Bugs within bugs: symbiotic bacteria in garden insects

Many insects are garden pests, and wreak havoc on both ornamental plants and crops. **Angela Douglas** reveals the unseen role of microbes in helping garden insects to survive.

Why is your garden green?

Macrobiologists would give a microbe-free answer to this question. The animals consuming garden plants – mostly insects – are kept in check by a combination of plant defences which make the plants unpalatable or downright toxic, and natural enemies such as ladybirds and other predatory beetles, parasitic wasps and fungal pathogens. This approach neglects a key weapon of phytophagous (plant-feeding) insects in their fight back against the near-inedible plants and army of natural enemies: symbiotic micro-organisms.

Many of the microbes requisitioned by phytophagous insects in their unending war with plants and natural enemies cannot be cultured by traditional laboratory methods. However, the recent development of molecular techniques to study unculturable forms is permitting many new discoveries to be made about these micro-organisms.

Why symbiotic micro-organisms are used by phytophagous insects

Micro-organisms are metabolically versatile, while animals are metabolically impoverished. This difference is key to understanding why symbiotic micro-organisms are exploited by phytophagous insects. Much plant material is deficient in nutrients that these insects cannot synthesize, including essential amino acids and vitamins. It may contain poisonous secondary metabolites that insects cannot detoxify. These metabolic deficiencies can be made good by forming symbioses with micro-organisms possessing these capabilities. Microbial symbioses in insects vary widely in their intimacy, from complex and ever-changing microbial communities in the gut lumen to highly specific relationships involving single bacterial taxa restricted to particular insect cells.

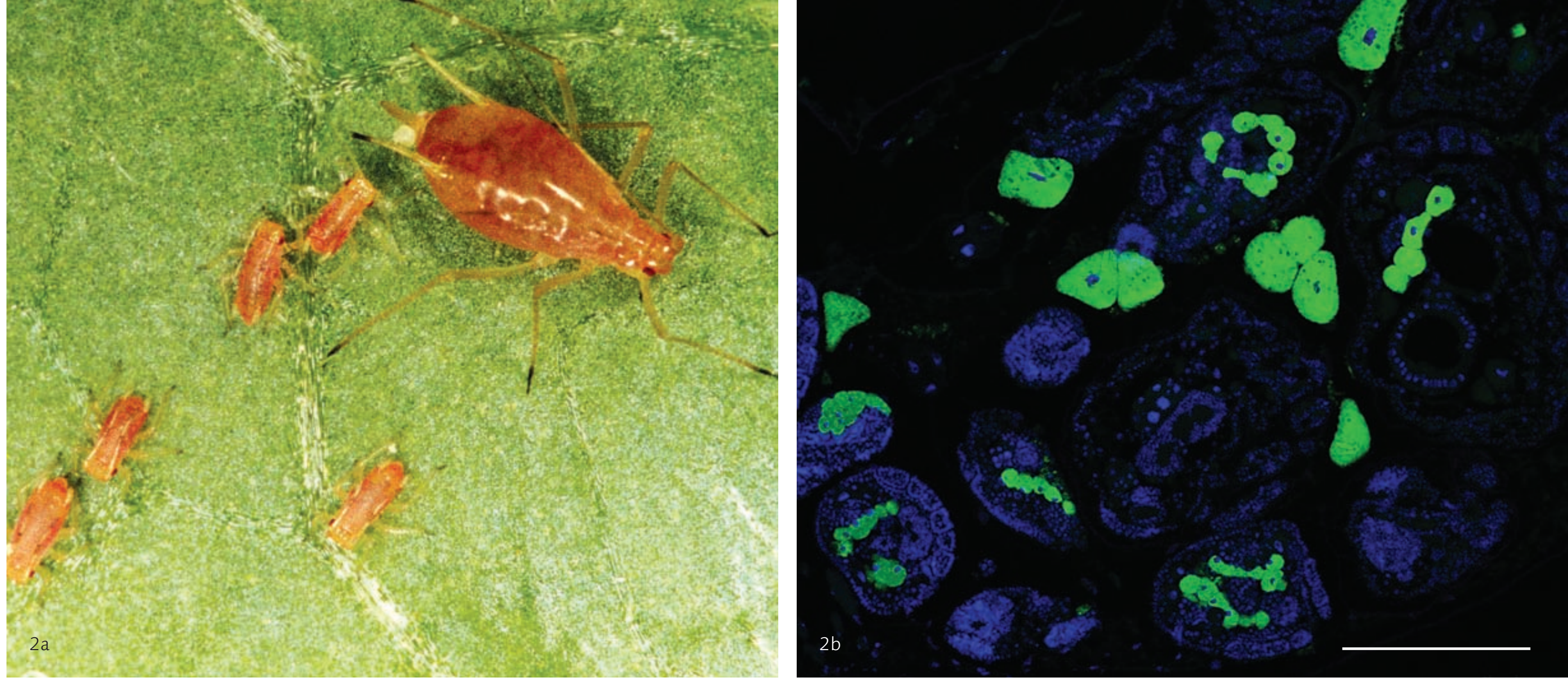
Insect herbivores can be divided into two functional groups: the 'chewers', such as caterpillars, grasshoppers and leaf beetles, which consume plant tissues wholesale; and the 'suckers', such as whitefly, aphids and spittlebugs, which imbibe plant sap. I will consider these two groups separately.

The microbiology of chewing insects: why caterpillars are not mini-cows

The traditional, but inaccurate, perception of microbial symbioses in chewing insects comes from our understanding of microbial associations in cows and other vertebrate herbivores which have cellulose-degrading bacteria in their guts. Wood-feeding lower termites – I trust absent from gardens in the UK – conform broadly to this paradigm (although their cellulolytic micro-organisms are protists rather than bacteria), but among phytophagous insects symbiotic cellulose degradation is exceptional. Some insects produce

▲ North American lupin aphids, *Macrosyphum albifrons*, feeding on a lupin stem. The large female on the left is giving birth to a live nymph. *Vaughan Fleming / Science Photo Library*

◄ A caterpillar on a blueberry leaf. *Derek Shimmin / Science Photo Library*



▲ Fig. 1. Bacterial symbiosis in weevils. (a) A weevil of the genus *Sitophilus* feeding on grain. (b) The symbiotic bacteria in *Sitophilus*, informally known as SOPE, are located in the cytoplasm of special insect cells, known as bacteriocytes. *G. Febvay and A. Heddi*

▶ Fig. 2. Bacterial symbiosis in aphids. (a) The aphid *Macrosiphum euphorbiae* colonizes potato plants and many garden plants. (b) Its symbiotic bacteria, assigned to the genus *Buchnera*, are restricted to insect cells, bacteriocytes, in the aphid body cavity. In this section of an adult reproductive female aphid, the *Buchnera* are localized using a *Buchnera*-specific FITC-labelled probe (green). Bar, 200 µm. *A.C. Darby, S.M. Chandler and A.E. Douglas*

their own cellulase enzymes. Others appear to be cellulase-free herbivores that eat lots of plant food, digest the starch, protein, etc., and void the greater part of the plant material as frass (faeces).

The biosynthetic capabilities of symbiotic micro-organisms are, however, exploited by some chewing insects, especially those feeding on 'tough', slowly growing plant tissues of low nutrient content. The micro-organisms are believed to provide the insects with essential amino acids and vitamins, supplementing the supply from their plant diet. The main focus of recent research on this type of relationship is the *γ-Proteobacteria* required by the economically important weevil pests, *Sitophilus*, of grain stores. The symbiotic micro-organisms with biosynthetic functions in the many other chewing phytophagous insect pests are a 'rich seam' for future microbial research.

The microbiology of sucking insects: symbioses without parallel

Insects feeding on the phloem or xylem sap of plants include whitefly, aphids, spittlebugs, leafhoppers, jumping lice (psyllids), scales and cicadas. They are major garden pests, depressing plant growth and flower production, transmitting plant viruses and producing unsightly sticky honeydew that is substrate for sooty moulds and other fungi. Without exception, these insects bear micro-organisms, usually bacteria, in cells separated from the gut. The bacteria are *α-*, *β-* or *γ-Proteobacteria*, varying between insect groups.

The best studied of these associations is between the *γ*-proteobacterium *Buchnera* and aphids. *Buchnera* provide the aphid tissues with essential amino acids, which are deficient in plant phloem sap. The association is very ancient, originating some 200 million years ago. In other words, *Buchnera* has been transmitted from mother to offspring through the generations of aphids since the early Mesozoic and perhaps earlier. The genome of *Buchnera* in three aphid species has been sequenced completely, and their genomes are tiny, about 650 kb, with gene complements that are a subset of the genes in the more familiar, related *γ*-proteobacterium *Escherichia coli*. Gene loss has been substantial, including all of the energy-producing TCA cycle apart from the gene for *α*-ketoglutarate dehydrogenase (required for synthesis of the essential amino acid lysine), many genes required for the cell envelope (e.g. no capacity to synthesize lipopolysaccharides), and most transcriptional regulatory sequences. Despite this, *Buchnera* has retained the genes for essential amino acid biosynthetic pathways, and there is strong evidence that they can synthesize these compounds, releasing 50 % or more to the aphid tissues.

It is widely assumed, but has not been demonstrated, that the relationship of phloem- and xylem-feeding insects generally is similar to the aphid–*Buchnera* symbiosis. These symbioses are without parallel, because the animal hosts, all hemipteran insects, are the only animals that can utilize plant sap as sole food throughout their life cycle; and the repeated evolution of symbioses with micro-organisms has been crucial to this lifestyle.

Microbiology and chemical warfare in the garden

Chemical warfare is rife in the garden. Plant secondary compounds deter or are toxic to phytophagous insects, and some phytophagous insects are chemically protected against natural enemies. Micro-organisms associated with phytophagous insects have been suggested repeatedly to detoxify plant secondary compounds or synthesize secondary compounds effective against natural enemies. To date, the evidence has been slight. Perhaps the best example is the rove beetle *Paederus*, with bacteria allied to *Pseudomonas aeruginosa* that protect the beetle with a polyketide informally known as pederin.

There are, however, indications that bacteria may intervene in aphid interactions with plants and natural enemies. The bacteria, informally known as secondary bacteria, are

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γ-Proteobacteria distinct from *Buchnera*, and not present in all aphids. They promote aphid resistance to parasitic wasps, perhaps by producing chemicals that kill the parasitoid eggs deposited into the aphid body, and can alter the range of plants utilized by aphids. The general implication is that these horizontally transmissible bacteria not required by the insect may have a dramatic impact on the outcome of aphid interactions with other macro-organisms in terrestrial communities, including the growth rate and persistence of aphid colonies on garden plants. It is not known if other phytophagous insects have comparable microbial relationships.

Plant defences revisited

So far, I have not specified the mode of action of plant chemical defences against phytophagous insects. Many of the known ones are exploited in chemical insecticides that target the nervous and digestive systems of the insects. However, for the many insects dependent on microbial symbioses, there is a further crucial target – the symbiotic micro-organisms and their interactions with the insect. Research on plant defences with symbiotic micro-organisms as the primary target is in its infancy, but there is now evidence that the *Buchnera* symbiosis may be targeted by factors in certain plants, causing reduced essential amino acid production, disrupted regulation of the *Buchnera* population, and depressed aphid performance. Further research is required to establish the symbiosis-active compounds, the incidence of such plant defences and the insect/microbial gene products that are targeted. This approach may offer the opportunity to replace the relatively non-specific chemical insecticides, widely used in gardens despite the great collateral damage to natural enemies, by precision insecticides targeted to specific microbe–insect relationships.

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Useful website

www.york.ac.uk/depts/biol/units/symbiosis/intro.htm

Further reading

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