The potential for biological control of the two invasive *Rubus species*, *R. ellipticus* var. *obcordartus* and *R. niveus* in Hawaii (Phase 2)

Final report (January 2014 – June 2016)

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In collaboration with

The National Bureau of Agricultural Insect Resources

The Indian Council for Agricultural Research

Cover photo: *Rubus ellipticus* infected with the *Pseudocercospora/Pseudocercosporella* leafspot pathogen in its native Indian range.

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1. Executive Summary

The research conducted during the second phase of the project assessing the potential for biological control of the Hawaiian-invasive species *Rubus ellipticus* and *Rubus niveus* built on the first project phase which was undertaken as a scoping study in 2012. During this first phase survey sites were identified in the Shimla district and the Kullu valley/India and a range of damaging arthropods and fungal pathogens associated with the target weeds were collected and deposited. The second project phase reported on here also benefitted from a scoping study conducted in parallel for the Galapagos Islands (funded by the Ecuadorian Government) to investigate potential biological control agents of *Rubus niveus* in its native range for control of this species which is invasive in the Galapagos Islands.

During the second project phase altogether three field surveys were conducted in the Asian native range of the two *Rubus* species; two surveys in India covered previously visited as well as new sites in the Shimla district and the Kullu valley, and additionally new sites in the Nilgiri Hills in southern India; one survey in China covered sites in the provinces Yunnan, Guizhou and Sichuan. Voucher specimens were deposited with the respective nationally mandated institutes/organizations. Identification of arthropod species collected in India was undertaken by Indian taxonomists, while identification of fungal specimens was based on field observations and preliminary laboratory studies undertaken at NBAIR. Arthropod and fungal specimens collected in China were preliminarily assessed using the CABI facilities in Beijing.

Based on identifications, literature searches and field observations selected natural enemies have been prioritized for further evaluation as potential biocontrol agents. From India these comprise seven arthropod species including a leaf rolling moth [*Acleris enitescens*] and beetles species in the genera *Aphthona, Apoderus, Oomorphoides*, *Sibinia* and *Coraebus*) as well as three fungal pathogens including two *Phragmidium* spp. and a *Pseudocercospora* sp / *Pseudocercosporella* sp. leafspot. From China these encompass with respect to the arthropods the beetle *Coraebus* cf *quadriundulatus,* a currently unidentified leaf-rolling beetle (Attelabidae), an unidentified sawfly (Tenthredinoidea) and an unidentified tortricid moth as well as a rust pathogen, *Phragmidium* sp. and a *Pseudocercospora* sp /

Pseudocercosporella sp. leafspot. Timely export permission for prioritized agents could not be secured from the respective Indian authorities due to internal organizational politics, thus it was not possible to undertake preliminary host specificity assessments of prioritized agents within the frame of this project phase. Official permission for export of the insects *Aphthona piceipes*, *Oomorphoides* sp. *Sibinia* sp. and *Coraebus coerulens* from India was granted by the respective authorities on April 25th 2016 thereby enabling future export and evaluation. Export of potential agents from China was not within the remit of this project phase, however, in the future export procedures can be facilitated through the CABI China office in Beijing. Inoculation studies with a *Phragmidium* rust, prioritized as a result of the field work conducted in China, was undertaken at the CABI China laboratory and showed the genotype of *R. ellipticus* invasive in Hawaii to be susceptible to the pathogen. These studies furthermore provided initial evidence for the existence of *formae speciales* (host-adapted strains) within this *Phragmidium* rust.

Initial molecular studies carried out as part of the scoping study conducted for Galapagos to elucidate the geographic origin of their invasive *R. niveus* genotype also including Hawaiian samples of this plant species. While neither conclusive nor comprehensive preliminary findings from these studies indicate that India rather than China might be the source of the *R. niveus* introduction into Hawaii. Comparable work to elucidate the origin of the *R. ellipticus* introduction into Hawaii has not yet been undertaken.

2. Acronyms and Abbreviations

BCA	Biological Control Agent
DARE	Department of Agricultural Research and Education
DLNR	Department of Land and Natural Resources
HISC	Hawaii Invasive Species Council
ICAR	Indian Council of Agricultural Research
MTA	Material Transfer Agreement
MoU	Memorandum of Understanding
NBPGR	National Bureau of Plant Genetic Resources (India)
NBA	National Biodiversity (India)
NBAIR	National Bureau of Agricultural Insect Resources (India)
NBAII	National Bureau of Agriculturally Important Insects (India)
NBAIM	National Bureau of Agriculturally Important Microorganisms (India)
PPI	Plant Protection Institute (China)
USDA-FS	United States Department of Agriculture, Forest Service

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The author of this report would like to thank the Department of Land and Natural Resources, State of Hawaii as well as the Hawaiian Invasive Species Council for continued funding of this research. Further we would like to acknowledge USDA FS for coordinating the funds, and in particular, we would like to thank Tracy Johnson for his support throughout the project. In addition, we would like to thank all of our colleagues at the CABI India office and the CABI China office for the support and help with the logistical components of the surveys and our valued collaborators at NBAIR for their invaluable professional and pragmatic support. Thanks goes also to Harry Evans for his advice and to Carol Ellison and Kate Pollard for their support and for making the results of the scoping study for biological control of *R. niveus* undertaken for the Galapagos Islands available to feed into this report.

3. Project Background

Rubus ellipticus (var. *obcordatus*), yellow Himalayan raspberry, is classed as one of the world's 100 worst invasive species (IUCN). Native throughout south-east Asia, including the Himalayan region of Pakistan and India, *R. ellipticus* was first introduced into Hawaii around 1961, where it naturalized in the vicinity of Volcano. A single plant can grow into a 4m tall impenetrable thicket and, with its recurved prickles and sturdy stems, poses a problem for livestock and humans alike. In addition to invading pastures and disturbed areas, *R. ellipticus* has penetrated deeply into pristine wet forests throughout the Island of Hawaii. Plants can establish in dense shade and grow to overtop adjacent hapu'u. Longer range spread is considered inevitable and there is generally little doubt that, in time, *R. ellipticus* has the ability to become established throughout the Hawaiian Islands. The invasive congener, *Rubus niveus*, introduced into Hawaii in the 1960's, is present on the islands of Hawaii, Kauai and Maui, where it is most problematic. The ecological and economic impacts of both *Rubus* species are similar, and the species are now considered to be beyond management using traditional control methods.

Previous research into the potential for classical biological of *R. ellipticus* focussed on natural enemies from China (Wu *et al*., 2013). In 2012, an initial scoping study, funded by the USDA Forest Service, Pacific Southwest Research Station, allowed exploratory field work for natural enemies to be extended into the Indian Himalaya region. Survey work found a range of damaging, potentially specific arthropods and plant pathogens associated with *R. ellipticus.* Selected Lepidopteran and Coleopteran specimens were identified to species level and deposited in national Indian collections as part of the staged process to secure export under a material transfer agreement. Detailed results of this scoping study have been reported in the final report of Phase 1 of the project (Tanner, 2013). Field studies conducted in 2012 also found that *R. niveus* grows in close proximity to *R. ellipticus* in the field, and shares a similar suite of natural enemies. It was thus proposed to target both *Rubus* species simultaneously in order to reduce cost and effort, and *R. niveus* was subsequently included in the research conducted during the second phase of the project (2014-2016) reported on here. The Hawaiian-funded research was co-funded by a separate scoping study for classical biological control of *R. niveus* which was conducted by CABI for the Galapagos Islands, where this species is highly invasive.

4. Phase 2 Detail

4.1 Background

This final report covers the work conducted for the second phase of the project on biological control of the two invasive *Rubus* species, *R. ellipticus* and *R. niveus*, in Hawaii (2014 to 2016) and incorporates previous progress reports. It also draws on results from the scoping study undertaken for the Galapagos Islands focussing on *R. niveus*. Funding for this research phase was provided initially by DLNR/State of Hawaii with additional funds granted by HISC for FY 2015 at a later stage. The grant has been administered through USDA-FS. The additional HISC funding was incorporated into the existing CABI-USFS contract leading to a modification of the original grant agreement and an extension of this project phase from 6/30/15 to 6/30/2016. Given that the HISC funding level award was reduced by almost 2/3 compared to the initial bid (to U$ 80,000 from a requested U$112,902 for the *Rubus* work package), the original work package 'Objectives' and 'Measures of Effectiveness' were revised accordingly.

4.2 Objectives and Measures of Effectiveness

Objectives

- To maintain/renew collaborations with NBAIR/ICAR to facilitate survey permits and deposition and export of identified and prioritized natural enemies
- To conduct surveys for natural enemies of *R. ellipticus* var. *obcordatus* and *R. niveus* in the in the India native range
- To coordinate expert identifications of insect/pathogens species and to deposit voucher specimens with respective national authorities conditional for future export
- To prioritize insect/pathogen species for export and research as potential biocontrol agents
- To secure export permissions from Indian authorities facilitated by Indian collaborators
- To conduct preliminary assessment of prioritized *Rubus* agents

- To provide a comprehensive report detailing all research to date and building the case for prioritized agent(s)

Measures of Effectiveness

- Ongoing effective collaboration with Indian counterparts
- Surveys conducted and natural enemies collected, identified and deposited
- Insect and pathogen agents on *Rubus* species prioritized for future research
- Export permission secured and agents shipped to the UK
- Data produced on host specificity of selected agents
- Comprehensive report produced

4.3 Administration

Personnel

CABI-UK

Dr Rob Tanner, in his role as project manager, has been responsible for the project from the start until his secondment to EPPO/Paris at the beginning of 2015. Following his departure **Dr Marion Seier** took over the project management working in close collaboration with **Dr Carol Ellison**, who managed the scoping study on biological control of *R. niveus* undertaken for the Galapagos Islands. **Kate Pollard** provided scientific support, particularly during survey work, while **Nikolai Thom** was responsible for plant propagation and technical support to the project.

CABI-South Asia, New Delhi, India

Dr Ravi Khetarpal held the position of Country Director India until October 2015, when he was appointed to the new role of Regional Advisor, Strategic Science Partnerships, South-Asia and **Gopi Ramasamy** took over the directorship. In addition to leading the development of a South Asia science strategy and establishing strategic science partnerships, Ravi will be overseeing the establishment of an ICAR-CABI Joint Laboratory modelled on the one CABI has established in China. Both Gopi and Ravi were involved in liaising with ICAR institutes and DARE at the highest levels to obtain endorsements for collaborative agreements and project specific work plans, as well as notify relevant authorities of CABI surveys.

CABI –China

Dr Feng Zhang, Country Director of the CABI-China Office housed by the Chinese Academy of Agricultural Sciences, provided logistic support for the Rubus field survey conducted in China in 2014. **Huanhuan Wan** supported the survey scientifically and technically and conducted subsequent inoculation tests.

NBAIR (formerly NBAII and Project Directorate for Biological Control)

In June 2014 NBAIR in Bangalore became the principal collaborative institute taking over from NBPGR based in Delhi. **Dr Abraham Verghese**, Director of NBAIR, has since overseen the project collaboration and **Dr Chandish Ballal** (Principal Scientist & Head, Division of Insect Ecology) continued in her role as Principal Investigator supported by **Dr Prashanth Mohanraj**, Head of the Dept, Biosystematics and **Dr Sunil Joshi** (Principle Scientist, Entomology). The various taxonomists who have identified collected specimens are acknowledged by name in the respective sections of this report. Several curators at NBAIR facilitated deposition and maintenance of voucher specimens.

NBAIM

NBAIM, based in Mau, holds the national mandate to deal with fungal organisms and initial contact has been made with **Dr Sushil K Sharma** (Principle Scientist, Agricultural Microbiology) for deposition and identification of any field-collected pathogen material conditional to any future application for export.

Collaboration - India

ICAR/ NBAIR, DARE and NBA

With the transfer of responsibility from NBPGR to NBAIR all paperwork and specimens pertaining to the project changed hands and work plans for 2014 were submitted and approved. In 2015 new work plans, pro-formas and collaborative budgets were submitted timely for approval by DARE/ICAR, but concerns were raised by NBAIR regarding the CABI ICAR MoU. It was highlighted that new incumbents in government and at senior levels in DARE and ICAR would be less aware of the historical links with CABI and of the mutual benefits of genetic exchange for biocontrol. Despite assurances and endorsements given by DARE in 2014 that the existing MoU did not need modification to reflect the shift in responsibilities between bureaux and would sit under an overarching CABI–ICAR MoU, a “rechristening” of the project collaboration was requested. A new MoU was submitted, together with supporting documentation specific to biocontrol in India and access and benefit sharing, naming the ongoing project to identify potential biocontrol agents for *Rubus* spp. (*Rubus ellipticus* and *Rubus niveus*) as one of the collaborative projects and listing all of the collaborating bureaux. As the year progressed, all of CABI's collaborative activities in India came under scrutiny and retrospective documentation and justification for all aspects of work in country were called for by ICAR/DARE. As a result, authorisations for research work and approval of the submitted work plans were stalled which also impacted on the ability to obtain export permits for natural enemies collected during the survey conducted in September 2015. By early 2016 CABI-ICAR work plans for invasive species projects had been approved retrospectively, and NBAIR is awaiting written confirmation of this so funds can be transferred.

MTAs for the insects *Aphthona piceipes*, *Oomorphoides* sp. *Sibinia* sp. and *Coraebus coerulens,* four of the agents collected in India prioritized for further research into their potential as biocontrol agents for *R. ellipticus and R. niveus* (see 7.4 agent prioritization), had been submitted in 2015 and have since been approved. Official permission for export of these four agents was granted on 25th April, 2016. Collaboration with NBAIM is less advanced compared to the long-standing working relationship with NBAIR and thus no export permits have been obtained for prioritized fungal pathogens as yet.

A request for official collaborations with the respective institutes to continue has been made in May 2016.

Collaboration - Hawaii

A pre-proposal bidding for U$120K to support ongoing work in the invasive *Rubus* species in the 2017 fiscal year was submitted to HISC through USDA's Forestry Service (Tracy Johnson) in November 2015. Whilst this is not a prerequisite for funding, it is intended to provide a preview of future biocontrol objectives on which to build more robust budget requests. Following on from this the full proposal has been submitted in response to the HISC call according to the deadline of 1 July. A previous bid for HISC funding in FY16 had been unsuccessful.

4.4 Conferences and Presentations

- In September 2015, the 13th International conference on Ecology and Management of Alien Plant Invasions (EMAPI) was held in Hawaii and attended by Corin Pratt who gave an oral presentation ("Tackling two of the toughest") on the progress of CABI's Rubus (*R. ellipticus* and *R. niveus*) and Hedychium initiatives.

- During the survey visit to India in September 2015 Marion Seier presented a talk to NBAIR staff titled "Use of biodiversity to tackle invasive weeds" which highlighted the collaborative *Rubus* project.

4.5 Shipments

Plants

Seeds of the Hawaiian biotype of *R. ellipticus* as well as of the two Hawaiian-native *Rubus* species, *R. hawaiiensis* and *R. macraei* were shipped by Tracy Johnson (USDA-FS) to the UK in June 2015. Seeds were germinated in the CABI greenhouses and currently several plants of *R. ellipticus* and *R. hawaiiensis* are being held at CABI available for future host-specificity assessments. To date seeds of *R. macraei* have failed to germinate, and a new supply would be needed for inclusion of this critical non-target species. One common UK commercial variety of each blackberry (*Rubus fruticosus*) and raspberry (*Rubus idaeus*) have also been purchased for inclusion into testing of prioritized agents.

Leaf material

Leaf material of *R. ellipticus* and *R. niveus* was collected by Tracy Johnson from a site in Volcano, Hawaii (19*28'29"N, 155*15'34"W, 1242 m.a.s.l.) and sent as dried samples to CABI/UK. This material is to be used for comparative molecular studies with leaf samples of the two target species from their native range. It will be important to establish the geographic origin of the introduced populations invasive in Hawaii in order to target collection and testing of natural enemies to achieve the best match between biotypes of potential biocontrol agents and those of the exotic *Rubus* species.

5. Surveys

Building on the first phase of the project more thorough field surveys were conducted in the native Indian and, additionally, in the Chinese range of *R. ellipticus* and *R. niveus* in order to study and collect natural enemies associated with the two species and to deposit voucher specimens for identification and reference at the relevant national institutes. This protocol needs to be followed in order to obtain high-level permission for export of agents to the CABI quarantine facilities in the UK for further studies. Field work was also used to make initial assessments of the potential of natural enemies as classical biological control agents by evaluating the degree and type of damage caused to the host. Also assessed was their apparent field host specificity by evaluating whether related plant species, i.e. species belonging to the Rosaceae family, or plant species sharing the same habitat also come under attacked. During the course of the survey fungal specimens were collected as infected leaf or stem material and dried and stored in a plan press. Arthropod specimens were either kept in tubes for subsequent pinning and identification or stored in 70 % alcohol (for adults) or, when immature stages were collected, kept alive for subsequent rearing to adult stage and identification in the laboratory of the collaborating national institute.

The selection of survey areas and sites was made based on the field work undertaken in the initial scoping study and first project phase in 2012, collection details recorded for plant specimens of the two *Rubus* species held at the Kew Herbarium (London), as well as logistics derived from surveys undertaken previously by CABI staff in the region. In total three surveys were conducted in the native range throughout the course of the second phase of the project. Details are given below.

5.1 India

In India two surveys were conducted covering three regions: the Kullu Valley and the area around Shimla in the Himalayan foothills (northern Indian State of Himachal Pradesh), where both *R. ellipticus* and *R. niveus* are considered to be native; the Nilgiri Hills south of Mysore (southern Indian State of Tamil Nadu), since *R. niveus* is also commonly called Mysore Raspberry and there is a possibility that this southern region may also be a centre of diversity of this *Rubus* species. The first survey was conducted by Rob Tanner and Kate Pollard from 27 July-14 August 2014 covering all three regions; the second survey was conducted by Marion Seier from 6-17

September 2015 and concentrated only on the Shimla area. Both surveys were undertaken with approval of NBA and ICAR and supported by staff from NBAIR (Drs Chandish Ballal, Prashanth Mohanraj and Sunil Joshi). Figure 1 shows the regions covered during the surveys.

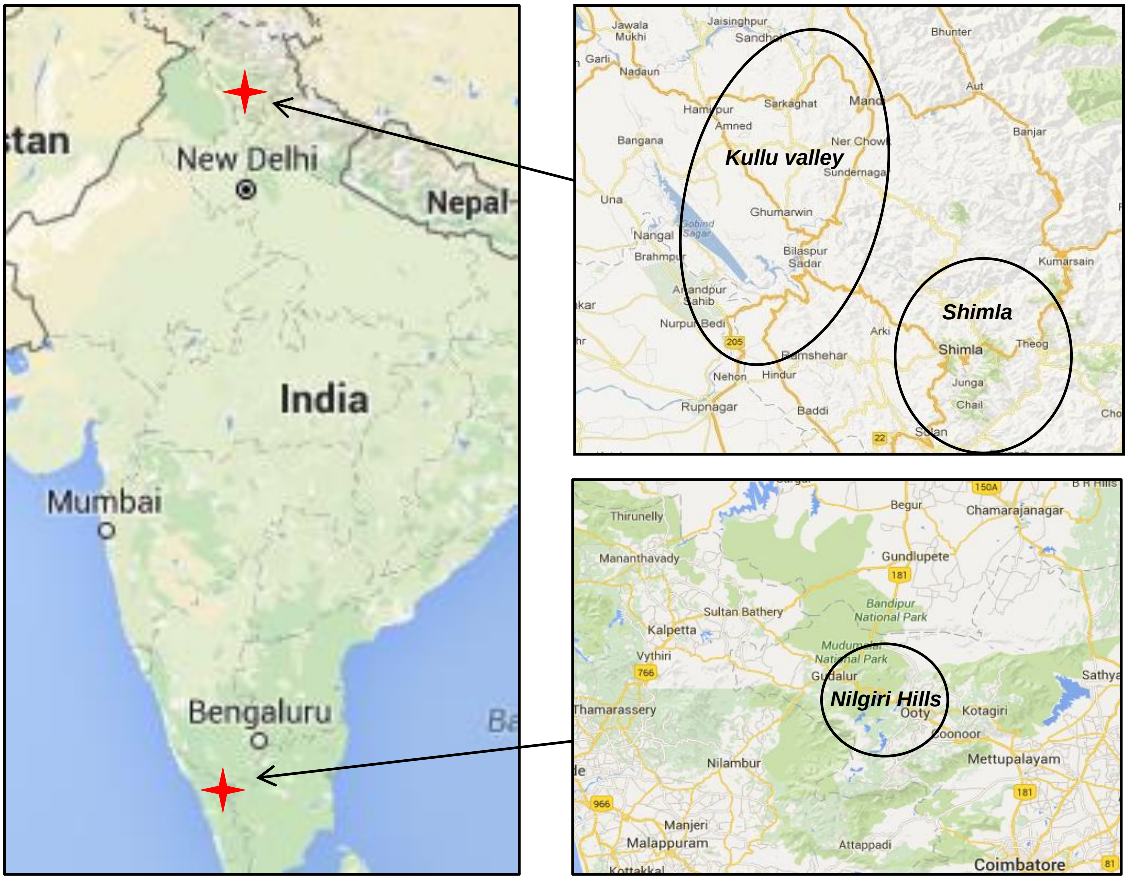

Figure 1: Geographic regions surveyed in India in 2014/15

During both surveys a wide range of field sites was visited and extensive sampling was undertaken for both arthropod and fungal natural enemies (Fig. 2a). Populations of *R. ellipticus* were found at altitudes ranging from 975 m.a.s.l. (previously reported also 789 m.a.s.l. see Tanner, 2013) and 2593 m.a.s.l., while *R. niveus* populations were present at the higher altitudes between 1493 to 2593 m.a.s.l..

Collected field material was deposited at NBAIR (arthropods) and NBAIM (pathogens) as the nationally recognized repositories. Furthermore, leaf material of various populations of *R. ellipticus* and *R. niveus* was deposited at NBPGR for future molecular analysis (Fig.2b).

Figure 2: a) Drs Ballal and Joshi assessing *Rubus niveus* for natural enemies present (India); b) Carol Ellison sampling leaf material of *Rubus niveus* for molecular studies (China)

Table 1 give the details of all survey sites visited, the natural enemy collections made and arthropod identifications provided by Indian expert taxonomists , as applicable. Official identifications for the fungal pathogen material deposited are yet to be received from the respective taxonomists at NBAIM, but tentative identifications have been made based on field observations and initial laboratory evaluation. An assessment of the biocontrol potential of individual agents is given under section 7.1 (“Natural Enemies”).

Table 1. Indian Survey 2014 / 2015

Date	Site	Co-ordinates	Alt. (m)	Location / region	Site description	Insects		Pathogens		Identification
						R. niveus	***R. ellipticus***	***R. niveus***	***R. ellipticus***	
29/07/2014	29-1	N 30° 57.086' E 077° 07.312'	1493	Shimla	On track to railway line. Mixed forest of pine and deciduous trees, large understory with bracken etc.	no	Different lepidopteran larvae; 1 brown coleopteran species; white leaf hopper species	Leafspot 1 (tan in colour) & Rust 1 (uredinia) on older leaves	Leafspot 1 (tan in colour) & Rust 1 (uredinia) on older leaves	One lepidopteran species identified as *Thyatira batis*
30/07/2014	30-1	N 31° 02.138' E 077° 07.356'	1795	Shimla	Path near to railway line. Pine forest not densely covered, open understory therefore less humid.	no	Different lepidopteran larvae;1 black-brown weevil species; 1 coccinellid species; 1 mealybug; 1 chrysomelid species; 1 small black coleopteran species; 1 dipteran species	no	Leafspot 2 (red in colour)	Weevil species tentatively identified as *Myllocerus rufescens;* Chrysomelid species identified as *Colasposoma semicostatum*
	30-2	N 31° 00.464' E 077° 05.721'	1710	Shimla	Shimla to Solan, approx 20km from Shimla. On roadside on edge of pine forest with a few deciduous trees.	no	Several coleopteran species; 1 chrysomelid species; different lepidopteran larvae; 1 coccinellid beetle; 1 leaf hopper; 1 stink bug species	Rust 1 (uredinia)	Leafspot 1 + 2 (tan and red) Rust 1 (uredinia)	Chrysomelid species identified as *Colasposoma semicostatum;* 1 coleopteran species identified as member of Galerucinae, 1 coleopteran species as *Apoderus* sp.; 1 lepidopteran species as member of Crambidae, 1 lepidopteran species as member of Tortricidae; leaf hopper species identified as *Kolla paulula;* stink bug species identified as *Paracritheus* sp.
	30-3	N 30° 57.001' E 077° 07.206'	1510	Shimla	Mixed forest of pine and deciduous trees, large understory with bracken etc. Area more open at railway line and more shrubby hillside	1 hemipteran species; 1 lepidopteran (larva)	1 chrysomelid species	Rust 1 (uredinia) & leafspot	Leafspot	Lepidopteran species identified as *Thyatira batis*; chrysomelid species identified as *Oomorphoides* sp.

Date	Site	Co-ordinates	Alt. (m)	Location / region	Site description	Insects		Pathogens		Identification
						R. niveus	*R. ellipticus*	*R. niveus*	*R. ellipticus*	
	30-4	N 30° 56.130' E 077° 08.272'	1295	Shimla	Pine mixed forest at edge, *Rubus ellipticus* along roadside in grassy area	*Plant not present*	1 leaf hopper species; 1 tree hopper species	*Plant not present*	Leafspot 1 (tan) & Rust 1 (uredinia)	Leaf hopper species identified as *Kolla paulula;* tree hopper species identified as *Gargara* sp.
	30-5	N 31° 02.740' E 077° 07.912'	1842	Shimla	Grassland with shrubby species and few pine trees along train track and up hillside next to it, area fairly open.	*Plant not present*	Several lepidopteran species; 1 brown weevil species; 2 chrysomelid species	*Plant not present*	Leafspot 1 & 2 (tan & red)	Weevil species tentatively identified as *Myllocerus rufescens;* chrysomelid species identified as *Colasposoma semicostatum* and *Trichotheca hirta*
31/07/2014	31-1	N 31° 06.539' E 077° 12.415'	2202	Shimla	Near to Halli. Present along path in open area at edge of forest on main slope, not present within the forest.	Stem borer; 1 lepidopteran (larva); 2 coccinellid species	no	no	no	Lepidopteran species identified as *Thyatira batis;* one coccinellid species identified as *Coccinella septempunctata*
	31-2	N 31° 07.142' E 077° 13.851'	2384	Shimla	Road on the way to Runparh, 20km from Shimla to Kufri. On edge of road on side of mixed forest, not within forest	Several lepidopteran species (larvae); 1 coleopteran species, aphids	*Plant not present*	no	*Plant not present*	Lepidopteran species tentatively identified as *Pandemis dumetana*; 1 coleopteran species tentatively identified as *Coraebus coerulens*
	31-3	N 31° 07.201' E 077° 14.138'	2497	Shimla	Pine forest.	2 lepidopteran species (larvae)	no	no	no	n/a
	31-4	N 31° 07.211' E 077° 14.350'	2505	Shimla	Open area to the side of the main footpath, in grassland/shrubland including clover and ferns.	2 lepidopteran species (larvae); 1 weevil species; 1 stem borer species	*Plant not present*	Rust 1 (uredinia) & Leafspot	*Plant not present*	One lepidopteran species identified as *Gonitis mesogona*; weevil species identified as *Sibinia* sp.
	31-5	N 31° 05.763' E 077° 16.723'	2593	Shimla	Road through pine forest. Present along roadside, not in forest only on track. Very good site	1 leaf miner species; 1 flea beetle species	1 flea beetle species	Rust 1 (uredinia) & Leafspot	no	Flea beetle species identified as *Aphthona piceipes*

Date	Site	Co-ordinates	Alt. (m)	Location / region	Site description	Insects		Pathogens		Identification
						R. niveus	*R. ellipticus*	*R. niveus*	*R. ellipticus*	
01/08/2014	1-1	N 31° 06.306' E 077° 07.467'	1926	Shimla	Just off bypass road towards Totu (1 km), can also see Barahi village from hill. Track through pine forest, with few other tree species.	2 lepidopteran species (larvae)	1 coleopteran species	no	Leafspot	Lepidopteran tentatively identified as *Pandemis dumetana,* coleopteran species tentatively identified as *Myllocerus rufescens*
	1-2	N 31° 07.094' E 077° 06.276'	1761	Shimla	Open hillside on edge of pine forest; not many insects, not a very good site.	*Plant not present*	No	*Plant not present*	Leafspot	n/a
	1-3	N 31°08.824' E 077° 04.949'	1588	Shimla	About 4km from Devnagar. Road through hillside of mixed forest and an area of open grass with fewer trees. Stream running through at the bottom of hill (pathogens here).	no	Fly larvae, one lepidopteran species	no	Rust 1 (uredinia) & Leafspot	Fly larvae identified as *Sisyropa* sp. (parasitoid)
02/08/2014	2-1	N 31° 07.888' E 077° 13.309'	2186	Shimla	Mashobara village. Along main track though open pine forested hill with understory.	Several lepidopteran species (larvae)	3 lepidopteran species larvae), 1 leaf miner species	no	no	One lepidopteran species ex *R. ellipticus* identified as belonging to the family Nolidae
	2-2	N 31° 10.406' E 077° 12.320'	2121	Shimla	Baldeyan, 2.5 km before Naldehra. Pine forest down slopes at top with bracken and other shrubs. Also open area of grassland with exposed rocks.	no	3 lepidopteran species	no	Leafspot 2 (red)	One lepidopteran species tentatively identified as *Acleris enitescens*
	2-3	N 31° 08.955' E 077° 12.869'	2199	Shimla	Close to Moshobara (2.8 km away). Pine forest on the edge of the roadside.	no	no	Rust 1 (uredinia)	Leafspot	n/a
03/08/2014	3-1	N 31° 42.290' E 077° 01.745'	975	Kullu	Hillside with forest with fairly dense cover at side and few shrubs. R. ellipticus present but very few plants, at edge of road. Fast flowing stream running downhill	*Plant not present*	3 lepidopteran species	*Plant not present*	Rust 1, leaf spot 1 (tan)	n/a
04/08/2014	4-1	N 32° 06.609' E 077° 10.996'	2217	Kullu	On slope with apple orchard and pine trees. R. niveus found in clearings.	no	*Plant not present*	Leafspot 1 (dark & large lesions)	*Plant not present*	n/a

Date	Site	Co-ordinates	Alt. (m)	Location / region	Site description	Insects		Pathogens		Identification
						R. niveus	***R. ellipticus***	***R. niveus***	***R. ellipticus***	
	4-2	N 32° 06.697' E 077° 10.965'	2175	Kullu	*R. niveus* found in gully and alongside rocky path. Apple and pine trees present. Also present in clearings with bracken.	One lepidopteran species (larva)	*Plant not present*	Rust 1 (uredinia) Rust 2 (telia – microcyclic) Leafspot 1 (Tan)	*Plant not present*	n/a
11/08/2014	11-1	N 11° 28.416' E° 076°40.936'	1650	Nilgiris	Main road to Ooty, edge of road and hill near covered with shrubs (inc. invasive species).	no	no	no	Leafspot 2 (red)	n/a
	11-2	N 11° 27.574' E 076° 41.032'	1850	Nilgiris	Marsikallu. Edge of agricultural.	no	*Plant not present*	no	*Plant not present*	n/a
	11-3	N 11° 27.326' E 076° 40.869'	2044	Nilgiris	Edge of the roadside, fairly open area.	No	*Plant not present*	Rust 1 (uredinia)	*Plant not present*	n/a
	11-4	N 11° 26.678' E 076° 40.322'	2144	Nilgiris	Thalakuntha (Gudalur junction). Edge of open pine forest, in grassy area at edge of road.	no	One leaf hopper species	no	Rust 1 (uredinia)	n/a
12/8/14	12-1	N 11° 24.820' E 076° 43.526'	2301	Nilgiris	On the way to Doddabetta from Ooty, Slightly shaded area with exposed bank few shrubs and large bushes on bank.	no	no	no	no	n/a
	12-2	N 11° 24.966' E 076° 43.855'	2357	Nilgiris	Open area on bank.	no	Aphids	Rust 1 (uredinia) Rust 2 (?telia – microcyclic rust species)	Leafspot 1 (tan)	n/a
	12-3	N 11° 24.520' E 076° 44.279'	2329	Nilgiris	Near Doddabetta On track at edge of road.	no	Thrips	Rust 1 (uredinia)	no	n/a
08/09/2015	8-1	-	2182	Pawoba, Shimla	Roadside exposed stand	*Plant not present*	Small black chrysomelid species, large brown chrysomelid species	*Plant not present*	no	Small black Chrysomelid *Aphthona simlaensis*
09/09/2015	9-1	N 31° 06.266' E 077° 09.130'	2173	Chaura Maidan, Shimla	Steep bank along a small road	*Plant not present*	1 leafhopper species; 1 small beetle species	*Plant not present*	Rust 1 (uredinia), Leafspot 1 & 2 (tan and red)	n/a

Date	Site	Co-ordinates	Alt. (m)	Location / region	Site description	Insects		Pathogens		Identification
						R. niveus	***R. ellipticus***	***R. niveus***	***R. ellipticus***	
	9-2	N 31° 05.935' E 077° 07.822'	2134	Bheemakali Mandir, Shimla	Exposed site on a slope and along railway line	no	1 small black chrysomelid; one large brown chrysomelid; one lepidoteran larva; aphids; lots of feeding damage but causal agent not seen	Rust 1 (uredinia) Leafspot 1 (tan)	Leafspot 1 (tan)	n/a
	9-3	N 31° 06.792' E 077° 06.397'	1850	Totu, Shimla	Stands along road	*Plant not present*	Leafminer damage	*Plant not present*	Leafspot 1 (tan)	n/a
	9-4	N 31° 06.811' E 077° 06.431'	1834	Heera Nagar, Shimla	Mixed forest in valley	one leafhopper species, whitefly, large metallic chrysomelid	no	Leafspot 2 (red)	Rust 1 (uredinia), Leafspot 1 & 2 (tan and red)	Large metallic chrysomelid ex *R. niveus* identified as *Altica himensis*
10/09/2015	10-1	N 31° 05.788' E 077° 16.729'	2569	Kufri, Shimla	On path away from main road, open habitat with *Rubus niveus* interdispersed with Buddleja on slope. Site 31-5 from 2014 survey.	Stem borer, 1 black large chrysomelid species	*Plant not present*	Rust 2 (telia?) Leafspot 1 (tan)	*Plant not present*	Chrysomelid species ex *R. niveus* identified as *Altica himensis*
	10-2	N 31° 07.201' E 077° 14.138'	2497	Charabra, Shimla	Pine forest. Site 31-3 from 2014 survey.	1 larval species, signs of stem borer, small chrysomelid species	*Plant not present*	Leafspot 1 & 2 (tan and red)	*Plant not present*	Chrysomelid species identified as *Altica himensis*
	10-3	N 31° 07.211' E 077° 14.350'	2505	Charabra, Shimla	Open area to the side of the main footpath, in grassland/shrubland including clover and ferns	no	*Plant not present*	Rust 1 (uredinia)	*Plant not present*	n/a
	10-4	N 31° 07.792' E 077° 13.527'	2396	Mashoraba, Shimla	Stand along path on slope	no	*Plant not present*	Leafspot 1 (tan) Stem canker	*Plant not present*	n/a
11/09/2015	11-1	N 31° 03.231' E 077° 08.594'	2226	Taradevi, Shimla	Exposed open slope, sunny, but cool and windy	Black chrysomelid species	Small black chrysomelid species, small brown beetle	Rust 1 (uredinia) Rust 2 (telia?)	Leafspot 1 (tan)	Small black chrysomelid ex *R. ellipticus* identified as *Aphthona simlaensis*

Date	Site	Co-ordinates	Alt. (m)	Location / region	Site description	Insects		Pathogens		Identification
						R. niveus	*R. ellipticus*	*R. niveus*	*R. ellipticus*	
	11-2	N 31° 03.152' E 077° 08.590'	2140	Taradevi,Shimla	Mixed pine forest, shady	*Plant not present*	Black chrysomelid species, also larval pupa on roots	*Plant not present*	Leafspot 1 (tan) Commencing rust 1 infection	n/a
	11-3	N 31° 02.663' E 077° 07.901'	1969	Shoghi, Shimla	Stand along the roadside and open slope with deciduous vegetation	*Plant not present*	Black larvae in leaf buds; aphids	*Plant not present*	Leafspot 1 (tan)	n/a
12/09/2015	12-1	N 31° 18.690' E 077° 26.434'	1870	Shimla region/ Jalori pass	Stand along roadside	Aphids	*Plant not present*	no	*Plant not present*	n/a
	12-2	N 31° 30.437' E 077° 25.376'	2386	Shimla region/ Jalori pass	Shady stand along the roadside	*Plant not present*	1 chrysomelid species	*Plant not present*	Leafspot	Chrysomelid species identified as *Pentamesa haroldi*
	12-4	N 31° 34.210' E 077° 22.134'	2890	Shoja	Stand along roadside in a little mountain village	no	*Plant not present*	Rust 1 (uredinia)	*Plant not present*	n/a
13/09/2015	13-1	N 31° 29.822' E 077° 25.259'	2324	Shimla region/ Jalori pass	Stand along mountain roadside	no	no	Rust 2 (telia?) but hyperparasitised Leafspot 1 (tan)	Leafspot 1 (tan)	n/a
	13-2	N 31° 20.780' E 077° 26.230'	2050	Shimla region/ Jalori pass	Stand along mountain roadside	*Plant not present*	1 black chrysomelid species; shoot rolling larva	*Plant not present*	no	Chrysomelid species identified as *Aphthona simlaensis*
14/09/2015	14-1	N 30° 57.281' E 077° 06.815'	1640	Near Solan, Shimla	Woodlands	*Plant not present*	3 different (lepidopteran) larvae; mealybug; bigger chrysomelid species (also found feeding on Impatiens species)	*Plant not present*	Leafspot 1 (tan) Rust 1 (uredinia)	n/a

5.2 China

In China one survey was conducted by Rob Tanner, Carol Ellison and Huanhuan Wan between 29 September – 5 October 2014, covering a range of sites in the three provinces Yunnan, Guizhou and Sichuan.

Specimens of field collected arthropod and fungal natural enemies, as well as leaf material of selected *R. ellipticus* and *R. niveus* populations were deposited at PPI. Figure 3 shows the regions covered during the surveys.

Figure 3: Geographic region surveyed in China in 2014

Details of all survey sites as well as the natural enemy collections made are given in Table 2. A basic assessment of the insect samples collected during the survey in China was undertaken using the CABI facilities in Beijing. Preliminary pathogen identifications are based on field observations as well as preliminary microscopic examination in the lab. Details of tentative identifications, as well as an assessment of the biocontrol potential of agents are given in Table 4 (section 7.2 "Natural Enemies").

Table 2. China Survey 2014

Date	Site	Co-ordinates	Alt. (m)	Location/ region	Site description	Insects *R. niveus*	Insects *R. ellipticus*	Pathogens *R. niveus*	Pathogens *R. ellipticus*
29/09/2014	29-1	N 26° 20.506’ E 106° 39.364’	1113	Guiyang City, Guizhou Province	Along roadside	Stem galls containing multiple larvae, Lepidoptera 1, Diptera, Hymenoptera, Coleoptera	*Plant not present*	Phytoplasma, rust (uredinia), leafspot (tan)	*Plant not present*
	29-2	N 26° 15.606’ E 106° 40.964’		Guiyang City, Guizhou Province	On the edge of a rocky hillside	Leaf miner, Chrysomelidae	*Plant not present*	Phytoplasma, rust (uredinia), leafspot (tan)	*Plant not present*
	29-3	N 26° 05.200’ E 106° 38.500’		Guiyang City, Guizhou Province		Lepidoptera, Hymenoptera	*Plant not present*	Leafspot (tan)	*Plant not present*
	29-4	N 26° 19.421’ E 106° 36’883’		YanLou Village	Plant on edge of forest by road. Beside an irrigation channel.	Stem galls and defoliating lepidopteran larvae 2, Diptera	*Plant not present*	Leafspot	*Plant not present*
30/09/2014	30-1	N 26° 35.571 E 106° 42.227’		Jin Hua Town, Guizhou Province	Site next to motorway, by toll-bridge	Diptera, stem borer, stem galls, defoliating lepidopteran larvae, Coccinellid, Heteroptera, Hemiptera, Coleoptera	*Plant not present*	Rust (uredinia), leafspot (tan)	*Plant not present*
02/10/2014	2-1	N 25° 46' 172’ E 100° 14.460’	2020	Erhai Lake, Dali, Yunnan	Next to lake park, semi natural vegetation, shade on steep hill	Sawfly damage	no	no	no
	2-2	N 25° 41' 958’ E 100° 16.063’	1976	Kunming , Yunnan	Edge of a lake	*Plant not present*	One lepidopteran larva	*Plant not present*	Reddish leaf spot
	2-3	N 25° 38' 286’ E 100° 00.791’	1534	Pingbo Town, Yunnan	Stand on the roadside	*Plant not present*	no	*Plant not present*	Rust (uredinia), reddish leaf spot
	2-4	N 25° 36' 578’ E 100° 02.277’	1448	Pingbo Town, Yunnan	Stand on the roadside	*Plant not present*	no	*Plant not present*	Leafspot Interveinal (with fruiting bodies)
04/10/2014	4-1	N 24° 82' 469’ E 102° 78.197’		Kunming, Yunnan	By the side of a lake	Lepidopteran larvae	*Plant not present*	Rust (uredinia)	*Plant not present*
05/10/2014	5-1	N 30° 40 845’ E 104° 09199’		Chengdu, Sichuan	Just off motorway, waste land	No	*Plant not present*	Leafspot interveinal	*Plant not present*
	5-2	N 30° 21' 303’ E 103° 52458’		Chengjia, Pujiang, Chengdu, Sichuan	Edge of tea plantation, mixed cropping, small holder.	Coleoptera (weevil)	*Plant not present*	Leafspot (tan)	*Plant not present*
	5-3	N 30° 21' 224’ E 103° 51355’		Chengjia, Pujiang, Chengdu, Sichuan	Steep bank next to irrigation channel	no	*Plant not present*	no	*Plant not present*

6. Molecular Studies

Leaf material from *R. ellipticus* and *R. niveus* was provided by Tracy Johnson from one population on Hawaii Island and was also collected from selected populations of the two *Rubus* species in their native range and deposited with the respective national repositories. Within the frame of this project as yet no comprehensive molecular studies have been undertaken to try and determine from where in the Asian range the genotypes of the two *Rubus* species invasive on Hawaii originate. However, some initial work has been carried out in this respect for *R. niveus* as part of the scoping study conducted for Galapagos by including the Hawaiian samples provided. DNA was extracted from Hawaiian leaf samples and compared, using three sets of primer pairs (*rbcL, trnL* and *LEAFY),* with that from samples collected in China. Permission for the export of the leaf samples from India has been delayed due to changes in Indian Council for Agricultural Research (ICAR) personnel, thus leaf samples taken from herbarium specimens collected during past surveys and held at CABI were used for this study. (Indian leaf samples collected during the 2014/2015 surveys will be analysed in India by a molecular laboratory and the results combined with those obtained in the UK in order to provide supplementary sequences for a more robust comparison, than that provided by the few herbarium samples currently included.) Also included in the analysis were DNA samples from plants in the UK and other species of *Rubus.* To date, findings from the study, although not conclusive, indicate that India rather than China might be the source of the *R. niveus* introduction into Hawaii. This will need to be verified by a more comprehensive analysis. As yet no equivalent molecular work has been undertaken for *R. ellipticus*.

7. Natural enemies

Arthropod specimens collected during the field surveys in India have been officially identified, many to species level, by collaborating Indian taxonomists (as credited). A number of the larval collections failed to pupate or hatch under laboratory conditions, thus their species identity and potential as biocontrol agent remains currently unknown. No formal identification reports had been received for the fungal specimens deposited with NBAIM/Mau at the time of writing this final 2nd phase project report, thus any identifications given are provisional and based on field observations, as well as on preliminary microscope studies conducted at NBAIR/Bangalore. As yet no agents have been exported from India for further evaluation. To date no official identification has been undertaken for arthropod and fungal specimens collected during the field survey in China. However, preliminary assessments were carried out using the CABI facilities in Beijing.

7.1 India

The following section lists the arthropod and fungal natural enemies collected from *R. ellipticus* and *R. niveus* in the surveyed Indian-native range in 2014 and 2015 and gives an initial assessment of their potential as biocontrol agents based on field observations and published literature.

Arthropods

Colleoptera

- *Altica himensis* Shukla/Chrysomelidae (ident. by Dr D. Prathapan) – collected ex *R. niveus;* this flea beetle is an important herbivore of *Rumex hastatus* and is confined to this host during the dry season; during the wet season the beetle expands its host range to a number of other herbaceous species (Shah & Jyala, 1998); no potential as BCA
- *Aphthona piceipes* Scherer/Chrysomelidae (ident. by Dr D. Prathapan) – collected from *R. niveus; Aphthona* species tend to be host specific; the genus includes *Euphorbia* specialists, used as biocontrol agents for invasive Euphorbia species in North America (Winston *et al.* 2014), but also pests; food plants of genus include: *Acacia, Acer, Asparagus, Carex, Euphorbia, Geranium, Helianthemum, Iris, Linum, Lythrum, Populus, Rosa, Rubus,*

Symphytum, Ulmus (Medvedev & Roginskaya, 1988); possibly potential as BCA (Fig. 4a)

- *Aphthona simlaensis* Konstantinov & Lingafelter/Chrysomelidae (ident. by Dr D. Prathapan) – collected from *R. ellipticus;* see comments for *Aphthona piceipes;* possibly potential as BCA (Fig. 5a)
- *Apoderus* sp./Attelabidae (ident. by Dr. V.V. Ramamurthy) – collected from *R. ellipticus*; *Apoderus minimus* and *A. nigroapicatus* recorded from *R. ellipticus* in China (Wu *et al*., 2013); possibly potential as BCA (Fig. 4b)
- *Coccinella septempunctata* Linnaeus/Coccinellidae (ident. by Dr. J. Poorani) – collected from *R. niveus*; predator; no potential as BCA
- *Colasposoma semicostatum* Jacoby/Chrysomelidae (ident. by A. Moseyko) – collected from *R. ellipticus*; recorded as a pest of the genera *Shorea* (Dipterocarpaceae) and *Terminalia* (Combretaceae); not a priority species for evaluation
- *Coraebus coerulens* Kerremans/Buprestidae (ident. by Dr. V.V. Ramamurthy) - collected from *R. niveus* and *R. ellipticus* (see also Tanner 2013, 1st phase report). *Coraebus quadriundulatus* Motschulsky has been recorded on *R. ellipticus* in China feeding on leaves of the plant (Wu *et al*., 2013, see also Table 4). Species within the genus develop mainly within woody species or the stems of plant species. No information available on the host range of *C. coerulens*, but species within the genus are recorded from a number of plant species (Soria *et al*., 1992 a,b; Iglesias *et al*., 1992); possibly potential as BCA (Fig. 4c)
- *Cryptocephalus dodecaspilus* Suffrian (possibly junior synonym of *C. sannio* Redtenba)/Chrysomelidae (ident. by Dr D. Prathapan) - usually specialists, seldom common, recorded on roses as minor pest (Fletcher 1917); not a priority for evaluation (not listed in Table 1)
- *Harmonia eucharis* Mulsant/Coccinellidae (ident. by Dr. J. Poorani) – predator; no potential as BCA
- *Myllocerus* cf *rufescens* Ramamurthy & Ghai/Curculinoidae (ident. by Dr. V.V. Ramamurthy) – collected from *R. ellipticus*; biology of species not known, genus includes pests and polyphagous species, broad-nosed weevils not well known as specialists; not a priority species for evaluation
- *Oomorphoides* sp./Chrysomelidae (ident. by Dr D. Prathapan) – collected from *R. ellipticus*; genus seems to specialise on Araliaceae, possibly also found feeding on *R. ellipticus* in Sikkim (field observation); possibly potential as BCA (Fig. 4d)

- *Pentamesa haroldi* Baly/Chrysomelidae (ident. by Dr D. Prathapan) – collected ex *R. ellipticus;* possibly oligophagous/monophagous rather than polyphagous, but unknown; not a priority species for evaluation
- *Sibinia* sp./Curculinoidae (ident. by Dr. V.V. Ramamurthy) – collected from *R. niveus*, subgenus *Sibinia* is mostly from the Old World, 100+ species, hosts in the plant families Caryophyllaceae, Plumbaginaceae, Santalaceae, Thymelaceae, Boraginaceae, Portulacaceae, fruit and flower bud feeders (Clark, 1978); possibly potential as BCA
- *Trichotheca hirta* Baly/Chrysomelidae (ident. by A. Moseyko) – collected from *R. ellipticus*; no information, seems to be a conspicuous widespread species; not a priority species for evaluation
- Two un-identified Galerucinae species

Hemiptera

- *Gargara* sp./Membracidae (ident. by C.A. Viraktamath) - possibly specific but not used as CBC before; not a priority species for evaluation
- *Kolla paulula* (Walker)/Cicadellidae (ident. by C.A. Viraktamath) – vector of diseases; no potential as BCA
- *Matsumuraja capitophoroides* Hille Ris Lambers & *Matsumuraja rubifoliae* Takahashi/Aphididae – recorded from *Rubus* spp., species in the genus *Matsumuraja* attack *Rubus* species; the two named species have alternate hosts (Banerjee *et al.,* 1991); no potential as BCA
- *Paracritheus* sp./Pentatomidae (ident. by C.A. Viraktamath) – not a priority species for evaluation
- One unidentified species/ Erythroneurini/Typhlocybinae/Cicadellidae (not listed in Table 1)
- one unidentified species/Meenoplidae (not listed in table 1)

Lepidoptera

- cf *Acleris enitescens* Meyrick/Tortricidae – collected from *R. ellipticus,* the larvae are known to feed on Rubus species; possibly potential as BCA
- *Gonitis mesogona* Walker/Erebidae – collected from *R. niveus*; known from other plant species; not a priority species for evaluation
- cf *Pandemis dumetana* Treitschke/Tortricidae – collected from *R. niveus*; highly polyphagus; no biocontrol potential

- *Somena scintillans* Walker/Erebidae – highly polyphagus; no biocontrol potential (not listed in Table 1)
- *Soritia pulchella* Kollar/Zygaenideae – polyphagus; no biocontrol potential (not listed in Table 1)
- *Thyatira batis* L./Drepanidae - polyphagus; no biocontrol potential
- *Trabala vishnou* Lefèbvre/Lasiocampidae - highly polyphagous; no biocontrol potential
- One unidentified species/Tortricidea

Detailed information about all identified Lepidopteran species, crediting the taxonomists responsible, is given in the identification report below (Table 3).

Table 3: Lepidopteran identification report for Indian field collected specimens

S. No	Species	Site No.	Image	Taxonomist views	Comments
1.	? *Acleris enitescens* (Meyrick) Tortricidae	R14-97		Tentative identity by Dr Ankita Gupta Dr. Roger Kendrick views: Dissection needed for species confirmation	*Acleris* spp. tend to be polyphagous, although *A. enitescens* only recorded from Rubus (Robinson et al. 2001).
2.	*Gonitis mesogona* Walker Erebidae	R14-71		Identified by Dr Ankita Gupta and identity confirmed by Dr. Roger Kendrick	http://www.mothsofborneo.com/part-15-16/scoliopterygini/scoliopterygini_7_3.php The host plant is usually *Rubus* (Rosaceae), but the larva has also been recorded from *Rosa* in the same family and from *Lantana* in the Verbenaceae (Robinson *et al.*, 2001).
3.	? *Pandemis dumetana* Treitschke Tortricidae	R14-68		Tentative identity by Dr Ankita Gupta Dr. Roger Kendrick views: Dissection needed for species confirmation	*Pandemis dumetana* is highly polyphagous including Rubus (Robinson et al. 2001).
4.	*Somena scintillans* (Walker) Erebidae	CABI coll.35		Identified by Dr Ankita Gupta and identity confirmed by Dr. Roger Kendrick	Highly polyphagous (Robinson et al. 2001).
5.	*Soritia pulchella* (Kollar) Zygaenidae	R14-80		Identified by Dr Ankita Gupta and identity confirmed by Dr. Roger Kendrick	Polyphagous, including Rubus (Robinson et al. 2001).

S. No	Species	Site No.	Image	Taxonomist views	Comments
6.	*Thyatira batis* (L.) Drepanidae	R14-39		Identified by Dr Ankita Gupta and identity confirmed by Dr. Roger Kendrick	Several Rubus spp. (Robinson et al. 2001). NZ investigated and rejected for Rubus biocontrol.
7.	*Trabala vishnou* (Lefèbvre)	HP Shimla		Identified by Dr Ankita Gupta and identity confirmed by Dr. Roger Kendrick	Highly polyphagous (Robinson et al. 2001).
8.	Unidentified	R 14-1		Dr. Roger Kendrick views: Erebidae. The labial palps suggest Hypeninae or Herminiinae. Perhaps a *Bertula* sp. (Herminiinae), though not at all confident about this. Photo of the moth when it was alive needed!	Only Bertula food plant record Poaceae (Robinson et al. 2001).
9.	Unidentified	R14-30		Dr. Roger Kendrick views: Dissection required. Looks close to the genus *Nosophora* (Crambidae, Spilomelinae), which is desperately in need of review.	Only one food plant record for Nosophora, viz. Theaceae (Robinson et al. 2001).
10.	Unidentified	R14-91		Dr. Roger Kendrick views: Dissection advisable. Is a Nolini (Nolidae, Nolinae); none too sure about the genus (never mind the species) - based on the plates in Moths of Thailand (vol 3, part 2; Kononenko & Pinratana, 2013), could be in *Manoba*, *Meganola*, or *Nola* (or one of the smaller genera!).	(Robinson et al. 2001): Manola no records; Meganola several families; Nola several families; N. internella recorded from Rubus shoots (and from other plant families).

Fungal Pathogens

- Leafspot 1 (tan) – collected regularly from *R. ellipticus* and *R. niveus;* the pathogen was commonly present causing substantial damage at some individual sites; tentatively identified as either *Pseudocercospora* sp. or *Pseudocercosporella* sp. (asexual stage of *Mycosphaerella* sp., Mycosphaerellaceae, Ascomycota); it is currently unknown whether the respective pathogens collected from *R. ellipticus* and *R. niveus* are identical; Wu *et al.* (2013) listed *Pseudocercosporella arcuata* S.K. Sing, P.N. Singh & Bhalla and *Pseudocercospora heteromalla* (Syd.) Deighton as pathogens of *R. ellipticus* in China, the latter with a prospective narrow host range; Wu et *al.* (2013) and Guo (1997a) list *Mycosphaerella confusa* F.A. Wolf (asexual stage *Pseudocercospora rubi* (Sacc.) Deighton) as associated with *Rubus* species, including *R. ellipticus* and *R. niveus,* in Asia but having a broad host range; Ellison and Barreto (2004) list a *Pseudocercospora* sp. associated with *R. niveus* in China; fungal pathogens representing the conidial, asexual stages of the *Mycosphaerella* genus have been previously used as BCA, i.e. *C. piaropi* against invasive water hyacinth (*Eichhornia crassipes*) in South Africa and *Septoria passiflorae* against invasive banana poka (*Passiflora tripartite*) in Hawaii, their impact has been variable (Winston *et al*., 2014); possibly potential as BCA (Fig. 4.e)
- Leafspot 2 (red) – collected regularly from *R. ellipticus* and *R. niveus;* tentatively placed in the genus *Phomopsis* (asexual stage of *Diaporthe*, Diaporthaceae, Ascomycota) or *Septoria* (asexual stage of *Mycosphaerella/Sphaerulina*., Mycosphaerellaceae, Ascomycota); Wu *et al.* (2013) list *Septoria darrowii* Zeller and *Mycosphaerella rubi* Roark (asexual stage *Septoria rubi S. rubi* Berk. & M.A. Curtis) as pathogens of *Rubus* species in China; Guo (1997b) lists *Sphaerulina westendorpii* (Westend.) Verkley, Quaedvl. & Crous (asexual stage *Septoria rubi* Westend*)* as a ubiquitous pathogen associated with species in the genus *Rubus*; *S. westendorpii* and *M. rubi* are considered teleomorph synonyms *(*www.mycobank.org); low potential as BCA
- Other leafspot (?) – collected from *R. ellipticus* and *R. niveus*; fungal leafspots which could not easily be assigned to either leafspot 1 or 2 based on macroscopic symptoms observed in the field and could represent an additional species; will need tentative identification

- Rust 1 (uredinia) – collected regularly from *R. ellipticus* and *R. niveus;* tentatively identified as *Phragmidium* sp./ Phragmidiaceae, Basidiomycota; it is currently unknown whether the respective rust pathogens collected from *R. ellipticus* and *R. niveus* represent the same species; a range of *Phragmidium* species have been recorded as associated with *R. niveus* in its native range, some of these may upon closer assessment prove to be identical, comparatively fewer *Phragmidium* species have been reported from *R. ellipticus* (Farr and Rossman, 2016; Wu *et al*., 2013); many of these rusts have also been recorded from other *Rubus* species, however, host specific *formae speciales* adapted to individual host species might exists within individual rust species; species belonging to the genus *Phragmidium* are autoecious, thus complete the whole life cycle on host species; historically rust fungi have provided good control of a number of other *Rubus* species (Winston *et al.,* 2014); possibly potential as BCA (Fig. 4f)
- Rust 2 (telia, possibly microcyclic) – collected only from *R. niveus;* tentatively *Phragmidium* sp./ Phragmidiaceae, Basidiomycota; the rust genus *Phragmidium* does accommodate microcyclic species, which are characterized by lacking two spore stages (aecial and uredinial) from their life cycle, possibly potential as BCA (Fig. 5b)

Previously, the rust *Hamaspora* sp,/ Phragmidiaceae, Basidiomycota. had also been informaly identified from *R. niveus* material collected in the Nilgiris by the pathologist Dr. Sreeramkumar (NBAIR).

7.2 China

Table 4 gives a summary of the arthropod and fungal natural enemies collected from *R. ellipticus* and *R. niveus* in the surveyed Chinese-native range in 2014, as well as initial assessment of their potential as biocontrol agents based on field observations and published literature. An identification of the collected lepidopteran larva could not be obtained as they could not be reared through to the adult stage.

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Table 4: Assessment of arthropod and fungal pathogen natural enemies collected on *Rubus ellipticus* and *Rubus niveus* in China, 2014

Classification	**Collected from**	**Comments**	**Biocontrol Potential**
Arthropods			
Coraebus cf *quadriundulatus* Motschulsky C: Insecta O: Coleoptera F: Buprestidae Jewel beetle	*R. niveus*	One adult specimen and 1 larvae of a *Coraebus* species were collected. Wu *et al.* (2013) reported *C. quadriundulatus* from *Rubus ellipticus* in China and it is possible that the species collected here belongs to the same taxon. Another Asian species recorded from *Rubus* spp. is *C. niponicus* Lewis,1894 (Japanese Buprestidae 2015). It is feasible that *C. quadriundulatus* develops inside the roots of *Rubus* similar to the European *C. rubi*. Adults will feed on leaves but may not be as host specific as the larvae as observations from other plant genera suggest (Natural-Japan.net 2015). *C. rubi* has previously been tested as a control agent for *Rubus fruticosus* in NZ but turned out not to be host specific enough (Julien *et al.,* 2012). A narrower specialisation of *C. quadriundulatus* inside the genus *Rubus* is, however, possible.	medium
1 unidentified species C: Insecta O: Coleoptera F: Coccinellidae Ladybird beetle	*R. niveus*	One unidentified Coccinellid species was collected from *Rubus niveus*. In all likelihood this will be a predatory species feeding on other phytophagous insects such as aphids on *R. niveus* and is as such not a suitable candidate for biological control.	very low
cf *Dactylispa* sp. C: Insecta O: Coleoptera F: Chrysomelidae Leaf-miner	*R. niveus*	In contrast to several species of this family recorded from *R. ellipticus* in China (Wu *et al.,* 2013) only one species belonging to the Hispinae was found during this survey on *R. niveus*. The taxon belongs to *Dactylispa* or a closely related genus. *Dactylispa parbatya*, occurring in the Eastern Himalayas and China, is a leaf-miner of *Rubus* and has so far only been reported from this plant genus (Santiago-Blay, 2004). Other *Dactylispa* species recorded from *Rubus* are *D. insulicola*, *D. rubus* and *D. nemoralis* (from Java). On genus level *Dactylispa* is polyphagous with records from many plant families (Santiago-Blay, 2004). No information is available about the host specificity of these within the host genus *Rubus*.	low
1 unidentified species C: Insecta O: Coleoptera F: Attelabidae Leaf-rolling weevil	*R. niveus*	Several specimens of a leaf-rolling weevil belonging to the family Attelabidae, subfamily Attelabinae were collected. Wu *et al.* (2013) report four different Attelabinae feeding on *Rubus ellipticus* in China (*Apoderus minimus*, *A. nigroapicatus*, *Henicolabus hypomelas*, *Phymatapoderus latipennis*). *A. minimus* is supposedly an orange coloured species (Wu *et al*., 2013) and available pictures of *P. latipennis* differ significantly from the species recorded during this survey. It is however possible, that the specimens belong to one of the other two species. It seems worthwhile to follow up the collected taxon with regards to its suitability as a biological control agents for *R. niveus*. Leaf-rolling weevils often need a high degree of specialisation to adapt their leaf-rolling skills to their host plants.	high

Classification	Collected from	Comments	Biocontrol Potential
1 unidentified species C: Insecta O: Hemiptera F: Aphididae Aphid	*R. niveus*	At least one unidentified species of aphids was collected. This family Aphididae contains both polyphagous and monophagous species. A number of aphid species are known to feed on *Rubus* spp. and whilst the majority are polyphagous some seem to be host specific to a relatively high degree. For example, *Amphorophora rubi* feeds on the underside of leaves of blackberry (*Rubus fruticosus* agg.) and related *Rubus* species, but not on raspberry (*Rubus idaeus*) (Blackman & Eastop, 2015a). A species recorded to feed on the undersides of leaves of *Rubus* sp. in China is *Paraphorodon omeishanaensis* Tseng & Tao (Blackman & Eastop, 2015b). In addition, 14 east Asian species of the genus *Matsumuraja* Schumacher are associated with *Rubus* (Blackman & Eastop, 2015c).	low
1 unidentified species C: Insecta O: Hemiptera F: Coccoidea, cf Diaspididae / Scale insect	*R. niveus*	One species of scale insects was found in the stems of *R. niveus*. The taxon possibly belongs to the family Diaspididae and may be closely related or identical with *Aulacaspis rosae* Bouché, a widespread, cosmopolitan species feeding on Rosaceae.	very low
1 unidentified species C: Insecta O: Hemiptera F: Pentatomidae Pentatomid bug	*R. niveus*	Several specimens of a pentatomid bug were collected from *R. niveus*. The species resembles *Nezara viridula*, which is known to be very polyphagous. Assumed to be of African origin this species has now a cosmopolitan distribution.	low
cf *Lasioptera rubi* (Schrank) C: Insecta O: Diptera F: Cecidomyiidae	*R. niveus*	Numerous stem galls were collected from *R. niveus* containing larval stages of several unidentified fly species. The galls on *R. niveus* are consistent with galls formed in other *Rubus* spp. by *Lasioptera rubi* (Cecidomyiidae). In Europe this species is reported from *R. fruticosus, R. idaeus, R. loganobaccus, R. caesius, R. wahlbergii,* and *R. discolor* (Barnes, 1948). In Japan *L. rubi* has been recorded from *R. parvifolius, R. idaeus, R. phoenicolasius, R. trifidus,* and *R. crataegifolius* (Yukawa *et al*., 2014). *L. rubi* has previously been tested as a control agent against *R. fruticosus* in NZ but turned out not to be host specific enough (Julien *et al.,* 2012). *Lasioptera* is a species rich genus and the host range of this genus is spreading over a wide range of different plant families. *L. rubi* feeds on hyphae of a fungus lining the galls (ambrosia galls) and it is often accompanied by inquilines (in the case of this species other gall midges).	medium
1 unidentified species C: Insecta O: Diptera F: Muscidae House/stable fly	*R. niveus*	One unidentified species of house/stable flies has been observed on *R. niveus*. It is unlikely that its biology is linked to this species.	very low

Classification	Collected from	Comments	Biocontrol Potential
2 unidentified species C: Insecta O: Diptera F: Syrphidae	*R. niveus*	Adult specimens of two unidentified hoverfly species were collected from *R. niveus*. Larvae of Syrphidae are generally predatory and adults are flower visiting nectar and pollen feeders not suitable as biological control agents for weeds.	none
Larval stage of several unidentified fly species C: Insecta O: Diptera	*R. niveus*	Stem galls collected from *R. niveus* contained the remains of larval stages belonging to several unidentified fly species. Some galls contained living fly larvae and subsequently produced a number of as yet unidentified adult flies. These can belong to gall midges (Cecidomyiidae, see above) but possible also to saprophytic species or inclines belonging to other fly families.	medium
1 unidentified species C: Insecta O: Hymenoptera SF: Tenthredinoidea Sawfly	*R. niveus*	Larvae of one unidentified sawfly species were recorded to feed on *R. niveus* leaves. A number of sawfly species have been described to develop on Rubus, for example in Europe *Arge cyanocrocea* on *R. fruticosus* and *R. idaeus* but also on *Sanguisorba officinalis*. In South East Asia *Arge hasegawae* is reported to feed on *Rubus crataegifolius* (Shinohara *et al.,* 2011). In China one unidentified *Arge* sp. has been recorded from *R. ellipticus* (Wu *et al*., 2013). Larvae found during this survey possibly also belong to the family Argidae and even the genus *Arge*. However, superficially the specimens recorded resemble caterpillars of *Cibdela janthina* (Argidae), a species, which has been released on La Réunion for the control of *Rubus alceifolius* (Le Bourgeois *et al.,* 2011). Currently six species belonging to this genus are recorded from China but no information on their host range is available (Wei *et al.,* 2006). As sawflies often have a restricted host range and have been successfully used as biocontrol agents in the past, we recommend to assess the potential of the here recorded taxon for the control of *R. niveus* in more detail.	high
Fungal Pathogens			
Rust (uredinia) Tentatively identified as *Phragmidium* sp. P: Basidiomycota C: Urediniomycetes O: Pucciniales F: Phragmidiaceae	*R. niveus* / *R. ellipticus*	It is currently unknown whether the respective rust pathogens collected from *R. ellipticus* and *R. niveus* represent the same species; see comments made for *Phragmidium* sp. for field collections made in India	high

Classification	Collected from	Comments	Biocontrol Potential
Leafspot (tan) Tentatively identified as *Pseudocercospora* sp. or *Pseudocercosporella* sp. P: Ascomycota C: Dothideomycetes O: Capnodiales F: Mycosphaerellaceae	*R. niveus*	See comments made for *Pseudocercospora* sp./ *Pseudocercosporella* sp. for field collections made in India	high (but see comment under "Agent prioritization")
Reddish leafspot Tentatively identified as *Mycosphaerella* sp. P: Ascomycota C: Dothideomycetes O: Capnodiales F: Mycosphaerellaceae	*R. ellipticus*	The pathogen was tentatively placed in the genus *Mycosphaerella* based on spore morphology, it is possibly that this sexual spore stage is linked to asexual stage of *Septoria,* as mentioned for the red leafspot listed for the field collections made in India; Wu *et al*. (2013) list *Mycosphaerella confusa* F.A. Wolf as associated with *R. ellipticus* in China, but state that the pathogen has a broad host range; other *Mycosphaerella* species associated with the genus *Rubus*, but not specifically listed as associated with *R. ellipticus*, are *M. fragariae* (Tul.) Lindau (broad host range) and *M. rubi* Roark (narrow host range) (Wu *et al*., 2013); *Mycosphaerella* species can have detrimental impacts on crop plants, i.e. *M. fijiensis* and *M. musicola* (black and yellow sigatoka) affecting cultivated and wild banana and plantain (genus Musa); *Mycosphaerella polygoni-cuspidati* is being evaluated as a control agent for Japanese knotweed (*Fallopia japonica*) in the UK and Canada, as yet *Mycosphaerella s*pecies have not been used as biocontrol agents.	medium
Interveinal leafspot Tentatively identified as anamorph, (possibly *Diplodia* sp.), of *Botryosphaeria* sp. P: Ascomycota C: Dothideomycetes O: Botryosphaeriales F: Botryosphaeriacea	*R. niveus / R. ellipticus*	The pathogen was tentatively identified from diseased field material of *R. ellipticus*. It is currently unknown if the intraveinal leafspot collected from *R. niveus* is caused by the same species. Most *Diplodia/ Botryosphaeria* have a broad host range and species of the genus have not been used as BCAs.	low

Classification	Collected from	Comments	Biocontrol Potential
Interveinal leafspot Tentatively identified as *Colletotrichum* sp. (possibly two species), of *Botryosphaeria* sp. P: Ascomycota C: Sordariomycetes O: Incertae sedis F: Glomerellaceae		The pathogen was also tentatively identified from diseased field material of *R. ellipticus* showing interveinal lesions. Potentially two different species of Colletotrichum were associated with the lesions. It is currently unknown if *Colletotrichum* sp. is also present in the intraveinal leafspot lesions collected from *R. niveus. Colletotrichum* species have been used as biological control agents, mostly developed as a mycoherbicide based on host specific forms (*formae speciales*), i.e. northern jointvetch (*Aeschynomene indica*) (Collego™), round-leaved mallow (*Malva pusilla*) (BioMal ®), *Hakea sericea* (Hakatak®). Use as a classical agent include *C. clidemiae* against *Clidemia hirta* on Hawaii, *C. gloeosporioides* f.sp. *miconiae* against *Miconia calvescens* in French Polynesia and Hawaii. Impact has been variable (Winston *et al.*, 2014); since *Colletotrichum* sp. has not been recorded previously as a pathogen of *R. ellipticus*, it is likely that the one or two species listed here are primarily endophytes rather than primary pathogens.	low
Leafspot (other)	*R. niveus*	A fungal leafspot not easily assigned to either of the other leafspots mentioned above based on macroscopic symptoms observed in the field; could represent a different species	unknown

P = phylum; C = class; O = order; SF = superfamily, F = family

Figure 4: Prioritized natural enemies collected in India a) *Aphthona piceipes;* b) *Apoderus* sp.; c) *Coraebus coerulens*; d) *Oomorphoides* sp.; e) *Pseudocercospora* sp./*Pseudocercosporella* sp.; d) *Phragmidium* sp.(uredinia)

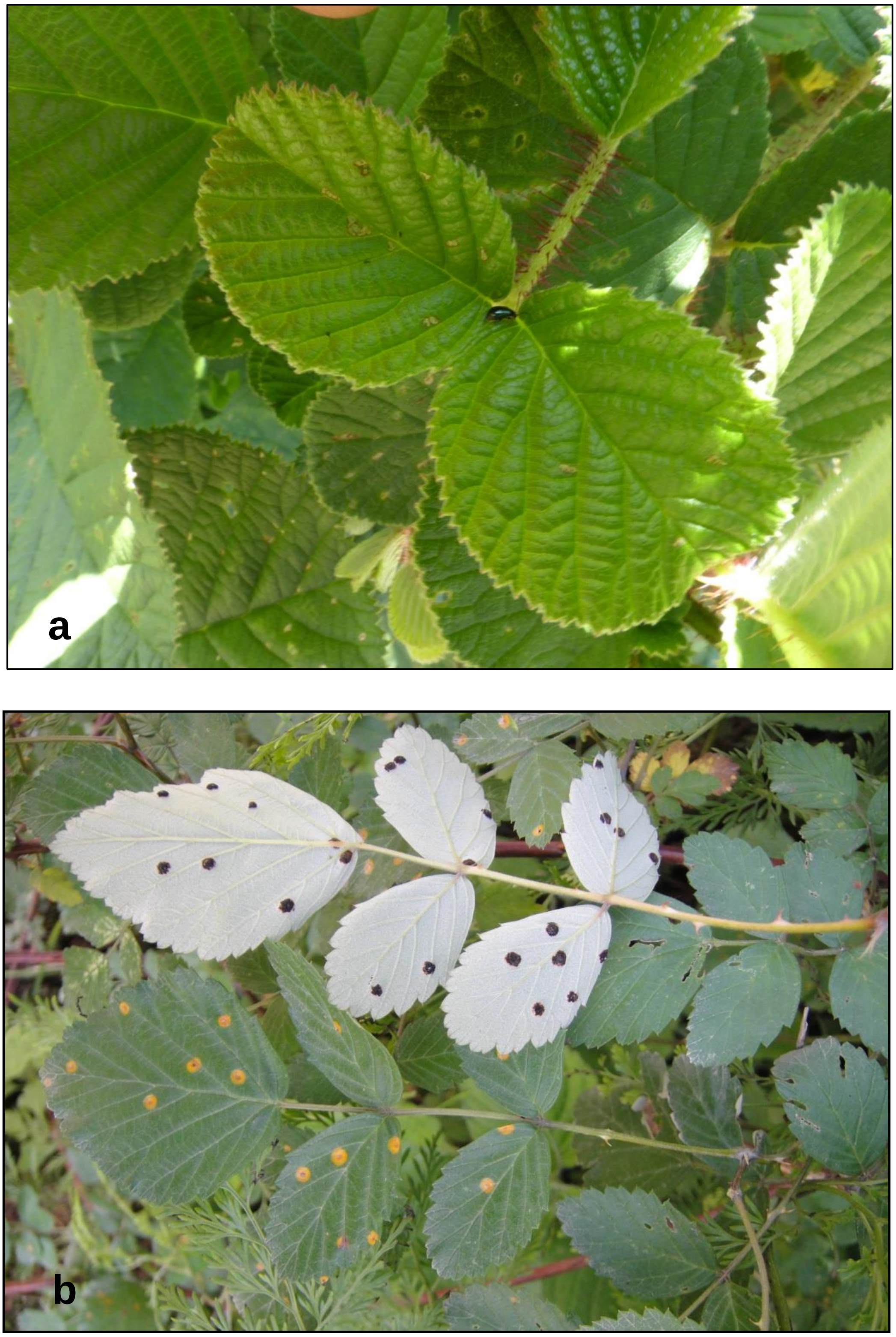

Figure 5: Prioritized natural enemies collected in India a) *Aphthona simlaensis ex R. ellipticus;* b) *Phragmidium* sp. (microcyclic?) ex *R. niveus*

7.3 Inoculation studies

Natural enemies have as yet to be exported from India or China for further evaluation under quarantine conditions in the UK. However, Hawaiian plants of *R. ellipitcus* were sent to China in order to assess their susceptibilty towards the uredinial stage of the rust (tentatively identified as *Phragmidium* sp.). Inoculations were carried out in the CABI facilities in Bejing by Huanhuan Wan, who showed that the Hawaiian genotype is susceptible to the rust with sporulation occurring between 14-18 days after inoculation. Cross inoculation using the rust ex *R. ellipticus* to infect *R. niveus* resulted predominantly in leaf chlorosis and only very limited sporulation on *R. niveus*. Conversely, inoculation of *R. ellipticus* with the rust ex *R. niveus* did not result in sporulation. This would indicate that if the rust ex *R. ellipticus* and *R. niveus* belong to the same species, *formae speciales* within this species could exist which are adapted to individual hosts within the *Rubus* genus.

7.4 Agent priorizitation

Based on literature research, field observations as well as consultation with biocontrol experts the following natural enemies have been prioritized for export and preliminary specificity assessments and evaluation as BCAs for *R. ellipticus* and *R. niveus.*

Arthropod species

Arthropods agents could be assessed for their biocontrol potential for either of the two invasive *Rubus* species.

From India

- *Aphthona piceipes/* Chrysomelidae (ex *R. niveus*)
- *Aphthona simlaensis/* Chrysomelidae (ex *R. ellipticus*)
- *Apoderus* sp./ Attelabidae (ex *R. ellipticus*)
- *Coraebus coerulens*/ Buprestidea (ex *R. ellipticus* and *R. niveus*)
- *Oomorphoides* sp./ Chrysomelidae (ex *R. ellipticus*)
- *Sibinia* sp./ Curculionidae (ex *R. niveus*)
- ? *Acleris enitescens*/ Tortricidae (ex *R. ellipticus*)

To date official export permits have been granted by the Indian authorities for *A. piceipes*, *C. coerulens*, *Oomorphoides* sp. and *Sibinia* sp.

From China

- *Coraebus* cf *quadriundulatus/* Buprestidea (ex *R. niveus*)
- Unidentified leaf-rolling beetle/Attelabidae (ex *R. niveus*)
- Unidentified sawfly/ Tenthredinoidea (ex *R. niveus*)
- Unidentified species/ Tortricidae (ex *R. niveus*)

Additional insects ex China previously identified for having potential as BCAs (Wu *et al*., 2013; see section 10, Supplementary Materials, Table 5).

- *Chlamisus setosus/* Chrysomelidae (ex *R. niveus* and *R. ellipticus*)
- *Chaetocnema* spp./ Chrysomelidae (ex *R. niveus* and *R. ellipticus*
- *Epiblema tetragonana/* Tortricidae (ex *R. niveus* and *R. ellipticus*
- *Involvulus* sp./ Curculionidae (ex *R. niveus* and *R. ellipticus)*

Export of agents from China can be facilitated through the CABI China office in Beijing.

Fungal pathogens

From India

- *Phragmidium* sp. (uredinial species) (ex *R. ellipticus* and *R. niveus*)
- *Phragmidium sp.* (microcyclic? species) (exclusively ex *R. niveus*)
- *Pseudocercospora/ Pseudocercosporella* sp. (ex *R. ellipticus* and *R. niveus*)

From China

- *Phragmidium* sp. (uredinial species) (ex *R. ellipticus* and *R. niveus*)
- *Pseudocercospora* sp./ *Pseudocercosporella* sp. (ex *R. ellipticus* and *R. niveus*)

Additional pathogens ex China previously identified for having potential as BCAs (Wu *et al*., 2013)

- *Hamaspora benguetensis* Syd. (ex *R. ellipticus)*
- *Hamaspora rubi-siboldii* (Kawagoe) Dietel (ex *R. ellipticus*)

Rusts species dominate the recorded fungal natural enemies from *R. niveus* and as part of the scoping study for biological control of *R. niveus* on Galapagos the species *Phragmidium himalense* J.Y. Zhuang and *Phragmidium mysorense (= Phragmotelium mysorense* Thirum. & Mundk.*)* have been identified as the primary candidates for evaluation as BCAs based on their reported narrow host ranges and damage caused to the host (see section 10, Supplementary Materials, Table 6). For *R. ellipticus* only two *Phragmidium* rusts have been recorded, *P. bulbosum* (Fr.) Schltdl. and *Phragmidium orientale* Syd. & P. Syd. (Farr and Rossman, 2016; Wu *et al*., 2013), however two *Hamasporium* species also have been highlighted previously as potential BCAs (Wu *et al*., 2013).
The damaging pathogen tentatively identified as *Pseudocercopsora* sp. or *Pseudocercosporella* sp. could have potential as BCA for *R. ellipticus* since the species *Pseudocercospora heteromalla* (Syd.) Deighton, recorded from *R. ellipticus* in India (Farr and Rossman, 2016), is reported to have a narrow host range (Wu *et al*., 2013). Conversely, the cercosporoid pathogen collected from *R. niveus* would not be suitable as a biocontrol agent should its identity be confirmed as *Pseudocercospora rubi* (Sacc.) Deighton, recorded from this host in Asia and known to have a wide host range. It will be crucial to obtain formal taxonomic identification of the pathogens deposited to prioritize their evaluation accordingly.

8. Recommendations for Future Research

Building on the results of the second project phase, as well as on research conducted by Wu *et al.* (2013) and as part of the scoping study undertaken for Galapagos further research into the potential of selected natural enemies as biological control agents for *R. ellipticus* and *R. niveus* on Hawaii is recommended.

Export permits for four prioritized agents from India have been granted and additional permits, including permits for fungal pathogens, should be pursued in order to commence host specificity evaluations. Export procedures of agents from China could be facilitated by the CABI China office in Beijing.

Further survey work at different times in the growing season should be undertaken in the native Himalayan region in collaboration with national institutions, primarily to re-collect prioritized insect and pathogen species for export, as well as to compile more comprehensive data on the natural enemy guild of the two *Rubus* species. Additional surveys would also facilitate the collection of leaf material from of a range of native *R. ellipticus* and *R. niveus* populations to be used for a molecular comparison with material of exotic populations on Hawaii to establish the origins of introductions. This will be important to achieve optimal match between biotypes of potential biocontrol agents and those of the invasive *Rubus* species.

Preliminary host-range testing of exported agents against closely related non-target species (i.e. *Rubus macraei* and *Rubus hawaiiensis*) should be conducted in the UK, together with studies focusing on agent life-cycle and biology. This will aid the selection of the most promising agent(s) for more comprehensive assessments.

A proposal spelling out work to be conducted based on these recommendations has been submitted to HISC for FY 2017.

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10. Supplementary Materials

The subsequent information concerning *R. niveus* was compiled by Carol Ellison, Kate Pollard and Norbert Maczey for the final report of the scoping study "Investigation of potential biological control agents in the native range of mora (*Rubus niveus*), for control of the species in the Galapagos Islands" funded by the Government of Ecuador.

Table 5. List of arthropod natural enemies with classical biological control potential recorded from *R. niveus* from the literature*

Species name and classification	Geographical distribution	Host range	Comments and biocontrol potential
Chlamisus setosus C: Insecta O: Coleoptera F:Chrysomelidae Warty beetle	China	Possibly restricted to the genus *Rubus.* Feeding of larvae assessed, fed on: *R. ellipticus, R. niveus, R. sumatranus. R. wallichianus, R. irenaeus R. lambertianus, R. tephrodes*	Congeners are known to be monophagous or oligophagous (Tan *et al.,* 1980, Reu and Del-Claro, 2005). In the field, adults and larvae fed on leaves, often producing feeding scars on the abaxial surfaces.
Chaetocnema spp. C: Insecta O: Coleoptera F: Chrysomelidae Flea beetle	China	Possibly restricted to the genus *Rubus.* Feeding of adults assessed, fed on: *R. ellipticus, R. niveus, R. wallichianus, R. irenaeu.* No feeding occurred on plants of other genera.	Two morphological forms of species in the genus *Chaetocnema* were collected. Adults consumed a great deal of leaf material. Species from this genus have been used in successful weed biocontrol (Winston *et al.,* 2014)
Epiblema tetragonana C: Insecta O: Lepidoptera F: Tortricidae Leaf-rolling moth	China (widely distributed) and Europe	Possibly restricted to the genus *Rubus*. In no-choice host tests, larvae showed a narrow host range, surviving only on species in the genus *Rubus.* Larvae fed on: *R. amabili, R. ellipticus, R. niveus, R. lambertianus, R. reflexus.* No feeding occurred on plants of other genera.	Larvae heavily damage twigs and appeared to suppress the growth of branches and buds, which could reduce the size and affect the architecture of the plants. This species has been recorded in both Asia and Europe. Species from this genus have been used in successful weed biocontrol (Winston *et al.,* 2014)
Involvulus sp. C: Insecta O: Coleoptera SF: Curculionidae weevil	China	Possibly restricted to the genus *Rubus*. Feeding of adults assessed, fed on: *R. ellipticus, R. niveus, R. wallichianus.* (Note: only three species tested in the genus *Rubus* and all were fed on; but no feeding occurred on plants of other genera.)	Colourful, shiny bronze weevil that feeds on *R. niveus* buds. The damage to buds causes them to wither, suggesting that it may effectively reduce plant stature. Preliminary tests showed a narrow host range based on adult feeding, and only three *Rubus* species were fed upon. Note: *Involvulus* is now separated into three separate genera – *Apoderus, Attelabus* and *Rhynchites*.

* Data from Wu, *et al.* (2013). C = Class; O = order; F = family; SF = superfamily

Table 6. List of fungal natural enemies with classical biological control potential recorded from *R. niveus* from the literature and data bases

Species name and classification	Geographical distribution	Host range	Comments	Key references and biological control potential
Hamaspora acutissima P: Basidiomycota C: Urediniomycetes O: Pucciniales F: Phragmidiaceae	Australia, Far East Asia (China, Indonesia, Japan, New Caledonia, Papua-New Guinea, Philippines, Taiwan)	Widespread in Asia, restricted to the genus *Rubus*; recorded from at least 19 species (*R. alceifolius, R. calycinoides, R. elmeri, R. formosensis, R. fraxinifolius, R. glomeratus, R. laciniatostipulatus, R. oluccanus, R. nantoensis, R. nesiotes, R. niveus, R. parkeri, R. pectinellus, R. pyrifolius, R. rolfei, R. rosaefolius, R. setchuenensis, R. swinhoei, R. tagallus.)*	Does not appear to be very damaging. Spermogonia and aecia unknown; therefore could be heteroecious (may have a secondary host). Has distinctive very long, threadlike teliospores	Monoson, 1969; Yun, 2016a. Low
Hamaspora longissima P: Basidiomycota C: Urediniomycetes O: Pucciniales F: Phragmidiaceae	Asia (China, India, Java, Taiwan) plus one report from Africa (Ethiopia)	Rust, widespread in Asia, restricted to the genus *Rubus*; recorded from at least 14 species (*R. australis, R. ellipticus, R. friesiorum, R. lambertianus, R. ludwigii, R. moluccanus, R. niveus, R. pinnatus, R. rigidus, R. steudneri, R. taiwanianus, R. transvaliensis, R.Volkensii, R. xanthoneurus.)*	Does not appear to be very damaging. Spermogonia and aecia unknown; therefore could be heteroecious (may have a secondary host). Has distinctive elongate teliospores.	Monoson, 1969; Yun, 2016b. Low
Phragmidium assamense P: Basidiomycota C: Urediniomycetes O: Pucciniales F: Phragmidiaceae	China (Yunnan), India (Assam)	Restricted to the genus *Rubus*; recorded from at least 7 species (Rubus coreanus, Rubus flosculosus, Rubus idaeopsis, Rubus lasiostylus, Rubus mesogaeus, Rubus niveus)	Synonym = *Phragmidium coreanum* Uredinia and telia only described	Cao *et al.,* 2000. Medium/high
Phragmidium barclayi P: Basidiomycota C: Urediniomycetes O: Pucciniales F: Phragmidiaceae	Asia (China: Nyalam, Yunnan; India Nepal, Pakistan)	Attacks *Rubus* species native to Asia. Recorded mainly from *Rubus* subgenus *idaeobatatus* (*R. austrotibetanus, R. hoffmeiterianus, R. niveus*), also *Rubus* subgenus *Rubus*, specifically R. koehleri.	Uredinia and telia recorded. Taxonomic entity unclear, possibly: *Phragmidium barclayi = Phragmidium rubi* sensu Barclay which may = *P. microsorium = P. bulbosum* (current name)	Kakishima *et al.,* 2002. Medium **Requires further investigation**
Phragmidium coreanum P: Basidiomycota C: Urediniomycetes O: Pucciniales F: Phragmidiaceae	China	Recorded from *R. coreanus, R. coreanus* var. *tomentosus, R. innominatus, R. niveus*	Synonym = *Phragmidium assamense*	Guo, 1989. Medium/high
Phragmidium griseum P: Basidiomycota C: Urediniomycetes O: Pucciniales F: Phragmidiaceae	Asia (China, Japan, Korea, Taiwan, USSR)	Restricted to the genus *Rubus*, subgenus *Idaeobatus;* recorded from at least 18 species (specifically *R. aculaetiflorus, R. conduplicatus, R. croceacanthus, R. crataegifolius, R. illecebrosus, R. micophyllus, R. mingetsensis, R. minusculus, R. morifolius, R. nesiotes, R. niveus, R. palmatus, R. rosifolius, R. rosifolius* var. *maximowiczii, R. rosaefolius var. tropicus, R. spectabilis, R. trianthus, R. wrightii*)	Uredinia and telia recorded.	Yun, 2016c; Wei, 1988. Medium/low

Species name and classification	Geographical distribution	Host range	Comments	Key references and biological control potential
Phragmidium himalense P: Basidiomycota C: Urediniomycetes O: Pucciniales F: Phragmidiaceae	Nujiang District, Yunnan Province, China; Tibet	Recorded only from *Rubus niveus*	Spermogonia, aecia and uredinia unknown. Telia pulvinate and compact. (Could be microcyclic species found in Kulu valley, India?)	Zhuang, 1986 High
Phragmidium incompletum P: Basidiomycota C: Urediniomycetes O: Pucciniales F: Phragmidiaceae	Himalaya, Nepal	Recorded from *Rubus niveus*, *R. paniculatus*	Taxonomic entity unclear, not included in Species Fungorum **Requires further investigation**	Singh & Palni, 2011. Possibly high, but taxonomic entity unclear
Phragmidium microsorum P: Basidiomycota C: Urediniomycetes O: Pucciniales F: Phragmidiaceae	India	Taxonomic entity unclear Species Fungorum states that *Phragmidium microsorum* is a synonym for *P. bulbosum* (current name) with wide host range (recorded from 28 *Rubus* species, not including *R. niveus*). Widespread Eurasian species.	Urediniospores and teliospores only described. **Requires further investigation**	Patil *et al.,* 2004; Sarbhoy & Agarwal, 1990. Probably low, but taxonomic entity unclear
Phragmidium mysorense (mysorensis) P: Basidiomycota C: Urediniomycetes O: Pucciniales F: Phragmidiaceae	Pakistan	*Rubus niveus* is only recorded host	Aecia found in Pakistan (uredinia and telia not found) Taxonomic entity not included in Species Fungorum, not even as a synonym. Assumption *Phragmidium mysorense* = *Phragmotelium mysorense*	Muhammad Asim Sultan *et al.,* 2006. Probably high, but taxonomic entity unclear **Requires further investigation**
Phragmidium nambuanum P: Basidiomycota C: Urediniomycetes O: Pucciniales F: Phragmidiaceae	China, Japan, Taiwan	Recorded from at least 8 *Rubus species: Rubus* subgenus *Idaeobatus* (specifically *R. crataegifolius*, *R. innominatus*, *R. kinashii*, *R. mesogaeus*, *R. phoenicolasius*, *R. pirifolius*, *R. simple)* and *Rubus* subgenus *Cylatis* (specifically *R. xanthocarpus*).	Uredinia and telia described	Wei, 1988; Yun, 2016d. Medium
Phragmidium rubi-thunbergii P: Basidiomycota C: Urediniomycetes O: Pucciniales F: Phragmidiaceae	China Himalaya, Japan	Recorded from *Rubus* subgenus *Idaeobatus* (5 species), specifically *R. hirsutus*, *R. idaeus*, *R. nishimuranus*, *R. niveus*, *R. parvifolius*, *R. pileatus*	Probably = *Phragmotelium rubi-thunbergii* (current name) Uredinia and telia described	Yun, 2016e. Medium
Phragmidium shensianum P: Basidiomycota C: Urediniomycetes O: Pucciniales F: Phragmidiaceae	China	Recorded from 5 species of *Rubus* (*R. idaeus, R. niveus, R. parvifolius, R. rosaefolius and R. triphyllus*)	Uredinia and telia described	Zhuang, 2005. High/medium

Species name and classification	Geographical distribution	Host range	Comments	Key references and biological control potential
Phragmidium shogranense P: Basidiomycota C: Urediniomycetes O: Pucciniales F: Phragmidiaceae	Pakistan (Kaghan valley, Shogran; Azad, Kashmir)	Recorded from at least 4 species of *Rubus* (*R. pungens*, *R. fruticosus, R. niveus,* and *R. penduculosus)*	Telia described (spermogonia, aecia and uredinia not found). Telia scattered not in typical pulvinate structure of microcyclic rusts.	Afshan *et al.,* 2011. High/medium
Phragmidium tibeticum P: Basidiomycota C: Urediniomycetes O: Pucciniales F: Phragmidiaceae	China, Tibet	Recorded from 2 Rubus species (*R. cockburnianus* and *R. niveus*)		Zhuang , 2012. High/medium
Phragmotelium mysorense P: Basidiomycota C: Urediniomycetes O: Pucciniales F: Phragmidiaceae	India (Nandi Hills, Mysore State)	Recorded from 2 *Rubus* species: *R. niveus* and *R. rugosus* (unresolved name) = *R. formosensis* (accepted name)	? = *Phragmidium mysorense* All spore stages recorded from *Rubus niveus* **Requires further investigation**	Ragunathan & Ramakrishnan, 1973; Proceedings of the Indian Academy of Sciences, 1942. Probably high, but taxonomic entity unclear
Sphaerulina westendorpii (anamorph *Septoria rubi*) P: Ascomycota C: Dothideomycetes O: Capnodiales F: Mycosphaerellaceae	Ubiquitous	Pathogen recorded from many countries across the globe; from at least 46 species of *Rubus*.	**Requires further investigation**	Guo, 1997a. Probably low, but taxonomic entity unclear
Mycosphaerella confusa (anamorph *Pseudocercospora rubi*) P: Ascomycota C: Dothideomycetes O: Capnodiales F: Mycosphaerellaceae	Largely Asia	Largely recorded from *Rubus* species from Asia (28)	**Requires further investigation**	Guo, 1997b. Probably low but taxonomic entity unclear
Meliola rubiella var. *indica* P: Ascomycota C: Sordariomycetes O: Meliolales F: Meliolaceae	India (Tamil Nadu)	?Recorded only from *Rubus niveus*	Potential host specific, but does not appear particularly damaging to its host **Requires further investigation**	Hosagoudar, 2006. Unknown

P: Phylum; C = Class; O = order; F = family

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