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Seed viability of *Persicaria chinensis* (L.) H.Gross in Aotearoa / New Zealand

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Abstract

The pest plant *Persicaria chinensis* (Chinese knotweed) is an unwanted organism under the Biosecurity Act 1993 and is listed on the Ministry for Primary Industries (MPI) Official New Zealand Pest Register (ONZPR). Chinese knotweed is currently managed through a species-led eradication programme in Tāmaki Makaurau / Auckland. In Aotearoa / New Zealand it is believed Chinese knotweed is spread by vegetative means; however, little is known about the viability of the seeds and their role in distribution. This study examined the viability of Chinese knotweed seeds in Aotearoa / New Zealand. Sixteen fruit were used in a simple germination test that was set up to determine viability of the seeds. The results showed that the seeds are viable and under ideal conditions the seeds will germinate. Locations in Tāmaki Makaurau / Auckland are mostly evidenced as human induced; however, there is circumstantial evidence that suggests dispersal by birds may also be likely.

Keywords

Chinese knotweed, *Persicaria chinensis*, seed germination, dormancy, tetrazolium, unwanted organism

Introduction

Persicaria chinensis (L.) H.Gross, family *Polygonaceae*, is a fast-growing, multi-branched scrambling shrub-like herbaceous perennial (Figure 1) with stout rhizomes, and given support of surrounding vegetation can grow to varying heights (Galloway & Lepper 2010; eFlora 2008). It has the common name Chinese knotweed and is native to tropical and sub-tropical Asia, where it grows in a range of environmental conditions from sea level to 3000 m (Galloway & Lepper 2010). It has a status of unwanted organism in Aotearoa / New Zealand and is managed jointly between MPI and Auckland Council (Auckland Council 2020). It is related to other invasive *Persicaria* species such *P. orientalis* (L.) Spach, *P. capitata* (Buch.-Ham. Ex D.Don) H.Gross and *P. perfoliata* (L.) H.Gross (Hignell 2018). *P. chinensis* is one of many *Polygonaceae* species used in traditional medicines (Hao et al. 2015).

In 2009, Chinese knotweed was identified as the plant found growing rampantly along the boundaries of a few properties in the Tāmaki Makaurau / Auckland suburb of Glenfield. This discovery became the first record of this species in Aotearoa / New Zealand. An investigation by the Ministry of Agriculture and Forestry Biosecurity New Zealand (MAFBNZ) took place at the time to find the origin of this incursion. The investigation concluded it was likely imported as Chinese medicine ingredients (Galloway & Lepper 2010). Contractors were engaged by MAFBNZ to carry out control of the plants in the properties affected. By the end of 2010 it was reported that the area was cleared, with no further regrowth (Galloway & Lepper 2010). However, since then the species had been recorded from approximately

Figure 2. Flowers and 'fruit' of *Periscaria chinensis* (Chinese knotweed). Photo: R. Gilchrist, 2017

Figure 1. Example of *Persicaria chinensis* (Chinese knotweed) coverage, 2022. Photo: K. Wootton

40 locations in Auckland, three in Hamilton and one in Palmerston North (source: internal Auckland Council data base and Plant Health and Environment Laboratory [PHEL] Laboratory Information Management System, 2023). It is unknown whether this spread is directly related to the original site, but it is most likely the spread has been facilitated by people sharing plant material, or general dumping of garden waste. The other possibilities for the distribution include multiple importation events or via natural seed dispersal, or all the above.

In Aotearoa / New Zealand, Chinese knotweed was initially believed to spread by vegetative means only (Galloway & Lepper 2010), but there have been reports of fruit and seed production (R. Gilchrist, personal communication, August 8, 2023). Flowers are frequently seen (Figure 2), and fruit also, but to a lesser extent, on Chinese knotweed populations under Auckland Council management. There are also reported unexplained occurrences of the plant, such as in a bush area where it would be unlikely to have been placed intentionally or accidentally, and another being across the road from the main infestation site, under a phoenix palm in a residential garden (R. Gilchrist, personal communication, August 8, 2023). The fruit of Chinese knotweed are technically dry achenes which are included in fleshy, bluish-black perianths. The achenes are black, trigonous, 2.8–4.0 × 2.0–3.0 mm, dull, and minutely punctuate (Ogle et al. 2020). For the purposes of this paper, the achenes will be referred to as seeds. The eFlora of China notes Chinese knotweed flowers from June to November and fruits from July to December (eFlora 2008). Observations from the Auckland Council have unofficially shown flowers and fruit form between April and October; flowers in April, May, June, July, August, September, and fruit in June and October (R. Gilchrist, personal communication, August 8, 2023).

There is a gap in the understanding of the seed ecology of Chinese knotweed in Aotearoa / New Zealand. Studies overseas have indicated the flowers are insect pollinated (Wong et al. 2015), and the seeds are dispersed by animals (Partasasmita & Ueda 2005). Seed dormancy triggers and germination conditions for this species are unknown, however closely related species have been studied internationally (Smith et al. 2014; Justice 1941; Timson 1965). *Polygonum / Persicaria* have seeds that are enclosed in a thick, hard coat consisting of the cutinised epidermal layer, and the embryo and the endosperm are surrounded by a thin layer of compressed integuments (Ransom 1935). The linear embryo is curved around the outer edge of the seed and free from the endosperm (Ransom 1935; Neudorf et al. n.d.). Research also found that some *Polygonum / Persicaria* species exhibit dormancy and require a chilling to break this (Smith et al. 2014; Justice 1941; Timson 1965).

MPI PHEL Botany was approached by Biosecurity Response, MPI, to undertake a viability test on seeds that the Auckland Council had collected from fruit on a vacant site in Tāmaki Makaurau / Auckland. The aim of this study was to determine whether Chinese knotweed in Aotearoa / New Zealand produces viable seed.

Methods

Plant material

The seeds were collected from a site that was a moderate–steep slope running down to a reserve with a stream running through it. The population was of mature plant cover of about 60 m^2, creeping along the ground, and covering existing vegetation.

An MPI Chief Technical Officer (CTO) approval was granted to undertake the viability testing at PHEL (permission under Sections 52[D] and 53[2] of the Biosecurity Act 1993), and resulting seedlings were vouchered to the PHEL herbarium, including a DNA sequence reference. Sixteen seeds were used in viability and germination studies.

Tetrazolium viability testing

The tetrazolium (TZ) test method was based on the International Rules for Seed Testing (ISTA), 'Chapter 6 – The tetrazolium test' (using the imbibing in water method) (International Seed Testing Association 2022). Using a Sigma 10 ml vial of 2,3,5-Triphenyl-tetrazolium chloride solution (TTC), two seeds were soaked in water for 24 hours at 21 °C. The distal ends of the seeds were chipped and placed in the TZ solution in a vial wrapped in aluminium foil to block light. The vial was placed in a HeraTherm incubator at 30 °C for 24 hours. The seeds were removed from the vial, washed, and dissected to reveal the embryos. To excise the embryos of the TZ seeds, a seed was placed in a small decline in a piece of eraser so it could not move, and a fine scalpel blade was used to dissect into the seed coat and endosperm to excise the embryo. The seed was evaluated for viability by observing whether the embryo had been stained red/pink.

Stratification

Dormancy breaking was based on research found on *Polygonum / Persicaria* species from various authors (Smith et al. 2014; Justice 1941; Timson 1965), such as *Persicaria perfoliata* being chilled in moist peat moss at 1–4 °C for four months.

Fourteen seeds were placed into a chiller at 4 °C for pre-chilling (stratification) in the dark. The seeds were kept in a Petri dish with damp commercial-grade peat, with an estimated chilling period of up to three months (Smith et al. 2014).

Germination

The germination test method is based on the International Rules for Seed Testing's 'Chapter 5 – The germination test' (International Seed Testing Association 2022). There are no specific guidelines for *Persicaria* or *Polygonum* in the International Rules for Seed Testing. There is insufficient data in the scientific literature to be found specifically for germination of Chinese knotweed.

Five days into stratification, four of the ten seeds were taken out and cleaned with sterile water. They were then placed on top (TP) of the damp germination paper (38# germination blotting paper from Anchor Paper) in a sealed Petri dish inside an environmental growth chamber (PHCbi MLR-352H) at 21 °C with 24/7 light.

After 28 days from the start of the test, the remaining seeds had begun to germinate in the peat when observed at the second inspection. Eight germinating seeds were removed from the chiller and the peat. These germinating seeds were placed onto the surface of a mix of peat and commercial-grade potting mix in a propagating cell tray. The cell tray was placed into a plastic bag, sealed and labelled, then placed inside a containment greenhouse with a set temperature of 21 °C. The seeds were checked daily and watered as required with a hand-held mister.

DNA sequencing and analysis

Nucleic acid extraction, PCR and sequencing were undertaken following Dharmaraj et al. (2022). A total of 0.3 g of plant tissue from a seedling was ground together with 3 ml of cetyltrimethylammonium bromide (CTAB) lysis buffer prior to incubation. The internal transcribed spacer (ITS) region of nuclear ribosomal DNA was amplified using primers ITS5 and ITS4 (White et al. 1990) and the *trnL-F* cpDNA region using the primers 'c' (Taberlet et al. 1991) and 'f' (Sang et al. 1997). PCR products were sequenced and searched against NCBI Genbank using BLASTn to confirm species identity.

Results

Of the 16 seeds received, the two seeds tested with TZ were viable. When excised, the pink-stained embryo was found on the outer edge of the seed (Figure 3). Of the remaining 14 seeds, 12 germinated successfully; three reached cotyledon stage, one developed to radicle emergence, and the remaining eight seedlings survived to produce four to six true leaves. The four seeds taken out of stratification after five days germinated within five days at 21 °C. Fungal mycelium growth developed on one seedling. No further observations occurred, and the seedlings were disposed of.

The remaining seeds in the chiller were checked twice more, and the second check (28 days from the start of the test) revealed that many of the seeds had germinated. The Petri dish was removed from the chiller and inspected, showing that all but two seeds had germinated. The eight germinated seeds were removed and placed in small propagating cell trays with peat and potting mix, and placed in a plastic bag to maintain humidity in a temperature-controlled greenhouse unit set to 21 °C. All eight seedlings developed cotyledons and two true leaves (Figure 4) before DNA was extracted from one seedling.

The seven remaining seedlings were potted on into horticulture growing tubes and continued to develop, producing more intact true leaves and robust root systems (Figure 5). At the end of the grow-out period the average seedling height was 7.8 cm and the average root length 12.4 cm.

The entire test was completed after 102 days. This was from the first day of chilling to last seed germination, at 28 days. Then there were another 74 days of seedling growth until the seven seedlings were processed and vouchered to the PHEL herbarium reference number PHEL00241.

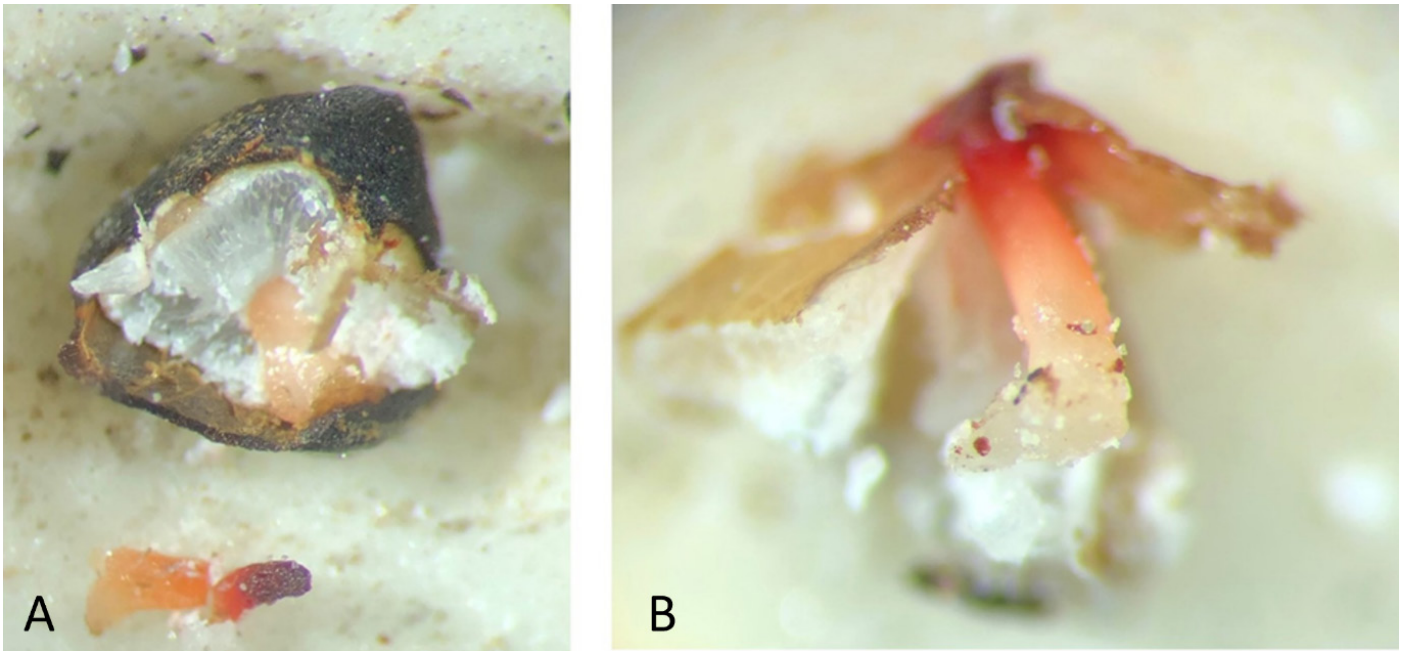

Figure 3. TZ evaluation of *Persicaria chinensis* (Chinese knotweed) seeds. (A) Part of embryo removed from Seed 1. (B) Embryo excised from Seed 2. Photos: C. Elliott, 2023

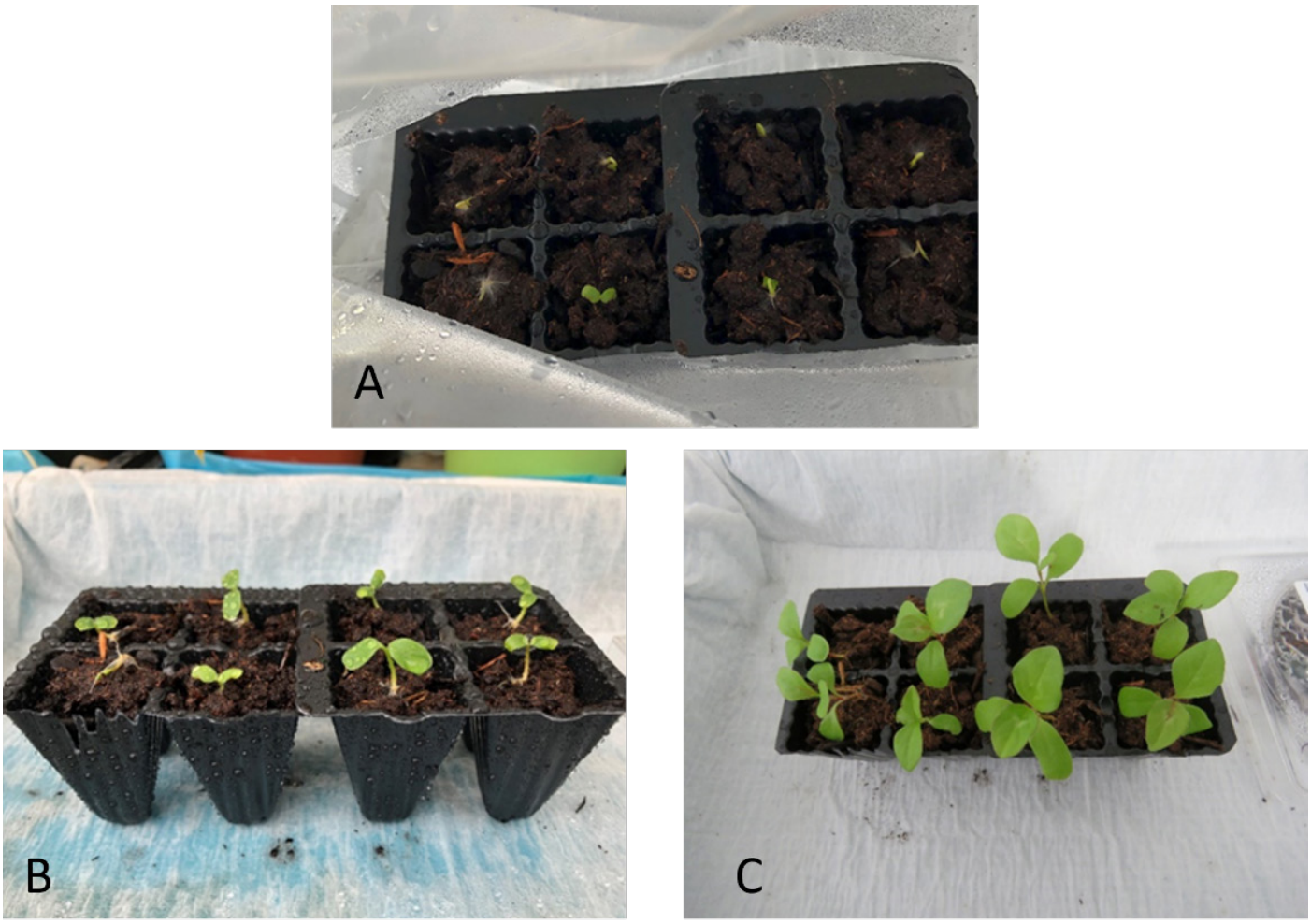

Figure 4. Eight *Persicaria chinensis* (Chinese knotweed) seedlings post-germination. (A) Removed from petri dish. (B) At 10 days' growth. (C) At 33 days' growth. Photos: C. Elliott, 2023

Figure 5. *Persicaria chinensis* (Chinese knotweed) seedlings. (A) Seven plants at end of the test. (B) Measuring height. (C) Root and shoot measuring. Photos: C. Elliott, July 2023

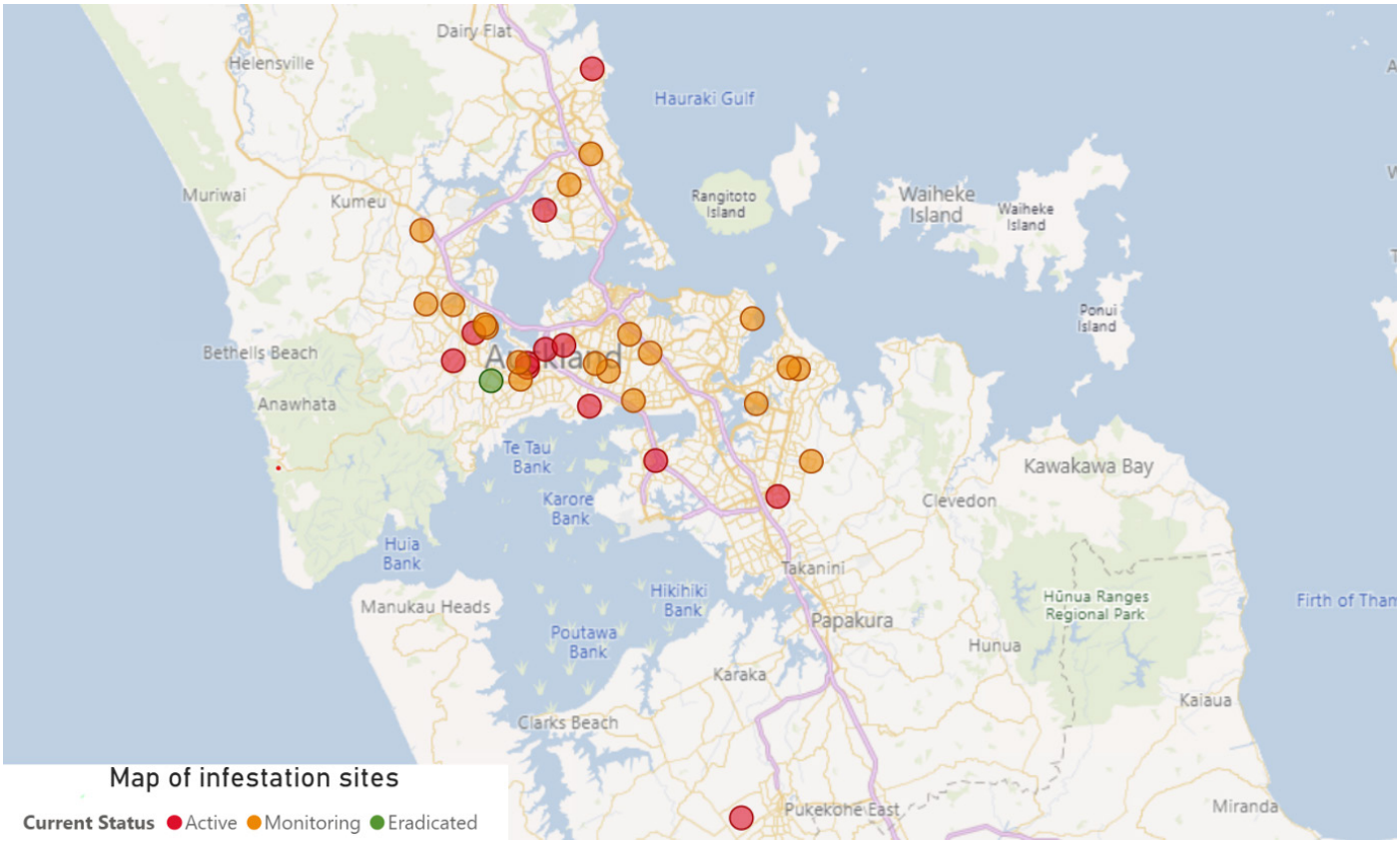

Figure 6. Map of the Tāmaki Makaurau / Auckland isthmus with infestation locations of *Persicaria chinensis* (Chinese knotweed). Source: Auckland Council, 2023

Discussion

The germination test described in this study indicates that (a) Chinese knotweed produces seed; (b) the seeds are viable; and (c) they may germinate in cold conditions. However, without a comprehensive control, it is not clear whether chilling is a requirement for germination or how the seeds will respond in Tāmaki Makaurau / Auckland conditions. If dormancy is not released, the question remains: how long will the seeds remain viable and therefore a hidden seed bank? Two references indicate that a close relative, *P. perfoliata*, can remain viable for up to three years (Global Invasive Species Database 2023) and *Polygonum persicaria* has been reported viable for as long as 20 years (Timson 1965).

It was discovered after the successful germination that the submitted 12 seeds were not fresh, and the initial collection and storage of the fruit was approximately four months. From the Tāmaki Makaurau / Auckland site, a branch from a Chinese knotweed plant was removed with fruit intact and placed into a paper bag, then placed inside a plastic bag and stored in a refrigerator from December 2022 to March 2023 at an Auckland Council location. This treatment is similar to a treatment called after-ripening (Justice 1941; Ransom 1935), which is loosely described as a period of time in which mature harvested seeds are stored at a particular temperature and state, e.g., wet or dry, to aid dormancy release and promote germination.

With only a small number of seeds available to determine viability, an extensive repeatable trial could not be undertaken using a range of treatments such as germination temperatures and dormancy-breaking techniques. For this germination test, the decision was made to remove some seeds from the estimated two- to three-month dormancy time as an early indicator of germination.

Chinese knotweed has now been recorded from approximately 40 locations (some of which may contain multiple neighbouring addresses) in 29 suburbs across the Tāmaki Makaurau / Auckland region, from Pukekohe in the south to Torbay in the north, Whenuapai and Massey in the west, to Pakuranga in the east (Figure 6).

Birds are the likeliest natural dispersal method, with stem fragments in garden waste disposal, and intentional cultivation and distribution also probable dispersal pathways (R. Gilchrist, personal communication, August 8, 2023; Rojas-Sandoval & Acevedo-Rodríguez 2014). Birds are known seed dispersers of Chinese knotweed and other *Persicaria / Polygonum* species (Partasasmita

& Ueda 2005; Rojas-Sandoval & Acevedo-Rodríguez 2014). An Aotearoa / New Zealand study found that eight bird species have a key role in spreading non-native environmental weeds (Wootton & McAlpine 2015).

This germination test has confirmed that, in Tāmaki Makaurau / Auckland, Chinese knotweed seeds are viable and do germinate. The authors also consider it reasonable to assume that bird dispersal of the seeds is occurring, along with the known human-mediated dispersal.

Future research on the seed ecology of Chinese knotweed, including the possible existence of a seed bank, could enhance the effective planning for its control (Copeland & McDonald 2001) and future management in Tāmaki Makaurau / Auckland.

Author Contributions

Lydia Starr: Conceptualisation (50%); data curation (30%); investigation (10%); methodology (5%); validation (5%); visualisation (30%); writing – original draft (10%); writing – review and editing (20%)
Carol Elliott: Conceptualisation (50%); data curation (70%); investigation (90%); methodology (95%); validation (95%); visualisation (70%); writing – original draft (90%); writing – review and editing (80%)

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