THE BLACKCURRANT GALL MITE (*CECIDOPHYOPSIS RIBIS*): ITS BIOLOGY AND STRATEGIES FOR CONTROL

S C GORDON, R M BRENNAN, A T JONES, B FENTON, A N E BIRCH

Scottish Crop Research Institute, Invergowrie, Dundee DD2 5DA, Scotland

ABSTRACT

Blackcurrant gall mite (*Cecidophyopsis ribis*) and reversion disease are the two most important problems of blackcurrants (*Ribes nigrum*). Factors affecting mite behaviour, dispersal and migration are discussed, as are chemical and plant breeding strategies for their control.

INTRODUCTION

The most serious problems facing blackcurrant growers world-wide are the increasing incidence of blackcurrant gall mite (*Cecidophyopsis ribis* Westw.) and reversion disease, the agent of which is transmitted by the mite (Adams and Thresh, 1987). The lack of gall mite-resistant cultivars in Western Europe coupled with limited efficacy of available chemical control measures has led to heavily infested plantations being grubbed after as little as 5-6 years cropping, a situation that means decreased economic returns for the grower and higher costs for end-users.

TAXONOMY AND IDENTIFICATION

The blackcurrant gall mite belongs to the Eriophyid group of mites, which are characterised by their small size, *ca* 0.15 mm long, the presence of four functional legs, cigar shaped body and their close association with their plant hosts. The taxonomy of Eriophyid mites colonising *Ribes* spp. is complex and accurate identification is problematic. Recent studies using light and scanning electron microscopy have shown that at least five *Cecidophyopsis* spp. can be distinguished on different *Ribes* spp (Amrine *et al.*, 1994). Three species colonise cultivated *Ribes*; *C. ribis* on blackcurrant, *C. grossulariae* on gooseberry and *C. selachodon* on red currant (Amrine *et al.*, 1994). Molecular techniques using Polymerase Chain Reaction (PCR) to amplify the very small quantities of DNA recovered from mites followed by the use of Restriction Fragment Length Polymorphism (RFLP) analysis to separate the above species have been developed at the Scottish Crop Research Institute (SCRI) (Fenton *et al.*, 1993). This technique has shown that mites recovered from galled blackcurrants grown in Scotland, England, Poland, Finland and Sweden were indistinguishable but mites obtained from other *Ribes* host species were distinct (Fenton *et al.*, 1993 and unpublished data).

MITE BIOLOGY

Many Eriophyids can alter their host to provide a suitable microenvironment in which to develop. Blackcurrant gall mites colonise developing blackcurrant buds in

late spring and their feeding initiates prolific growth of cells within the bud resulting in the characteristic 'big bud' disorder. These modified buds, which usually become sterile, may contain many thousands of mites by the onset of winter and, in plantations where roguing is not practiced, individual infested bushes may have in excess of 100 infested 'big buds'. However, in the spring these buds begin to desiccate and die and the mites must vacate them. Although the damage to plants is extensive and the number of mites great, little is known about the detailed biology of this mite species, e.g. the number of generations and their duration.

DISPERSAL, MIGRATION AND BEHAVIOUR

Migration of blackcurrant gall mites may be over relatively short distances, along the stem to the new spring growth, or over greater distances to adjacent plants. To colonise new sites, some distance from the original source of infestation, mites either make use of air currents by extending themselves on their caudal setae and launching into wind currents, or by attaching themselves to foraging insects. It is not known if only adult mites migrate in the spring but recent scanning electron microscopy studies of overwintering galled blackcurrant buds suggest that all motile mites are of a similar size, indicating that these mites may overwinter as adults (G H Duncan pers. comm.). If this observation is confirmed, it would suggest that only adult mites are present in the buds in the spring and they are responsible for migration to, and colonisation of, the new buds. Some of the factors influencing spring migration of blackcurrant gall mite were recently investigated at SCRI using a laboratory wind tunnel (Jepson and Healy, 1988) operating at a velocity of *ca* 1.1 ms^{-1}. Preliminary results indicate that temperature is more important than humidity in initiating aerial dispersal from infested blackcurrant buds (S C Gordon, A N E Birch and S Pluta, unpublished data). There was a linear increase in the numbers of mites caught on target slides *ca* 1 m downwind with an increase in temperature between 20-25^{o}C, but over the same temperature range, doubling the RH from *ca.* 20% to 40% did not influence the number of mites caught. Laboratory studies on the behaviour of walking mites in relation to light, humidity and gravity showed that only 1-6% of the mites emerging from the buds in winter were attracted to light compared with 50% emerging in May. Also, the majority of mites emerging from buds in spring climbed upwards (i.e. were negatively geotropic) and were attracted to areas of high humidity (Herr, 1987). All these factors enable the mites to migrate and colonise new buds in the spring.

REVERSION DISEASE

Reversion is the most important virus or virus-like disease of *Ribes* spp., with a world-wide distribution apart from the Americas. The disease causes sterility in infected bushes and despite over 50 years' research the agent of reversion remains uncharacterised. Details of its acquisition, transmission and interactions with the mite vector and the host plant are unknown.

Three forms of reversion have been reported, each with different symptoms which can take up to 2 years to develop (Adams and Thresh, 1987; Jones, 1994). In all three forms, infected leaves of most blackcurrant cultivars are usually characterised by a decrease in marginal serrations and a less clearly defined sinus at the petiole. However,

the most reliable symptoms of reversion are in the flower buds as they open in early spring. All strains, including the common European form, cause a marked decrease in the density of hairs on the flower buds, producing a much darker appearance. The more severe 'Russian' form, occurring in Finland and countries of the former Soviet Union, in addition causes floral malformation and doubling of the floral parts, with increased pigmentation. The third form, occurring in Poland, is similar to the European strain but in addition shows a proliferation of the shoot tips and floral branches (Jones, 1994).

Leaf and flower symptoms in redcurrant are less noticeable than in blackcurrant (Adams and Thresh, 1987), while gooseberry appears to be immune to infection. The blackcurrant gall mite is the only known vector of reversion agent. Other Eriophyid mite species such as *C. grossulariae* and *C. selachodon* have been identified on other *Ribes* species (Keifer, 1975; Amrine *et al.*, 1994), but it is not clear if these mite species can act as vectors of the reversion agent.

Electron microscopy of ultrathin sections of leaves, flowers and buds of plants infected with the European and Russian strains of reversion have not identified any virus-like particles, structures of other pathogens or ultrastructural abnormalities nor have they been identified in sections of mites feeding on reverted plants (Roberts *et al.*, 1993).

Initial studies to analyse nucleic acid from reverted plants by Jones *et al.* (1986) detected several dsRNA species in reverted blackcurrant plants. Subsequent studies have shown that dsRNA species were also present in some reversion-free plants but not in virus-tested plants of several blackcurrant cultivars held in isolation at SCRI (Jones, 1994). Work is in progress to isolate, clone and produce probes specific to each of the dsRNA species in reverted plants and to determine which, if any, are associated with the disease.

Recently, claims have been made that the severe form of reversion in Finland may be caused by a nepo-like virus (Lehto and Lemmetty, 1994), but the nature and role of this virus in the disease process still remains to be verified.

CONTROL OF MITES AND REVERSION DISEASE

Chemical

Currently, chemical control of blackcurrant gall mite relies mainly on the accurate timing of the organochlorine insecticide, endosulfan. This product, which has a degree of bee safety, gave adequate control when tested in Denmark as did the systemic carbamate insecticide, oxamyl (Nielsen, 1987). None of several other products tested were effective. The synthetic pyrethroid insecticide fenpropathrin also controlled blackcurrant gall mite (Nicholls *et al.*, 1986) and it has now obtained approval for use on blackcurrants in the UK. As the main migration period coincides with blackcurrant flowering, there is always a risk of the acaricidal spray harming foraging bees. Although direct contact with fenpropathrin kills bees, bees are strongly repelled by

treated crops (Wael and Laere, 1989) which may greatly reduce overall bee mortality. Any new product developed will require to be equally innocuous to bees.

Sources of gall mite resistance

Genetic resources are available for the development of gall mite resistant cultivars with juice quality acceptable to processors. They are those containing i) the resistance gene *Ce* (Knight *et al.*, 1974); ii) resistance gene *P* (Anderson, 1971), and iii) hitherto unexploited sources of resistance carrying undefined resistance genes. The preferred source of resistance used in the SCRI breeding programme is the gene *Ce* derived from gooseberry, since it appears to offer a stronger resistance, with mites unable to infest the buds of *Ce*-carrying plants. Plants containing gene *P* derived from *R. nigrum* var *sibiricum*, *R. ussuriense*, *R. pauciflorum* and *R. petiolare* have been used in other breeding programmes. In these selections, buds infested by mites become necrotic rather than form galls and mites fail to survive. However, galling has been reported in some *P*-gene carrying genotypes (M M Anderson, pers. comm.; R M Brennan, unpublished data), suggesting mite reproduction and survival under some circumstances. Some gene *P*-containing plants become infected with reversion.

Further studies have identified *R. cereum*, *R. glutinosum* and *R. janczewskii* as possible sources of gall mite resistance.

Development of markers for mite resistance

After crosses are made to incorporate mite resistance genes, it is necessary to identify and select resistant segregants. Normally, this has involved the use of field infestation plots with mite-infested spreader rows, but this is slow and problematic in several respects, as it is time-consuming, poses a risk of 'escapes' and is potentially hazardous to neighbouring blackcurrant plantations. Recent studies at SCRI have examined a more rapid laboratory screening protocol based on the chemical composition of blackcurrant buds (Brennan *et al.*, 1992) and attempting to link specific compounds with resistance or susceptibility. The terpenoid chemical profile of the bud extracts from a range of resistant and susceptible plants were compared using linear discriminant analysis which classified the genotypes with 88% success. This shows considerable promise as a diagnostic tool in the breeding and screening process, and further refinements using bud extracts from segregating progenies are in development.

Herr (1987) investigated the role of phenolic components in mite susceptible blackcurrants and in redcurrant and gooseberry. He suggested that the flavonols were of importance in regulating the gall forming process by altering the IAA inhibitor/activator ratios and they might be important in initiating the onset of galling as a result of mite feeding. More recently, capillary zone electrophoresis has been used to analyse the phenolic acids and flavonoids from *Ribes* genotypes (J B Fernandez *et al.*, unpublished data), to examine the role of these compounds in mite resistance. Preliminary results indicate that mite infested buds contain altered ratios of certain phenolic acids and flavonoids compared with uninfested buds on galled plants. Further investigations are underway to understand the biological significance of these changes.

Molecular studies at SCRI designed to identify markers linked to important agronomic traits are in progress (Brennan *et al.*, 1993); particular importance is placed on the development of markers closely linked to gall mite resistance genes. The tagging of such genes in this way will significantly increase the efficiency with which they can be introgressed into new cultivars of blackcurrant.

Plant resistance to reversion

The main donors of reversion resistance genes are *R. dikuscha* and associated cultivars such as the Russian cv. Golubka (Brennan *et al.*, 1993). Several agronomically acceptable reversion-resistant seedlings have been identified by graft inoculation, and the first of these are already in advanced trials. The nature of reversion resistance and its genetic control are unclear, although Knight (1985) has suggested that `Golubka' is heterozygous for a single dominant resistance gene. However, at SCRI, graft inoculation of segregating progenies with reversion suggests a more complex situation. Another source of reversion resistance being used in the SCRI breeding programme is *R. pauciflorum* and its derivatives such as the Russian cultivar `Pilot A. Mamkin'.

CONCLUSIONS

Further research is required in virtually all areas of mite biology, but especially on infestation, dispersal, plant resistance mechanisms and mite/reversion agent relationships. Information is also required on mite populations to ascertain the likelihood of resistance-breaking strains, and there is a need for further exploration for new sources of resistance. The use of molecular markers to investigate both mite and plant genotype is forming an important part of the research at SCRI.

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A NOVEL TECHNIQUE FOR CULTURING THE BULB SCALE MITE (*STENEOTARSONEMUS LATICEPS*) AND ITS IMPLICATIONS FOR STUDIES ON BIOLOGY AND CONTROL

S M T LYNCH, A BEDI

Central Science Laboratory, MAFF, London Road, Slough, Berks., SL3 7HJ

ABSTRACT

A culturing technique has been developed for *Steneotarsonemus laticeps* (the bulb scale mite) on its natural food medium, bulb scales. This has been modified to enable studies of the life history to be made. Implications for the control, and prevention of the build-up, of infestation are discussed.

INTRODUCTION

The bulb scale mite, *Steneotarsonemus laticeps*, has become a major problem in cultivated narcissus because the conditions under which the bulbs are grown, in order to achieve maximum output of flowers and bulbs, would appear to favour rapid mite population growth within the bulb. Infested bulbs which are forced exhibit the most serious and obvious signs of damage, with a considerable reduction in flower yield and quality. Infested bulbs grown normally do not usually display such a high level of damage but there may be a long term cumulative effect. Hodson (1934) reported that warm, dry weather during May and June usually exacerbates the problem. In the United Kingdom during 1989 and 1990 there was a greater incidence of rejections than in previous years, by the Plant Health and Seeds Inspectorate (PHSI), of bulbs for export because of *S. laticeps* infestation. In 1990 2.6% of UK stocks (PHSI figures) were rejected for this reason with an estimated loss of £75,000. The actual problem during these years may have been greater than this, in that stocks known by the growers to have been infested would presumably not have been presented for export. The reason for the sudden upsurge of infestations during this period is not known, but a combination of factors may have been involved. These might be, for example, a failure to detect initial low levels of infestation combined with the temperature and humidity conditions needed for the rapid increase of the mite.

The most detailed observations on the life history of *S. laticeps* were made by Blattney (1933) and Hodson (1934) and these have been summarised in a review by Lynch (1993). The studies made by Hodson (1934) were the more detailed, but were hampered by the lack of a consistent culturing method and controlled conditions. Hodson's initial unsuccessful attempts to monitor the life history were by using either bulb scales isolated in glass cells or by applying glass cells containing mites to the outside of skinned bulbs or bulb foliage. His most successful observations entailed isolating single female mites on very small, skinned, dormant bulbs but even with this latter method the data obtained were minimal, and obtained under uncontrolled and fluctuating conditions.

There appear to have been no subsequent published observations on the biology of *S. laticeps* and very little is known about this important pest. This is because of the special difficulties involved in making observations on mites which live principally between the fleshy scales within the bulb, and would, therefore, not normally be visible during their reproductive cycles. Further information is required on the factors that control its survival and breeding rates and, in particular, on the effects of low temperatures in depressing reproduction and the determination of the maximum reproductive potential at higher temperatures. Such information is essential when considering the development of a physical control strategy during storage and might explain why *S. laticeps* is a problem in certain years on narcissus grown in the open. Fox-Wilson (1939) quoted an upper thermal death point of between 38°C and 49°C but did not say how this was determined.

To obtain accurate information on the biology and effects of various physical conditions on the mite it is necessary to develop a method of culturing *S. laticeps* in order to yield reliable supplies of mites of known age for experimental work. Unfortunately the living bulb does not make an easy or consistent medium on which to culture mites for laboratory investigation. A further complication is that any food medium used needs to allow for the fact that the mites have piercing mouthparts which are used to feed through the epidermal layer. It would also be reasonable to presume that because of its adaptation to living a large amount of its life history within the bulb, it probably has a much lower tolerance to fluctuations in humidity. This again would make it more sensitive to experimental procedures which do not take this into account. The present study was undertaken to develop a culturing method representing conditions within the normal habitat of the mite (i.e. the bulb) which could be developed for life history studies under a range of physical conditions.

MATERIALS AND METHODS

The successful techniques described below evolved from a number of different trials including variations of the methods used by Hodson (1934). The food medium used was individual bulb scales obtained from non-infested bulbs ('Golden Harvest' cultivar of narcissus, supplied by HRI Kirton). To ensure that the bulbs were not infested, a random selection was cut open, the scales separated and checked under a light microscope. The mites used were obtained from heavily infested narcissus bulbs of the cultivars 'Ice King' and 'St Patricks Day' from a local garden centre. The mites were either picked out from between dissected bulb scales by hand using mounted needles or a single haired artists' paint brush, or were washed out of the bulbs with distilled water and filtered on to black filter paper (Schleicher & Schull nr 551, 100 mm) using a Buchner Funnel. Approximately twenty mites from each bulb used were mounted on microscope slides and their identity confirmed as *S. laticeps*.

For mite culturing purposes bulbs were separated into individual scales and the cut ends sealed with wax. Each scale was gently flattened and sandwiched between a glass microscope slide and a black perspex strip 50 mm long and 25 mm wide. Each perspex strip contained, offset to one side, a hole 18 mm in diameter and 3 mm deep, with gently sloping sides. This hole formed a cell, the floor of which was made by the bulb scale . The join between the base of the perspex cell walls and the floor was sealed with wax to prevent the mites escaping, leaving a central area of bulb scale on which the mites could live and feed. The edges of the perspex and

glass 'sandwich' were also sealed with wax. Approximately twenty mites were placed in each cell and the cells were sealed using a glass microscope slide held at each corner with a "Bulldog " clip. Ten cells containing mites were placed in a desiccator containing potassium hydroxide solution giving an equilibrium relative humidity of 90%. Ten cells without mites were placed under identical conditions in another desiccator in order to observe any deterioration of the bulb scales in the absence of mites. Observations were made on a daily basis. The desiccators were kept in the dark in a constant environment room maintained at 20°C, and removed daily for observations.

For observations on the life history the same experimental procedure was followed using identical perspex strips, but this time containing two equidistant cells 10 mm in diameter and 1.5 mm deep. Five female mites of unknown age were placed in one of the cells in each strip and allowed to lay up to 50 eggs before being moved to the second cell in the strip where they could lay further eggs. The cells were placed in desiccators in environment rooms maintained at five temperatures. All were kept at 90% relative humidity. The temperatures used were 10°C, 15°C, 20°C, 25°C and 30°C. Initially seven cells were set up at each temperature, but because of high mortality at the two highest temperatures, a further five cells were set up at 25°C and ten cells at 30°C. Again cells without mites were set-up in order to observe any deterioration of the scales .

RESULTS

For the culturing experiment scales in the cells containing no mites remained in good condition, with no apparent signs of deterioration, for six to eight weeks. Of the cells containing mites, four showed signs of fungal growth and were discarded. In the remaining six cells there was evidence of feeding lesions and the mites appeared to be healthy. After nine days there were 50+ eggs laid per cell and at this time the original females were removed. Numerous larvae appeared from day 11 onwards and by day 25 most had become adults. These F1 adults were allowed to lay eggs before they, in turn, were removed and development followed through the egg, larval, and quiescent "pupal" to the F2 generation adult. At this time (55 days) the F2 females were moved into new cells as the original scales were showing multiple feeding lesions and signs of deterioration. The observations were carried on until the F4 generation appeared, after which time the cultures were allowed to continue without removing the new females. The entire cultures were transferred to new cells as the bulb scales deteriorated, approximately every 6-8 weeks.

TABLE 1 Life history observations

	10°C	15°C	20°C	25°C	30°C
Mean eggs/cell	15.5	23.9	30.3	13.0	0.4
Maximum % hatch in one cell	82.6	92.8	81.8	27.2	0
Minimum time from egg lay to adult (days)	51	22	15	-	-
Maximum adult longevity (days)	60+	60+	37	31	28

Table 1 shows the mean number of eggs laid per cell at each temperature, the maximum

observed percentage hatch, the minimum time taken for the first adult progeny to appear from the time the first egg was observed, and the maximum observed longevity of the female parents.

No adult progeny were produced at the two highest temperatures, 25°C and 30°C. At 30°C a total of six eggs was produced in seventeen cells and none hatched. At 25°C eggs were laid (see Table 1) but overall few (7%) hatched, and none reached the "pupal" pre-adult stage. There was also a problem with wax lifting away from the bulb at 25°C and 30°C, possibly because of the temperature. The bulb scales at these temperatures appeared enlarged and dry in both the cells with and without mites. At 10°C the bulb scale in one of the cells became badly discoloured at an early stage and the cell was discarded. In four of the remaining six cells a total of 17 eggs were produced but only four (24%) hatched and no adult progeny were produced. In the two remaining cells 76 eggs were produced and 55 (72%) hatched. Adult progeny were produced in both of these cells. At both 15°C and 20°C eggs were produced in all the cells, and in sufficient numbers that, for each, a duplicate cell had to be set-up. Five of the seven cells kept at 15°C produced adults, as did four of the seven cells kept at 20°C. At 10°C, 15°C and 20°C the bulb scale in cells containing mites again showed evidence of feeding lesions. Those without mites remained healthy for 6-8 weeks at 20°C and 8-10 weeks at 10°C and 15°C.

DISCUSSION

A simple method of culturing and accurately observing the life history of *S. laticeps* under controlled conditions has been developed. The use of whole bulbs by Hodson (1934) must have made accurate observations extremely difficult because of the large and varied surface over which the mites could spread and remain concealed. The technique described here overcomes these problems and allows populations of *S. laticeps* to be reared on a continuous basis using their natural food medium and, to some extent, mimics their natural habitat within the bulb scales. It provides them with their natural breeding surface, although the air space above the mites in the culture cells would be larger than that between scales in the bulb, and the glass cover would also not allow the retention or availability of such a high relative humidity as living bulb material. The method has proved successful over several generations, although it could, no doubt, be further refined by investigations of the best conditions for maximum productivity. Cultures can be reared in the same cells for 6-8 weeks at 20°C, and for 2-3 weeks longer at 15°C, before being transferred to new cells.

The life history results suggest that, taking into account the mean number of eggs laid, the percentage hatch and the adult longevity, the optimum temperature for development of these mites is around 20°C, and that at 25°C and above no development takes place. The results at 10°C suggest some heterogeneity in response, with some females being able to reproduce and others not. At 15°C and 20°C the life history was completed more quickly than the 49 days recorded by Hodson (1934). At all temperatures the maximum observed adult longevity was equal to or longer than that found by Hodson (1934) but in both cases the adults were of unknown age at the beginning of the experiments so the actual longevity was longer than that observed. Whilst Hodson (1934) did not observe males until at least the third generation, males were observed here from the first generation onwards.

Although these results confirm what Hodson (1934) suspected, that the life history could be considerably shortened at the high temperatures used during the forcing process, they should not be used as a definitive set of values for *S. laticeps*. The present work should be considered a feasibility study. There were insufficient replicates for a complete life history study and further work needs to be carried out in order to confirm and improve on the results obtained to date. In particular the results at higher temperatures need further investigation. The higher mortalities and failure to complete the life cycle may be the result of failure to maintain a sufficiently high relative humidity or they may be a function of the effect of the higher temperatures on the bulb scale tissue. Although large numbers of eggs can be laid by some females and the life cycle completed at 10°C, further data is also needed at this and at lower temperatures which would be relevant to the prevention of a build up of infestation during storage.

The development of a successful culturing method has a number of implications for control recommendations and provides the possibility of looking at the efficacy of present treatments. Following harvesting in July, narcissus bulbs are frequently stored outdoors for a considerable period (2-3 months). External ambient temperatures during this period can in some years be quite high (18°C), temperatures approaching ideal conditions for the rapid build up of *S. laticeps* populations. Even at 10°C, large numbers of eggs can be laid and the life cycle completed. Bulbs for forcing, which may have already been stored outside at near optimum conditions for the mites, are "vernalised" by placing them at less than 9°C for 9 weeks prior to forcing. There is, therefore, not only the potential for a large build up of the infestation during the outside storage period, but also for a continued slow but steady increase of this population during "vernalisation". Depending on the market, forcing occurs over a range of times (2-6 weeks) and temperatures which can be as high as 21°C. This could result in a further large increase in an already rapidly increasing infestation.

Our results show a very high mortality and cessation of development at around 25°C. Bulbs for replanting outside are usually hot water treated at temperatures of 43-44°C, often resulting in damage to the bulbs. It would appear that such high temperatures may not be necessary to control *S. laticeps*.

The results indicate a considerable variation in the duration of the egg period, the longest being 49 days. In the past, experiments on control by fumigation have been considered successful if, on examination, no eggs were found to have hatched after 14 days (Gurney and Gandy, 1974). It has also been suggested (Mackie *et al*, 1942) that treatments are repeated after 14 days to ensure complete control, this being considered the limit of duration of the egg stage. Our results show that this is not necessarily true, and that there may still be the potential for build up of infestation long after this period. This culturing method could be used not only to monitor post-treatment egg survival and hatch, but also to assess the success of the hot water treatment as there appears to be no confirmation of the efficacy of this method.

In conclusion the development of the culturing technique has made further biological studies possible and could provide important information on the apparent failure of control measures in the past.The information gathered could also be used to develop a more effective strategy to control mites when ambient conditions predict exacerbation of an existing problem

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Session 6A

Recent Developments in the use of Biological Control Agents against Pests

Chairman	Mr S C Gordon
Session Organiser	Dr R J Chambers
Papers	6A-1 to 6A-4

BIOLOGICAL CONTROL OF PESTS: CURRENT TRENDS AND FUTURE PROSPECTS

W.J. RAVENSBERG

Koppert BV, Veilingweg 17, P.O.Box 155, 2650 AD Berkel en Rodenrijs, The Netherlands

ABSTRACT

The use of beneficial organisms for biological control of pests has increased considerably over the last decade. This is due to increasing resistance of pests against pesticides, new imported pests, more research efforts, the increased availability of natural enemies and a growing concern about the use of pesticides. The application of natural enemies in European horticulture is described covering protected crops, field vegetables, hardy ornamental stock and soft fruit. Natural enemies are mainly used in vegetables under glass. Use under polythene covers and in field crops is slowly increasing, particularly in southern Europe. The success of biological pest control is demonstrated by the expansion of the biocontrol industry, however there is a worrying tendency to supply products with inadequate information on their use. Legislation is being developed by an increasing number of countries for both microbiological pesticides and beneficial arthropods. The current regulations and the need for harmonisation are discussed. A view of the future of biocontrol is given focussing on the main limiting and promoting factors.

INTRODUCTION

Beneficial organisms are used widely for biological control of pests in commercial horticultural crops. Many beneficial arthropods (parasites and predators) as well as insect-parasitic nematodes are released in a variety of crops. Research on microbial pesticides based on bacteria, fungi or viruses is being conducted and some have reached the market as products. This paper outlines the current status of biological control in European horticulture and discusses both positive and negative developments and their effects on future prospects.

Commercial applications of biological control agents are described in a general sense within a European context. Information from countries belonging to the former Soviet Union is not included since reliable data is hard to obtain. This overview concentrates on the use of natural enemies in protected crops and in soft fruit, vegetables and ornamentals grown outdoors. Only natural enemies that are readily available to professional growers are mentioned. All commercial applications are directed towards the control of insect and mite pests; natural enemies for control of other pests like nematodes, slugs or rodents are not available. With increased use of biological control the associated industry has undergone considerable expansion and a view of that industry is given with emphasis on the role it plays in the success of biocontrol.

Until recently the use of beneficial arthropods was exempt from registration. This is gradually changing and current legislation is discussed as are possible future developments in this area. In general a very bright future is predicted for biological control. Whether this is a realistic view is outlined by discussing present barriers for the practical use of biocontrol and promotional factors that could generate an increase in its use.

NATURAL ENEMIES AND THEIR COMMERCIAL USE

History of biological control in greenhouses

The commercial application of biological control in European horticulture spans a period of more than 25 years. It started in 1968 with the use of *Phytoseiulus persimilis* for the control

of two-spotted spider mites (*Tetranychus urticae*) in glasshouse cucumber. This was followed by the application of the parasite *Encarsia formosa* for the control of the greenhouse whitefly (*Trialeurodes vaporariorum)* in tomato. The area on which they were applied increased gradually and today thousands of growers are using these beneficials. Because they were used as part of integrated pest management programmes the chemicals used to control other pests and diseases often interfered with the biological control. Consequently, the search for natural enemies of pests such as leafminers, thrips and aphids was initiated and one by one new natural enemies began to be mass-produced and incorporated into IPM programmes.

Natural enemies were used predominantly in tomato and cucumber until about 1985. Since then biocontrol has been used in sweet pepper, eggplant, bean, melon and strawberry. Only relatively recently have natural enemies been used in greenhouse ornamentals, outdoor vegetables, soft fruit and hardy ornamental nursery stock (Ravensberg, 1992). Growers use biological control methods mostly because the efficacy and costs are comparable with conventional methods. Use for "philosophical" reasons such as "green" or "organic farming" only accounts for a very small percentage of the users.

Commercially-available natural enemies

Over the past 25 years more than 25 species of natural enemy have been taken into production and sold commercially for biological pest control (Table 1). The first two species have already been mentioned, the first microbial pesticide based on *Bacillus thuringiensis* came on the market for use in protected crops at the beginning of the seventies. It took quite a long time before they were joined by other natural enemies. The search for new beneficial arthropods and microbials was and is necessary mainly for two reasons. Chemical control of secondary pests interferes with the natural enemies in use. Of greater importance are accidental introductions of new pest organisms to Europe which are resistant to a large number of chemical pesticides. The main threats for existing IPM programmes were the establishment in Europe of *Liriomyza trifolii*, *Frankliniella occidentalis*, *Aphis gossypii* and *Bemisia tabaci* (van Lenteren et al., 1987). These dictated the need for research into and subsequent production of the beneficial organisms to control leafminers, thrips, aphids and whitefly. Most recently a new aphid species poses a threat to biocontrol in sweet pepper in the Netherlands. Initially thought to be a red form of *Myzus persicae*, it is resistant to pirimicarb and difficult to control with other chemicals. It has now been identified as *Myzus nicotianae* (Guldemond & Dieleman, 1994).

With the expansion of biocontrol into other crops such as ornamentals, soft fruit and field vegetables natural enemies of mealybugs, Sciaridae and *Otiorhynchus* spp. have been taken into production. Many new natural enemies are currently being evaluated for future use.

Microbial pesticides

The number of microbial pesticides available on the market for control of insects is still very small. *Bacillus thuringiensis* is available in most countries for the control of caterpillars; in some countries *Bacillus thuringiensis* var. *israelensis* is available for control of mosquitoes and is occasionally used to control Sciaridae in ornamentals. Commercial formulations of the entomopathogenic fungus *Verticillium lecanii* are available on the market for the control of whitefly and aphids. These are Mycotal, registered in the UK, the Netherlands and Switzerland, and Vertalec, registered in the UK and Switzerland (Ravensberg et al., 1994). A third product, Microgermin Plus, is used, as both other products, in Denmark and Sweden without a registration.

A product has been developed based on the fungus *Metarhizium anisopliae* for the control of the black wine weevil (Stenzel et al., 1992). It has been registered in Germany and Austria, but is not available on the market. One virus-based product is registered in the Netherlands (Spod-X), for the control of *Spodoptera exigua* in greenhouse ornamentals (Smits & Vlak, in press).

Research on some entomopathogenic fungi for the control of several greenhouse pests is being conducted. These are *Aschersonia aleyrodis*, *Beauveria bassiana* and *Paecilomyces fumosoroseus* (Meekes et al., in press). It is difficult to predict when these will become available on the market. Viruses of *Mamestra brassicae* and *Spodoptera* spp. are also being investigated (Fargues et al., 1991) and in 1993 the *Mamestra* NPV was registered in France for control of cabbage moth on cabbages.

Areas with commercial applications of natural enemies

Natural enemies are used in horticulture in almost all European countries. The crops in which they are applied are mainly tomato, cucumber and sweet pepper. These three crops probably make up more than 80% of the total area utilising biological control. Other crops include bean, eggplant, melon, watermelon, strawberry and various cut flowers and ornamental plants. Biocontrol is predominantly used in heated greenhouses where crops are cultivated for periods of 8 –10 months or more. Usage under polythene and in field crops are increasing. Protected crops in Europe cover an area of about 65,000 ha, of which 17,000 ha are glasshouses (van Lenteren & Woets, 1988). Biocontrol is now applied on an estimated 6,000 ha. Chemical control is still used on a large area, though this is slowly but steadily expected to decrease in favour of integrated control.

TABLE 1. Commercially-applied natural enemies for control of horticultural pests

Natural enemy	Target pest	In use since
Phytoseiulus persimilis	*Tetranychus urticae*	1968
Encarsia formosa	*Trialeurodes vaporariorum* (1)	1972
Bacillus thuringiensis	caterpillars	1972
Opius pallipes (2)	*Liriomyza bryoniae*	1980
Dacnusa sibirica	*Liriomyza bryoniae* / *L. trifolii* (3)	1981
Amblyseius barkeri	*Thrips tabaci* (4/5)	1981
Diglyphus isaea	*Liriomyza bryoniae* / *L. trifolii* (3)	1984
Heterorhabditis spp.	*Otiorhynchus* spp.	1984
Steinernema spp.	Sciaridae	1984
Amblyseius cucumeris	*Thrips tabaci* (5)	1985
Chrysoperla carnea	aphids	1987
Aphidoletes aphidimyza	aphids	1989
Aphidius matricariae	*Myzus persicae* (6)	1990
Verticillium lecanii	whitefly / aphids	1990
Orius spp.	*Frankliniella occidentalis, T. tabaci*	1991
Aphidius colemani	*Aphis gossypii, Myzus persicae*	1992
Trichogramma evanescens	caterpillars	1993
Aphelinus abdominalis	*Macrosiphum euphorbiae*	1993
Leptomastyx dactylopii	citrus mealybug	1993
Cryptolaemus montrouzieri	mealybugs	1993
Hippodamia convergens	aphids (7)	1993
Macrolophus caliginosus	whitefly	1994
Amblyseius degenerans	thrips	1994
Spodoptera exigua NPV	*Spodoptera exigua*	1994

1) since 1988 also against *Bemisia tabaci*
2) only until 1982, other natural enemy available
3) since 1990 also against *Liriomyza huidobrensis*
4) only until 1990, other natural enemy available
5) since 1986 also against *Frankliniella occidentalis*
6) only until 1992, other natural enemy available
7) not mass-produced, but field collected.

THE BIOCONTROL INDUSTRY

When the commercial use of natural enemies started two companies were mass-producing insects and mites. This number increased slowly during the early eighties, but over the last decade the number of producers has grown to over thirty. There are three large companies (> 100 employees), most of the others are smaller (< 30 employees). Some of the companies only concentrate their activities in the fields of biological control and pollination (bumblebees are used for pollination of tomatoes), others are part of larger organisations with different kinds of interests in or even outside the horticultural sector. Only the larger companies produce the whole range of natural enemies and bumblebees, most of the smaller ones only produce part of the range. Large chemical companies now show serious interest in this area and one of them has recently started distributing natural enemies.

As the application of and research into biological control increased, many countries felt that they needed a local producer. In many cases the establishment of a producer has been stimulated by governmental or regional subsidies, as for instance in Germany and Italy. This has sometimes caused unequal competition.

Due to the increasing number of producers and a relatively small market, fierce competition has arisen over the last couple of years and has resulted in lower prices for the growers. Bumblebee prices were the first to decrease, closely followed by natural enemies. Initially growers profited from the lower prices. However, technical advice given with the natural enemy is included in the product price and is becoming harder and harder to justify economically because of price reductions and lower margins. Whether this is a good development in the long run is very doubtful since the success of biological control largely depends on the combination of product plus advice.

Another development is also tending to loosen the link between product and advice. As the competition for customers becomes ever stronger, not only producers of natural enemies but also distributors are trying to promote themselves to the growers by offering "new" natural enemies. In many cases these new products are not accompanied with proper instructions; applied research is only performed by a small number of companies and national research institutes cannot keep up with these developments. The natural enemies are often shipped from other countries or even other continents and proper investigations on their use have not been conducted at all or not for the local situation. For example the predatory mite *Amblyseius fallacis* is now promoted for control of spider mites in Dutch greenhouses. This predator is used in Canada on outdoor crops such as apples and strawberries. Whether this mite is useful in greenhouse crops, besides the already available predatory mites, has not been investigated. This could lead to failures and resultant mistrust of the growers. The image of biocontrol carefully built up over 25 years is now under threat because of these new market developments. The growers may hope to profit from the fierce competition, however in the long run it could lead to biocontrol failures and an associated reduction in the use of biocontrol.

The industry should voluntarily prepare self-regulating guidelines to prevent unwanted developments. For instance, a certification system could be developed certifying those companies that deliver qualitatively good products accompanied with proper advice. The latter could also apply to distributors. If not, local or European regulations may be needed to keep biocontrol safe, reliable and effective. At this time nobody can afford a reduction of the successes of biocontrol. The future for biocontrol looks bright, but we have to guard against uncontrolled growth that might result in biocontrol failures and bad publicity.

BIOLOGICAL CONTROL AGENTS AND LEGISLATION

Registration of micro-organisms

In general biological control products based on micro-organisms such as bacteria, fungi,

viruses and protozoa need to be registered through similar procedures as those used for chemical pesticides. Until recently Sweden and Denmark were the exception and biological products could be sold without any kind of registration. Legislation dealing with registration of micro-organisms has now been drafted and was implemented in Denmark in July 1994 and is due to come into force in Sweden from January 1995.

Although in many countries the authorities encourage the development of safer pesticides such as those containing micro-organisms, special criteria and guidelines for applying for the registration of these products have hardly been developed. Only in the Netherlands has an application form for biologicals complete with guidelines and requirements been developed. This became available in 1992 after more than 10 years of discussion. Another factor is the cost of registration. Some countries charge lower registration fees for biological products than for chemical products. For example in the UK registration of a chemical product (a new active ingredient) costs £ 60,000 whereas a biological product costs £ 13,600; in Denmark registration of microbials is free until 31 December 1995. However, from this year (1994) in the Netherlands both categories of insecticides have costed Dfl. 43,000 to register. A cost differential is still being discussed. The cost of registration of microbials, which are generally very specific and only aimed at a small market, deters their development in most European countries. This is often in contradiction with political aims in this area.

Registration of macro-organisms

Registration of macro-organisms such as mites, insects and nematodes has not been required by most European countries until now. Some countries ask for import permits for non-indigenous organisms (see below). In Switzerland it is necessary to register these types of products. There is however no special administrative procedure and/or application form for biological control agents. In Hungary registration is officially required, however the procedure is still under evaluation and waiting for EU guidelines. Natural enemies are sold without registration at present. Austria introduced regulations for macro-organisms in 1991 but only implemented them in 1992. France and Sweden are preparing legislation. Many countries are discussing whether or not they need registration for these macro-organisms and some have started to develop guidelines and requirements for applications.

In the European Union "macroscopic biocontrol agents" are exempt from evaluation under the new pesticide legislation (Directive 91/414/EEC). It seems that the EU also wants to develop legislation to regulate macro-organisms but presumably this will not be within the forseeable future. In general the situation differs considerably from country to country and this leads to confusion about the information required.

Non-indigenous organisms

For micro-organisms the use of non-indigenous species is covered by the registration procedure. More questions are usually asked than for indigenous species, especially on likely environmental impacts. For macro-organisms, countries have different criteria to allow importation and release and information on possible environmental impact has to be provided before an import permit is granted.

In the UK, Germany and Denmark some existing legislation seems to apply to the import of these organisms. In the UK the Wildlife and Countryside Act prohibits the release into the environment of non-indigenous organisms. This is backed up by the Plant Health Order which lists species of organisms posing a threat to plant health that are notifiable. Provision has recently been made for the inclusion of non-indigenous species of natural enemies and an import licence can be applied for from the Department of the Environment. Special concern is given to the environmental impact of new species (establishment, competition, effects on non-target organisms, etc.). The procedure still seems to be under review. In Germany similar legislation through the "Bundesnaturschutzgesetz" seems to apply to non-indigenous natural enemies. The procedure to obtain a permit in this case is unclear. Denmark recently enabled a

new Act on the protection of the environment and releases of non-indigenous organisms are no longer permitted. The consequences for biocontrol organisms are not yet clear.

Code of conduct

A code of conduct for the import and release of biocontrol agents is being developed under the auspices of the FAO and the IOBC. Organisations addressed are governmental bodies, research institutes, industry, trade associations and the public sector. The aim of this is " .....to set forth responsibilities and to establish voluntary standards of conduct for all public and private entities engaged in or affecting the distribution and use of biological control agents, particularly where national legislation to regulate their use does not exist or is inadequate" (FAO, 1992). At first the Code mainly dealt with releases for classical biological control, but augmentative releases are being given more attention in each round of discussions. The goal of this code is to harmonise regulation, to prevent unnecessary and complicated national legislation and to prevent undesirable, harmful effects of releases as much as possible.

The development of quality control criteria

Inundative and seasonal inoculative biological control are based on regular introductions of mass-produced natural enemies. The success of the programmes rely largely on the quality of the natural enemies. But what is quality and how can it be measured? Producers of natural enemies and research workers in the global IOBC Working Group "Quality Control of Mass Reared Arthropods" are collaborating to develop quality control criteria and tests. The aim of quality control should be to determine whether a mass-reared natural enemy is still in a condition to properly control the pest. Criteria refer to product control, i.e. to the end product that leaves the producer and are designed to be able to define and measure quality; this will give an indication of quality to producers, distributors and users (van Lenteren, 1993a). The biocontrol industry should voluntarily develop these criteria to be able to guarantee a standard quality to its customers. This will demonstrate that the industry wants to be taken as seriously as any other. It is hoped that quality control criteria will deter inexperienced operators who may cause biological control failures.

Hopefully this IOBC initiative will prevent the development of all kinds of criteria by different legislative authorities. The Working Group expects that within two or three years these criteria will be developed and accepted by the biocontrol industry as standard guidelines. How these criteria will fit into national or European legislation is not yet clear.

Legislation and consequences for the use of biocontrol agents

After some 25 years of commercial application more and more national authorities feel the need to regulate biocontrol agents to conform with the use of other control compounds. This has some positive and negative influences on biological control.

Registration of microbial pesticides is without doubt necessary because of toxicological and environmental issues. However, problems are created by the enormous variation in registration requirements between countries and it is very hard to compile one set of data that allows application in many countries. At the same time this makes estimations of costs very difficult. A unified procedure is necessary. In the EU Agrochemical Registration Directive common criteria are being established for evaluation of products. These uniform principles aim to ensure that all member states will have the same requirements for registration including microbials. A more detailed overview of the foreseen registration routes for microbial pesticides is given by Marshall (1992).

Regulation of the use of macro-organisms is to some extent desirable, both from the point of view of governments and the biocontrol industry itself. Governments would like to know what is happening and to satisfy this need a duty to report uses and imports of natural enemies could be considered. This could be included with information on the host range,

biological parameters and possible environmental effects to be provided by the distributor of the natural enemies. If objections arise from these data more information could be requested.

Most people involved in biocontrol do not see the need for strict registration of macro-organisms by the national authorities. Why should efficacy be proven in all kinds of crops and all kinds of circumstances by using very specific protocols? Good companies will only market reliable products and therefore conduct voluntarily their own efficacy test. If natural enemies do their job, growers will use them, if not they will not buy them. Let general market mechanisms take care of this. It is in the interest of the industry itself to develop some kind of code, certification or quality mark to assure reliability to its customers. Quality control criteria are a step in this direction.

Strict registration leads to a number of problems, especially related to efficacy testing. With "well known" macro-organisms test protocols are rather easy to develop, but with "new" species this is more difficult. It usually takes several years to build up enough experience to be able to give the most appropriate instructions for using a natural enemy. To attempt to prove efficacy in their first year of use could mean failure to register them. A remarkable example of the problems associated with registration procedures is seen in Morocco and Japan, countries without any history of commercial use of natural enemies. Efficacy data are required for each natural enemy on every crop and tests have to be done by the authorities over several seasons. Test protocols are not very appropriate and lead to failures which waste time. The applicant often needs to guide the authorities intensively. Since many natural enemies are used in IPM programmes it will take a long time to register them all, even for one crop, and so growers will have to wait before they can be offered reliable pest control programmes. This hampers the implementation of biocontrol enormously.

The uncertainties about importation, registration and release of natural enemies and all the different requirements hamper and delay the use of natural enemies. For some countries this really forms a threshold in developing IPM programmes. In the case of microbials it certainly means that companies are very reluctant to start registration procedures. Only if the market is relatively will registration be pursued.

FUTURE PROSPECTS

Biological control has expanded considerably over the past decade. However the increase in area using biocontrol has not been as spectacular (3,000 ha in 1985, 6,000 ha in 1994) as the range of natural enemies in use. In the glasshouse sector the role of biocontrol in IPM programmes has become increasingly important with the result that most insect and mite pests are controlled by beneficial organisms. This has led to a reduction in the use of chemicals. But why has biocontrol not expanded more, for instance into other crops, in polythene tunnels, etc.? The most important reasons for this are discussed below. Van Lenteren (1993b) also discusses present barriers to the practical use of IPM. Factors which could promote biocontrol in the near future are also listed.

Limiting factors

Costs of natural enemies

As the number of natural enemies used in a certain crop increase biocontrol can become prohibitively expensive to the grower. This has been particularly evident in cucumber over the past few years. Although thrips and aphids can be controlled in this crop by using natural enemies the number needed makes the programme too costly. This is emphasised even more by the tendency to plant two or three crops in one year, each new crop requiring introductions of beneficials. With the availability of new chemicals with a relatively broad spectrum, biocontrol is simply outcompeted. This happened in the Netherlands when abamectin became available for the control of thrips, spider mites and leafminers. The same thing is expected to

happen when imidacloprid comes onto the market for control of whitefly and aphids. With the appearance of compounds such as these the grower soon abandons biocontrol, especially when prices of vegetables are as low as they have been over the past three years.

IPM is complicated

A large number of the growers that have protected vegetables still use chemical control. It is an old strategy that is easily comprehended and allows them to make independent decisions on when and how to use it. In contrast, IPM requires much more attention to detail. More knowledge of the pests and the natural enemies is required as well as of integration with other control methods. In addition, monitoring the pest and the natural enemy requires greater discipline from the grower and the ability to react to specific situations. Often an adviser is needed to analyse the situation with the grower. This makes IPM more complicated and not easily adopted by growers. This is especially seen in southern Europe where large areas are still treated by chemical control alone. Research, extension and education of growers is desperately needed to stimulate IPM.

Lack of tolerance to pest damage

Many countries have a zero tolerance to signs, presence and damage of arthropods on imported plant material, especially in ornamentals. This instills in the growers the need for a very intensive chemical control programme. In this way import restrictions imposed by government bodies imply extensive use of chemicals and limit the use of biocontrol. The quarantine status of some pests (e.g. leafminers or *Bemisia tabaci*) force growers to over-use pesticides. The lack of tolerance, even to slight cosmetic damage, in wholesale trade channels also results in the use of chemicals. As a result there is little or no room for biocontrol in pot plants and cut flowers where, in contrast to vegetables such as tomatoes, the whole plant or a large part of it is sold. For ornamental crops these restrictions form a large barrier to biological control.

Growing conditions

Most biocontrol is used in heated greenhouses. However the largest area of protected crops lies in southern Europe where no heating systems are normally used. This causes quite different climatic conditions compared to northern and middle Europe. Moreover, greenhouse constructions are very different of which possibly the most important aspect is their open structure. This makes migration of pests into the crop from fields or weeds around the greenhouse more likely. Growing periods and management practices also differ greatly in southern Europe and this also has an impact on the possibilities for biocontrol. These conditions make the implementation of biocontrol more difficult and different strategies will have to be developed, possibly including other natural enemies which are better adapted to the region. It is clear that this will take time.

Promoting factors

Resistance to pesticides

The main driving force to use biological control comes from the resistance of pests to chemicals. Many imported pests have high levels of resistance. Moreover pests continue to develop resistance to pesticides, particularly in horticultural crops where frequent applications of insecticides exert a strong selection pressure on insects. A useful selective whitefly compound, buprofezin, was lost through resistance within a few years (techn. comm. Zeneca). When reasonable results with chemicals can no longer be achieved, biological alternatives will be used. This has been seen on a large scale in many situations. Striking examples are the problems caused by *Liriomyza trifolii*, *L. huidobrensis*, *Frankliniella occidentalis*, *Aphis gossypii* and *Bemisia tabaci*. Most of these can now be controlled by using natural enemies: usually the only viable option.

Accidentally-imported resistant pests have caused a great deal of trouble in existing IPM programmes. In another sense it has helped biological control by proving so clearly to growers that biocontrol works better and longer than chemical control.

Increasing availability of natural enemies

The number of natural enemies available to growers has increased rapidly and is expected to continue. Biocontrol systems are also becoming more reliable and sophisticated and possibilities exist for preventive use. *Orius* spp. and *Amblyseius cucumeris* are two such examples. These can feed and reproduce in crops containing pollen but change their diet to the pest organism as soon as it appears. Another development is the use of slow-release-systems from which predators migrate into the crop over a period of weeks. These equate to a small in-crop rearing system. Beside the available arthropods, insect-parasitic nematodes are sold on the market and will presumably soon be followed by slug-killing nematodes (Wilson et al., 1993).

Micro-organisms are still rarely used as control agents. Interest in the development and production of microbial pesticides is shown by large chemical companies. The number of products that can be expected to appear in the near future is, however, still limited. The availability and use of bumblebees for pollination promotes the application of biological control, especially in tomato.

Environmental concern

The development of new pesticides is becoming more and more expensive, due partly to environmental concern which results in tighter registration requirements. Consequently fewer pesticides are likely to become available to the glasshouse market and so more opportunities for biocontrol will arise. Although this is the broad trend, however, with the release of compounds such as abamectin and imidacloprid, biocontrol does not gain ground.

Environmental concern has become a political issue and many European governments want to reduce the use of chemicals. This could result in a greater demand for alternatives to the currently used chemical methods. Reduction in chemicals does not, however, automatically lead to more use of natural enemies. Many other methods can be used to reach that goal, such as hygienic measures, supervised chemical control, the use of resistant cultivars, crop rotation, etc.

The changing attitude of consumers and supermarket chains towards produce treated with chemicals is the main market driving force that stimulates IPM. Although consumers are hardly willing to pay more for products not treated with chemicals, the competition between growers' cooperatives and between supermarkets now strongly favours biological control. This is probably the most direct driving force that could, slowly but steadily, lead to further expansion in the use of natural enemies.

Concluding remarks

Is the future for biological control really so bright as is often said? We have seen that many barriers still exist that do not favour the practical use of biocontrol. However, many developments do support the use of natural enemies and will definitely facilitate an increase in biocontrol in the future. The determination of governments to reduce the use of chemicals will cause demands for biological alternatives as will the growing concern of consumers on the use of chemical products in the production of their food. Since these are relatively slow processes the role of parasites, predators and insect pathogens is not expected to increase dramatically. Biological control will not replace chemical control, but it will become an important component of pest control programmes.

The role that governments play is very important. To make biological control more widely

accepted more emphasis should be placed on research, extension, education and promotion. Various European countries have come up with plans to reduce the use of chemicals. Intentions are very good and offer possibilities for the use of biocontrol, but until now the effects have been rather small. Alternative control methods have to be developed by research workers with the biocontrol industry and then promoted through good education and extension programmes. This needs considerable funding from governments which is generally lacking. Promotion through specific legislative measures could be a good tool. Such measures could include; taxes on chemicals, withdrawal of chemicals when reliable biological alternatives are available, more control over chemical use through prescription systems, perhaps in combination with certification of pest control advisers, clear and harmonised registration procedures for microbial pesticides.

At present it is clear that the real incentive for growers to use biological control is the market. The supermarket chains and the consumer increasingly ask for food and ornamental plants produced safely and in an environmentally-friendly way. All over Europe this has a greater influence on growers decisions on how to control their pests. This is of course the best way in which biocontrol could expand. Providing growers a wide enough range of effective biological solutions which can be relied upon is the challenge to research, extension and the biocontrol industry for the near future.

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CONTROL OF *OTIORHYNCUS SULCATUS* IN SOFT FRUIT USING DRENCH TREATMENTS OF *STEINERNEMA CARPOCAPSAE*

A. C. SAMPSON

Ciba Bunting Limited, Westwood Park, Little Horkesley, Colchester, Essex, CO6 4BS

ABSTRACT

Drench treatments of *S. carpocapsae* achieved 66-85% control of vine weevil larvae in strawberries, and 34-66% control in blackcurrants when applied at soil temperatures above 12°C. Treatments brought weevil populations down to an average of 2 per plant in strawberries and 3.5 per plant in blackcurrants. As a result of reduced weevil populations, plant growth in treated plots was 17 to 100 percent greater than in untreated plots. The amount of regrowth varied with number of weevils per plant, plant variety and the cultural techniques employed. The percentage control declined steadily with temperatures below 15°C and treatment timing was critical in order to combine adequate temperatures with the presence of larvae in the soil. Therefore, treatments in early May and late August are recommended in the UK. A rate of 5 x 10^9 nematodes per treated hectare is recommended following replicated rates trials.

INTRODUCTION

The pest status of the black vine weevil, *Otiorhynchus sulcatus* (F.), has increased since the withdrawal of persistent organochlorine compounds and the uptake of cultural practices which favour weevil development and survival (Moorhouse *et al.*, 1992). Vine weevil can be found frequently in nursery suppliers from where it is distributed widely into gardens throughout the country. Being polyphagous and parthenogenetic the pest establishes rapidly in new areas.

Vine weevil is now the most damaging strawberry pest in the UK and an increasing problem in blackcurrants. Larvae feed on roots and can cause plants to wilt and die. Chemical control options are limited in both number and efficacy. The broad spectrum chemicals available for larval control cannot be used during cropping because of residues; they also harm natural enemies such as ground beetles (Evenhuis, 1983). Viable alternatives are sought and entomopathogenic nematodes are considered one of the most promising options for soft fruit.

Nematodes of the genera *Steinernema* and *Heterorhabditis* and their associated bacteria *Xenorhabdus* spp. kill vine weevil under suitable environmental conditions. They have the advantages of being easy to apply and are effective against a wide range of soil-inhabiting pests with a rapid kill equivalent to insecticides (Kaya and Gaugler, 1993). In addition, they are safe to vertebrates and have little impact on non-target arthropods (Georgis *et al.*, 1991) and can therefore be applied before and during cropping. Through recent advances in production technology *Steinernema carpocapsae* (Weiser) can now be produced more cheaply than other nematode products by liquid fermentation. Improved formulations

protect the infective juvenile stage and provide a consistent high quality to the end user with a shelf life of up to five months at room temperature or up to twelve months under refrigeration (Georgis, 1992). Trials were carried out to test the efficacy of *S. carpocapsae* and determine optimum timing and application rates for the control of *O. sulcatus* in soft fruit under UK conditions. The results have been used to make commercial recommendations for the product EXHIBIT.

METHODS

Field trials were carried out in commercial strawberry and blackcurrant crops in Kent, Sussex and East Anglia between 1990 and 1993. Each consisted of a 3 or 4 replicate randomised block design with equal numbers of treated and control plots. Plots varied in size, the minimum being 200 blackcurrant bushes or 400 strawberry plants.

An alginate gel formulation of *S. carpocapsae* was applied through commercial spray equipment as a high volume drench. The spray was drenched over the top of the strawberry plants or directed to the base of blackcurrant bushes from both sides of the row. Where available, irrigation was applied before and after application unless the soil was already moist through rainfall. Soil temperatures were measured at a depth of 5cm on the day of application at all sites. Application of nematodes through T-tape irrigation was also trialed in collaboration with Reading University (Kakouki *et al.*, 1994).

In strawberry trials, different treatment timings of *S. carpocapsae* were compared. Applications were made in spring or summer and at varying soil temperatures. From four to twelve weeks after treatment plants were randomly selected for sampling, then removed with the root ball and surrounding soil of dimensions 30 x 30 x 15cm. The soil was sifted by hand and vine weevils removed and counted. Between 10 and 40 plants were sampled per plot. In the 1993 trials additional assessments were made to determine the effect of treatment on plant vigour. The number of new leaves were counted from 50 randomly selected plants per plot, two months after treatment.

Blackcurrants trials were carried out in 1992 and 1993. Applications were not made at soil temperatures below 12°C following poor results in the strawberry trials at low temperatures. In addition to spring and summer trials, trials 6B and 7B were treated in summer 1992 and spring 1993. Vine weevil numbers were assessed as above with 10 plants sampled per plot. Plant growth response was measured in summer 1993. The number of new basal shoots per bush and length of regrowth on a randomly selected side branch was measured on 100 plants per plot.

Replicated rates trials were carried out in 1993. In the summer strawberry trials, rates of 2.5 and 5 billion nematodes per treated hectare were trialed. In spring blackcurrant trials, rates of 2.5, 5 and 10 billion nematodes per treated hectare were tested. As it was a cool spring, applications were made while soil temperatures were still below optimum at 12°C. It was decided to go ahead with treatment as the weevil population had started to pupate.

The site details for the strawberry and blackcurrant trials are given in Tables 1 and 2. Analysis of variance (ANOVAR) was carried out on weevil numbers and plant growth between treated and untreated plots within each trial using log (n+1) for normalisation.

TABLE 1. Site details for the strawberry trials

Trial number	1S 1990	2S 1990	3S 1991	4S 1992	5S 1992	6S 1993	7S 1993
Soil type	Sandy loam	Clay	Peat bags	Loam	Sandy loam	Loam	Sandy Loam
Cultivar	Elsanta	Elsanta		Elsanta	Elsanta	Dominal	Hapel
Rate (No/treated ha)	5×10^9	5×10^9	2.5×10^9	5×10^9	5×10^9	5×10^9♦	5×10^9♦
Treatment date	17.9.90	21.9.90	24.4.91	8.5.92	10.9.92	17.8.93	23.8.92
Volume of water/ plant (ml)	100	100	100	280	360	160	150
Sprayer	Knapsack	Knapsack	Hand	Claxton	Claxton	Claxton	Claxton
Soil temperature °C	12	10	19	10	13	16	15
Samples per plot (reps)	40(4)	40(4)	10(3)	30(4)	30(4)	50(3)	40(4)
Weevil range/plant	0-33	0-45	0-14	0-14	0-37	0-45	0-30
Weevils/plant untreated	9.6	4.5	2.1	0.75	8.5	11.8	17.5
Weevils/plant treated	3.45	2.7	0.4	0.48	2.8	2	3.1
% increase in growth	-	-	-	-	-	33	17
ANOVAR weevil nos. F=	5.86*	1.29NS	5.1*	0.3NS	27.6***	5.2*	10.6**

TABLE 2. Site details for the blackcurrant trials

Trial number	1B 1992	2B 1992	3B 1992	4B 1993	5B 1993	6B 1992-3	7B 1992-3
Soil type	Sandy	Clay	Silty	Sandy	Silty	Silty	Clay
Cultivar	Nevis	Ben Lomond	Nevis	Nevis	Nevis	Nevis	BenLomond
Rate (No/treated ha)	5×10^9	5×10^9	5×10^9	5×10^9✞	5×10^9✞	5×10^9✞	5×10^9✞
Treatment date	7.5.92	19.8.92	17.8.93	13.5.93	16.5.93	17.8.92 16.5.93	19.8.92 15.6.93
Volume of water/ plant(ml)	1500	2000	1700	1600	1700	1700	2000
Sprayer	Munckhof	Munckhof	Smallford	Munckhof	Smallford	Smallford	Munckhof
Soil temperature °C	14	15	15.4	13	12.5	12	12
Samples per plot (reps)	10(4)	5(4)	5(4)	10(4)	10(4)	10(4)	10(4)
Weevil range/plant	0-25	0-38	0-46	0-21	1-35	0-23◆	0-11◆
Weevils/plant untreated	8.7	7	7.9	5.1	9.6	5.1◆	4.3◆
Weevils/plant treated	3	3	4.5	2.6	6.3	3.2◆	2.2◆
% increase in growth	-	63	30	28	20	76	100
ANOVAR weevil nos. F=	25.2***	2.19	1.13NS	4.2*	14.4***	10.2**	16.5***

♦ Rate of 2.5×10^9 were also trialed.

✞ Rates of 2.5×10^9 and $10^9 \times 10^9$ were also trialed.

◆ Values only given for the spring re-treatment.

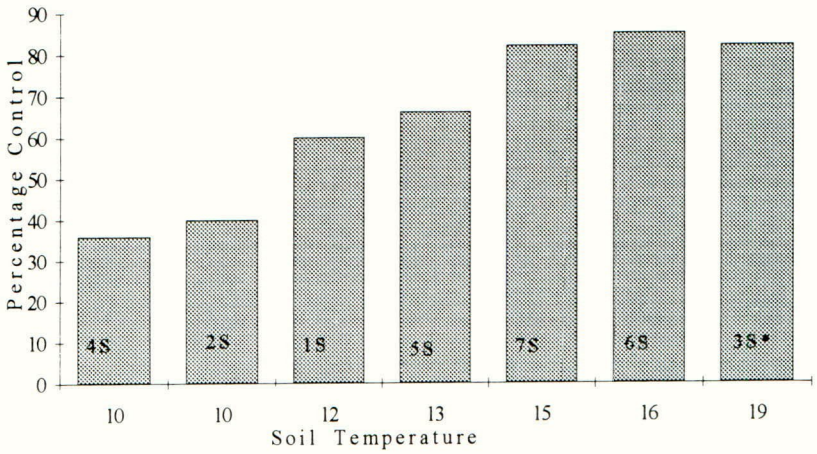
RESULTS

Efficacy and treatment timing

Strawberries

The percentage control achieved was greatly dependent on the soil temperature at the time of application (Fig 1.). At a rate of 5 billion per treated hectare and a soil temperature of 10°C, less than 50% control was achieved. At 15°C to 19°C, the level of control improved to 82-85%. At 10°C the level of control was similar whether nematodes were applied in summer against younger larvae (40% in trial 2S) or in spring against older larvae (36% in trial 4S). In 1993 trials, the 82% and 85% reduction in the vine weevil numbers corresponded with a 17% (Trial 7S) and 33% (Trial 6S) increase in new leaf material as compared to untreated plots.

Figure 1. Percentage vine weevil control at different soil temperatures in strawberries.
* Trial number.



Blackcurrants

The level of control achieved with the 5 billion rate in blackcurrants varied between 34% and 66% (Figure 2). The overall effect of combined summer and spring treatments on weevil numbers could not be determined because of movement between plots over-winter. Although the percent control was lower than in strawberries, nematode treatments reduced larval root damage with resulting increase in both the number of new canes initiated and the length of side shoot growth in the following summer. These factors were multiplied together to give a growth index. Summer treatments promoted 63% and 30% more growth (trials 2B and 3B), spring treatments promoted 28% and 20% more growth (trials 4B and 5B) and plants treated in both summer and spring had 76% and 100% more growth than untreated

plants (trials 6B and 7B). Treated plants were both measurably and visibly less stunted (Figure 3).

Figure 2. Average number of larvae per plant in treated and untreated blackcurrant plots using a rate of 5 billion nematodes per treated hectare.

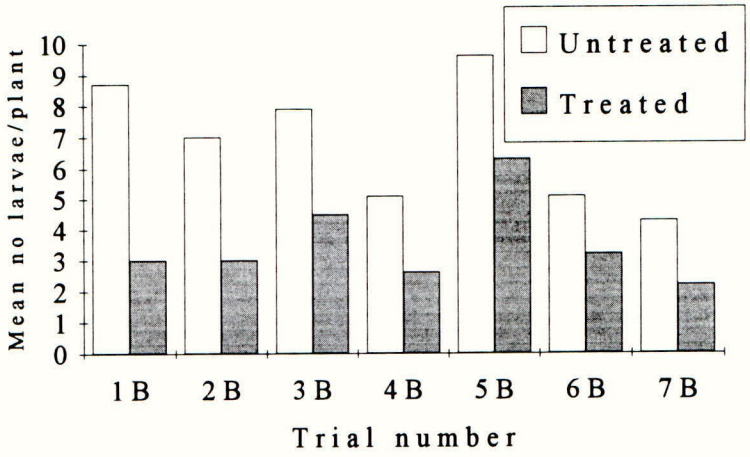
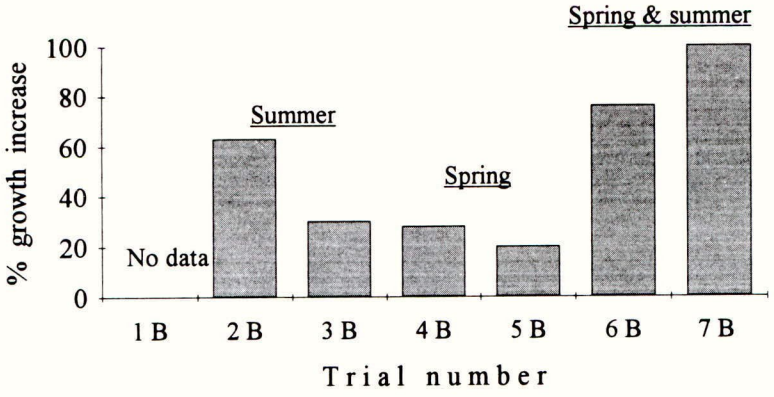


Figure 3. Percentage increase in plant growth as a result of nematode treatments in blackcurrants. Growth measured as no. of new shoots multiplied by length of regrowth.



Root damage caused by *O. sulcatus* had the greatest impact on new shoot development (Table 3). The number of new shoots was inversely proportional to the average number of vine weevils per bush.

TABLE 3. Impact of larval feeding on cane initiation and length of side shoot growth in two blackcurrant trials.

BEN LOMOND (7B)		NEVIS (6B)	
Mean no. larvae/bush (n=40)	No. of new canes/plant (n=100)	Mean no. larvae/bush (n=40)	No. of new canes/plant (n=100)
2.2	1.31 ± 0.36	3.2	3.33 ± 0.35
2.3	1.24 ± 0.35	5.3	2.65 ± 0.37
2.8	1.26 ± 0.34	6.3	2.06 ± 0.32
3	1.43 ± 0.25	7.7	2.10 ± 0.34
5.5	0.93 ± 0.22	8.6	1.84 ± 0.28
7	0.86 ± 0.23	9.5	1.94 ± 0.32

Application rates

In the spring 1993 blackcurrant trials, a rate of 5 billion per treated hectare provided significantly better control than the 2.5 billion rate ($p=0.05$). The 10 billion rate resulted in equal or reduced control compared to the 5 billion rate (Table 4).

TABLE 4. Rate response following spring applications in blackcurrants.

Rate per treated hectare	Percentage Larval Reduction				
	Trial 4B	Trial 5B	Trial 6B	Trial 7B	Average
2.5 Billion	11	10	40	26	22
5 Billion	48	34	36	49	42
10 Billion	24	20	35	49	32

In the summer 1993 strawberry trials there was no significant difference between rates of 2.5 and 5 billion per treated acre in trial 7S, but the level of control reduced from 83% at the 5 billion rate to 38% at the 2.5 billion rate in trial 6S.

DISCUSSION

S. carpocapsae achieved 82-85% larval control in UK strawberries when applied at 5 x 10^9 nematodes/treated hectare and temperatures above 14°C. 100% control is unlikely with a single treatment as some larvae will be protected inside the strawberry crown or located

outside the treatment zone. A few will develop before or after soil temperatures are suitable for control. The average number of weevils following treatment was two per plant (Table 1) which is considered to be below the damage threshold (Penman and Scott 1976). The overall effect of *S. carpocapsae* treatments was to bring the weevil population down enough to prevent stunting, so allowing a significant increase in plant growth and ultimately yield.

The poorer control in blackcurrants at the same rate can be explained by the larger root ball and the difficulty in applying nematodes throughout the weevil infested zone. However, blackcurrants have a higher damage threshold (Penman and Scott, 1976) and *S. carpocapsae* brought populations down to an average 3.5 larvae per bush (Table 1) while the greatest impact on plant growth was seen above five larvae per bush (Table 4). Where there is a bad weevil infestation the results show the benefits of two treatments a year in summer and spring. The total effect of two treatments applied in August and May on weevil numbers could not be determined in these trials because over nine months the weevil population had moved between plots. The plant growth effect was significant however ($p<0.001$) increasing from 76% with a single treatment to 100% with two treatments in trial 6B and from 30% to 63% in trial 7B. At these sites untreated bushes were severely stunted which explains the spectacular regrowth in treated bushes. Weevil damage had the greatest impact on the number of new canes initiated which will have a direct effect on the following year's yield as bushes fruit on the previous year's regrowth. With repeated use, nematode treatments will improve both the productivity and longevity of a plantation.

In both blackcurrants and strawberries, a rate of 5 billion per treated hectare gave the most consistent results. At higher rates the nematodes clump together and distribution is patchy (Biosys, pers. comm.). At the lower rate of 2.5 billion, the control achieved was just over half that of the 5 billion rate except in trials 7S and 6B where the two rates were not significantly different. Both sites were on sandy or silty land, and *S. carpocapsae* move better and persist longer in sandy soils than in clay soils (Kung *et al.*, 1990). At 15°C in a sandy loam soil the 2.5 billion rate may be sufficient as long as the soil is kept moist to enhance nematode movement and survival. Unless ideal treatment conditions are present, a rate of 5 billion per treated hectare is recommended.

Because efficacy is reduced below 15°C, treatment timing is critical. Adequate soil temperatures must be combined with the presence of larvae. In most years summer treatment against young larvae provides the best control, timed in the second half of August when the majority of weevil eggs have been laid and soil temperatures are still high, averaging 17°C. Through September, temperatures decline from 16°C to 12°C. With severe pest pressure a second treatment is recommended in spring. Soil temperatures rise above 10°C in the first week in May and increase steadily to reach 14°C by the end of May. However, the rise in temperature above 12°C also stimulates vine weevil larvae to pupate from mid-May (Garth and Shanks, 1978). By the beginning of June, when soil temperatures are optimal for nematode use, adults are starting to hatch out and the target population is lost. As infective juveniles persist at high levels in the soil for four weeks (Kung *et al.*, 1990) spring treatments are best timed when soil temperatures reach around 13°C, usually in the second week in May, when the first pupae are hatching and the soil temperatures are rising.

It is recommended that *S. carpocapsae* be applied at a rate of 5 billion per treated hectare in late August and early May when soil temperatures exceed 12°C for maximum impact on vine weevil populations.

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