Bloodworms - the search for the 'X-factor'

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IN A RICE HULL

- Bloodworms release a compound into the water of rice fields which deters adult rice bloodworm midges looking for suitable egg laying sites – adult midges respond by laying their eggs in other areas where there will be less competition
- Identifying the compound involved in deterring midges may help to provide an alternative approach for bloodworm management in establishing rice crops

Bloodworms are the larvae of midges, and are major pests of aerially-sown rice crops. At least one preventative insecticide treatment has to be applied each season to protect seedlings during crop establishment.

Many species of bloodworms can be found in establishing rice crops, and the biology of some species is still poorly known. In contrast, the biology of the rice bloodworm (*Chironomus tepperi*, Figure 1), which is the most serious pest species during early establishment, is now fairly well understood.

Colonisation patterns

When we used traps to collect adult *C.tepperi* midges emerging from untreated rice fields we found that only a single emergence peak occurs for this species. Timing of this peak depends on temperature, but it generally occurs around 16 days after flooding, with all adult emergence occurring within about two to three days either side of the peak. No further emergence of this species occurs.

We know from laboratory development studies that the eggs giving rise to this emergence of adults must have been laid in the first two to three days after flooding. If the midges that emerge during the first peak were to mate and lay their eggs back into the same field, another peak of emergence should occur approximately 16 days later – but it doesn't.

Given that each female midge has the potential to produce over 800 eggs in her brief adult life, it is hard to imagine that adults from the first emergence lay their eggs back into the same field and that no larvae whatsoever successfully develop to maturity and emerge in a second peak. In fact, there is no obvious reason for adult emergence to occur in discrete peaks at all – in many species of bloodworms, recruitment and emergence are both continuous, with larval populations consisting of mixed-age individuals at all times.

This pattern of emergence indicates that female *C.tepperi* adults can somehow 'sense' when rice bays are newly flooded, and only lay their eggs at this time. By laying their eggs early when there are no other bloodworms present, female *C.tepperi* midges are ensuring their offspring have the best chance of survival, as they will not have to compete for food and other resources. The adults that emerge approximately 16 days later recognise the established rice field as an unsuitable habitat for their offspring, and look elsewhere for a more suitable site to lay their eggs.

Biological 'signals'

So how do *C.tepperi* females recognise a newly-flooded bay from an older one?

They respond to some sort of chemical signal in the water – either an attractant present immediately after flooding that subsequently vanishes, or a repellent chemical that is initially absent and appears in the water later on.

We developed a simple test to evaluate the response of female *C.tepperi* midges to different water samples.

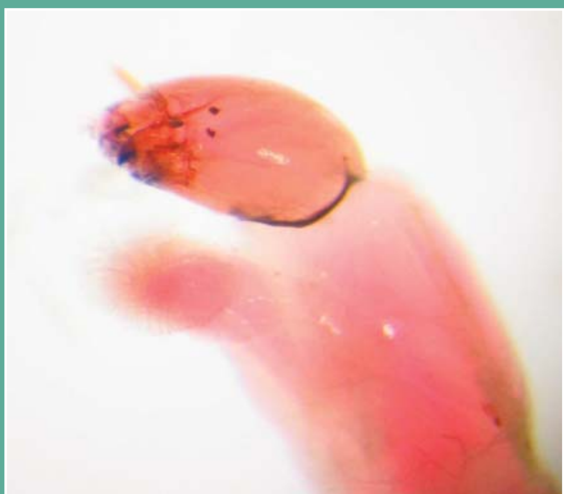

Figure 1 *The rice bloodworm,* Chironomus tepperi

Two plastic cups, one containing a test solution and one a control solution (essentially plain water) are placed in an empty aquarium and 30 anaesthetised adult *C.tepperi* (15 females and 15 males) are placed in a dish in the centre (Figure 2). The aquarium is covered and left for 24 hours and the number of egg masses in each cup is counted. The test is repeated 15 times for each test solution.

Based on Japanese research, we initially looked at a range of nitrogen compounds, but found these had little influence on choice of egg laying site. We then changed direction, and looked at what is missing from newly flooded rice fields compared to older fields. The obvious answer was 'bloodworms', so we tested the idea that female *C.tepperi* might avoid sites where bloodworms are already present.

Figure 2 *Testing system used to evaluate the egg laying preferences of female rice bloodworm midges*

We ground up *C.tepperi* bloodworms (1 per 100 mL of water) and tested the effect – almost 90% of egg masses were laid in the control water (Figure 3), indicating that *C.tepperi* larvae contain a chemical that deters egg laying by adults of the same species. When we ground up bloodworms of a different species (*Polypedilum nubiferum*), we got a similar result with *C.tepperi* adults – strong avoidance. To see just how sensitive the response is, we placed single live bloodworms in 100 mL of water overnight, and then removed them and tested the 'bloodworm-conditioned' water they had lived in. Female *C.tepperi* avoided laying their eggs there as well. Interestingly, when we ground up adult *C.tepperi* midges in water, there was no significant response from the adults (Figure 3).

These results indicate that bloodworms release one or more chemicals into the water that discourage egg laying by *C.tepperi*, the most significant pest species of bloodworm affecting the NSW rice industry.

So how can this information be used?

If we can identify the compound responsible, we could use it to 'trick' the rice bloodworm midges into not laying their eggs into rice crops in the first place. From the midges' perspective, we could make a newly flooded field appear to be an older one, already occupied by a significant bloodworm population. The females would then look for other sites to lay their eggs.

Identifying the 'X-factor'

Identifying the deterrent compound is, unfortunately, easier said than done. Chemical analysis of 'bloodworm-conditioned' water offers, on the surface, a rapid approach to identifying the 'X-factor', and we have been collaborating with chemists at Charles Sturt University who are trying to detect the mystery compound using a technique called gas

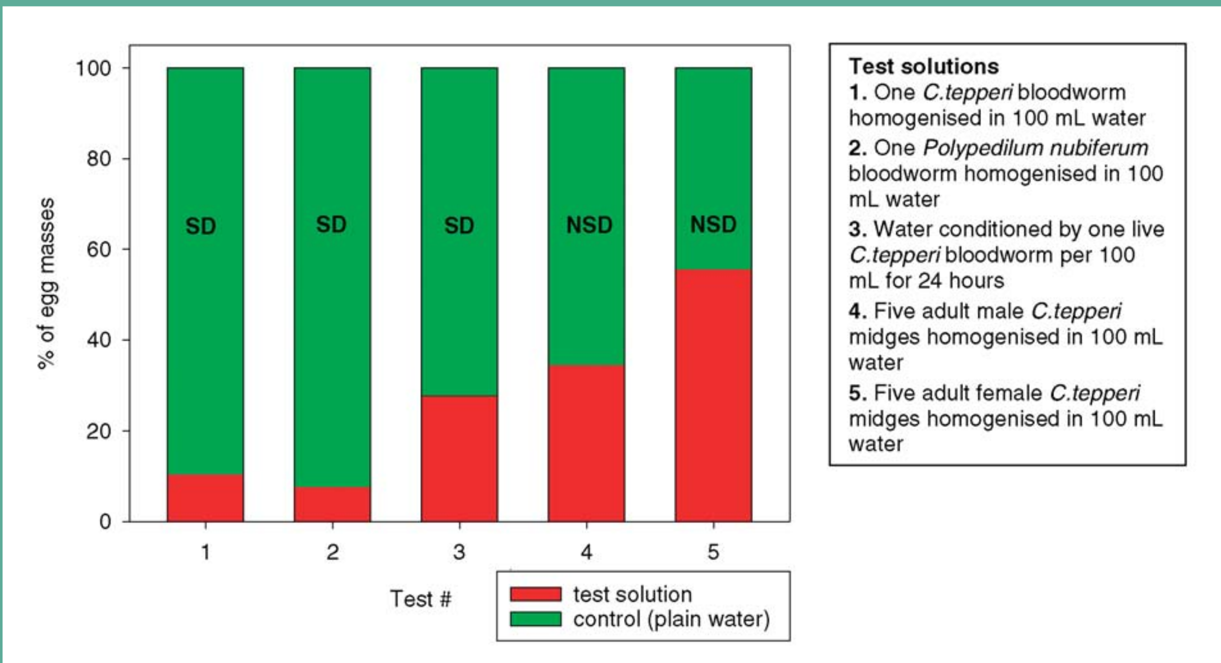

Figure 3 *Response of female rice bloodworm adults in two-choice tests for preferred egg-laying sites. SD indicates a significant difference between the test solution and the control. NSD indicates no significant difference between treatments.*

chromatography (GC). Although we have had some encouraging results, there are problems associated with analysing extracts from bloodworm-conditioned water. The main problem is that there are a very large number of different compounds in the water, most of which are probably inactive. This makes it difficult to interpret the GC results. Also, because our information about the compound is still limited, we don't know if the extraction and analysis techniques we're using are really optimal for detecting it.

The approach we are now taking involves fractionating the compounds present in bloodworm-conditioned water, and identifying which fractions are still biologically active. This process tells us more about the nature of the 'X-factor', and also provides samples for chemical analysis that are less 'cluttered' with inactive compounds. We know from earlier work that the compound is stable when heated, so it isn't a protein. When we remove the organic compounds from bloodworm-conditioned water using a technique called C18 reverse-phase extraction, the water loses its ability to deter egg laying – so we know the compound isn't an inorganic salt (which would flow through the extraction column unimpeded). Difficulties associated with removing the active compound from the extraction column using organic solvents suggest the mystery compound is a relatively large and insoluble organic molecule. We are currently trying different sorts of extraction columns in an effort to find out more about the chemical characteristics of the compound. Biological testing of the fractionated samples is, however, a very slow process.

Although the 'X-factor' is very stable under laboratory conditions, we know it breaks down fairly quickly in the field, suggesting it may be susceptible to either UV or bacterial degradation. This suggests that the 'X-factor' itself may not be suitable as an egg-laying deterrent. However, once the 'X-factor' has been identified, it may be possible to identify a related chemical that produces a similar response in female rice bloodworm midges whilst showing improved persistence in the field. This compound – a more persistent analogue of the 'X-factor' itself – could be used as the basis for an environmentally friendly commercial deterrent for use against the rice bloodworm.

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