SESSION 9C

NEW RESEARCH IN BIOLOGICAL CONTROL AND PLANT BREEDING

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PRESENT STATUS OF BIOLOGICAL CONTROL OF THE COFFEE BERRY BORER HYPOTHENEMUS HAMPEI

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ABSTRACT

Two of the three known parasitoids of Hypothenemus hampei have recently been shipped to Mexico and Ecuador. The third cannot be reared in the laboratory at present and cannot be shipped directly because of the risk of spreading coffee diseases. Beauveria bassiana and entomogenous nematodes are pathogenic and their integration into control schemes requires investigation.

INTRODUCTION

The coffee berry borer, Hypothenemus hampei, is the most serious insect pest of coffee in many coffee-producing countries. It was first recorded from West Equatorial Africa in the first decade of the century, then from East Africa and by 1925 it was also known from Indonesia and Brazil (Le Pelley, 1968). More recently it has been confirmed from the Caribbean, Central America and Ecuador (Reid, 1983; Quezada, 1985; Klein Koch et al 1987). Now, Colombia is probably the only major coffee producing country where it is absent but its arrival from Ecuador is imminent.

The female beetle enters the coffee berry and bores tunnels in which eggs are laid. The larvae cause further damage by feeding. During this time the berry may fall from the bush. Although a second generation may occur within the berry, brood females usually leave to find new berries to attack.

Economic losses are due to increased fall of young berries and, more importantly, yield loss and quality reduction in the harvested crop. Up to 90% of berries may be attacked (Le Pelley, 1968; Reid, 1983) and yield losses can reach 40-80% (Le Pelley, 1968). Recently Reid & Mansingh (1985) showed that 21% of the Jamaican crop is lost to export because of H. hampei damage.

CONTROL MEASURES

Pesticides Pesticides such as gamma HCH and dieldrin are used, with endosulfan increasingly being shown as one of the most effective. However, resistance to endosulfan has been reported in New Caledonia (Brun & Ruiz, 1987). Other problems with pesticides include tainting of the coffee, cost, residues in the crop and the practical difficulties of machinery availability and effective application in difficult terrain; all are compounded by the need

for frequent application.

Cultural Many cultural methods have been used (Le Pelley, 1968), of which the most effective is total removal of all berries from ground and bushes after the main harvest, to interrupt the reproduction of the pest. This is often too labour intensive to be practical and is not viable, except for collection of fallen berries, where coffee production is continuous. Increasing the harvesting frequency also limits pest reproduction, but this too is labour intensive (Koch, 1973).

Plant resistance Le Pelley (1968) states that Coffea arabica is the most susceptible followed by C. canephora (Robusta) with C. excelsa and C. liberica being less attacked. However these relative susceptibilities can vary as may susceptibility between cultivars within species (Koch, 1973).

Quarantine H. hampei has limited dispersal ability and its spread has been due mainly to human activity. Quarantine has been inadequate, as shown by the worldwide spread of the pest. Colombia is aware of the quarantine requirements but the pest is likely to enter from Ecuador where it occurs close to the border and where quarantine services are limited.

Classical biological control Three hymenopteran parasitoids of H. hampei are known; two bethylids, Prorops nasuta and Cephalonomia stephanoderis, and a braconid, Heterospilus coffeicola. Earlier this century P. nasuta was shipped from East Africa to Indonesia, Sri Lanka and Brazil and in 1962 to Peru. In Brazil the climate was thought unfavourable for its survival and the use of gamma HCH worked against it. Even so it was recovered recently (Yokoyama et al., 1978) and infested berries containing parasitoids are being moved to other coffee producing areas (C. Klein Koch, pers. comm.), indicating that its presence is thought to be of some value, although it has not achieved significant control.

C. stephanoderis was discovered in Côte d'Ivoire by Ticheler (1961) who recorded parasitism of up to 50%. (Koch, 1973) found that the parasitoid reduced H. hampei populations by 20-30% towards the end of the coffee season, and by only 5% between seasons.

H. coffeicola has not been reared in the laboratory so far, thus an introduction would entail using field-collected material without rearing under quarantine. Since many insects including parasitic wasps can transmit plant diseases, and the major coffee disease Colletotrichum coffeanum (Coffee Berry Disease) occurs in East Africa but not elsewhere, direct shipment of H. coffeicola cannot be considered at present.

Little consideration has been given to the introduction of pathogens to control insect pests in outbreak areas. Forest scolytids are known to harbour a number of specific nematodes and protozoa (Mills, 1983) and the exploitation of these organisms as classical biological control agents is worth considering, but none are known at present from Hypothenemus hampei. Mills (1983) did

not note virus diseases in his review of natural enemies of forest scolytids and none are known from H. hampei.

Beauveria bassiana has frequently been recorded from H. hampei in the Old and New World and has been suggested as a viable mycopesticide (Pascalet, 1939) but advances in application techniques appear to be required.

RECENT DEVELOPMENTS IN COFFEE BERRY BORER CONTROL AT CIBC

CIBC is supplying Ecuador and Mexico with parasitoids. Since quarantine measures are vital, parasitoids are shipped only after being reared for a generation in CIBC's quarantine facilities in UK.

In 1987 P. nasuta was shipped from Kenya, via UK to Ecuador where rearing, releases and recovery are progressing well (C. Klein Koch, pers. comm). Early in 1988 C. stephanoderis and P. nasuta were reared from material collected in Togo where P. nasuta was considered rare (Ticheler, 1961; Koch, 1973). C. stephanoderis was shipped to Ecuador and Mexico and is rearing well in Ecuador (C. Klein Koch, pers. comm.) This was the first use of C. stephanoderis in a biological control programme. The interaction between it and P. nasuta populations derived from East Africa where C. stephanoderis does not exist will be very interesting.

Work is being conducted on Heterospilus coffeicola by CIBC in UK and Kenya. Another Heterospilus sp. can retain Colletotrichum gloeosporioides spores on its body in the laboratory and these remain viable for at least 14 days (Nemeye, 1988). Experiments on the ability of this Heterospilus sp. to transmit C. coffeanum are currently in progress. This justifies the precaution of demanding either the rearing of a generation under quarantine or developing measures to clear the pathogen spores from the insect's body. In Kenya CIBC has managed to mate H. coffeicola in captivity as a first step in developing a laboratory rearing system so that it can be reared in UK quarantine and then shipped to outbreak areas.

The nematode Heterorhabditis sp. can kill larval and adult Hypothenemus hampei and complete its life cycle in adults and older larvae (G. Allard & D. Moore, in prep.). It may be possible to use nematodes to treat fallen berries, obviating the need to collect these and so allowing them to remain as a mulch. Dispersing H. hampei adults may also spread the nematodes within the pest population.

Work with B. bassiana showed that water suspensions of the fungus killed H. hampei in 5-7 days depending on dosage. At the high rates the insect may die before laying eggs, but use of a lower rate might allow dissemination of the fungus by dispersing adults.

POTENTIAL DEVELOPMENTS IN BIOLOGICAL CONTROL OF H. HAMPEI

The known parasitoids sustain themselves in the pest

population but now that successful introductions have been made into new environments where the pest is severe, the possibility of mass-rearing to augment normal parasitoid levels should be considered. This could be particularly valuable where a pronounced cropping season occurs and attempts are made to "clean up" all remaining berries at the end of the main harvest. Berries on the bush contain more parasitoids than those on the ground and these berries could be used to build up colonies of parasitoids in readiness for an augmentative release at the start of the next season's pest outbreak. Such parasitoid culture would be extremely simple, cheap and suitable for smallholder farmers. Allied to cultural methods, such as removal of shading which inhibits parasitoid attack, control by parasitoids could be greatly enhanced.

Fungal pathogens recorded from H. hampei include Metarhizium anisopliae, Paecilomyces tenuipes (=Spicaria japonica), Nomuraea rileyi (=Botrytis rileyi) and Beauveria bassiana, but attention has focussed on the last, which is the most frequently recorded. Within the original range of H. hampei the recorded incidence of B. bassiana varies greatly. Pascalet (1939) noted it was the most effective natural control agent in Cameroon and recommended using it for control, but Ticheler (1961) found it relatively rarely in Côte d'Ivoire and it is also uncommon in Kenya (S. Murphy, pers. comm.). High levels of the pathogen have been observed in the field in Mexico (J. Barrera, pers. comm.) and Ecuador (Klein Koch et al., 1987). While these differences may be due to climatic factors, it is also possible that the strains of B. bassiana attacking H. hampei in the New World are not co-evolved African ones, but local strains which have moved onto the pest from native hosts. No comparison of the virulence of African and Latin American isolates has been carried out, but instances are known where strains of entomophagous fungi from other hosts are more virulent to the target pest than those from the host itself (e.g. Alves et al., 1984).

B. bassiana causes epidemics on borers and high mortality when climatic conditions are suitable but natural outbreaks are not reliable enough to give effective control. It has been appreciated recently that an important factor restricting the infectivity of naturally occurring fungal pathogens is their limited capacity to spread. Uniform dispersal of infective formulations by efficient spray application equipment overcomes this constraint and this realisation has resulted in the development of several effective mycopesticides.

H. hampei appears to be a very suitable target for B. bassiana when applied as a myco-insecticide, since the pest is reported to be more severe under wet conditions, which are exactly the conditions under which B. bassiana is likely to be more effective, the pathogen is non-tainting to the coffee berries and the disease can spread inside berries to destroy pest colonies. The fungus can be applied using conventional machinery such as hydraulic knapsacks or motorised mistblowers. The fungus could be prepared on cheap, locally available materials, with a consequent saving of foreign exchange expenditure on pesticides.

CIBC has isolates of B. bassiana from Jamaica, Mexico, Ecuador, Togo, Kenya and Sri Lanka. It is easy to grow on simple media such as rice grains or maize, and there is much literature on its use against pests in many countries. It is interesting that the most extensive use has been in China against corn and forest pests where the fungus has been grown by farmers in very simple production units (Hussey & Tinsley, 1981).

DISCUSSION

No studies have been carried out on H. hampei levels when all three parasitoids are present. Heterospilus coffeicola attacks during the early stage of the life cycle but the two bethylids are more active at a later stage and therefore the parasitoids would probably not compete with each other. From present knowledge, pest populations may be reduced by 20-30% by Cephalonomia stephanoderis and perhaps by 20% by P. nasuta in areas of continuous production where parasitism is optimal. However, it is unlikely (although possible) that the combined effect of all three parasitoids would reduce populations by more than 50% unless their levels were artificially augmented, and this has not yet been tried. Thus pesticide application may also be necessary to give acceptable control, but conventional pesticides are inimical to parasitoid success. By contrast, some strains of B. bassiana are host specific and their pathogenicity may be confined to one order or even family of insects (Keyserlingk, 1980).

Recent progress on spray machinery, such as the development of Ultra Low Volume (ULV) application methods gives new scope for the fungus as a potential control agent, when allied to recent work on formulation. B. bassiana is over 30 times as infective when applied in oil compared with water, perhaps because the oil spreads the conidia more effectively over the insect cuticle (Prior et al., 1988). Oil formulation would allow the pathogen to be applied using ULV techniques with a consequent saving in labour and water.

There would appear to be good prospects for an integrated control programme for Hypothenemus hampei involving parasitoids, perhaps with augmentative release, entomopathogenic nematodes for suppression of populations in fallen berries, and applications of B. bassiana as a mycopesticide to the aerial parts of the plant. One possible problem is the use of fungicides, particularly copper, to control coffee leaf rust. Copper is toxic to B. bassiana, but recent work in Colombia (T. Wiles, pers. comm.) has demonstrated that leaf rust control is possible at very much lower rates of copper than are used at present. This improvement, combined with careful spray timing, perhaps by simply applying the B. bassiana a few days before the fungicide, could eliminate the problem.

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HIRSUTELLA SPHAEROSPORA AS A POTENTIAL BIOCONTROL AGENT OF RASTROCOCCUS INVADENS WILLIAMS

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ABSTRACT

The mealybug Rastrococcus invadens was accidentally introduced recently into West Africa, where it has become a serious pest of fruit trees and ornamental plants. The entomopathogenic fungus Hirsutella sphaerospora was isolated from dead specimens of the pest collected in Togo. The fungus grows on a variety of simple defined media and produces both conidia and sclerotia. Laboratory trials showed a high level of mortality in R. invadens following inoculation with fragmented mycelia; treated populations of mealybugs declined rapidly and subsequent populations also became infected by the fungus. H. sphaerospora appears to be a promising biocontrol agent.

INTRODUCTION

In 1982 an unidentified mealybug of the genus Rastrococcus was found infesting fruit trees and ornamental plants in Ghana. It has since become an increasingly serious pest, especially of mango and citrus, in Ghana, Togo, Benin and Cote d'Ivoire, but occurring on plant species from at least 22 different plant families (Agounke et al., in press). The mealybug was identified as a new species, Rastrococcus invadens, originating from the Oriental Region (Williams, 1986). Because of its severity and problems with conventional control, the Food and Agriculture Organisation of the United Nations funded a biological control project, which began in June 1986. One component was the study of the entomopathogen Hirsutella sphaerospora, isolated on two separate occasions from R. invadens collected in Togo. H. sphaerospora was first described on larvae of Eriococcidae in the Galapagos Islands (Evans & Samson, 1982), but there have been no studies of its pathogenicity.

Relevant details of the life cycle of R. invadens are as follows. The adult female produces about 180 young, most of which emerge in the first three weeks of her reproductive period. At 25°C the young pass through two instars in about 20 days and then 75-80% develop as males through two pupal stages and the remainder develop to adult females in another 10 days. Before the males enter the pupal stages there are no obvious differences between the young that will develop as males and those that will develop as females. The adult female has a pre-reproductive stage of between 12-20 days. (Willink & Moore, in press). In the present work, as the pupal stage is not affected by H. sphaerospora, the male population as represented by pupae or adult males is ignored

when counting live mealybug numbers. The mealybug population is assessed by counting first and second instars, which may develop as male or female, and third instar and adult females. Hence the initial population cycle is of a rapid increase in numbers of young followed by a decline which is a result of most second stage larvae developing as males. This in turn is followed by an increase as new adult females begin producing young.

The present work demonstrates pathogenicity of the fungus against R. invadens at high and low pest densities and persistence of the pathogen on the host plant.

METHODS

Production of fungal inoculum

Pure cultures of H. sphaerospora were grown at 25°C in a synthetic medium of $CaCl_2$ (0.01 g/l), dried $MgSO_4$ (0.5 g/l) dried KH_2PO_4 (1.5 g/l) glucose (10 g/l), yeast extract (5 g/l) and peptone (0.5 g/l). The cultures were produced while held in a rotary action flask shaker at 150 rev/min.

The fungal material used for the inoculum consisted of fragmented dried mycelium produced by filtering the fermentation media and discarding the liquid filtrate. The mycelium was suspended in an equal volume of water which was then removed by vacuum. The resultant mat was sprayed with a 10% (w/v) aqueous solution of glucose to saturation and then incubated at 22-26°C and 20-40% RH for 4-5 hours. This was followed by incubation at 5°C and 95-100% RH for 18 hours, rapid drying at 20-26°C and grinding of the mycelium.

Inoculation

For inoculation the powder was added to water containing Tween 20 wetting agent at a concentration of 0.01% and the suspension sprayed onto plants infested with R. invadens. Humidity was maintained at 100% by enclosing the plants in plastic bags.

Culture of R. invadens

The mealybugs were reared on citrus plants grown in a quarantine glasshouse and the experiments were done at 25±1°C, 80±5% RH and a light intensity of 10,000 lux in a light regime of 16 h light and 8 h dark. Infestations for the experiments were achieved by placing eight adult females onto each plant and leaving them for three weeks to produce young.

Effects of multiple application on mortality

To determine the effect of the number of applications on mortality, three plants infested with mealybugs were sprayed with a 4% (w/v) solution of fragmented mycelia. One of them was sprayed once, another twice and another three times, with an

interval of fifteen days between applications. Another infested plant was used as a control but there was no replication. The plants were examined weekly for two months after the first inoculation and the total number of live insects (all first and second stage, third stage and adult females only) per plant recorded. The test was done at both high and low initial mealybug infestations.

Importance of pathogen persistence on the plant surface

Plants were infested in the usual way and treated with 4% wv suspensions of H. sphaerospora which eradicated the mealybugs. Reinfestation was then done by adding eight adults to each plant. The plants were checked weekly for two months to score the numbers of live insects. Again males were not assessed.

RESULTS

Figure 1 demonstrates the effect of the number of fungal applications on mortality. For both trials the control results are very similar with a decline in numbers over about a three week period, resulting from development of the males, followed by an increase as females become adult and begin producing crawlers.

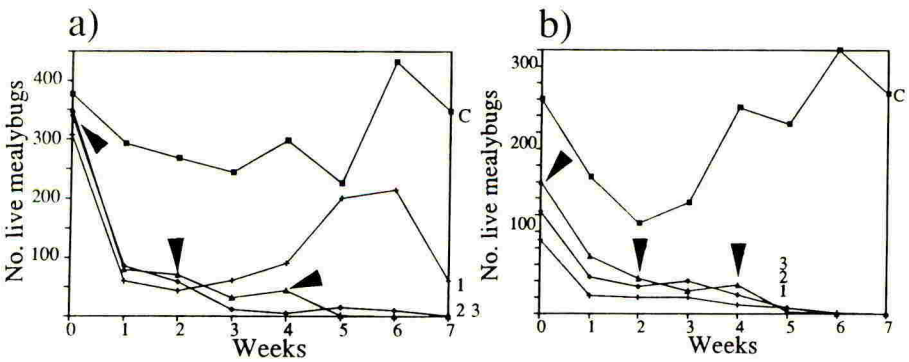

Fig. 1. The effect of different numbers of applications of H. sphaerospora on numbers of R. invadens.
a) High initial host density.
b) Low initial host density.
C = Control
1,2,3 = Number of applications.
◄ = Time of application.

Another feature of both graphs is that the numbers of live mealybugs on the plants treated with H. sphaerospora dropped rapidly after treatment. The reduction in live mealybugs was due to the parasitic nature of the fungus; scanning electron microscopy clearly showed conidiophores of H. sphaerospora

bursting through the cuticle of the host after spreading through the body of the mealybug (E. Fernandez Garcia, in prep.). In both experiments, and for all plants treated with the fungus, most mortality was caused in the first week after treament. In experiment 1 (Fig. 1a), where initial mealybug populations were high, the populations on the plants treated once recovered to some extent and began increasing again at four weeks after application, then decreased due to further infections. There was no difference in the mealybug populations treated twice or three times nor was there any definite recovery amongst the multiply-treated populations.

In the second experiment (Fig. 1b) initial mealybug populations were relatively low (the high levels in the control were an unfortunate consequence of purely random selection of plants for treatments). Again the pathogenic action of the fungus had its effect quickly, mainly within the first week. In this instance there was no recovery in the populations on the plants receiving the fungus and by week five the mealybug populations had been totally eliminated.

In the third experiment the mealybug populations were again eliminated by the fungus (Fig. 2). For experimental purposes three applications were made to ensure total kill. After reinfesting the plants with eight reproducing adults, mealybug numbers did begin to rise, but seven weeks later R. invadens numbers were held at a low level by fungus attack, showing that sufficient inoculum had persisted to establish another epizootic.

In all experiments, first and second stage R. invadens were more susceptible to the fungus than were the third stage and adult females.

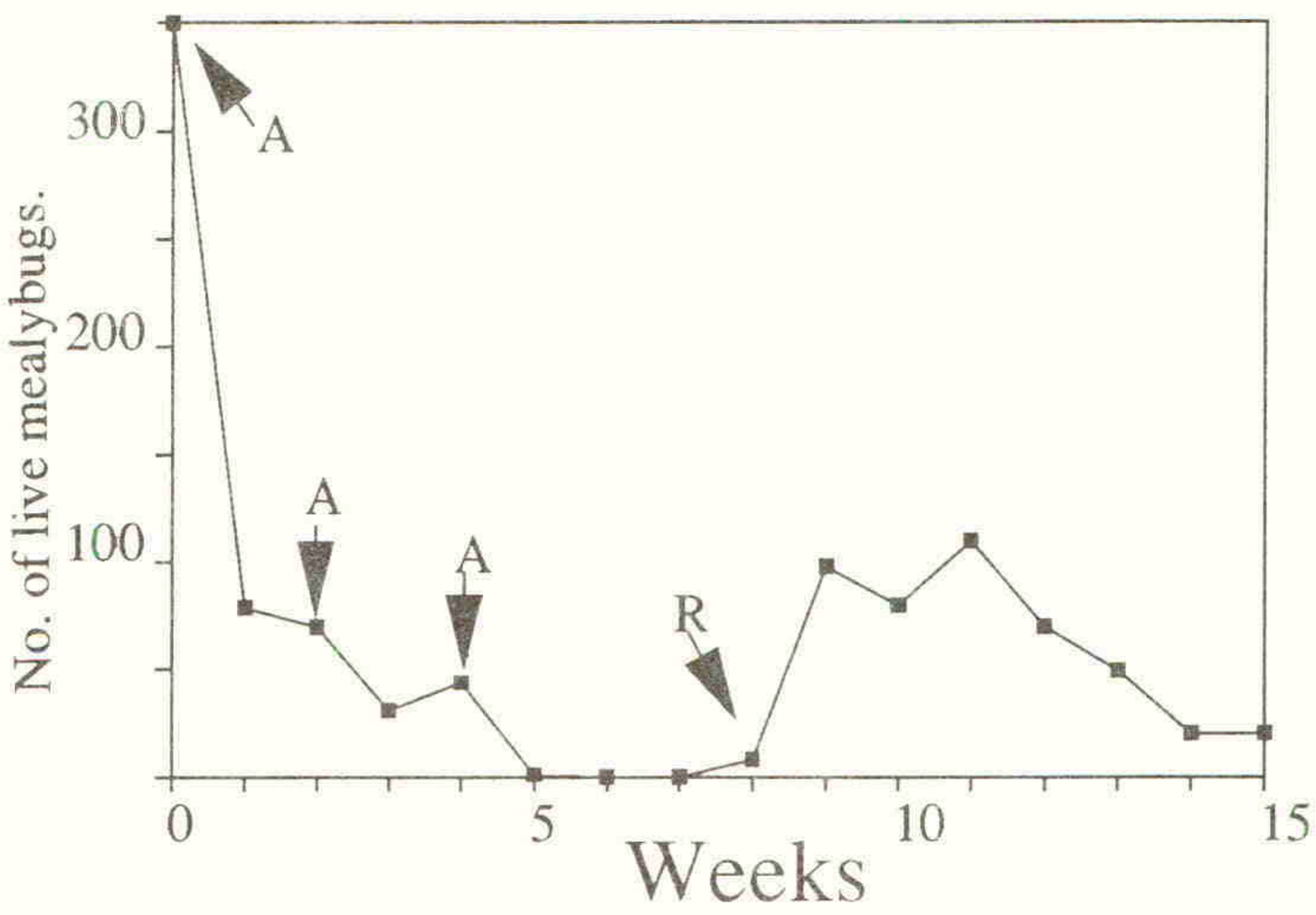

Fig. 2. The effect of residual H. sphaerospora on subsequent R. invadens populations.
A = time of application.
R = time of re-infestation.

DISCUSSION

One aim of this work was to demonstrate the parasitic nature of the fungus. A number of fungi have been recorded as pathogenic to mealybugs (e.g. Dick, 1969), but many of these records are old, were made by entomologists rather than pathologists and a number are dubious. For example Le Ru et al. (1985) have recorded only three species of fungi in the Entomophthorales as being pathogenic to mealybugs, expressed reservations about two of them and considered only one, Neozygites fumosa, to be truly parasitic. This was recorded from the cassava mealybug, Phenacoccus manihoti, by Le Ru (1986) who considered it to be the most significant indigenous natural control agent. H. sphaerospora is thus one of very few fungi proven to be pathogenic to mealybugs.

The work also suggested that one or two applications could exert control over the mealybug populations on a single plant for an appreciable time (given high humidity), probably due to subsequent populations becoming infected from fungal propagules bursting out from dead hosts.

The young may be more susceptible to the fungus because their cuticle has a thinner wax layer than adults.

H. sphaerospora has many of the desirable attributes of a microbial control agent (Burges & Hussey, 1971) and may have potential as a mycopesticide. It produces both asexual spores and resistant vegetative propagules on simple defined media and both mycelia and sclerotia are capable of infecting R. invadens under optimal conditions (E. Fernandez Garcia, in prep.). It is relatively easy to culture and appears suitable for mass production, possibly on a commercial scale. It is likely to show a high level of specificity and hence should be environmentally safe: in specificity tests H. sphaerospora failed to infect a number of other Pseudococcidae. The centre of origin of the fungus is not known, but it might have originated from the Far East (the area of origin of R. invadens) and could have arrived with the mealybug. However, it is more likely to have transferred from an insect already present in West Africa (H.C. Evans, pers. comm.). In the latter case the fungus would obviously not be strictly specific to R. invadens.

Further work is required, especially under field conditions, but preliminary evidence does suggest that H. sphaerospora is a potentially useful agent against R. invadens and possibly one open to commercial exploitation.

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LIFE TABLE ANALYSIS OF TRITROPHIC INTERACTIONS: CASSAVA, *MONONYCHELLUS PROGRESIVUS* AND *TYPHLODROMALUS LIMONICUS*

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ABSTRACT

Life tables were constructed for the tetranychid mite *Mononychellus progresivus* on 14 cassava clones. Mites reared on clones which resulted in the most extreme life table parameters were offered as prey to the phytoseiid predator *Typhlodromalus limonicus*. Survival and development of *T. limonicus* on prey from each clone were compared. Clones which were most favourable as hosts for *M. progresivus* produced the lowest quality prey for *T. limonicus*.

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) evolved in the neotropics under highly localized biological and physical influences (Lozano & Bellotti 1980) which led to the formation of characteristic arthropod complexes in each of the major edaphoclimatic zones where it is grown. Most arthropods which feed upon cassava attained major pest status after accidental introduction to previously uninfested areas, or as a consequence of replacement of traditional cultivation practices by extensive monoculture. Two major mechanisms of suppressing arthropod populations are built into traditional cassava cultivation in the neotropics: 1) the existence of locally-adapted gene pools which resulted from the selection of superior clones by farmer-breeders over a period of centuries and, 2) the presence of a large complex of natural enemies associated with each of the major cassava pests. Cassava's natural history suggests that the most effective way of dealing with pest outbreaks both outside and within the neotropics is to recreate as closely as possible the conditions which formerly maintained pests below economically important levels.

The present paper evaluates the effect of cassava clone on the most widely-distributed and specialized natural enemy known (Acari: Phytoseiidae *Typhlodromalus limonicus* Garman & McGregor) of the cassava green mite (CGM) (Acari: Tetranychidae *Mononychellus progresivus* Doreste), which became a serious pest after accidental introduction to Africa in the 1970's.

MATERIALS AND METHODS

Fourteen cassava clones were selected from the CIAT cassava germplasm bank including six whose degree of field resistance to CGM was known. Life tables were constructed for CGM reared on each clone. Colonies of CGM were subsequently established on five clones with extreme CGM life table parameters and life tables were constructed for *T. limonicus* offering CGM reared on each clone as prey. Since *T. limonicus* were taken from a laboratory colony on the clone CMC 40, life tables were constructed for parents and for their F_1 offspring in order to be able to separate effects of the previous host. All experiments were conducted at 25°C; 70 ± 5% r.h.;

12L:12D photoperiod. Specific methods for each species are detailed below. Analysis of variance was performed where appropriate and logarithmic transformation was used where necessary; however, only untransformed data are present.

CGM life tables

Colonies of CGM were established in the screenhouse (30 ± 5°C; 70 ± 5% r.h.) on potted plants of the clone CMC 40. Gravid females from the colonies were transferred to detached lobes of cassava leaves of each clone placed on water-saturated sponges in plastic petri dishes (15 cm diam, 2 cm height). After 6 hours, the females were removed and their eggs were observed every 3–4 hours between 8 a.m. and 8 p.m. until completion of development. Cohorts of 100 eggs were observed per clone. At the teliochrysalis stage, each leaf lobe was supplied with an adult male. After copulation, each female was transferred to a transparent plastic vial (2 cm diam; 1 cm height) lined with a moistened disc of filter paper under a leaf disc of the same clone on which she had been reared. The vials were sealed with adhesive plastic and the number of eggs laid was recorded each day until the death of the female. Mites were transferred to fresh leaf discs every 2 days.

T. limonicus life tables

Gravid female T. limonicus were transferred from a laboratory colony to detached lobes of cassava leaves of each clone as described above for CGM. After 12 hours the females were removed and 100 eggs per clone were placed individually in the plastic vials described for CGM. These were lines with discs of each clone cut from leaves infested with CGM. Observations were made every 3–4 hours from 8 a.m. to 8 p.m. and supplemental M. progresivus were supplied to mobile stages of T. limonicus as necessary to ensure that prey were available in non-limiting quantities. Individuals were transferred to new leaf discs every 2 days. Females were mated after emergence from the teliochrysalis and oviposition was recorded daily until the death of the female. One hundred eggs per clone were placed individually in plastic vials containing leaf discs of the same clone on which their mother had been reared, and all observations were repeated for the F_1 generation.

RESULTS

CGM life tables

Percent survival of CGM from egg to adult was 85% or greater in tolerant and susceptible clones and 76% or less in clones known to be resistant. Development time was significantly shorter ($P < 0.05$) on tolerant and susceptible than on resistant clones (Table 1). CGM reared on clones which resulted in early first reproduction and in long-lived adult females produced significantly more eggs per female (Table 2). The resistant check clones tended to delay reproduction, and to produce short-lived females with low fecundity, whereas the susceptible and tolerant checks resulted in the opposite. There was a nine-fold difference in per female egg production between the resistant clone MCUB 72 and the susceptible CMC 40. CGM sex ratio was also affected by clone with the most extreme values in CM507-37 (1.9 females/male) and in CM696-1 (7.2 females/per male). The average sex ratio was 4.0 (S.D. = 1.7).

TABLE 1

Effect of cassava clone on survival and development of Mononychellus progresivus

Clone	Resistance rating	% survival egg to adult	Egg to adult development time (days)
MBRA12	T	99	9.9 H
CG5-79	T	95	9.9 H
CMC 40	S	94	10.3 F
MCOL 22	S	87	10.6 DE
MCUB 74	U	86	11.5 B
MMEX 59	U	84	10.2 FG
CM1091-2	U	82	10.5 E
CM696-1	U	82	10.0 GH
CM723-7	U	82	10.2 FG
CG427	U	81	10.9 C
CM507-37	U	77	11.5 B
MCUB 72	R	76	12.2 A
MCOL 1351	R	58	10.7 CD
MECU 85	U	56	11.3 B

T = tolerant, S = susceptible, R = resistant, U = unknown. Values followed by different letters are significantly different (Duncan's Multiple Range Test; $P < 0.05$).

TABLE 2

Effect of cassava clone on reproduction of Mononychellus progresivus

Clone	Resistance rating	Age of first reproduction (days)	Adult female longevity	Total eggs per female
MCUB 72	R	13.9 A	5.4 E	6.3 E
CM507-37	U	13.5 B	11.4 BC	7.3 E
MCUB 74	U	13.2 C	9.8 C	16.4 CD
MECU 85	U	13.1 C	10.9 C	13.9 D
MCOL 1351	R	12.7 D	7.8 D	6.6 E
CG427	U	12.2 E	11.4 BC	13.0 D
MCOL 22	S	11.9 F	12.6 BC	15.4 D
CM1091-2	U	11.6 G	8.2 D	16.3 D
CM723-3	U	11.6 G	11.1 BC	38.4 B
CM696-1	U	11.6 G	9.6 C	22.1 C
MMEX 59	U	11.5 G	9.9 C	29.5 B
CMC 40	S	11.1 H	26.1 A	59.3 A
MBRA 12	T	11.0 H	13.1 B	26.9 B
CG5-79	T	11.0 H	11.8 BC	36.0 B

T = tolerant, S = susceptible, R = resistant, U = unknown. Values followed by different letters are significantly different (Duncan's Multiple Range Test; $P < 0.05$).

Net reproductive rate was highest in susceptible and tolerant clones and lowest in resistant clones. Mean generation time did not show a clear pattern with respect to resistance status; however population doubling times ($\log_e$ [2]/r) were short on susceptible and tolerant clones, and long on resistant clones suggesting that clone had a greater influence on reproduction than on development of CGM (Table 3).

TABLE 3

Effect of cassava clone on life table parameters of Mononychellus progresivus

Clone	Resistance rating	R_O	T	DT
CMC 40	S	36.5	19.5	3.3
CG5-79	T	27.8	16.0	3.3
MBRA 12	T	21.6	16.1	3.6
CM723-3	U	17.2	15.8	3.9
MMEX 59	U	15.8	15.1	3.9
MCOL 22	S	12.0	17.8	5.0
CM696-1	U	10.3	15.8	4.7
MCUB 74	U	9.1	17.8	5.6
CG427	U	7.9	16.4	5.5
CM1091-2	U	7.6	16.2	5.5
CM503-37	U	4.4	18.6	8.7
MECU 85	U	4.0	16.4	8.2
MCUB 72	R	2.1	17.1	16.1
MCOL 1351	R	1.9	15.6	16.2

R_O = net reproductive rate, T = mean generation time (days), DT = population doubling time (days)

T. limonicus life tables

At the time of writing, data are available only for percent survival and development time of T. limonicus. Comparison of percent survival and development time for parents and their offspring suggests that there is a strong effect of the initial host clone CMC 40. The initial cohort of females which provided the eggs from which the parent generation developed were reared on CGM which had fed on CMC 40. Percent survival to adulthood was higher in parents than in F_1 offspring on all clones except MCUB 72. In this clone F_1 survival increased to 90% from 77% in the parental generation. Development time was longer in the parents than in the F_1 on all clones except CM507-37. The effect of clone on development time was significant ($P < 0.05$). In both the parents and the F_1, development was slower in the tolerant check CG5-79 than in the resistant MCUB 72 (Table 4).

TABLE 4

Effect of cassava clone on survival and development time of Typhlodromalus limonicus

Clone	% survival egg to adult		Egg to adult development time (days)	
	Parents	F_1	Parents	F_1
CM507-37	64	54	129 D	139 A
CM696-1	70	59	145 C	140 A
CG5-79	71	59	166 A	145 A
MCUB 72	77	90	155 B	127 B
MMEX 59	87	46	141 C	125 B

Values followed by different letters are significantly different (Duncan's Multiple Range Test; $P < 0.05$)

DISCUSSION

Complete life tables for T. limonicus could not be constructed because some individuals of both the parental and F_1 generations were still being followed; however the available data suggest that clones which were the most favourable hosts for CGM (tolerant and susceptible) were not favourable for its predator T. limonicus. Survivorship and development of T. limonicus was favoured by the resistant clone MCUB 72. Although these data do not permit generalization about how resistance status affects T. limonicus, they suggest that migration between clones could have a strong effect on survivorship of this predator. The differences in developmental rate observed when T. limonicus is offered prey reared on different host clones suggest that there is an effect on the host plant on prey quality. Life tables will be constructed for T. limonicus on each clone when the data become available and the effect of prey quality on fecundity and longevity will be determined.

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INTERSPECIFIC COMPETITION OF FALL ARMYWORM PARASITES

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ABSTRACT

Interspecific competition by the parasitoids Cotesia (= Apanteles) marginiventris Cresson, Microplitis manilae Ashm., and Chelonus insularis Cresson in fall armyworm (FAW) (Spodoptera frugiperda) (J.E. Smith) larvae were studied. C. marginiventris was a superior competitor relative to C. insularis but C. insularis was a superior competitor to M. manilae. M. manilae females significantly altered their behaviour in terms of examination of the host, probes and apparent ovipositions when C. insularis-parasitized larvae were presented. However C. marginiventris did not discern the presence of C. insularis in the host. The parasitization by C. marginiventris in FAW larvae parasitized by C. insularis placed on greenhouse-grown corn, sorghum, Bermuda grass (Cynondon dactylon (L)) and itch grass showed a more than two-fold increase in percentage parasitization on corn compared with sorghum and a more than four-fold increase compared with Bermuda and itch grass. M. manilae females altered their behaviour with C. insularis-parasitized host larvae and were not competitive with C. insularis. But C. marginiventris females oviposited in both C. insularis-parasitized and non-parasitized larvae and were superior competitors compared with C. insularis.

INTRODUCTION

The fall armyworm (FAW), Spodoptera frugiperda is a serious pest of many graminaceous crops throughout the southeastern United States. Estimates of average annual crop losses exceed $300 million (Mitchell 1979). Since overwintering occurs only in the southern portions of Florida and Texas (Lunginbill 1928), incrasing FAW mortality in the south should lower the numbers of adults participating in the northward migration each spring.

Knowledge of parasitoid inter-relationships within FAW larval populations will increase our understanding of the factors that affect the dynamics of this pest, as well as contribute to biological control efforts. In endemic host-enemy associations, interspecific competition appears to play a crucial role in structuring the parasitoid guild (Force 1974), and may influence the entire natural enemy complex as well. Parasitoids of the FAW have similar life cycles and so provide a relevant model for the study of interspecific competition within the host larva. Fifty-three species of parasitoids have been reared from field-collected larvae (Ashley 1979). The concept of interspecific competition during parasitoid development is illustrated by the presence of a density dependent pattern in percent parasitization between Chelonus insularis Cresson and Temelucha difficulis Dasch. (Ashley et al. 1982).

The present study assesses interspecific competition between two species of larval parasitoids, Microplitis manilae (Ashm.) and Cotesia (= Apanteles) marginiventris Cresson, and an egg-larval parasitoid

C. insularis. It also describes the host finding and ovipositional sequence for C. marginiventris for hosts already parasitized by C. insularis. In addition data are presented on host acceptance by M. manilae and C. marginiventris of larvae already parasizited by C. insularis.

RESULTS AND DISCUSSION

Experiment 1

Results of interspecific competition experiments demonstrated that C. insularis was more competitive than M. manilae (Fig. 1). However, when C. marginiventris was substituted for M. manilae it emerged more frequently than C. insularis. Combined parasitization rates ranged from 73-78%, which suggested that multiple parasitization did not affect FAW larval mortality. FAW larvae parasitized by any two of the three parasitoid species only produced a single parasitoid adult, which suggests the destruction of one parasitoid larva by another. Salt (1961) and Vinson & Ables (1980) reported that when multiple parasitism occurred, all but one species was usually eliminated through physical attack, physiological suppression, or both. In a few instances, especially with gregarious parasitoids, some individuals of both species may survive (Miller 1982, Weselch 1983).

Experiment 2

The proportions of C. marginiventris and M. manilae adults that emerged from parasitized and non-parasitized hosts did not differ (Table 1). C. marginiventris parasitized more hosts than M. manilae. Larvae that were not parasitized by either parasitoid and emerged as FAW adults displayed significant differences in all four treatments. C. marginiventris did not appear to discriminate against hosts parasitized previously by C. insularis as there were no significant differences between C. insularis x C. marginiventris and C. marginiventris only treatments.

TABLE 1

Mean percentage emergence of Chelonus insularis (Ci), Cotesia marginiventris (Cm) and Microplitis manilae (Mm) from FAW exposed and not exposed as eggs to C. insularis, mean percentage for emergence of FAW adults, larvae dying because they refused to eat the diet, and larvae not pupating

Treatment	Mean emergence			FAW	Refused diet	Did not pupate
	Ci	Cm	Mm			
Ci x Cm	32.5	56.5		5.0	0.0	0.0
Cm only		52.5		30.0*	4.3	6.3
Ci x Mm	48.0		37.5	13.0	0.0	0.5
Mm only			44.5*	28.5*	11.5	16.5*

Treatments replicated nine times with 20 larvae/treatment. Data analysed by Student's t-test (* = significantly different at the 5% level).

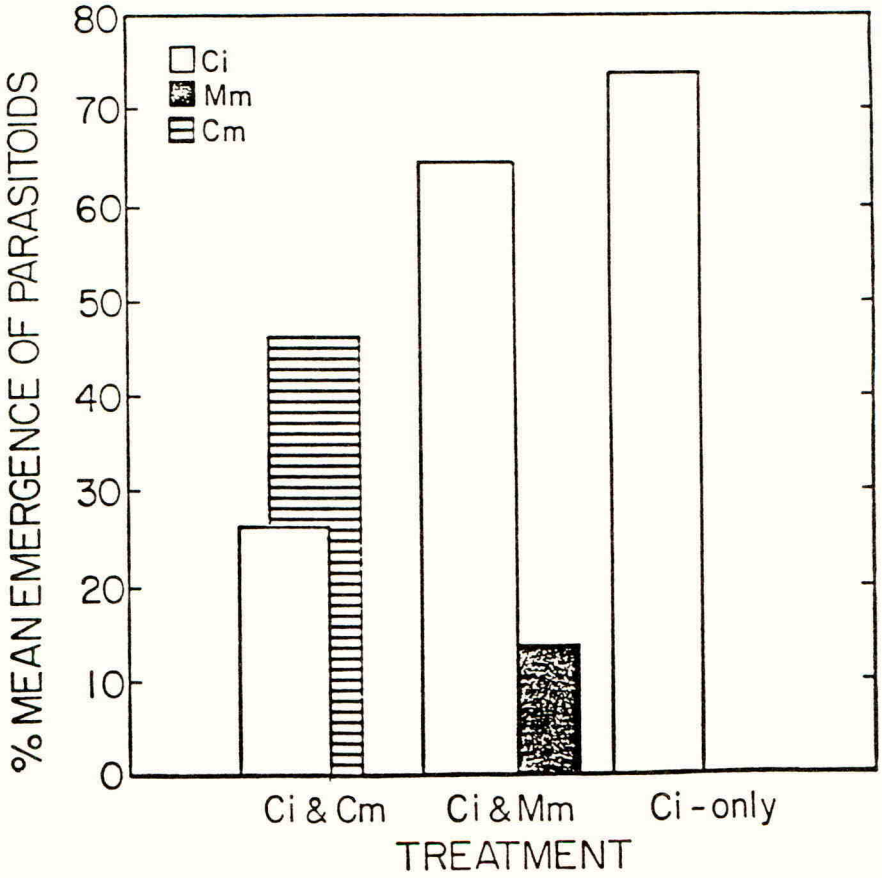

Fig. 1. Mean percent emergence of C. insularis (Ci), M. manilae (Mm), and C. marginiventris (Cm) from fall armyworm larvae exposed to multiple parasitization

The percentage parasitization by C. marginiventris showed more than a two-fold increase in corn compared to sorghum and more than a four-fold increased over Bermuda grass and itch grass (Fig. 2). Sixty percent of the larvae were parasitized by C. marginiventris in corn after an 80 min host exposure period. There was no increase in parasitization for Bermuda grass and itch grass after 20 min. Ashley et al. (1983) found that parasitization rates for C. insularis and Temelucha spp. were substantially higher in corn than in Bermuda grass and paragrass Brachiarie mutica (L.). C. marginiventris parasitized the highest proportion of hosts of Bermuda grass and paragrass. The differences in parasitization rates between these parasitoids may reflect a host plant preference (Ashley et al. 1983).

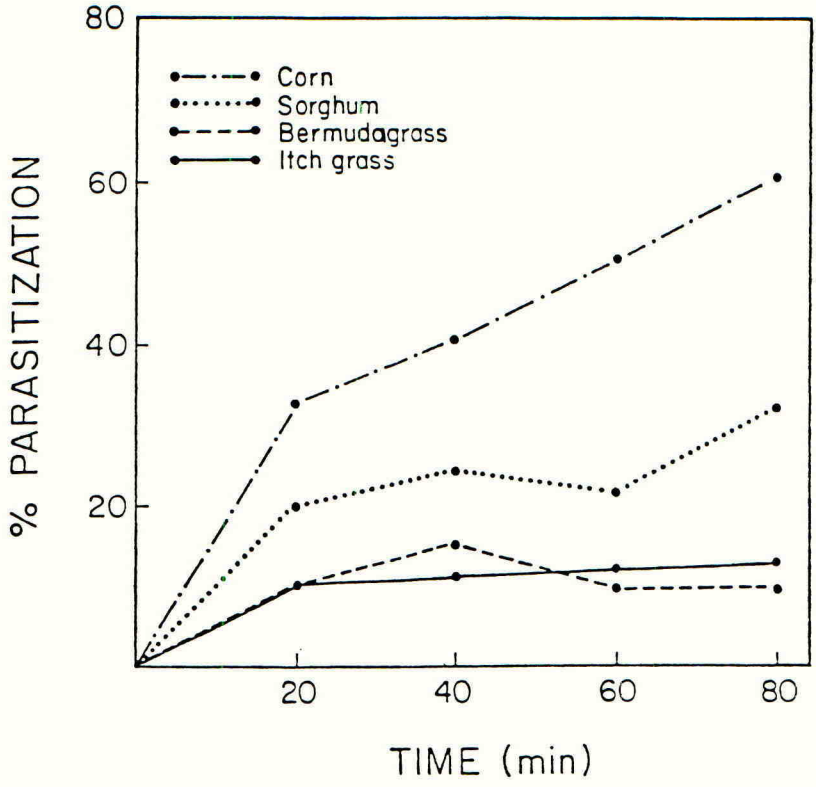

Fig. 2. Change in percentage parasitization of fall armyworm larvae by C. marginiventris with time on four armyworm foodplants.

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THE EFFECT OF PESTICIDES ON TRICHOGRAMMA JAPONICUM

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ABSTRACT

A study was made of the effect on the egg parasite, Trichogramma japonicum (Chalcidoidea), of various pesticides commonly used in paddy fields. In areas where large quantities of pesticides were used, the percentage parasitism of eggs of the pyralid moth, Tryporyza incertulas (Yellow Paddy Stem Borer), by T. japonicum was 1.2 - 5.3%, whereas in areas of low pesticide usage it reached 23.7%. Of the eight pesticides tested, piperiphos caused the greatest mortality. The emergence rate of T. japonicum was reduced by 35% compared with untreated controls. Fujithion, azinphos-methyl and tetrochlorvinphos caused less mortality. Application of pesticides by broadcast and incorporation was less damaging than the use of liquid sprays. Adult T. japonicum were more sensitive to pesticides than larvae and the pupae were less sensitive than the larvae.

INTRODUCTION

In China, pest control by utilising natural enemies has developed rapidly in recent years. There are, at present, two ways to use them; one is to protect natural enemies, the other is to mass-rear them artificially and then release them in fields at an appropriate time for pest control. Because it uses natural enemies more effectively, the former method has received world-wide attention. Among the factors affecting the population dynamics of natural enemies, the chemical used is a major one. Because large quantities are applied, natural enemies are badly affected. Consequently, the ecological equilibrium between pests and natural enemies is destroyed and pests can resurge. In the present system of integrated pest management, the problem needing to be solved most urgently, is how to combine chemical control with protection by natural enemies. In the present study, the effects of pesticides on T. japonicum were investigated, to determine which active ingredients and application methods were least harmful, as a contribution to putting the integration of pesticides and natural enemies on a sound scientific basis.

MATERIALS AND METHODS

Effect of various frequencies of pesticide application on T. japonicum in farmed paddies

Paddy fields were classified according to the frequency (high, medium and low) of pesticide application; random samples of T. incertulas egg masses were collected from each field 3-4 d after the moth's oviposition peak (fourth generation) and percentage parasitism by T. japonicum determined.

Effects of various pesticide application rates and methods on populations of T. japonicum in experimental paddies

The pesticides used were piperiphos (25% WP), chlormephos-ethyl (75% EC), fujithion (50% EC), azinphos-methyl (20% EC), tetrachlorvinphos (15% EC), pirimiphos-ethyl (50% EC), methamidophos (50% EC) and 1.5% parathion-ethyl plus 30% gamma HCH dust; they were applied by hydraulic sprayer (30 kg per mu), with a ULV sprayer and by broadcast incorporation (chemical in 40 kg fine soil per mu). Random samples of T. incertulas egg masses were collected from all treatments and retained in tubes to record percentage emergence of T. japonicum. Samples of eggs were also dissected to look for evidence of dead larvae, pupae and adults of T. japonicum and enable the calculation of the mortality of each developmental stage.

Effect of piperiphos on survival/oviposition of adult T. japonicum

Samples of T. incertulas eggs were taken daily from the field and the percentage parasitism, by T. japonicum, of recently-laid eggs was determined (host oviposition date was deduced from time of hatching in the laboratory in relation to known host development rates). The field was sprayed on 21 June with piperiphos (0.2 kg 25% emulsion in 30 kg water per mu) and egg samples taken a day later and treated as above. Differences in percentage parasitism of recently-laid host eggs, pre- and post-spray, provided a quantitative estimate of the mortality (or effect on oviposition behaviour) of adult T. japonicum attributable to the spray.

RESULTS

Effect of various frequencies of pesticide application on T. japonicum in farmed paddies

Table 1 shows that T. japonicum was adversely affected in fields receiving repeated applications of various pesticides. Fields at the Crop Institute (Jiangxi Academy of Agricultural Sciences) typically received 7-8 applications per year and the percentage parasitism here ranged from 1.2% to 5.3%. At this level, borer populations would not be controlled. Conversely, very few pesticides were applied to fields at Fulin village and the percentage parasitism here reached 23.7%, a level that would result in reduction of borer numbers and damage. Pesticide usage on fields of the Animal Husbandry Institute was intermediate and the percentage parasitism was correspondingly intermediate (13.9%).

Effects of various pesticide application rates and methods on populations of T. japonicum in experimental paddies

Table 2 shows that pesticide application adversely affected emergence of T. japonicum, the severity of this effect varying according to pesticide active ingredient and application method. Piperiphos had the greatest effect, with T. japonicum emergence being reduced by 50% compared with controls. Chlormephos-ethyl and parathion-ethyl plus gamma HCH also had

severe effects. Emergence in the fujithion, azinphos-methyl and tetrachlorvinphos treatments was not significantly different from controls. Soil-incorporated pesticides caused less T. japonicum mortality than liquid sprays, (e.g. percentage emergence for pirimiphos-ethyl was 41.5% and 8.2% respectively). In the methamidophos trial, there was no significant difference in emergence rate between application by hydraulic sprayer and ultra low volume application. Sensitivity to pesticides varied with the developmental stage of T. japonicum; pupae were least affected (7.4% - 23.3% mortality), adults (including those still present in the host) were very sensitive (44.6% - 70.1%) and larvae were intermediate (15.8% - 43.6%).

TABLE 1

Parasitization percentage of T. japonicum in T. incertulas eggs in paddies with different level of chemical application

Level of application	Site	Sample date	No. of egg masses	No. of eggs Observed	Parasitized	% parasitism of eggs
High	The Crop Institute	14 Sept	32	3 280	173	5.3
		19 Sept	50	4 900	60	1.2
		28 Sept	36	3 621	191	5.3
Middle	The Institute of Animal Husbandry	15 Sept	59	7 423	1 029	13.9
Low	Fulin Village	14 Sept	45	3 948	938	23.7

Effect of piperiphos on survival/oviposition of adult T. japonicum

Table 3 shows that there was a noticeable effect on adults shortly after application, causing a reduction in percentage parasitism from a mean of 28% before spraying down to 10% after spraying. This could have resulted from direct mortality of adults, or a disruption of oviposition behaviour, or a combination of these efffects.

TABLE 2

The effect of pesticides on the emergence of T. japonicum from T. incertulas eggs and on the development of T. japonicum

Pesticide	Application Methods	Amount (kg/mu)+	No. of Egg masses	No. of Eggs	No. of Trichogramma emerged	Emergence rate (%)	No. of dead Trichogramma: Total	Adults No.	Adults %	Larvae No.	Larvae %	Pupae No.	Pupae %
piperiphos	I*	0.2	15	989	255	25.8	734	385	52.2	276	37.6	73	9.9
	FCS**	0.2	19	1 353	319	23.6	1 034	470	45.5	451	43.6	113	10.9
fujithion	I	0.2	33	2 400	1 174	48.9	1 226	580	7.3	404	32.9	242	19.7
	FCS	0.2	23	1 308	494	37.8	789	510	63.9	135	16.9	152	19.2
azinphos-methyl	I	0.2	24	1 840	840	45.7	1 141	603	52.8	385	34.8	153	12.4
	FCS	0.2	31	1 913	772	40.4	1 000	646	44.6	285	23.5	119	11.9
pirimiphos-ethyl	I	0.2	27	1 660	688	41.5	972	499	51.3	247	25.4	226	23.3
	FCS	0.2	26	1 661	137	8.2	1 479	1 037	70.1	230	15.8	207	13.9
tetrachlorvinphos	I	0.25	29	1 972	948	48.1	972	465	47.8	322	33.1	185	19.0
	FCS	0.25	28	1 654	851	51.5	1 003	513	51.1	330	32.9	160	15.9
methamidophos	ULVS***	0.13	14	950	363	38.2	587	333	56.7	198	33.7	56	9.5
	FCS	0.13	21	1 327	567	42.7	760	387	50.9	317	41.7	56	7.4
chlormephos-ethyl	I	0.1	19	1 243	465	36.7	-	-	-	-	-	-	-
	FCS	0.1	16	1 046	267	26.3	-	-	-	-	-	-	-
pirimiphos-ethyl	I	0.13	22	1 092	329	37.4	713	394	55.3	300	42.1	64	8.9
+ gamma HCH	FCS	0.13	17	1 005	114	11.3	892	541	60.7	166	18.6	185	20.9
Control	-	-	23	1 501	771	51.4	-	-	-	-	-	-	-

I* Incorporation
FCS** Full coverage spray (hydraulic)
ULVS*** Ultra low volume spray
+ 15 mu = 1 ha

TABLE 3

Effect of 25% piperiphos applied on 21 June on survival/oviposition of adult T. japonicum

Date of borer oviposition (deduced)	No. of egg masses	No. of eggs Observed	No. of eggs Parasitized	% parasitism of host eggs
22 June	6	729	74	10.1
20 June	17	2 326	726	31.2
19 June	8	10 188	262	25.7
17-18 June	9	1 097	310	28.2
16 June	7	710	194	27.3

DISCUSSION

For successful integrated pest management it is necessary to reduce to a minimum any harmful effects of pest control measures on the agroecosystem. Not only should the effect of the measure on the target pest and crop be considered, but the direct and indirect effects on other pests and natural enemies also has to be taken into account. Although pesticides are currently a very effective means of suppressing pests, they have the disadvantage of also killing natural enemies. Extensive use of pesticides is reducing the diversity of insect communities in agroecosystems and is disrupting the ecological equilibrium between natural enemies and their hosts in such a way that pest resurgence becomes more likely. Hence it is important to fully exploit natural enemies as the first line of pest control and only use pesticides when expedient. There is therefore an urgent need to find effective ways of combining chemical and biological control.

A desirable pesticide should have a high efficiency and selectivity, but a low persistence in the environment; this would minimise any adverse effects on the natural enemy fauna. Practical ways of reducing the detrimental effects of pesticides include careful choice of active ingredient, time and method of application, and restricting the treated area more precisely to coincide with the pest. In the case of T. japonicum, fudithion, sarathion and tetrachlorvinphos were fairly safe, soil incorporation was safer than liquid application and pesticides applied at the pupal stage of T. japonicum caused less mortality than when applied at other developmental stages. Information such as this is useful in the development of robust and economic integrated pest management programmes.

Editor's note: 15 mu = 1 ha

HONEYDEW AS A KAIROMONE FOR APHID PARASITOIDS AND HYPERPARASITOIDS

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ABSTRACT

The responses of some primary parasitoids and hyperparasitoids of cereal aphids to honeydew were tested on filter paper discs. All showed an increase in time spent searching honeydew-impregnated discs compared with untreated controls. *Aphidius rhopalosiphi* females were shown to habituate to the honeydew. Their response to the honeydew increased with increasing concentration up to 0.25 mg/µl, but with no further increase at 0.5 mg/µl. *A. rhopalosiphi* was shown to respond to some non-host aphid honeydew as strongly as to that of its hosts.

INTRODUCTION

Sitobion avenae, and rarely *Metopolophium dirhodum*, may cause significant losses of both quantity and quality in wheat crops. Vorley and Wratten (1985) have shown that aphidiid parasitoids may greatly reduce numbers of aphids in the field.

This present study investigates the kairomonal properties of aphid honeydew, with a view to using kairomones to enhance parasitoid action in the field, possibly in conjunction with inundative release. *Aphidius rhopalosiphi* has been shown to respond to chemicals from wheat and host aphids in an olfactometer (Powell and Zhang, 1983). Also, longer searching times by *A. rhopalosiphi* females on honeydew-contaminated wheat plants compared with clean plants have been demonstrated (Gardner and Dixon 1985).

MATERIALS AND METHODS

Adult parasitoids were collected from a field of winter wheat using a D-vac. Males and females of the following species were tested for their response to honeydew: primary parasitoids *Aphidius picipes, A. rhopalosiphi, Aphidus ervi* (females only), *Praon volucre*: hyperparasitoids *Dendrocerus carpenteri, Alloxysta victrix, Alloxysta macrophadna* (males only), *Phaenoglyphis villosa. A. rhopalosiphi* was cultured in the laboratory on *S. avenae* for use in further tests.

Honeydew was collected following the method of Bouchard and Cloutier (1984). Large numbers of aphids were allowed to build up on their host plants and plastic sheets covered with Parafilm were placed underneath to collect the honeydew. After 2-3 days the sheets were dried in an oven at approximately 35°C. The honeydew was then scraped off and dissolved in water to a concentration of 0.25 mg/µl. This solution was then filtered and sealed under nitrogen in ampoules before storing at -20°C. Honeydew was collected from *S. avenae, M. dirhodum, Rhopalosiphum padi, Acyrthosiphon pisum, Elatobium abietinum* and *Microlophium carnosum*.

The parasitoids were tested on filter paper discs similar to those used by Zaborski *et al.* (1987). The centre of a 15 cm filter paper disc was treated with 100 µl of honeydew solution. This produced a treated area of approximately 4 cm in diameter. This was ringed in pencil, and further circles with diameter 8 cm and 12 cm were drawn concentrically on the same paper. Control discs were produced by treatment with 100 µl of water. Filter papers were used betwen 2 and 6 hours after preparation, and were used repeatedly for different individuals. There was no evidence for any effect of previous searching by parasitoids on the searching time of later parasitoids on the same paper.

Each test consisted of a parasitoid being released onto the central treated area of a disc. Their movement was video-recorded until they had walked or flown beyond the 12 cm circle. Tests were carried out under a single overhead incandescent bulb at a temperature of 20 ± 1°C. The time spent searching (i.e. walking) in the inner, middle and outer regions, delimited by the circles, was calculated, and the number of visits made to each region was noted. Analysis was by ANOVA either on $\log_e$ (time + 1) spent searching in each region or on $\log_e$ (total time + 1) spent searching.

The field-collected parasitoids were tested on *S. avenae* honeydew-treated and control papers. At least 45 min were allowed between tests with the same individual. *A. rhopalosiphi* individuals were tested twice on each honeydew-treated and control paper; individuals of the remaining species were tested once on each. More than five individuals of each sex were tested in most species.

In the remaining tests 2-3 day old virgin, inexperienced females of *A. rhopalosiphi*, from a laboratory culture, were used. The effect of concentration of the honeydew on the parasitoids' behaviour was tested by measuring the response to papers treated with *M. dirhodum* honeydew solutions of 0.031, 0.062, 0.125, 0.25 and 0.5 mg/µl, as well as to a control paper. Twenty parasitoids were individually tested on each paper.

Honeydew from the three main cereal aphids *S. avenae*, *M. dirhodum*, and *R. padi* (which are hosts of *A. rhopalosiphi*) was compared for its effects on the parasitoids' searching. Twenty parasitoids were tested on discs prepared from the honeydew of each species of aphid. Similarly, in further tests, discs treated with *M. dirhodum*-honeydew and water were compared with discs treated with honeydew from *A. pisum*, *M. carnosum* and *E. abietinum*, which are not hosts of *A. rhopalosiphi*.

To assess the effect of exposure to honeydew immediately before testing, parasitoids were confined for 300 seconds between two pieces of *M. dirhodum* honeydew-treated filter paper. As a control, parasitoids were confined between water treated papers for the same length of time. As further controls, parasitoids with no pre-treatment were tested on honeydew-treated and control discs. Twenty parasitoids were tested in each treatment.

RESULTS

All the parasitoids and hyperparasitoids showed similar responses to the honeydew (results for *A. rhopalosiphi* and *D. carpenteri* are illustrated in Fig. 1). The effect of treatment was significant ($P < 0.001$ in all cases except that of *A. macrophadna* where $P < .05$). In all species females showed

longer searching times in response to honeydew than males, but the effect of sex was only statistically significant ($P < .05$) for *A. rhopalosiphi* and *P. villosa*. *A. rhopalosiphi* females searched longer than any of the other parasitoids, apart from *P. villosa* females, on honeydew-treated papers. However, only two *P. villosa* females were tested and therefore the result should be treated with caution. The number of visits to the central area was higher in the honeydew trials than in the controls for all the species, and significantly so for most.

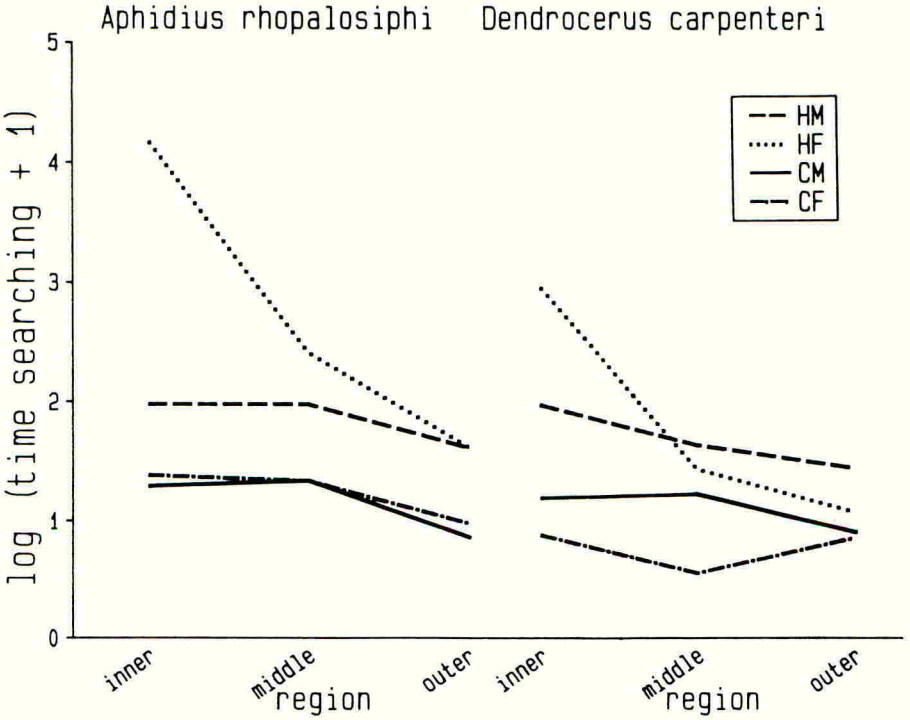

FIG. 1. The searching time of parasitoids to control and honeydew-treated discs. HM=Honeydew, male HF=Honeydew, female, CM=Control, male, CF=Control, female

A. rhopalosiphi increased its searching time on discs treated with increasing concentrations of honeydew, up to the standard concentration of 0.25 mg/µl (see Fig. 2). Further increase in concentration produced no increase in response.

There was no significant difference in response to honeydew from the three cereal aphids *S. avenae*, *M. dirhodum* and *R. padi* (Table 1). Honeydew from *M. carnosum* was as effective as that of *M. dirhodum* ($P > .05$), and that from *A. pisum* was less effective ($P < .05$), but still elicited a response. Honeydew from *E. abietinum* elicited a similar response to that from *A. pisum*, lower than to *M. dirhodum* but higher than to the water control ($P < .05$).

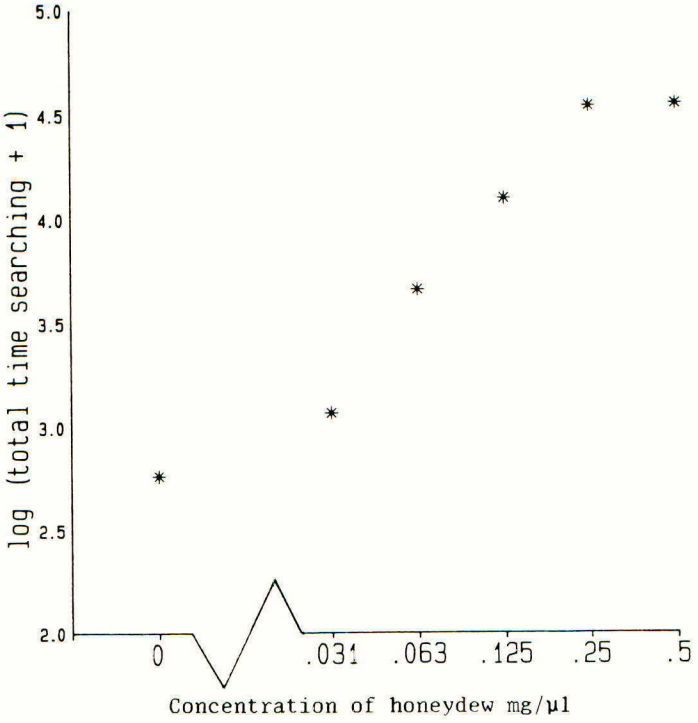

FIG. 2 The effect of concentration of honeydew on the total time spent searching by *A. rhopalosiphi* females.

TABLE 1

Means of $\log_e$ (total time + 1) spent searching on various honeydews.

Aphid	Experiment No. 1	2	3
M. dirhodum	4.7	4.5	4.8
S. avenae	5.1		
R. padi	4.8		
M. carnosum		4.4	
A. pisum		3.9	
E. abietinum			4.0
water		2.9	3.1
standard error of the mean	0.22	0.25	0.29

Parasitoids exposed to honeydew for 300 s immediately prior to testing hardly responded to honeydew (mean of the $\log_e$ (total time + 1), x = 3.41, water control x = 2.67) but those given 300 s exposure to water responded normally (x = 4.55, honeydew control x = 4.57)

DISCUSSION

The response of *A. rhopalosiphi* to honeydew was similar to that of *A. nigripes* (Bouchard and Cloutier, 1984). The response of *A. nigripes* to honeydew of different concentrations from *Macrosiphum euphorbia* on filter paper discs was similar to that in Fig. 2, but with no increase in response above 0.09 mg/µl. Bouchard and Cloutier (1984) measured the response of parasitoids to honeydew on successive visits to a treated paper. They showed that on the second visit (immediately after the first) the response was absent, but that one hour later the parasitoids responded normally. In more extensive experiments (Budenberg, unpul. results) *A. rhopalosiphi* has been shown to lose its response to honeydew after 120 s, and to fully regain it in 90 min. In our tests a normal visit by *A. rhopalosiphi* to a treated paper lasted 120 s, and therefore it appears that both species habituate and recover in the same manner. Habituation prevents parasitoids being arrested for a long time in honeydew-contaminated areas not containing aphids. This is a situation likely to occur with the artificial application of kairomones early in the year when aphid numbers are small.

A. rhopalosiphi responded to non-host aphid honeydew of *M. carnosum* as strongly as to that of host aphids, but it showed smaller responses to the honeydew from *A. pisum* and *E. abietinum*. *A. nigripes* responded to the honeydew of two non-host aphids, *R. padi* and *Myzus persicae*, when searching potato plants. This suggests that the response of aphidiids to honeydew is not to species-specific chemicals, but is either to chemicals which occur in a wide range of plants, or to chemicals synthesized in the aphid gut.

Honeydew has previously been shown to be a kairomone for aphidiid parasitoids by Gardner and Dixon (1985) and Bouchard and Cloutier (1984), but this is the first report of it as a kairomone for aphid hyperparasitoids. Honeydew has also been reported as a kairomone for syrphids (Bombosch and Volk, 1966), coccinellids (Evans and Dixon, 1986, Carter and Dixon, 1984) and cecidomyiids (Wilbert, 1974). Any use of chemicals derived from it for enhancement of parasitoid action in the field must therefore be assessed for its effect on hyperparasitism and on these other natural enemies. Indeed, in field trails in June 1988, designed to increase parasitism by applying honeydew to wheat plants, parasitism was not increased (probably due to an inappropriate pattern of application) but syrphid eggs were laid in response to the honeydew, and there was a suggestion of a higher predation rate of aphids associated with the honeydew (Budenberg, unpubl. results).

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ASSESSING THE CEREAL APHID CONTROL POTENTIAL OF GROUND BEETLES WITH A SIMULATION MODEL

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ABSTRACT

The effect that ground-searching carabids have on cereal aphid population development is difficult to assess due to a lack of data on consumption rates, absolute predator densities and aphid availability. Much of these data have become available recently and have been incorporated into a simulation model. The model was used to predict the effect of predation by ground beetles on a cereal aphid population, using field data from southern England in 1987. When predation was omitted from the model, the predicted aphid population reached a damaging level. When predation by ground beetles was included, at the maximum rate, the predicted aphid population was similar to the observed population. However, when predation rates were reduced to estimates of rates likely to occur in the field, by discounting a proportion of the beetle population assumed not to consume aphids, the predicted aphid population again over-estimated observed levels. The role of ground beetles in controlling aphid populations is discussed in relation to these results.

INTRODUCTION

Field studies have shown that ground beetles (Carabidae) can reduce the rate of increase of aphid populations in cereal crops (Edwards, Sunderland and George, 1979; Chiverton, 1986). However, until recently their relative effectiveness has been difficult to assess as little information was available on their densities and consumption rates and because of the presence of other natural enemies such as spiders, coccinellids, syrphids and parasitoids. Data are now available and have been incorporated into a simulation model which describes the population dynamics of the grain aphid, *Sitobion avenae* on winter wheat (Carter *et al.*, 1982). Simulation modelling is useful in assessing the effect of different predator groups because separate sub-models may be constructed which allow each predator group to be evaluated independently.

The model was used to predict the potential impact of ground beetles on *S. avenae* populations in winter wheat.

BIOLOGICAL BASIS

Gut dissection (Sunderland and Vickerman, 1980) and ELISA (Sunderland *et al.*, 1987) studies have shown that ground beetles consume cereal aphids. Ground beetles are generally poor climbers, and so it is likely that the majority of aphid predation takes place on the ground. The rate at which aphids are predated by ground beetles is dependent on two factors;

(i) The rate that aphids reach the ground.

(ii) The rate that these aphids are consumed.

Aphids fall to the ground due to disturbance by predators (Holmes, 1983), adverse weather conditions, such as heavy rain (Watson and Carter, 1983), or they may die. Aphids also walk between plants across the soil (Holmes, 1983). The rate aphids return to plants is unknown but as they are capable of climbing wheat shoots and the density of shoots is usually high it is likely that few aphids would die before encountering another shoot, in the absence of predation.

The rate at which aphids are consumed on the ground is dependent on the densities of alternative prey items, predator densities, the searching strategies of these predators, and temperature. The combination of these factors make accurate estimation of aphid consumption rates in the field difficult. Maximum consumption rates have been measured for 10 species of carabid at up to 4 temperatures (12.3°C, 16.6°C, 20.6°C, 23.6°C) in the laboratory (Sopp and Wratten, 1986; Sopp. pers comm.). Studies of the gut contents of ground beetles collected from the field using ELISA suggested that only a proportion of a particular population contain aphids (Sunderland *et al.*, 1987), which would result in a restricted aphid consumption rate below the maximum rate possible for that population.

THE MODEL

1. Aphid population dynamics

The population development of *S. avenae* was simulated using a model which incorporated aphid immigration, development, survival, reproduction, morph determination, and crop development (Carter *et al.*, 1982). The model required data input for initial numbers of aphids obtained from counts, estimates of aphid immigration rates based on catches from 1.5 m suction trap, and daily maximum and minimum temperatures obtained using a Grant Squirrel temperature recorder. Data were collected in a winter wheat field (cv. Moulin) at Leckford Estate, Hampshire during 1987.

2. Ground beetle predation sub-model

A predation sub-model, which calculated the rate at which aphids fell off plants and the rate at which they were consumed was incorporated into the aphid population model.

(i) Aphid fall-off

The rate aphids fell to the ground (Gr, numbers falling m^{-2} day^{-1}) was measured in the field using horizontal sticky traps and was dependent on aphid density (Pl, numbers m^{-2}):

$$Log_{10}(Gr)=1.0392*Log_{10}(Pl)-1.1465 \quad (r=0.93,\ P<0.01,\ n=19)$$

It was assumed in the model that aphids fell at a constant rate throughout the day, and that all instars fell at a rate in proportion to their numbers on plants. Numbers of aphids were converted into mg of fresh aphid material using a conversion factor for each instar. No account was taken in the model of aphids walking from plant to plant without falling off.

(ii) Aphid consumption

Daily consumption rates (c_i, mg beetle^{-1} day^{-1}) were calculated for each predator species (i) present in the field using a multiple regression equation derived from the data of Sopp and Wratten (1986) and Sopp (pers. comm.) with two independent variables; mean daily temperature (t, °C), and mean fresh weight of the beetle species (W_i, mg). The equation was used to estimate consumption rates of species of known weights which were not included in the original regression analysis:

$$Log_{10}(c_i)=0.5290*Log_{10}(W_i)+0.0559*t-1.5601 \quad (r^2 = 0.89,\ P<0.01,\ n=429)$$

The maximum daily consumption rate (M_i, mg m^{-2} day^{-1}) was calculated for each predator species by multiplying the daily consumption rate (c_i) by the predator density (d_i, numbers m^{-2}):

$$M_i=c_i*d_i$$

Predator density for each species (d_i) was measured using destructive surface searches by removing soil to a depth of 5 cm. This method probably underestimated density because large ground beetles burrow deeply and would have been missed. *Bembidion obtusum*, *Bembidion lampros*, *Agonum dorsale*, *Nebria brevicollis*, *Notiophilus biguttatus* and *Trechus quadristriatus* were present in the samples.

Estimated field consumption rates (E_i, mg m^{-2} day^{-1}) were calculated for each predator species by multiplying the maximum daily consumption rate (M_i) by the proportion of individuals that had consumed aphids per day (P_i):

$$E_i=M_i*P_i$$

P_i was obtained for each species by dividing the proportion of animals tested by ELISA that had eaten aphids in a previous study by the maximum detection period of prey antigen measured by Sunderland *et al.*, (1987). These values were calculated using data from a different field site, collected in previous years and were used as an estimate of the values of P_i.

The activity pattern of each species was categorized as either nocturnal, diurnal, or indeterminate (Luff, 1978), and it was assumed that each species consumed aphids at the appropriate time of day. Consumption was limited either by the weight of aphids falling or by satiation of the predators. Aphids which fell but were not consumed were assumed to return to plants.

SIMULATION RESULTS

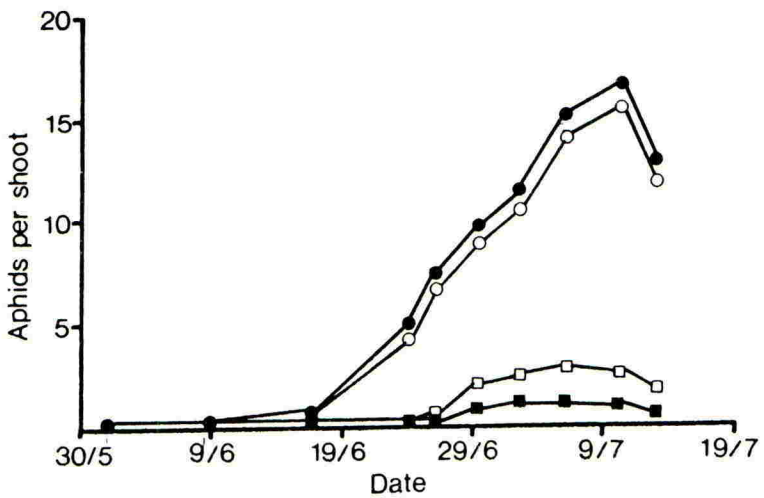
At the end of each day, the number of aphids per shoot, the number of aphids dying due to background mortality excluding predation, and the number of live aphids consumed were printed. The model was run in three ways. Firstly, predation was excluded which resulted in maximum aphid numbers, secondly, predation was included at a maximum rate and thirdly, predation was included using estimates of field consumption rates.

The maximum proportion of the ground beetle population which could contain aphids consumed live was calculated by dividing the total number of aphids eaten m^{-2} day^{-1} by the total ground beetle density (total numbers m^{-2}). The proportion of the ground beetle population which could contain aphids which had died due to background mortality was calculated by dividing the total numbers of aphids dying m^{-2} day^{-1} by the total ground beetle density (total numbers m^{-2}). It was assumed that all aphids dying due to background mortality fell to the ground.

Observed numbers of aphids reached a peak of 1.5 aphids $shoot^{-1}$ (Figure 1). The model predicted peak aphid numbers of 17.1 aphids $shoot^{-1}$ when the effects of predation were excluded. When the model was run with estimated field predation rates, predicted numbers were very close to those predicted without predation. When maximum predation was included numbers peaked at 2.6 aphids $shoot^{-1}$, which was close to those observed. The effects of predation had little effect on the timing of the peak numbers of aphids.

FIGURE 1

Aphid population development observed in the field (■), predicted excluding ground beetle predation (●), predicted including ground beetle predation at a maximum rate (□) and predicted including estimated ground beetle field consumption rates (○).



When maximum consumption rates were included, a higher proportion of ground beetles could contain aphids consumed live rather than dead, because the number of aphids falling m^{-2} day^{-1} was greater than the number dying m^{-2} day^{-1} (Table 1). The proportions of ground beetles which could contain aphids consumed live or dead when estimated field consumption rates were used were limited by the values of P_i. These proportions were lower than those predicted when maximum predation rates were used.

TABLE 1

Proportion of ground beetle populations which could contain aphids consumed live or dead during the early aphid increase phase when maximum consumption and estimated field consumption rates were used.

Date	Maximum consumption rate		Estimated consumption rate	
	Dead	Live	Dead	Live
1/6	0.072	0.154	0.007	0.007
8/6	0.019	0.24	0.006	0.006
16/6	0.01	0.082	0.006	0.006
24/6	0.031	0.178	0.005	0.005
26/6	0.044	0.23	0.005	0.005

DISCUSSION

As the aphid population model with no predation overestimated observed numbers of aphids, mortality factors other than background mortality must have been operating in the field. Ground beetles feeding at the maximum rate could have consumed enough aphids to account for the small numbers of aphids. However, this rate would have resulted in higher proportions of ground beetles consuming aphids each day than those observed in earlier field trials (Sunderland *et al.*, 1987), suggesting that ground beetles did not consume aphids at a rate high enough to account for the observed aphid population.

When the ground beetles were allowed to consume aphids at estimated field consumption rates they had little effect which indicates that ground beetles on their own were ineffective at controlling aphid populations. However, they are usually less abundant than other polyphagous predator groups such as Staphylinidae and Araneae, and may play a role in conjunction with these predators. ELISA-based assessments of the proportion of a predator group containing a particular prey type are likely to be under-estimates (Sopp, unpublished). However, a doubling of this proportion in a new run of the model did not markedly change the effect on the aphid population.

It is not known whether aphids consumed in the field are eaten live or are scavenged. Simulation results suggested that the proportion of beetles containing aphids obtained from field studies could be achieved solely by scavenging or solely by consuming live aphids. All species present consume both live and freshly killed aphids (Sunderland *et al.* 1987). The relative importance of live and dead aphids would depend on the rate at which they reach the ground, the time they spend there, the preference for live or dead aphids and the rate at which other predators consume them. At present little is known about these factors. If it is demonstrated that scavenged aphids make up a high proportion of ground beetles aphid consumption, then the value of ground beetles as aphid control agents would be reduced.

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RESISTANCE IN MAIZE TO THE AFRICAN ARMYWORM, SPODOPTERA EXEMPTA (WALKER) (LEPIDOPTERA: NOCTUIDAE).

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ABSTRACT

Resistance in maize to the African armyworm, Spodoptera exempta is manifested as increased larval mortality, inhibition of larval growth, and reduction in larval feeding. Larval growth and amounts of leaf tissue consumed were dependent on the age of the tissues used, e.g. of the 2nd or 4th leaf, the latter was the most preferred in all cultivars tested. cv. Bastille supported the lowest larval growth, reduced feeding and high mortality. Michoacan 12 was the most preferred cv. supporting high larval growth and consumption. Sequential extracts of cv. Bastille with solvents of increasing polarity were made. Bioassay showed that feeding deterrent and toxic activity were present in the hexane extract, while phagostimulatory activity was present in the water fraction and extracts.

INTRODUCTION

Maize (Zea mays L.) is a staple food crop in Africa, accounting for 35.7% of the region's total cereal production (FAO 1986). One of the most important groups of insect pests attacking maize are the armyworms, Spodoptera spp. (Ortega et al., 1980); the African armyworm (AAW), Spodoptera exempta (Walker) (Lepidoptera: Noctuidae) being the most prevalent and damaging in Africa, south of the Sahara (Haggis, 1986; Brown, 1962).

Because of their 'plague' nature it is thought that varietal resistance would not be an effective means of crop protection. Studies on the potential use of resistant varieties in the control strategies against the African armyworm are therefore still in their infancy.

This paper presents results of a study conducted with a view to identifying antibiotic resistance effects in four selected maize cultivars and in their extracts on S. exempta based on food consumption, larval growth and mortality. A knowledge of the nature of the mechanisms involved would allow for the selective breeding of varieties with an inherent resistance. Susceptible plants could be made resistant by genetic manipulation techniques. Their use in areas prone to armyworm invasion could reduce crop damage and thus provide a useful aid in armyworm management.

METHODS

Larval food consumption, growth and mortality

Newly hatched first instar larvae were provided with known fresh weights of 2nd or 4th leaf sections of 1st stage plants (Hanway, 1963). Amounts consumed, larval growth as measured by larval fresh weight increment, and mortality were recorded after 12h and every 24h thereafter for 156h. Larvae were provided with fresh food at these times. Dry weight of food consumed was calculated by the method of Waldbaurer (1968). 5 replicates of each cultivar (10 larvae/replicate) were used. Data was analysed by ANOVA and means separated by the SNK test. Mortality was transformed to $\sqrt{n + 0.5}$.

Extracts

200 g cv. Bastille were extracted with solvents of increasing polarity in the following sequence: 1. Hexane (filtrate partitioned between hexane and water). 2. Hexane-insoluble matter extracted with ethyl acetate. 3. Ethyl acetate-insoluble matter extracted with acetone. 4. Acetone-insoluble matter extracted with methanol, filtrate evaporated *in vacuo* and partitioned between ether and water. 5. Methanol-insoluble matter extracted with neutral water. All extract filtrates were evaporated *in vacuo* and finally dried under N_2. The dried extracts (10mg/g medium) were fed to last instar larvae in 2% agar discs containing 5% sucrose, a known phagostimulant of *S. exempta* (Ma, 1976). Deterrent activity of the extracts was determined by comparison of the amounts eaten (by weight) with that of the control discs after 24h. 5 replicates (1 larva/ replicate) were used for each dried extract.

RESULTS

Food consumption

Fig. 1 shows mean amounts of leaf sections consumed. Differences in the amounts of 2nd and 4th leaf sections consumed were not apparent in the early stages but were exhibited in the later stages of the experiment. Overall, cultivars could be ranked according to the amounts of leaf sections consumed as : Michoacan 12 > BS 13 > Pool 6 > Bastille for 2nd leaf sections, and Michoacan 12 > Pool 6 > Bastille > BS 13 for 4th leaf sections. Within all cultivars, sections from the older 2nd leaf were consumed less than those from the younger 4th leaf.

Larval growth

Fig. 2 shows larval growth as measured by mean larval fresh weights. Ranking of the cultivars based on the ability to support growth appears to be Michoacan 12 > Pool 6 > BS 13 > Bastille on both 2nd and 4th leaf sections. Differences in weight between larvae fed 2nd or 4th leaf sections were significant at 36 and 108h for Michoacan 12, 60 to 156h for Pool 6, 132 to 156h for BS 13, and 108 to 156h for Bastille.

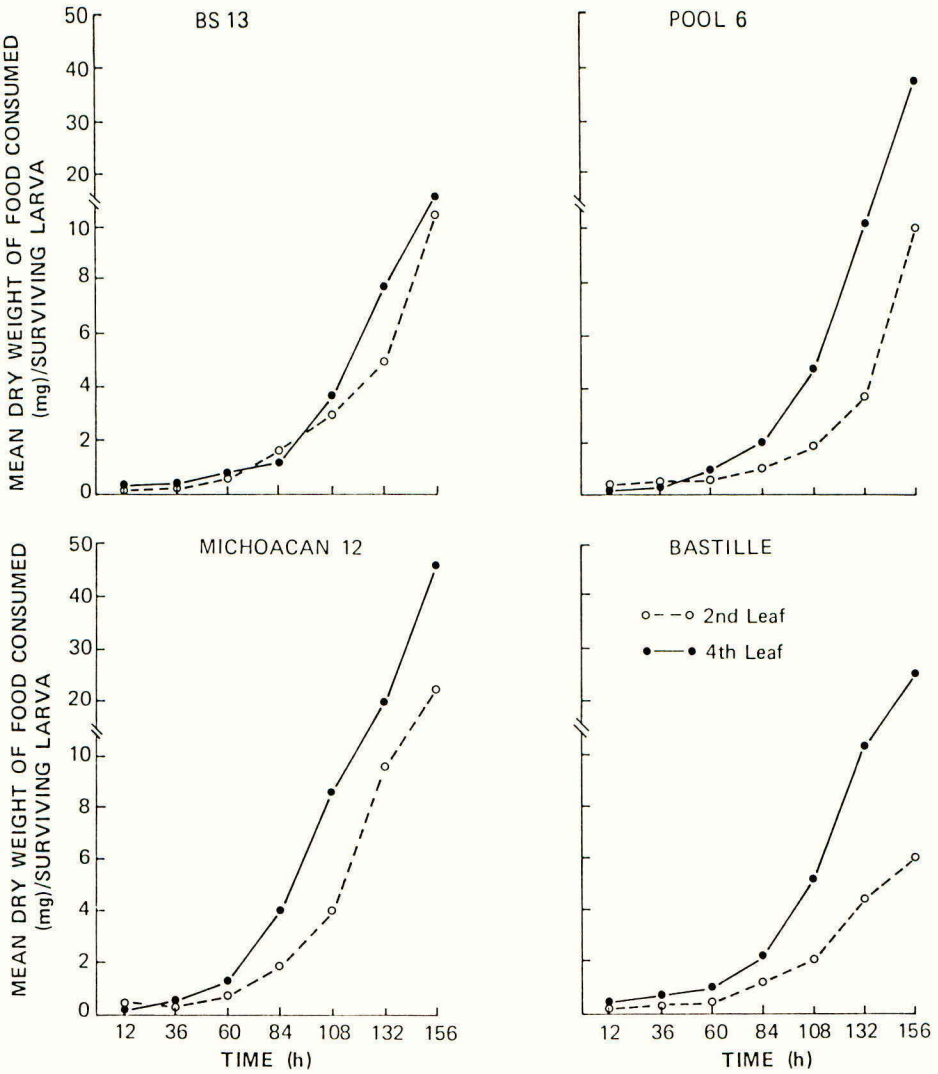

Fig. 1 Mean cumulative amounts of food consumed.

Larval mortality

Fig. 3 shows mean larval mortality at the end of the experimental period (156h). Larval mortality was highest on Bastille and Pool 6 for the older 2nd leaf sections. On the 4th leaf sections Bastille still had the highest mortality. BS 13 had the lowest mortality on both leaf sections. No significant differences in mortality on BS 13 were detected between larvae fed 2nd or 4th leaf sections.

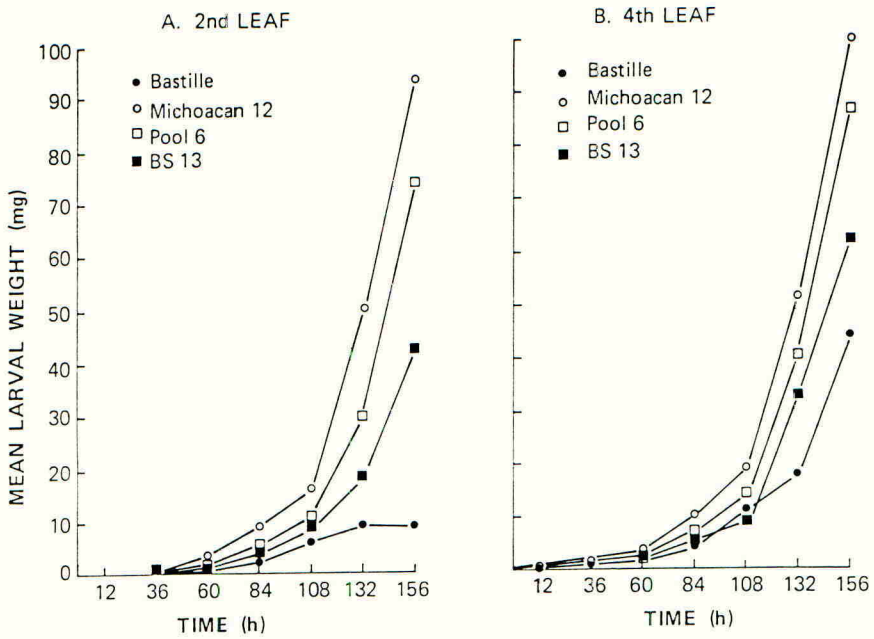

Fig. 2. Larval growth as measured by mean larval fresh weights.

Extracts

The hexane (lipophilic) extract was found to have feeding deterrent activity (Fig. 4). Toxic activity was also noted as the larvae died within the 24h of the experiment. The water fraction and water extracts were phagostimulatory. Bernays et al., (1974) showed that an unidentified lipid-soluble component(s) of maize leaves suppressed feeding in acridids.

Comparison of the water fraction and sucrose activities on a weight to weight basis (5%) showed the water fraction to be the more phagostimulatory (Fig. 5). Ma and Kubo (1977), working on maize of an unstated resistance rating, found the hexane extract caused biting activity and the water fraction phagostimulatory activity, largely due to sucrose and adenosine.

CONCLUSIONS

1. There is much variation in the susceptibility of maize cultivars to the AAW. Such preferences could be exploited to reduce armyworm damage by planting varieties that are to some extent resistant.

2. Resistance in maize to the AAW is manifested as increased larval mortality, inhibition of larval growth and reduction in feeding.

3. Larval growth and amounts of leaf tissue consumed are dependent on the tissues provided.

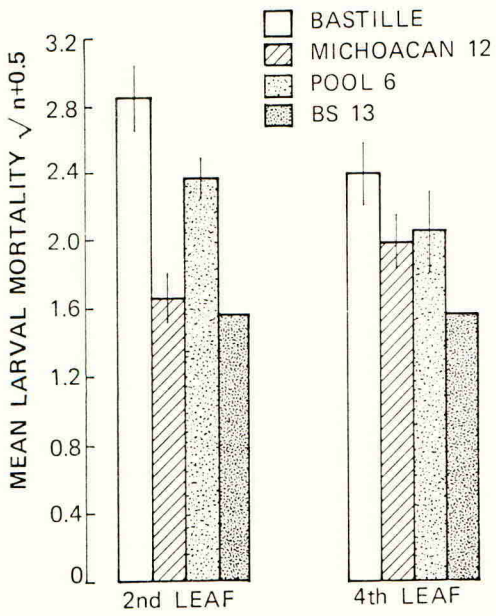

Fig. 3 Mean larval mortality n = 10; bars represent Standard Errors.

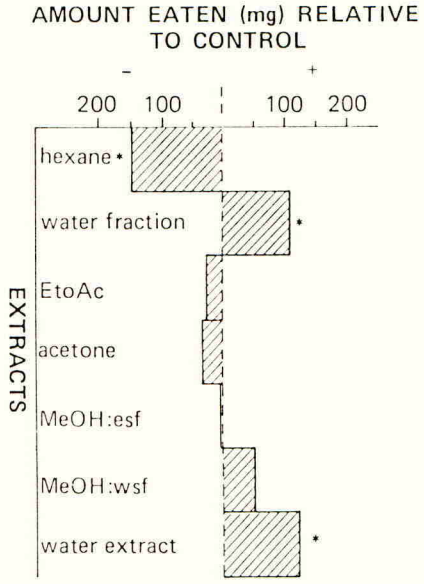

Fig. 4 Feeding response of larvae to extracts. *Significant from control.

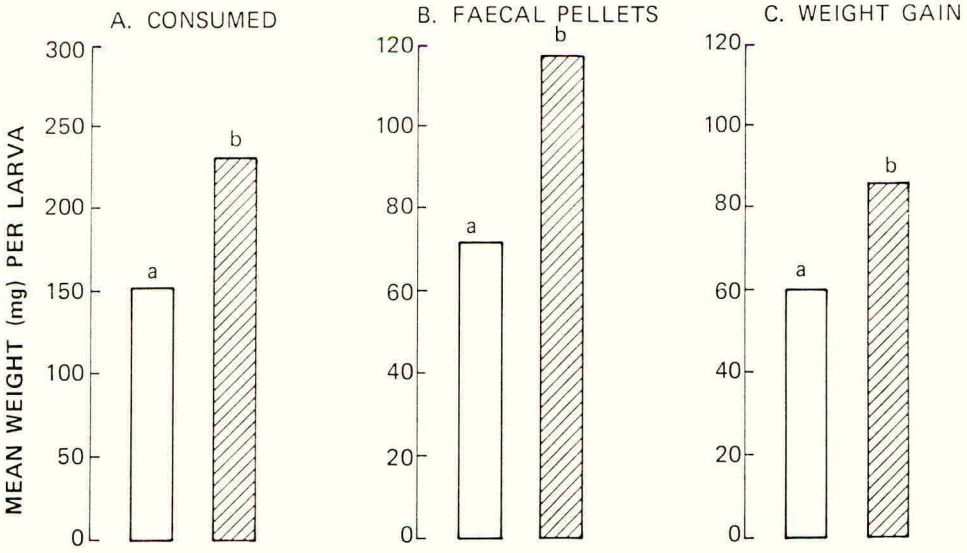

Fig. 5 Comparison of effects of the water fraction, 5% ▨ and sucrose, 5% □ . Means followed by different letters (a,b) are significantly different at the 5% level by T-test.

4. Of the four cultivars Bastille was the least preferred - causing low larval growth, reduced feeding and high mortality. Michoacan 12 was the most preferred - allowing high larval growth and food consumption.

5. Bioassay shows that feeding deterrent and toxic activity are present in the hexane extract of cv. Bastille.

6. Phagostimulatory factor(s) other than sucrose may be present in the water fraction or water extracts.

ACKNOWLEDGEMENTS

This paper forms part of Ph.D. studies by E. J. Okello-Ekochu who particularly wishes to thank The Committee of Vice-Chancellors and Principals of the U.K., The Africa Educational Foundation, The Sidney Perry Foundation, and The Kulika Foundation, who, amongst others, have provided financial support. Appreciation is expressed to CIMMYT for supplying Michoacan 12 and Pool 6 seeds, and to W.A. Russel, Department of Agronomy, Iowa State University, for BS 13 seeds.

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THE ROLE OF HYDROXAMIC ACIDS IN RESISTANCE OF GRAMINEAE TO APHIDS

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ABSTRACT

Antibiotic resistance to the aphid Sitobion avenae was assessed in relation to levels of hydroxamic acids (Hx) in a wide genetic range of cultivars and species of Triticum and Aegilops. Within hexaploid and tetraploid Triticum material total plant Hx concentration explained a significant proportion of the variation in intrinsic rate of increase (r_m) of S. avenae. However, with "primitive" Triticum and Aegilops taxa, the relationship was not significant although Hx concentration was generally highest in these groups. Whilst total plant Hx concentration declined during seedling growth, Hx concentration in newly-emerging leaves remained high in plants of all ages, including in the emerging flag-leaves of mature plants.

INTRODUCTION

Partial host-plant resistance could make a substantial contribution to reducing the damaging effects of cereal aphids and therefore to reducing insecticide use, although aphid resistance has not been deliberately bred into any U.K. wheat variety (Vickerman & Wratten 1979). The progress of plant breeders in their search for resistant genes is however restrained by the absence of a reliable, rapid and convenient assay for resistance, and by the lack of information on the mechanisms of resistance when it is found, and on its genetic origins.

Naturally occuring hydroxamic acids (Hx), in particular the compound 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), have been shown to be involved in the resistance of cereals against bacteria (Corcuera et al. 1978), fungi (ElNaghy & Linko 1962) and several insects (Klun et al. 1967). Previous entomological studies have concentrated mainly on research into insect resistance in maize; however Hx have been implicated as resistance factors in several wheat cultivars to the aphid species Metopolophium dirhodum, Schizaphis graminum and Rhopalosiphum maidis (Corcuera et al. 1982). Sitobion avenae has been investigated in this context only in preliminary work by Bohidar et al. (1986), where 96 percent of the variance in the resistance of seedlings of six wheat cultivars was explained by Hx concentrations.

The objectives of the present investigation were a) to assess antibiotic resistance to the cereal aphids, S. avenae and R. padi in relation to levels of Hx in a wide genetic range of cultivars and species of the genera Triticum and Aegilops and b) to attempt to explain some of the residual variation by analysis of the temporal and spatial distribution of Hx within the plant.

MATERIALS AND METHODS

Choice of plant material

The seed material used in this study was chosen using criteria based on a knowledge of the phylogeny of Triticum aestivum (Riley 1965). Representatives of each stage in the proposed evolution of modern wheat were selected, including both Triticum and Aegilops species. For the examination of the temporal and spatial variation of Hx, the following taxa were used: Ae. speltoides line A, T. durum cv. SNA3, T. aestivum cv. Likay and T. monococcum line A. These cultivars were chosen for their high, medium, low and very low Hx concentrations respectively.

Assessment of the relationship between Hx concentrations and r_m .

The methods used for producing test insects and plants for assessment of Hx concentration and aphid performance were similar to those used by Bohidar et al.(1986), with the exception that recording of fecundity over five days, when nymph production was highest, rather than 10 days, was found to be sufficient to give consistent and reliable differences in the values for the intrinsic rate of natural increase (r_m ; Birch 1948). The r_m values for Sitobion avenae on each cultivar were calculated using a program incorporating the "Jack-knife" technique to give the standard error (Birch & Wratten 1984). Aphids were introduced onto plants at the early two-leaf stage (GS 11-12; Zadoks et al. 1974), about seven days after seedling emergence, at which stage Hx concentrations were determined in uninfested plants of the same cohort. The relationship between r_m and Hx concentration in seven-day-old seedlings for each variety was tested using regression analysis, with all varieties tested as one collection, and also tested in groups of related material.

Temporal and spatial variation in Hx concentrations

To study temporal and spatial variation of Hx concentration in selected taxa, Hx concentration was assessed in individual leaves of different ages in two-, three-, four- and five-leaved plants. Seeds were sown on the same day and grown under identical conditions as in earlier experiments. A proportion of the seedlings within each cohort were harvested as each new leaf emerged, at the two-, three-, four- and five-leaf stages, enabling samples at each growth stage to be analysed for temporal variation in total Hx concentration. Before analysis the plants were stripped of their leaves and those leaves of similar ages (or positions on the plant) at each growth stage were amalgamated into four 1-g samples for analysis. In a subsidiary experiment flag-leaves were collected from field-grown T. aestivum plants (cv. Mission) of known growth stages. Four 1-g samples were used in the analysis of flag-leaves at each growth stage.

RESULTS

Estimates of r_m for Sitobion avenae were calculated at daily intervals. The total Hx concentration of the plant explained a significant proportion of the variation in intrinsic rate of increase of S. avenae on hexaploid and tetraploid Triticum material ($\log y = 0.57 - 0.16 \log x$; $r = -0.58$; $p < 0.01$) (Figure 1), but not in "primitive" Triticum and Aegilops taxa.

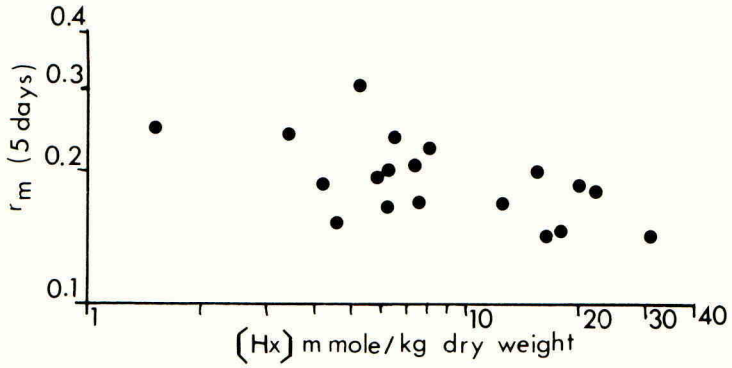

Fig. 1. The relationship between r_m of Sitobion avenae and Hx concentration in seven-day-old seedlings of hexaploid and tetraploid Triticum.

The temporal and spatial variation in Hx concentration seen in four cultivars is shown in Figures 2a-2d. Hx concentration is highest in the emerging leaves of seedlings but declines sharply as the leaf ages. At all seedling ages, the last leaf to emerge has the highest concentration of Hx. This can be seen even in seedlings of T. monococcum A which have very low total plant Hx concentration. Similarly, in mature plants of T. aestivum cv. Mission previously thought to be very low in Hx concentration, the newly-emerging flag leaf has relatively high concentrations of Hx, which decline rapidly during anthesis (Table 1).

TABLE 1

Hydroxamic acid concentration in the flag leaves of wheat cv. Mission.

Crop stage	[Hx] m moles/kg dry weight	95 % confidence limits	
		lower	upper
Half of inflorescence emerged	15.3	14.9	15.8
Emergence complete	13.7	12.4	15.0
Anthesis complete	8.9	7.9	9.9
Early milk	3.7	3.3	4.2

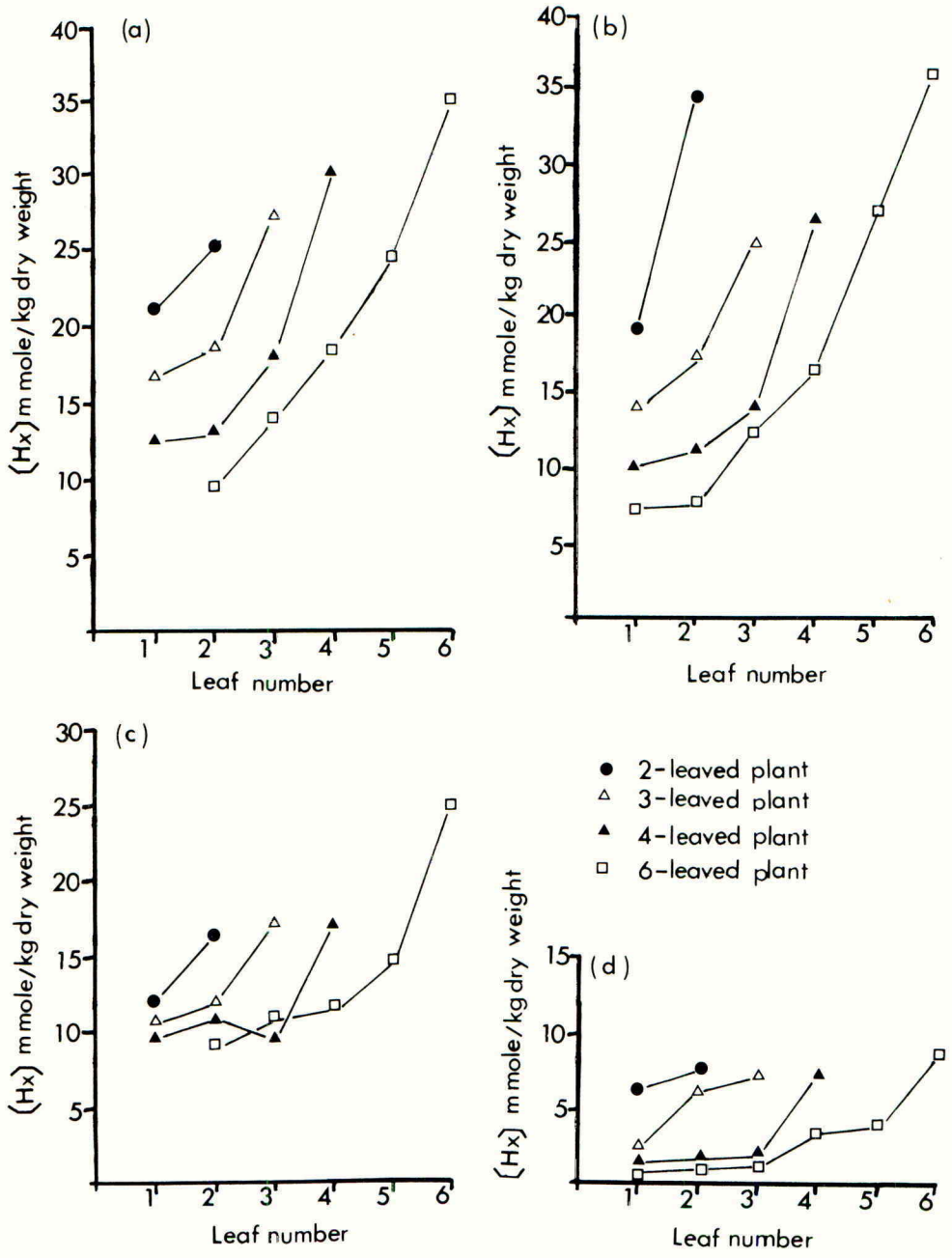

Fig. 2. The Hx concentration of individual leaves of (a) Triticum durum cv. SNA3, (b) Aegilops speltoides line A, (c) Triticum aestivum cv. Likay and (d) Triticum monococcum line A, at different growth stages. The leaf number refers to the order of leaf emergence in seedlings. ie. leaf 1 and leaf 3 are the first and third leaves to emerge respectively.

DISCUSSION

Although resistance to aphids in modern wheat cultivars is generally very low, a wide range of resistance within the genus Triticum can be seen when the genetic range of plant material screened is increased. Hydroxamic acids (Hx) appear to explain 25 percent of the resistance in the tetraploid and hexaploid wheats; however, the relationship between Hx concentration and r_m is less clear in the "primitive" Triticum and Aegilops taxa. It is doubtful that any example of plant resistance to an insect can be explained on the basis of a single, simple biological characteristic of the plant; however, a greater understanding of the behaviour of the different hydroxamic acids which differ in their toxicity and of their effects on aphid feeding behaviour may explain some of the variation in the relationship between Hx concentration and r_m for primitive wheats. In future studies, high performance liquid chromatography (HPLC) will be used to distinguish between different Hx in plant material. Future work will also take place under carefully controlled environmental conditions since fluctuations in light intensity and in water availability may also have contributed to the high degree of variation (Manuwoto & Scriber 1985).

Whilst it was previously thought that Hx concentration decline rapidly during seedling growth, reaching very low levels in maturing plants, the present study has shown that Hx concentration is relatively high in the newly emerging leaves of a number of cultivars of different ages, even in the emerging flag-leaves of a mature, modern wheat cultivar. In a tillering plant a fairly high proportion of leaf material might therefore be high in Hx concentration, thus giving a greater degree of protection to the plant than was previously supposed. The localisation of Hx in areas of new growth must have important implications for the plant's resistance to aphid damage. Higher levels of Hx in more primitive species of wheat such as some lines of Triticum durum, suggests that plant breeding programes may have selected against this characteristic.

From this study, a useful matrix of data giving the taxonomic pattern of resistance within Triticum and Aegilops has evolved, and it is hoped that the increasing knowledge of Hx behaviour in the plant might lead to the use of Hx analysis in plant-breeding programmes, both as a standard when selecting promising lines for resistance to cereal aphids, and also in the isolation of resistant genes.

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A NOVEL APPROACH TO INDUCING FUNGAL RESISTANCE IN ONIONS

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ABSTRACT

A treatment of Allium cepa bulb scales with elicitor molecules from mycelium of the fungus Fusarium solani resulted in accumulation of onion phytoalexins which we called tsibulins (from Ukrainian "tsibulya" = onion). The tsibulins are non-volatile fungitoxic substances. The analysis of their mixture and separate preparative fractions by HPLC, plus UV, IR, PMR and chromato-mass spectra revealed a set of common structure features, in particular carbohydrate chains, sulfoxide and disulfide units, uncoupled carbonyl and hydroxyl groups. A fungal elicitor from F. solani was purified by gel filtration chromatography. Both lipid and carbohydrate fractions were shown to be associated with elicitor activity. Experimental field studies over 3 years demonstrated that induced resistance by prior treatment of onion seeds or plants with biotic elicitor at a very low concentration (0.0025%) resulted in highly effective protection against a downy mildew agent Peronospora destructor.

INTRODUCTION

The ability of fungal metabolites to elicit host defence mechanisms in a number of plant-parasite interactions is now well established (Metlitskii & Ozeretskovskaya 1984, Darvill & Albersheim 1984). Work on the induction of phytoalexin biosynthesis has centred mainly on isoflavonoid compounds in the Leguminosae and sesquiterpenoids in the Solanaceae (Bailey 1982).

Active research on the identification of new phytoalexins and their role in disease resistance continues in many laboratories. Although post-infectional increases in concentrations of antifungal compounds have been shown (Stewart & Mansfield 1984) no phytoalexins have previously been identified from onions.

In this paper we report on the discovery of onion phytoalexins which we called tsibulins (from Ukrainian "tsibulya" - onion) and on the purification of an abiotic elicitor from F. solani mycelium and its application to induce resistance against a downy mildew agent Peronospora destructor.

MATERIALS AND METHODS

Fungi and plants

Botrytis allii, B. cinerea, F. solani, F. oxysporum, F. moniliforme and Cladosporium spp. were maintained and cultured as previously described (Dmitriev et al. 1984). Bulbs of cv. Skvirskii were purchased in September and stored at 4°C until required. Fleshy scales dissected from onion bulbs were inoculated with 25 ul droplets of sterile distilled water containing 10^3-10^5 conidia/ml and incubated in humid chambers in the dark at 20°C.

For mycelium inoculation bulb scales were placed with the abaxial epidermis uppermost on moist tissue paper in plastic sandwich boxes. Small agar pieces (c. 7 mm in diameter) containing 2-3 day-old fungal mycelium were mounted onto the epidermis. After incubation in closed boxes in the dark at 20°C for 48 h, tissue was scraped from inoculation sites with a scalpel blade and analysed for the presence of antifungal compounds.

Extraction of onion antifungal compounds

Excised tissues from beneath mycelial inocula were collected and homogenized in re-distilled EtOH 96% (at least 10 ml EtOH/g fresh wt tissue). The extract was dried and evaporated *in vacuo* at 40°C. The aqueous phase was re-extracted with benzol (inoculum droplets were immediately extracted with benzol). The benzoic fraction was evaporated up to removal of water and suspended in a solution of hexane-diethyl ether (3:1). The residue was removed by centrifugation (3000 g) and the supernatant filtered and stored under oxygen-free nitrogen at -20°C.

Isolation of onion antifungal compounds

The compounds were isolated from extracts by a combination of TLC and HPLC (Dmitriev *et al.* 1987). The hexane-ether extract was fractionated by HPLC and each fraction evaporated to dryness and resuspended in 0.25 ml EtOH. Volumes of 25 ml (the extract from 0.1 g fresh wt tissue) were spotted onto a TLC plate and assayed with *Cladosporium* spp. and selected fractions were subsequently assayed against *B. allii* and *B. cinerea* sporelings. The preparative isolation of pure individual compounds from extract was rather difficult due to their numerous and close chromatography properties.

Accumulation of onion antifungal compounds

Accumulation of total antifungal activity in hexane-ether extracts of infected tissue was visualized on chromatograms by the *Cladosporium* spp. bioassay. Bands of inhibition on TLC plates were ascribed to individual compounds according to R_f and colour. Following inoculation of bulb scales with conidia, inoculum diffusates (inoculum droplets) were collected and extracted with EtOH and other extraction agents at daily intervals for 3 days. Tissues from beneath mycelial inocula were collected at 1, 2 and 3 days after inoculation and extracted.

Preparation of elicitors

Mycelium (approx. 105 g wet wt) was collected from 14-day-old bottle cultures and homogenized three times in 200 ml of deionized water in a Sorval omnimixer at 14500 r/min for 2 min, in an ice bath, centrifuged at 3000 g for 30 min, and extracted by water or 70% EtOH. Extracts were filtered, EtOH removed by evaporation *in vacuo* at 40°C and the aqueous residue adjusted to the original volume and purified by gel filtration chromatography on Sephadex G-50 (Dmitriev *et al.* 1988). Culture filtrate extracts were prepared by staining cultures through 4 layers of washed muslin and filtering through Whatman glass fibre/C discs.

RESULTS

Isolation and identification of onion antifungal compounds

The hexane-ether extracts prepared from bulb scales bearing limited lesions were fractionated by HPLC. The fractions were visible under UV light (254 nm) and eluted in 9 major groups (Fig. 1). TLC plate bioassays revealed their antifungal activity: growth of Cladosporium spp. was strongly inhibited. Thus, a total of 9 antifungal compounds was indicated. We called them tsibulins (Tsl) from Ukrainian "tsibulya" - onion. Tsl inhibited the germ-tube growth of B. allii and B. cinerea. LD_{50} was between 12 and 37 μg/ml. Further purification of Tsl by column re-chromatography and HPLC revealed their similar mass spectra, UV, IR and PMR-spectra (Dmitriev et al. 1987). Tsl were characterized as low MW (150-500) carbohydrate chains containing a set of common structural elements: sulphoxide and disulfide units, uncoupled carbonyl and hydroxyl groups and differing in length and amount of double links.

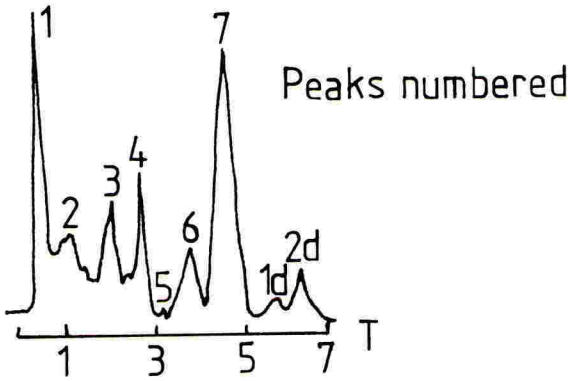
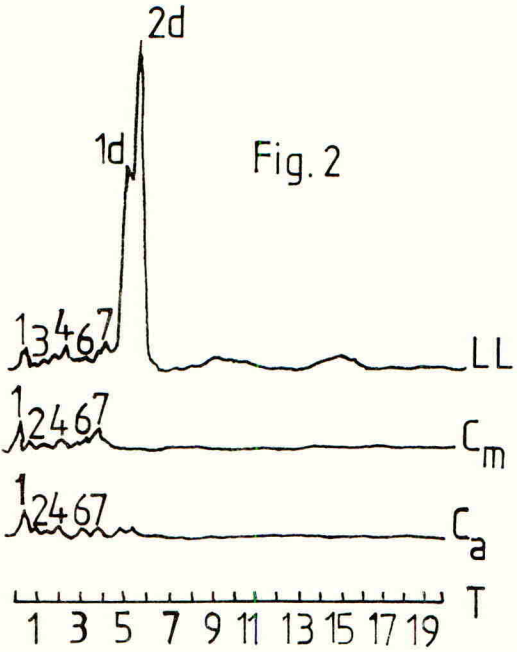


Fig. 1. Separation of tsibulins in an extract of onion bulb tissue by HPLC. All peaks are antifungal. Detection: UV Vis LCD 2563. T - elution time (min)

Two antifungal compounds - Tsl 1d and 2d were not detected in healthy bulbs but appeared in appreciable amounts after inoculation with pathogenic or nonpathogenic fungi (Fig. 2). The important role of Tsl 1d and 2d as markers of onion active defense reactions was confirmed by experiments with inoculum droplets (Fig. 3). Following inoculation with conidia B. allii or B. cinerea only these two compounds were accumulated in diffusates. The slight antifungal activity in extracts from water droplets appear due to difficulty with ensuring total sterility.

There is no doubt that Tsl 1d and 2d are produced by the host tissue, because they accumulated during treatment of bulb scales with 10^{-3}-10^{-4} M $HgCl_2$. The possibility that Tsl 1d and 2d were produced by simple alterations of endogenous precursors is unlikely since they were not detected in homogenized healthy tissue that had been incubated for several hours.



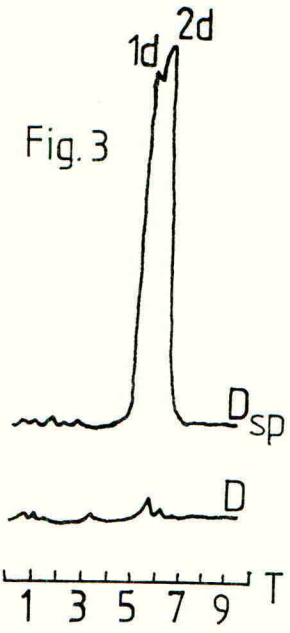


Fig. 2. HPLC of tsibulins from onion bulb tissues. C_a - absolute control (intact bulb scales); C_m - methodological control (uninoculated bulb scales maintained in humid chambers); LL - limited lesions; T - elution time (min).

Fig. 3. HPLC of tsibulins from drop-diffusates with B. allii conidia (5×10^2–5×10^3 spores/ml) (D_{sp}) and water control (D).

Time course of Tsl accumulation

The accumulation of Tsl 1d and 2d was detected in cv. Skvirskii-B. cinerea (limited lesions-highly resistant combination) as early as 8-10 h after inoculation. At this time cytoplasmic vesicles containing phenolic compounds had formed near the penetration site. The concentration of Tsl 1d and 2d increased rapidly after 24 h. At 48 h after inoculation, when fungal growth within bulb tissues had competely ceased, the total amounts of Tsl reached 600 ug/g fresh wt of tissue.

Localization of Tsl accumulation

Large amounts of Tsl accumulated in the inoculated area, while zero to very low levels of Tsl were detected in tissues neighbouring the uninoculated area of the same scale. An intense fluorescence and a UV-absortion spectrum characteristic of Tsl were detected by microspectrophotometry of the cells surrounding the infected sites only. These results strongly suggest that accumulation of Tsl occurs in cells at the infection site and that Tsl produced in these cells can diffuse into intercellular spaces where the fungal hyphae are growing.

Induction of Tsl by live conidial suspensions, mycelial fractions and culture filtrates

Laboratory experiments on Tsl-inducing properties were carried out with Fusarium. Different species differed in their ability to induce Tsl (Table 1). Application of crude culture filtrate from Fusarium to bulb scales gave a small elicitor activity. Water extracts of F. solani mycelium purified by gel filtration chromatography showed the maximal phytoalexin-inducing ability. Treatments of the purified elicitor fraction with either sodium periodate or the nonspecific protease preparation, pronase, substantially reduced its activity as an elicitor of Tsl 1d and 2d formation. The results indicated that both protein and carbohydrate were associated with the most effective elicitor fraction.

TABLE 1

Tsibulin induction in onion bulb scales by different fungi

Pathogen	Elicitor	Content in 1 ml	Tsibulins 1d+2d (µg/g fresh wt)
EtOH-control	-	-	-
F. solani	Spores	$2x10^5$	82
	Mycelium extract - water	2.0 mg	79
	- EtOH	2.0 mg	58
	Culture filtrate	-	33
F. oxysporum	Spores	2×10^5	74
	Mycelium extract - water	2.0 mg	66
	Culture filtrate	-	21
F. moniliforme	Spores	2×10^5	68
	Mycelium extract - water	2.0 mg	54
	Culture filtrate	-	19

Induced resistance of onion by biotic elicitor in field studies

An elicitor fraction from F. solani in a very low concentration (0.0025% of fresh wt), which elicited only traces of Tsl 1d and 2d accumulation, was used to induce disease resistance. Onion was treated by elicitor in two ways: pretreatment of seeds just before sowing or spraying of plants up to 2 days before artificial inoculation with P. destructor. Experimental field studies over 3 years showed (Table 2) that spraying of growing plants by elicitor provided a reduction in the percentage of downy mildew. In elicitor-treated plants the disease symptoms were expressed as isolated, small necroses (c. 3-4 mm in diameter) and sporulation was confined to a narrow strip (2-3 mm) around them. In contrast, on untreated or water-treated controls the necroses occupied one third of the upper part of leaves and sporulation was abundant. The increased resistance remained in elicitor-treated plants under field conditions during the whole vegetation period after artificial inoculation with P. destructor.

TABLE 2

Ability of biotic elicitor to induce resistance in Allium cepa

Type of treatment	Cultivar	% incidence of disease July	% incidence of disease August	% extent of disease July	% extent of disease August
Water control	Strigunovskii	79.6	100.0	5.7	72.6
Elicitor	"	37.1	100.0	2.0	56.3
Water control	Skvirskii	92.7	100.0	7.4	74.6
Elicitor	"	56.1	100.0	2.3	62.9
Water control	Oktyabrskii	86.4	100.0	6.1	76.2
Elicitor	"	47.7	100.0	2.1	58.2

CONCLUSION

Two phytoalexins were demonstrated to occur in onion bulb scales as antifungal subtances responsible for resistance to fungal infection. These are newly synthesized Tsl 1d and 2d. Phytoalexins are characterized as products which are synthesized via gene depression or activation of a latent enzyme system. A fungal elicitor from F. solani, purified by gel filtration chromatography, induced highly effective protection in onion against the most harmful P. destructor. This represents a novel approach to plant immunization by low-risk compounds - biotic elicitors which are non-toxic for plants, animals and man. Application of biotic elicitors isolated from micro-organisms to induce disease resistance in plants does not pollute the environment and does not disturb the ecological balance in the biosphere.

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