Tales of the unexpected: research discoveries that occurred by accident

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Tales of the unexpected: research discoveries that occurred by accident

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Abstract
Data from four experimental research projects are presented which have in common that unexpected results caused a change in direction of the research. A plant growth accelerator caused the appearance of white black bean aphids, a synthetic pyrethroid suspected of enhancing aphid reproduction proved to enhance plant growth, a chance conversation with a colleague initiated a search for fungal DNA in aphids, and the accidental invasion of aphid cultures by a parasitoid reversed the aphid population ranking of two Brussels sprout cultivars. This last result led to a whole series of studies on the plant odour preferences of emerging parasitoids which in turn revealed the unexpected phenomenon that chemical cues to the maternal host plant are left with the eggs at oviposition. It is pointed out that, too often, researchers fail to follow up unexpected results because they resist accepting flaws in their hypotheses; also that current application criteria for research funding make it hard to accommodate unexpected findings.

Key words: *Aphidius colemani, Aphis fabae,* benomyl, broad beans, Brussels sprouts, cypermethrin, *Myzus persicae,* olfaction, plant growth regulators, symbionts.

Introduction
It is a pleasure and a privilege to have been invited to contribute an article to this special issue of *Acta Societatis Zoologicae Bohemicae* published to mark the 60th birthday of Dr Alois Honek. In considering what form my article should take, I had to accept that coccinellids have had only minor parts to play in my research and that most of my work has been on the aphids they consume or more recently the behaviour of parasitoids and not aphid predators.

An important characteristic of Alois Honek is his ability to "think outside the box". This 21st century colloquialism refers to his habit of testing rather unexpected hypotheses or identifying phenomena in data that they were not originally designed to reveal.

In my 50 year research career, I count myself very lucky to have had several unexpected phenomena revealing themselves while testing a different hypothesis. I therefore decided that my contribution suitable for the occasion would be to combine these unexpected research results into a single paper. Of course students were usually involved in the work, and most of it has been published; I therefore acknowledge their contribution in the literature citation accompanying the heading for each topic.

White black bean aphids (Honeyborne & van Emden 1975)
When I first started research in the early 1960s on the host plant relationships of aphids, the agrochemical company Cyanamid asked me to test whether chlormequat chloride (Cycocel) had any effect on aphids. Cycocel was marketed as a plant growth retardant, especially to reduce straw height and prevent lodging in cereals. As a growth retardant obviously affects plant physiology profoundly, it seemed likely that its effect on the plant

might also result in some affect on aphid growth and reproduction. So it proved; populations of the cabbage aphid (*Brevicoryne brassicae* (L.)) increased significantly more rapidly on untreated than on Cycocel-treated Brussels sprouts (van Emden 1964). So a plant growth retardant also retarded aphid growth. I was also aware that another paper (Carlisle at al. 1963) had reported that treatment of locusts the plant growth accelerator gibberellin had shortened their instar periods. So we had the hypothesis that chemicals affecting the rate of plant growth might have parallel effects on insects. We tested four plant growth regulators including gibberellin, but none of them had any identifiable effect on the reproduction of the aphids in our test system, the black bean aphid (*Aphis fabae* Scop.) on broad bean (*Vicia faba* L.). However, ethylene-*bis*-nitrourethane (EBNU), an experimental plant growth accelerator, had the remarkable effect of producing white *A. fabae*.

To investigate this further we sowed broad beans every two weeks in order have a succession of plants aged 6-8 weeks over a long period of time. When 8 cm high, half the plants in each batch were sprayed with an aqueous suspension of 0.02% EBNU while the other half were sprayed with water. We originated two lines of aphids on each treatment. One line remained on the same treatment throughout the experiment: the other was switched at each generation as newly born nymphs to the alternative treatment. The colour of the eventual adults and their nymphs was recorded as "dark" or "pale"; no intermediates were found.

The results are presented diagrammatically (Fig. 1) and are unambiguous. Adults and their nymphs kept their dark or pale colour when kept respectively on control or EBNU treated plants from one generation to the next. Nymphs transferred to the alternative treatment kept their coloration for their lifetime, but the colour of their nymphs now reflected the treatment to which the mother had been transferred.

Had the production of pale aphids been due to a lack of pigment precursors in EBNU-treated plants, one would have expected a nymph transferred to a control plant to regain pigmentation. This did not happen, making it more likely that an inhibition of pigmentation was involved.

An unexpected source of plant growth regulation (Hutt et al. 1994)

Beginning in the late 1970s, there were reports (e.g. Highwood 1979, Iftner & Hall 1983, Jones & Parella 1984, Wilson et al. 1988) that the use of synthetic pyrethroid insecticides increased populations of pest mites. These effects were variously attributed to kill of predators, direct effects on the mites and indirect effects through their nutrition from the plant.

We decided to see whether there were similar effects on aphids in the glasshouse situation, using the cypermethrin based insecticide Cymbush ™ (Zeneca Agrochemicals, now Syngenta) with broad bean and *A. fabae* as the experimental system. Cymbush is formulated as a 10% emulsifiable concentrate (EC) and the chemical was applied at 50 ppm a.i. to the beans with a hand sprayer. The experiment was a failure, since the blocking by glass of much of the UV in sunlight prolonged the residual life of the synthetic pyrethroid (cf. van Emden & Hadley 2011) to the extent that plants had flowered, formed pods and the leaves were beginning to senesce before aphids would survive when caged on the leaves in clip cages. What was noticeable, however, was that these advanced stages of plant development were limited to Cymbush-treated plants and were not seen in control plants sprayed only with tap water.

The research project therefore switched from a study on aphids to one on the effect of Cymbush on plant development. To measure the effects on plants of the active ingredient (cypermethrin) itself, it was necessary to compare the effects of the complete

product (Cymbush) with those of the formulation without the cypermethrin. Zeneca agreed to supply the blank formulation, but asked that the work should be switched from broad bean to cowpea (*Vigna unguiculata* (L.) Walpers.

Cowpea seeds were sown and the plants finally potted into 18 cm diameter pots. When the plants were six weeks old, they were placed individually on a rotating turntable and sprayed with a hand sprayer to give even coverage of the leaves without run-off. Eight plants were assigned to each of three treatments: distilled water, distilled water containing 500 ppm blank formulation or a dilution of the complete product containing 50 ppm cypermethrin. The aerial fresh weight of the plants was recorded when they were 9, 11 and 12 weeks old (Fig. 2B). The expected result, that cypermethrin accelerated plant growth, was not obtained. Instead, it was clear that the growth acceleration was a property of the blank formulation, as this gave the same significant increase of 10-20% in aerial plant weight as the complete product.

We reverted to broad beans for the remaining experiments. Twenty plants at the four leaf stage were sprayed with either distilled water, 500 ppm of the blank formulation, or two logarithmic dilutions thereof (250 and 125 ppm). The aerial fresh weight and increase in plant height were recorded when the plants were four weeks old, and both were significantly greater in plants sprayed with 250 and 5000 ppm blank formulation than in the plants sprayed with distilled water (Fig. 2C).

Cymbush has the following components as w/v (Fig. 2A): cypermethrin 10%, anionic emulsifier 2.5% non-ionic emulsifier 5.5% and solvent (Solveso 100) 82%. The three formulation components were available separately from Zeneca, and 20 broad bean plants were sprayed with each component at the appropriate rate equivalent to that in a spray of Cymbush at a dose of 50 ppm cypermethrin. The results (Fig, 2D) clearly showed that the growth-promoting effect came from the non-ionic emulsifier, and this was confirmed by a further experiment in which several more growth statistics were compared for broad beans sprayed with distilled water, blank formulation or non-ionic emulsifier (Fig. 3). Blank formulation and non-ionic emulsifier gave similar and significant increases over distilled water for plant height, stem and leaf fresh weight and leaf area, confirming that the non-ionic emulsifier accounted for all the growth stimulation given by the complete blank formulation.

The study now returned to aphids, and an experiment to see whether the blank formulation and non-ionic emulsifier increased reproduction of *A. fabae*. Twenty broad beans at the four-leaf stage were sprayed with each of the three treatments in the previous experiment. One newly moulted adult aphid was caged (in a small clip cage) on each plant and five offspring were retained to develop and reproduce. The resulting aphid population on each plant was counted visually after 7 and 14 days (Fig. 4). At both counts, the mean aphid population was remarkably similar on plants sprayed with blank formulation and non-ionic emulsifier and aphid numbers were about twice those on plants sprayed with distilled water.

A final experiment was conducted to check that the entire Cymbush product also stimulated the reproductive performance of *A. fabae,* and so the study returned full circle to the original aim, which had been diverted to work on plant growth phenomena. Given the short-lived nature of broad bean plants, they were sprayed at the very early growth stage of one pair of true leaves. Sixty plants were selected; half were sprayed with tap water and the other half with Cymbush. To minimise the residual life of the pyrethroid, a low Cymbush dose of only 10 ppm cypermethrin was combined with keeping the plants outside the glasshouse to maximise degradation of the cypermethrin residue by photochemical oxidation. Adult aphids were put onto leaves in clip cages 11 days after spraying, and one newly born nymph retained per plant. The nymphs were monitored

daily till they began reproducing. The number of nymphs born in 11 days (the rounded down integer of development time from birth to reproduction) was recorded and this datum could be combined with development time to calculate the intrinsic rate of natural increase (*rm*) of the aphids (Wyatt & White 1977). Development time was not affected by Cymbush, but total fecundity in 11 days was increased by 22% (Fig. 5), even with such a low dose of the product. This increase resulted in aphids on Cymbush-treated plants having a higher *rm* than those on plants sprayed with water.

It is only because of the failure of our first experiment that we spotted the acceleration of growth caused by the product; otherwise the association of an increase in aphid performance with the anionic emulsifier would never have been made and we would have attributed that increase in performance by the aphid to the cypermethrin itself.

Many other insecticides are formulated as ECs, and it is likely that those formulations contain similar ingredients to those in Cymbush. Our speculation is that other active ingredients in ECs have some phytotoxicity which cancels out the growth-promoting potential of their blank formulation in a way pyrethroids, derived from natural plant products, do not.

The three DNAs in the aphid (Akhtar & van Emden 1996)

Soon after its introduction in the 1970s, there were anecdotal reports that the wide-spectrum fungicide benomyl (methyl 1-butylcarbamoyl benzimidazol-2-yl carbamate) also gave good control of aphids. Akhtar & van Emden (1992) found that even low doses of soil-applied benomyl (50 and 100 ppm of active ingredient (a.i.) when 200 ppm is a relatively high but not exceptional field dose) reduced the weight of *Rhopalosiphum padi* (L.) on wheat plants.

Work was in progress in several institutions based on the hypothesis that the effect on the aphids was an indirect one resulting from changes in the physiology of the host plant. Although our plan was to look at these changes in terms of soluble nitrogen, we realised no one had looked at the possibility of direct toxicity of the fiungicide. We therefore applied benomyl topically at 200 ppm in butanone, and obtained 80% mortality of *R. padi* (Akhtar & van Emden 1992).

This unexpected result changed the direction of our research, but was only a preliminary to the real surprise which was to come later. Was the fungicide affecting the essential microbial symbionts in the aphid? After all, these were bacteria and thus more likely targets for a fungicide than the aphids themselves. This hypothesis eventually showed that we were rather naïve in matters of micro-organisms but, had we not made this error of thinking, an interesting piece of scientific detective work – with its remarkable conclusion - would never have materialised.

The normal appearance of the mycetome in *R.padi* is shown in Fig, 6A. The nucleus of a mycetocyte cell (under the letter "A") is clearly defined, as are the dense symbiotic bacteria in the mycetocyte cytoplasm. Figure 6B shows a similar section from an aphid killed by feeding on a plant given a soil drench of 200 ppm benomyl. The nucleus in the bottom right corner of the photograph has largely broken down as has the cytoplasm, and empty vacuoles have appeared.

Of course the symbionts cannot survive in dead aphids, so the next question was – were the bacteria dying because benomyl had killed the aphids by some other mechanism, or were the aphids dying because the benomyl had first killed the symbionts? To answer this we killed aphids with 6.25 ppm a.i. pirimicarb as a 50% a.i. granular formulation applied to the soil. Pirimicarb is a systemic insecticide to which aphids are particularly sensitive. The electronmicrographs (Fig. 6C) showed that the mycetome in the aphids killed by pirimicarb is of normal appearance. The insecticide was clearly killing the

aphids before there was an effect on the symbionts, suggesting that with benomyl the symbionts were dying before the aphids.

At this point in the study the results seemed pretty clear; benomyl kills the symbionts and the aphids consequently die. We were ready to publish. Fortunately I thought the late Dr Roland Fox, a microbiologist on our staff, might be interested and so I told him of our work. To my surprise, he was quite certain that our conclusions were wrong. Benomyl has no effect on bacteria; its mode of action is by inhibiting the assembly of microtubules (Davidse 1973), which are major components of the cytoskeleton and unique to eukaryotic cells.

The research now took on a new direction – electron micrography to find microtubules. We found microtubules (Fig 9A) in the mycetocyte cytoplasm in which the symbiotic bacteria are embedded, but microtubules were never observed in aphids killed with a soil drench of 200 ppm benomyl (Fig. 9B).

It was now clear that the primary step in the toxicity of benomyl to aphids was the breakdown of the cytoskeleton of the mycetocyte cytoplasm which acts as the interface through which the aphid passes nutrients to sustain the symbiotic bacteria. These bacteria then die, which in turn deprives the aphids of essential amino acids and causes death. If Dr Fox's advice that the action of benomyl on microtubules is restricted to fungi, then the mycetocytes in the aphid may contain fungal DNA acquired by horizontal transfer (Xiong & Eickbush 1990) or from the assembly of eukaryotes from assemblages of micro-organisms (the controversial endosymbiosis theory of Margulis & Fester 1991).

The structure of aphids may therefore involve three diverse taxa of the living world, animal, bacterial and fungal. However, the discovery of this novel possibility depended on the accident of mentioning what appeared to be a cut-and-dried result to a microbiologist colleague.

Aphid resistant plants have larger aphid populations than more susceptible ones
(van Emden 1978; Douloumpaka & van Emden 2003)
For several years, work in my laboratory used two cultivars of Brussels sprout to study partial plant resistance to aphids. The more susceptible cultivar “Winter Harvest” (WH) was contrasted with the cultivar “Early Half Tall” (EHT), on which populations of *B. brassicae* increased a little more slowly (Dodd & van Emden 1979).

In one project we set up an aphid culture on several plants of both cultivars in a single large muslin-covered cage. After some six-weeks, we noticed that the EHT plants had noticeably more aphids than those of WH. Closer inspection revealed many more parasitoid mummies on WH than on EHT. Parasitoids had accidentally managed to enter the cage, and a more lightly but more slowly increasing aphid population on EHT had overtaken a potentially faster growing but heavily parasitised population on WH. A comparison of the population growth of aphids on the two cultivars (Fig. 10) confirmed this conclusion. After 47 days, the number of aphids on the partially resistant EHT was about double that on the intrinsically more aphid-susceptible WH.

Chemical analysis of the leaves showed that EHT had less glucosinolate (a phagostimulant for *B. brassicae*) than WH, and olfactometer studies revealed that the parasitoid (*Diaeretiella rapae* (M’Intosh)) preferred the odour of WH to that of EHT. As a result, over three times as many mummies were formed in 10 days on WH than on EHT (Fig. 9).

This serendipitous accidental invasion of our culture cages by *D. rapae* spawned what was probably the most productive period of research in my research group at Reading. A series of papers was published on the constancy of aphid parasitoids to the odour of the plant species/cultivar on which they had developed, and on when and where

these odour preferences were acquired during development and emergence from the mummy. Other work manipulated this acquisition process and the placing of the preferences in long and short term memory (van Emden et al. 2008).

Here I shall move to a second accidental discovery in our work, which moved it in a new direction.

What we were planning to achieve was to produce parasitoids without any plant odour preferences, and the obvious approach was to parasitise aphids and rear out the parasitoids to adult on artificial diet (van Emden, 2009) devoid of plant secondary compounds. *Aphidius colemani* Viereck from Brussels sprout were allowed to parasitise the aphid *Myzus persicae* (Sulzer) on the diet, and the offspring were tested in a 4-way olfactometer for any preference for the odour of aphid-free Brussels sprout (cv. "Bedford Winter Harvest"), cabbage (cv. "Hispi"), Chinese cabbage (cv. "Wong Bok") and wheat (cv. "Maris Huntsman). No preference for any odour was shown (Table 1, first row).

Now came the next accidental discovery. Powell & Wright (1992) had shown that more aphids were parasitised when the host plant was present than when it was absent. Our previous work suggested that allowing parasitisation to occur with the young aphids on a plant leaf for only 24h should not affect subsequent odour preferences of the parasitoid offspring. We therefore re-tested the preferences of the parasitoids emerging from diet-reared aphids (parasitized by females from Brussels sprout) for the odours of the same four plant types as before, but this time when each of the plant types had also been used as the substrate for the aphids for 24h during parasitisation. The result was completely unexpected (Table 1, rows 2-5). An odour preference was now shown, but only by one group of parasitoids. The mothers of this group had parasitised aphids on a Brussels sprout leaf, i.e plant material with the same chemistry as the plant on which they themselves had been reared.

The same phenomenon was shown when parasitoids reared on cabbage were used (Table 1, rows 6-9). Thus the underscored bold type in Table 1 shows that the only parasitoids emerging from aphids reared on artificial diet devoid of any secondary plant chemistry that showed any odour preference were ones where the plant substrate on which parasitisation had occurred matched the plant which was the source of their parent generation, and it was the odour of this plant for which the preference was shown.

This can only be explained by postulating that the mother parasitoid leaves a chemical cue for the host plant on which she developed in or with the egg at oviposition. Later work (van Emden et al. 2014) has shown that, within Brussels sprouts, this cue is so cultivar-specific that it must take the form of enough of the secondary chemistry spectrum to "identify" the cultivar. This later work also indicates high mortality of parasitoids in aphids on diet when the mothers were themselves reared on diet – i.e. they had no chemical cue to leave with their offspring. The main secondary compounds of brassicas are toxic glucosinolates It seem likely that the inclusion of these compounds at oviposition induces the production of enzymes in the offspring to detoxify them. It could loosely be said that *A. colemani* invented immunisation before the Victorian physician Edward Jenner!

In every olfactometer test, between 30 and 40 percent of the parasitoids remained in the central arena of the apparatus without entering or gathering at the entrance of any of the four arms within 15 min. As each arm was releasing a different odour to create a mixture of olfactory signals in the arena, such a high proportion of "non-responders" was to be expected.

Conclusions

All four of the research projects reported here began with the aim of relating the reproductive performance of aphids with changes in host plant chemistry (particularly components of soluble nitrogen) obtained by varying the host plant variety or by inducing changes by experimental treatments to the plants. However, they all finished up very differently.

Before we had reason to analyse the chemistry of bean plants treated with different plant growth regulators, we had observed the reduction in pigmentation of *A. fabae* on plants treated with EBNU and switched the project to a study of how that effect changed with aphid transfers between control and EBNU treated plants.

In the experiments with Cymbush, we had expected to find a positive effect of the product on aphid reproduction, with the hypothesis that this was an effect of the product on the nitrogen compounds in the plant. However, the surprising observation that Cymbush treated bean plants flowered and podded earlier than untreated ones changed the project to experiments on plant growth to identify the component of the formulation responsible for the growth acceleration.

The critical accidental event in the study with benomyl was the mention to our microbiology colleague of our hypothesis that this fungicide directly killed the bacterial symbionts. If he had not refuted this, the work up to that point might well have been passed by referees for publication in an entomological journal. Not only would as false conclusion have been published, but the intriguing speculation about the incorporation of fungal DNA into the mycetocyte tissue would not have been raised.

The accidental invasion of our aphid cultures on Brussels sprouts by a parasitoid was without doubt the accidental event in my research career which had the greatest impact, as it moved our work away from aphids *per se* to an emphasis on parasitoid olfactory behaviour for over 25 years. This began with a long period of research on how host-plant related olfactory preferences of the parasitoids at emergence were determined and could be manipulated. However, the unexpected result of introducing a host plant leaf for 24 hr when the aphids to be parasitized were reared on artificial diet changed our direction for a second time. The revelation that, at oviposition, female *A. colemani* leave with their offspring a maternal cue to the secondary chemical spectrum of the plant on which they had developed, eventually revealed the cultivar-specificity of this cue and its potential importance in inducing defensive enzymes in the offspring.

In my career, I must have scrutinised several hundred research reports either as a referee or as an examiner for undergraduate, masters or doctoral theses. I have come across many examples where clear evidence of an exciting phenomenon was ignored because the researchers used every statistical technique they could think of to avoid accepting that the insects were telling them their hypothesis was probably wrong! The three statistical techniques most often used for this were logarithmic transformation, regression and the amplification of degrees of freedom by combining within- and between-plot variation in analysis of variance.

The second problem which gets in the way of looking out for the unexpected is the pressure on researchers today to fund their work by securing grants in a highly competitive environment. This increasingly requires a prediction of what the results will be and a statement of the stages ("milestones") en route to that goal. As far as research is concerned, these milestones are a straightjacket. The projects I have described in this paper are quite different; the students involved had been awarded funding with no strings attached or (in one case) were self-funded. The progress of the work could thus follow the directions suggested by the insects rather than by the requirements of grant-awarding state-funded research councils.

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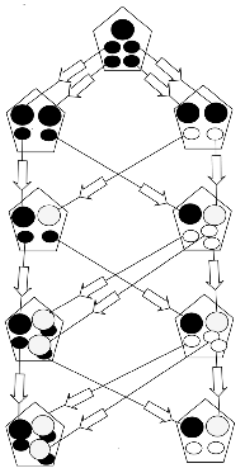
Table 1. Mean per cent female *Aphidius colemani* responding positively within 15 min. to each of four odours (Brussels sprout, cabbage, Chinese (Ch.) cabbage and wheat) in a four-arm olfactometer. The mother parasitoid generation had been reared on either Brussels sprout or cabbage and 24 hr parasitisation took place on each of the four plant species used in the olfactometer. Otherwise the host aphids (*Myzus persicae*) were from a culture on artificial diet and then further reared on that diet after parasitisation.

Source of parasitoid	24h parasitisation	Sprout	Cabbage	Ch. cabbage	Wheat	No choice
Sprout	Diet	17.2a	18.6a	15.1a	18.3a	*30.8*
Sprout	**Sprout**	**36.0**b	10.0a	12.4a	10.0a	*31.6*
Sprout	Cabbage	13.0a	15.6a	17.2.a	13.6a	*40.6*
Sprout	Ch. cabbage	17.4a	18.8a	16.0a	15.6a	*31.2*
Sprout	Wheat	18.2a	19.0a	8.0a	14.8a	*40.0*
Cabbage	Sprout	13,5a	18.6a	16.0a	10.8a	*40.8*
Cabbage	**Cabbage**	12.3a	**30.0**b	16.8a	7.6a	*38.8*
Cabbage	Ch. cabbage	17.6a	15.2a	17.4a	11.0a	*38.8*
Cabbage	Wheat	17.6a	12.0a	15.8a	15.8a	*38.8*

The data were analysed by analysis of variance of four replicates, each of 18-32 parasitoids. Means with the same letter within rows are not significantly different (P=0.05).

The bold and underlined entries are explained in the text.

Legends for figures



Untreated Treated with EBNU

Fig. 1. Transfer of *Aphis fabae* between untreated plants and plants treated with ethylene-*bis*-nitrourethane (EBNU). Large circles, adults; small circles, nymphs; black circles, dark aphids; white circles, pale aphids.

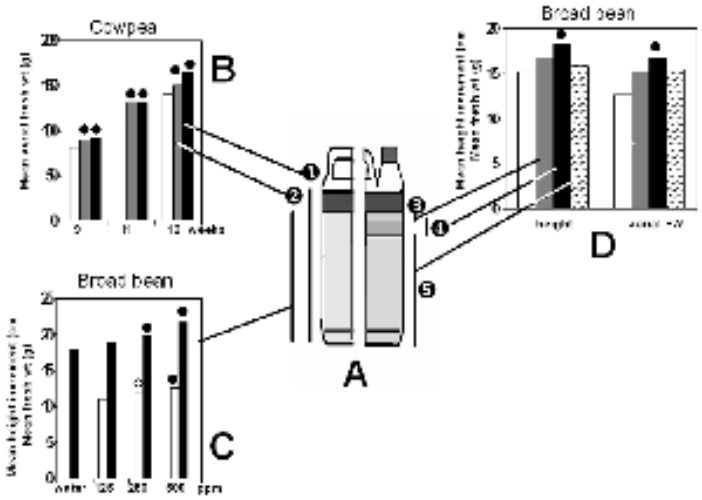


Fig. 2. The components of Cymbush and their effect on plant growth.

A, Diagram of the proportions of the different components. The dark band shows the proportion of the active ingredient (cypermethrin).

1, entire product.

2, blank formulation comprising:

3, ionic emulsifier.

4, non-ionic emulsifier

5,solvent.

B, Mean aerial fresh weight of cowpea sprayed with blank formulation (grey) or the complete product (black). Circles show means differing significantly ($P<0.001$) from plants sprayed with distilled water (white).

C, Mean increase in height (white) and mean fresh weight (black) of broad bean plants sprayed with distilled water or increasing concentrations of the blank formulation. Circles show means differing significantly (black, $P<0.001$; white, $P<0.05$) from plants sprayed with distilled water.

D, Mean increase in height and mean fresh weight of broad bean plants sprayed with ionic emulsifier (grey), non-ionic emulsifier (black) and solvent (hatched). Circles show means differing significantly ($P<0.001$) from plants sprayed with distilled water (white).

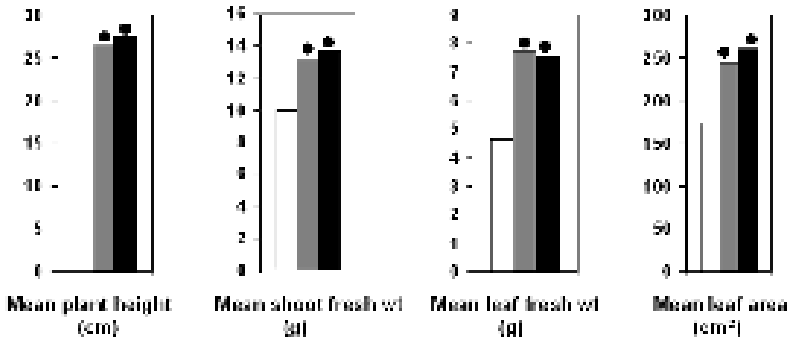


Fig. 3. The effect on various growth measurements of broad bean plants sprayed with the entire blank formulation (grey) or \non-ionic emulsifier alone (black).). Circles show means differing significantly (P<0 001) from plants sprayed with distilled water (white).

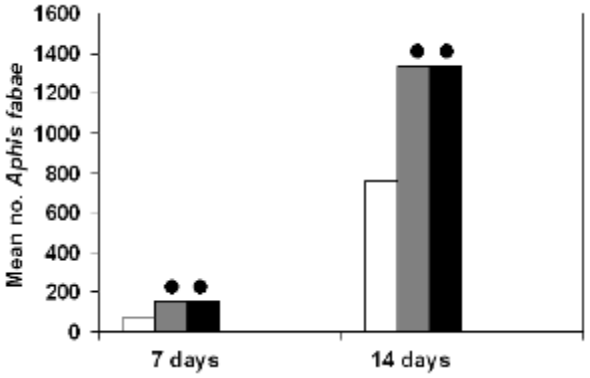


Fig. 4. Mean no. *Aphis fabae* developing on broad bean plants sprayed with the entire blank formulation (grey) or non-ionic emulsifier alone (black).). Circles show means differing significantly (P<0.001) from plants sprayed with distilled water (white).

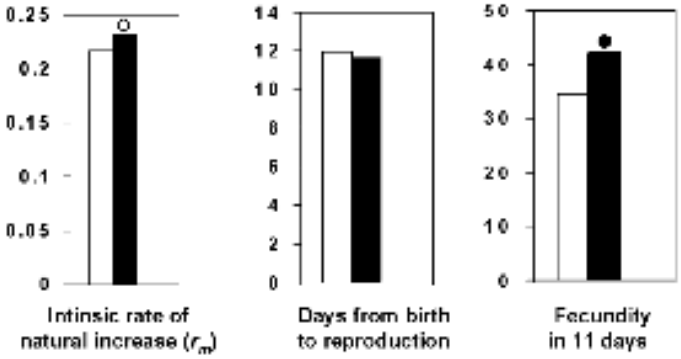
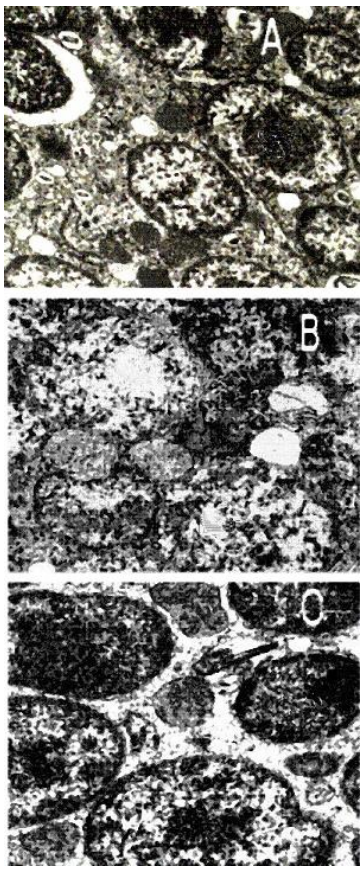


Fig. 5. The intrinsic rate of natural increase (r_m) and its components of *Aphis fabae* on broad bean plants sprayed with the entire product at a concentration of 10 ppm cypermethrin. Circles show means differing significantly (black, P<0.001; white, P<0.05) from plants sprayed with distilled water.



sections of part of the mycetomes of *Aphis fabae*. A, normal aphid; B, aphid killed by the fungicide benomyl; C, aphid killed by the insecticide primicarb. Smb, symbiotic bacterium embedded in mycetocyte cytoplasm.

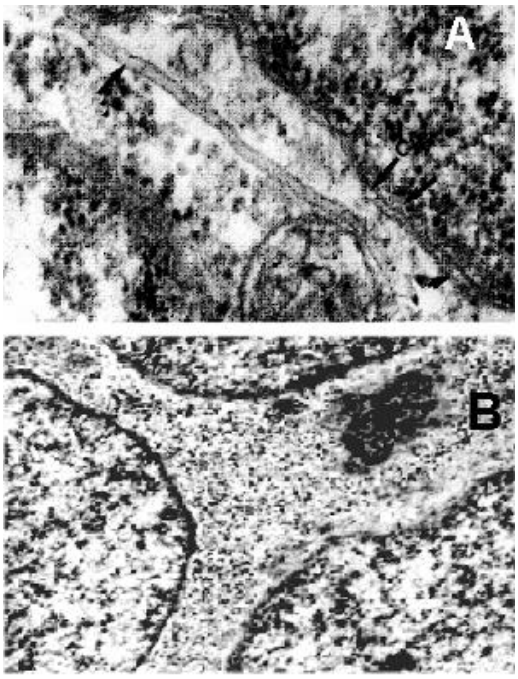


Fig. 7. Electron micrographs of section of equivalent areas of mycetocyte cytoplasm in the mycetome of *Aphid fabae* to illustrate the presence of tubulin in normal aphids and its absence in aphids killed by the fungicide benomyl.

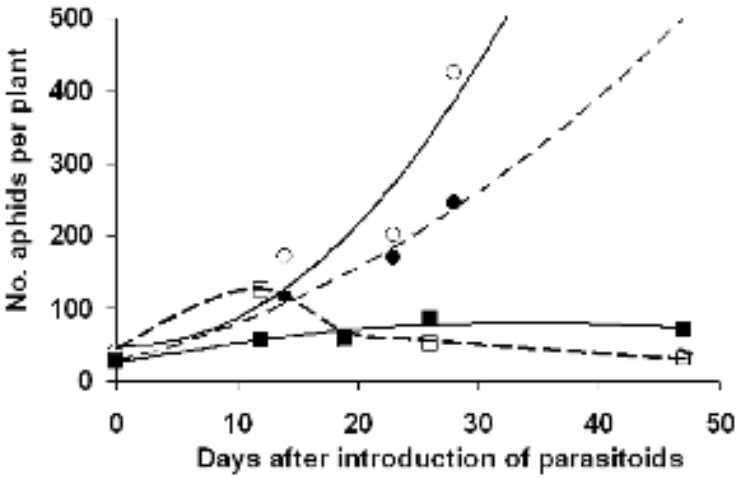


Fig. 8. Changes in *Brevicoryne brassicae* populations on two Brussels sprout varieties, Winter Harvest (white symbols) and Early Half Tall (black symbols), both with (squares) and without (circles) parasitoids.

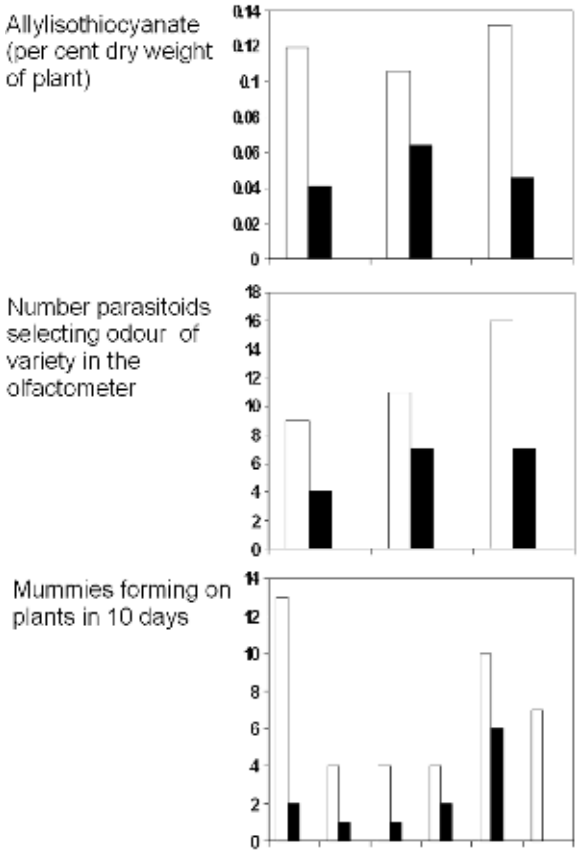


Fig. 9. Contrast between the Brussels sprout varieties Winter Harvest (white) and Early Half Tall (black) in allylisothiocyanate concentration and the behaviour of the parasitoid *Diaretiella rapae*. Each pair of columns represents a different replicate of the same comparison.