



General News

Using Predatory Mites to Disseminate *Beauveria* Spores

A news report from China highlights an innovative approach that uses predatory mites to disperse an entomopathogen: *Neoseiulus cucumeris* and *Amblyseius swirskii* 'dusted' with *Beauveria bassiana* conidial spores are being successfully used for biological control of mite and insect pests.¹

Predatory mites are important biocontrol agents for pest mites and small insect pests in integrated pest management programmes. But predatory mites cannot control larger insects or life stages. For example, the Asian citrus psyllid *Diaphorina citri*, which is native to China, vectors the bacterium that causes huanglongbing – arguably the worst problem in citrus trees worldwide. Predatory mites feed on *D. citri* eggs and sometimes first-instar nymphs, but not later life stages. In China, control of *D. citri* commonly relies on insecticides.

A solution to the problem has been pioneered by Professor Zhang Yan-Xuan (Fujian Academy of Agricultural Sciences). Prof. Zhang led the introduction and commercialization of predatory mites for biological control in China during the 1990s, starting with *N. cucumeris* and *A. swirskii*. Since then, mass-produced mites have become an integral part of pest control strategies in field and greenhouse crops in China. In 2005 she founded the Fujian Yan Xuan Biological Technology Co. Ltd. (www.zgbsm.com), which is located in Fujian Provincial Academy of Agricultural Sciences, Plant Protection Research Institute. The company has successfully overcome the major hurdles to commercializing the biocontrol agents: factory-level production, packaging, storage, long-distance transportation and field deployment. It has secured eight national patents for its technology, and produced three standards for Fujian Province. It now has an annual production capacity of 800 billion predatory mites for use in crops including citrus, cotton, bamboo, tea, fruit trees such as apples, pears and chestnuts, vegetables, strawberries, hops and flowers. In 2008 it began exporting to Europe, and it also exports to Canada. The company has won many awards, including the second prize of the National Award for Progress in Science and Technology, Ministry of Agriculture, and Ministry of Science and Technology awards for key new products.

In 2013, Prof. Zhang started to focus her attention on developing a predator-mediated entomopathogen disseminating system, which would enable biological control of a wider spectrum of pest species.² She developed the system on *D. citri*, later applying it more widely. Other studies, particularly in Mexico and Brazil as well as China, have suggested that a variety of fungal pathogens could have potential as biocontrol agents against *D. citri*. US scientists have been working on an application technology for green-

houses, but the potential for entomopathogens to control Asian citrus psyllid has not yet been realized.

Beauveria bassiana is used commercially in biological control worldwide. Studies have been conducted on its compatibility with various predatory mite species, with variable results and conclusions. Prof. Zhang and her team started by showing that adult female *N. cucumeris* were tolerant of *B. bassiana*.³ They then tested a range of spore concentrations on *N. cucumeris* and *A. swirskii* nymphs. They found that *B. bassiana* applied at 10^4 spores/ml gave approaching 100% mortality of *D. citri* within 7 days, but caused only low mortality of nymphs of both mite species (not significantly different to control treatment).²

They developed a 'dusting' method to inoculate adult female *N. cucumeris* and *A. swirskii* with *B. bassiana* conidia, and estimated that on average there were at least 10^4 spores per mite immediately after the dusting process. They next showed that both mite species could disseminate spores to *D. citri* on potted plants of *Murraya paniculata* kept in high humidity. They found that *D. citri* mortality rates were high when either species was used to disseminate the pathogen in this way: the mortality was similar to that achieved by spraying plants with a spore suspension. Dead insects of all life stages were often covered with white fungal mycelia, which could be cultured and used to re-infect new *D. citri*.

Studies by Prof. Zhang and her team were important in confirming a number of attributes of the mite-carrier system. They showed that both immature and adult mites were tolerant of the fungus. Their most recent study demonstrated that the mite-carrier system could achieve good mortality of *D. citri* on plants. An earlier study had shown that *N. cucumeris* could climb 15 m up bamboo plants to reach prey, which suggested mobility will be adequate at the orchard level. A question remained on the efficacy of *B. bassiana* in the field, because high humidity is required for germination, but successful greenhouse use seemed entirely possible. Finally, the predator-mediated delivery system potentially offered one substantial gain: protection of a tree is likely to be effective for fewer spores than recommended for spraying – by a factor of more than 10^4 per tree, which reduces the cost of treatment.

Prof. Zhang has now led field trials in more than 1.2 million hectares of over 20 crops in 20 provinces of China. According to the news article¹, she has invented and patented a container for releasing the pathogen-dusted mites, and has used drones on the vast cotton fields of Xinjiang Uygur Autonomous Region. She says pesticide use can be reduced by 40–50% percent by adopting the system. She and her team are also experimenting with other pathogens, and have screened 16 fungal strains. For example,

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they have shown *N. cucumeris* can disseminate *Paecilomyces fumosoroseus* for control of aphids on aubergine/eggplant, using the dusting method developed for *B. bassiana*.⁴

One crop where the system has been a success is tea, where pesticide use is traditionally high. Pesticide residue limits in China, and in export markets such as the European Union, can lead to rejection of tea consignments. The news article describes the situation in Beiliao village near the city of Zhangping in Fujian Province. Here a cooperative marketing shuixian tea guarantees hefty compensation for any tea it produces that is found to contain excessive pesticide residues – and it does not find itself paying out. Cooperative members have been implementing the mite predator-mediated *Beauveria* disseminating system for several years and no longer use pesticides.

¹ Wu, Y.-L. (2016) Predatory mites' new mission: carrying pathogens to infect and kill pest insects. Web: http://news.xinhuanet.com/english/sci/2016-12/02/c_135875408.htm

² Zhang, Y.-X., Sun, L., Lin, G.-Y., Lin, J.-Z., Chen X., Ji, J., *et al.* (2015) A novel use of predatory mites for dissemination of fungal pathogen for insect biocontrol: the case of *Amblyseius swirskii* and *Neoseiulus cucumeris* (Phytoseiidae) as vectors of *Beauveria bassiana* against *Diaphorina citri* (Psyllidae). *Systematic and Applied Acarology* 20, 177–187.

³ Zhang, Y.-X., Sun, L., Lin, J.-Z., Chen, X. and Ji, J. (2013) Virulence of *Beauveria bassiana* CQBB111 strain to *Diaphorina citri* and *Neoseiulus cucumeris*. *Chinese Journal of Biological Control* 29, 56–60.

⁴ Sun, L., Zhang, Y.-X., Zhao, L.-L., Lin, J.-Z., Chen, X. and Ji, J. (2015) Effect of *Neoseiulus cucumeris* carrying *Paecilomyces fumosoroseus* on the prevention of aphids and spider mites in eggplant. *Agricultural Science and Technology* 16, 2720–2724.

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Commercializing a Biocontrol Product for Africa: aflasafe

A new project to deliver a cost-effective, commercial product to reduce aflatoxin contamination in maize and groundnut was launched in Nigeria in December 2016, with plans to make it available in other countries in sub-Saharan Africa. The biocontrol product aflasafe™ is based on technology developed and in use for more than a decade in the USA. The products for Africa have been developed through a series of projects by the International Institute of Tropical Agriculture (IITA) and the US Department of Agriculture – Agriculture Research Service (USDA-ARS) in collaboration with several national institutions in other African countries, and with funding from several donors and national governments.

The latest project, the aflasafe Technology Transfer and Commercialization Project (aTTC), funded by the Bill & Melinda Gates Foundation and the US Agency for International Development (USAID), is being implemented in 11 countries: Burkina Faso, the Gambia, Ghana, Kenya, Malawi, Mozambique, Nigeria, Senegal, Tanzania, Uganda and Zambia. It is led by IITA with support from USDA-ARS, Chemonics, Dalberg Global Development Advisors, the Partnership for Aflatoxin Control in Africa of the African Union (PACA), various national institutions, and regional economic communities. The aTTC project places partnership with the private sector at the core, and is focused on delivering an affordable commercial product to African farmers. It will do this by fostering partnerships between the international/government research institutions involved in aflasafe development and business partners with expertise in manufacturing and distribution.

Aflatoxins are produced by *Aspergillus* fungi, notably *A. flavus* and *A. parasiticus*. They can contaminate crops at all points in the food chain, and particularly when growing plants are under stress (from temperature, drought, pests) or crops are kept in poor post-harvest conditions, particularly if they are not dried properly or humidity is high. Management measures must therefore be in place throughout the food production chain, and biological control is particularly suitable in this regard.

Aflatoxins cause acute and chronic symptoms, either of which may prove fatal. They are associated with immune system and liver disorders, including liver cancer, and stunting in children. Their presence in crops and food products is not readily apparent to farmers or consumers, and farmer and public awareness is low. The rural poor are chronically exposed to unsafe amount of aflatoxins. In Kenya, where a particularly toxic strain of *A. flavus* predominates, large quantities of maize are sporadically declared unfit for human or livestock consumption; epidemics have been reported three times in the past quarter-century and there may have been a number of undocumented deaths related to aflatoxin poisoning.

A breakthrough came in the USA in 1996 when scientists at USDA-ARS demonstrated that some *A. flavus* strains do not produce aflatoxins (atoxicogenic strains) and can outcompete strains that do. They used this biological control principle to develop two biocontrol products – AF36 and Afla-Guard – which have been successfully used in the USA for over a decade. The delivery system involves coating heat-killed small grains, such as hulled barley or wheat, with conidia of atoxicogenic *A. flavus*, which can then be applied to growing crops in the field. Farmers in Texas, Arizona and California have found the strategy also has continuing effects post-harvest because the beneficial fungi persist in the harvested crop, and it has multi-season, area-wide impacts because they persist and spread through the environment.

This technology is particularly suitable for the African context because it addresses the source of aflatoxin – the fungus in the soil – before it can contaminate the crop prior to harvest. In 2007, IITA

scientists identified four suitable Nigerian atoxigenic strains of *A. flavus*. Over the next year they showed these could reduce aflatoxin levels by up to 100%. The biocontrol product aflasafe was approved for provisional registration in Nigeria in 2009 and full registration in 2015. Sorghum is used as the carrier for the fungus: grain is coated with atoxigenic *A. flavus* and provides a medium and food source for it. The coated grain is applied on fields 2–4 weeks before crop flowering. Specialized application equipment is unnecessary: hand scattering works well over small areas. Once deployed, the atoxigenic fungi multiply on the sorghum carrier, and displace aflatoxin-producing strains. The altered community structure becomes more benign, and the potential for contamination decreases.

Farmers conducted field trials in maize in Nigeria in 2009–2012 and achieved reductions of 80–90%. Groundnut farmers obtained >80% aflatoxin reduction in Senegal paving the way for full registration of aflasafe SN01 in 2016. Construction of a manufacturing plant in Nigeria began in 2012 and it became operational in 2014. Research was expanded to other countries in West, East and southern Africa in order to develop biological control infrastructure and staff training, and to screen in-country *A. flavus* populations for atoxigenic strains for regional products. At the same time, initiatives have sought to raise public awareness of aflatoxins as a health issue and to build a market for the product.

In Kenya, a biological control laboratory was established at the Kenya Agricultural and Livestock Research Organization in 2015. Native atoxigenic strains were isolated and found to reduce aflatoxin levels by up to 98%. These were developed as the biocontrol product aflasafe KE01™, which was launched in late 2016. It is expected to reduce aflatoxin contamination in farmers' fields by over 70%. Commercial production of the Kenyan product will begin at a recently completed small-scale regional manufacturing plant by mid-2017. In the meantime, as in Nigeria, the focus is on building linkages with the business community.

Once the technology is successfully tested and commercialized in Nigeria and Kenya, the lessons learned will be used in the partner countries where work is in progress. In all, aflasafe products are registered or in the process of becoming nationally registered in 11 countries.

Protection with aflasafe is estimated to cost US\$12–20/ha and farmers could achieve up to 500% return on investment. It will reduce crop losses that result from detection of contamination and has potential for reducing aflatoxin contamination long-term. Its use should also translate into enormous health benefits for both farmer families and the wider community, not to forget livestock that will not be fed contaminated foodstuff.

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Further reading: Bandyopadhyay, R., Ortega-Beltran, A., Akande, A., Atehnkeng, J., Mutegi, C.K., Kaptoge, L., *et al.* (2016) Biological control of aflatoxins in Africa: current status and potential challenges in the face of climate change. *World Mycotoxin Journal* 9, 771–779.

Decisions about Biological Control: Rice Farmers in Iran

A study among rice farmers of Iran's northeastern province of Golestan provides behavioural insights into why farmers do not adopt, adopt and persist, or adopt but discontinue with biological control.¹ The authors note that most studies investigating integrated pest management (IPM) adoption look at socio-economic variables, while they have used three behavioural models to examine decision-making drivers: diffusion of innovation theory, the theory of planned behaviour and the technology acceptance model.

Smallholder Iranian farmers may spend a significant part of their resources on controlling the stem borer *Chilo suppressalis*, the dominant pest of rice in Iran. An IPM programme based on use of *Trichogramma* was first rolled out in the country in 1990. Although the egg parasitoid was shown to provide up to about 80% control of the stem borer, farmer uptake was hampered by inadequate technical facilities and support services, poor farmer skills and climate incompatibility with the biological control technology. More recently, government-funded programmes have promoted use of *Trichogramma* by subsidizing costs and providing the parasitoid for free, and by farmer education via farmer field schools and extension activities. In Golestan, where rice is grown on more than 55,000 ha, uptake of the technology was high, with production areas using biological control increasing twenty-fold, from 2100 ha in 1996/97 to nearly 42,000 ha in 2012/13.

The analysis was based on 415 questionnaire responses (via farmer interviews) from 22 villages where (i) some farmers had at least 3 years' experience with biological control and (ii) all three categories of farmers were present: adopters (continue to apply *Trichogramma* for stem borer control – 25.3%), discontinuers (dropped use of *Trichogramma* when government incentives stopped – 30.1%) and non-adopters (used pesticides instead – 44.6%). The questionnaire process collected socioeconomic data together with farmers' perceptions about (i) the technology (how it fits with existing values, prior experiences, and needs), how useful and easy it is to use, and belief in their own ability to implement it, and (ii) views on pros, cons and safe use of pesticides, and trust in agro-input dealers. The responses were used for a multi-nominal logistic model.

Attributes of the biological control technology itself most affected whether biological control was adopted in the first place. If farmers perceived biological con-

trol as effective and easy to apply, and had belief in their understanding and ability to implement it, they were more likely to adopt it. Education, off-farm income and source of information (extension rather than informal channels such as neighbours) also affected uptake: better-educated, better-informed farmers who had confidence in the technology and their ability to apply it, and with another source of income, were most likely to try it. Yet many farmers who adopted biological control abandoned it and returned to using pesticides. This was most strongly related to perceptions of its usefulness and ease of use once they had tried it. While farmers continued to implement biological control if they found that it was, as they expected, effective and easy to implement, discontinuation seemed to follow quickly if it did not meet farmer expectations. The smaller-scale farmers were particularly likely to stop using it. Rejection of biological control altogether was associated with attitudes to pesticides: biological control was less-often adopted by farmers who saw pesticides as effective and the way they applied them as safe. Equally, farmers producing high yields and farm income were less likely to try biological control.

The authors acknowledge that this study, with mostly experienced, middle-aged male farmers and for an incentivized scheme in one province, crop and biological control technology, may not be generalizable beyond or even throughout Iran, but argue that it raises issues that policy makers should consider and that merit further research. Long-term adoption of the biological control technology rested mostly on what happened *after* adoption. The authors suggest prolonging extension and training support after initial roll-out to encourage long-term use. They also advise monitoring abandonment rates and continuing with incentives if drop-out rates are high. With regard to promoting adoption in the first place, they view reliable information as key, and recommend education programmes by plant protection staff before biological control is rolled out, e.g. with farmer study groups, to ensure they have a thorough and realistic understanding of the technology. To encourage adoption among the least well-off and smaller-scale farmers, they recommend considering these farmers specifically during development of biological control technologies, and note that increasing off-farm employment opportunities may encourage them to try novel pest control methods.

¹Abdollahzadeh, G., Damalas, C.A. and Sharifzadeh, M.S. (2017) Understanding adoption, non-adoption, and discontinuance of biological control in rice fields of northern Iran. *Crop Protection* 93, 60–68.

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Cryptic Species: Implications for Biological Control

The results of a morphological and genetic study of a biocontrol agent released in South Africa against

water hyacinth (*Eichhornia crassipes*) has implications for biological control programmes planning to introduce a natural enemy species from separate, allopatric populations.¹

The mirid *Eccritotarsus catarinensis* was imported into South Africa from two geographically isolated populations more than 3500 km apart (southern Brazil and northern Peru). The decision to introduce the Peruvian population was made after a severe genetic bottleneck was encountered in the Brazilian population in South Africa: all but one gravid female were lost shortly after importation into quarantine for host-specificity testing. Nevertheless, the progeny were subjected to host-specificity testing, cleared for release, and then mass reared and released throughout South Africa in 1996. They established widely and contribute to water hyacinth control, notably when periodic outbreaks at some sites cause weed mats to collapse. Various factors may have limited the impact of *E. catarinensis* in South Africa including water nutrient levels and temperature, but low genetic diversity was believed to be a major constraint. The Peruvian population was introduced with the intention to interbreed it and the Brazilian population to increase genetic diversity – a not uncommon procedure in biological control programmes. The Peruvian population was imported and released in 1999, although its establishment has yet to be confirmed.²

The latest study shows that the two populations have now been identified as reproductively isolated cryptic species. They had been identified as the same species on morphological criteria, both contemporary with the 1999 release and on recent re-examination of the material. Yet comparison of the mitochondrial cytochrome oxidase subunit 1 gene (COI) and inter simple sequence repeat (ISSR) data had indicated much more divergence than in other mirids that have been studied. Another examination of the original material and additional specimens for the two populations was conducted, which revealed consistent but subtle morphological differences – but no more than might occur between populations of a single mirid species. The authors of the new study note that genetic evidence can be misleading, so they conducted breeding experiments to try and settle the matter. Their results indicated that the two populations are reproductively isolated: no-choice experiments (where insects were given the chance to mate with insects from the other population only) led to a very few hybrid offspring, but none were produced in choice experiments (where insects could choose to mate with either population). They excluded the possibility of *Wolbachia* infection (which can interfere with reproduction and therefore breeding experiments) and concluded that they were looking at two separate species. Preliminary studies suggest that premating behavioural barriers may be involved. The authors say that this is the first time that an introduced weed biocontrol agent population has been confirmed to comprise reproductively isolated, cryptic species. The Peruvian population will be described as a new species.

Cryptic species are a significant issue for biological control researchers to address. There is increasing

evidence from DNA barcoding to suggest that they are more common than previously thought. Some cryptic species have been suspected on the basis of lineages with different host plant preferences, and confirmed by genetic analysis.³ This can be exploited by selecting the lineage with the most suitable host range and impact on the target weed. In general, though, few biocontrol agents have been screened using genetic tools before release. Although it is accepted practice to import material specifically to increase genetic diversity, re-testing of a 'new' population is often not deemed necessary if it is identified to be the same species as the biocontrol agent already released. Fortunately, in the case of *E. catarinensis*, both populations were subjected to host-specificity testing. Their preferences did differ, but both were considered safe to release in South Africa.

The authors say that importation of multiple consignments of the same species for biological control should be conducted with caution, and that host testing needs to be standard where allopatric populations of a natural enemy are being introduced for biological control.

¹ Paterson, I.D., Mangan, R., Downie, D.A., Coetzee, J.A., Hill, M.P., Burke, A.M., *et al.* (2016) Two in one: cryptic species discovered in biological control agent populations using molecular data and crossbreeding experiments. *Ecology and Evolution* 6, 6139–6150.

² Taylor, S.J., Downie, D.A. and Paterson, I.D. (2011) Genetic diversity of introduced populations of the water hyacinth biological control agent *Eccritotarsus catarinensis* (Hemiptera: Miridae). *Biological Control* 58, 330–336.

³ Tošveski, I., Jović, J., Krstić, O. and Gassmann, A. (2013) PCR-RFLP-based method for reliable discrimination of cryptic species within *Mecinus janthinus* species complex (Mecini, Curculionidae) introduced in North America for biological control of invasive toadflaxes. *Biocontrol* 58, 563–573.

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Exotic Parasitoid Provides a 'Lifeline' for Native Parasitoids

A paper in *Ecology and Evolution*¹ follows up on an earlier study^{2,3} that described how the invasion of the brown marmorated stink bug, *Halyomorpha halys*, has created an 'evolutionary trap' for parasitoids of native stink bugs in recently invaded areas in North America and Europe. The earlier studies demonstrated that native stink bug parasitoids accept *H. halys* egg masses as a host but produce no viable progeny.

The newly published study examined interspecific interactions between a native European egg parasitoid, *Trissolcus cultratus*, and an Asian parasitoid, *Trissolcus japonicus* (a candidate for the biological control of *H. halys*), by providing *H. halys* egg masses to *T. cultratus* at various time intervals following the

initial parasitization by *T. japonicus*. The results showed that the native species *T. cultratus* can act as a facultative hyperparasitoid of the exotic *T. japonicus*. Although this is only possible during certain stages of *T. japonicus* development, the presence of the introduced parasitoid may reduce the impact of the evolutionary trap for indigenous parasitoid species, providing an 'invasional lifeline' for *T. cultratus*. The occurrence of facultative hyperparasitism between scelionid parasitoids associated with stink bugs may be common. This intraguild predation could promote preservation and stabilization of native communities by impacting the diversity and population dynamics of native stinkbugs and their parasitoids (e.g. native parasitoids avoid wasting reproductive effort on unsuitable hosts), or reduce success of biological control programmes (e.g. by reducing the population size of the exotic parasitoids used as biocontrol agents against the pest). Some native parasitoids may learn to recognize and seek out cues indicative of acceptability and developmental suitability of previously parasitized *H. halys* eggs during the limited time window, to avoid an evolutionary trap.

¹ Konopka, J.K., Haye, T., Gariépy, T., Mason, P., Gillespie, D. and McNeil, J.N. (2016) An exotic parasitoid provides an invasional lifeline for native parasitoids. *Journal of Applied Ecology* 7: 277–284. DOI: 10.002/ece3.2577.

² Abram, P.K., Gariépy, T.D., Boivin, G. and Brodeur, J. (2014) An invasive stink bug as an evolutionary trap for an indigenous egg parasitoid. *Biological Invasions* 16, 1387–1395.

³ Haye, T., Fischer, S., Zhang, J. and Gariépy, T.D. (2015) Can native egg parasitoids adopt the invasive brown marmorated stink bug, *Halyomorpha halys* (Heteroptera: Pentatomidae), in Europe? *Journal of Pest Science* 88, 693–705.

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Biological Control of Carp in Australia

Viral pathogens have been successfully used as vertebrate biocontrol agents only three times, and never before for fish. Australia's Invasive Animals Cooperative Research Centre has been navigating uncharted waters to research an exotic virus as a biocontrol agent for carp (*Cyprinus carpio*) in the Murray–Darling Basin. It has, however, been able to take on board lessons from earlier virus-based projects (including two against rabbits in Australia), as well as learn from epidemiological studies of the disease in natural and farmed carp populations. The team has now completed phase 3 of the project and in doing so has cleared most of the technical barriers.

Carp are native to central Asia. They have been introduced in many parts of the world as food and recreational fish, including the ornamental koi carp. First introduced to Australia in the nineteenth century, they have been established since at least the 1920s and are now found in all states except the Northern Territory. They are tolerant of many habitats, omnivorous and can be highly prolific. Carp can be unpopular with anglers, especially since flooding in the 1970s allowed the escape of a more-recently imported aquaculture strain into the Murray–Darling Basin catchment of south-eastern Australia. They are now the most abundant large fish in this river system, in some areas accounting for 90% of the fish biomass. They have contributed to degradation of the natural aquatic ecosystem and are associated with a major environmental problem. Impacts include reduced water quality and adverse effects on other fish species, invertebrates and plants, and they may be implicated in algal blooms, riverbank erosion and disease.

Over the last decade, biological control has emerged as a leading contender in an integrated management programme for carp in Australia. A viral disease of carp was first described in Israel in 1998 and spread rapidly to many countries (but not Australia). It was subsequently identified as cyprinid herpesvirus 3 (CyHV-3) and as a potential biocontrol agent.

The biological control project began in the mid-2000s. Research focused on gaining an in-depth knowledge of carp biology and the epidemiology of the virus. Also critical was determining the host specificity of the virus. Once an Indonesian strain of CyHV-3 was selected, the emphasis in the first two phases of the project was on biology and epidemiology: investigating the clinical course of disease and patterns of mortality (important for animal welfare considerations) and the susceptibility of carp of different ages to CyHV-3 and fish–fish transmissibility to assess its suitability as a control agent. A start was also made on host-specificity testing – this continued in phase 3 and is now almost complete.

Phase 3 focused on whether the virus would be safe and effective as a biocontrol agent, and on its deployment and long-term use. By the end of this phase, CyHV-3 had been tested against a list of native and introduced fish and other species including crustaceans, frogs, turtles, birds and mammals: clinical, molecular and histological observations were used to confirm the virus was specific to *C. carpio*, so spill-over infections and ‘species jumps’ are exceedingly unlikely. A few additional native fish species remain to be tested. The efficacy of the virus depends on the transmissibility–virulence relationship. From project research and information from natural outbreaks, it was concluded that close contact between infected and uninfected carp will lead to best transmission, i.e. where carp aggregate regularly and latent virus can be reactivated.

In this phase, work also began on a release strategy, in particular, the development of an epidemiological model to determine when and where virus might be released for maximum impact, and also assessing methods for storage and actual release of the virus

into waterways. The final model will be generalized for the whole Murray–Darling Basin and will predict optimum timing for releases (what season and what part of the El Niño Southern Oscillation cycle), the best location (upstream or downstream or in recruiting grounds), what supplementary control is best (e.g. another broad-scale approach to control, plus regional controls like fishing, trapping, netting) and when is it best to do this (before or after virus release).

Work is also planned on tools to monitor impacts of the virus on populations. The team expect the initially high virulence to decline after release as surviving carp give rise to disease resistance, so more virulent strains or additional control measures will be needed in time. The virus was sequenced to help understand the host–virus interaction and to identify a marker for persistent/latent infection in carp that survive infection. Further work on a genetic strategy is on the horizon.

A critical step now is public engagement to garner further understanding and awareness for an eventual release in the future. The angling community and conservation groups are largely supportive. Their views are being taken into account, along with concerns expressed by the fertilizer business (which uses carp as an input), animal welfare groups, and individuals concerned with the potential impact of many dead carp in the waterways. In May 2016, the Australian Government pledged Au\$15 million over 2.5 years for a National Carp Control Plan to fund the additional research and obtain regulatory approval as well as engagement. It is funding a consultation process, facilitated by a National Coordinator working with a wide range of stakeholders (governments, industry, community and environmental groups, research organizations) to understand the issues and bring together a comprehensive plan, based on evidence from research done during the project and a risk assessment, together with a sound understanding of community views. The aim is to release CyHV-3 in the Murray–Darling Basin by the end of 2018 but before this happens, a lot more work is required.

Main source: McColl, K. (2016) Final Report: *Phase 3 of the Carp Herpesvirus Project (CyHV-3)*. PestSmart Toolkit publication, Invasive Animals Cooperative Research Centre, Canberra, Australia.

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Schistosomiasis, Dams and Migratory Prawns

Dams have often been associated with increased human schistosomiasis. A group of US, Senegalese and Israeli scientists have demonstrated a causal link between elevated levels of schistosomiasis and catastrophic declines in *Macrobrachium* migratory prawn populations upstream of a dam in West Africa. River prawns are widely but not universally distributed. A review of information for dams in other subtropical and tropical countries found that increases in schistosomiasis have been greater for dams in areas where historically *Macrobrachium* prawns have been recorded. These recent findings were summarized in a presentation at the American Geophysical Union meeting in San Francisco in December 2016. Presenters suggested that restoring native migratory prawn populations to waterways upstream of dams could be combined with current drug-based programmes to reduce and in some locations eliminate the disease. River prawns are native to large areas of the world, and the presenters suggested some 35–48% of the 800 million people worldwide at risk of schistosomiasis stand to benefit.¹

Schistosomiasis is a chronic subtropical and tropical disease caused by freshwater parasitic *Schistosoma* worms. Schistosomes are dependent on both snail and human hosts to complete their life cycles. Water becomes contaminated by human faeces or urine containing parasite eggs, which hatch and infect the snails. The snails subsequently release worm larvae into the water, which penetrate the skin to infect people using the waterbody. According to the World Health Organization, more than 61.6 million people were treated for the disease in 2014. It notes that the disease is most prevalent in poor, rural areas, particularly agricultural and fishing communities, and children are particularly at risk.

The schistosome life cycle was discovered in the early twentieth century and snail control became the prime target for reducing disease incidence. Forty years ago, the anti-schistosome drug praziquantel was developed and snail control fell from favour. The drug is effective but short-lasting: it rids the body of schistosomes but people can be quickly re-infected. A review of control programmes concluded that over the past century, snail control has been most effective in reducing human schistosomiasis incidence.² Researchers argue that combining the old with the new – snail control plus drug use – could reduce disease incidence where native migratory prawn populations have been decimated by dam building, and even eliminate the disease.

Field research to find evidence for why damming a waterway increases schistosomiasis incidence began

in the Senegal River basin.^{3,4,5} The Diama Dam was built across the river estuary to prevent seawater incursion inland during the dry season and assure a reliable supply of freshwater for irrigation and other uses. After its completion in 1988 there was a massive outbreak of urinary schistosomiasis. This was accompanied by the arrival of a second *Schistosoma* species presenting as a different form of the disease and with a different snail host. The new schistosome species spread rapidly, while the ‘resident’ species became much more prevalent and also spread to new foci. Schistosomiasis has remained at epidemic levels ever since with infection rates higher than 90% in some areas. The Senegal River basin is now one of the most heavily infected areas in the world, while before the construction of the dam it was largely free of this disease.

Several factors could have contributed to a surge in the disease. Slower, year-round freshwater provides a better habitat for snails. The dam facilitated the expansion of agriculture: irrigation channels and an expanded area of rice cultivation increased snail habitat. Agriculture attracted more workers and the population increased, which enhanced disease transmission. But, another factor was the decimation of populations of predatory river prawns.

The river prawn *M. vollenhovenii*, which is native to the west coast of Africa from Angola to Senegal, was found up to 400 km inland in the Senegal River before the Diama Dam was built. It depends on brackish, estuarine water to complete its life cycle. The dam prevents the female prawns from migrating to the estuary, where they reproduce, and also prevents young prawns from migrating back upstream. Researchers conducting field studies in Senegal deployed baited prawn traps, inspected fishermen’s catches and conducted interviews, which revealed a common story: very few prawns were found upstream of the dam. Almost 6300 hours of baited trapping caught two adult prawns. Fishermen’s catches were low (but indicated seasonal variation, with proportionately more prawns caught upstream during the dry season). Interviews revealed that catches had fallen dramatically after the dam had been built.

Laboratory aquarium experiments showed that *M. vollenhovenii* is a voracious predator of the snail species that act as schistosome hosts in the Senegal River. Other snail natural enemies were considered, but there was no evidence of their potential as snail biocontrol agents. The exception, with high potential for success, was the Louisiana crayfish, *Procambarus clarkii*. This exotic species was introduced to Kenya for aquaculture purposes, escaped, and is now established in the wild. Its efficacy in reducing schistosomiasis in children has been shown in field trials in Kenya.⁶ But environmental concerns regarding its introduction to Senegal meant that the project proceeded with the native *M. vollenhovenii*.

Freshwater crustacean predators – prawns and crayfish – are generalists but experiments have shown that they are voracious feeders on freshwater snails. Calcium may underlie a preference for snails: freshwater crustaceans with their strong calcified shells

live in an environment where calcium ions are scarce (cf. seawater), so 'edible' calcium in the form of snails and their shells could make them preferred food.

The scientists conducted a pilot field experiment to assess impact of *M. vollehovenii* in a village community on the Senegal River. They designated an upstream control village and downstream intervention village. Prawns were introduced into a net enclosure where the intervention villagers accessed the river bank. Drug treatment was given to eliminate schistosomiasis at the outset, and snail populations and snail and human schistosome infections were recorded at both villages for the next 18 months.

Snails were less abundant by an average of 50% and were an average of 80% less infected in the intervention village. Although the intervention village started with a significantly higher prevalence of schistosomiasis in humans, the re-infection rate was lower than in the control village after restocking with prawns; infection intensity, e.g. output in urine or faeces, (the best proxy for human disease) was also lower. The team used a mathematical model of the system to show how re-stocking with prawns in combination with infrequent mass drug treatment could eliminate schistosomiasis from sites of high disease transmission.

The next question is how to restore the prawn population. One option being explored is modifying the dam to re-establish natural recruitment and migration. A prawn ladder, modelled after eel ladders in Europe, could be constructed alongside the Diama Dam to facilitate this. Two factors are in its favour: (i) the Diama Dam is a low dam, only about 8 m high, and (ii) *M. vollehovenii* has a benthic lifestyle more like crayfish, and the prawns have been observed climbing up small waterfalls in Cameroon. The downside in terms of impact on snails (and schistosomiasis) is that river prawn populations are currently so depleted that recovery (and impact on disease) might take a very long time.

Another option (they are not mutually exclusive) is to couple re-stocking with aquaculture. A village-level prawn industry extending far inland was an early victim of the dam. This could be revitalized to breed prawns for release at schistosomiasis foci. The researchers envisage a win-win situation: small juvenile prawns are more voracious and therefore effective as snail predators, while large mature prawns could be harvested. To circumvent the problem of disruption to the migratory life cycle, researchers have proposed a technique for producing all-male offspring: sexual differentiation is determined by hormones at the larval stage. This is already being manipulated by (non-transgenic) molecular means for the Malaysian species *Macrobrachium rosenbergii*, which is common in aquaculture.

Schistosomiasis is a debilitating and potentially lethal disease affecting millions of people, mostly the rural poor. The health benefits of restoring predatory prawn populations could be enormous. An additional enticement is the prospect of coupling re-stocking

with development of aquaculture industries to benefit these communities. In Senegal, where current work is being carried out with permission from relevant Senegalese government ministries (Health, Agriculture, Environment), the added value of an economic motivation for augmenting prawn populations is attractive to both the authorities and villagers. But risks also need to be considered and a decision made on the basis of the balance of benefits and risks. If the focus is on restoring *Macrobrachium* species in their native ranges, countries without relevant national legislation can follow guidelines for inundative and inoculative release of a native biocontrol agent, for example OECD⁷ (appendix 2) and IOBC⁸ (section 4). In Senegal, however, domestication of the native species *M. vollehovenii* is challenging and it could take several years to achieve sustainable production. An alternative route could be to introduce the exotic species *M. rosenbergii*, but release male-only progeny into the river system. It is reasonable to assume risks would be significantly less with an introduction of material unable to reproduce and therefore establish. Risk assessment would cover this aspect, and, again, countries without relevant national legislation who are contemplating this approach can follow IPPC guidelines for introducing exotic biocontrol agents.⁹

It is beyond the scope of the article to address, but an important issue this project exposes is potential difficulties in reconciling risks and benefits to agriculture, the environment and human health: what is the 'right' balance and how can it be assessed?

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ment of schistosomiasis control over the past century shows targeting the snail intermediate host works best. *PLoS Neglected Tropical Diseases* 10, e0004794. DOI: 10.1371/journal.pntd.0004794.

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⁹ IPPC (2005) *Guidelines for the Export, Shipment, Import and Release of Biological Control Agents and other Beneficial Organisms*. International Standards for Phytosanitary Measures No. 3. International Plant Protection Convention, FAO, Rome.

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