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AQUATIC WEEDS

Natural enemies from South Africa for biological control of *Lagarosiphon major* (Ridl.) Moss ex Wager (Hydrocharitaceae) in Europe

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Abstract The non-native invasive plant, *Lagarosiphon major* (Hydrocharitaceae) is a submersed aquatic macrophyte that poses a significant threat to water bodies in Europe. Dense infestations prove difficult to manage using traditional methods. In order to initiate a biocontrol programme, a survey for natural enemies of *Lagarosiphon* was conducted in South Africa. Several phytophagous species were recorded for the first time, with at least three showing notable promise as candidate agents. Amongst these, a leaf-mining fly, *Hydrellia* sp. (Ephydridae) that occurred over a wide distribution causes significant leaf damage despite high levels of parasitism by braconid wasps. Another yet unidentified fly was recorded mining the stem of *L. major*. Two leaf-feeding and shoot boring weevils, cf. *Bagous* sp. (Curculionidae) were recorded damaging the shoot tips and stunting the growth of the stem. Several leaf-feeding lepidopteran species (Nymphulinae) were frequently recorded, but are expected to feed on a wide range of plant species and are not considered for importation before other candidates are assessed. The discovery of several natural enemies in the country of origin improves the biological control prospects of *L. major* in Europe.

Keywords *Hydrellia* · *Bagous* · Leaf-mining fly · Phytophagous organisms · Classical biological control · Native survey · Ireland

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Introduction

African curly leaved waterweed, *Lagarosiphon major* (Ridl.) Moss ex Wager (Hydrocharitaceae) (Oxygen weed, African Elodea) is a submersed non-native macrophyte found in several countries in Europe (Symoens & Triest, 1983; Preston et al., 2002; Reynolds, 2002; Stokes et al., 2004; van Valkenburg & Pot, 2007) and New Zealand (McGregor & Gourlay, 2002). It was first recorded in Ireland in 1966 (Symoens & Triest, 1983) and has become noticeably invasive in the past decade. The discovery of substantial infestations in Lough Corrib in 2005 has identified the need to control this species in Ireland (Gavin et al., 2007; Caffrey et al., 2008). Both mechanical and chemical methods are used to control *L. major* with variable degrees of success (Caffrey et al., 2008). However, the applications of such control methods in environmentally sensitive areas are unsustainable as a long-term solution since many herbicides are being de-registered and the application of chemicals in waterways is considered unsuitable (see Water Framework Directive, CEC, 2000; Shaw, 2007). Classical biological control in this case may provide an ideal strategy to control *L. major* in a variety of freshwater habitats.

Invasive, free-floating and emergent aquatic weeds are amongst the weed species in Europe considered as good targets for classical biological control (Sheppard et al., 2005). A number of the world's worst aquatic invasive species have been successfully controlled in other parts of the world using biological control (Cruttwell McFadyen, 1998; Charudattan, 2001; Hill, 2003; McConnachie et al., 2004). The control of submersed plants presents a particular challenge as most biocontrol programmes have focused on terrestrial and free-floating aquatic plant invaders, with comparatively little attention given to submersed plant species in the past. An exception is *Hydrilla verticillata* (L.f.) Royle, where two curculionid beetles *Bagous affinis* Hustache and *Bagous hydrillae* O'Brien, and two ephydrid flies *Hydrellia pakistanae* Deonier and *Hydrellia balciunasi* Bock were released as biocontrol agents in the United States (Balciunas & Burrows, 1996; Grodowitz et al., 1997; Julien & Griffiths, 1998). A native weevil *Euhrychiopsis lecontei* (Dietz) has also been used to control *Myriophyllum spicatum* in northern United States and Canada (Julien & Griffiths, 1998; Johnson & Blossey, 2002). Many submersed weeds do present a significant threat to aquatic and riparian habitats (Charudattan, 2001) and warrant further attention, despite a general lack of published information on the natural enemies associated with submersed plants in countries of origin (Bennett & Buckingham, 2000). Many species in the family Hydrocharitaceae, such as *Egeria densa* Planchon, *Elodea canadensis* Michx, *Elodea nutallii* (Planchon), and *Hydrilla verticillata* are invasive in Europe (Preston et al., 2002; DAISIE, www.europe-aliens.org; Anonymous, 2009). Biological control investigations have been initiated on these and other submersed weeds, such as *Cabomba caroliniana* Gray, with surveys in the countries of origin revealing some promising candidate agents (Schooler et al., 2006). Prospects for the biocontrol of *L. major* was considered for New Zealand, but no biological control programme was initiated as other aquatic plants were considered to be of higher priority (McGregor & Gourlay, 2002), and no natural enemies were known from the region of origin of the weed. However, as Sheppard et al. (2005) indicate, species like *L. major* may become suitable target species for biological control if we know more about the natural enemies in the country of origin. Indeed, a short-term survey completed at two sites in South Africa recorded the presence of some natural enemies on *L. major* (Schutz, 2007).

The genus *Lagarosiphon* is native to sub-Saharan Africa, and nine species are described with variable distribution ranges throughout the continent including Madagascar (Wager, 1928; Symoens & Triest, 1983). In southern Africa, *Lagarosiphon muscoides* Ridley and *L. major* are the most common species encountered, with most of the records of *L. major* occurring south of Zambia (from the South African Cape provinces to Zimbabwe). Both *L. major* and *L. muscoides* are considered noxious weeds in South Africa (Obermeyer, 1964, 1966), and often proliferate in man-made impoundments. As a result of the extensive natural distribution range in southern Africa, a short-term survey was conducted to determine the phytophagous organisms associated with *L. major* in particular. Results of this survey are presented in this article, and the prospects of initiating a biological control programme in Ireland are discussed.

Materials and methods

A field survey was undertaken in South Africa in November 2008. Distribution records from the South African National Biodiversity Institute (Fig. 1) and the published literature (Symoens & Triest, 1983) were used to target localities where *L. major* had been recorded, and priority was given to sites with recent records. The survey included sites from some of the most southerly records in Eastern Cape Province (~750 m a.s.l.) to high-altitude sites (1,400–2,000 m a.s.l.) in Mpumalanga Province. In suitable climatic areas,

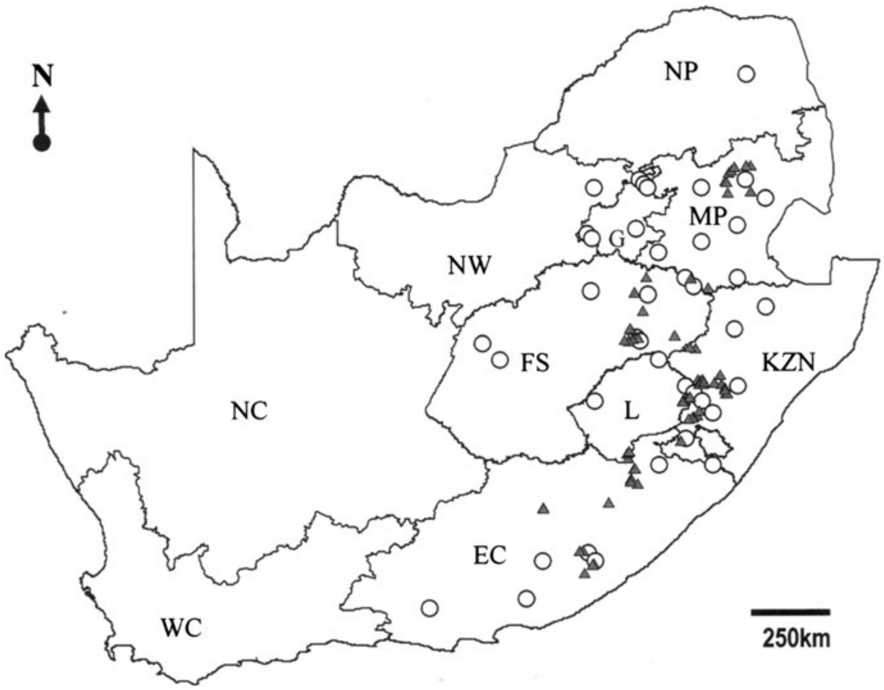

Fig. 1 Distribution records of *L. major* in South Africa from South African National Biodiversity Institute (SANBI) (*open circles*), and sites surveyed for *L. major*/*L. muscoides* (*closed triangles*). *GT* Gauteng, *MP* Mpumalanga, *NP* Northern, *NW* Northwest, *KZN* Kwa-Zulu Natal, *EC* Eastern Cape, *WC* Western Cape, *NC* Northern Cape, *FS* Free State, *L* Lesotho

further impoundments were surveyed, and additional site records held by Rhodes University were also visited. At each site, plants were assessed for damage symptoms. Damaged plants were collected for dissection and the rearing of natural enemies. Arthropod specimens were assigned a Rhodes University accession number and sent to the South African National Collection of Insects (ARC-PPRI Pretoria) for identification. The plant material collected at each site was identified in the field using Cook (2004) and sent to Lesley Henderson (Weeds Division, ARC-PPRI Pretoria) for confirmation of identification. At each site, hydrochemical parameters, including temperature (°C), pH, conductivity (μS/s), total dissolved solids (ppm), sodium chloride (NaCl, ppm), and Dissolved Oxygen (mg/l and % saturation) were recorded with a hand-held meter to characterise the water.

Results

The survey coincided with the early summer period, and 34 of 65 sites visited were surveyed in some detail (Fig. 1). Both *L. major* and *L. muscoides* were regularly encountered, and plants were growing vigorously showing evidence of healthy growth over the winter period. Most stands were accessible from the bank occurring in less than 1.5-m depths, with some ponds only having a depth of about 0.5 m. Plants were accessible whilst wading, and all plant parts were inspected (with less time spent on rhizomes and roots). Sites surveyed included mostly man-made dams and reservoirs, natural lakes and also some rivers and streams. Stands of *L. major* ranged from small clumps amongst beds of *L. muscoides* along the edges of dams, to large beds occupying the entire water column of small impoundments.

Hydro-chemical results served to characterise the sites, and some parameters are worth noting. Although temperature fluctuated on a daily basis, water temperatures were warmer (29.1°C max., mean ~22°C) than those recorded during summer in Ireland. High altitude sites (1,500–2,000 m a.s.l.) are cool during winter, at which time plants were noted to be growing well (subsequent observations by authors). Conductivity levels were high (250–330 μS/s), and all sites had pH levels well over 8.00. Many impoundments were maintained as salmonid fisheries, and the dissolved oxygen levels were characteristic of clean water conditions, but highly vegetated small ponds reached oxygen levels of over 160%.

Many aquatic invertebrate groups were found amongst the plant material, but the survey focused on likely phytophagous organisms. Organisms encountered included mostly insect groups (Table 1), and in the absence of some confirmed identities, six types of damage were noted. Specimens sent for identification are lodged at the National Collection of Insects (ARC-PPRI) in Pretoria.

Leaf-mining fly

The larvae mined the leaves, and pupated within the leaf tissue from which the adults emerged. The larva fed internally leaving the upper and lower leaf epidermal tissue intact, but plants were probably unable to photosynthesise (Plate 1a). The larvae (Plate 1b) moved between leaves and fed on an excess of 15 leaves before pupating. Early larval instars burrowed into the leaves at the shoot tip, and stem elongation resulted in the damage being obvious further down the stem. The pre-pupa and pupa were noticeable within the leaf (Plate 1c). Adult flies (Plate 1e) emerged and inhabited the water surface and emergent plants. The eggs were small (Plate 1d), laid singly or in small clusters on any emergent plant material. Leaf mining damage was recorded on both *L. muscoides* and *L. major*. The fly larvae and pupae were noticeable and often abundant at most of the sites surveyed. The fly was tentatively identified as an ephydrid fly, prob. *Hydrellia* sp. (Diptera: Ephydridae) (Biosystematics Division (BD) PPRI), and similar flies reared from *L. muscoides* await identification.

Braconid parasitoids were noticeable at almost every site, and many pupae collected in the field were parasitised. Adult parasitoids were observed in the field searching under water and probing the fly larvae. The adults held a bubble of air in the wings and walked over the plant material with reasonable speed whilst searching. Specimens reared from one site have been identified as *Ademon* spp. (Dr Gerhard Prinsloo, BD-PPRI). About ten species are known from this genus from the USA, Europe and the Orient, and the known hosts of some of these include ephydrid flies. Other specimens have been identified by Dr Kees Van Achterberg (Nationaal Natuurhistorisch Museum Naturalis, Netherlands) as belonging to the genus *Chaenusa* Haliday, a fairly large, widespread group known to be exclusively parasitic on ephydrid flies, but had not previously been recorded in Africa.

Stem-mining fly

At a single site, larval specimens were found burrowing down the stems of *L. major* plants (Plate 2a) (Lydenberg, Mpumalanga Province). A small number of affected stems were found at the site but only hymenopteran parasitoids were reared from the specimens collected. This damage did not seem to

Table 1 Phytophagous natural enemies and the characteristic damage recorded on *L. major* during a survey in South Africa

Natural enemy	Herbivorous life stage	Damage characteristics	Distribution[a]			
			EC	KZN	FS	MP
Coleoptera (Curculionidae)						
Bagous spp. (sp. 1 & 2)	Adult & larva	Shoot-tip & stem borer (larva) and leaf-chewer (adult)	*			
Diptera (Ephydridae)						
Hydrellia spp.	Larva	Leaf-miner	**	**	**	**
Diptera						
Unidentified sp.	Larva	Stem-miner				*
Lepidoptera (Crambidae)						
Nymphulinae sp. 1 (cf. *Synclita*)	Larva	Leaf-chewer	**	**	**	**
Nymphulinae sp. 2 (cf. *Parapoynx*)	Larva	Leaf-chewer	**	**	**	**

Individuals recorded as rare (*) or common (**) at majority of sites surveyed

EC Eastern Cape, *KZN* Kwa-Zulu Natal, *FS* Free State, *MP* Mpumalanga

Plate 1 Typical damage induced by the leaf-mining fly (Ephydridae: Diptera) on *L. major* (**a**), fly larva feeding within the leaf tissue (**b**), fly larva pupating within the leaf (**c**), eggs laid on emergent material (**d**) and ephydrid adult fly *Hydrellia* sp. (**e**)

Plate 2 Stem damage and pupa under a thin epidermal layer of a fly collected on *L. major* (**a**), characteristic feeding damage of various Lepidoptera collected on *L. major* and *L. muscoides* (**b**) and larva of prob. *Synclita* sp. (**c**)

stunt the growth of the main stem, but only a few specimens were collected and stems may support more than one larva at higher fly densities.

Leaf-feeding lepidopterans

Damage attributed to leaf-feeding lepidopteran larvae was readily encountered at survey sites. The leaves were cropped to near the base, leaving areas leafless along the length of the stem (Plate 2b). Feeding larvae at times damaged the stem and older feeding sites were evident in places, but the stem was rarely entirely severed. Although identifications are not complete, it is suspected that at least two species were collected. These may include prob. *Synclita* sp. (see Plate 2c) and prob. *Parapoynx* species, which had been recorded on *L. major* by Schutz (2007). All the adults reared were confirmed as belonging to the family Crambidae (Lepidoptera: Nymphulinae) (identity confirmed by Vivienne M. Uys, BD-PPRI).

Leaf and shoot feeding weevils

Two curculionid beetles were encountered at separate sites during the survey. These were quite distinct in size, with the smaller of the two (Plate 3a) collected at two of the sites (Kubusi Dam near Stutterheim and Wriggleswade Dam, EC) and the larger weevil (Plate 2c) at only one site (roadside pond, EC). Both were only encountered in the Eastern Cape, and subsequent collection trips (conducted by Rhodes University JC, MH and GM) at Wriggleswade Dam proved unsuccessful in recovering any additional specimens of the small species. However, seven individuals of the larger weevil were collected from the roadside pond. At the sites where the weevils were found, the shoot tips of the plants were damaged (Plate 3b). The crowns of the main stems in many instances were damaged to such an extent that side shoots were produced below the shoot tip (Plate 3d). It is expected that the larvae of the weevils burrow into the main stem, as eggs were laid amongst the whorl of leaves at the shoot tip when weevils were maintained during the field trip. Adults fed on the leaves, and feeding damage was noticeable as elongated holes along the length of the leaf. Specimens were sent for identification and were confirmed to be a *Bagous* sp. (Coleoptera: Curculionidae, Bagoini) (Riaan Stals, BD-PPRI).

Discussion

The survey resulted in the discovery of numerous species that fed and caused significant damage to *L. major* in the country of origin. It is the first large scale survey conducted on this target weed in South Africa. As with many other submerged invasive plant species, like *Egeria densa*, *Hydrilla verticillata* and *Cabomba caroliniana*, dedicated surveys in the country of origin have revealed a complex of phytophagous natural enemies (Balciunas & Center, 1981; Schooler et al., 2006). The most promising of these on this survey include the leaf-mining ephydrid fly *Hydrellia* sp. (Diptera: Ephydridae). Flies of this genus were found to occur throughout the geographic range surveyed (during the field trip), and the characteristic damage to leaves was observed on many of the pressed specimens in the National Herbarium in Pretoria (South Africa).

Hydrellia flies have been recorded on several species in the family Hydrocharitaceae in different continents (evidence of co-evolution) and have been released as biological control agents. Two *Hydrellia* species have been deliberately released as part of a biological control programme and established on *Hydrilla verticillata* in the United States (Balciunas & Burrows, 1996; Grodowitz et al., 1997; Julien & Griffiths, 1998). These cause significant damage to weed infestations in the field (Balciunas et al., 2002) and under experimental conditions (Doyle et al., 2002, 2007). In addition, impact surveys in South America show that a *Hydrellia* sp. on *Egeria densa* has significant impact on the leaves (Cabrera Walsh et al., 2007). It is encouraging to note that biocontrol programmes on similar submerged plants exist and that the types of agents that are performing well have now been discovered on *L. major* in South Africa. A *Hydrellia* sp. culture has been maintained at Rhodes University (following further field collections) and initial biology and host specificity studies have been initiated. Furthermore, a culture of the fly has been imported into a quarantine facility at University College Dublin (Ireland) to initiate comparable studies on the biology and conduct the host specificity testing.

Tried and tested techniques are already developed to rear and test similar flies under laboratory conditions (Balciunas & Center, 1981; Buckingham, 1988; Buckingham et al., 1989, 1991; Balciunas & Burrows,

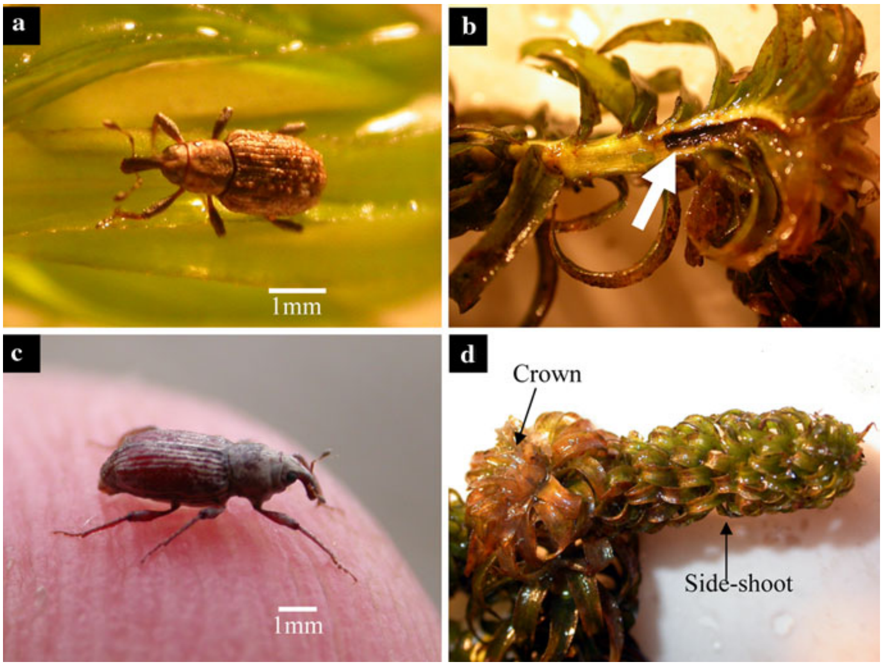

Plate 3 Crown and stem-mining weevils collected on *L. major* in South Africa. Smaller of the two adult weevils, cf. *Bagous* sp. A (**a**), crown damage probably due to larval feeding damage (**b**), larger of the two adult weevils, cf. *Bagous* sp. B (**c**) and side-shoot stimulated by crown damage on the main stem (**d**)

1996; Grodowitz et al., 1997). These will improve the screening efficiency of candidate agents on *L. major*. The biocontrol programme in the United States has been implemented since the 1990s and provides reassuring evidence that agents like *Hydrellia pakistanae* and *Hydrellia balciunasi* are likely to be host specific and pose no significant threat to native plants. However, the host specificity of every candidate must be determined before the risks of a potential release in Europe can be assessed (see Bigler et al., 2005; Shaw, 2007).

The complex of natural enemies found on *L. major* is notably similar to that discovered on *Hydrilla verticillata* on the Asian and Australian continent. This included the leaf-mining flies and two weevils, all of which were released as biocontrol agents in the United States (Balciunas & Burrows, 1996; Grodowitz et al., 1997; Julien & Griffiths, 1998). Small beetle species have been particularly successful as biocontrol agents and have resulted in the effective control of some of the world's worst aquatic and terrestrial weeds. These include the control of water hyacinth, *Eichhornia crassipes* (Mart.) Solms-Laubach (Center et al., 1999; Hill, 2003), *Myriophyllum aquaticum* (Cilliers, 1999); water lettuce *Pistia stratiotes* L. and salvinia *Salvinia molesta* D.S. Mitchell (Hill, 2003). A small weevil, *Stenopelmus rufinasus* Gyllenhal has provided some of the most spectacular control of *Azolla filliculoides* Lamarck in South Africa where it was released as a biocontrol agent (McConnachie et al., 2004). It also controls this floating weed in Ireland, where it arrived inadvertently (Baars & Caffrey, 2010), and in other European countries. This weevil poses no threat to native plants in any of these countries. It is so encouraging, therefore, that two *Bagous* species were collected during the field survey, and observations on at least one of the species suggested that the damage induced was sufficient to stunt the growth of the main stem of *L. major*. It may be difficult, however, to definitively identify the species as the taxonomy of the Afrotropical Bagoinae is poorly developed. Only four species are described from South Africa, and recent revisions from Australia, Japan, India and western Palaearctic reveal the presence of numerous species and as-yet more undescribed species (Riaan Stals pers. comm.). With so few species described from South Africa, it is possible that the specimens collected from *L. major* are as yet undescribed. Of the few known species from South Africa, none appear to be of economic importance. Moreover, as a result, the urgency of the review of this genus is low, with no current research being undertaken.

Many aquatic plants in Ireland are fed on by lepidopteran larvae. The leaf-feeding moth, *Parapoynx stratiotata* (L.) has been recorded in southern parts of the UK (NHM, UK) and Ireland (Karsholt & van Nieukerken in Fauna Europaea), with known hosts including *Ceratophyllum* spp. (Ceratophyllaceae), *Elodea* spp. (Hydrocharitaceae), *Nuphar* spp. (Nymphaeaceae) and *Potamogeton* spp. (Potamogetonaceae). Similar host records suggest that lepidopteran species in the subfamily Nymphulinae have a wide host range, and as a result, are of no use from a biological control perspective. However, Balciunas et al. (2002) still encourage the consideration of lepidopteran species collected on *Hydrilla* in the countries of origin.

The use of classical biological control has a history of very safe and successful programmes throughout the world, and its implementation in Ireland needs to be considered for its growing number of invasive weeds. Aquatic invasive plants present a particular threat to Irish native habitats, and the use of mechanical and chemical control methods does not present a long-term solution. Indeed, recurrent costs to control alien invasive species using anything other than biological control are unsustainable and expensive (Langeland, 1996; Balciunas et al., 2002; Johnson & Blossey, 2002; McConnachie et al., 2004). Although not all alien invasive species can be targeted using biological control, many are considered suitable (Sheppard et al., 2005). Following the discovery of some promising natural enemies on *L. major* in South Africa, investments should be made to initiate a biological control programme against this plant. *Lagarosiphon major* has no closely related native plant species in Europe (with similar growth habit), improving the chances of finding a host-specific agent. There are, however, some very significant factors that will potentially slow down or obstruct the biological control of plant species in Ireland and Europe, despite a long history of biological control of arthropod pests. These include the public misconception of the use of biological control, lack of a coherent legislative framework for the release of biocontrol agents, lack of ownership by a single national authority to provide permission for release, and the availability of long-term funding to provide continuity to research programmes (see Shaw, 2007). As we work to resolve some of these appreciable constraints, considerable progress has been made with the introduction of candidate agents into quarantine for research purposes.

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