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NANOTECHNOLOGY FOR FLYSTRIKE AND LICE CONTROL

AUTHORS

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SUMMARY

New chemical formulations for flystrike control are required to support the phaseout of mulesing and because of the development of resistance to the most widely used flystrike control compounds. Control of sheep lice has suffered similar resistance problems and remains an issue in the sheep industries. Nanotechnology offers a means of providing extended and 'softer' protection of sheep against flystrike and lice. This project is designing and testing unique silica nanocapsule formulations with spikes on the particle surface and purpose-designed release characteristics to give prolonged periods of protection against flystrike and lice, with minimal residues and off-target effects. This will provide new, labour efficient, options for managing flystrike in unmulesed sheep and countering resistance in sheep blowflies and lice.

Background

With ongoing requirements to increase production efficiency and constraints on the availability of labour livestock producers increasingly favour parasite treatments that can provide extended periods of protection. For this reason there has been much interest in controlled release technology such as long-acting injectable formulations for internal and blood feeding ectoparasite control, slow release polymer matrix devices such as ear tags and collars for prolonged buffalo fly control in cattle and flea control in dogs and cats, rumen capsules for helminth and tick control and more recently, microencapsulated and nanoparticle formulations.

Whereas traditional formulations of pesticide depend for prolonged action on a single initial high level treatment so that control is maintained until concentrations decay below effective levels, controlled release systems aim to release pesticides in steady amounts at active levels or to release only at times of infestation risk. This approach has a number of advantages in addition to prolonged control. Doses need not be as large so there is less risk of tissue residues. There is generally a lower risk to the operator and of environmental contamination and there is a reduced chance of subclinical toxicity or accidental poisoning of animals. In addition, there are a number of 'softer' chemistries, including plant extracts that have been shown to have activity against *Lucilia* spp. These compounds are often favoured in pest control, particularly by organic producers, because of their rapid degradation in the environment and lower potential for tissue residues but are of limited practical usefulness because of their limited persistence. Suitable controlled release systems may enable the use of insecticides which have not previously been suitable for use because of poor persistence in the fleece. Micro or nanoencapsulation technology can protect these compounds against environmental degradation and release them strategically at times of flystrike risk, or over an extended period of time to provide practically significant periods of protection against flystrike.

A wider choice of insecticides would be valuable in providing additional options in planning insecticide usage programs to minimise resistance development. In addition, controlled release systems that maintain insecticide at high concentration and then give a rapid residue decay avoiding resistance-selecting 'decay tails' (Anderson et al 1989), particulate controlled release system that could sit inert in the fleece and only release in the presence of moisture, systems that maintain high levels of insecticide through the fly season and then decay during the winter when no flies are present, or systems containing insecticides that degrade rapidly once released could also reduce the risk of resistance development.

Major innovations in the area of nanotechnology have led to the development of a variety of nanoparticle-based pesticide formulations, including polymeric/cellulose nanocrystals and lipid nanoparticles. By encapsulating active ingredients into nanocapsules, breakdown due to environmental pesticides can be reduced and chemical can be delivered at steady active levels over a prolonged period or designed to release only at times and sites where they are needed. Nanoencapsulated formulations also have the important attribute that they can generally be applied using existing application equipment.

UQ Silica nanoparticles

The UQ silica nanoparticles are a patented technology to fabricate novel hollow silica (SiO_2) nanocapsules that can be loaded with active molecules to enable superior protection against insect pests (Australian Patent Appl No. 2015901379). The nanocapsules have a large hollow cavity and porous silica shell which protects the internal active payload against degradation, while pores in the shell allow easy active loading into the hollow cavity and sustained release of the active compound. A number of designs of particle have been tested. The basic design is the smooth nanoparticle (SNP) as described above. However, a number of more recent designs of rough-surface nanoparticles (RNP) have a more pollen grain like topology (Figure 1a) with silica spikes (or 'whiskers') covering the nanocapsule outer surface. Similar to pollen grains, these spikes aid retention of the capsules on surfaces. The characteristics of these particles are 'tunable' and the particles can be designed with different characteristics such as with different chemical payloads, different size, different wall thicknesses and pore sizes, and different silica 'whisker' characteristics to optimise their functionality for different uses. This project is developing and testing silica nanocapsule formulations that can potentially provide prolonged, safe and residue free protection against sheep flystrike and lice and provide new, labour efficient, options for managing these pests. The UQ nanocapsules also possess advantages compared to other types of nanoparticles for translation to a viable commercial product. Polymer or lipid nanocapsules are often expensive or unstable under field conditions, whereas silica has been well recognized as inert and abundant in the environment with good bio-compatibility and is approved by Food and Drug Administration (FDA) for oral delivery. Moreover, the UQ patented technology provides a relatively simple approach to the fabrication of nanocapsules, employing cheap industrial chemicals, which is ideal for large scale commercial oriented production.

Three types of silica nanoparticles were initially studied in this project, smooth surface silica nanoparticles (SNP's), silica nanoparticles with silica spikes on the surface (RNP's) and RNPs with a surface modification to provide hydrophobic surface characteristic (RNP-C18) (Figure 1). The initial particles were 200-300 nm in diameter, but a number of other diameter particles with diameter from 180 – 800 nm have been fabricated and tested.

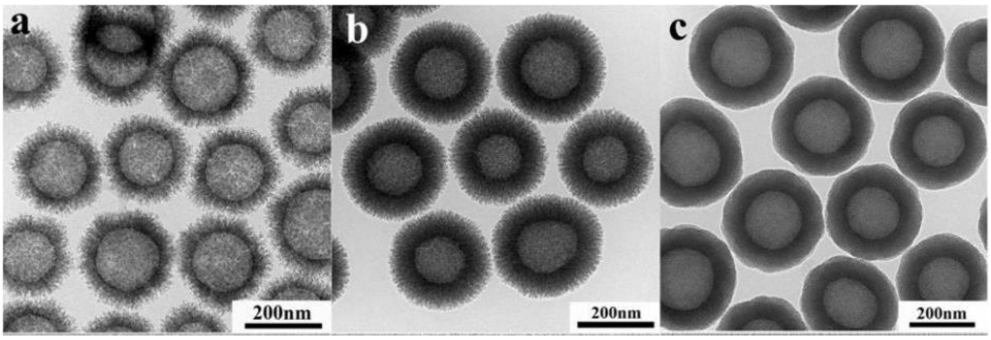

Figure 1. Transmission electron micrograph images of (a) rough nanoparticles; (b) rough particles after C18 surface modification and (c) smooth nanoparticles.

More recently, two new types of particles (FNS-60 and FNS-60-H) with-hydrophobic surface characteristics have been developed and are being tested. The FSN-60 particles have a higher pore volume than the previous formulations allowing higher chemical loading which, depending on release dynamics, is expected to provide further improvements in longevity of effect.

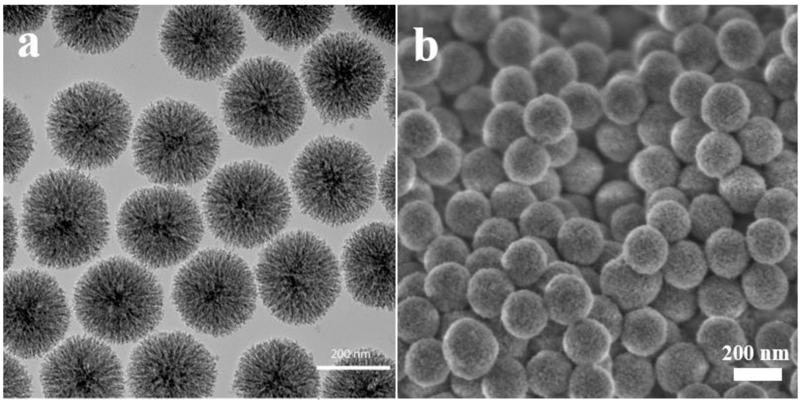

Figure 2. a) Transmission electron micrograph and, b) scanning electron micrograph images of the FSN-60 silica nanoparticles.

As noted above, it is expected that the silica nanoparticles will be able to provide greater persistence of protection by protecting encapsulated chemicals from environmental breakdown and in the case of the rough nanoparticle types, superior adherence to wool and to the cuticle of insects. Adherence to wool fibres is shown below. The electron micrographs (Figure 3) show the nanoparticles adhering to the wool fibres after water rinsing. This effect appears to be most marked with the C18 nanoparticles (Figure 3c) with the remaining particles more evident than with the smooth and rough particles.

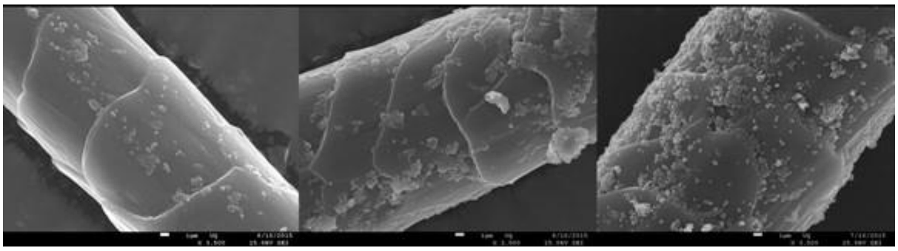

Figure 3. Electron microscope images of nanoparticles adhered to wool after water rinsing; (a) smooth nanoparticles (b) rough nanoparticles (c) RNP- C 18 nanoparticles.

We have also investigated the distribution and cuticular adherence of the different silica nanoparticles following treatment of *L. cuprina* larvae using fluorescence microscopy. Blowfly larvae were exposed to the fluorescein-labelled nanoparticles using a standard larval wool assay whereas sheep body lice were exposed by either being placed in wool that had been dipped in the nanoparticle solutions or by exposing them to a lice diet that had been treated with the nanoparticles. Figure 4 shows a high density of fluorescein-labelled particles (RNP) in the guts of both first stage blowfly maggots and lice. This indicates that both the insects are ingesting significant amounts of the labelled particles. The feeding habits of both insects would seem to favour active accumulation of particles but whether the particles are attaching to gut lining or peritrophic membrane, or just accumulating as the insects feed is currently unclear.

Cuticular adhesion was also noted in the assays with both blowfly larvae and lice, but the fluorescence was much lower, than in the gut. This is expected as ingestion of particles occurs actively as the insects feed whereas the particles on the cuticle would be acquired passively and presumably more slowly as the larvae or lice contact particles as they move through the wool or on the skin surface. Cuticular electron micrographs for both blowflies and lice suggest that the C18 and rough nanoparticles both adhere more strongly to cuticle than the smooth particles and that the C18 particles adhere more strongly than the rough particles. These results suggest that best effect against both blowfly larvae and lice is likely to be achieved when the nanoparticles are administered with the objective of oral toxicity. However, the rough or C18 particles could also be expected to add to the toxic dose delivered, particularly with purpose designed chemical payload and release characteristics.

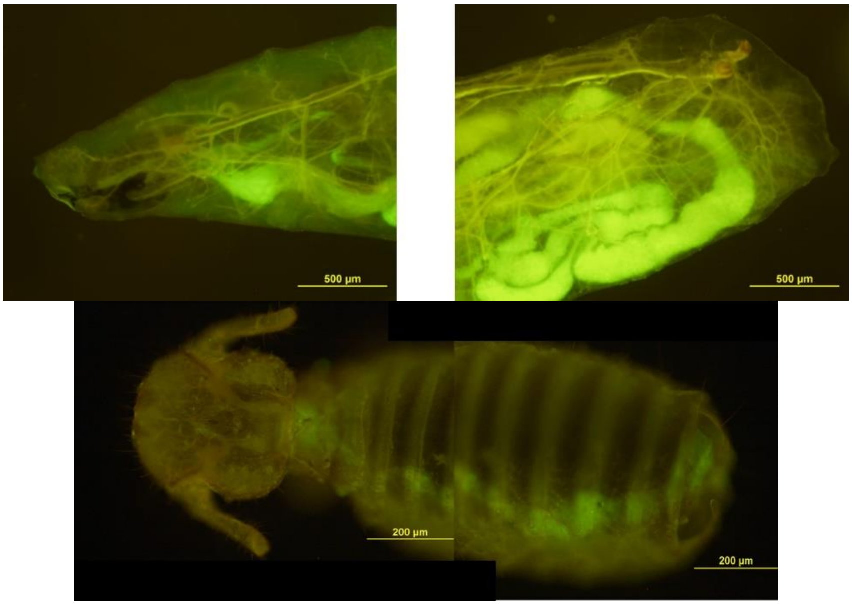

Figure 4. Fluorescein-labelled rough nanoparticles ingested during feeding in assays with first stage sheep blowfly larva (fluorescence in the anterior and posterior sections of the gut shown) and an adult sheep louse.

Testing against sheep blowflies

To test the relative efficacy of different formulations in the presence of environmental influence such as photo-degradation and leaching from the fleece by rainfall a series of laboratory tests with *L. cuprina* larvae have been conducted. Formulations for the tests were dispersed in the carrier compound (water for lipophilic pesticides, hexane for water soluble pesticides) by ultrasonication for 1 hour and applied to wool staples collected from a Merino fleece known to have had no previous chemical treatment. First stage blowfly maggots were then exposed to the treated wool using standard larval assays. To test the effects of photodegradation with the different nanoparticle formulations the treated wool samples were first exposed to ultra-violet radiation by two methods, an artificial UV exposure regime in the laboratory, or extended exposure to natural sunlight on the roof of the EcoSciences precinct in Brisbane (Figure 5).

The incorporation of water soluble chemicals may offer potential for development of a formulation that is strategically released under moist conditions, but which remains inert in the fleece when there is no moisture and therefore no flystrike risk, or which is only released in the insect gut following ingestion. That is, a formulation with a longer presence in the fleece and designed to release only at times and in sites where control is needed.

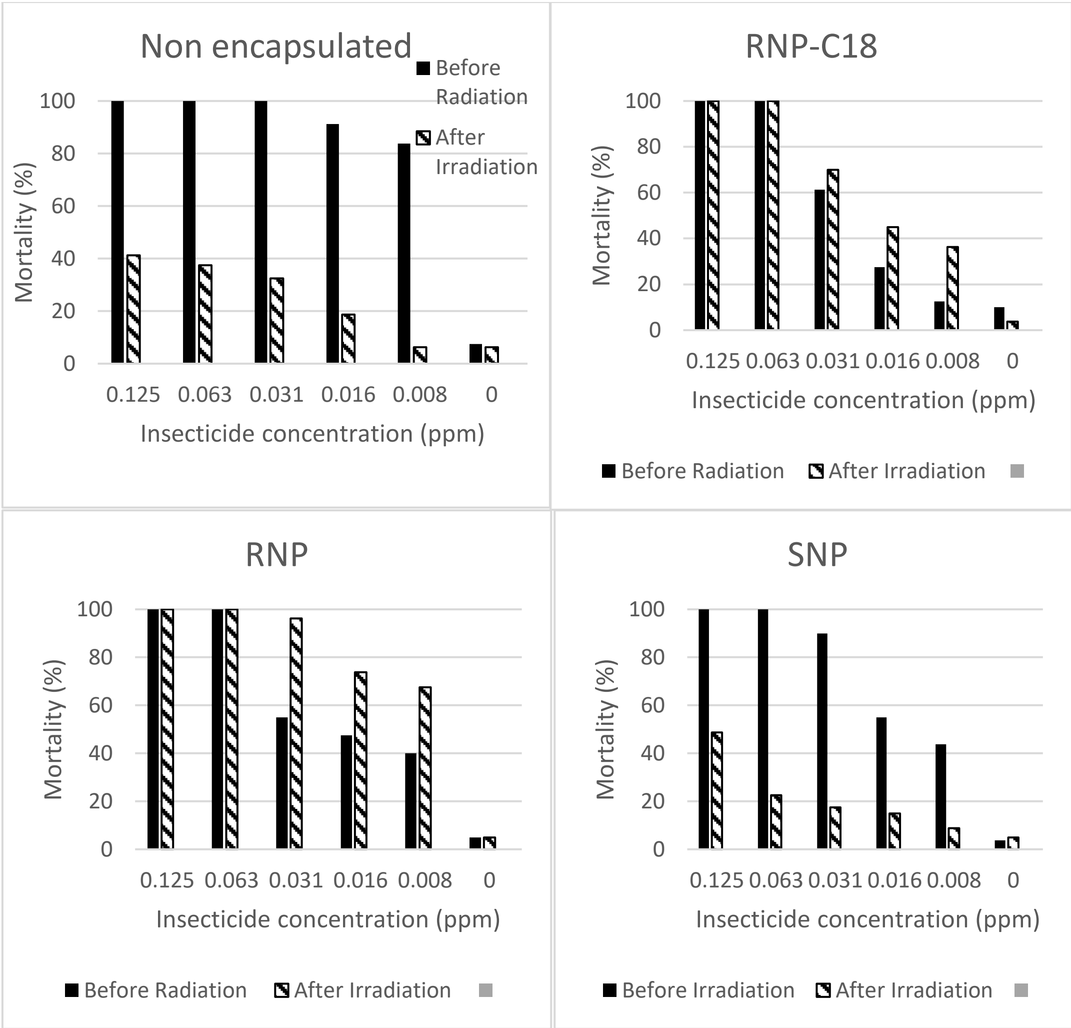

Figure 5. Effect of UV exposure on efficacy of nanoparticle formulations of lipophilic insecticide and a commercial formulation in larval assays.

Figure 5 shows the mortality of larvae exposed to wool treated with nanoparticles containing a lipophilic chemical following exposure of the wool to high-level UV radiation. As with most of the assays conducted, the rough nanoparticle formulations suffered much less degradation, and remained effective against the exposed larvae whereas the effectiveness of the unencapsulated chemical and the smooth nanoparticle formulations larvae was considerably reduced after irradiation.

Figure 6 suggests that the rough-surface particles also assist in reducing leaching of water-soluble chemical from the wool. After the wool samples had been exposed to approximately 6 cm of simulated rainfall on two occasions there was a significant decrease in efficacy of the unencapsulated chemical whereas the decrease was relatively small with the FSN-60 and RNP chemicals.

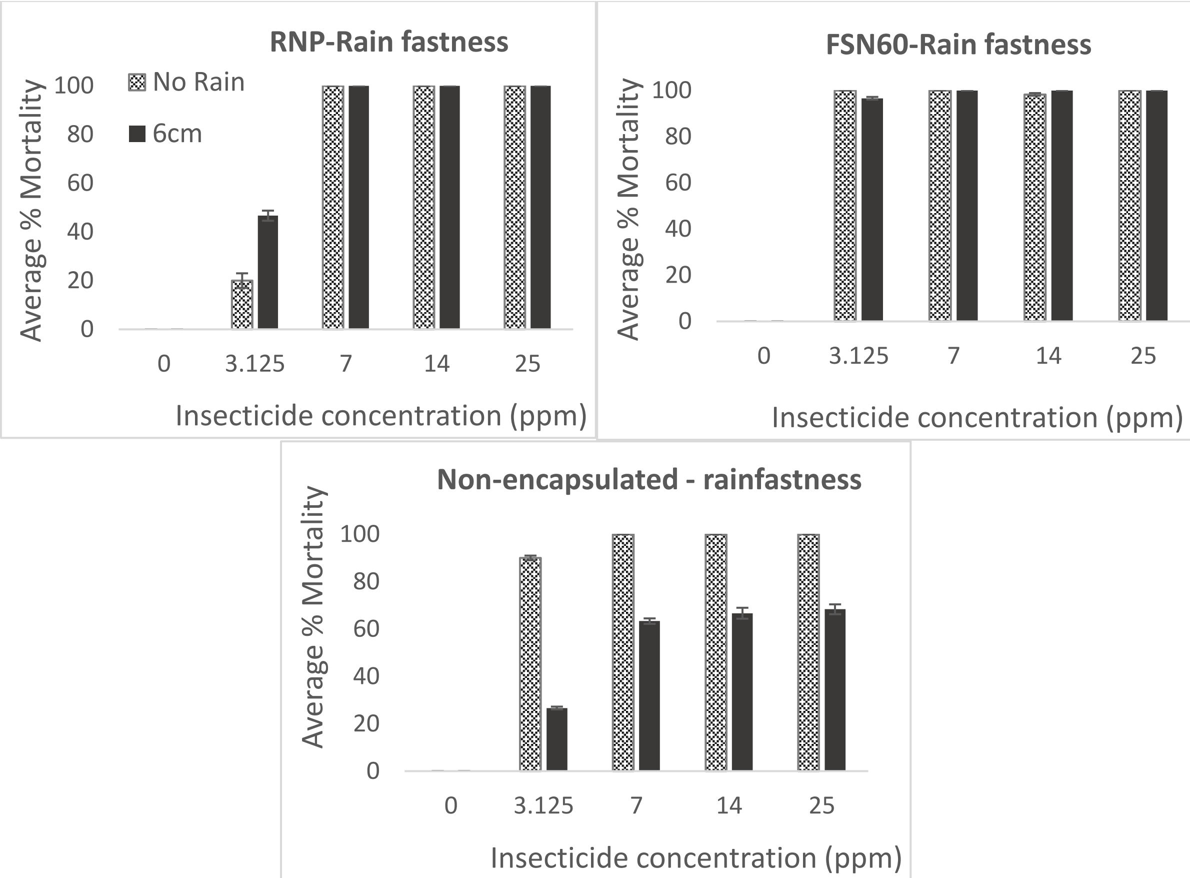

Figure 6. Larval toxicity in assays for rain fastness with wool treated with different formulations of water-soluble pesticide then exposed to simulated rainfall on two occasions.

Low residue chemicals and plant extracts

A large range of plant extracts and other chemical compounds have been shown to have insecticidal and repellent effects against *L. cuprina*. Although these compounds can often give short term protection, their effectiveness is usually rapidly lost due to volatilisation and environmental degradation. However, our results to date suggest that degradation can be significantly reduced by incorporation in rough silica nanoparticles and that appropriate formulation may be able to make their decay profile more favourable for practical use (Figure 7).

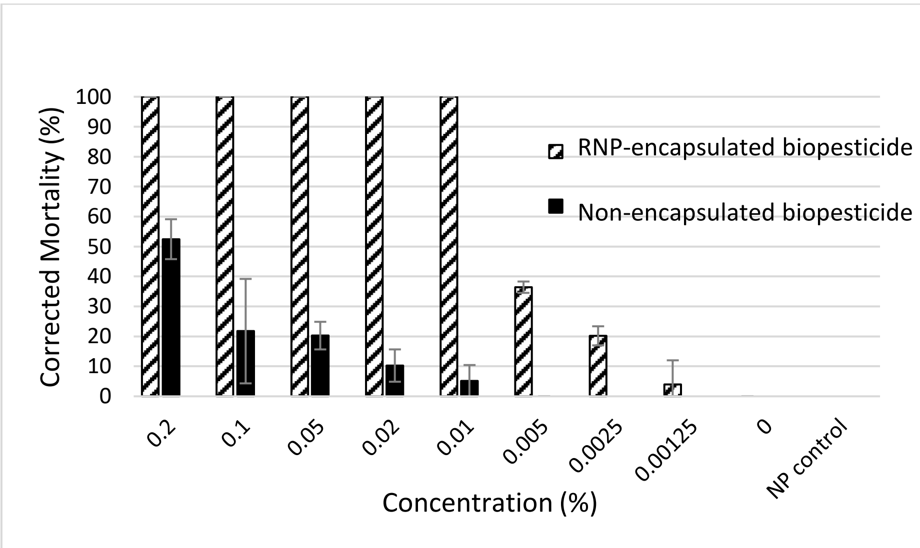

Figure 7. Mortality induced by a photo-labile volatile plant compound presented as free plant extract and encapsulated in rough nanoparticles in first instar *L. cuprina* larval assays.

CONCLUSION

Huge advances in controlled release technology for a wide range of applications, and in particular nanotechnology, offer significant opportunities for the development of new or enhanced sheep blowfly and lice control strategies. Although there have been some studies in this area in the past (Anderson et al. 1989, James et al. 1990, 1994, Rugg et al. 1998) for a range of reasons these have largely not been pursued.

The silica nanoparticles described here are environmentally degradable, have low health risk and importantly can be applied by conventional application equipment. As shown here they provided better protection in the presence of environmental challenge in laboratory tests. Studies are now required to test the behaviour of the particles in the sheep wool-skin environment to see if extended protection can be obtained from these formulations under more practical conditions.

What has long been considered the cardinal rule of toxicity, 'dose makes the poison' has been attributed to Paracelsus, a 15th century Swiss physician. This has more recently been elaborated to 'Dose makes the poison – but formulation is the key'. Nowhere would this seem to be more appropriate than with the possibilities presented by nanotechnology.

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