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Mass production of *Trichogramma chilonis*: an economical and advanced technique

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ABSTRACT

The use of technological advances to enhance the economical production of *Trichogramma chilonis* indicated that radiation increased the incubation period of the eggs of Angoumois grain moth, *Sitotroga cerealella,* which proved useful to increase the parasitic potential of *T. chilonis*. Results showed that a radiation dose of 25 Gy can be effectively used to enhance parasitic potential and to decrease the age effect of host eggs for parasitization. The parasitism on F_1 eggs, obtained from the parents irradiated as one-day old eggs at 25 Gy, was higher than that of normal or irradiated P_1 eggs. The use of nuclear techniques in the mass rearing facility of the parasitoids also enhanced the production of females who play an important role in the augmentative biocontrol programme. Studies revealed that Angoumois grain moth eggs, after irradiation at 25 Gy, can be effectively stored at 10 °C for up to 40 days without disturbing the quality of the eggs for parasitization. This offered potential benefits of providing flexibility and efficiency in mass production of the parasitoids.

INTRODUCTION

The continuous and indiscriminate use of insecticides has created several problems which jeopardize their efficacy. The most important of these are the evolution of insect resistance to insecticides and disturbance of biological equilibrium in an agroecosystem. Interest has therefore, resurfaced in using the biological control which has long been recognized as an important pest management component. Beneficial insects have been successfully manipulated with a variety of augmentation and conservation strategies (Mohyuddin, 1991; Ashraf *et al.,* 1999) These mainly involve periodic releases and environmental management, such as providing food or hosts during times of low prey density. Production of natural enemies in an efficient and economical way is a prerequisite for biological control programmes. Considerable technological advances have been made in mass rearing of parasitoids and predators for augmentative biological management of pests. Irradiation may play a significant role in the production of natural enemies. It has been reported that parasitization was increased in the progeny of irradiated lepidopterous pests (Mannion *et al.,* 1995; Carpenter *et al.*, 1995 & 1996). Marston & Ertle (1969), tested the acceptability of irradiated moth eggs to *Trichogramma minutum* Rile, and reported that irradiated eggs were as suitable as control eggs for parasite development. Eggs of *Ephestia kuehniella* (Zeller) killed with ultraviolet irradiation were suitable for mass rearing of *Trichogramma* spp. (Voegele *et al.*, 1974). Lewis & Young (1972), reported that when adult males of *Heliothis*

zea (Voddic) were sterilized with 320 Gy of gamma irradiation and paired with untreated females, the eggs produced were as suitable as control eggs for attack and development of *T. evanescens*. The studies reported here were designed specifically to evaluate the feasibility of using nuclear techniques for efficient and economical production of egg parasitoid *Trichogramma chilonis,* for releases in the field.

MATERIALS AND METHODS

Effects of irradiation of the host eggs on parasitization.

Eggs of Angoumois grain moth, *Sitotroga cerealella,* were collected from moths reared in controlled conditions in 2.5 litre glass burneys. For this purpose, large numbers of 1- to 2-day old adults were collected from stock cultures and placed in inverted 1 litre plastic jars with screen bottoms. Eggs that fell through the screen bottoms were collected by sifting them with the help of a 70 mesh screen. Egg cards were prepared with approximately. 2000 eggs/card by sprinkling them onto cards having a patch of gum. After an hour, the cards were exposed to radiation at different doses (5,10,15,20,25,30,35,40,45 and 50 Gy) and then the eggs were exposed to the parasite *T. chilonis* in conical flasks at different ages of the host eggs. Each card was exposed to twenty pairs of the parasite and the eggs were removed from the flasks after 24 hours. The rate of parasitism on each card and the effect of age of the eggs for parasitization were recorded.

Effect of storage of the host eggs on parasitization.

The glued fresh eggs of Angoumois grain moth (approx. 2000/cards) were irradiated at the dose of 25 Gy and stored in an incubator at 10°C temperature for 10, 20, 30, 40 and 50 days. Un-irradiated eggs were also stored at the same temperature for the same period. After storage for the mentioned period, eggs were exposed to the parasitoids at ambient temperature for up to 6 days. The suitability of the eggs for parasitization by *T. chilonis* was observed.

RESULTS AND DISCUSSION

Effects of irradiation of the host eggs on parasitization.

The effects of radiation on percentage hatch and adult emergence are presented in Table-1. Results indicated that radiation significantly reduced the percentage hatch of the eggs irradiated at different doses. The percentage hatch decreased as the radiation dose increased and it was negligible at the dose of 50 Gy. The egg hatch was statistically identical with the control at the doses ranging from 5 to 15 Gy and thereafter, it decreased gradually with the increase in dose rate. Similarly, the maximum number of adults of Angoumois grain moth emerged in the untreated control followed by the 5 Gy dose of gamma radiation. emergence gradually decreased with the increase in dose, until less than one percent of adults successfully emerged from the eggs treated at the dose of 50 Gy. The sex ratio in the moths emerged from the irradiated eggs, was skewed in favour of males at higher doses which may

be because the females are more sensitive than males and some females might have died in the embryonic stage. The results (Table 2) showed that the parasite preferred the fresh eggs for parasitization and that parasitization decreased as the age of the host eggs increased. However, the parasitization was comparatively higher at 20 and 25 Gy dose of radiation. Moreover, irradiation of host eggs also decreased the age effect and significantly higher numbers of eggs were parasitized on 2-, 3- and 4-day- old irradiated host eggs as compared to normal eggs. On irradiated eggs parasitization was recorded up to 6-days of age whereas, on normal eggs it occurred up to 4-days of age. Results revealed that radiation doses of 20 and 25 Gy can be effectively used to enhance parasitization as well as to decrease the age effect of host eggs of the parasitoids.

Table 1. Effect of Gamma Radiation on the eggs of Angoumois Grain Moth.

Dose (Gy)	Hatch (%)	Pupal recovery	Sex ratio	
			Male	Female
5	88.6	64.3	24	18
10	80.0	62.0	22	18
15	81.6	66.6	27	15
20	76.3	51.3	25	9
25	72.3	50.3	24	9
30	68.0	40.3	18	6
35	40.3	27.6	14	5
40	19.6	12.3	7	0
45	3.6	2.0	3	0
50	0.6	0.6	0	0
Control	90.3	68.3	25	22

Effect of storage of host eggs on parasitization.

Results revealed that eggs were effectively parasitized after they had been kept in an incubator at 10°C for 50 days. Parasitization was higher in irradiated eggs as compared to normal stored eggs (Table 3). Moreover, parasitization was recorded at up to 7 days of age in the irradiated eggs, as compared to 4 days on normal un-irradiated eggs, when both had been stored for up to 20 days at 10 °C and offered to the parasite at ambient temperature (27±2 °C). However, the maximum number of eggs were parasitized on the 1st day of their exposure to the parasitoids. It was observed that storage of eggs after irradiation at 25 Gy did not disturb the sex ratio of the parasite. Moreover, the sex ratio was slightly in favour of females in both irradiated and un-irradiated stored eggs. Studies revealed that Angoumois grain moth eggs, after irradiation at 25 Gy, can be effectively stored for up to 40 days at 10 °C temperature and offered to the parasite for parasitization without disturbing any potential. The irradiated and normal eggs of different ages, exposed to the parasitoids for parasitization did not show any storage effect.

Eggs of Angoumois grain moths are a suitable host for *Trichogramma chilonis* (Morrison *et al,* 1976). Eggs irradiated at 20 or 25 Gy had good percentage parasitization as compared to the control. These finding are in close conformity with Brower (1982), who observed that eggs of Indian meal moths, irradiated at a dose of 500 or 1000 Gy were preferred by *T. pretiosum to* normal untreated eggs. He observed that Indian meal moth eggs killed by irradiation could be used for the rearing and releases of the parasitoids. Studies revealed a great promise for the use of ionizing radiation in support of the development of improved mass rearing methods. The regulatory climate is becoming much more stringent, and nuclear techniques may help to facilitate acceptance of biological control by preventing the accidental release of reproductively viable pest organisms along with their natural enemies (Delfosse, 1997; Hill, 1997)

Table 2. Effect of irradiation of host eggs on parasitization by *Trichogramma chilonis*.

Dose	Parasitization potential in host eggs at different ages (days)						
(Gy)	1	2	3	4	5	6	7
5	19.2	16.4	11.2	6.4	0.6	0	0
10	23.2	21.0	17.6	11.0	0	0	0
15	20.2	17.0	12.6	11.8	0.8	0	0
20	23.6	20.0	15.2	13.2	9.0	3.8	0
25	24.4	19.8	13.2	9.2	4.2	1.4	0.8
30	19.2	16.6	13.2	9.4	4.8	1.8	0
35	17.8	15.6	14.6	5.4	0.8	0	0
40	15.2	13.4	8.4	1.4	0	0	0
45	13.4	7.6	5.4	0.2	0	0	0
50	11.6	7.6	1.4	0	0	0	0
Control	18.8	11.2	7.2	2.0	0	0	0

The present investigations indicated that the egg parasitoids *T. chilonis* can be reared effectively on irradiated host eggs. The irradiation also increased the parasitic potential of the parasitoids and decreased the age effect of the host eggs. This will not only reduce the cost of the rearing but also facilitate the supply of parasitoids out of season. Moreover, the excess production of the eggs of Angoumois grain moth can be stored at 10 ^{0}C after irradiating them at 25 Gy without damaging their quality for the rearing of the parasitoids. Furthermore, for obtaining the optimum number and good quality of the parasitoids, the standard conditions should be given due consideration.

ACKNOWLEDGMENTS

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Table. 3 Effect of storage time on host eggs for parasitization.

Storage time (days)	Age at ambient temp. (days)	No. of eggs parasitized		Emergence percentage			
		Irradiated	Normal	Irradiated		Normal	
				Male	Female	Male	Female
10	1	18.83	15.10	37.16	43.36	36.2	46.6
	2	19.16	13.8	34.78	43.48	36.1	37.3
	3	17.17	4.8	33.98	40.78	24.1	27.5
	4	14.67	1.0	34.09	38.64	33.3	16.6
	5	11.00	--	30.30	37.87	--	--
	6	7.50	--	26.67	33.23	--	--
	7	3.00	--	25.00	30.00	--	--
20	1	20.66	15.6	38.70	41.93	34.0	39.7
	2	18.00	15.0	32.88	41.14	32.2	37.7
	3	15.83	12.6	37.89	38.94	27.6	33.2
	4	15.50	9.6	38.70	36.55	25.8	31.0
	5	11.16	13.3	35.82	37.31	22.5	25.0
	6	6.16	1.6	37.83	35.13	20.0	00.0
	7	2.33	--	--	--	--	--
30	1	18.83	16.6	36.28	39.82	36.0	38.0
	2	18.33	15.3	34.54	38.18	35.8	40.2
	3	17.66	9.0	33.0	33.96	25.9	31.4
	4	13.66	9.0	28.0	32.92	27.7	29.6
	5	8.16	6.6	24.48	32.65	20.0	30.0
	6	3.0	1.1	33.33	16.66	--	--
40	1	17.5	16.5	34.28	39.04	33.6	36.3
	2	16.33	15.5	30.61	36.73	33.3	39.7
	3	15.16	10.5	35.16	24.17	26.9	28.5
	4	12.00	6.1	33.33	22.22	24.3	21.6
	5	11.83	--	32.39	19.71	--	--
50	1	7.8	13.8	27.65	42.55	32.5	36.1
	2	6.66	10.8	30.00	37.5	27.6	30.1
	3	5.83	7.6	37.14	25.71	21.6	23.9
	4	4.83	3.1	31.03	20.68	10.52	10.52

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Bemisia argentifolii parasitoids on poinsettia

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ABSTRACT

Biological control of introduced pests using parasitoids needs a comprehensive knowledge of the host-parasitoid relationship. *Bemisia argentifolii*, a pest of ornamentals has recently been introduced to Europe, therefore development of its biological control is an urgent task. Studies at the Department of Entomology of Wageningen University evaluated host searching behaviour of five parasitoid species/strains. *Eretmocerus mundus* proved to be the most efficient, while the other species showed only medium performance.

INTRODUCTION

The silverleaf whitefly (*Bemisia argentifolii*) (Homoptera: Aleyrodidae), also referred to as the B-biotype of *B. tabaci*, is a severe pest world-wide, causing damage especially on ornamentals like poinsettia. The chemical control of this pest has not been successful for several reasons. Growers have very low tolerances for whitefly nymphs, adults and honeydew on ornamental plants, therefore a more effective solution is needed. Since the mid 1960's, the number and scope of studies on the natural enemies of *B. tabaci* have greatly increased (Greathead & Bennett, 1981). Most of the research has been conducted with parasitoids belonging predominantly to the genera *Encarsia*, *Eretmocerus* and *Amitus* (Barro, 1995). The host-searching efficiency of natural enemies is an important parameter in the evaluation of their potential for biological control of insect pests (van Roermund *et al.*,1997). Because host-densities are typically low under a successful biological control program, the probability of encountering hosts for parasitization is extremely important. Species with high fecundity but inefficient host-searching behaviour may never encounter hosts to deposit their eggs. This could be compensated by releases of large numbers of parasitoids, which increases the probability that at least some parasitoids will encounter the pest insects, but this is expensive. It is therefore economically more efficient to select for parasitoids with good searching capacities (Drost *et al.*, 2000). The whitefly-working group of the Department of Entomology of Wageningen University selected five parasitoid species and strains for this long-term project. The first choice was *Encarsia formosa*, which is an obvious choice because of its success against *T. vaporariorum*. However previous data showed that the commercially reared *Encarsia formosa* (NL) was not effective as a biological control agent of *B. argentifolii* (Boiselair *et al.*, 1990), and this strain was compared to the *E. formosa* strain from Beltsville (MD,USA) that has been reared for many years on *B. argentifolii*. Many species recovered from *B. argentifolii* belong to the genus *Eretmocerus*. From this genus, the species *Er. eremicus* was selected because it was already studied by USA researchers and it is commercially available. The species *Er. mundus* has been selected because it is very abundant in southern Spain and Italy, parasitizing *B. tabaci*. Finally, the species *Amitus hesperidum* has been selected because there is no literature on this species, although it is recovered in many samples in Central America and it is uniparental (Drost *et al.*, 2000).

MATERIALS AND METHODS

Origin and rearing procedures of insect materials

The whitefly originated from a population that entered the Netherlands on poinsettia cuttings from California in 1989. Laboratory colonies were maintained by infesting poinsettia plants with whitefly adults. *Encarsia formosa* (NL) probably originates from a population discovered in England in 1926. Parasitoids were delivered every week as black pupae on paper cards. *Encarsia formosa* (MD), the Beltsville strain was developed from wasps initially found attacking *Trialeurodes vaporariorum,* but subsequently reared on *Bemisia argentifolii* with poinsettia as the host plant at USDA-ARS laboratory Beltsville, Maryland (Bentz, 1993). A starting colony of *Amitus bennetti* was obtained from a population on *Bemisia argentifolii*, on cotton, kept at the University of California, Riverside, USA, by T.S. Bellows and B. Orr. *Eretmocerus eremicus* parasitoids were obtained from the same company as *E. formosa* (NL). The species was reared on *Trialeurodes vaporariorum* on tobacco and originated from Arizona. Pupae were delivered every week in bottles with fine vermiculite (Drost *et al.*, 2000). *Eretmocerus mundus* was taken from a stock originally obtained from a commercial company in Italy, that reared the parasitoids on *Bemisia tabaci* (unknown biotype), on courgette. Colonies of *E. formosa* (MD) and *E. mundus* were established using poinsettia plants with third and/or fourth instar nymphs of *B. argentifolii*; for *A. bennetti* first and/or second instar nymphs were used. Parasitized pupae were left to emerge in sleeve cages containing a poinsettia plant with whitefly nymphs. Parasitized pupae of *E. formosa* (MD) and *E. mundus* were collected after 18 days and those of *A. bennetti* were collected after 28 days and kept in a glass Petri-dish or vial with a drop of honey until emergence (Drost *et al.*, 2000).

Experimental set-up

Poinsettia (*Euphorbia pulcherrima* cv. Goldfinger) plants were used as host plants. Every day 2-3 leaves were infested with *B. argentifolii* adults, by using small clip-cages. The infested plants were kept in a separate cage without whitefly adults in a greenhouse compartment. In this way there was a continuous supply of leaves with desired nymphal stages to be used for experiments: 10-11 day old pupae of *B. argentifolii* for *Amitus*, 12-13 day old pupae for *Eretmocerus* species and 13-15 day old pupae for *Encarsia* strains. Before observation the leaf with the desired nymphal stage was removed from the plant and kept in small plastic container with water. Only four larvae were left on the underside of the leaf, the others were removed to assure low host density. The observation was carried out in a climate room. Five healthy poinsettia plants were used to imitate the light conditions of a crop, providing the parasitoid with ample opportunity to hop or fly to another leaf (Roermund & van Lenteren, 1995). Parasitoid pupae were left to emerge in glass petri-dishes or vials in the presence of honey; female parasitoids of 1-2 days of age for *Encarsia* strains and up to 1 day of age for *Amitus* and *Eretmocerus* species. At the time of the experiment they had had no previous experience in oviposition and all parasitoids were used once in an experiment (Drost *et al.*, 2000).

The experiment started when a female parasitoid was released on the upper side of the leaf and started walking (Roermund & van Lenteren, 1995). The parasitoid was observed through a stereo microscope.

To analyse the foraging behaviour of parasitoids, all behavioural elements and positions on the leaf were recorded and analysed using the Observer 3.0 (Windows version) of Noldus Technology (Wageningen, the Netherlands).

RESULTS

Residence time

The total residence time (time spent on the leaf) was the highest with *Amitus bennetti*, less with *Encarsia formosa* Beltsville strain and *Eretmocerus mundus*, and the lowest with *Encarsia formosa* (NL) and *Eretmocerus eremicus*. When the parasitoids did not discover any of the hosts, the residence time was much lower than in the case of encounters. When hosts were encountered the residence time increased 1.6-1.8 fold, except with *Er. eremicus* (2.7 fold) (Figure 1.).

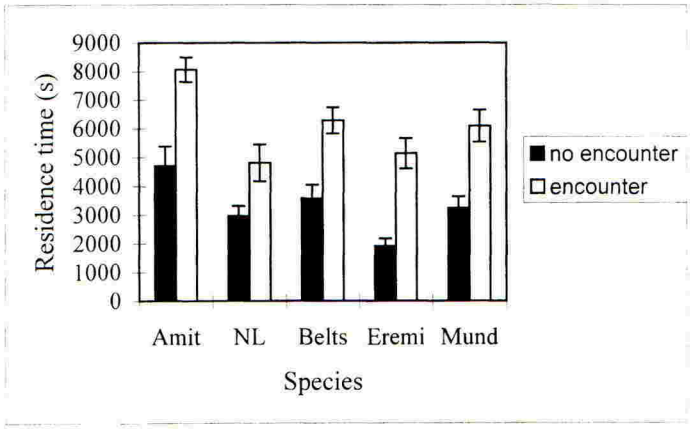

Figure 1. Residence time (sec) with and without encounters. Error bars indicate standard errors.

These long residence times were not caused by parasitoids spending more time sitting still or preening or handling hosts. They spent most of the time on the leaf surface without being in contact with hosts. Host handling time took only 1-16% of the total residence time. The host handling time was the highest for the *E. formosa* strains (11 and 16% respectively) and was much less with the other species (1-4%). The searching activity, walking while drumming and drumming as a percentage of the total time spent on the leaf, excluding host handling time, was above 60% for all the five species. They were searching for hosts most of the time.

Encounters and ovipositions

The average number of encounters and ovipositions in hosts were rather low, which can be explained by the release method; the first method when parasitoids were released on the upper leaf side was not successful at all, so later parasitoids were released on the under side of the leaf. In the case of the first method, parasitoids spent more than half of their residence time on the upper leaf side, so the probability of encountering a host was much lower than in the case of the second method. The difference in results between the two methods is obvious (Figure 2.) especially with *E. formosa* (NL); in this case the total number of encounters was 14.6 times higher with method 2 than with method 1. For the other species the measure of increase was only 1.6-3 fold and with *Er. eremicus* a decrease can be seen in the number of encounters with method 2. *Er. mundus* was the only species which had a relatively high number of

encounters even with method 1. This number was as high as the number of *E. formosa* (NL) with method 2.

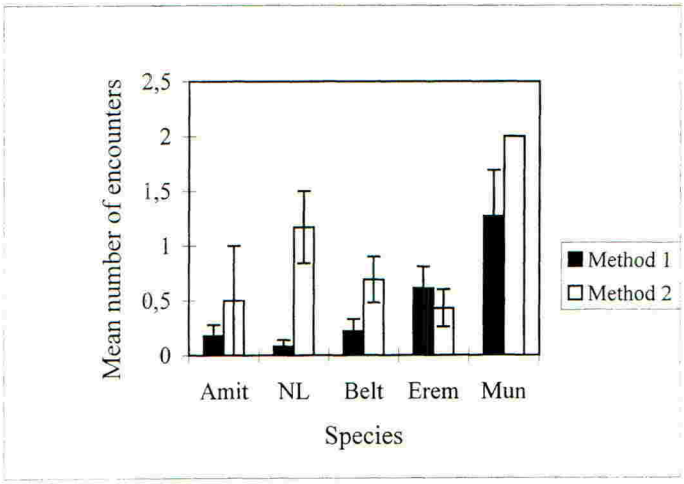

Figure 2. Difference in number of encounters when releasing on the upper leaf side (method 1) or on the under leaf side (method 2). Error bars indicate standard error.

During the experiment not all of the encounters ended with oviposition; sometimes the parasitoid examined an already parasitized host and rejected it, or just ignored it and walked over it. To give a measure of how big this difference is one should use the success ratio, which parameter has a significant impact on the reduction of the pest organism (Roermund, 1995). The success ratio gives the ratio between the number of ovipositions and the number of encounters; thus how many encounters end with oviposition. The results in Table 1. show a 100% success ratio for *A. bennetti*, which means that every time the female found a host, it oviposited in it. *E. formosa* Beltsville strain, *Er. eremicus* and *Er. mundus* showed almost the same ratio, which was between 75 and 79%. Only *E. formosa* (NL) showed a ratio under 60%. Although the success ratio is almost the same for the three species mentioned above, there is a large difference between them in number of encounters and ovipositions. *Er. mundus* had the highest number of ovipositions and had many more encounters than the other four species; the number of encounters was almost double that of the other species.

The number of encounters has a great impact on the time allocation; encounters and especially ovipositions arrested the parasitoid on the leaf, increasing the total residence time. In the case of *E. formosa* with the greenhouse whitefly it has been proven that each encounter with a host leads to an increase in total residence time. In our case a great influence of the number of ovipositions on the time allocation was also observed. All the species except *E. formosa* (NL) show an increase in residence time after each oviposition (Figure 3). The rate of increase is different for all of the species. For *A. bennetti* to find the first host takes much more time than for the rest of the parasitoids. With *E. formosa* (NL) there is a big fluctuation; no clear effect of the ovipositions can be seen.

Table 1. Total number of encounters and ovipositions of the 5 parasitoids and their success ratio. Standard error and number of replicates are given between brackets.

Parasitoid species	*Amitus bennetti*	*Encarsia formosa (NL)*	*Encarsia formosa(MD)*	*Eretmocerus eremicus*	*Eretmocerus mundus*
Encounters with hosts	0.16 a (0.09;19)	0.45 a (0.15;42)	0.38 a (0.11;40)	0.35 a (0.10;37)	0.93 a (0.30;28)
Ovipositions in hosts	0.16 a (0.09;19)	0.26 a (0.08;42)	0.30 a (0.08;40)	0.27 a (0.07;37)	0.68 a (0.21;28
Success ratio(%)	100	58	79	77	73

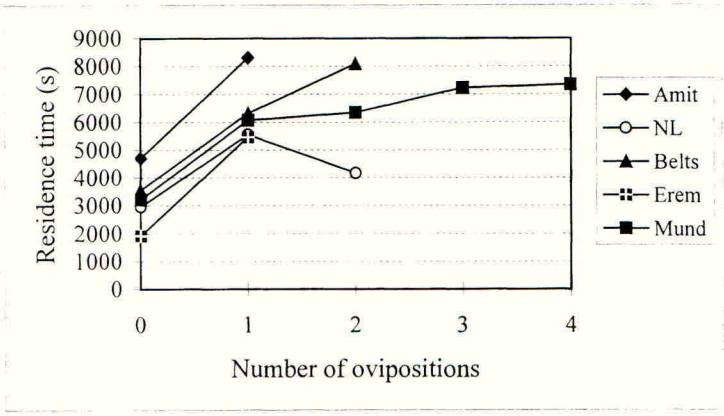

Figure 3. Influence of the number of ovipositions on the time allocation

Position on the leaf

Parasitoids change from one leaf side to another while searching. When released onto the upper leaf side, parasitoids spent more than the half of their residence time on the upper side of the leaf, except *Er. eremicus* which spent only a third of its residence time on the upper side.

When released onto the under side of the leaf, parasitoids spent more than 80% of the residence time on this side, where hosts were present. This percentage is big especially with the two *Eretmocerus* species, which spent 93-95% of the residence time on the under side of the leaf.

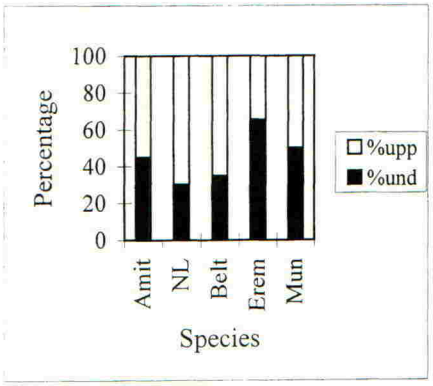

Figure 4. Division of residence time between upper and under leaf side with method 1.

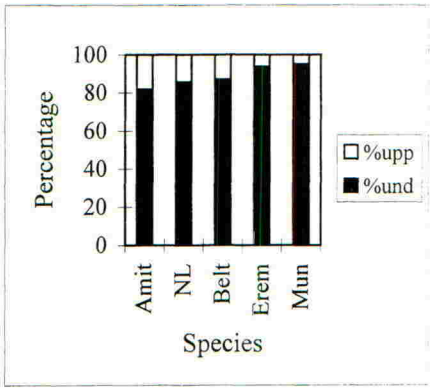

Figure 5. Division of residence time between upper and under leaf side with method 2.

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Field evaluation of genetically modified *Helicoverpa armigera* nucleopolyhedrovirus in cotton bollworm control

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ABSTRACT

Cotton is host to several species of Heliothine moths, whose larvae can cause devastating crop losses and trigger therefore considerable investments in crop protection in the form of crop breeding (Bt-based molecular resistance), frequent pesticide sprays, or the application of biologicals. A host-specific viral pathogen, *Helicoverpa armigera* nucleopolyhedrovirus (HaSNPV), has been developed as a commercial biopesticide to control Heliothine pests in China. To improve its insecticidal properties, HaSNPV has been genetically modified by deletion of the ecdysteroid UDP-glucosyltransferase (*egt*) gene from its genome and insertion of an insect-selective scorpion toxin (AaIT) gene at the *egt* gene site. In 2000 and 2001, the efficacy of these HaSNPV recombinants to control bollworm was evaluated in cotton. The results indicated that the *egt* deletion recombinant of HaSNPV (HaCXW1) did not show significant improvement compared to wild-type viruses. On the other hand, the AaIT-recombinant, HaWHL4a, gave substantially improved control, as shown by a lower number of surviving larvae and percentage of damaged squares, flowers and bolls in the plots treated with this recombinant. When cotton was sprayed with HaSNPV mutants or a chemical insecticide standard to control natural infestation of bollworm over a whole cotton growing season, the yield of cotton lint in plots treated with HaWHL4a was significantly higher than that in the plots treated with wild-type HaSNPV. These results indicate that modification of HaSNPV by expressing an insect-specific toxin can significantly improve the control of the cotton bollworm, and can be useful in the future to control this pest in Chinese cotton.

INTRODUCTION

Cotton is one of the most important cash crops in China, and plays a significant role in economic and societal developments. The Chinese textile industry needs stable and sustainable production of cotton in a changing world. In the past 15 years, an average of 5.6 million ha of cotton were grown annually in China, yielding an annual production of 4.5 million tons of cotton.

Heliothine bollworms (*Helicoverpa armigera* and *H. zea etc.*) are amongst the most serious pests around the world (Fitt, 1989). As one of the key pests on important crops in China, cotton bollworm (*H. armigera*) is widely distributed in the major cotton-producing regions. Control of *H. armigera* on cotton in South China has depended almost exclusively on chemical pesticides such as Esfenvalerate, Endosulfan and Pyrethroids. In North China, transgenic cotton incorporating the toxin genes from *Bacillus thuringiensis* has been introduced on a substantial scale (~ 0.5 million ha in 2000). However, *H. armigera* has a strong ability to develop resistance to chemical insecticides (McCaffery & Walker, 1991) as well as to the toxins present in the genetically modified cotton varieties (Liu *et al.*, 1999; Shelton *et al.*, 2000). Therefore there are increasing and urgent demands for Integrated Pest Management (IPM) or biocontrol alternatives.

HaSNPV has been registered as a biological pesticide in China since 1993. In the past few years, HaSNPV has been used on approximately 100,000 ha annually to control Heliothines, including *H. armigera, H. assulta*, and *H. zea,* on cotton, tobacco, and hot pepper (Zhang, 1989; Zhang, 1994) and demand is much larger than the supply of the virus. However, like other baculoviruses, HaSNPV suffers the disadvantage of having a relatively slow speed of action as compared to chemical insecticides and a low virulence for older instar larvae. These disadvantages limit the use of HaSNPV on an even wider scale.

In order to compete with chemical insecticides, approaches are being sought to improve the efficacy of HaSNPV by genetic engineering. Several recombinant HaSNPV mutants have been generated. One recombinant is an ecdysteroid UDP-glucosyltransferase gene (*egt*) deletion mutant (HaCXW1), which lacks the coding sequence of *egt* and contains instead a GFP (Green Fluorescent Protein) gene under the control of the HaSNPV polyhedrin promoter as a marker (Chen *et al.*, 2000). The other recombinant HaWHL4a is based on HaCXW1 and contains an insect-selective scorpion toxin (AaIT) gene under control of a chimaeric HaSNPV polyhedrin / p6.9 promoter (Sun *et al.* 2001). In laboratory bioassay, the LD_{50} (median lethal dosage) values of these recombinants were similar to those of wild-type HaSNPV, when tested on the 1^{st}-5^{th} instar larvae of *H. armigera*. The LT_{50} (median lethal time) and FT_{50} (median time of feeding cessation) values of the recombinants were significantly shorter than those of wild-type HaSNPV (Sun *et al.*, unpublished data).

In 2000 and 2001, the control efficacy of these HaSNPV recombinants was evaluated in cotton with approval from the Safety Administration Office for Agricultural Biological Genetic Engineering, the People's Republic of China.

MATERIALS AND METHODS:

Materials: Two HaSNPV recombinants (HaCXW1 and HaWHL4a) were compared in cotton field with wild-type HaSNPV, 'Kungfu' (2.5% ι -Cyhalothrin EC (w/v)) (Zeneca Agrochemicals) or *Bacillus thuringiensis* subsp. *Kurstaki* wettable powder (32,000 IU/mg) (Keluo Bio-insecticide Corporation, Hubei, PR China). Virus stocks were formulated as suspensions that contained 2×10^9 PIBs/ml with water, emulsifier (5%, v/v) and glycerine (20%, v/v).

Field trial 1 (Anyang, Henan, 2000): A field experiment was conducted on 'Zhongmiansuo #35' cotton grown near China Cotton Research Institute, Anyang, Henan. HaSNPV-wt, HaCXW1, HaWHL4a at 2.4×10^{12} PIB/ha and 2.5% ι -Cyhalothrin EC at 2,252 ml/ha were compared for efficacy against a natural infestation of *H. armigera*. Formulated materials were diluted in tap water. Treatments were applied to plots (7 rows by 8 m) of cotton which was planted on April 24, 2000 with 80 cm row spacing. Treatments and non-treated controls were replicated 4 times in a randomised complete block design. Applications were performed on 20 and 25 August, 2000 with the appropriate dose of 1125 liter/ha using a backpack sprayer with a 0.5 mm diameter nozzle at 3×10^5 Pascals. In each plot, a total of 25 plants were labelled. Labelled plants were only in the centre 3 rows of the plots and not in the terminal 2 m of each row. On 25, 28 and 30 August, the number of surviving larvae was inspected on labelled plants. On 30 August, the number of squares, flowers and bolls on labelled plants which were damaged due to feeding by bollworm was determined as well. Meanwhile the number of undamaged squares, flowers and bolls was also counted.

Field trial 2 (Qianjiang, Hubei, 2000): A field experiment was conducted on 'Ekangmian #5' cotton grown in Qianjiang County, Hubei. The recombinant HaWHL4a at 2.1×10^{12}, 1.5×10^{12} and 9.0×10^{11} PIB/ha, the wild-type HaSNPV at 2.1×10^{12} PIB/ha and *B. thuringiensis* WP at 4.8×10^{10} IU/ha were compared for efficacy against natural infestation of *H. armigera*. Treatments were applied to plots (50 m^2) of cotton which was planted on 8 April with 80 cm row spacing. Treatments and non-treated controls were replicated 4 times in a randomised complete block design. Applications were performed on 8 August with the appropriate dose of 1125 liter/ha using a backpack sprayer with a 0.5 mm diameter nozzle at 3×10^5 Pascals. In each plot, 25 plants were labelled. Labelled plants were only in the centre 3 rows of the plots and not in the terminal 2 m of each row. On 8, 11 and 15 August, the number of surviving larvae was inspected on labelled plants in each plot. On 15 August, the number of squares, flowers and bolls on labelled plants which were damaged due to feeding by larvae was determined as well. Meanwhile the number of undamaged squares, flowers and bolls was also counted.

Field trial 3 (Anyang, Henan, 2001): A field experiment was conducted on 'Zhongmiansuo #38' cotton grown near the China Cotton Research Institute, Anyang, Henan. HaSNPV-wt, HaCXW1, HaWHL4a at 2.4×10^{12} PIB/ha and 2.5% ι -Cyhalothrin EC at 2,252 ml/ha were compared for efficacy against two generations of natural infestation of *H. armigera*. Treatments were applied to plots (11 rows by 8 m) of cotton which was planted on 28 April,

2001 with 80 cm row spacing. Treatments and non-treated controls were replicated 4 times in a randomised complete block design. Plots were sprayed on 26 and 30 June, 3, 16 and 19 July with the appropriate dose of 512.5 ~1125 liter/ha (depending on the size of cotton plants) using a backpack sprayer with a 0.5 mm diameter nozzle at 3×10^5 Pascals. In each plot, 25 plants were labelled. Labelled plants were only in the centre 5 rows of the plots and not in the terminal 2 m of each row. The number of surviving larvae and squares, flowers and bolls which were damaged due to feeding by bollworm was inspected at 3~4 day intervals. Cotton lint from 150 plants in the centre of each plot was collected on 15 September and 10 October, 2001.

Statistic Analysis: Significant differences among treatments in the number of surviving larvae, percentage of damaged squares, flowers and bolls and the yield of cotton lint (cotton lint collected on 15 September and 10 October was pooled) were determined by ANOVA. Treatment means were separated by Duncan's multiple range test (SPSS, v10.0, 1999). Percentile data were arcsine-transformed for statistical analysis.

RESULTS AND DISCUSSION:

In experiment 1, statistically significant effects of recombinant viruses on the number of surviving larvae were absent, except at 10 days after the initial spray (30 August) (Table 3). Meanwhile the percentage of damaged squares, flowers and bolls in the plots treated with HaWHL4a observed on 30 August were significantly lower than those in plots that had been treated with the wild-type virus. The data also showed that HaWHL4a provided control of bollworm *H. armigera* equivalent to the chemical standard, 2.5% ι -Cyhalothrin EC. The *egt*-minus recombinant (HaCXW1) did not produce better control than the wild-type in this experiment.

Table 1: Control of bollworm *H. armigera* in Experiment 1 (Anyang, Henan, 2000)

Treatment	Dosage per ha	Surviving larvae / 25 plants			% damaged squares, flowers and bolls on 30 August
		25 August*	28 August	30 August	
HaSNPV-wt	2.4×10^{12} PIB	15.8 a	13.5 bc	17.5 b	10.9 b
HaCXW1	2.4×10^{12} PIB	30.0 b	17.3 c	21.0 b	15.1 bc
HaWHL4a	2.4×10^{12} PIB	8.8 a	7.8 ab	3.8 a	4.1 a
2.5% ι -Cyhalothrin EC	2,252 ml	3.5 a	2.5 a	3.3 a	4.7 a
Control	-	34.5 b	25.8 d	22.3 b	18.4 c

*Applications were made on 20 and 25 August.

In Experiment 2, at 3 days after spray, the number of surviving larvae in the plots treated with HaWHL4a started to be significantly lower than those in the wild-type virus and Bt-WP treated plots (Table 2). At 7 days after spay, the percentage of damaged squares, flowers and bolls in the plots treated with HaWHL4a was significantly lower than those in the wild-type

virus and Bt-WP treated plots. Thus, in this experiment, the control provided by the AaIT-recombinant virus was superior to that provided by the wild-type virus or *Bacillus thuringiensis*.

Table 2: Control of bollworm *H. armigera* in Experiment 2 (Qianjiang, Hubei, 2000)

Treatment	Dosage per ha	Surviving larvae / 25 plants			% damaged squares, flowers and bolls on 15 August
		8 August*	11 August	15 August	
HaSNPV-wt	2.1×10^{12} PIB	20.8 a	13.8 b	9.8 c	11.2 b
HaWHL4a	2.1×10^{12} PIB	19.0 a	4.8 a	2.8 a	7.6 a
HaWHL4a	1.5×10^{12} PIB	22.5 a	12.0 b	6.8 b	12.5 bc
HaWHL4a	9.0×10^{11} PIB	19.3 a	11.8 b	8.3 bc	14.3 c
B. thuringiensis WP	4.8×10^{10} IU	19.0 a	12.3 b	8.0 bc	10.7 b
Control	-	20.8 a	19.5 c	18.5 d	17.5 d

*Applications were made on 8 August.

In Experiment 3, over the period of the experiment, the number of surviving larvae and damaged squares, flowers and bolls in the plots treated with HaWHL4a was lower, but not significantly different from those in the wild-type virus and 2.5% ι -Cyhalothrin EC treated plots with the exception of one observation (19 July) (Data not shown). The final yield of cotton lint in the plots treated with HaWHL4a was significantly higher than that in the wild-type virus and HaCXW1 treated plots, but not significantly different from the yield in 2.5% ι -Cyhalothrin EC treated plots (Table 3).

Table 3: Control of *H. armigera* in Experiment 3 (Anyang, Henan, 2001)

Treatment	Dosage per ha	Yield of cotton lint (kg/ha)
HaSNPV wt	2.4×10^{12} PIB	1023± 34 b
HaCXW1	2.4×10^{12} PIB	951± 37 b
HaWHL4a	2.4×10^{12} PIB	1250± 68 a
2.5% ι -Cyhalothrin EC	2,252 ml	1084± 49 ab
Control	-	931±109 b

Laboratory bioassays indicated that the *egt* deletion mutant of HaSNPV (HaCXW1) killed their *H. armigera* hosts significantly faster than HaSNPV-wt (Chen *et al.*, 2000; Sun *et al.*, 2001). However, in these field trials, HaCXW1 did not provide better control of the host larvae than the wild-type virus. A similar lack of response of control efficacy to deletion of the *egt* gene was found for *Autographa californica* multicapsid NPV (AcMNPV) (Treacy *et al.*, 1997). In our field release, the insertion of the AaIT gene into the *egt* gene locus clearly improved the insecticidal properties of HaSNPV on cotton. This finding confirms studies by Cory *et al.* (1994) and Gard (1997), who demonstrated improved field efficacy of AcMNPV-AaIT recombinants as compared to the wild-type virus on cabbage and cotton.

CONCLUSION:

The results from these field experiments indicate that modification of HaSNPV by expressing an insect-specific toxin can significantly improve the control efficacy of the cotton bollworm and can be useful in controlling this pest in Chinese cotton in the future.

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The role of parasitoids in decreasing the number of Diamond Back Moth (*Plutella xylostella*) in horticultural crops

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ABSTRACT

Two years of observations on the occurrence of *P. xylostella* as well as on the composition and the effectiveness of larva and pupa parasitoids were carried out in Mydlniki near Krakow, Poland. In 2000 the number of *P. xylostella* larvae was three times higher than in 2001. In the first year, 10 species and in the next year 7 species of parasitoids were found with *Diadegma fenestralis* Holmgr as the most numerous. In both years the parasitisation of larvae and pupae was very high, reaching 80%.

INTRODUCTION

Plutella xylostella L. is one of the most important pests of cabbage crops in Poland and all over the world. Its quick development rate as well as growing resistance to pesticides and biological agents based on *Bacillus thuringensis* are the reasons why commonly applied protective methods often fail (Free, *et al.*, 1991; Shelton, *et al.*, 1993). The use of natural enemies of *Plutella xylostella* might become the effective alternative methods of pest control in the near future.

Among numerous parasitoids connected with *P. xylostella* larvae and pupae the most important are ichneumonid wasps belonging to the genus *Diadegma* (Pimental, 1961; Rahn & Chevallerau, 1996). Other parasitoids, mostly Braconidae and Eulophidae are also mentioned as effective in decreasing the number of *P. xylostella* larvae (Tobias, 1971; Mushtaque & Mohyuddin, 1987; Telekar, 1996).

MATERIALS AND METHODS

Observations of the occurrence, development and parasitoids of *P. xylostella* were carried out in the Plant Protection Experimental Station in Mydlniki near Krakow (southern Poland) in 2000-2001. *P. xylostella* larvae and pupae were collected from three plots with early cabbage cv. Drago, late cabbage cv. Balaton (both as monocrop) and late cabbage cv. Balaton undersown with white clover (local cultivar). No chemical treatments had been applied on the plots.

After the first appearance of larvae on the plants, the analysis of 100 randomly selected plants was carried out every week, from each plot. Pupae and larvae (in the last developmental stage) were picked from the plants and placed in the rearing chamber (18^0C, 85% r.h., 16

hours daylength). The emerged parasitoids were collected every day, the species were identified, and dominance (in %) calculated.

RESULTS

In the middle of May, 2000 the first larvae of *P. xylostella* had already been observed on early cabbage and the pupae of this moth were being collected until the middle of September (Figure 1). In 2001 the first appearance of larvae on the plants took place as late as in the middle of June whereas the last effective analysis was carried out at the beginning of September (Figure 2). The occurrence of *P. xylostella* larvae on plants was about 6 weeks longer in 2000 in comparison with the following year, and the number of larvae and pupae collected was three times higher (Table 1 and 2).

Table 1. *Plutella xylostella* moths and parasitoids reared out from collected larvae and pupae (2000)

Number of specimens	Date					total
	9-30.05	6-27.06	4-25.07	1-29.08	5-27.09	
Plutella xylostella	31	29	48	37	28	142
Diadegma fenestralis	-	150	686	195	138	1200
Mesochorus sp.	-	4	17	-	-	21
Diadromus collaris	-	-	-	-	1	1
Hemiteles sp.	-	-	1	1	-	2
Gelis nigricornis	-	-	1	1	-	2
Cotesia fuliginosus	-	3	10	1	-	14
Cotesia longipalpis	-	-	1	-	-	1
Cotesia sp.	-	-	3	-	1	4
Habrocytus sp.	-	-	31	8	-	39
Eupteromalus sp.	-	-	5	1	-	6
Dead	2	15	105	46	14	182
Total	33	201	907	290	182	1614

From the end of June until the middle of July 2000 and in the middle of July 2001 the number of *P. xylostella* larvae was higher than the economic threshold of 6 larvae/10 plants (Kempczynski, 1983). The great number of larvae as well as the lack of control did not cause the loss of cabbage yield. Neither did we observe the multiheaded plants which often appear after heavy attack by *P. xylostella* larvae.

Table 2. *Plutella xylostella* moths and parasitoids reared out from collected larvae and pupae (2001)

Number of specimens	Date 11-25.06	2-30.07	6-27.08	3.09	Total
Plutella xylostella	8	41	1	3	55
Diadegma fenestralis	30	372	22	2	426
Diadegma semiclausum	-	1	-	-	1
Diadromus collaris	-	2	-	-	2
Habrocytus sp.	-	1	1	-	2
Cotesia fuliginosus	-	5	-	-	5
Cotesia longipalpis	1	5	-	-	6
Cotesia sp.	-	1	-	-	1
Mesochorus sp.	-	3	-	-	3
Dead	2	52	4	-	58
Total	41	485	28	5	559

In both years the parasitisation of *P. xylostella* larvae approached 80% - regardless of the crop. (Table 3). In 2000 the first appearance of *P. xylostella* was estimated to be as early as the beginning of May, and the first pupae were collected in June. From that moment until the end of the season parasitisation was at a similarly high level from 71-83%.

Table 3. The results of rearing *Plutella xylostella* larvae and pupae (2000-2001)

		Year 2000		2001	
		No.	%	No.	%
Early cabbage	Moths	45	6,8	22	10,3
	Parasitoids	519	78,6	172	80,4
	Dead	96	14,6	20	9,3
	Total	660	100	214	100
Late cabbage with white clover	Moths	61	11	12	10,6
	Parasitoids	440	79,6	92	81,4
	Dead	52	9,4	9	8
	Total	533	100	113	100
Late cabbage in monoculture	Moths	35	8,8	22	9,5
	Parasitoids	322	81,1	182	78,4
	Dead	40	10,1	28	12,1
	Total	397	100	232	100

In the following year the first generation of the pest was observed in June. 75% of the population was parasitised. In 2000, on the whole, about 10 species of parasitic wasps belonging to the families *Ichneumonidae* (5 species), *Braconidae* (3 species) and *Pteromalidae* (2 species) were observed. In the following year three of the above mentioned species did not appear (Table 4).

Table 4. The composition of *Plutella xylostella* parasitoids

Species		Year 2000		2001	
		No.	%	No.	%
Diadegma fenestralis	*Ichneumonidae*	1200	93	426	95,5
Diadegma semiclausum		0	0	1	0,2
Mesochorus sp.		21	1,6	3	0,7
Diadromus collaris		1	0,05	2	0,5
Hemiteles sp.		2	0,2	0	0
Gelis nigricornis		2	0,2	0	0
Cotesia fuliginosus	*Braconidae*	14	1,1	5	1,1
Cotesia longipalpis		1	0,05	6	1,3
Cotesia sp.		4	0,3	1	0,2
Habrocytus sp.	*Pteromalidae*	39	3	2	0,5
Eupteromalus sp.		6	0,5	0	0
Total		1290	100	446	100

Both in 2000 and in 2001 the dominant species among the *Plutella* parasitoids turned out to be *Diadegma fenestralis* Holmgr. - as it constituted about 90% of all of the wasps reared. The species such as *Mesochorus* sp., *Hemiteles* sp., *Gelis nigricornis*, *Habrocytus* sp. and *Eupteromalus* sp. belonging to parasitoids of the second degree, as well as braconids belonging to the genus *Cotesia* were less numerous, totalling about 10% of the population.

Growing resistance of *Plutella xylostella* to synthetic pesticides (Liu et al., 1981; Tabashnik, 1986) stimulated this research on parasitoid composition and effectiveness in the hope of finding an alternative method of pest control. In Poland the first research on *Plutella* parasitoids was carried out by Łagowska in 1981. Also observations of the dominant parasitoid species - *Diadegma fenestralis* were made by Wiech and Jankowska (1999). This ichneumonid wasp was carried from Europe to India where it became the natural enemy of *P. xylostella* (Devi and Raj 1995; Usha et al. 1997). In Poland the two other species which are used as the means of biological pest control were also found. These are *Diadegma semiclausum* and *Diadromus collaris*.

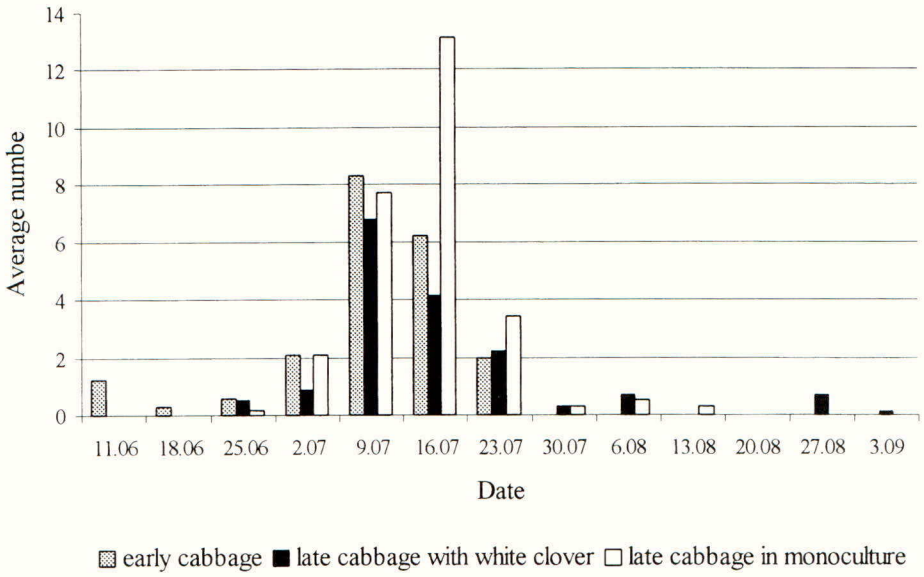

Figure1. The number of *Plutella xylostella* larvae (mean on 10 plants) (2000)

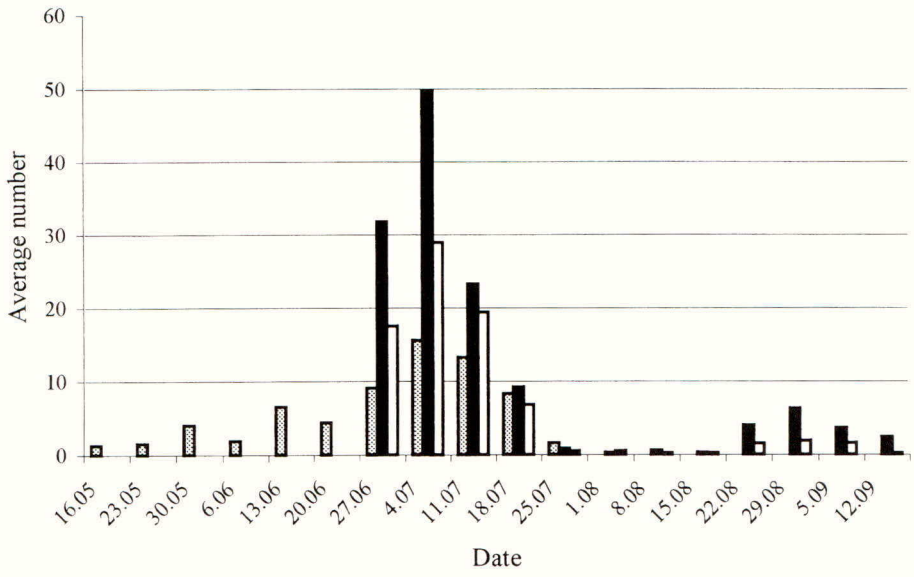

Figure 2. The number of *Plutella xylostella* larvae (mean on 10 plants) (2001)

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Development of a biopesticide for the coconut mite in India

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ABSTRACT

The eriophyid mite, *Aceria guerreronis*, which has been known to be present in the Americas, Western Africa and the Asia Pacific for a long time, appeared on an extensive and unprecedented magnitude on coconuts in India for the first time in the southern state of Kerala in the late 1990s. Within three years it turned out to be a major outbreak in south India including the Lakshadweep Islands. The losses range from 30-60% in terms of yield. The mite has been spreading towards the north, and the neighbouring coconut-growing countries are also likely to be affected by the pest. The originally recommended chemicals, such as monocrotophos and dicofol, did not find favour with the growers because of poor performance. Because of the environmental hazards associated with pesticide use studies were commenced on the indigenous natural enemies of the mite for the possible exploitation of these as biocontrols. *Hirsutella thompsonii,* a proven fungal parasite of eriophyid mites, has been found throughout south India, affording natural control of the pest. Investigations through laboratory bioassays and limited field experiments on the potential of the fungus resulted in the development of the first Indian mycoacaricide based exclusively on *H. thompsonii.* The powder formulation is based on the strain MF(Ag)5 [IMI 385470], the best performer among 15 monoconidial isolates, with a potency of 2.5 x 10^8 CFU/g. A schedule of 3 applications of the product at 1% concentration with 15-day intervals can give a mean mortality of over 80%. The fungus could get itself established in a particular coconut garden within 20 days offering sustained control of the pest. This paper is intended to relate the havoc that is being caused by the coconut mite in India and to present the data on the natural abundance of *H. thompsonii* in association with the mite, its isolation, characterization, laboratory evaluation and the subsequent development of a biopesticide based on the fungus for field use.

INTRODUCTION

Aceria guerreronis, a nut-infesting eriophyid mite, has been reported to be present in many coconut-growing regions of the Americas, Africa and the Asia Pacific (Howard *et al.*, 2001). The South Asian countries, India and Sri Lanka, have been suffering from this menace since 1998. The sudden emergence of the mite as a serious pest of coconuts coupled with a steep fall in the prices of the coconut and its products in India has left the coconut farmers with few options.

A. guerreronis has moved at a very rapid pace over the last four years from Ernakulam district of Kerala, where the pest was noticed on a large-scale for the first time in India (Sathiamma *et al.*, 1998), to all the major coconut-growing districts of Tamil Nadu (TN), Pondicherry, Karnataka, Andhra Pradesh (AP), Goa and Maharashtra in peninsular India. Apart from the mainland it has also been serious in the Lakshadweep Islands (LI). Coconuts in the coastal states of Maharashtra and Gujarat in the west and Orissa and West Bengal in the east as well as in the Andaman & Nicobar Islands (ANI) are also reported to be suffering from the mite. There is a significant threat to the other coconut-growing countries in Asia and Oceania, too (Moore, 2000). The sudden emergence of the mite as the number one pest of coconuts, causing losses between 30-60% in yield, led to the unprecedented and unusual use of plant protection chemicals in the coconut ecosystem. This is in contrast to the large-scale and successful use of biological means to control the established major pests, especially the black-headed caterpillar (*Opisina arenosella*).

The recommendations have varied from state to state over the past few years. Systemic and contact insecticides/acaricides, particularly monocrotophos, dicofol, triazophos and carbosulfan are extensively applied as a spray or applied to the palms by feeding the roots or by injecting through the trunk. Azadirachtin and a neem oil-garlic mixture are also popularly used. Micronized elemental sulphur, a popular acaricide/fungicide in the form of a WP formulation, is also in widespread use, but because of its toxicity to *H. thompsonii* (Kumar & Singh, 2002) its use is discouraged.

In spite of the association of a number of acaropathogenic fungal genera with the coconut mite in south India, *Hirsutella thompsonii* is the best natural regulator of the pest (Kumar & Singh, 2000). Chandler *et al.* (2000) have also highlighted the potential of *H. thompsonii* as a mycoacaricide against the coconut mite and other eriophyids. We therefore began a programme of studies at the Project Directorate of Biological Control (PDBC) to develop a biopesticide based on *H. thompsonii*.

NATURAL ABUNDANCE OF *H. THOMPSONII*

Surveys were undertaken in Kerala, Karnataka, TN, AP, Pondicherry and LI during 1999-2001. The method described by Kumar *et al.* (2001) was followed to find out the incidence of *H. thompsonii* in each location. Sampling was done only from the coconut trees that had not received any pesticide treatment before. Samples were processed within 48 hours of collection. As in the case of other eriophyids, bacterial, viral or protozoan pathogens have not been encountered so far. Fungal pathogens, mostly mitosporic fungi, were frequently noticed in alliance with the mite either as pathogens or as saprophytes. None of the pathogens appeared to be as potent as *H. thompsonii* in terms of severity of infection. The mite-specific fungus *H. thompsonii* is an extensively studied mycoacaricide candidate and is a pathogen of both eriophyids and tetranychids (Chandler *et al.*, 2000).

On site observations of both fallen and harvested nuts had indicated the association of *H. thompsonii* with the mite in 10-25% of the samples. The incidence of the pathogen was the highest in TN and the lowest in Pondicherry and LI (Table 1). In Coimbatore district of TN alone, 17.19 per cent of samples yielded the fungus during October 1999- February 2000 (Kumar *et al.*, 2001). In the case of Kerala, the incidence was slightly higher during the monsoon season. However, the incidence of *H. thompsonii* was erratic. The fungus has also

been associated with the coconut mite in tropical America and West Africa (Moore and Howard, 1996).

Table 1. Natural incidence of *H. thompsonii* during 1999-2001

State/ Union Territory	Number of districts/ islands surveyed	Nuts with coconut mites infected with *H. thompsonii* (%)
Kerala	9	3.58
Tamil Nadu	3	5.45
Karnataka	10	2.50
Andhra Pradesh	3	3.00
Pondicherry	1	1.00
Lakshadweep Islands	1	1.00

ISOLATION AND CHARACTERIZATION OF *H. THOMPSONII*

The procedure reported by Kumar *et al.* (2001) was adhered to for the isolation and purification of fungal pathogens from the coconut mite. The characteristic features such as monophialides bearing spherical and verrucose conidia of about 3.5 µ across were taken into consideration for identifying *H. thompsonii* in culture. Isolates were grouped based on synnemata production. The synnematous isolates were classified as *H. thompsonii* var. *synnematosa.*

LABORATORY EVALUATION

A total of 15 monoconidial isolates of *H. thompsonii* were purified and evaluated against the coconut mite in the laboratory. Infection by the fungus was initiated by conidia, which adhered to the body of the mite. Inside the body of the mite, the hyphae multiplied initially in the central area of the haemocoel as oval bodies and then became chainlike as they grew anteriorly or posteriorly along the inner body wall. At an advanced stage multinucleate spherical chlamydospores were present. In both infected nymphs and adults, hyphae came out through the natural apertures, leg joints and sideways through the body wall. Conidia were produced on solitary phialides arising from external mycelia growing away from the cadaver. The isolates MF(Ag)5, 6, 7 and 21 were found to be the best among the collection. Sampedro and Rosas (1989) performed pathogenicity tests in Mexico with 7 strains of *H. thompsonii* at different conidial concentrations and obtained the highest mortality (88.36%) with the strain HtMOR.

PRODUCT DEVELOPMENT

The most promising *H. thompsonii* isolate MF(Ag)5 (IMI 385470), which was derived from an infected mite collected at a coconut farm in Pollachi (Coimbatore district, Tamil Nadu) on 19 February 2000 was taken up for product development because it possessed the most desirable characteristics, including the best sporulation, fastest growth rate, etc. Based on a series of limited field trials we developed a powder formulation called 'Mycohit' (Table 2) for

field use (Kumar and Singh, 2000). The biomass (mycelia and conidia) of *H. thompsonii* obtained through liquid culturing (stationary, shake or fermentation) was used for preparing the formulation. The mycoacaricide should be applied at 1% concentration. For about 50 trees, 1 kg of the product is mixed in 100 litres of water and used at around 2 litres per tree. Three treatments at 15-day intervals should be given for controlling the mite and to get the fungus established in the garden. Hindustan Antibiotics Limited (HAL) in Pune has been approached for commercialization of the mycoacaricide. The experimental batches from HAL have also been found to be equally effective as the original PDBC version of the mycoacaricide.

Table 2. Product details for the mycoacaricide

Parameter	Description
a.i.	Mycelia and conidia of *H. thompsonii*
Cfu	2.5×10^8 /g
M.C.	12 %
Form	Powder
Colour	Off white

FIELD EVALUATION

The mycoacaricide has been proved to be effective in field studies in stimulating disease outbreaks in the mite populations. Under field conditions, artificial epizootics could be created within 20 days of application of the product. Limited trials have been conducted in Karnataka, Kerala, TN and AP. Consistently high results were obtained so far in Karnataka and the product has been found to be superior to monocrotophos, dicofol and wettable sulphur and several other commonly employed chemicals.

During June-July 2001, a field experiment was laid out for comparing mycoacaricide (PDBC) and 'Mycohit-T' (T-04) of HAL with 'Sultaf 80W' (Rallis India Ltd., Mumbai), a WP formulation of micronized elemental sulphur, at Chinnanayakpalya near Bangalore (Bangalore Urban district, Karnataka). Both the versions of the mycoacaricide were used at 1% concentration and wettable sulphur was tested at 0.4%. All the three products were applied thrice at 15-day intervals at the rate of 2 litres of spray suspension per tree. Control trees received only plain water. Observations on the mortality of the mite population were recorded from three nuts plucked randomly from each tree every week from the start of the experiment.

Highly significant differences were observed between treatments and the two *H. thompsonii* formulations were far better than wettable sulphur (Table 3). Both versions of the mycoacaricide showed the highest mortality of 98.80% (PDBC) and 96.62% (HAL) in a single nut by the end of the third week i.e., 7 days after the application of the product the second time. The maximum mean mortality figures obtained were 88.00, 81.23 and 64.48% with mycoacaricide (PDBC), mycoacaricide (HAL) and wettable sulphur, respectively (Table 3). Tender nuts were randomly harvested in January 2002 and were graded based on the percentage of damage on the surface of the nuts (1: no damage; 2: 1-10% damage; 3: 11-25% damage; 4: 26-50% damage; and 5: > 50% of mite damage with reduction in size and great distortion). The grades were 1.37, 1.63, 2.77 and 3.87 for mycoacaricide (PDBC), mycoacaricide (HAL), wettable sulphur and control, respectively.

Table 3. Effect of two versions of the *H. thompsonii*-based mycoacaricide and a chemical on the mortality of the coconut mite

Treatment	Mortality of the coconut mite (%)										
	BS*	7 DAT	14 DAT	21 DAT	28 DAT	35 DAT	42 DAT	49 DAT	56 DAT	63 DAT	70 DAT
Mycoacaricide (PDBC)	2.00[a]	25.59	88.00	33.14	39.49	58.51	74.67	70.54	78.34	84.40	81.58
	7.87[b]	29.99	70.81	35.05	38.85	50.04	61.45	57.23	62.31	67.08	64.74
Mycoacaricide (HAL)	1.86	29.02	60.88	63.73	57.88	55.79	67.82	81.23	76.96	69.82	70.24
	7.82	32.06	52.76	53.03	49.54	48.41	55.48	64.70	61.62	56.75	57.04
Wettable sulphur	3.20	64.48	61.35	34.41	34.38	31.89	28.69	37.11	26.84	59.01	57.22
	10.27	53.55	51.59	35.73	35.74	34.15	32.34	37.25	31.09	50.22	49.16
Control	2.00	3.98	4.48	3.58	2.69	5.00	4.67	4.55	4.00	3.64	4.44
	8.05	11.47	12.21	10.86	9.32	12.92	12.47	12.30	11.50	10.89	12.14
SEM	0.42	1.98	2.79	1.29	1.88	1.93	2.34	1.94	1.19	1.16	0.89
CD (p=0.05)	1.22	5.75	8.08	3.75	5.45	5.59	6.78	5.64	3.46	3.36	2.59

DAT- Days after treatment (first); BS- Before spray
15 DAT - second spray was given
30 DAT - third spray was given * Sampling done just before spraying
[a] Original value; [b] Arc-sine transformed value

As in the present study, Becerril and Sanchez (1986) reported 25-75% mortality of the coconut mite in field investigations conducted in 1982 and 1983 in Mexico. Conidiation is an important factor for achieving high mortality of the mites under field conditions (Moore *et al.*, 1989) and this has been proved by our studies. Once *H. thompsonii* gains entry to the perianth it is capable of assuming epizootic proportions among mites present below the bracts (Hall *et al.*, 1980).

CONCLUSIONS

Indian plant protection specialists and coconut farmers have now come to the conclusion that chemicals cannot give a sustainable solution to the problem of the coconut mite, and that an integrated approach giving more emphasis to biologicals and botanicals along with a proper nutrient management is needed to control the pest. A pesticide moratorium has already been declared to conserve the natural enemies and the work on the biological control of the mite has been given more importance. *H. thompsonii* is being tested on a large-scale at many locations all over south India and the results should give an idea as to what kind of strategy needs to be followed for its effective usage for long-term benefits.

ACKNOWLEDGEMENTS

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Putative biological control agents of *Microdochium nivale* isolated from compost

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ABSTRACT

Nectria inventa and a *Pythium oligandrum*-like oomycete were isolated from 24- and 1-month-old compost respectively, and tested for antagonism against *Microdochium nivale*, *in vitro*. Both organisms were able to inhibit the pathogen's mycelial growth; *N. inventa* appeared to cause inhibition through the production of diffusible antifungal metabolites, whereas the *Pythium* species appeared to inhibit *M. nivale* mycelium only when in direct contact with the pathogen. The ability of these organisms to significantly inhibit the growth of *M. nivale in vitro* highlights them as putative biological control agents of *M. nivale* seedling blight.

INTRODUCTION

Fusarium seedling-blight caused by *Microdochium nivale* is an important seed-borne disease of winter wheat in the UK. The percentage of seed samples tested by the Official Seed Testing Stations in England and Scotland, with incidence of *M. nivale* greater than the recommended 5% treatment threshold (Cockerell, 1995), averaged 86% in 1997 and 1998 (Cockerell & Jacks, 2002; J. Thomas, pers. comm.). Plant establishment from untreated, infected seed has been observed to correlate significantly with the percentage incidence of *M. nivale* on the seed (Humphreys *et al.*, 1995).

Chemical seed treatment for the control of Fusarium seedling blight is limited, following the withdrawal of organo-mercury fungicides in 1992 (Paveley *et al.*, 1996) and the development of resistance by *M. nivale* to benzimidazole fungicides (Parry *et al.*, 1995). Successful development of biological treatments for the control of certain seedling diseases has led to EPA registration of products such as MycoStop® and SoilGard® (Lumsden *et al.*, 1995), but there is currently no commercial biological control product for Fusarium seedling-blight.

Composted bark has been found to naturally suppress a number of seedling diseases when incorporated into potting media (Hoitink & Fahy, 1986). Pathogens inhibited by bark compost have included *Rhizoctonia solani*, *Phytophthora cinnamomi* and several *Fusarium oxysporum* pathotypes. In the field, Tilston (2000) observed a reduction of Take-All symptoms on winter wheat, caused by *Gaeummanomyces gramini* var. *tritici*, when composted green-waste was used as a soil amendment. Disease suppression by compost can be chemical and/or biological in its nature, and is thus affected by the maturity of the compost as this reflects the decomposition stage of the parent material. Research on the use of compost in agriculture is currently being driven by the EC Landfill Directive 1999/31/EC and the Department for Environment, Food and Rural Affairs' strategies for sustainable and organic agriculture (website: http://www.DEFRA.gov.uk).

This paper reports the findings of preliminary *in vitro* investigations into the potential of a fungus and an oomycete, isolated from composted tree and grass trimmings of different maturities, to act as biological control agents against *M. nivale*.

MATERIALS AND METHODS

Compost

Tree and grass trimmings were collected from local amenities and composted in open windrows according to standards set by the UK Composting Association. Following the screening, peak-heating and cooling periods of the composting process, the composted material was stock-piled to mature and stabilise. The composts used in these investigations were 1- and 24-month maturities (the 24-month-old compost had been stock-piled for 12 months and stored in cool, moist conditions at Harper Adams University College for a further 12 months).

Fungi

Microdochium nivale was isolated from a seedling of winter wheat cv. Equinox, grown from infected seed. One hundred seeds were surface sterilised and plated, four per plate, on half-strength potato dextrose agar (PDA). Seed-plates were incubated in the dark at 15°C until germination and under near UV lighting at 18°C during seedling development. Sporodochia were isolated from a seedling showing Fusarium seedling-blight symptoms and identification of *M. nivale* was confirmed by colony characteristics and spore morphology.

Nectria inventa was isolated from 24-month-old compost plated onto a *Gliocladium* selective medium (Park *et al.*, 1992) without benomyl, using the soil-washing technique for isolation of fungi from organic particles (Williams *et al.*, 1965). Plates were incubated in the dark at 15°C for development of mycelia and under near UV lighting at 18°C for sporulation. Initial identification of *N. inventa* was made according to Domsch *et al.* (1993) and has been confirmed by the International Mycological Institute identification service (IMI No. 388637).

Oomycete

A *Pythium oligandrum*-like species, was isolated from 1-month-old compost by preparing 'well-plates'. A 'well' was made on Oxoid No. 3 agar plates (9 cm diam.) by removing an arc-shaped section of the agar measuring 1 cm in from the edge of the plate and 5.5 cm in length, using a flamed scalpel. The 'well' was filled with compost level to the agar surface and the compost was solidified using molten agar. A mycelial plug of *M. nivale* was inoculated 5 cm from the edge of the compost. Plates were inverted and incubated at 15°C in the dark until fungal colonies grew from the compost towards the *M. nivale* inoculum. One colony that consistently grew from the compost and inhibited *M. nivale* was pure cultured and provisionally identified as *P. oligandrum* according to Domsch, *et al.* (1993). Isolates of this species were randomly chosen from four of the replicate well-plates to further test for their ability to inhibit *M. nivale* in dual culture.

Dual Culture Plates

Dual culture plates of *M. nivale* and *N. inventa*, using half-strength PDA, were inoculated using the following treatments:

1. *M. nivale* and *N. inventa* inoculated at the same time and spaced 5 cm apart.
2. *M. nivale* inoculated 5 days before *N. inventa* and spaced 5 cm apart.
3. *N. inventa* inoculated 5 days before *M. nivale* and spaced 5 cm apart.
4. *M. nivale* and *N. inventa* inoculated at the same time and adjacent.
5. *M. nivale* inoculated 5 days before *N. inventa* and adjacent.
6. *N. inventa* inoculated 5 days before *M. nivale* and adjacent.

Six replicate plates were prepared per treatment. Once inoculated, the plates were inverted and incubated in the dark at 10°C. Mycelial radial growth was recorded every other day from the 5th day of incubation until *M. nivale* had colonised the plate in any treatment. This occurred on day nine. Percentage inhibition of *M. nivale* by *N. inventa* was calculated as ((r1-r2)/r1) x 100) and zones of inhibition were also measured (Fokkema, 1993) (Figure 1).

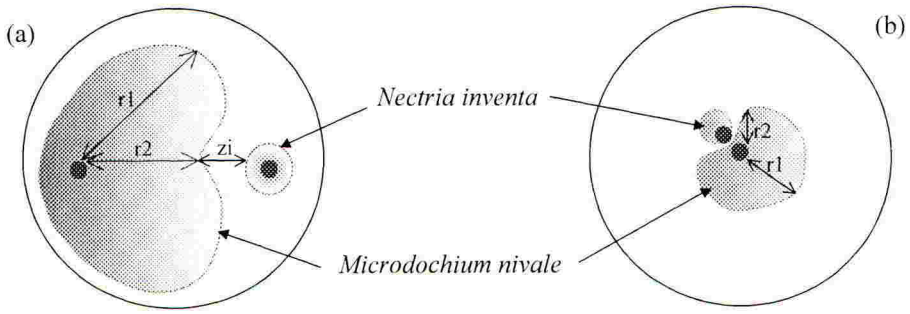

Figure 1. Diagram of dual culture plates of *Microdochium nivale* and *Nectria inventa* placed either apart (a) or adjacent (b). Parameters for inhibition are the % inhibition of radial growth ((r1-r2)/r1) x 100) and the width (cm) of the zone of inhibition (zi).

Dual culture plates of *M. nivale* and the *P. oligandrum*-like isolates were prepared on half-strength PDA by inoculating the plates with a mycelial plug of *M. nivale* and of the respective *P. oligandrum*-like isolate, opposite one another at a distance of 5 cm. Twenty replicate plates were prepared per isolate plus 20 of *M. nivale* alone as a control treatment. Plates were inverted and incubated in the dark at 10°C until colonisation of the plate in any treatment. This occurred on day fourteen. After 5 days' incubation, measurements were taken as per the *M. nivale* and *N. inventa* plates. As the *P. oligandrum*-like isolates inhibited the r1 radius of *M. nivale* as well as the r2 radius, % inhibition was calculated in this experiment using the r1 radius of *M. nivale* in the control plates in the calculation % inhibition = ((r1-r2)/r1) x 100).

Data Analysis

Data was analysed using GenStat® (IACR-Rothamsted). Analysis of variance was used to compare data at the 0.05 confidence limit.

RESULTS

Inhibition of *Microdochium nivale* by *Nectria inventa*

Percentage inhibition of *M. nivale* mycelial growth was significantly greater ($P < 0.001$) on day 9, when *N. inventa* was either inoculated 5 days prior to, or at the same time as, *M. nivale* compared to when *M. nivale* was inoculated 5 days prior to *N. inventa*. This occurred both when the fungi were placed 5 cm apart or adjacent (Table 1).

The zone of inhibition for *M. nivale* and *N. inventa* placed 5 cm apart, on day 9, was 0.97 cm if *M. nivale* was inoculated 5 days prior to *N. inventa*, 1.03 cm if both fungi were inoculated at the same time, and 1.56 cm if *N. inventa* was inoculated 5 days prior to *M. nivale*. Inoculating *N. inventa* 5 days before *M. nivale* resulted in a significantly ($P < 0.001$) wider zone of inhibition compared to the two other inoculation timing treatments.

Table 1. Percentage inhibition of *Microdochium nivale* mycelial growth by *Nectria inventa* on dual culture plates with different inoculation timing and spacing treatments. Values are means of 6 replicates per treatment.

Treatment	Fungi spaced apart*	Fungi placed adjacent
M. nivale inoculated five days before *N. inventa*	0.96[a]	2.91[a]
M. nivale and *N. inventa* inoculated at the same time	12.15[b]	13.20[b]
N. inventa inoculated five days before *M. nivale*	10.69[b]	41.97[c]
	lsd 6.89	cv% 42.4

* Values with the same letters within columns are not significantly different.

Inhibition of *Microdochium nivale* by *Pythium oligandrum*-like isolates

On day 14, the overall mean percentage inhibition of *M. nivale* mycelial growth by the *P. oligandrum*-like isolates was 34.3%. All isolates were able to inhibit the pathogen but percentage inhibition varied between isolates (Table 2), and this was associated with their radial growth. Zones of inhibition were not formed on the *M. nivale-P. oligandrum*-like plates.

Table 2: Percentage inhibition of *M. nivale* mycelial growth by *P. oligandrum*-like isolates on dual culture plates. Values are means of 20 replicates per treatment.

Treatment	Percentage Inhibition of *M. nivale* mycelium*
M. nivale plus *P. oligandrum*-like isolate 7	37.00[b]
M. nivale plus *P. oligandrum*-like isolate 10	42.06[a]
M. nivale plus *P. oligandrum*-like isolate 14	23.08[c]
M. nivale plus *P. oligandrum*-like isolate 18	35.06[b]
	lsd 2.82 cv%16.3

* Values with the same letters are not significantly different.

DISCUSSION

Mycelial growth of *Microdochium nivale* was inhibited, *in vitro*, by fungal organisms isolated from 24-month- and 1-month-old composted tree and grass trimmings. *Nectria inventa* is able to degrade lignin, whereas *Pythium* species are associated more with the utilisation of less complex sugars (Domsch, *et al.*, 1993). The nutritional requirements of the two organisms are represented by the respective maturities of the compost from which they were isolated.

Under the conditions of these experiments, *N. inventa* appeared to inhibit *M. nivale* by production and excretion of diffusible antifungal metabolites. Inhibition was increased when *N. inventa* was pre-incubated for a period of 5 days before the substrate was inoculated with *M. nivale*. This suggests a stronger competitive ability by *N. inventa* if it is present on the substrate prior to colonisation by *M. nivale*, and/or a weaker competitive ability by the pathogen if the substrate is already colonised by *N. inventa* (Garrett, 1970). Isolates of a *P. oligandrum*-like species appeared to antagonise *M. nivale* only when in direct contact with the pathogen's mycelium. Competition, antibiosis and mycoparasitism are all forms of antagonism, often operating synergistically, which are regularly associated with mechanisms of biological control (Faull 1988; Jeffries & Young 1994).

The nutrient content of a growth medium can affect fungal activity, which therefore may not reflect fungal interactions under natural conditions (Campbell, 1989). Further *in vitro* studies to elucidate the mechanisms involved in these interactions have used very low nutrient agar amended with compost extract and/or seed exudates in order to represent more natural nutritional conditions. Findings from this series of *in vitro* experiments will be used to develop *in planta* assays to test the ability of *N. inventa* and the *P. oligandrum*-like species to control *M. nivale* seedling blight when applied as seed treatments.

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Controlling infection of cereal grain by toxigenic *Fusarium* species using fungal competitors

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ABSTRACT

Strains of fungi, including non-pathogenic *Fusarium* spp., were tested in controlled-environment and field experiments for their ability to control the most important pathogenic and toxigenic *Fusarium* spp. that cause ear blight on cereals. They were tested at both the inoculum production and ear infection stages. Some fungi effectively decreased growth of *Fusarium* spp. on wheat straw and maize stem pieces (representing an inoculum source for ear or cob blight). The most effective of these were fungi (mainly non-*Fusarium* spp.) that had only small effects against *F. culmorum* at the ear infection stage on barley, oats or wheat. Isolates of non-pathogenic *Fusarium* spp. were more effective against ear infection, however, decreasing ear blight and deoxynivalenol in the harvested grain as much as the standard fungicide, tebuconazole. Experiments to test for control at these two stages in the disease cycle are continuing.

INTRODUCTION

Fusarium ear blight (head blight, scab) and associated contamination of grain with mycotoxins are causing considerable concern in all cereal-growing areas of the world. The damage has been particularly severe in North America in recent years (Windels, 2000). Pre-harvest control of the disease is an objective of a major EU-funded project aimed at preventing *Fusarium* mycotoxins entering the human and animal food chains. Biological control is being attempted at two stages in the life cycle of the pathogens: during colonisation of, or spore production on, infested crop residues (the inoculum source) and during infection of the flowering spikes. Eliminating or decreasing a pathogen at the inoculum source is an ideal way of controlling disease. In the case of *Fusarium* diseases of cereals, infection can sometimes be decreased by burial of the infested debris during ploughing (e.g. Yi *et al.*, 2001). Deep cultivation is not always desirable or practical, however. The ear infection stage is a particularly suitable target for direct control by biological agents. This is because

application of the agent to the ear can be timed precisely (during anthesis) and it needs to survive and compete for a relatively short period of time in a habitat consisting of new plant material occupied by a very limited natural microbial community.

Much published research on biological control of ear blight has concentrated on antagonistic bacterial strains (e.g. Luz *et al.*, 2002). The emphasis in our research is on strains of fungi that occur naturally in the habitats in which they are required to operate, and so may have a competitive advantage. Results of experiments are described in which the effects of fungi, previously selected in controlled-environment and glasshouse screens, on colonisation of crop residues by *Fusarium* spp., and on ear infection and mycotoxin production, were determined.

MATERIALS AND METHODS

Isolates of pathogenic *Fusarium* spp. from cereal crops in The Netherlands, Italy and the UK were used in experiments in the respective countries. More than 100 isolates of other fungi, including yeasts, and actinomycetes were obtained mostly from cereal plants and identified to species. They were screened for antagonistic or competitive activity against pathogenic *Fusarium* spp. by testing, for example, their ability to inhibit *Fusarium* sporulation on straw or to suppress ear blight on glasshouse-grown wheat (Dawson *et al.*, 2002; Köhl *et al.*, 2002). On the basis of these screens, a collection of isolates was selected for further testing of their abilities to inhibit colonisation of straw or infection of ears and cobs, first in repeated controlled-environment or glasshouse experiments and, subsequently, in field experiments. Some of the controlled-environment and field experiments are described. The experiments described include examples of fungi that were tested for effects on both straw colonisation and ear infection. Fungi 1-6 are isolates of five different fungal species. Fungi 7-19 are isolates of five species of non-pathogenic *Fusarium*.

Controlled-environment experiments tested effects on colonisation of wheat straw pieces or maize stem pieces sterilised by γ-irradiation. They were first sprayed with water (control) or spore suspensions (10^4 conidia ml^{-1}) of *Fusarium*. Wheat straw was treated with conidia of *F. culmorum* or *F. graminearum*, whilst maize stem pieces were treated with *F. graminearum*, *F. verticillioides* or *F. proliferatum*. After 6 h, they were sprayed with water or suspensions of conidia of test fungi (10^6 conidia ml^{-1}). The straws and stem pieces were incubated at 15°C in a high-humidity chamber for 21 days. Conidia were washed from the wheat straws by shaking the five 5-cm pieces per replicate for 10 min in 10 ml washing fluid (20% ethanol and 0.01% Tween 80 in water). Maize stem pieces were processed similarly using appropriate volumes. The effect of each test fungus was calculated as the difference between the number of conidia produced (counted using a haemocytometer) on the control straws or stem pieces and the number produced on those treated with the test fungus, expressed as a percentage.

In field experiments to test effects on infection, groups of 10 ears per treatment, in four randomised blocks per experiment, were inoculated during anthesis in crops of autumn-sown barley, oats and wheat. Conidial suspensions of test fungi (10^6 ml^{-1}) were sprayed onto the ears, which were then covered with a polyethylene bag for 3 days. A conidial suspension of *F. culmorum* (10^4 ml^{-1}) was then applied and the ears were again covered for 3 days. Ear blight was assessed as the percentage of each spike (ear) affected. In the wheat experiments, ear blight developed slowly and became indistinguishable from ripening. The incidence of diseased grains was therefore assessed after harvest. Grain weights per ear and per 1000

grains (results not shown) and their mycotoxin contents were determined. Results are shown only for deoxynivalenol (DON), the predominant mycotoxin.

RESULTS

Most of the test fungi decreased sporulation of *Fusarium* spp. on wheat straw and maize stem pieces (Table 1). Non-*Fusarium* test fungi tended to have more effect than the non-pathogenic *Fusarium* isolates.

Table 1. Summary of effects of fungal strains on sporulation by *Fusarium* spp. on inoculated wheat straw or maize stem pieces in controlled environments

	% decrease compared with *Fusarium*-only control				
	Wheat		Maize		
Fungus	*Fc*	*Fg*	*Fv*	*Fp*	*Fg*
1	93*	96*	73*	81*	71*
2	99*	96*	96*	98*	97*
3	57	72*	91*	62*	49
4	100*	94*	-	-	-
5	98*	94*	14	90*	69
6	49	68	90*	86*	82*
7	41	79	36	74*	64*
8	-	-	0	95*	97*
9	92	99*	58	43	0
10	84	98*	21	38	0
11	95	96	12	75*	36
12	31	74	-	-	-
14	-	-	81*	0	0
15	60*	65*	-	-	-
16	71*	76*	-	-	-
17	17	48	85*	21	0
18	71*	75*	-	-	-
19	78*	82*	0	53	71*

Fc, *F. culmorum*; *Fg*, *F. graminearum*; *Fv*, *F. verticillioides*; *Fp*, *F. proliferatum*.
*, Significant effect ($P \leq 0.05$).
-, not tested.

In field experiments on ear infection, severe disease did not develop on barley or oats and there were significant effects of treatments on ear blight only in oats, in which it was decreased by the fungicide but increased by some fungi (Table 2). DON production was significantly decreased in barley by tebuconazole and fungus 7. The percentage of diseased grains is shown for wheat, since ear blight was not clearly distinguishable from natural senescence. Effects on visibly diseased grain were not significant. All fungi tended to suppress DON production in this wheat experiment, but less than the fungicide tebuconazole, which suppressed DON production to a level similar to that in the untreated (no *F. culmorum*) control.

In a separate field experiment, four isolates (11, 12, 13 and 15) of non-pathogenic *Fusarium* spp. decreased the incidence of diseased grains as effectively as tebuconazole (Table 3). DON production was suppressed significantly by isolates 8 and 13 when analysed after log-transformation, but by isolates 12 and 13 when not transformed, and by tebuconazole.

Table 2. Effects of fungal strains on ear blight caused by *Fusarium culmorum* and deoxynivalenol (DON) content of cereal grain in field experiments

	Barley		Oats	Wheat	
Test fungus	Logit % ear blight[a]	DON (log mg kg^{-1})[ab]	Logit % ear blight[a]	Logit % diseased grains[a]	DON (mg kg^{-1})[b]
None, no *Fus.*	0	4.55 (0.09)	-2.19 (1.2)	-1.38 (5.9)	*1.77*
None	-1.54 (4.4)	6.98 (1.07)	-1.41 (5.6)	-1.00 (12.0)	*10.86*
1	-1.51 (4.6)	*0.42*	-1.01 (11.8)	-0.99 (12.2)	*2.81*
2	-1.59 (4.0)	*0.23*	-1.02 (11.6)	-1.17 (8.9)	*3.97*
3	-1.77 (2.8)	6.12 (0.45)	-1.19 (8.5)	-1.35 (6.3)	*2.97*
4	-1.71 (3.2)	6.01 (0.41)	-1.02 (11.6)	-1.14 (9.3)	*5.57*
7	-1.58 (4.0)	4.93 (0.14)	-1.07 (10.5)	-1.21 (8.2)	*3.86*
Tebuconazole	-1.92 (2.1)	5.26 (0.19)	-2.02 (1.7)	-1.49 (4.9)	*1.78*
SED [df]	0.201 [42]	0.724 [18]	0.205 [45]	0.273 [45]	-
P	0.2	0.02	<0.001	0.4	-

[a]Back-transformed means are shown in parentheses.
[b]Actual values are shown in italics for treatments that were not included in the analyses because samples were bulked (and so non-replicated).

DISCUSSION

There were significant effects of fungal strains on colonisation (assessed by spore production) by *Fusarium* spp. of wheat straw and maize stem pieces. The non-pathogenic *Fusarium* spp. tended to be less effective than the other fungi at this stage. Preliminary results from similar experiments (not shown) in the field show similar trends, although *F. graminearum* has so far tended to be more susceptible than the other pathogens to suppression. Suppression in the field may also be short-lived and so delayed or repeated applications of such treatments may be an option. Subsequent field experiments are also testing straws naturally infested with *Fusarium* spp., which are being placed in wheat crops to determine the effects on ear blight and mycotoxin development.

Fungi from six genera showed potential as antagonists of ear blight in preliminary glasshouse screens on wheat (Dawson *et al.*, 2002). Seventeen fungal isolates, which were able to decrease ear blight by more than 70% in glasshouse screens, were then tested in field experiments. However, some fungi that showed potential in the glasshouse were ineffective under field conditions (complete results are not shown here). Isolates of non-pathogenic *Fusarium* spp. were effective in the glasshouse and field.

The most promising fungal strains so far tested on infested crop residues had little effect when applied to ears. It is therefore apparent that the best strains will be different for different stages in the life cycles of the fungi. Non-pathogenic *Fusarium* spp. are particularly

promising for direct protection of the ears. Some have been shown to decrease DON production by *Fusarium* pathogens *in vitro* (Cooney *et al.*, 2001). Although DON was not decreased below the proposed EU standard (0.75 mg kg^{-1}; Prickett *et al.*, 2000) where disease was most severe, some isolates were as effective as tebuconazole. In less extreme conditions, where severe disease has not been encouraged artificially, DON may be decreased to permitted levels. The most promising isolates are being tested against ear blight of wheat in larger-plot experiments that are mist-irrigated to ensure disease development (*cf.* Lacey *et al.*, 1999). Control of infection of maize cobs is also being tested in Italy. A further objective is the integration of biological control at the two key stages of inoculum production and ear infection.

Table 3. Effects of non-pathogenic strains of *Fusarium* spp. on ear blight of wheat caused by *Fusarium culmorum* and deoxynivalenol (DON) content of grain in a field experiment

Test fungus	Logit % diseased grains (back-transformed mean)	DON (mg kg^{-1})	DON (log mg kg^{-1})
None, no *F. culmorum*	-2.00 (1.8)	0.05	3.72 (0.04)
None	-0.31 (35.0)	16.78	9.61 (14.94)
6 (non-*Fusarium*)	-0.25 (37.6)	21.91	9.92 (20.34)
8	-0.84 (15.8)	8.79	8.36 (4.25)
9	-0.59 (23.6)	14.96	9.56 (14.24)
10	-0.32 (34.7)	18.40	9.66 (15.67)
11	-1.10 (9.9)	10.12	8.77 (6.44)
12	-1.22 (8.0)	6.70	8.65 (5.73)
13	-1.51 (4.6)	4.47	8.26 (3.87)
14	-0.59 (23.5)	20.97	9.95 (20.90)
15	-0.90 (14.2)	8.03	8.98 (7.94)
16	-0.34 (33.8)	18.19	9.78 (17.63)
17	-0.67 (20.7)	14.44	9.50 (13.32)
18	-0.52 (26.0)	10.52	9.23 (10.20)
19	-0.36 (32.8)	19.86	9.87 (19.30)
Tebuconazole	-1.14 (9.2)	9.59	8.19 (3.61)
SED (45 df)	0.281	4.535	0.585
P	<0.001	<0.001	<0.001

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