POSTER SESSION 4A

ADVANCES IN PEST AND DISEASE MANAGEMENT IN TROPICAL AND SUB-TROPICAL CROPS

Session Organiser: Dr R Black
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Poster Papers: 4A-1 to 4A-4

Management of *Pythium aphanidermatum* in greenhouse cucumber production in the Sultanate of Oman

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ABSTRACT

A rapid rural appraisal of greenhouse cultivation in Oman showed that the principal constraints to increased yields include salinity and lack of available water for irrigation, and diseases. The main causes of preventable yield loss are damping-off disease caused by *Pythium aphanidermatum* and downy mildew. In response farmers may make up to 20 fungicide applications per season. Disease progress in damping-off incidence appears to be a two-phase process: randomly placed foci become established from primary inoculum and then expand, probably via secondary inoculum. Fungicide trials showed that propamocarb provides the most effective control of damping-off; the commercial biocontrol agent *Trichoderma harzianum* gave a level of control equivalent to metalaxyl, the most widely used fungicide in the Sultanate.

INTRODUCTION

The Ministry of Agriculture and Fisheries (MAF) in the Sultanate of Oman has identified greenhouse crop production as a priority sector for expansion. Recent surveys conducted by MAF indicate that there are over 1000 greenhouses in the country, mostly located close to areas of high population (Muscat municipality) or close to the border with the United Arab Emirates (Figure 1).

The majority of greenhouses are constructed as single-span metal frames covered with plastic sheeting with a length of 30m, width of 9m, and a centre height of 3m. Greenhouse temperature control is typically achieved by using fans at one end of the house to pull air into the greenhouse across a water-soaked pad located at the opposite end. Over 95% of greenhouses are used to cultivate cucumbers, usually producing three crops per year with planting dates of August, December and March. Some farmers cultivate an additional summer crop although temperatures in excess of 40°C create substantial and prohibitive cooling costs for the majority of growers in such circumstances.

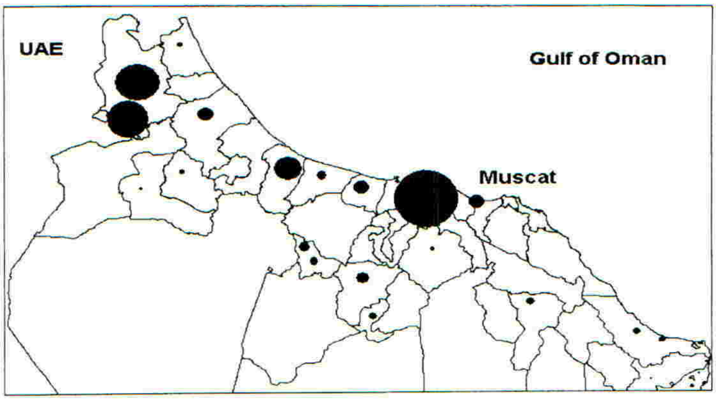

Figure 1. Distribution of greenhouses in northern Oman (large circles indicate a high concentration of greenhouses in an administrative authority).

The major constraints to further expansion of the greenhouse production system are water availability, salinity of irrigation water and soil borne disease, especially damping off, caused by *Pythium aphanidermatum*. In the absence of these constraints growers may achieve harvests of 5t per greenhouse per season. Typical yields are in the range 3-4t but many farmers produce less than 2t. In view of the generally poor yields a research programme was initiated to identify, from the range of cultivation practices and disease management techniques used by farmers, those factors that contribute most significantly to disease reduction. One overall goal of the research programme was to investigate the principal components of potential integrated disease management strategies for greenhouse production systems within the Sultanate.

MATERIALS AND METHODS

Survey of greenhouse crop production practices

A rapid rural appraisal of 100 farms was conducted to ascertain the extent of crop losses and the utilization of fungicides as well as other non-chemical intervention techniques for disease limitation, such as soil ridging, drip irrigation line placement and soil solarisation. The farms were grouped according to size and ownership; data were also collected on cucumber varieties, production limitations due to salinity, irrigation practices and sources of crop management information and advice.

Spatiotemporal distribution of disease

A database of natural *P. aphanidermatum* induced damping-off and wilt epidemics is currently being established. A random selection of farms representing different farm types and regions within the Sultanate was made. At the start of each cropping season individual plants in selected greenhouses were labelled, disease assessments were made each day and the location of diseased plants over time was mapped. In addition, temporal disease progress was evaluated by constructing disease progress models for each epidemic to allow estimations to be made of the rate of disease progress and final disease incidence.

Biological control of *P. aphanidermatum* using *Trichoderma harzianum*

The scale of fungicide use in greenhouse cucumber production suggested that reductions could be achieved if alternatives could be shown to have an efficacy at least equivalent to the commonly applied chemical products. Consequently, a series of trials were established to determine the efficacy of a commercial *T. harzianum*-containing product against *P. aphanidermatum*. *Trichoderma* was compared to commercially available and, according to the survey data, the extensively used active ingredients propamocarb, metalaxyl and hymexazole. Cucumber seeds (cv. Printo) were sown in peat moss and transplanted into a greenhouse after 10 days. The commercial products were applied 3 times during the season (on the day of transplanting and 14 and 21 days after transplanting). Rates of application were according to label instructions: propamocarb ('Previcur') as soil drench at 30ml per 20l; metalaxyl ('Ridomil') broadcast post emergence at 10kg per feddan (4200 m^2); hymexazole ('Tatchgreen') as a post emergence drench at 3l per m^2. *Trichoderma* was mixed with peat moss prior to seed sowing and was subsequently applied as a soil drench 14 and 21 days after transplanting. The dose of *Trichoderma* was 200g per m^3 of soil. A randomised block design was used to incorporate four replicates of each treatment. Each replicate consisted of 35 plants in rows containing 140 plants in total. Damping off and wilt disease incidence were assessed at seven-day intervals from the time of planting until the end of the season.

RESULTS

Survey of crop protection practices

The rural appraisal showed cucumber production to account for over 95% of greenhouse crop production in Oman. The most widely grown cultivars were Luna (14.7% of greenhouses) and Hanna (13.7%). The average yield was 3.2t per greenhouse per season with a minimum of 1.0t and maximum of 5.9t (1.0t is roughly equivalent to 37 t/ha). There was a wide variation in incidence and severity of disease between locations. Damping-off and downy mildew were reported as important constraints to production by 74% and 79% of farmers respectively. In response to disease, all of the surveyed farmers reported the use of fungicides. The average number of applications per season (approximately 110 days) was 5.2, with a minimum of 1 application and a maximum reported frequency of 20 times per season. In addition, greenhouse crop farmers reported an average application frequency for insecticides of 6.9 times per season. The frequency of insecticide and fungicide applications ranged from 2 per season (1 fungicide and 1 insecticide application) to 43 (23 insecticide and 20 fungicide applications). Over 23% of farmers reported using solarisation to reduce disease intensity and 24% indicated that they change the soil in their greenhouses at least once per year in order to prevent soil borne pathogen build-up. Over 85% of farmers reported using soil ridging rather than flatbed cultivation to improve drainage and reduce disease.

Temporal and spatial distribution of disease

Daily analysis of the spatial distribution of diseased plants in greenhouses appeared to indicate a two-phase process in disease development. The establishment of initial foci of infection was followed by the expansion of certain focal points to encompass groups of up to 10 neighbouring plants on either side of the plant that was first infected (Figure 2). For the farmer this was expressed as gaps of dead seedlings in the rows of cucumber plants.

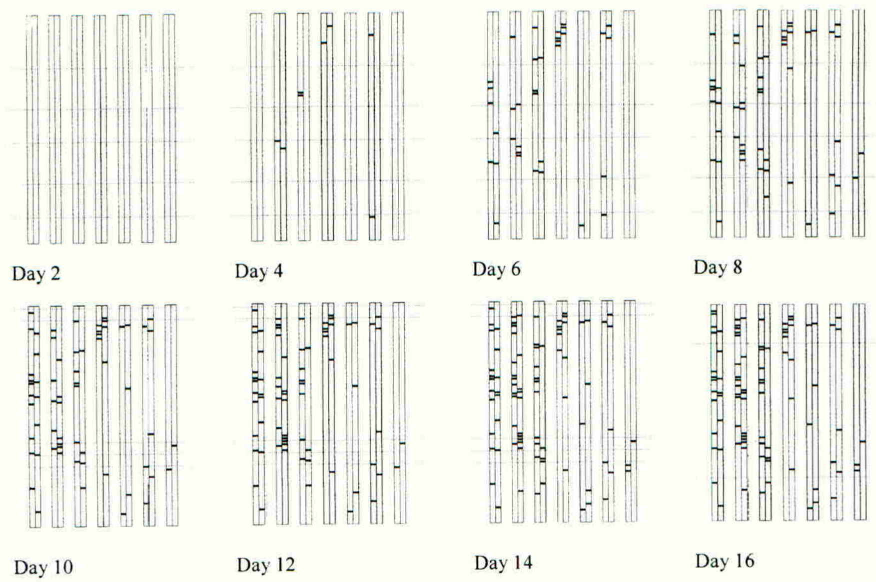

Figure 2. Pattern of the spatial distribution, at 2-day intervals, of transplanted cucumber seedlings infected with *P. aphanidermatum* during natural disease epidemics.

Figure 3 shows the temporal analysis of the greenhouse cucumber damping-off epidemic represented by Figure 2. As with most of the epidemics analysed to date, a Gompertz model, $y = K * (\exp(\log(y_o / K) \exp(-r * \text{time})))$ (Neher & Campbell, 1997) gave the best fit to the data. In the case of the epidemic illustrated in Figures 2 and 3 the model suggested parameter values of $K = 0.087$, $r = 2.9\text{x}10^{-4}$ and B $(-\ln(y_0 / K)) = -0.331$.

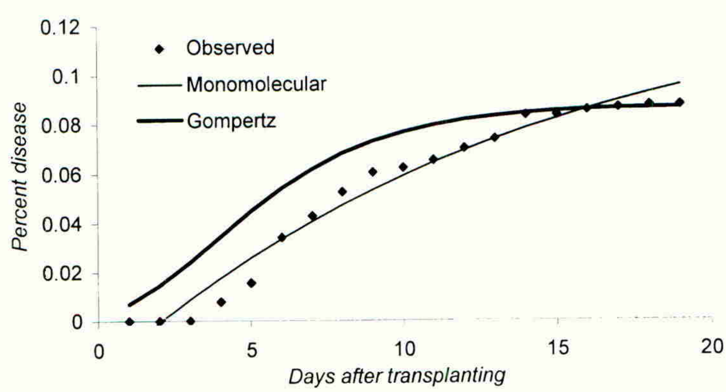

Figure 3. Observed progress of cucumber damping-off caused by *Pythium aphanidermatum* together with non-linear regression models.

Chemical and Biological control of *P. aphanidermatum*

The mean incidence of damping-off and wilt disease in the different treatments is shown in Figure 4. Treatment with propamocarb gave the most satisfactory results with a disease incidence of 1.6% 42 days after transplanting. Treatment with *Trichoderma* gave a level of control (18.0% incidence after 42 days) similar to that achieved with metalaxyl (14.9%). The disease incidence after 42 days was highest (40.7%) in those plants receiving no chemical or biological control agent.

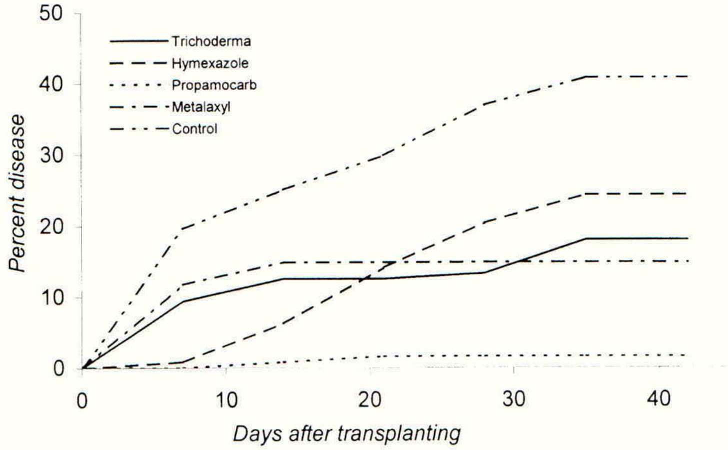

Figure 4. Incidence of damping-off and wilt disease in greenhouse cucumbers treated with *Trichoderma* and chemical fungicides

Crop yields (Figure 5) were similarly highest in those plants treated with propamocarb (695kg) and lowest in the control plants (292kg). Plants treated with Trichoderma had similar yields (539kg) to those plants receiving applications of metalaxyl (561kg).

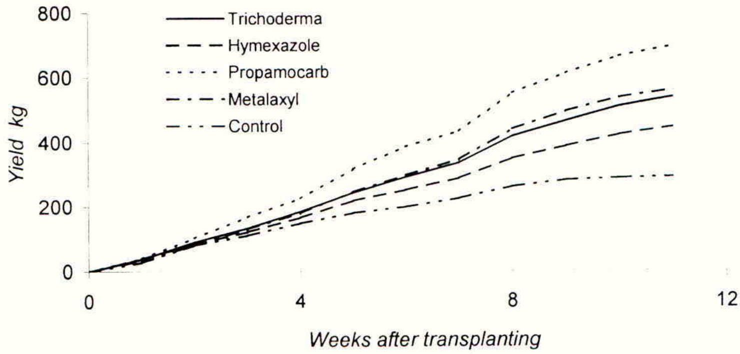

Figure 5. Cumulative fruit yields in greenhouse cucumbers treated with *Trichoderma* and chemical fungicides

DISCUSSION

Damping-off is a major constraint to increased cucumber production in the greenhouses of Oman. Previous, albeit circumstantial evidence for yield reductions of 15-20% because of damping-off was confirmed during the rural appraisal. In the fungicide trial reported here losses of 14.9% were observed in those plants treated with metalaxyl, the active ingredient most widely used by greenhouse cucumber producers in Oman. The most effective fungicide was propamocarb, the treatment recommended by the Ministry of Agriculture. However, as a result of cost this product is not as extensively used as metalaxyl. The widespread use of metalaxyl in the greenhouse production system, especially where such high reapplication rates are used, is a cause for concern regarding fungicide resistance. Evidence has recently emerged for the presence in Oman of metalaxyl-resistant strains of *P. aphanidermatum* (Al Saadi & Deadman, unpublished). The general effectiveness of *T. harzianum* against *Pythium* species is well known (for example, Bell *et al*., 2000). Commercial formulations of *Trichoderma* are not currently available in Oman. However, the data presented here indicate a good potential for the reduction in the use of chemical fungicides were such products to become available in future.

The rural appraisal also indicated that many farmers used solarisation techniques to reduce the impact of soil borne diseases. However, analysis of the survey data failed to indicate a reduction in disease severity following solarisation. Closer questioning of farmers revealed that the reported use of solarisation was merely the exposure of the soil (rather than covering with plastic) during the summer months. Similarly, the rural appraisal failed to indicate that soil ridging had a reducing effect on disease incidence. Further controlled experiment trials are in progress to evaluate solarisation and soil ridging techniques. Disease distribution patterns appear to show the involvement of primary and secondary inoculum in epidemic progress. In-depth analysis is in progress and appears to show rapid epidemic development and quenching causing the epidemic to be quickly slowed (C. Gilligan, personal communication).

Overall, the data suggest strategies for improving disease management within greenhouse cucumber production systems in Oman. The high levels of pesticide use that were recorded on some of the farms surveyed are often typical of those found in developing agricultural economies with sub-tropical climates (Thacker *et al*., 2000). However, the data also indicate that many farmers take an integrated approach to disease management by incorporating different agronomic practices within their crop production systems. Research is currently underway to evaluate which of these practices have the greatest potential to contribute to disease control in greenhouse crop production in Oman.

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The effects of cultivation practices and pre-treatment of tubers with sodium hypochlorite on the incidence of blackleg, *Erwinia carotovora* and tuber moth, *Phthorimea opercullela* in potato production in the Sultanate of Oman

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ABSTRACT

Two experiments were carried out to examine the role of cultivation practices and pre-treatment of tubers with sodium hypochlorite (NaOCl) in suppressing the damage caused by blackleg disease (*Erwinia carotovora*) and by potato tuber moth (*Phthorimea opercullela*) to potato crops in the Sultanate of Oman. Increasing the planting depth of tubers was found to cause a significant reduction in the level of tuber moth infestation in harvested tubers. Pre-treating infested and uninfested tubers with NaOCl had a significant impact upon the incidence of blackleg in the subsequent crop. In addition, seed that was infested with tuber moth showed higher levels of blackleg damage in the subsequent crop. However, this difference was not statistically significant. Overall, the data indicate that pre-treatment with NaOCl and the manipulation of tuber planting depth may make a significant contribution to blackleg and tuber moth suppression within potato production.

INTRODUCTION

Although potato is still a relatively new crop in Oman, in less than 10 years total production of potatoes has more than doubled from 5,600t in 1996 to over 16,000t in 2001 (Figure 1). During the same period average yields have increased from 22t/ha in 1996 to 30t/ha in 2001 while the area under potato production has increased from 250ha to 550ha.

Although yields in excess of 30t/ha are now routinely achieved (Deadman *et al.*, 2000), there remain a number of important constraints to potato production within the Sultanate. In common with a number of agricultural operations in Oman, limited water availability is a severe restriction to increased potato production. In addition, increasing salinisation of irrigation water has become a serious constraint. The main biotic limitations to increased yields include insect pests such as the peach potato aphid *Myzus persicae,* the whitefly *Bemisia tabaci,* and the potato tuber moth *Phthorimea opercullela.* Important diseases of potato include early blight *Alternaria solani,* leaf spot caused by *Stemphylium solani*, and blackleg caused by *Erwinia carotovora* (Moghal, 1993). Recent research has highlighted the interaction between the leafminer *Liriomyza trifolii* and the leaf spot pathogen *A. alternaria* in reducing potato yields in Oman (Deadman *et al.*, 2000).

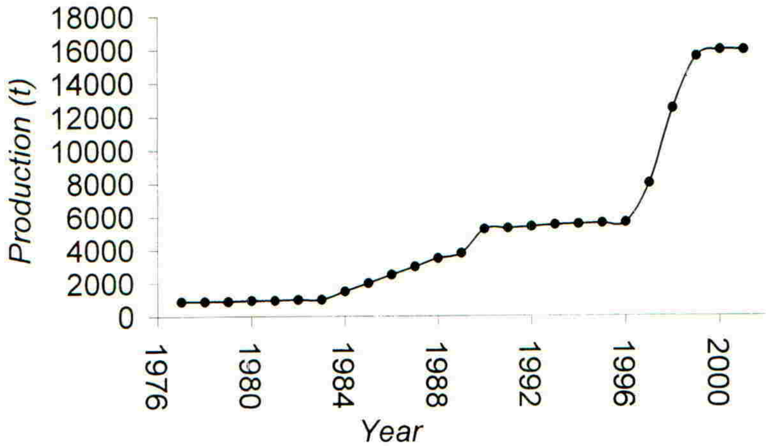

Figure 1. Change in potato production in Oman 1977 – 2001. Source: FAO Statistical database at http://apps.fao.org.

The bulk of the potato production in Oman is initiated from certified seed tubers, imported primarily from Western Europe. One consequence of this pattern of seed supply is that it largely dictates that the growing season commences in November when imported tubers become available. However, there is evidence that a November planting date may not be optimal for Oman, both in terms of yield and of efficient land use (Khan *et al*., 2000). Altering the planting time for potato cultivation however, would require either the identification of new sources of seed tubers and/or the establishment of an indigenous facility for high quality certified seed production.

Two important barriers, both to the short-term objective of improving crops yields, and to the long-term aim of establishing local seed tuber production systems, are blackleg disease (*E. carotovora*) and potato tuber moth (*P. opercullela*). Both represent extremely serious limitations to potato production in Oman. For example, farm surveys have shown that in extreme cases blackleg has been responsible for complete crop failure while routine inspection of tuber stores frequently shows damage by tuber moth to be in excess of 25% (Deadman and Khan, unpublished).

The purpose of the research described in this paper was to quantify in detail the importance of blackleg as a disease of potato in Oman and to establish the degree of any interaction between tuber moth infestation in stored seed and the establishment of blackleg infections in subsequent crops. Preliminary research had indicated that yield losses caused by the blackleg pathogen and by the insect pest might be synergistic. The research also attempted to identify potential cultural methods for reducing tuber moth infestation levels and the utility of pre-treating tubers with sodium hypochlorite (NaOCl) for pathogen control. Research undertaken elsewhere has already shown that pre-treatment with NaOCl may reduce disease incidence in potato crops (e.g. Errampalli & Johnston, 2001).

MATERIALS AND METHODS

A series of field experiments were established to examine the effect of cultural practices and the effect of pre-treatment with NaOCl on the incidence of blackleg damage and on the level of tuber moth infestation in harvested tubers. Cultural practices were evaluated principally by varying planting method. The potato varieties Aida and Lady Rosetta were used in the experiments, as determined by availability from importers.

Planting depth

To control the depth of the seed tuber, three planting methods were used. In the first, seed was placed in position on the flat soil surface. The soil was then ridged over the tubers so that they were approximately 10cm below the soil surface. In the second method, tubers were planted by hand in pre-ridged soil. The soil was then mechanically re-ridged so that the top of the tuber was approximately 5cm below the soil surface. In the third method, tubers were planted by hand without re-ridging so that there was less than 5cm of soil above the tuber. The potato variety Aida was used in these experiments as imported, certified seed. Two replicate rows (70 tubers per row) were used for each of the three planting methods. Plant emergence was measured weekly, as was the incidence of blackleg symptoms.

Pre-planting tuber treatment

A second experiment was designed to determine the interaction between tuber moth infestation and incidence of blackleg in a subsequent crop. Potato tubers, of the variety Lady Rosetta, were harvested from the 1999-2000 season and were kept in cold store prior to grading according to the presence or absence of tuber moth damage. Each cohort was divided into two: half were soaked in 5% NaOCl for 5 minutes while the rest were soaked in water for the same period of time. Tubers soaked in NaOCl were then washed twice in water. All tubers were planted on 20 September 2000 in a randomized block design with 15 replicate tubers per block and 4 replicate blocks. Plant emergence was assessed weekly from the date of planting for 8 weeks. Blackleg incidence was assessed after 5, 6 and 8 weeks.

RESULTS

Planting depth

The percentage plant emergence through time for the variety Aida with respect to planting depth is shown in Figure 2. Although the final plant densities were equivalent, plant emergence was delayed when the planting depth was increased above 5cm. The greatest delay in emergence was when a planting depth of 10cm was used.

The percentage incidence of blackleg in those tubers with a planting depth of less than 5cm was 12.5%. For the 5-10cm depth it was 11.8%, and for the 10cm planting depth, the final disease incidence was 9.1%. These relatively small differences were not statistically significant ($P > 0.05$). The final level of potato tuber moth infestation on the harvested tubers, however was significantly lower for the 10cm planting depth (3.7%) compared to the 5-10cm depth (7.8%) and the planting depth of less than 5cm (18.6%).

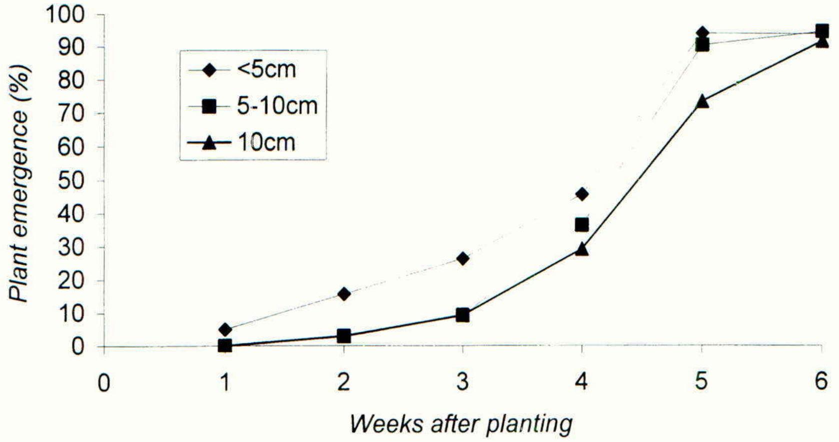

Figure 2. Emergence of potato plants (variety Aida) following planting at different soil depths.

Pre-planting tuber treatment

The percentage emergence of plants that were pre-treated with NAOCl is shown in Figure 3. The percentage incidence of blackleg that was recorded on these plants is shown in Figure 4.

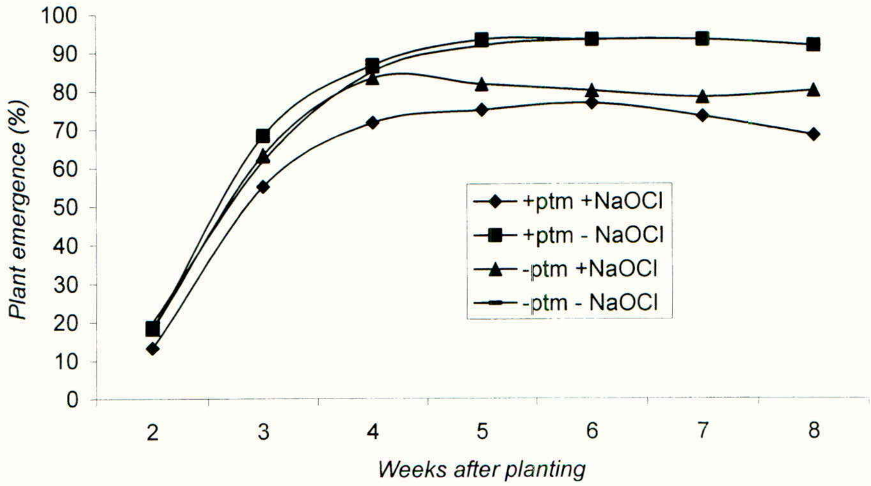

Figure 3. Percentage emergence of potato variety Lady Rosetta after pre-planting treatment with NaOCl on tuber moth infested and uninfested tubers. Emergence declines in some cases because of post-emergence mortality. (ptm = potato tuber moth).

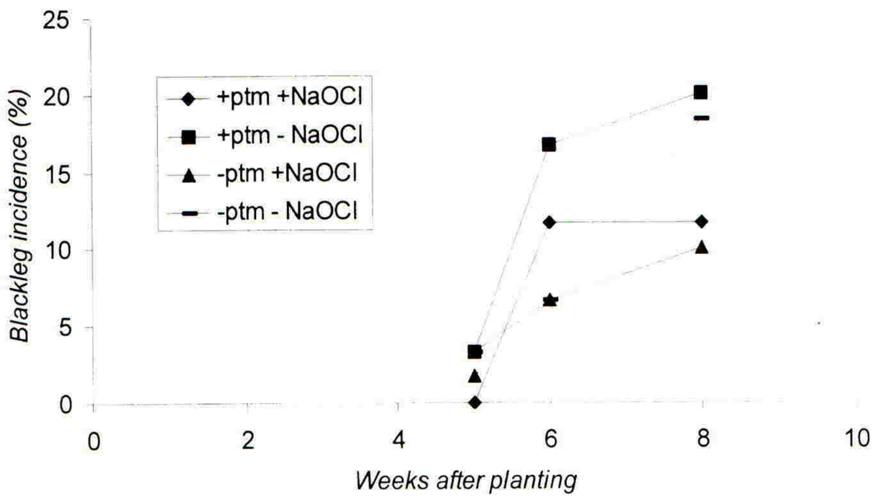

Figure 4. Incidence (%) of blackleg in tubers grown from seed infested and uninfested with potato tuber moth that had been pre-treated with NaOCl or with water. (ptm = potato tuber moth).

The percentage emergence of potato plants was similar for all treatments for the first three weeks. Subsequently, plants emerging from tubers treated with NaOCl showed lower final stand densities than were recorded in plots with untreated tubers. Pre-treatment with NAOCl may therefore have an impact on successful tuber development. There was no difference in the level of emergence between tuber moth infested and uninfested seed in the tubers not treated with NaOCl. However, for the treated seed the final stand density was lower where the seed had been infested with tuber moth. These small differences in emergence were not significant.

There was a clear difference in the final incidence of blackleg between the various treatments. Disease incidence was significantly higher ($P < 0.05$) where plants were emerging from tubers that had been soaked in water compared with those that had been pre-treated with NaOCl. Where plants had been treated with NaOCl, there was a marginal and non-significant ($P > 0.05$) higher incidence of blackleg in plants emerging from infested seed compared to those emerging from uninfested seed. Similarly, where seed was soaked in water the incidence of blackleg was higher where the seed had been infested with tuber moth compared to seed that had not. However, as above, this difference was not statistically significant.

DISCUSSION

Increasing the planting depth of the seed was found to have a significant impact upon the level of subsequent tuber moth infestation. Tubers planted at a depth of approximately 5cm recorded infestation levels of 18.6% while tubers planted to a depth of approximately 10 cm recorded infestation levels of 3.7%. These data are therefore consistent with those of Ali (1993) who observed similar trends in Sudan. In these experiments Ali (1993) found that increasing the planting depth of tubers and more frequent ridging subsequently reduced the infestation level from 16% (planting depth 5cm) to 3.3% (10cm planting depth). Siddig (1988) has also reported similar results from experiments that were also carried out in Sudan. Greater planting depth causes subsequent tuber initiation events to occur at greater depth. One consequence of this is that the developing tubers, which are at a greater depth in the soil, reduce the potential for the adult female moth to gain access to these tubers and lay eggs. Tubers produced in shallow soil are more likely to afford ready access to female moths that lay eggs on the surface of the developing tuber.

Surface sterilizing potato seed tubers with NaOCl (5%) reduced the final level of plant emergence, presumably because of some mild phytotoxic effects. However, there was also a significant reduction, of approximately 50%, in the level of blackleg disease incidence following this pre-planting treatment. Previously NaOCl has been used as a pre-storage treatment. For example, Romdhani and El Mahjoub (1991) found that soaking potato tubers in 1.2% NaOCl for 10 minutes reduced the storage losses by 30-66% depending on the particular potato variety. However, they also found that this pre-treatment failed to eliminate the bacterium from suberized lenticels or from the vascular tissue. There was also a small but non-significant reduction in the level of blackleg disease when plants developed from seed that had been established from non-tuber moth infested tubers (15.8% blackleg incidence) compared to those that developed from infested tubers (14.1% incidence). Overall the data presented in this paper therefore support those collected elsewhere. The data show that both planting depth and pre-treatment with NaOCl may impact on the severity of yield losses caused by blackleg and potato moth to potato crops grown in Oman. Evidence for synergistic losses caused by these two agents was only partial and was not statistically significant.

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Monitoring of Thysanoptera in tropical crops in S. Tomé e Príncipe

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ABSTRACT

The attraction of *Selenothrips rubrocinctus* in relation to yellow, white and blue sticky traps was tested, in cacao, avocado, guava and mango in S. Tomé e Príncipe. There were no significant differences between the traps. However, it is concluded that yellow traps must be avoided: they caught a significantly higher number of potentially beneficial small Hymenopteran parasitoids and yellow traps therefore threaten these taxa. *Megaphragma* sp. (Trichogrammatidae), a parasitoid of thrips, was very abundant. *S. rubrocinctus*, for many years considered the most important thrips pest in these crops in the country, was not the most abundant species. In cacao it constituted 9% against 69% of *Ceratothripoides revelatus*. In fruit crops, *Megalurothrips sjostedti* was the most abundant species (94% in mango, 88% in avocado and 69% in guava). Larvae, pre-pupae and pupae were not detected in the soil, indicating that the pupation process of the species present in the crops studied does not occur there.

INTRODUCTION

São Tomé e Príncipe is an African country constituted by two main islands and several small islets, located in the Golf of Guinea, with an agriculture mainly dominated by cacao production.

For many years, *Selenothrips rubrocinctus* has been considered one of the most serious cacao pests in this country, and the main thrips pest species. Recently, it has also been suspected of causing damage in avocado, guava and mango. If fact, the literature indicates these crops as hosts of *S. rubrocinctus*, along with cashew (Dupont, 1993; Steyn, 1993; Woin *et al.*, 1995; Lewis, 1997; Patel *et al.*, 1997; Young & Poffley, 1997 and Ojelade, 1998). Sanchez-Soto (1998) studying host plants of this species, in Mexico, determined a total of 21 host families corresponding to 34 species.

Damage is caused by larval and adult forms feeding on both the leaf and fruit surfaces and extracting chlorophyll, which results in the browning of the plant tissue (Steyn *et al.*, 1993).

Drops of excreta dry and form brownish mouldy spots. Symptoms in cacao include leaves with widespread interveinal yellow and brown patches, and bronzed pods (Lewis, 1997). The dirty brown colour of pods makes it difficult to determine when they are ripe (Urquhart, 1955). In avocado fruit, severe infestations may cause skin cracking and it was reported that excreta of thrips are visible on discoloured parts of the pericarp, which makes the fruits unattractive, with the consequent loss of commercial value (Lewis, 1997). According to Castel-Branco (1963) and Lewis (1997), *S. rubrocinctus* prefers mature leaves to young ones. The damaged leaves will die if the attack is severe, and trees may be defoliated. Repeated attacks may kill a tree (Urquhart, 1955).

To determine the best colour to be used in sticky traps for monitoring of this pest, its colour preference was tested, as well as the one of the small hymenopteran species present in the crops, which are potential parasitoids of thrips and of other pests. The presence of immatures in the soil was also evaluated, in order to determine if control measures can be directed to the soil, or just towards the canopy.

MATERIALS AND METHODS

Research took place in different cacao areas and in an orchard of mango, guava and avocado, in the island of S. Tomé at the end of the dry season in September 2000, during two periods of 5 days.

Coloured traps

Traps used were 15x10 cm coloured acrylic plates (Plexiglas), 3 mm thick. A transparent polyacetate sheet was stuck to each face of the plate with sticky tape on the top and bottom edges, and then it was covered by a thin layer of Napvis glue (Figure 1a) The colours tested were: white (Plexiglas no. 199), yellow (Plexiglas no. 566) and blue (Plexiglas no. 326).

Traps were hung vertically in the trees, at the lower level of the canopy, in a complete block design: each block, in each tree, with three traps (one of each colour) at approximately the same distance from each other. In cacao, fourteen blocks were constituted (seven in each period of five days). In the orchard there were six blocks in each one of the three crops (three in each period of five days).

The traps were removed from the trees five days later, and in the laboratory the traps were handled according to a methodology developed by Mateus (1998): (1) a sheet of white paper was put over the glue on each trap face (Figure 1b); (2) the insects captured in the traps were therefore lodged between the polyacetate sheet and the white paper; (3) the upper pieces of sticky tape were cut and one of the pairs paper-polyacetate was rotated 360° in relation to the other pair (Figures 1c and d); (4) four layers (polyacetate-paper-paper-polyacetate) per trap were obtained, external faces being those of the polyacetates sheets without glue (Figure 1e); (5) these sets of sheets were stored inside newspapers for some time before being analysed, and the acrylic plates were re-used immediately; (6) immediately before counting the insects, a white paper sheet was put between the other two (already transparent because they were impregnated with glue) (Figure 1f), giving opacity for several minutes, therefore allowing captures in each face of the trap to be separately counted; (after some minutes this paper also becomes transparent owing to the glue); (7)

thrips were extracted from the glue by cutting a small square of polyacetate-paper around each individual (Figure 1g), which was immersed in some drops of commercial petroleum for approximately 10 min to dissolve the glue. This methodology allows the traps to be quickly re-used and the insects to be stored in the glue for some time before analysis. The "traps" can also be easily transported between newspapers or other layers of paper.

ANOVA was conducted for *S. rubrocinctus* in cacao and guava and for Hymenoptera in cacao and the orchard together, at a 5% level of significance. *S. rubrocinctus* numbers were not analysed in mango and avocado, because captures were very low. Data were transformed for normality and homoscedasticy (homogeneity of variance): log (x+1) for thrips and log (x) for Hymenoptera. Means were compared by the Tukey test, at a 5% level of significance, when indicated by ANOVA.

Immatures

Ten mature leaves of cacao were randomly collected in each of six trees, and ten dead leaves fallen on to the floor beneath those trees were also collected randomly. The leaves were washed in water with some drops of detergent, which was afterwards filtered. Thrips larvae, pre-pupae and pupae were counted.

RESULTS

S. rubrocinctus was not the most abundant thrips species in any crop. The highest percentage of *S. rubrocinctus* was observed in cacao (9%). The most abundant species were: *Ceratothripoides revelatus* in cacao (67% of the thrips captured) and *Megalurothrips sjostedti* in the fruit crops (69% in guava, 94% in mango and 88% in avocado) (Table 1).

For S. rubrocinctus, in cacao and in guava, there were no significant differences between thrips captures in the three trap colours. For Hymenoptera there were significant differences between yellow traps and the other, the yellow traps being the most attractive (Table 2).

Table 1. Trap data for thrips. Mean number of thrips per trap, mean number of *Selenothrips rubrocinctus* per trap, and its percentage in relation to the total thrips and most abundant thrips species captured.

Crop	Thrips/ trap	*S. rubrocinctus*/ trap	Most abundant species
Cacao	47.0	4.3 (9%)	*Ceratothripoides revelatus* (67%)
Guava	14.9	1.1 (7%)	*Megalurothrips sjostedti* (69%)
Mango	79.8	0.1 (0%)	*Megalurothrips sjostedti* (94%)
Avocado	27.6	0.7 (3%)	*Megalurothrips sjostedti* (88%)

Standard deviations for thrips and *S. rubrocinctus*: cacao (81.9 and 4.5), guava (7.5 and 2.6), mango (122.6 and 0.2) and avocado (35.1 and 1.5)

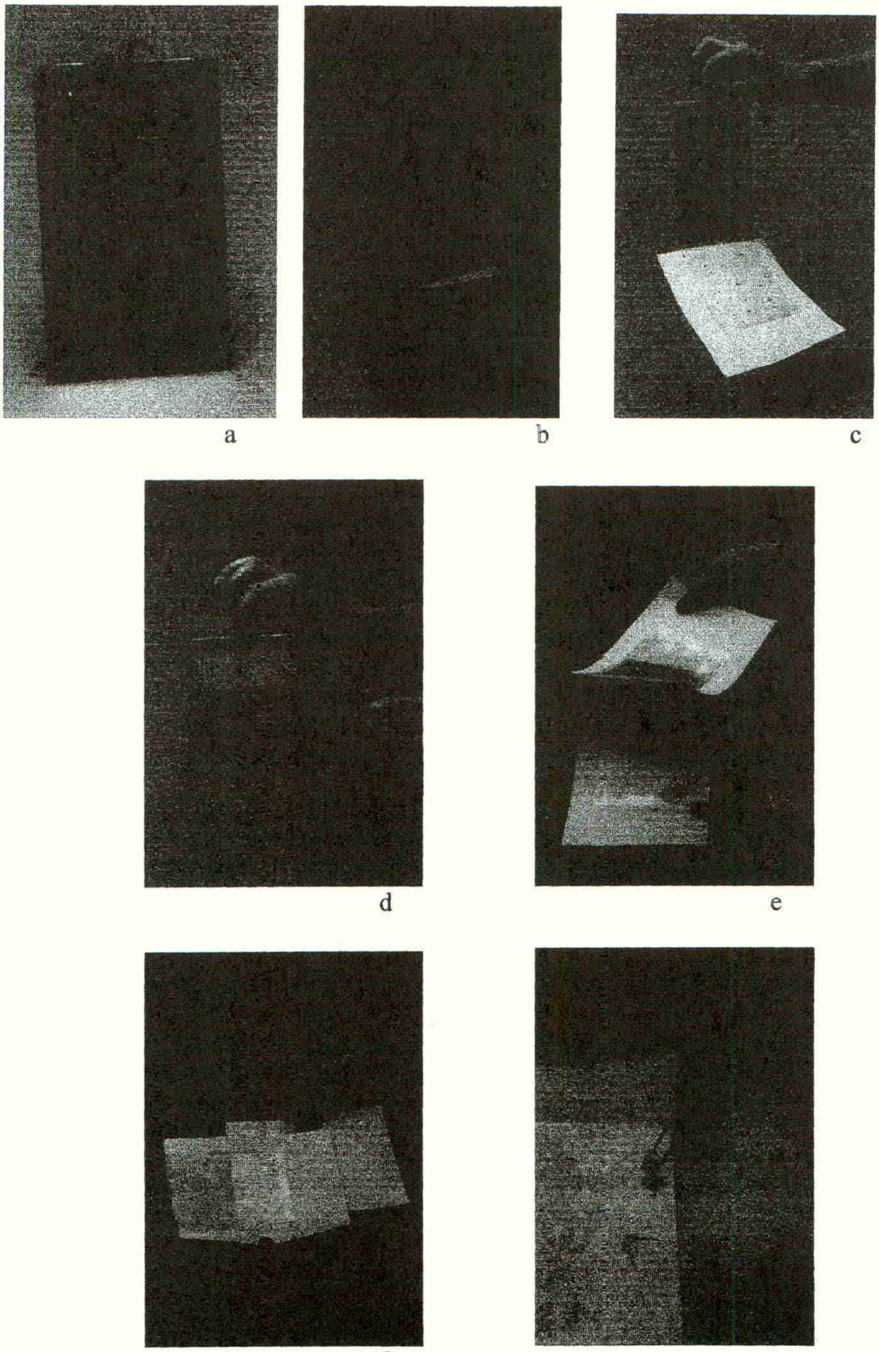

Figure 1. Different steps of traps handling in the laboratory. See text for explanation.

Table 2. Mean number of *Selenothrips rubrocinctus* and Hymenoptera per trap.

Insect species	Crop	Trap colour		
		Yellow	White	Blue
S. rubrocinctus	Cacao	4.3	4.2	4.4
	Guava	2.8	0.2	0.3
Hymenoptera	Cacao + Orchard	27.4 *	15.6	17.3

The mean followed by the * is significantly different from those in the same line at a 5% level. Standard deviations for *S. rubrocinctus* in cacao (3.9 in yellow, 4.7 in white and 5.2 in blue traps); in guava (3.9 in yellow, 0.4 in white and 0.5 in blue traps) and for Hymenoptera (38.4 in yellow, 13.7 in white and 17.4 in blue traps)

No larvae, pre-pupae or pupae were detected in the leaves fallen on to the floor (a total of 60 leaves). By contrast, 59 larvae and 20 prepupae or pupae were collected from the leaves of the trees (a total of 60 leaves), a mean of 1.0 larvae/ leaf and 0.3 pre-pupa or pupa/ leaf. Among them, there were immatures of *S. rubrocinctus*.

A high diversity of small Hymenopteran parasitoids of Diaspididae, Aleyrodidae and Lepidoptera were captured (data not published). *Megaphragma* sp. (Trichogrammatidae), which is a parasitoid of thrips eggs, was very abundant.

DISCUSSION

S. rubrocinctus has been traditionally held responsible for damage by thrips in the cacao crop in S. Tomé e Príncipe, and more recently in fruit crops like guava, avocado and mango. The studies conducted for choosing the best colour to be used in sticky traps for monitoring purposes revealed that this species is equally attracted by the shades of blue, yellow and white tested. So, in what concerns this thrips species, any of these traps could be used with the same catch efficiency. However, in terms of the preservation of the diversity of the parasitoid fauna, yellow traps should be avoided in monitoring programmes, because they were the most attractive ones for Hymenoptera.

Among the Hymenoptera captured, the thrips eggs parasitoid *Megaphragma sp.* was very abundant. This may be explored in the future for biological control actions.

It was also observed that the pupation process of the thrips found in the ecosystems studied does not occur in the soil, and so management strategies must be oriented to the canopy. The use, for example, of soil nematodes or chemical treatments in the soil will have no effect against those species.

Anyway, bearing in mind that *S. rubrocinctus* has been considered the most serious thrips pest in cacao, it was expected to be more abundant. In this crop, it only reached 9% of the thrips captured. *C. revelatus* was the most abundant thrips species in cacao, and *M. sjostedti* was the most abundant in fruit crops. More work must be conducted in order to clarify this situation. The low number of *S. rubrocinctus* captured during the monitoring process may be explained by a seasonal effect, and so monitoring should be continued throughout the year. There may be also a behavioural effect: it is necessary to understand whether the low numbers of *S. rubrocinctus* in traps are due to a low flight activity. The rate of *S. rubrocinctus* damage in relation to other species should also be determined.

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Augmentation of parasitoids in conjunction with pheromones to manage cotton bollworms

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ABSTRACT

The cotton crop is subjected to the attack of three major bollworm species in the Sindh province of Pakistan, viz., pink bollworm, *Pectinophora gossypiella;* spotted bollworm, *Earias vittella*; and spiny bollworm, *E. insulana.* Chemical insecticides provide excellent control in many instances. However, due to the development of resistance in cotton bollworms, alternatives to insecticides are receiving more attention. Management studies of cotton bollworms in the field indicated that augmentative releases of egg parasitoids, *Trichogramma chilonis* and *Trichogrammatoidea bactrae* in conjunction with pheromones, suppressed the bollworms infestation to sub-economic levels. Separate treatment with pheromones or parasitoids was less effective, requiring supplemental measures. Establishment of the parasitoids in the field was low in the hot months of June and July, thereafter gradually increasing during August and September. Due to successive generations, the maximum number of the parasitoids was observed in the month of October. The application of pheromones, PB/SB-ROPE in combination with the parasitoids also cost less than insecticides to manage the bollworms.

INTRODUCTION

Cotton bollworms have become the most difficult pests to control because of the concealed feeding habits of the damaging stage. Chemical insecticides provide an excellent control in many instances. However, resistance in cotton bollworms has been reported against many pesticides (Bariola, 1985). This has required the use of increased amount of insecticides. In addition, pesticides also kill many of the natural enemies of the pests, rendering effective control even more difficult. Van Steenwyk *et al.* (1975) reported that season long application of insecticides in cotton fields, resulted in destruction of the beneficial insects thus accelerating the emergence of secondary pests. Therefore, there is a mounting need to develop alternate, economical and environmentally friendly methods to suppress the infestation of cotton bollworms. Among non-polluting insect management methods, biological control through parasitoids alone (Suh *et al.*, 2000, Orr *et al.*, 2000) or as an adjunct to other management tactics has been successfully used to combat many insect pests (Ahmad *et al.*, 2001). The use of *Trichogramma* wasps as a biocontrol agent is a recognized alternative to insecticides and has been applied successfully for the management of many insect pests (Suh *et al.*, 2000). Biocontrol technology alone has proven successful for the management of sugarcane borers (Ashraf *et al.*, 1999), but for cotton bollworms experience has reinforced the belief that single component strategies may not prove successful and that supplemental measures are required. Application of pheromones is also a very compatible component of biocontrol technology and integration of both these techniques may prove effective for the management of cotton bollworms. Therefore, the present studies were conducted to develop effective, economical and environmentally friendly insect pests management technique for the management of cotton bollworms with particular reference to behavioural and biological control tactics.

MATERIALS AND METHODS

Studies were conducted at farmers field in a semi-isolated area on 100 hectares of cotton during the 1999-2000 and 2000-2001 seasons. The area was divided into five different blocks (approximately 20 hectares) and each block was given a separate treatment. One block was treated with egg parasitoids, *Trichogramma chilonis* and *Trichogrammatoidea bactrae* reared on the eggs of Angoumois grain moth in the laboratory maintained at 25 ± 2 °C and 60-70 % relative humidity. The eggs of Angoumois grain moths were glued on white paper cards and exposed to parasitoids for 24 hours. The parasitoids prior to adult emergence were released by attaching the cards on the lower side of the cotton leaves at fortnightly intervals at the rate of approximately 20,000 parasites per hectare. Two thousand parasitoids were released at ten uniformly distributed locations per hectare. A second block was treated with both pheromones and parasites. The pheromones were applied once in a season at the start of square formation stage of the crop at the rate of 1000 ropes per hectare. The parasitoids were applied at the same rate and interval mentioned earlier starting from the square formation stage of the crop. The pheromone used was experimental, produced by the Shin-Etsu Chemical Company, Japan and supplied by the Karachi office of the Mitsubishi Corporation; this contained the pink bollworm pheromone, gossyplure, a 1:1 mixture of (Z,Z)- and (Z,E)7,11-hexadecadienyl acetate and a 10:1 mixture of the (E,E)-10,12-hexadecadienal and(Z)-11 hexadecenal components for the two *Earias* spp. The formulation is named as PB/SB-ROPE. This 'twist-tie' formulation consisted of a wire-reinforced hollow polyethylene tube, 20 cm in length, sealed at both ends, containing normally 38.5 mg a.i. of gossyplure plus 40 mg of *Earias* pheromone. The pheromones were applied once in a season at the start of square formation stage of the cotton crop. The third cotton block was treated with conventional insecticides. A total of six sprays were applied during the whole cotton season by the farmer himself. The farmer applied two sprays each of organophosphate and pyrethroid groups of insecticides. The fourth block was treated with an egg parasitoid, *Trichogramma chilonis*, alone at the same rate and time as mentioned earlier. The fifth block was treated with pheromones alone at the same rate and time as mentioned above. The infestation of pink bollworm and *Earias* spp. was recorded at weekly intervals. The establishment of the parasitoids were determined by placing Angoumois grain moth eggs in the field for 24 hours. These cards were brought into the laboratory after 24 hours exposure in the differently treated blocks and parasitoid emergence recorded. The cost of the treatments was recorded and the cost benefit ratio for each treatment was calculated.

RESULTS AND DISCUSSION

Results indicated that the infestation of pink bollworm (Table 1) and *Earias* spp. (Table 2) was least in blocks treated with pheromones PB/SB-ROPE in conjunction with egg parasitoids, *Trichogramma chilonis* and *Trichogrammatoidea bactrae*.

Table 1 Mean infestation of pink bollworm in cotton treated with different environmentally friendly treatments.

Treatments	Buds	Flowers	Green bolls
Trichogramma chilonis + *T. bactrae* + Pheromone (PB/SB-ROPE)	3.80^{c}	3.96^{c}	3.58^{c}
Pheromone (PB/SB-ROPE)	6.20^{b}	9.57^{a}	5.67^{b}
Trichogramma chilonis+ *T. bactrae*	5.52^{b}	6.49^{b}	6.84^{ab}
Trichogramma chilonis	6.28^{b}	8.47^{a}	5.76^{b}
Insecticides (control)	11.58^{a}	6.71^{b}	8.48^{a}

Mean values followed by similar letters are non-significant ($P \leq 0.05$)

Table 2 Mean infestation of *Earias* spp. in cotton treated with different environmentally friendly treatments.

Treatments	Buds	Flowers	Green bolls
Trichogramma chilonis + *T. bactrae* + Pheromone (PB/SB-ROPE)	4.27^{c}	3.77^{d}	4.72^{c}
Pheromone (PB/SB-ROPE)	6.40^{ab}	12.70^{a}	6.29^{b}
Trichogramma chilonis+ *T. bactrae*	7.65^{a}	7.00^{c}	5.65^{bc}
Trichogramma chilonis	5.41^{b}	8.02^{c}	8.76^{a}
Insecticides (control)	7.17^{a}	10.99^{b}	7.90^{ab}

. Mean values followed by similar letters are non-significant ($P \leq 0.05$)

However, the infestation in blocks treated with pheromones and egg parasitoids separately was higher as compared to insecticide treated blocks. The infestation of pink bollworm and the two *Earias* spp. remained low in the pheromone and parasitoids treatments indicating their efficiency in the field. However, the establishment of parasitoids was low during the hot cotton growing months. The parasitoid population started increasing in the cotton field as the temperature and relative humidity became favourable (Table 3). The population of the parasitoids gradually started increasing in the month of August and peak parasitization in the field was recorded in the month of November, the harvesting time of the crop. The maximum parasitization recorded in the month of November may be attributed to the successive generations produced by the parasitoids, favourable environmental conditions and presence of the host eggs in the field. Qureshi *et al.* (1985, 1989) observed the peak population of pink and spiny bollworms in the field during the month of October. Jones *et al* (1979) observed that crop microclimate is influenced by plant size, density and architecture. This has been shown to have a substantial effect on *Trichogramma* (Kot 1979; Orr *et al.*, 1997). In the present studies we observed that when the cotton plants were small in the field, establishment of the parasitoids was low but it started increasing as the development of cotton plants increased and more favourable microclimatic conditions of the cotton field prevailed.

Table 3 Mean percentage parasitization of the egg parasitoids in the field during different cotton growing months.

Months	Parasitism percentage	Mean Temp. (^{0}C)	Mean R.H. (%)
June	0.25^e	33.60	63.0
July	0.26^e	31.87	74.8
August	1.57^d	30.65	74.1
September	10.89^c	30.10	74.3
October	23.50^b	27.10	66.5
November	38.51^a	20.90	62.8

Mean values followed by similar letters are non-significant ($P \leq 0.05$)

The successful experimental management of cotton bollworms through pheromones reported here is not in conformity with the results of Henneberry *et al.* (1981). They reported that the infestation of pink bollworm was significantly less in pheromone treated areas as compared to insecticides on large isolated areas in the United States of America. This discrepancy in the results may be due to our cotton blocks not being well isolated (approximately 500 m apart) and consequently there may be some interaction between the treatments. The pheromones dispenser applied only once on the young plants may quickly lose their effectiveness because they would be below the canopy. Shorey *et al.* (1976) observed that dispensers of hexalure placed at the tops of cotton plants gave respectively 82 and 97 % better disruption of pink bollworm communication than dispensers placed mid-way down the cotton plant or on the ground. This might be the reason that in our studies pheromones alone were not as effective as combined treatment with pheromones and parasitoids. However, the amount of pheromones used in the present studies may have been sufficient to reach the upper canopy and achieve mating disruption of the bollworms. Gillespie *et al.* (1982) observed that the amount of gossyplure was more important in reducing trap catches and the disruption of mating of the pink bollworm than the number of point sources dispensing the pheromones. On the basis of these results, placement of the dispensers does not seem to be critical for disruption, if sufficient quantities of the pheromones are used.

Beneficial insects have long been recognized as an important tool, and in many cases they have controlled the pests well enough to eliminate the need for further treatment. However, besides such success this classical management strategy has not always provided adequate control (Caltagirone 1981), because of the need for periodical releases and environmental management, such as providing food or hosts during time of low prey density. The potential of management techniques of pheromones or biocontrol strategies against cotton bollworms may be increased by applying them together in Pakistan due to the lack of natural barriers.

Table 4. Cost economics of different environmentally friendly techniques and insecticides for the management of cotton bollworms.

	Treatments				
	T.. chilonis+ *T. bactrae*+ Pheromones	Pheromones	*T.chilonis* + *T.bactrae*	*T.chilonis*	Insecticides (control)
Cost of treatment/ ha (Rs.)	5200	4000	1200	600	4375
Application charges/ha (Rs.)	75	50	25	12.5	1250
Total cost (Rs.)	5275	4050	1225	612.5	5625
Seed cotton yield/ ha (Kg)	2900	1900	17500	1350	2462.5
Total income/ ha (Rs.)	26100	17100	15750	14400	22162.5
Net income/ha (Rs.)	59975	38700	38150	35387.5	49781.25
Benefit over insecticide (Rs.)	+ 10193.75	- 11081.5	- 11631.25	-14393.75	--
Cost economic ratio over insecticide	1 : 1.20	1 : 0.77	1 : 0.76	1 : 0.71	--

Studies revealed that more yield was achieved from the integrated treatment of cotton as compared to the separate treatments and the control insecticide treatment (Table 4). Although, the management cost was higher in the integrated treatment, the benefit achieved was also higher. The application costs of pheromones and parasitoids were very low as compared to insecticides. Pheromones are much more costly than insecticides in themselves but these are applied once in a season and their application costs only Rs. 50/- per ha. In terms of monetary benefits, Rs. 14245 more were obtained by integrated treatment of pheromones and the parasitoids as compared to insecticides treatment. Moreover, integration of these environmentally friendly techniques are species-specific and non-hazardous which did not disturb the beneficial insect fauna.

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