a significant relationship between numbers of pupae/metre and of adults collected 7 days later. The equation describing the relationship predicted that at infestation levels of 0.3 larvae/m it would take to almost 81 hours to collect 100 adults and ca 17 hours when 15% of plants were infested.

Conclusions.

It was concluded that vacuuming infested crops is impractical as a means of providing adults to implement autodissemination. However adults sourced from commercial suppliers of biological control agents may prove a viable alternative and should be considered if results from a concurrent investigation of autodissemination prove satisfactory.

Final Report

Introduction and Literature Review

Cruciferous vegetables are economically important crops worldwide; approximately 2.2 million ha are grown annually, a considerable proportion of which is produced in Asia. The diamondback moth (DBM), *Plutella xylostella* L. (Lepidoptera: Plutellidae), is the most significant and widespread pest of crucifers, with an estimated annual management cost of US$1 billion (Shelton et al., 1997). This pest status is related to the high reproductive potential of the moth (up to 20 generations a year), to the disruption or lack of natural enemies caused by excessive insecticide use and the ability of DBM to develop resistance to all currently available insecticides, including toxins of the microbial agent *Bacillus thuringiensis* Berliner (Tabashnik et al., 1993; Shelton et al., 1993a, b). More sustainable integrated pest management (IPM) strategies are urgently required (Talekar and Shelton, 1993; Shelton et al., 1997; Lim et al., 1997; Roush, 1997).

The entomopathogenic fungus, *Zoophthora radicans* Brefeld (Zygomycetes: Entomophthorales), is an important member of the natural enemy complex attacking DBM. Epizootics commonly develop in larval populations and can reduce local populations to zero (e.g., Ooi, 1981; Yamamoto and Aoki, 1983; Riethmacher et al., 1992). Such epizootics are unpredictable and often only occur in large host populations too late in the crop season to maintain damage below economic thresholds. To maximize the potential of *Z. radicans* for DBM management, epizootics must be initiated early in the season and in low-density moth populations. It may be possible to do so by a technique known as autodissemination, of which there are two kinds:

(i) Inoculative, in which males are attracted to specially designed inoculation chambers in response to synthetic female sex pheromone. Once inside the chamber they become contaminated with infective fungal conidia and then return to the crop, disseminating the pathogen amongst their own population.

(ii) Inundative, in which adults are either cultured or collected from an infested crop, infected with the pathogen and then released back into the crop to initiate an epizootic.

A large, multi-national trial to develop and evaluate **inoculative** autodissemination, in which the author is involved, commenced in June 2003 and will finish in 2005. As an addendum to that trial a small research project was established to investigate **inundative** inoculation, with the specific objective of determining whether the collection of adult DBM from infested crops using a vacuum sampler was a practical means of providing adults to be infected with *Z. radicans.* This report is an account of the research.

Given that growers are likely to apply control measures once DBM densities exceed infestation thresholds, two issues of particular relevance to collection of adults by vacuuming were investigated:

(i) Can vacuuming at pest densities at or below grower-acceptable thresholds of 0.3 larvae/metre of row or, alternatively, when 15% of plants are infested, yield sufficient adults for inoculation?

(ii) Does vacuuming affect the longevity and dispersal ability of adults? (If so then their capacity to distribute fungus throughout the field will be compromised and render the technique less effective).

As vacuuming will capture both male and female moths, it is also appropriate to ask:

(iii) Can infected females be included in the release without significantly contributing to resident DBM populations? Whilst this is not such an issue if adults are being returned to the crop from which they were collected (i.e. no additional females are being released), it may be relevant if they have been derived from a different site or from a DBM culture.

(Note: Trials designed to answer this question were incorporated in the Research Proposal. However, after the Proposal had been approved, the author discovered that Furlong et al. (1997) had previously demonstrated that infected adult females laid significantly fewer eggs than healthy females. For this reason no further investigations were conducted).

Materials and methods.

1. Feasibility of collecting adult moths from infested crops by vacuuming.

Establishment of crop and DBM infestation. A crop of ca 500 potted brocolli plants was established within a field cage measuring 16.4 x 16.4 x ca. 2.4 m (l x w x h) at Indooroopilly, QLD. Plants were spaced 40 cm apart within and 50 cm apart between rows. When ca 10 cm tall they were deliberately infested with DBM by releasing male and female moths into the cage over 3 consecutive days. The adults were sourced from cultures at the CSIRO laboratories.

Sampling. On 16 occasions at approximately weekly intervals, all plants within 5 randomly selected 1 m lengths of row were examined for the presence of DBM larvae and pupae. The larvae were classified as either small (1^{st} and 2^{nd} instars) or large (3^{rd} and 4^{th} instars). The proportion of sampled plants infested with DBM was also calculated. On the same day that the plants were sampled a Warrior® garden blower/vacuum (Fig. 1) was used to collect adult DBM from the crop for 10 minutes, during which time ca 100 plants were sampled.

Data analysis. Regressions of larval count (sum of early and late instars) versus number of adults captured 7 and 14 days later and of numbers of pupae versus adults 7 and 14 days later were calculated. Similarly, regressions of the proportion of plants

infested versus numbers of adults captured on the day of the survey and 7 and 14 days later was calculated.

Fig. 1. Vacuum sampling for adult DBM

2. Effect of vacuuming on adult longevity.

DBM pupae from the CSIRO laboratory culture were placed in a muslin-covered emergence cage (l x b x h = 28 x 38 x 60 cm). Within two days of emergence, 20 adults (males and females) were captured individually with a glass vial and 10 transferred to each of two 850 ml plastic container provisioned with 5 % sugar solution. These adults served as the controls. The remaining adults were removed from the emergence cage with a Warrior® garden blower/vacuum and subjected to 10 minutes of the airstream created by the vacuum. Twenty were then transferred to plastic containers as previously described for the control insects. When transfers had been completed all containers were inspected daily and the number of dead adults noted. Observations ceased when all adults were dead.

3. Effect of vacuuming on the dispersal ability of adults.

As the only practical means of recapturing released adults was with pheromone traps, female dispersal was not measured. Male 3rd and 4th instar larvae were extracted from the laboratory cultures and allowed to pupate and emerge as moths. Within 3 days of emergence, 108 males were removed from the emergence cage with a Warrior® garden blower/vacuum and subjected to 10 minutes of the airstream created by the vacuum before being transferred to an 850 mL plastic container in which a small quantity of yellow Dayglo® powder (DayGlo, Cleveland OH 44103, USA) had been placed. The powder marked the vacuumed moths so that they could be distinguished from the 89 control moths, which had been removed individually from the emergence cage, transferred to a separate 850 mL plastic container and dusted with blue Dayglo® powder. Both containers were provisioned with water.

On the afternoon of the day that the moths were marked, they were released from the central point of a 90 m x 21 m plot of cabbages in which a grid of pheromone traps baited with synthetic diamondback moth pheromone had been set out. The grid

consisted of 10 rows, each containing 2 traps. Rows were spaced 5 m apart and traps were 13 m apart within the rows (Fig. 2) such that no trap was more than 14 m from its nearest neighbour. Trap catch was read 4 days after release and individual moths identified as being treated (yellow), controls (blue) or feral (no colour). Mean distance between the central release point and recapture point was determined for both groups.

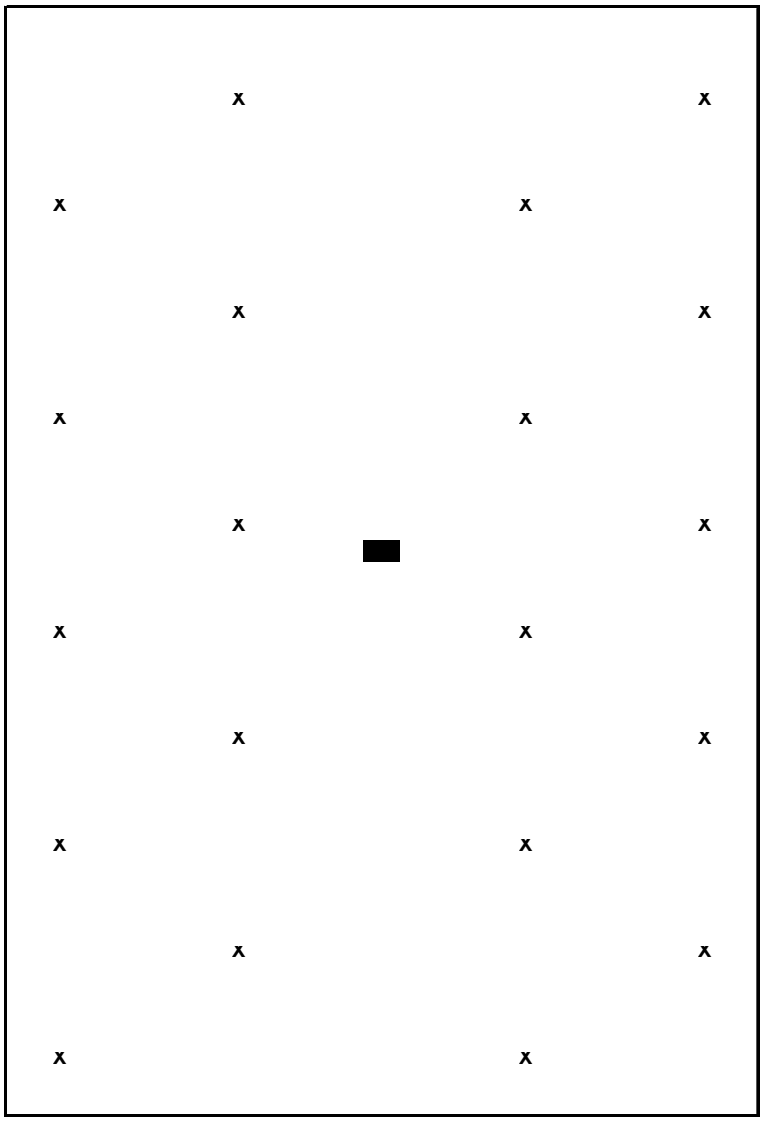

Fig. 2. Distribution of pheromone traps (**X**) within a 90 x 21 m plot of cabbages. Marked male DBM were released from the point indicated by the solid rectangle.

Results

Feasibility of collecting adult moths from infested crops by vacuuming

The relationship between numbers of immature stages per metre of row and numbers of adults collected by vacuum over a 10 minute period and between % plants infested by DBM and adults collected by vacuum over a 10 minute period is shown in Table 1.

Measure	Regression	r^2	Significance
All larvae/m vs adults captured 7 d later	y = 0.1056x	0.482	n.s.
All larvae/m vs adults captured 14 d later	y = 1.2688x	0.0852	n.s.
All larvae/m vs adults captured 21 d later	y = 1.1999x	0.02	n.s.
Large larvae/m vs adults captured 7 d later	y = 6.6731x	0.1844	n.s.
Large larvae/m vs adults captured 14 d later	y = 3.5557x	0.0675	n.s.
Pupae/m vs adults captured 7 d later	y = 2.476x	0.8715	P < 0.001
Pupae/m vs adults captured 14 d later	y = 0.8584x	0.2661	n.s.
% plants infested vs adults captured same day	y = 0.4489x	0.0296	n.s.
% plants infested vs adults captured 7days later	y = 0.4489x	0.2269	P < 0.01
% plants infested vs adults captured 14 days later	y = 0.2859x	0.2097	n.s.

Table 1. Relationship between DBM infestation and adults collected by vacuuming the crop for 10 minutes. n.s. = not significant..

The only significant relationships identified were those between numbers of pupae/metre and numbers of adults collected 7 days later (Fig. 3), and between the proportion of plants infested and adults collected 7 days later (Fig. 4). On the basis of these data the predicted time it would take to collect 100 adults is shown in figures 5 and 6.

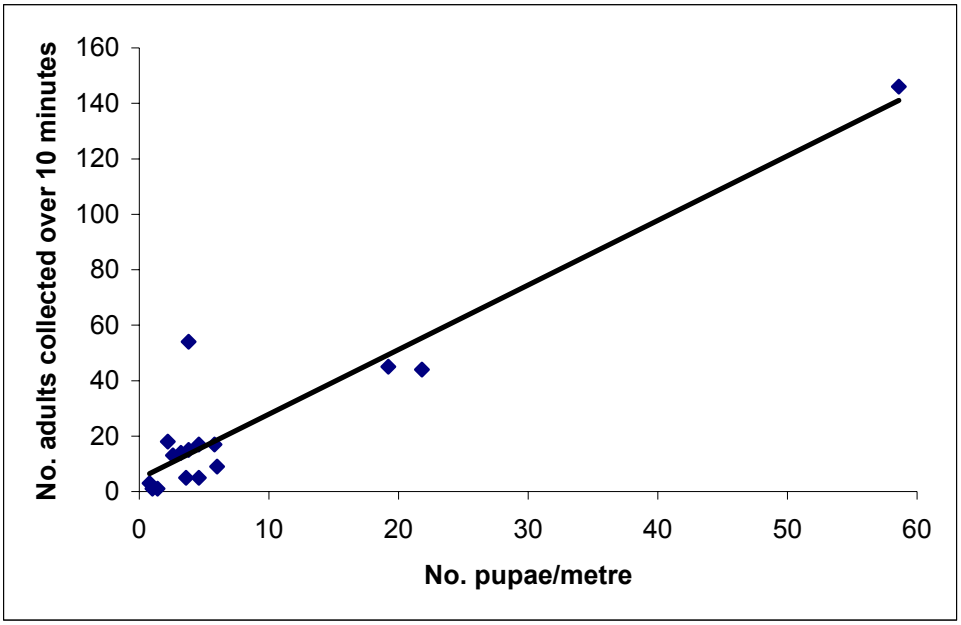

Fig. 3. Relationship between number of pupae/metre and yield of adult DBM by vacuuming the crop for 10 minutes, seven days after measuring pupal density.

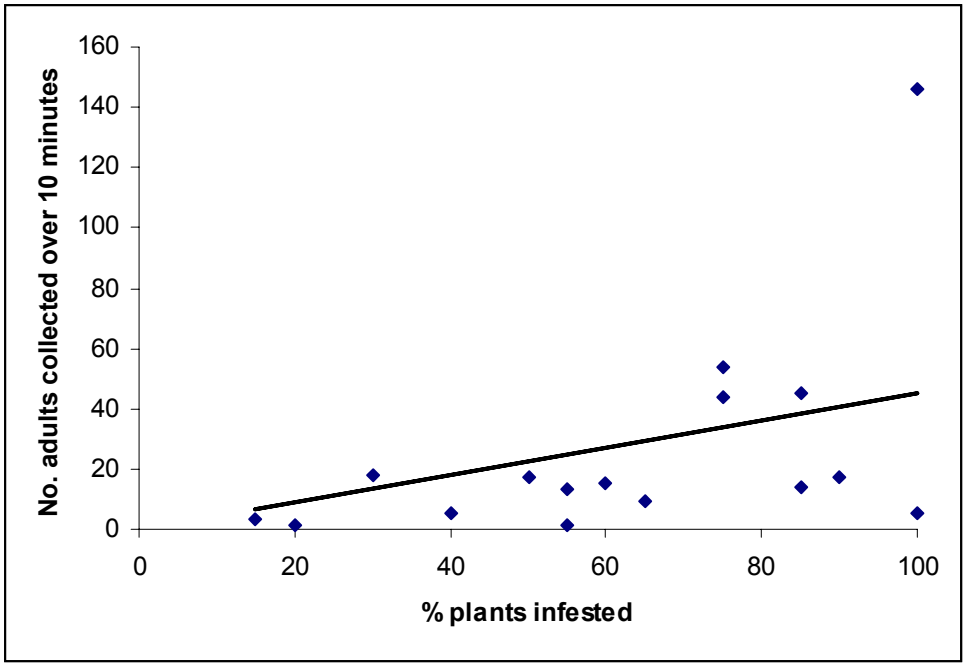

Fig. 4. Relationship between percentage plants infested and yield of adult DBM by vacuuming the crop for 10 minutes, seven days after % infestation was determined.

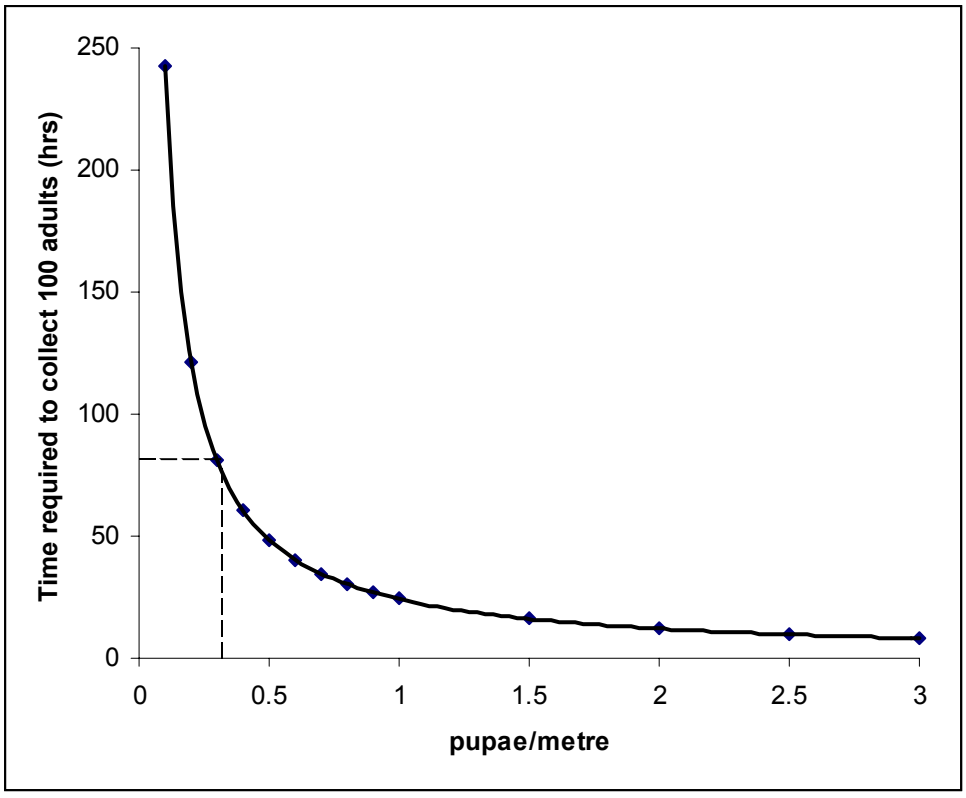

Fig. 5. Predicted time (hours) to collect 100 adults by vacuuming crop at various pupal densities. Broken lines indicate readings for a pupal density of 0.3/metre and show that it would take almost 81 hours to collect 100 moths.

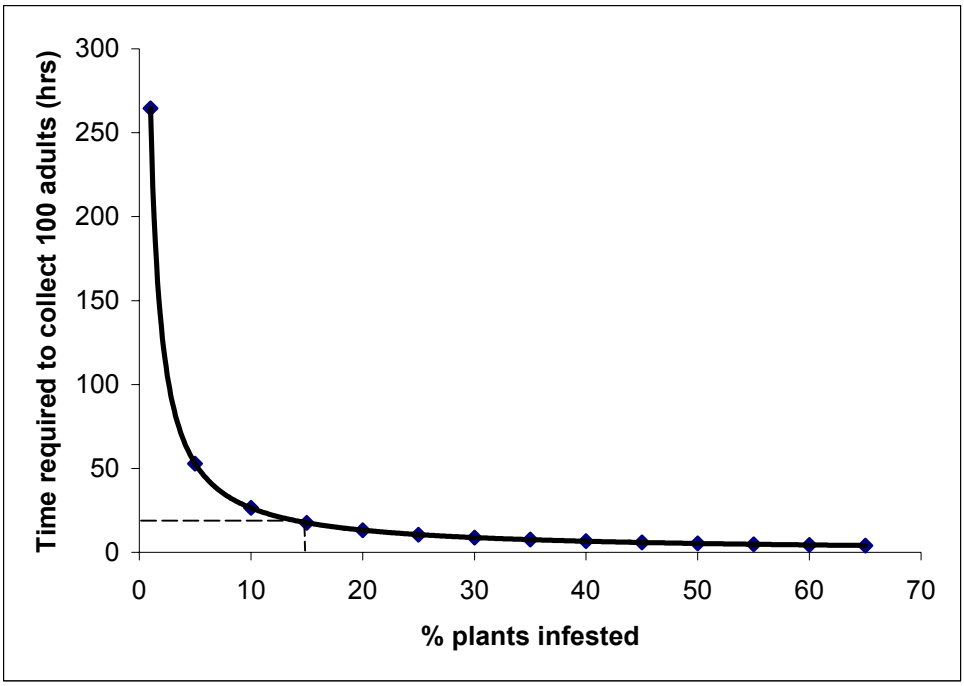

Fig. 6. Predicted time (hours) to collect 100 adults by vacuuming crop at various pupal densities. Broken lines indicate readings for a crop infestation level of 15% and show that it would take 17.6 hours to collect 100 moths.

Effect of vacuuming on adult longevity

Vacuuming had no effect on adult longevity. Mean longevity of vacuumed and control adults following imposition of the treatments were, respectively, 4.9 days and 4.3 days. The means were not significantly different.

Effect of vacuuming on dispersal

There was no significant difference between the dispersal ability of male treated and control moths as measured by trap catch (Table 2).

Treatment	No. released	No. recaptured	% recaptured	Mean distance (m) of recaptures from release point ± S.D.
Vacuumed	108	22	20.4	10.6 ± 15.5
Control	89	15	16.9	8.5 ± 8.4

Table 2. Mean distance travelled by male DBM following exposure to vacuum for 10 minutes

Discussion

Little information is currently available to enable estimates to be made of how many *Zoopthora* infected adults, expressed in terms of numbers/plant, one should release in order to establish an epizootic. However Furlong & Pell (2001) found that the greater the density of cadavers, the higher the transmission frequency. They demonstrated

significant increases in transmission rate from sporulating cadavers to larvae when the number of cadavers per plant was increased from 1 (mean transmission rate = 14%) to 3 (52%). In proof-of-concept trials designed to demonstrate that epizootics of *Z. radicans* could be artificially induced, Vickers et al. (2004) detected infected larvae within four days of releasing 960 infected adults in a broccoli crop of 560 plants. This equates to 1.8 adults per plant.

Although vacuuming has no detrimental effects on either adult longevity or dispersal ability, on the basis of data generated in the present trials, collecting sufficient adults by vacuum to enable a release rate of one per plant is clearly not a practical proposition. When DBM larval densities are at or near action thresholds, for example 0.3 larvae/metre (Mo et al., 2001), it would take at least 81 hours to collect enough moths to treat 100 plants, and that is an under-estimate given that it is based on pupal rather than larval density. If a threshold of 15% infested plants were used (Hamilton & Schellhorne, 2004) it would take over 17 hours to collect 100 adults.

Although it is probable that an epizootic could be established with the release of fewer infected adults/plant, the rate at which it became established would be commensurately slower, perhaps too slowly to provide effective control in some instances. However even if the ratio adopted for the purposes of this report of one infected adult for every 100 plants were to prove satisfactory, their collection by vacuum remains prohibitive in terms of the time it would take in low-density DBM infestations.

Nevertheless, inoculative autodissemination may still have promise. The technique is currently being investigated within another research project with which the author is associated, but which will source adults from cultures of the insect rather than directly from infested crops. This should be a much more efficient means of providing large numbers of adults for infection and release and is one that could be readily undertaken by commercial suppliers of biological control agents.

Technology transfer

There is no technology to transfer from this project. A related project to evaluate autodissemination is currently being conducted and provides for transfer of the technology to Australian growers if the technique proves effective.

Recommendations

That the provision of adult DBM from cultures established by commercial suppliers of biological control agents (rather than harvesting infested crops by vacuum) be promoted if inundative autodissemination proves effective in trials currently being conducted in a related project.

Acknowledgments

It is a pleasure to acknowledge the Australian Brassica growers and HAL for their financial support, Andrew White, Shama Khan and Karryn Waterworth (CSIRO Entomology) for their contributions to establishing and maintaining insect and plant

cultures, infestation surveys and day-to-day operations of the trials and Mike Furlong (University of Queensland) and Bronwyn Walsh (Queensland Department of Primary Industries) for allowing the use of a cabbage plot at Gatton Research Station for mark-recapture studies.

Bibliography

Furlong, M.J., J.K. Pell & G.V.P. Reddy, 1997. Premortality effects of *Zoopthora radicans* infection in *Plutella xylostella*. Journal of Invertebrate Pathology **70**, pp 214 – 220

Hamilton A. & Schellhorn N. 2004. Diamondback moth sampling plan. Victorian Dept. Primary Industries website: http://www.dpi.vic.gov.au/dpi/nrenfa.nsf/LinkView

Lim, G.S., A. Sivapragasm & W. H. Loke, 1997. Crucifer insect pest problems: trends, issues and management strategies, in: Sivapragasm, A., W. H. Loke, A. K. Hussan & G. S. Lim, (Eds.), Proceedings of the Third International Workshop on the Management of Diamondback Moth and other Crucifer Pests, Kuala Lumpur, pp. 3-16.

Mo, J., G. Baker & M. Keller, 2001. Evaluation of sequential binomial plans for decision making in the management of the diamondback moth (*Plutella xylostella*) (Plutellidae: Lepidoptera). Final Report to the Horticultural Research and Development Corporation, Project VG 97014. Advancing the integrated management of diamondback moth (DBM) in *Brassica* vegetables, pp 15-22.

Ooi, P.A.C. 1981. Microbial control of the diamondback moth in Cameron Highlands, Malaysia. Malaysian Applied Biology 10: 49-56.

Riethmacher, G.W., M. C. Rombach & J. Kranz, 1992. Epizootics of *Pandora blunkii* and *Zoophthora radicans* (Entomophthoraceae: Zygomycotina) in diamondback moth populations in the Philippines, in: Talekar, N.S. (Ed.), Proceedings of the Second International Workshop on Diamondback Moth and other Crucifer Pests, Tainan, Taiwan. Asian Vegetable Research and Development Centre, Shanhua, Taiwan, pp 193-199

Roush, R.T., 1997. Insecticide resistance management in diamondback moth: *quo vadis*? in: Sivapragasm, A., W. H. Loke, A. K. Hussan & G. S. Lim, (Eds.), Proceedings of the Third International Workshop on the Management of Diamondback Moth and other Crucifer Pests, Kuala Lumpur, pp. 21-24.

Shelton, A. M., J. L. Robertson, J.D. Tang, C. Perez, S. C. Eigenbrode, H. K. Preisler, W. T.Wiley & R. J. Colley, 1993a. Resistance of diamondback moth to *Bacillus thuringiensis* subspecies in the field. Journal of Economic Entomology 86: 697-705.

Shelton, A. M., J. A. Wyman, N. L. Cushing, K. Apfelbeck, T. J. Dennehy, S. E. R. Mah & S. D. Eigenbrode, 1993b. Insecticide resistance of the diamondback moth in North America. Journal of Economic Entomology 86: 11-19.

Shelton, A. M., C. J. Perez, J. D. Tang & J. D. Vandenberg, 1997. Prospects for novel approaches towards management of the diamondback moth, in: Sivapragasm, A., W. H. Loke, A. K. Hussan, & G. S. Lim, (Eds.), Proceedings of the Third International Workshop on the Management of Diamondback Moth and other Crucifer Pests, Kuala Lumpur, pp17- 20

Tabashnik, B.E., N. Finson, M.W. Johnson & W.J. Moar. 1993. Resistance to toxins from *Bacillus thuringiensis* subsp. *kurstaki* in the Diamondback Moth (Lepidoptera: Plutellidae). Applied Environmental Microbiology 59: 1332-1335.

Talekar, N.S. & A. M. Shelton, 1993. Biology, ecology and management of the diamondback moth. Annual Review of Entomology 38**:** 275-302.

Yamamoto, M. & J. Aoki, 1983. Periodicity of conidial discharge of *Erynia radicans*. Transcripts of the Mycological Society of Japan 24**:** 487-496

Vickers, R.A., Furlong, M.J., White, A. and Pell, J.K. (2004). Initiation of fungal epizootics in diamondback moth populations within a large field cage: proof of concept for auto-dissemination Initiation of fungal epizootics in diamondback moth populations within a large field cage: proof of concept for auto-dissemination. *Entomologia Experimentalis et Applicata* **111** (1), 7-18.