Application to introduce *Puccinia xanthii* for biological control of *Xanthium pungens* (cockleburr) in the Cook Islands

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On behalf of the Cook Islands Ministry of Agriculture:
Ara Tapu,
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Contents

1. Summary

The Cook Islands Ministry of Agriculture seeks approval for the release of the plant pathogen *Puccinia xanthii Schw.* (Pucciniales: Pucciniaceae) into Rarotonga for biological control (biocontrol) of the introduced plant cockleburr *Xanthium pungens* Wallr. (syn. *Xanthium strumarium*; *Xanthium occidentale* Bertol.) (Asterales: Asteraceae).

Cockleburr is an annual herbaceous plant that can grow up to 2.5 m tall. It is native to North America and now has a cosmopolitan distribution. Its origin in the Cook Islands is uncertain, but it is thought likely to have been accidentally introduced from Australia (Wilder, 1931), where it is also an introduced weed. It was introduced into Australia as a contaminant of cotton seed and it is often spread by burrs, which adhere to animal fur (Parsons and Cuthbertson, 1992).

Cockleburr is a major weed of row crops such as maize, groundnuts, cotton and soya beans. It can also invade pastures and grazing lands causing reductions in forage production and its seedlings contain the poison, xanthostrumarin which is toxic to most domestic animals, particularly pigs (http://cookislands.bishopmuseum.org/species.asp?id=6790). It has caused death in humans: it was responsible for at least 19 deaths and 76 illnesses in Sylhet District, Bangladesh, 2007 when people ate cockleburr plants, because they were starving during a monsoon flood and no other plants were available (Gurley et al., 2010).

Cockleburr is wind pollinated and produces copious pollen, which is allergenic, causing hay fever and it can also cause contact dermatitis in humans and farm animals (Parsons and Cuthbertson, 1992). In the Cook Islands it is a common weed on Rarotonga and is also present on Mauke (http://cookislands.bishopmuseum.org/species.asp?id=6790). It is possible that there will be future requests to release *P. xanthii* on Mauke.

Herbicides are difficult to apply without risking non-target impacts on the crops that *X. pungens* infests and negative impacts due to herbicide run-off. Mechanical control (hand-pulling and/or cultivating) can be done, but is time consuming and plants can resprout if the shoots are not severed from the roots.

Biocontrol is preferable over current control methods because once established, *P. xanthii* will persist, offering the potential for permanent weed suppression by reducing *X. pungens* abundance and, therefore, the negative impacts of this weed. This could greatly reduce the need for repeated use of chemical or mechanical weed control of *X. pungens*. In addition, *P. xanthii* has the potential to disperse and control *X. pungens* in wasteland and fallow areas where it is currently not being controlled, reducing the opportunities for this weed to re-infest areas from uncontrolled infestations.

Evidence is provided that *P. xanthii* is a highly host-specific plant pathogen is likely to reduce the harmful impacts of *X. pungens* in the Cook Islands and poses no threat to other plant species growing in the Cook Islands. Reductions in *X. pungens* will, therefore, benefit other plant species (native plants, crops or other weed species). The risk of a host-shift resulting in non-target attack on native species, or plants of economic or cultural importance in the Cook Islands is vanishingly small.

Puccinia xanthii is not present on islands in the Pacific region, although the introduction of *P. xanthii* into Fiji and Papua New Guinea, where *X. pungens* is also a serious weed, has been proposed (Julien et al., 2007). As well as Fiji and Papua New Guinea, *X. pungens* is also present on many other Pacific islands, including French Polynesia, Hawaii, New Caledonia and Tonga, where biological control using *P. xanthii* is likely to be feasible (Dodd and Hayes, 2009; Paynter, 2010).

As *X. pungens* is largely a weed of agricultural land, the most likely species to benefit from a reduction in *X. pungens* are crops.

This EIA has been prepared in accordance with Section 36 of the Cook Islands Environment Act 2003 (Box1, below) and section 68 of the Cook Islands Biosecurity Act 2008 (Box 2, below).

BOX 1. SECTION 36 OF THE COOK ISLANDS ENVIRONMENT ACT 2003
Environmental Impact Assessment – (1) No person shall undertake any activity which causes or is likely to cause significant environmental impacts except in accordance with a project permit issued under this section. (2) A person who proposes to undertake an activity of the kind referred to in subsection (1) shall apply to the permitting authority for a project permit in respect of the activity in accordance with the procedures (if any) prescribed by regulations. (3) Every application for a project permit shall be submitted to the Service and shall include an environmental impact assessment, setting out details of - (a) the impact of the project upon the environment and in particular - (i) the adverse effects that the project will have on the environment; and (ii) a justification for the use or commitment of depletable or non-renewable resources (if any) to the project; and (iii) a reconciliation of short-term uses and long-term productivity of the affected resources; and (b) the proposed action to mitigate adverse environmental effects and the proposed plan to monitor environmental impacts arising out of the project; and (c) the alternatives to the proposed project. (4) Every application for a project permit shall be accompanied by an application fee prescribed by regulations. (5) The Service shall undertake public consultation for the issuance of the project permit and in so doing – (a) publish details of the project in such a manner that these become accessible to the affected public; (b) make available copies of the environmental impact assessment report prepared by the project developer for review by the public; and (c) receive comments within 30 days from the date of public notice from the general public and other interested parties; (6) The Service shall request comments from any Government department or agency, or person affected by or having expertise relevant to the proposed

project or its environmental impact.

(7) After the permitting authority has reviewed and assessed the application and all relevant information including the environment impact assessment, it shall, subject to guidelines (if any) prescribed by regulations-

(a) issue a permit for the proposed project specifying the terms and conditions subject to which the permit is issued; or

(b) request the applicant to submit modifications regarding the proposed project; or

(c) where there are reasonable grounds to do so (taking particular account of the purpose of this Act), refuse to issue a permit for the proposed project and state the reasons for such refusal.

(8) The Service shall immediately convey to the applicant the decision of the permitting authority.

(9) Within 14 days of receiving notice of a refusal under subsection (7)(c) the applicant may by letter to the Minister, request that the Minister consider the permitting authority's decision. The Minister shall review the permitting authority's decision and all information relevant thereto and shall notify the applicant and the permitting authority in writing of the Minister's decision to either -

(a) uphold the permitting authority's decision to refuse a permit for the proposed project; or

(b) direct the Service to request that the applicant submit specified modifications to the Service regarding the proposed project for reconsideration by the permitting authority.

(10) If the Minister is required to make a decision under subsection (9) in any case where the Minister is the applicant for the permit, or is otherwise directly or indirectly interested in the permit application otherwise than as the reviewing authority, the Minister shall –

(a) with the concurrence of the permitting authority concerned, convene an independent panel to review the permitting authority's decision and submit a recommendation to the Minister; and

(b) follow that panel's recommendation in making the decision under subsection (9); and

(c) make those recommendations public.

(11) Every person commits an offence who, without reasonable excuse or lawful justification, fails or refuses to comply with subsection (1), and shall upon conviction be liable -

(a) in the case of a body corporate, to a fine not exceeding $100,000;

(b) in any other case, to a fine not exceeding $50,000.

(12) In addition to any penalty imposed under subsection (11), the Court may order that the person convicted –

(a) under the supervision and to the satisfaction of a person appointed by the Court, clear up and remove the damage caused to the environment as a consequence of the offence within such period and upon such conditions as may be specified in the order;

(b) pay such amount as the Court may assess in respect of the expenses and costs that have been or are likely to be incurred-

(i) in restoring the environment to its former state (its state immediately before the offence was committed); or

(ii) in removing or cleaning up or dispersing any oil or noxious liquid, or other harmful substance to which the offence relates.

(13) For the purposes of subsection (1), any designation, or issue or re-issue of approval of any land (whether by a Minister or any other public officer or authority, and whether under this or any other Act) for the disposal of any kind of waste is deemed to be an activity that is likely to cause significant environmental impacts.

BOX 2. SECTION 68 OF THE COOK ISLANDS BIOSECURITY ACT

68. Beneficial organisms and biocontrol agents –

(1) The Secretary[1] may in writing approve the release of beneficial organisms or biocontrol agents that he considers necessary or appropriate for the control or eradication of a particular pest or disease in the Cook islands.

(2) An approval under subsection (1) shall identify –

(a) the organism or agent;

(b) the pest or disease which it is intended to control;

(c) the area where it may be released;

(d) the period during which it may be released;

(e) the person or persons who may release it; and

(f) any conditions subject to which the approval is granted.

(3) No liability attaches to the Secretary, Director [2] or any public officer in respect of the release of organisms or biocontrol agents in accordance with this section, except on proof of negligence or malice.

(4) The Director shall keep a biosecurity register of –

(a) the names of any beneficial organisms or biological agents released under this section; and

(b) the place of and extent of release of such organisms and agents.

(5) In this section, "beneficial organism" and "biocontrol agent" mean a natural enemy, antagonist or competitor of a pest or disease, and any other self-replicating biotic entity used for pest and disease control.

[1] Secretary to the Ministry of Agriculture

[2] Director of Biosecurity

2. Background and aims of the proposal

Xanthium pungens is an annual herb native to North America. It has been introduced into many countries and territories in Europe, Africa, Asia and the Pacific region, where it has become a major invasive weed (http://www.cabi.org/isc/datasheet/56864).

Xanthium pungens is a major weed of row crops and has an economic impact in pastures, where cattle, sheep and pigs may be poisoned by eating young plants.

Xanthium pungens produces large amounts of highly antigenic pollen and the glandular hairs on the leaves and stem secrete a substance which causes contact dermititis in allergic individuals (http://www.cabi.org/isc/datasheet/56864).

The seeds are formed in burrs, which can be a problem when they become attached to the fur of livestock at pets.

Xanthium pungens has no reported beneficial properties in the Cook Islands.

FIGURE 1 A PATCH OF *XANTHIUM PUNGENS* GROWING ADJACENT TO A CITRUS GROVE IN RAROTONGA.

In the Cook Islands *X. pungens* is listed as a very common and serious weed in Rarotonga (http://cookislands.bishopmuseum.org/species.asp?id=6790), where it infests both cultivated land and pastures.

Xanthium pungens can be suppressed by herbicides, but extensive use of chemical herbicides threatens water resources and the fragile lagoon environment in the Cook Islands because, during heavy rains, runoff washes directly into the lagoon within minutes (Matepi et al., 2010). Control can be achieved through hand-pulling seedlings and cultivating. However, such methods are labour-intensive and plants may resprout if the roots are not severed from the stems. As noted above, contact dermatitis can occur in allergic individuals when hand-pulling plants.

Biological control is seen as the best option to control this weed in the Cook Islands, where it is a serious weed on Rarotonga and is also present on Mauke (http://cookislands.bishopmuseum.org/species.asp?id=6790).

The rust fungus *Puccinia xanthii*, which occurs throughout the native range of *Xanthium pungens*, is already known to be capable of suppressing *X. pungens* biologically: It was accidentally introduced into Australia, where it was first discovered infesting *X. pungens* plants near Brisbane in 1975 and has since dispersed throughout eastern Australia, where it is now responsible for complete control of *X. pungens* (van Klinken and Morin, 2012). Although *P. xanthii* is unlikely to eliminate *X. pungens*, it is likely to reduce it to a level where the impacts of this weed are much less harmful to the Cook Islands environment.

This proposal recommends collecting *P. xanthii* from *X. pungens* growing in Australia and shipping it to the Cook Islands, following processing at the Beever Plant Pathogen Containment Facility in Auckland, New Zealand, to ensure the pathogen is correctly identified and free of potential contaminants.

2.1 INFORMATION ON *PUCCINIA XANTHII*

Taxonomy

Phylum: Basidiomycota

Class: Urediniomycetes

Order: Uredinales

Family: Pucciniaceae

Genus: Puccinia

Species: *P. xanthii* Schw.

Native and Introduced Ranges of *P. xanthii*

Puccinia xanthii is a microcyclic, autoecious (i.e. all life cycle stages occur on the same host) rust fungus that is native to North America where, according to Morin et al. (1993) it is widespread and commonly infects *Xanthium* and closely-related *Ambrosia* spp. *Puccinia xanthii* is also present in Southern Europe and Australia, where it has been introduced (Morin et al., 1993). *Puccinia xanthii* affects the leaves and sometimes the stems of the host plants (van Klinken and Morin, 2012) causing large necrotic lesions (Fig. 2).

FIGURE 2 *PUCCINIA XANTHII* ON COCKLEBURR *XANTHIUM PUNGENS* (PHOTO: CSIRO HTTP://WWW.CSIRO.AU/PORTALS/MEDIA/NOOGOORABURR.ASPX)

2.2 HOST-RANGE TESTING

Scientific rationale for predicting the host-range of *P. xanthii*

A centrifugal phylogenetic method (Wapshere, 1974) has long been used to determine the host-range of a potential biological control agent by sequentially testing plant taxa most closely related to the target weed followed by increasingly distantly related taxa until the host-range has been circumscribed. This approach is supported by recent advances in molecular techniques: for example, host-shifts in lineages of specialist phytophagous insects are strongly linked to the evolution of host-plant lineages, and in particular plant chemistry. Such insects show a strong phylogenetic conservatism of host associations (Briese, 1996; Briese and Walker, 2002). This pattern of strong phylogenetic conservatism in diet suggests the non-target plants of greatest risk are those closely related to known hosts (Futuyma, 2000), and this has been validated by recent reviews of non-target attack by insect (Briese and Walker, 2002; Louda et al., 2003; Paynter et al., 2004; Pemberton, 2000) and fungal (Barton, 2004) weed biological control agents.

The target weed; nomenclature and phylogeny

Xanthium pungens Wallr. is also commonly referred to by the synonyms *Xanthium occidentale* Bertol. and *Xanthium strumarium* L.. Common names include cockleburr, Noogoora burr.

Xanthium pungens belongs to the family Asteraceae. The major clades of the Asteraceae are given in Figure 3; *X. pungens* belongs to tribe Heliantheae (subtribe Ambrosiinae-2 – see Fig. 4).

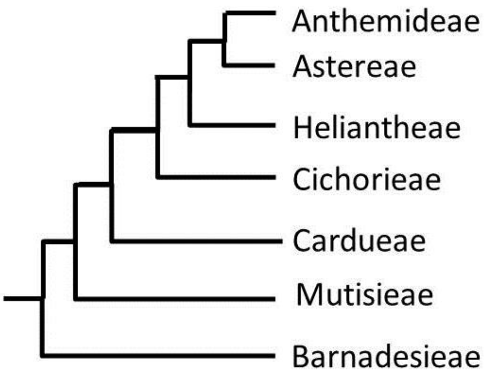

FIGURE 3 PHYLOGENETIC RELATIONSHIPS OF MAJOR CLADES OF ASTERACEAE BASED ON LIU ET AL. (2012).

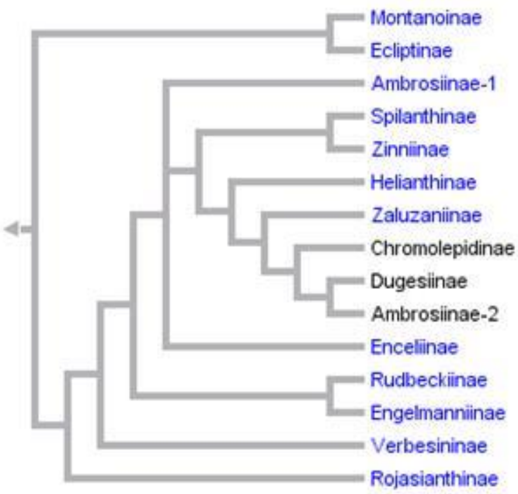

FIGURE 4 CHLOROPLAST DNA PHYLOGENY OF THE HELIANTHEAE AFTER PANERO (2008)

Summary of host-range testing results and field records

Host-range testing and field records indicate that *Puccinia xanthii* is only pathogenic to plants that belong to the tribe Heliantheae of the Asteraceae (Seier et al., 2009). Moreover, there is strong evidence that distinct host-specific rust populations occur, for example, a North American accession of *P. xanthii* that infected *Ambrosia trifida* did not infect *Xanthium strumarium* and an Australian accession of *P. xanthii* from *Xanthium occidentale* could not infect *Ambrosia artemisiifolia* (Seier et al., 2009).

The plant most closely related to cockleburr that is grown in the Cook Islands is sunflower *Helianthus annuus* L., which also belongs to the subtribe Helianthinae. Some sunflower cultivars were moderately susceptible to *Puccinia xanthii* in laboratory conditions, but most were resistant to infection (Morin et al., 1993; see Table 1, below). In field conditions, infection of sunflower is very rare, and the very low levels of infection reported had no discernible impact on sunflower growth and appearance (e.g. Alcorn and Kochman, 1976; Gulya and Charlet, 2002). The trivial damage to sunflowers has never been a problem for the sunflower industry in Australia (van Klinken and Morin, 2012). In contrast, this pathogen had a major impact on cockleburr in Australia: for example, contamination of wool by *X.*

strumarium burrs was reduced by 85% following the introduction of *P. xanthii* due to the dramatic reduction in weed populations (Chippendale, 1995).

Table 1. Host-range testing of *Puccinia xanthii* (adapted from Morin et al., 1993). Note *Xanthium occidentale* is a synonym of *X. pungens*. Key to symptoms: 0, immune, no symptoms; 1, resistant, only small necrotic or chlorotic flecks on leaves; 2, moderately resistant, necrotic flecks with few teliospores on leaves; 3, moderately susceptible, necrotic flecks, underdeveloped telia with some teliospores, few slightly swollen petiole and stem lesions; 4, fully susceptible, normal telia on leaves, several swollen petiole and stem lesions.

Family and species (Common name)	Family	Tribe (subtribe)	Symptoms
Xanthium occidentale Bertol. (Noogoora burr)	Asteraceae	Heliantheae (Ambrosiinae-2)	4
Xanthium cavanillesii Schouw	Asteraceae	Heliantheae (Ambrosiinae-2)	4
X. italicum Mor.	Asteraceae	Heliantheae (Ambrosiinae-2)	4
X. orientale L.	Asteraceae	Heliantheae (Ambrosiinae-2)	4
X. spinosum L.	Asteraceae	Heliantheae (Ambrosiinae-2)	1
Helianthus annuus L. (Sunflower)	Asteraceae	Heliantheae (Helianthinae)	
cv. Austed Advance			3
cv. DK 440			1
cv. DK 610			1
cv. Hysun 21R			2
cv. Hysun 25			1
cv. Hysun 33			1
cv. Hysun 35			3
cv. Hysun 44			3
cv. Hysun 45			1
cv. Hysun 55			2
cv. Pioneer F65			3
cv. Suncross 40R			3
cv. Suncross 60			2
Zinnia elegans Jacq. (Zinnia)	Asteraceae	Heliantheae (Zinniinae)	0
Ambrosia artemisiifolia L. (Ragweed)	Asteraceae	Heliantheae (Ambrosiinae-1)	0
Bellis perennis L. (English daisy)	Asteraceae	Astereae	0
Calendula officinalis L. (Calendula)	Asteraceae	Calenduleae	1
Carduus nutans L. (Nodding thistle)	Asteraceae	Cardueae	0
C. teniuiflorus Curt. (Winged slender thistle)	Asteraceae	Cardueae	1
C. pycnocephalus L. (Slender thistle)	Asteraceae	Cardueae	1
Carthamus tinctorius L. (Safflower)	Asteraceae	Cardueae	0
Cynara scolymus L. (Globe artichoke)	Asteraceae	Cardueae	1
Lactuca sativa L. (Lettuce)	Asteraceae	Cichoriae	0
Citrullus lanatus (Thunb.) Matsum. & Nakai var. caffer (Watermelon)	Cucurbitaceae		0
Glycine max (L.) Merrill cv. Forrest (Soybean)	Fabaceae		0
Pisum sativum L. cv. Yates telephone (Pea)			0
Gossypium hirsutum L. (Cotton)	Malvaceae		
cv. CS189			0
cv. Deltapine			0
cv. Siokra			0

Eucalyptus melliodora A. Cunn. ex Schauer (Yellow box)	Myrtaceae	0
Oryza sativa L. cv. (Amaroo Rice)	Poaceae	0
Sorghum bicolor (L.) Moench cv. E57 (Sorghum)	Poaceae	0
Triticum aestivum L. cv. Banks (Wheat)	Poaceae	0

Summary of host-range testing results and field records in relation to plant species of importance in the Cook Islands

Only four species that belong to the Asteraceae are native to the Cook Islands: *Wollastonia biflora* L (syn. *Melanthera biflora* (L.) Wild) (Tribe Heliantheae); *Fitchia speciosa* (Tribe Coreopsideae), *Tetramolopium mitiaroense* (Tribe Astereae) and *Adenostemma viscosum* (Tribe Eupatorieae).

Although *Wollastonia biflora* L. belongs to the tribe Heliantheae, this species is placed in the subtribe Ecliptinae (Fig. 2), which is relatively distantly related to *Xanthium* (which belongs to subtribe Ambrosiinae-2 – see Fig. 2). Therefore, it is more distantly related to *X. pungens* than both *Ambrosia artemisiifolia* and *Zinnia elegans*, which were immune to infection by *P. xanthii* (Table 1). Given that the even more closely-related sunflower *Helianthus annuus* was largely resistant to infection, the risk to *Wollastonia biflora* posed by *P. xanthii* appears to be very low indeed. Moreover, a search of fungal host plant databases (http://nt.ars-grin.gov/fungaldatabases/fungushost/FungusHost.cfm; http://nzfungi.landcareresearch.co.nz/html/search_hosts.asp; http://nzfungi2.landcareresearch.co.nz/default.aspx) indicates that there are no records of *Wollastonia* or *Melanthera spp.* being infected by *P. xanthii*, even in Australia, where *W. biflora* is a widely distributed (http ://bie.ala.org.au/species/urn:lsid:biodiversity.org.au:apni.taxon:634515) and "common coastal plant" http://www.anbg.gov.au/abrs/online-resources/flora/stddisplay.xsql?pnid=53138.

In order to show due diligence, additional host range tests were nevertheless conducted by Landcare Research using *W. biflora* plants that were collected from Rarotonga in August 2014. The tests were conducted in a mist-chamber using the optimal conditions (25 °C; 24 h dew point) described by Morin et al. (1993). Two tests were performed; the first was set up on 24th Nov 2014 and consisted of three *W. biflora* plants and three *X. pungens* control plants and a second test was set up on 26th Nov 2014 using four *X. pungens* control plants and two *W. biflora* test plants. The plants were monitored for symptoms over the next 14 days. After seven days, spores had formed on the *X. pungens* control plants in both tests, but none of the *W. biflora* test plants were infected. This difference was statistically significant ($P < 0.05$) and we conclude that, as expected, *W. biflora* is not a host of *P. xanthii*.

2.3 ALTERNATIVE CONTROL OPTIONS

2.3.1 NO CONTROL.

If *X. pungens* is not controlled, its negative impacts will undoubtedly persist.

2.3.2 CHEMICAL CONTROL.

X. pungens can be controlled by many soil-applied and foliar herbicides although populations resistant to imidazolinones and to the arsenical herbicides MSMA/DSMA have been reported in the USA (Heap, 1997).

2.3.3 MECHANICAL CONTROL.

Seedlings of *X. pungens* can be controlled by cultivation, but older plants often produce shoots from axillary buds if the root has not been severed**.** Hand-pulling of seedlings is also possible, but can be time consuming and can cause contact dermatitis in allergic individuals.

2.3.4 OTHER BIOLOGICAL CONTROL OPTIONS.

Three insects have been introduced and established in Australia for the biological control of *X. pungens:* A seed-fly *Euaresta aequalis* was introduced into Australia from North America in the 1930's. It became established east of the dividing range but only consumes c. 15% of seed (van Klinken and Morin, 2012). A stem-boring beetle *Nupserha vexator* was released in Australia in 1964 and is now quite common, but provides little control because most damage occurs after the plants have set seed (van Klinken and Morin, 2012). A multivoltine stem-galling moth *Epiblema strenuata* is widely established across Australia, but generally provides little control (van Klinken and Morin, 2012).

3. Environmental consequences

It is unlikely that the introduction of *P. xanthii* could be reversed. It is, therefore, important to determine the potential environmental consequences of its introduction.

- No action will result in continued invasion and a range of negative impacts such as costs to agriculture, water pollution (e.g. herbicide run-off), native species, medicinal plants and aesthetic values.
- Chemical and mechanical control are labour intensive and time consuming.
- Biocontrol, if successful, will result in leaves and stems being infected by rust pustules, resulting in dieback which may cause a short term aesthetic impact as dying plant material browns off and breaks down over time (but so would chemical control and slashing).
- Permanent reductions in *X. pungens* biomass could result in replacement by other invasive species (but note that the current MFAT-funded project will target other major weed species such as *M. micrantha* that have the greatest potential to replace *X. pungens*).

3.1 UNCERTAINTIES

Risk of non-target attack:

Non-target impacts of weed biological control are very rare: The vast majority of agents introduced for classical biological control of weeds (>99% of 512 agents released) have had no known significant adverse effects on non-target plants thus far (Suckling and Sforza, 2014). Moreover, the few cases where significant non-target attack has occurred (e.g. *Rhinocyllus conicus* on native thistles *Cactoblastic cactorum* on native cacti) were predictable from host-range testing and these introductions would not be permitted today (Suckling and Sforza, 2014).

There are no cases of weed biocontrol programmes where rapid evolution of an agent's host-range (i.e. a host-shift) has resulted in a changed fundamental host-range (Van Klinken and Edwards, 2002). Therefore, *P. xanthii* poses a negligible threat to native Cook Island species, which are outside of the fundamental host-range of *P. xanthii.* Moreover, although *P. xanthii* can complete its life cycle on some sunflower varieties in laboratory conditions, damage reported to occur on sunflowers in Australia is both very rare and trivial. For example, Alcorn and Kochman (1976) reported that an experimental breeding line of sunflowers being used to develop new hybrids was mildly susceptible to *P. xanthii* in field conditions, but that other lines in the same plots were not affected by *P. xanthii.* The long association (38 years) between sunflowers and *P. xanthii* in Australia and the absence of further records of non-target attack since then (Seier et al., 2011) indicate that the risk of serious damage to sunflowers is trivial.

3.2 PROPOSED PROTOCOL FOR INTRODUCTION OF *P. XANTHII*

Infected plant material with will be imported from Australia[1] into the Landcare Research Beever Pathogen Containment Facility in Auckland, New Zealand (note Landcare Research has all the necessary permits to propagate *X. pungens* and *P. xanthii* at the Beever Containment Facility). In Auckland, Landcare Research staff shall:

1. Within a mist-chamber: Inoculate potted *Xanthium pungens* plants that were obtained from the Cook Islands and shipped into containment in August 2014 by placing infected plant material obtained from Australia above them and allowing *P. xanthii* spores to 'rain' down on them. A New Zealand Ministry of Primary Industries inspector will examine the imported material shortly after importation from Australia, to ensure that no contaminants are present and the pathogen will be reared through at least one generation and symptoms will be observed to further ensure the culture is not contaminated with other plant pathogens;
2. Eliminate the source of contamination to ensure a clean culture, if contaminants are present;
3. Consult with fungal systematists to ensure the pathogen has been correctly identified. A Landcare Plant pathologist, Dr Mahajabeen Padamsee, who is an expert on rust fungus taxonomy, will formally confirm the identity of the fungus;

Once the fungal culture has been formally confirmed to be *P. xanthii* and determined to be free from any contaminants, *P. xanthii* will be shipped to the Cook Islands for mass-rearing field release (see below). Bare-rooted infected *X. pungens* plants will be shipped on a plane, in sealed containers within a sealed box.

4. Mass-rearing will be done in shade house conditions by Cook Islands Ministry of Agriculture staff (with the assistance of a Landcare Research plant pathologist, who will travel on the same flight as the shipment), prior to field releases.
5. Monitoring plots will be set up to assess impacts of *P. xanthii* in the Cook Islands.

[1]Note permission is not required to export *P. xanthii* from Australia to New Zealand.

Procedure for field release and monitoring.

Field releases and monitoring will be done by Cook Islands Ministry of Agriculture staff, with assistance from Landcare Research staff (who will accompany the first shipment of *P. xanthii* and make regular (annual) visits to assist monitoring and data analysis and train Cook Islands Ministry of Agriculture staff).

1. *Xanthium pungens* plants will be propagated by the Cook Islands Ministry of Agriculture approximately 4-5 weeks prior to importation of the rust.
2. These plants will be inoculated with *P. xanthii*, by suspending infected plant material over them and maintaining high humidity by surrounding the plants with plastic sheeting for approx. 24hr. Note the infected plant material that will be imported will originate from *X. pungens* plants that were collected in Rarotonga in August 2014 and grown in a quarantine glasshouse in New Zealand, prior to inoculation with *P. xanthii*.
3. The infected plants will be planted out among *X. pungens* infestations in Rarotonga.
4. A culture of *P. xanthii* will be maintained by Cook Islands Ministry of Agriculture staff in case further releases are required to ensure establishment of *P. xanthii*.
5. Monitoring will be done on a hierarchical basis. Initially, signs of establishment will be looked for by visually searching for pustules on *X. pungens* plants growing near to the release sites. If establishment is confirmed, then regular searches will be conducted along transects from the release site to investigate the rate of spread and permanent quadrats will be set up to investigate the impact of *P. xanthii* on *X. pungens* populations over time.

Summary of roles and responsibilities

Landcare Research will process the pathogen through the Landcare Research Beever Pathogen Containment Facility in Auckland, New Zealand, ensuring that:

(1) All relevant permits are obtained;
(2) That the pathogen to be shipped to the Cook Islands has been correctly identified and is free from contaminants.

Landcare Research will accompany the first shipment and assist/train Cook Islands Ministry of Agriculture Staff in how to mass-rear the fungus.

Landcare Research staff will assist/train Cook Islands Ministry of Agriculture Staff in how to set up monitoring plots to observe the spread and impacts of the pathogen.

The Cook Islands Ministry of Agriculture shall mass-rear the pathogen, carry out releases and conduct regular monitoring of its impacts, with assistance from farmers and other landowners who are willing to provide release and monitoring sites. The Cook Islands Ministry of Agriculture, together with Landcare Research, shall ensure that the general public is made aware of the project, through media releases and public consultation.

The National Environment Service is responsible for ensuring that the EIA is followed as described within this document.

The Technical Advisory Group, which consists of representatives from Landcare Research; Cook Islands Ministry of Agriculture; Cook Islands Natural Heritage Project; and the Cook Islands National Environment Service will help coordinate biocontrol release and monitoring activities and deal with operational problems that may arise

Areas where releases will be made

Release sites on Rarotonga will be selected by the Cook Islands Ministry of Agriculture, in consultation with the Technical Advisory Group, if required. It is anticipated that releases will commence within two months after the Environmental Impact Assessment permit has been granted. Once *P. xanthii* is established on Rarotonga, redistribution to outer islands affected by *X. pungens* (Mauke) will occur if they are considered to be desirable.

3.3 EDUCATION AND AWARENESS

It is recommended that some form of education and awareness programme be undertaken prior to and during the release of *Puccinia xanthii*, awareness beyond just the EIA to inform the public of the Ministry's intent to introduce. Education is more so important to inform the public of the expected impacts on *Xanthium pungens*, the expected consequences, a request not to tamper, destroy or export inoculated plants from the release sites. Although EIA are advertised in stores, libraries and online and notification of the availability of EIA for viewing is made in local papers, not everybody actually picks up and reads an EIA

To this end, the weed biocontrol project against *X. pungens* and other major weeds in the Cook Islands has been publicised on Cook Islands Radio, Television and in the Cook Islands News.

News articles can be seen here:

http://www.cookislandsnews.com/item/46267-biological-agents-to-control-weeds/46267-biological-agents-to-control-weeds

and here:

http://www.cookislandsnews.com/item/12777-weeds-list-highlights-biological-control/12777-weeds-list-highlights-biological-control

A video of a television article regarding the selection of plant targets for biological control is available here: http://www.youtube.com/watch?v=v1F8Bw2z3CE, but a more recent television and radio interview is unavailable to the author.

Further press releases are planned to coincide with the release of the EIA and (assuming the release is approved) the subsequent release of *P. xanthii* in the Cook Islands.

3.4 ECONOMIC CONSIDERATIONS

Agriculture accounts for 5.1% of the Cook Islands GDP(Anon, 2013). Although there is little published information on the impacts of weeds on agricultural production in the Cook Islands, data regarding herbicide usage are available. Herbicide use increased dramatically

(>400%) over the 15 years from 1995-2009, yet concurrent production of the three main production crops dwindled, indicating that weed problems are worsening (Fig. 5).

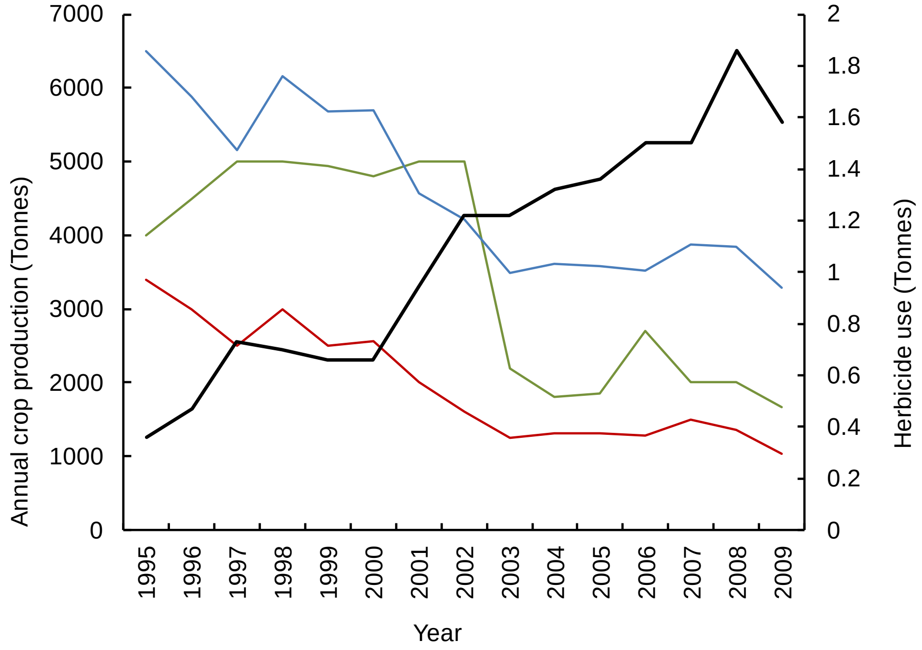

FIGURE 5. PRODUCTION OF THE THREE MAIN CROPS IN THE COOK ISLANDS: ROOTS AND TUBERS (BLUE LINE); COCONUTS (GREEN LINE); AND CASSAVA (RED LINE) AND HERBICIDE USE (BLACK LINE) IN THE FIFTEEN YEARS FROM 1995-2009 (DATA SOURCE: FAOSTAT: HTTP://FAOSTAT.FAO.ORG/SITE/424/DEFAULT.ASPX#ANCOR).

No formal cost-benefit analysis has been done for the introduction of *P. xanthii* in the Cook Islands because economic data regarding the cost of *X. pungens* (control costs and lost production) are lacking. In other countries, *X. pungens* can have major impacts on crop production: In Italy, the economic threshold of *X. strumarium* in soyabeans is only 0.05 plants per square m and higher densities have been reported to result in up to 80% yield loss (http://www.cabi.org/isc/datasheet/56864). Similar yield reductions due to *X. pungens* have been reported in groundnuts and horticultural row crops, such as snap beans (http://www.cabi.org/isc/datasheet/56864). Yield losses in maize are reportedly lower, but still significant (27%; http://www.cabi.org/isc/datasheet/56864). Yield losses due to *X. pungens* infesting crops in the Cook Islands are likely to be similar.

4. Conclusions

Xanthium pungens is already a major weed on Rarotonga and is also present on Mauke.

Successful biocontrol will not only reduce these impacts where *X. pungens* is already abundant, but has the potential to prevent *X. pungens* from becoming a major weed on islands where it is still in the early stage of invasion and reduce the risk of invasion of islands that are currently free of *X. pungens*.

The risks of conducting biocontrol using *Puccinia xanthii* are minor, compared to the potential benefits.

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