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Biological Control

BIOLOGY AND BIOLOGICAL CONTROL OF KNOTWEEDS

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Cover Photos: (a) Knotweed infestation, (b) *Aphalara itadori* adult, (c) *A. itadori* nymph, (d) *A. itadori* eggs (Credits: Fritzi Grevstad, Oregon State University)

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Biology and Biological Control of Knotweeds

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Contents

CHAPTER 1: INTRODUCTION

Overview

What we collectively refer to as "knotweed" is not a single plant species, but a complex of three species that can interbreed with each other. They include Japanese knotweed (*Fallopia japonica* [Houtt.] Ronse Decraene), giant knotweed (*F. sachalinensis* [F. Schmidt] Ronse Decraene), and their hybrid Bohemian knotweed (*F.* × *bohemica* [Chrtek & Chrtková] J.P. Bailey). The use of "knotweeds" throughout this manual encompasses all three species.

All three knotweeds are upright, herbaceous perennials that are easily recognized by their tall growth, large leaves, and clusters of small, white flowers (Figure 1a-c). Knotweeds propagate by seed and (most frequently) vegetatively through clonal fragmentation of stems and rhizomes (Grimsby et al. 2007). Japanese and giant knotweed are native to East Asia (Barney 2006). They were introduced to North America in the late 1800s as ornamentals and for erosion control before they escaped cultivation. The hybrid Bohemian knotweed has been intentionally cultivated and also naturally occurs in the field where both parent species overlap. Back-crossing with both parent species regularly occurs.

Figure 1-1. Knotweed plants a. Japanese knotweed; b. giant knotweed; c. Bohemian knotweed (Credits: a-c Fritzi Grevstad, Oregon State University)

In North America, Japanese and Bohemian knotweed are present in 42 states and eight provinces (Figure 1-2a). Giant knotweed occurs in fewer states and provinces (Figure 1-2b), but is locally just as invasive. All three species have become most abundant and problematic in the Northeast and in the Pacific Northwest, but they are also increasingly problematic in the interior regions.

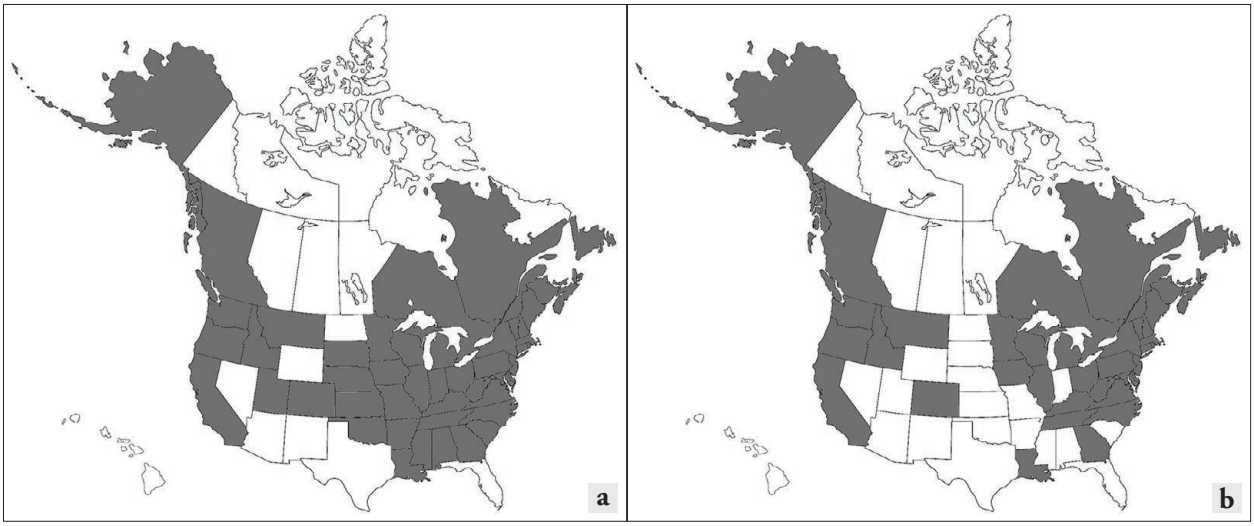


Figure 1-2. North American distribution of: a. Japanese and Bohemian knotweed; b. giant knotweed. Some states and provinces are more heavily infested than others (Credits: USDA PLANTS Database, EDDMapS)

Throughout their native and introduced ranges, knotweeds are frequently found in riparian, wetland, or lowland plant communities. They can inhabit a variety of sunlight, soil, moisture, and human disturbance conditions, but have become especially problematic in full sun locations along the banks and floodplains of rivers and streams and moist roadsides (Stone 2010).

Knotweeds are listed among the world's worst invasive species by the World Conservation Union, having also invaded Europe, New Zealand, and Australia (Lowe et al. 2000). Knotweeds compete aggressively for light, water, and nutrients, and they release compounds that are harmful to other plants (allelopathy) (Siemens and Blossey 2007, Murrell et al. 2011, Urgenson et al. 2012). Consequently, they are a major concern for displacing native and/or more desirable species in riparian areas and native and commercial forests. Their exclusion of trees along stream banks is potentially detrimental to fish and other stream inhabitants that benefit from shade. Knotweeds have no known value for wildlife, and they harbor fewer invertebrates compared to surrounding native vegetation, which has negative impacts on the food chain (Beerling and Dawah 1993, Maerz et al. 2005, Kappes et al. 2007, Gerber et al. 2008, McIver and Grevstad 2010). Knotweeds alter nutrient cycling in soil and streams (Dassonville et al 2007, Urgenson et al. 2009, Aguilara et al. 2010), and their lack of fine surface roots can lead to increased erosion (Child et al. 1992). Finally, knotweeds can live for decades, exacerbating their negative impacts.

Responding to the Threat of Knotweeds

Knotweeds are invasive species not native to North America whose introductions cause or are likely to cause economic or environmental harm. Knotweeds cost millions of dollars annually through the disruption of natural ecosystems, the devaluation of infested lands and housing, and in control and restoration efforts.

A general management response to the threat of knotweeds and other invasive species is based on four key elements or intermediate outcomes: prevention and preparedness, eradication, containment, and asset-based protection. In order to ensure a timely and appropriate management response, land managers must continually monitor, evaluate, and report/map new knotweed infestations and evaluate how knotweeds respond to each control effort. Research and development informed by the observations and needs of land managers will play a critical role in the eventual success or failure of knotweed prevention and management activities in their invaded range.

Prevention and Preparedness

Preventing high-risk invasive species from establishing is the most cost-effective approach to managing the threat they pose. Considerable resources and planning are required to maintain prevention of a large number of species. Preparedness encompasses all the activities and resources necessary to successfully manage new invasions.

Eradication

Eradication, getting rid of an invasive species completely, is generally only possible in the early stages of establishment when the distribution and abundance of the invasive species are low. Infestations that are eradicable are considered in the Early Detection/Rapid Response phase of invasion and should be addressed swiftly and aggressively. This approach can be almost as cost-effective as prevention.

Containment

Where an invasive species cannot be eradicated, there can be substantial net benefit gained from preventing its further spread. Containment involves measures to eradicate outlying (satellite) infestations and prevent spread beyond the boundaries of core infestations (those that are too large and well-established to eradicate). Obtaining a high degree of community support is a prerequisite for any long-term containment program.

Asset-Based Protection

An asset-based approach to managing an invasive species is appropriate once it has become so widespread that it would be inefficient to control the species everywhere it occurs and where containment would provide a low return on investment. The asset-based approach is used to manage the species only where specific highly-valued assets are in need of protection and/or restoration outcomes, such as the habitat of an endangered species or a site with cultural significance.

Monitoring, Evaluation, and Reporting

For science-based programs (such as invasive species management) monitoring, evaluation, and reporting are elements of adaptive management, whereby programs are continually reviewed and analyzed to ensure that their approaches are consistent with, and supportive of, any changes in environmental response, community expectation, or scientific knowledge.

Research and Development

The knowledge that comes from research and development is critical to implement evidence-based management approaches. In many cases, substantial advances in invasive species management will require development of new techniques and acquisition of greater and new knowledge. Investment in research needs to be sufficient to ensure future management is not seriously constrained by insufficient research and development support.

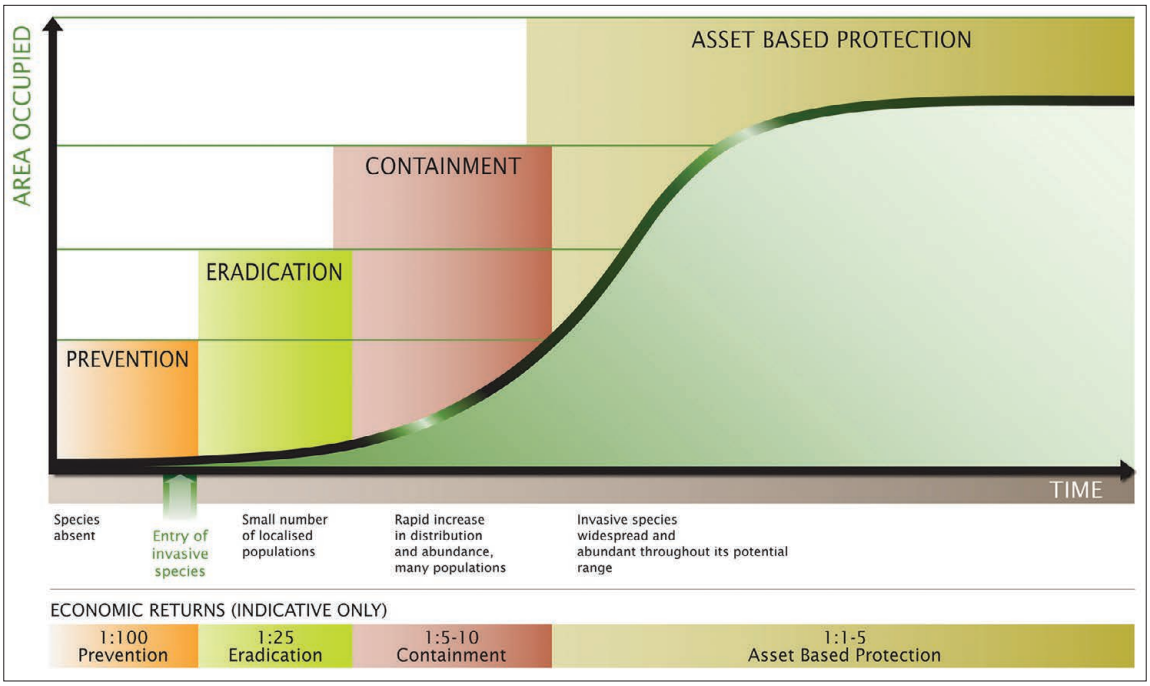


Figure 1-3. Generalized invasion curve showing actions appropriate to each stage (Credit: © State of Victoria, Department of Economic Development, Jobs, Transport and Resources. Reproduced with permission)

The Invasion Curve

The invasion curve (Figure 1-3) shows that eradication of invasive species such as knotweeds becomes less likely and control costs increase as the invasive species spread over time. Prevention is the most cost-effective solution, followed by eradication. If a species is not detected and removed early, intense and long-term control efforts will be unavoidable.

While knotweeds currently infest large areas in some regions, there are entire states and provinces where knotweeds are absent or are present at very low levels. The diversity of knotweed populations (from absent to widespread and abundant) throughout their potential range requires land managers to coordinate their management response to knotweeds across larger landscapes to prevent current infestations from spreading into uninfested areas.

Identifying where knotweeds are on the invasion curve in a particular area is the first step to taking management action. Inventorying and mapping current knotweed populations, coupled with research efforts to predict where knotweeds are most likely to inhabit, enables land managers to concentrate resources in areas where knotweeds are likely to invade, and then to treat individual plants and small populations before it is too late to remove them.

Though the knotweed biological control program is in its infancy, biological control options are readily available for many other invasive plant species. Biological control is just one of many control methods available to land managers, but biological control is generally not appropriate for areas on the left side of the invasion curve (species absent [prevention] - small number of localized populations [eradication]) because biological control alone will not result in weed eradication. Biological control as a control method is best suited to weed populations in the later phases of the invasion curve (rapid increase in distribution and abundance [containment] – widespread and abundant throughout potential range [asset-based protection]).

Management of Knotweed Infestations

Successful management of knotweeds is an intensive process which requires land managers to continuously inventory, map, and assess the extent and severity of knotweed infestations. Land managers must also understand the benefits and shortcomings of each weed control method available, alone and in combination, when applied to knotweeds. Chemical control (using herbicides) may be used to successfully control small knotweed infestations (left side of the invasion curve, Figure 1-3), or larger infestations when appropriate. However, in either case, land managers must be committed to annual monitoring and re-treatment due to repeated re-sprouting from the rhizomes. Chemical control can be very effective but may be impractical, prohibitively expensive, and outright banned from many of the riparian habitats knotweeds frequently invade. Where herbicides are permitted, care must be taken to avoid damaging desired vegetation and aquatic species. Hand digging small, individual knotweed plants may be feasible for small infestations on the left side of the invasion curve; however, hand digging that fails to remove all of the extensive rhizomes on larger plants may increase knotweed spread (Beerling 1991). Repeated mowing or cutting (twice monthly during the growing season for at least three years but typically longer) can be used in the containment and asset-based portions of the invasion curve by reducing knotweed vigor and seed production, but it may exacerbate the problem by triggering re-growth if performed less frequently (Prather et al. 2009). All roots, stems, flowers, and seeds should be securely bagged and taken to the trash or a transfer site to prevent possible knotweed vegetative growth or seed dispersal from cut material. Material should never be mulched or added to compost. Covering very small infestations with landscape fabric may successfully reduce stem numbers, but this requires open terrain and regular maintenance to prevent regrowth from puncturing the fabric. Sites must remain covered for at least three to five years and, once removed, will be devoid of all other vegetation.

Burning has typically yielded poor results for knotweed control due to their high moisture content and frequent proximity to water. Grazing knotweeds may reduce knotweed shoot density by up to 50%, but will not control or eradicate these species (Prather et al. 2009). Grazing can also be difficult and/or time-consuming, and may have severe negative, long-term consequences for plant communities. In restoration projects, knotweed populations must be substantially reduced before native plantings are successful. Typically, this requires three years of herbicide treatments before restoration efforts can begin. Monitoring and maintenance should continue for an additional 7+ years, perhaps indefinitely, once native vegetation begins to establish. Knotweed is likely to reinvade, undoing restoration activities if control work does not continue (Figure 1-4). Because chemical, physical, and cultural control methods are not universally effective in managing knotweeds throughout their invaded range, a biological control program was initiated in 2000. Biological control as a control method is best suited to knotweed populations in the later phases of the invasion curve. This manual discusses the biological control of knotweeds in North America.

Figure 1-4. Knotweeds re-invading an un-maintained restoration site (Credit: Fritzi Grevstad, Oregon State University)

The most effective weed management strategies are based on regular inventory and monitoring of target weed populations, application of one or many weed control methods, evaluation of treatment efficacy, additional inventory and mapping, and adjustment of control methods as needed to meet management objectives in response to changing weed populations through time. Integrated Weed Management (IWM) incorporates additional activities that enable land managers to address the threat of knotweed invasions in infested as well as uninfested areas across a landscape. IWM activities include education and outreach, inventory and

mapping, prevention methods, and control methods (physical control [hand digging or mowing], cultural control [revegetation, grazing, or fire], chemical control [herbicides], and biological control). IWM relies on the development of realistic weed management objectives, accurate weed identification and mapping, appropriate prevention and control methods, and post-treatment monitoring to ensure current weed-management activities are meeting knotweed management goals.

Land managers choose control methods, either alone or in combination that enable them to achieve their knotweed management goals or objectives in the most cost-effective manner. No single control method will enable managers to meet their knotweed management goals in all environments or instances. Control method(s) employed will depend on the size and location of the infested area and specific management goals (e.g., eradication vs. weed density reduction). Small patches of knotweeds may be eliminated through a persistent physical or chemical control program, but large infestations will often require the use of additional control methods. A combination of control methods consistently applied, evaluated, and adjusted through time is usually necessary to attain and maintain weed management goals for knotweeds.

Classical Biological Control of Weeds

Most invasive plants (weeds) in the United States are not native to North America; they arrived with immigrants, through commerce, or by accident from different parts of the world. These non-native plants are generally introduced without their natural enemies, the complex of organisms that feed on or attack the plant in its native range. A lack of natural enemies is thought to be one reason plant species become invasive weeds when introduced to areas outside of their native range (Keane and Crawley 2002).

Biological control (also called "biocontrol") of weeds is the deliberate use of living organisms to limit the abundance of a target weed. In this manual, biological control refers to "classical biological control," which reunites host-specific natural enemies from the weed's native range with the target weed in its introduced range (McFadyen 1998). Natural enemies used in classical biological control of weeds include different organisms, such as insects, mites, nematodes, and pathogens. In North America, most weed biological control agents are plant-feeding insects, of which beetles, flies, and moths are among the most commonly used.

Biocontrol agents may attack a weed's flowers, seeds, roots, foliage, and/or stems. Regardless of the plant part attacked by biocontrol agents, the aim is always to reduce populations and vigor of the target weed (Crawley 1998). Effective biological control agents seldom kill weeds outright. Rather, they work with other stressors such as moisture or nutrient shortages to reduce vigor and reproductive capability, or facilitate secondary infection from pathogens—all of which compromise the weed's ability to compete with other plant species.

Although weed biological control is an effective and important weed management tool, it does not work in all cases and should not be expected to eradicate the target weed. Even in the most successful cases, biocontrol often requires multiple years before impacts become noticeable. When classical biological control alone does not result in an acceptable level of weed control, other weed control methods (e.g., physical, cultural, or chemical control) may be incorporated to achieve desired results. The advantages and disadvantages of using biological control as a weed management tool are listed in Table 1-1.

To be approved for release in North America, weed biocontrol agents must be host-specific, meaning they must develop only on the target weed. Rigorous testing is required to confirm that biocontrol agents are host-specific and effective. Potential biocontrol agents often undergo five or more years of testing to ensure that rigid host specificity requirements are met, and results are vetted at a number of stages in the approval process.

Table 1-1. Advantages/disadvantages of classical biological control as a weed management tool.

Advantages	Disadvantages
Target specificity	Will not work on every weed in every setting
Continuous action	Permanent; cannot be undone
Long-term cost-effective; can provide sustained control at the landscape scale	Funding and testing candidate biocontrol agents is expensive; measurable impact may take years or even decades to materialize
Integrates well with other control methods	Approved biocontrol agents are not available for all exotic weeds
Generally environmentally benign	Like all weed control methods, non-target effects are possible, but pre-release testing reduces the risks
Self-dispersing, even into rough or difficult to access terrain	Unpredictable level of control; does not eliminate weed

The United States Department of Agriculture's Animal and Plant Health Inspection Service - Plant Protection and Quarantine (USDA-APHIS-PPQ) is the federal regulatory agency responsible for providing testing guidelines and authorizing the importation of biocontrol agents into the USA. The Canadian Food Inspection Agency (CFIA) serves the same regulatory role in Canada. Federal laws and regulations are in place to identify and avoid potential risks to native and economically valuable plants and animals that could result from exotic organisms introduced to manage weeds. The Technical Advisory Group (TAG) for Biological Control Agents of Weeds is an expert committee with representatives from USA federal regulatory, resource management, and environmental protection agencies, and regulatory counterparts from Canada and Mexico. TAG members review all petitions to import new biocontrol agents into the USA, and make recommendations to USDA-APHIS-PPQ regarding the safety and potential impact of prospective biocontrol agents. Weed biocontrol researchers work closely with USDA-APHIS-PPQ and TAG to accurately assess the environmental safety of potential weed biocontrol agents and programs. In addition, some states in the USA have their own approval process to permit field release of weed biocontrol agents. In Canada, the Biological Control Review Committee (BCRC) draws upon the expertise and perspectives of Canadian-based researchers (e.g., entomologists, botanists, ecologists, weed biological control scientists) from academic, government, and private sectors for scientific review of petitions submitted to the CFIA. The BCRC reviews submissions for compliance with the North American Plant Protection Organization's (NAPPO) Regional Standards for Phytosanitary Measures (RSMP) No. 7. The BCRC also reviews submissions to APHIS. The BCRC conclusions factor into the final TAG recommendation to APHIS on whether to support the release of the proposed biocontrol agent in the USA. When release of a biocontrol agent is proposed for both the USA and Canada, APHIS and the CFIA attempt to coordinate decisions based on the assessed safety of each country's plant resources.

Code of Best Practices for Classical Biological Control of Weeds

Biological control practitioners have adopted the International Code of Best Practices for Biological Control of Weeds. The Code was developed in 1999 by delegates and participants in the Tenth International Symposium for Biological Control of Weeds to both improve the efficacy of, and reduce potential negative impacts from, weed biological control. In following the Code, practitioners reduce the potential for causing environmental damage through the use of weed biological control by voluntarily restricting biocontrol activities to those most likely to result in success and least likely to cause harm.

International Code of Best Practices for Classical Biological Control of Weeds[1]

1. Ensure that the target weed's potential impact justifies release of non-endemic biocontrol agents
2. Obtain multi-agency approval for biocontrol target
3. Select biocontrol agents with potential to control target
4. Release safe and approved biocontrol agents
5. Ensure that only the intended biocontrol agent is released
6. Use appropriate protocols for release and documentation
7. Monitor impact on the target
8. Stop releases of ineffective biocontrol agents or when control is achieved
9. Monitor impacts on potential non-targets
10. Encourage assessment of changes in plant and animal communities
11. Monitor interaction among biocontrol agents
12. Communicate results to public

[1]Ratified July 9, 1999, by the delegates to the X International Symposium on Biological Control of Weeds, Bozeman, MT

There are several resources that provide additional information about general weed biocontrol practices and specific weed biocontrol systems, which can be found in the Chapter 1 references under Andreas et al. 2017, Coombs et al. 2004, Winston et al. 2014a,b, and Winston et al. 2017.

Biological Control of Knotweeds

The knotweed biological control program was initiated in 2000 as a joint USA – United Kingdom program sponsored by the USDA Forest Service Forest Health Technology Enterprise Team and various other European and USA agencies. Following surveys for natural enemies, both in the introduced ranges and overseas, several candidate species were identified and brought into insect containment laboratories at Oregon State University, Lethbridge Research Centre (Canada), and CABI (United Kingdom) for further testing. After extensive host specificity testing, the knotweed psyllid (*Aphalara itadori*, Figure 1-5) became the first biocontrol agent approved for release against invasive knotweeds. The psyllid was released in the UK in 2010 and in Canada in 2014. As of the date of this publication (2018), the knotweed psyllid is still under review by the USDA Animal and Plant Health Inspection Service for release into the USA.

Figure 1-5. An adult knotweed psyllid (*Aphalara itadori*) and eggs (Credit: Fritzi Grevstad, Oregon State University)

Is Biological Control of Knotweeds Right for You?

When biological control is successful, biocontrol agents increase in abundance until they suppress (or contribute to the suppression of) the target weed. As local target weed populations are reduced, their biological control agent populations also decline, due to starvation and/or dispersal to other target weed infestations. In many biocontrol systems, there are fluctuations over time with the target weed becoming

more abundant, followed by increases of its biocontrol agent, until the target weed/biocontrol agent populations stabilize at a much lower abundance.

As stated in Table 1-1, biological control is not effective in every weed system or at every infestation. The knotweed biological control program is also in its infancy and may require several more years before significant impacts are observed in North America. We recommend that you develop an integrated weed management program in which biological control is one of several control methods considered. Here are some questions you should ask before you begin a biological control program:

Is my management goal to eradicate the weed or reduce its abundance?

In some situations, knotweeds must be removed quickly and completely. For example, knotweeds might need to be removed before the sale or development of a piece of property or prior to a restoration project involving the planting of native plant species. Perhaps the knotweed invasion is a threat to an endangered species or it is in a location where it has potential to spread into a much larger area. In such cases, eliminating knotweed quickly is important. In general, biological control does not eradicate target weeds (McFadyen 2000), so it is not a good fit with an eradication goal. Depending on the target weed, which biological control agent is used, and land use, biological control can be effective at reducing the abundance and vigor of a large infestation of the target weed to an acceptable level. Other control methods may be better options for infestations and watersheds where eradication or rapid knotweed reduction is the goal, and/or where intensive control efforts are currently underway.

How soon do I need results: this season, one to two seasons, or within 5-10 years?

Biological control requires time and patience to work. Generally, it can take one to three years after release to confirm that biological control agents are established at a site, and even longer for biocontrol agents to cause significant impacts to the target weed. For some weed infestations, 5-30 years may be needed for biological control to reach its weed management potential.

What resources can I devote to my weed problem?

If you have only a small knotweed problem (<¼ acre [0.1 ha] or smaller), weed control methods such as hand digging and/or herbicides, followed by regular monitoring for re-growth and re-treatment when necessary, may be most effective. These intensive control methods may allow you to achieve rapid control and prevent the weed from spreading and infesting additional areas, especially when infestations occur in high-priority treatment areas such as travel corridors where the weed is more likely to readily disperse. If knotweeds are well established over a large area (>¼ acre, 0.1 ha), and resources are limited, biological control may be the most economical weed control option. A good example is a river system with many miles of infested banks that are difficult to access.

At knotweed infestations where multiple forms of control are planned, biocontrol may not be the best solution. There may be some situations where chemical, mechanical, and biological control can work in synergy on the same site; however, biocontrol is usually not compatible with these methods, especially in the early stages of biocontrol agent population expansion. Any physical treatment that kills off above-ground knotweed foliage will also reduce the biocontrol population and render it useless. Herbicide applications are likely to kill the knotweed psyllid, either through direct contact with the herbicide (especially the surfactant) or as an indirect effect of plant death. For the greatest biocontrol effect on the weed, it is best to leave biocontrol populations alone for several years, with the exception of monitoring.

The most effective means for following an integrated weed management approach for invasive plants in general includes using biological control at large infestations and chemical and physical control methods at smaller, new or satellite, or high priority populations (where immediate eradication is warranted) as well as to the edges of large infestations to prevent further spread. Knotweeds frequently infest watersheds, and infestations at the headwaters are a high priority for control in order to reduce the spread of propagules further down the system. In such watersheds, where headwaters are being intensively treated with chemical or physical control methods, biocontrol can be used at the lower end of the watershed, which is often a lower priority for intensive management until upstream populations are greatly reduced. Alternatively, in systems with less intensive management, biocontrol agents can be released in large patches throughout the watershed while smaller or high priority patches can be controlled using herbicides and physical control methods. Cultural control methods work to enhance the growth of more desirable vegetation and are best applied as complements to all other control methods.

Is the weed the problem, or a symptom of the problem?

Invasive plant infestations often occur where desirable plant communities have been or continue to be disturbed. Without restoration of a desirable, resilient plant community, and especially if disturbance continues, biological control is unlikely to solve your weed problems.

The ideal biological control program:

1. Is based upon an understanding of the target weed, its habitat, land use and condition, and management objectives
2. Is part of a broader integrated weed management program
3. Has considered all weed control methods and determined that biological control is the best option based on available resources and weed management objectives
4. Has realistic weed management goals and timetables
5. Includes resources to ensure adequate monitoring of the target weed, the vegetation community, and populations of biological control agents

About This Manual

This manual provides information on the biology and ecology of knotweeds and *Aphalara itadori*, the knotweed biological control agent currently approved in Canada and (at the time of this publication) under review in the USA. This manual also presents guidelines to establish and manage a knotweed biological control program. Throughout this manual, English units are given first for descriptions of plants and areas, followed by their metric system equivalents in parentheses. Metric units are the preferred and traditional reference for insects and are used throughout Chapter 3 for describing knotweed biological control agents. Table 1-2 provides English/metric conversions and abbreviations.

Chapter 1: Introduction provides introductory information on knotweeds (including their distribution, habitat, and economic impact) and classical biological control.

Chapter 2: Getting to Know Knotweeds provides detailed descriptions of the taxonomy, growth characteristics and features, invaded habitats, and occurrence of knotweeds in North America. It also describes how to differentiate knotweeds from each other and from look-alike species.

Table 1-2. English/metric conversion table

English System	Metric System
1/16 inch (in)	3.2 millimeters (mm)
1 inch (in)	2.54 centimeters (cm)
1 foot (ft)	30 centimeters (cm)
1 yard (yd)	0.9 meters (m)
1 mile (mi)	1.6 kilometers (km)
1 acre (ac)	0.4 hectares (ha)

Chapter 3: Biology and Host Specificity of Knotweed Biological Control Agents describes the knotweed psyllid *Aphalara itadori*, including details on its native range, original source of releases in North America, parts of knotweed plants attacked, life cycle, description, host specificity, known non-target effects, habitat preferences, and current status. It also describes candidate biocontrol agents that were previously studied but ultimately rejected.

Chapter 4: Implementing a Knotweed Biological Control Program includes detailed information and guidelines on how to plan, implement, monitor, and evaluate an effective knotweed biological control program. Included are guidelines and methods for:

- Selecting and preparing biological control agent release sites
- Collecting, handling, transporting, shipping, and releasing biological control agents
- Monitoring biological control agents and vegetation

The **Glossary** defines technical terms frequently used by those involved in knotweed biological control and found throughout this manual.

References lists selected publications and resources used to compile this manual.

Appendices:

I. *Aphalara itadori* Host Specificity Test Plant List

II. Troubleshooting Guide: When Things Go Wrong

III. Sample Biological Control Agent Release Form

IV. Knotweed Psyllid Monitoring Form

V. Knotweed Qualitative Monitoring Form

VI. Knotweed Quantitative Monitoring Form

CHAPTER 2: GETTING TO KNOW KNOTWEEDS

Taxonomy and Related Species

Knotweeds belong to the buckwheat family (Polygonaceae). Members of this family can be identified by their ocrea, which are structures found at leaf bases that sheath the stem (Figure 2-1). Members of this family also lack true petals. Their flowers consist of sepals that resemble petals. In other plant families, sepals are the parts of a flower that enclose the petals and are typically green and leaf-like. Many buckwheats also have swollen nodes (joints) along their stems. The buckwheat family has a worldwide distribution encompassing approximately 1,200 species (Freeman and Reveal 2005). It contains some familiar cultivated food plants such as rhubarb and buckwheat. In North America, native species in this family include docks (*Rumex* spp.), smartweeds (*Persicaria* spp.), and wild buckwheats (*Eriogonum* spp.).

Figure 2-1. An ocrea, a sheath surrounding the stem at the leaf joint, characteristic of the buckwheat family (Polygonaceae) (Credit: Martin Olsson)

Knotweeds were formerly included in the genus *Polygonum*, but more recent taxonomic groupings moved them to the genus *Fallopia* (Freeman and Reveal 2005). While some taxonomists have recently proposed another shift to the genus *Reynoutria* (Schuster et al. 2011), the shift is still being debated. The scientific names used in this manual are the ones used in the Flora of North America Vol. 5 (Freeman and Reveal 2005), which retains the genus *Fallopia*.

The genus *Fallopia* contains approximately 12 species worldwide. Two of these are native to the northeastern USA and eastern Canada, including the vines *F. scandens* (L.) Holub and *F. cilinodis* (Michx.) Holub. In addition to knotweeds, three other non-native *Fallopia* species have been introduced to North America: the weedy annual species *F. convolvulus* (L.) Á. Löve and *F. dumetorum* (L.) Holub, and the ornamental vine *F. baldshuanica* (Regel) Holub. Wirevines in the genus *Muehlenbeckia* are closely related to knotweeds and other species in *Fallopia*. None of the wirevines are native to North America, but two have been introduced as ornamental species in California (*M. complexa* [A.Cunn] Meisn. and *M. hastulata* (Sm.) I.M. Johnst.), and one has been introduced as an ornamental in Hawaii (*M. axillaris* [Hook. f.] Walp.). Knotweeds differ from all these species by being non-vining, perennial, and herbaceous. That is, they are long-lived plants that die-back and re-sprout anew each year from energy stored in the roots. Himalayan knotweed, *Persicaria*

wallichii Greuter & Burdet, is also introduced and invasive in North America and is sometimes lumped with the *Fallopia* species for management purposes. While in the same plant family, it is not closely related, and the biocontrol agent currently used against *Fallopia* knotweeds will not attack Himalayan knotweed.

The related *Fallopia* and *Muehlenbeckia* species and the unrelated Himalayan knotweed are listed in Table 2-1. Less related species in North America that have an appearance similar to knotweeds are also included, along with key characteristics that can be used to differentiate the look-alikes.

Classification

(In line with Freeman and Reveal 2005, Flora of North America Vol. 5)

Ranking	Scientific Name	Common Name
Kingdom	Plantae	Plants
Subkingdom	Tracheobionta	Vascular plants
Superdivision	Spermatophyta	Seed plants
Division	Magnoliophyta	Flowering plants
Class	Magnoliopsida	Dicotyledons
Subclass	Caryophyllidae	
Order	Polygonales	
Family	Polygonaceae	Buckwheat family
Genus	*Fallopia*	False-buckwheat
Species	*Fallopia japonica* (Houtt.) Ronse Decraene	Japanese knotweed
Species	*Fallopia sachalinensis* (F. Schmidt) Ronse Decraene	Giant knotweed
Species	*Fallopia* × *bohemica* (Chrtek & Chrtková) J.P. Bailey	Bohemian knotweed

Synonyms

Japanese knotweed: Japanese bamboo, Japanese fleeceflower, *Polygonum cuspidatum* Siebold & Zucc., *Reynoutria japonica* Houtt.

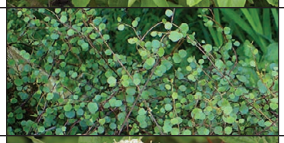
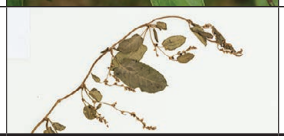
Giant knotweed: Sakhalin knotweed, *Polygonum sachalinense* F. Schmidt, *Reynoutria sachalinensis* (F. Schmidt) Nakai

Bohemian knotweed: hybrid knotweed, *Polygonum* × *bohemicum* (Chrtek & Chrtková) Zika & Jacobson, *Reynoutria* × *bohemica* Chrtek & Chrtková *F.* × *bohemica* [Chrtek & Chrtková]

Description

Knotweeds are upright herbaceous perennials that typically grow 3.3-10 feet (1-3 m) tall. They have a deep taproot and are also rhizomatous, meaning that they spread laterally through a network of subterranean stems that send up rapidly growing stalks in the spring (Figure 2-2a). Their rhizomes may extend up to 65 feet (20 m) laterally. Their above-ground stems are hollow, smooth, jointed and swollen at the nodes, and often woody at their base (Figure 2-2b). Their leaves are alternately arranged along the stem. Knotweed flowers are small, greenish to creamy-white, and have five petal-like sepals (Figure 2-2c). They grow in branched clusters from leaf axils near stem tips. Knotweed fruits are papery and have three wings (Figure 2-2d).

Table 2-1. Comparison of species present in continental North America that are either related to or resemble knotweeds, and key traits for differentiation.

Species	Image	Characteristics
Bamboo *Phyllostachys* spp. Grass family Exotic perennial grasses		Bamboo species are similar to knotweeds by having jointed, hollow stems and rhizomatous root systems. They differ by growing much taller (up to 40 feet or 12.2 m), lacking ocrea, having very narrow, lance-shaped leaves, and rarely flowering.
Black bindweed *Fallopia convolvulus* Buckwheat family Exotic annual vine		Black bindweed has similar ocrea and flowers. It differs from knotweeds by being a vine, growing as an annual, lacking rhizomes, having slender stems, and having smaller heart-shaped leaves up to 2.5 inches (6 cm) long.
Bukhara fleeceflower *Fallopia baldshuanica* Buckwheat family Exotic perennial vine		Bukhara fleeceflower has similar ocrea and flowers. It differs from knotweeds by flowering more profusely, growing as a vine, lacking rhizomes, having slender stems, and having smaller triangular leaves up to 4 inches (10 cm) long.
Climbing false buckwheat *Fallopia scandens* Buckwheat family Native perennial vine		Climbing false buckwheat has similar ocrea and flowers. It differs from knotweeds by growing as a vine, lacking rhizomes, having slender stems, and having smaller heart-shaped leaves up to 4 inches (10 cm) long.
Copse bindweed *Fallopia dumetorum* Buckwheat family Exotic annual vine		Copse bindweed has similar ocrea and flowers. It differs from knotweeds by being a vine, growing as an annual, lacking rhizomes, having slender stems, and having smaller heart-shaped leaves up to 2.5 inches (6 cm) long.
Fringed black bindweed *Fallopia cilinodis* Buckwheat family Native perennial vine		Fringed black bindweed has similar ocrea and flowers. It differs from knotweeds by growing as a vine, lacking rhizomes, having slender stems, and having smaller heart-shaped leaves up to 2.5 inches (6 cm) long with a fringe of hairs along their margins.
Himalayan knotweed *Persicaria wallichii* Buckwheat family Exotic perennial forb		Himalayan knotweed resembles knotweeds with its similar jointed, hollow stems and rhizomatous root system. It differs by having narrower, lance-shaped leaves up to 10 inches (25 cm) long by 2 inches (5 cm) wide. Its papery ocrea are also long and pointed.
Lilac *Syringa vulgaris* Olive family Exotic perennial shrub		Lilac resembles knotweeds in stem height and growth form. Lilac also has heart-shaped leaves, clustered flowers, and a suckering root system. It differs by having smaller, opposite leaves (up to 5 inches or 13 cm long), solid stems, and purple flowers.
Maidenhair vine *Muehlenbeckia complexa* Buckwheat family Exotic perennial vine		Maidenhair vine has similar ocrea and flowers. It differs from knotweeds by growing as a sprawling vine, lacking rhizomes, having slender stems, and having very small round, leathery leaves 0.4 inches (1 cm) long.
Redosier dogwood *Cornus sericea* Dogwood family Native perennial shrub		Redosier dogwood resembles knotweeds in stem height, growth form, and preferred riparian habit. It also has clusters of small white flowers and a spreading root system. It differs by having smaller, opposite leaves (5 inches or 13 cm long), solid stems, and berry fruit.
Wirevine *Muehlenbeckia hastulata* Buckwheat family Exotic perennial vine		Wirevine has similar ocrea and flowers. It differs from knotweeds by growing as a woody vine, lacking rhizomes, having slender stems, and having small oval to triangular leaves up to 1.5 inches (4 cm) long that are sometimes leathery.

Credits: Golden bamboo: © Eric Keith, iNaturalist.org; black bindweed: Olivier Pichard; Bukhara fleeceflower: Noebse; climbing false buckwheat: © Yasingi, iNaturalist.org; copse bindweed: Stefan.lefnaer; fringed black bindweed: © Mike V.A. Burrell, iNaturalist.org; Himalayan knotweed: Jennifer Andreas, Washington State University Extension; lilac: Georges Jansoone; maidenhair vine: Krzysztof Ziarnek, Kenraiz; redosier dogwood: Rob Routledge, Sault College, butgwood.org; wirevine: New York Botanical Garden, Steere Herbarium

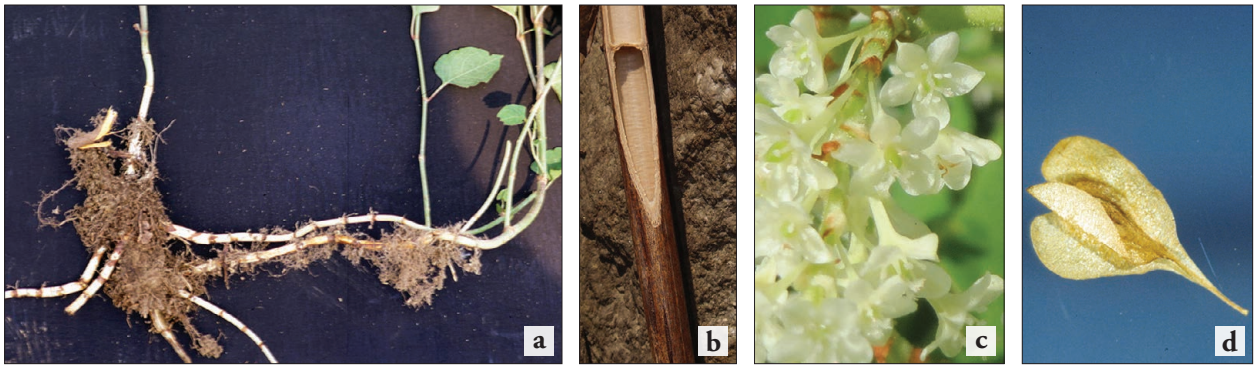


Figure 2-2. Traits characteristic of knotweeds (all images are of Japanese knotweed). a. rhizomes and new shoots; b. hollow stem; c. flowers with petal-like sepals; d. fruit (Credits: a Ohio State Weed Lab, Ohio State University; b Leslie J. Mehrhoff, University of Connecticut; c Andreas Rockstein; d Ken Chamberlain, Ohio State University)(a,b,d bugwood.org)

Differentiating Knotweeds

Although the three knotweeds are often lumped together under the common name "Japanese knotweed" or even just "knotweed," it is important to know the species identity of an infestation for management purposes. The species have different levels of vulnerability to herbicides and to different host races of the biological control agent (see chapter 3). The most reliable means for differentiating knotweeds is comparing their leaves. Leaf size, shape, and texture differ markedly between Japanese and giant knotweed. Because Bohemian knotweed is their hybrid, it has features intermediate between the two. Japanese knotweed has relatively small leaves, 3-7 inches (7.6-18 cm) long and 2-5 inches (5-12.7 cm) wide, with a squared-off base and abruptly pointed tip (Figure 2-3a). Japanese knotweed leaves are hairless with barely-visible bumps in place of hairs on their undersides Figure 2-3b). Giant knotweed has much larger leaves that are 6-12+ inches (15-30+ cm) long and 4-10 inches (10-25 cm) wide (Figure 2-3a). They have a heart-shaped base, tapering tip, somewhat wavy margins, and long, fine, wavy hairs on their undersides (visible along leaf veins, Figure 2-3c).

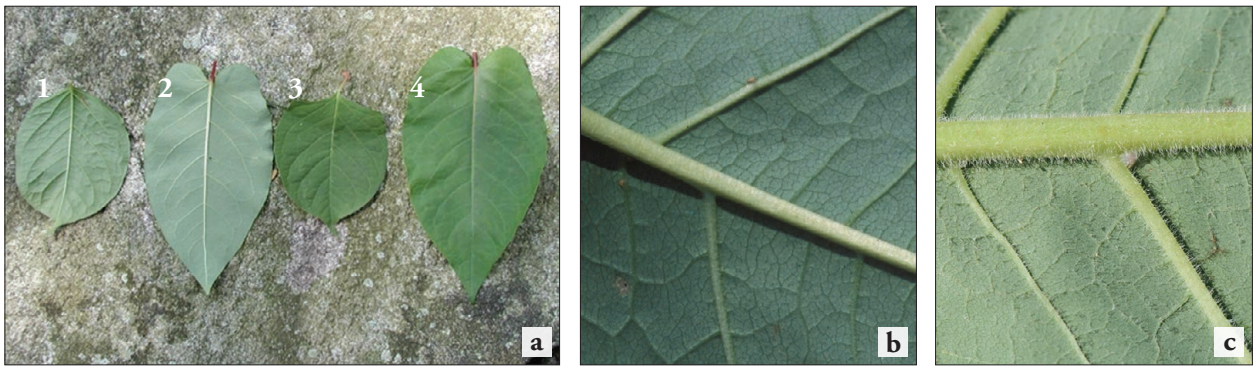


Figure 2-3. Comparison of Japanese and giant knotweed leaves. a. Japanese knotweed underside (leaf 1) and top side (leaf 3), and giant knotweed underside (leaf 2) and top side (leaf 4); b. Japanese knotweed leaf underside with no hairs; c. giant knotweed leaf underside with hairs (Credits: a Leslie J. Mehrhoff, University of Connecticut, bugwood.org; b James H. Miller, USDA Forest Service, bugwood.org; c © Cheryl Comeau Beaton, The Electronic Field Guide to the Invasive Plants of Nantucket, http://efg.cs.umb.edu/nantucket/)

There are a few other differences between the knotweed species, though not all of these are pronounced in all knotweed infestations. While Japanese knotweed typically only grows to 10 feet (3 m) tall, some giant knotweed and Bohemian knotweed plants may grow as tall as 13 feet (4 m). Japanese knotweed stems are often reddish when young, turning green with age (Figure 2-4a). Giant knotweed stems are more or less pale green at all stages (Figure 2-4b). Japanese flower clusters (inflorescences) are 3-6 inches (7.6-15 cm) long, and the leaf immediately beneath each flower cluster may be shorter than or the same length as the flower cluster (Figure 2-4c). Giant knotweed flower clusters are typically up to 4 inches (10 cm) long, and the leaf immediately beneath each flower cluster is always much longer than the flower cluster (Figure 2-4c). Japanese knotweed fruits are approximately 0.4 inches (10 mm) long while giant knotweed fruits are typically up to 0.6 inches (15 mm) long. Table 2-2 contains comparison images for select features of Japanese, Bohemian, and giant knotweed.

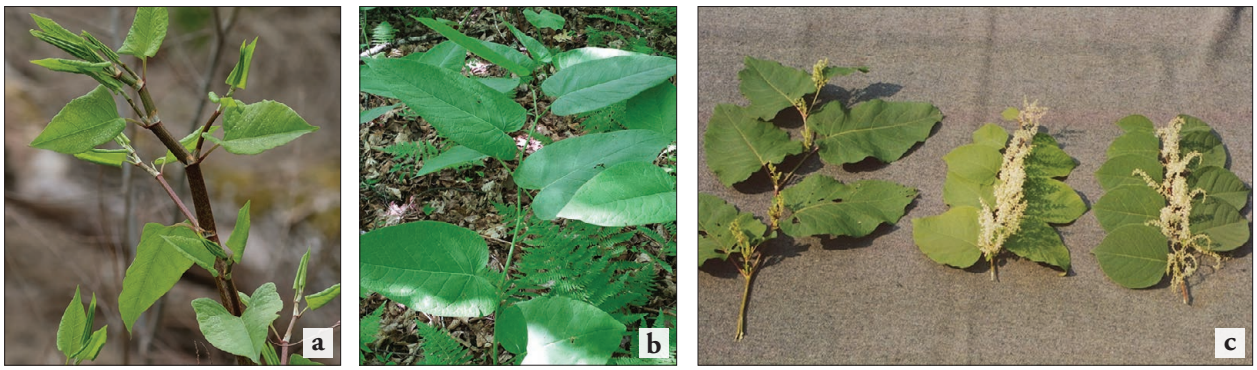


Figure 2-4. Comparison of knotweed traits a. young Japanese knotweed with reddish stem; b. young giant knotweed with green stem; c. flower clusters and subtending leaves of giant knotweed (left), Bohemian knotweed (center), and Japanese knotweed (right) (Credits: a © Mirko Schoenitz, iNaturalist.org; b © Tom Norton, iNaturalist.org; c Barbara Tokarska-Guzik, University of Silesia, bugwood.org)

Key to the Knotweed Species

(Adapted from Zika and Jacobson 2003. Use leaves from the middle of the stem for comparison as those at stem tips are most variable.)

1. Underside of leaves with fine hairs along leaf veins; base of leaves have heart-shaped bases; inflorescence is much shorter than its subtending leaf.....***Fallopia sachalinensis*** **(giant knotweed)**

1. Underside of leaves with very short spikes or bumps on the veins rather than hairs; leaf bases square to slightly heart shaped; inflorescence shorter or longer than subtending leaf.....**2**

> **2.** Underside of leaves with simple stout-based hairs or spikes; leaf base usually slightly heart-shaped; leaves are usually > 8 inches (20 cm) in length.....***Fallopia* × *bohemica*** **(Bohemian or hybrid knotweed)**
>
> **2.** Underside of leaves hairless or with only slight bumps in place of hairs; leaf bases squared off at base; largest leaves usually < 7 inches (18 cm) long.....***Fallopia japonica*** **(Japanese knotweed)**

Table 2-2. Comparison of knotweed plants, leaves, flowers, and fruits.

Japanese knotweed	Bohemian knotweed	Giant knotweed
		
		
		
		

Credits (Top to bottom): Japanese knotweed: 1 Jenn Grieser; 2 Jennifer Andreas, Washington State University Extension; 3 Bradley Kriekhaus, USDA Forest Service, 4 Leslie J. Mehrhoff, University of Connecticut (1,3,4 bugwood.org); Bohemian knotweed: 1 Sasha Shaw, King County Noxious Weed Control Program; 2 Wouter Koch, iNaturalist.org.; 3 Leslie J. Mehrhoff, University of Connecticut, 4 Barbara Tokarska-Guzik, University of Silesia (3,4 bugwood.org); giant knotweed: 1,2 Jennifer Andreas, Washington State University Extension; 3 Jan Samanek, Phytosanitary Administration; 4 Leslie J. Mehrhoff, University of Connecticut (3,4 bugwood.org)

History and Distribution of Knotweeds in North America

Japanese and giant knotweed were introduced to North America in the late 1800s as ornamentals and for erosion control before they escaped cultivation. The earliest herbarium record is from Yorkville, New York in 1873 (Barney 2006). Their hybrid Bohemian knotweed has been intentionally cultivated in North America and also naturally occurs in the field where both parent species overlap. Back-crossing with both parent species regularly occurs.

In North America, Japanese and Bohemian knotweed are present in 42 states and eight provinces (Figure 1-2a, repeated here in Figure 2-5a). Giant knotweed occurs in fewer states and provinces (Figure 1-2b, repeated here in Figure 2-5b), but is locally just as invasive. All three species have become most abundant and problematic in the Northeast and in the Pacific Northwest, but they are also increasingly problematic in the interior regions.

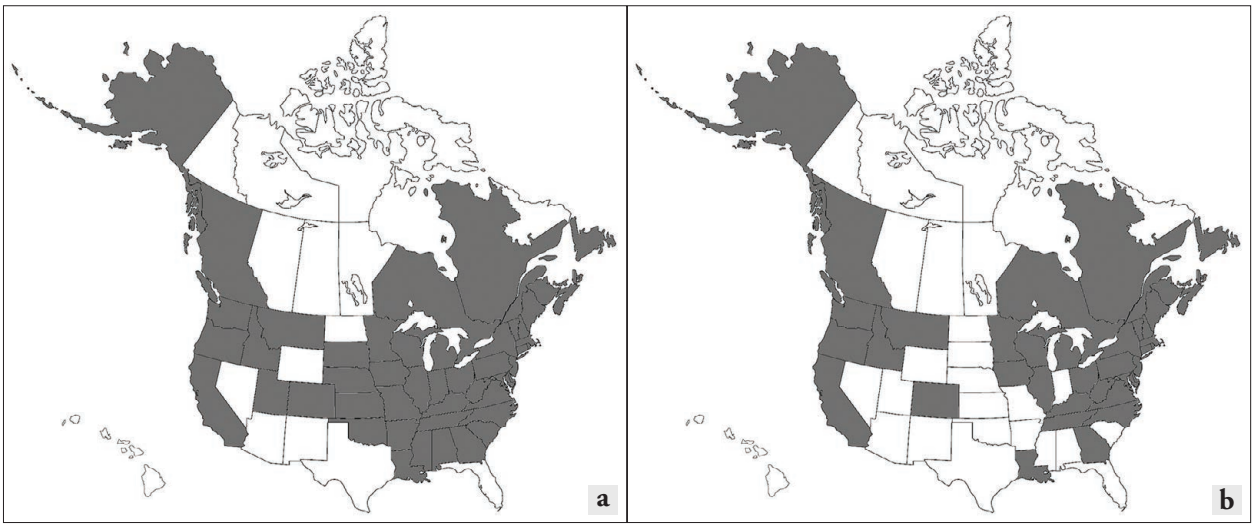


Figure 2-5. North American distribution of: a. Japanese and Bohemian knotweed; b. giant knotweed. Some states and provinces are more heavily infested than others (Credits: USDA PLANTS Database, EDDMapS)

Differentiation of knotweeds and their hybrid is challenging. Throughout North America, Bohemian knotweed is frequently misidentified as Japanese knotweed. In surveys of knotweed in the western USA, approximately 15% of the surveyed knotweed plants were pure giant knotweed, 15% were Japanese knotweed, and 70% were hybrids (McIver and Grevstad 2010, Gaskin et al. 2014). The northeastern USA appears to have a greater proportion of Japanese knotweed (Gammon and Kesseli 2010). In British Columbia, Canada, Japanese knotweed is the most common of the three species, based on records in the Invasive Alien Plant Program (IAPP 2012). The number of records for giant and Bohemian knotweed are approximately only 10% of the Japanese knotweed numbers.

Knotweeds are capable of growing in a wide variety of habitats (Figure 2-6a-l) including stream banks, river bars, and floodplains as well as human-disturbed sites such as roadsides, empty lots, and waste places. In their native Japan, knotweeds are also found at subalpine elevations on the scree slopes of volcanoes. Thus far, they have not invaded this habitat in North America. They can inhabit a variety of sunlight, soil, and moisture conditions, but have become especially problematic in full sun locations with moist soil (Stone 2010).

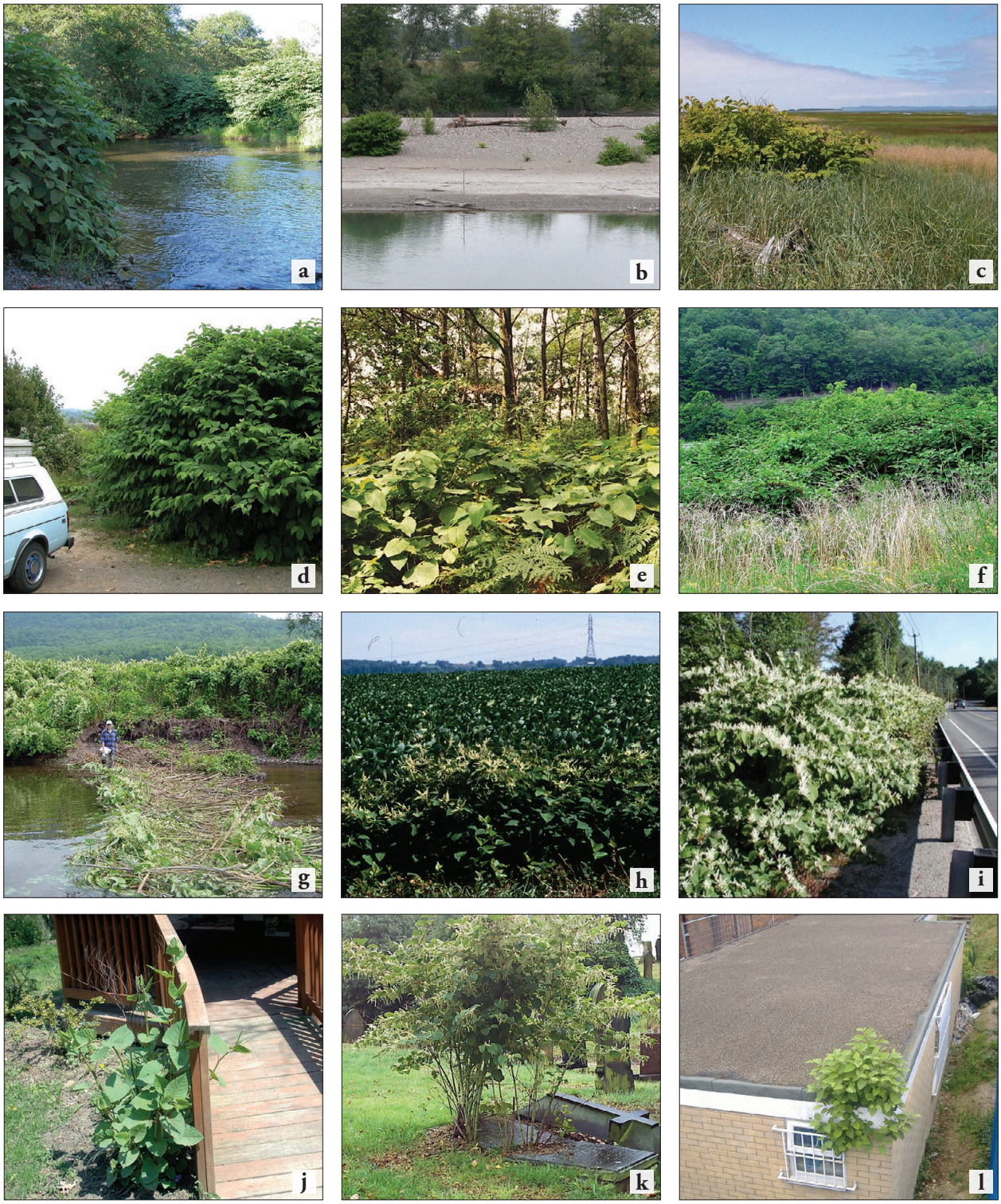


Figure 2-6. Knotweeds are capable of growing in a variety of habitats: a. river corridor; b. sand bar; c. edge of a coastal marsh; d. vacant lot; e. forest understory; f. open field; g. used in (and growing adjacent to) a beaver dam; h. edge of an agricultural field; i. roadside; j. park; k. emerging from a gravestone in a cemetery; l. gravel roof of a building (Credits: a-d Fritzi Grevstad, Oregon State University; e Barbara Tokarska-Guzik, University of Silesia; f Chris Evans, University of Illinois; g Mark Folsom; h Ohio State Weed Lab, Ohio State University; i Leslie J. Mehrhoff, University of Connecticut; j Chris Evans, University of Illinois; k,l Philip Rusted, Thurlow Countryside Management (R&D) (e-l bugwood.org)

Japanese knotweed's invasion of North America is still at an early stage. Using climate thresholds for knotweeds (based on their distribution in the United Kingdom), researchers were able to create a model showing the potential distribution of knotweeds in North America (Bourchier and Van Hezewijk 2010). Extended cold temperatures are one the of most important factors that limit the establishment and spread of knotweeds. In British Columbia, Japanese knotweed currently occupies only about half of the predicted suitable habitat in the province. For Ontario, the amount of suitable habitat has increased in recent years as average winter temperatures increased between 2000-2008. In the United States, the areas with large knotweed populations are likely to be more similar climatically to southern Ontario than to British Columbia, with generally warmer winter temperatures and longer growing seasons. As climate warming continues, a significant increase in the areas with Japanese knotweed in the United States is expected.

Knotweeds in their Native Range

Japanese knotweed is native to East Asia including Japan, China, Korea, and Taiwan. Giant knotweed is native to northern Japan and Sakhalin Island (Russia). The appearance of knotweeds in their native range varies much more than in North America, and there are entire forms in Japan that do not fit the descriptions by Zika and Jacobson (2003). For example, in Japan one can find giant knotweed plants without leaf hairs, Japanese knotweed plants with dense leaf hairs, and compact varieties that grow no taller than 3.3 feet (1 m) tall. Hybrids are present in the native range, but are not common. Bailey (2003) confirmed the presences of four subspecies of Japanese knotweed in Japan, and there are likely more. The variety that is invasive in North America is *Fallopia japonica* var. *japonica* (Houtt.) Ronse Decraene.

Knotweeds have many more herbivores attacking them in their native range than in their invasive range. In their native range, it is common to see plants that have leaves chewed up or deformed as a result of insect feeding or that are brown from disease (Figure 2-7, 2-8a-d). The plants in Japan also tend to be shorter in stature than they are in North America where they have fewer natural enemies. Japanese knotweed rarely forms large contiguous stands in Japan compared to its invaded range (although giant knotweed sometimes does so).

Figure 2-7. Knotweed attacked by native natural enemies in Japan (Credit: Fritzi Grevstad, Oregon State University)

Figure 2-8. In the native range, it is common to see knotweed leaves attacked by native insects and diseases (Credits: a-d Fritzi Grevstad, Oregon State University)

Reproduction and Life History

As herbaceous perennials, knotweeds sprout anew from rhizomes each spring, with shoots growing rapidly to a height of 10-13 feet (3-4 m) by mid-summer. Flowering occurs from late August to early September, and seeds ripen in October. Knotweeds in North America are usually reported as either dioecious (male and female flowers on separate plants) or gynodioecious (female and hermaphroditic flowers on separate plants) (Stone 2010). However, we have also found evidence for "leaky dioecy" in which some plants have female flowers (producing a large amount of seeds if there is a pollinator available), other plants have male flowers (producing no seeds), and others have hermaphroditic flowers (producing only a few seeds). Female flowers require pollen from a male or hermaphroditic plant, while hermaphroditic flowers can self-fertilize. Although the seeds have high germination rates in the laboratory (Figure 2-9a), seedling establishment in the field occurs infrequently (Forman and Kesseli 2003, Engler et al. 2011, Gaskin et al. 2014).

In North America, most knotweed infestations spread via clonal fragmentation of stems and rhizomes. Stem fragments as small as 1.6 inches (4 cm) have been observed regenerating (De Waal 2001, Figure 2-9b). Knotweeds spread readily along stream banks, where currents and flooding events cause erosion and fragmentation of rhizomes and stems that are subsequently dispersed downstream. Once a new plant establishes, it spreads by way of underground stems (rhizomes). Other common ways for knotweeds to spread include human redistribution of dirt and gravel, which may carry rhizomes or seeds with it (Figure 2-9c), or through roadside mowing and snowplowing.

Figure 2-9. Knotweed spread and reproduction a. knotweed seedling grown in a laboratory; b. stem fragment rooting and growing a new plant; c. soil pile contaminated with knotweeds (Credits: a,c Fritzi Grevstad, Oregon State University; b Timothy Miller, Washington State University)

Population Genetics

DNA sequence analyses of knotweeds collected throughout the Northwest (Gaskin et al. 2014) and the Northeast (Grimbsby et al. 2007, Gammon et al. 2007) reveal some interesting facts about knotweed diversity and distribution. Nearly all of the Japanese knotweed in North America is of a single female genotype that spread clonally through vegetative fragmentation (both naturally occurring and human assisted). Interestingly, this common genotype is identical to the Japanese knotweed genotype that invaded clonally throughout Great Britain (Gaskin et al. 2014, Hollingsworth and Bailey 2000). In contrast, giant knotweed and the hybrid Bohemian knotweed are genetically more diverse, which is likely the result of more frequent reproduction by seed.

Female knotweed plants require pollen from a separate male plant to set seed. The dominant Japanese knotweed clone, which bears only female flowers, can set seed but only when there are other knotweed plants bearing male flowers nearby, such as giant knotweed or Bohemian. Consequently, offspring of the North American Japanese knotweed are typically hybrids. Back-crossing between hybrids and parent species regularly occurs.

The ability of knotweeds to successfully spread by clonal fragmentation is apparent in some river systems that have extensive infestations of only one genotype. Examples include Big Creek (Oregon) with a single clone of giant knotweed, the Little Nestucca River (Oregon) with only the common female Japanese knotweed clone, and the Samish River (Washington) which was infested with a single male clone of Bohemian knotweed (Gaskin et al. 2014). The Bohemian clone of the Samish River also dominates several other river systems in the Northwest, and was by far the most common genotype, representing 69% of all Bohemian knotweed sampled and 55% of all knotweed plants sampled throughout the Pacific Northwest (Gaskin et al. 2014).

Ecology and Impacts

Invading knotweeds have impacts on existing plant and invertebrate communities and on properties of the soil. Dense knotweed thickets (Figure 2-10a,b) displace native plants through a combination of shading (Siemens and Blossey 2007), competition for nutrients, and the release of compounds that are harmful to other plants (allelopathy) (Murrell et al. 2011, Urgenson et al. 2012). Tree seedlings, for example, cannot grow in an established knotweed patch. The exclusion of trees along stream banks is potentially detrimental to fish and other stream inhabitants that benefit from the shade. While knotweeds provide shade along stream edges, tree shade extends much further into the stream or riverbed.

Figure 2-10. Dense knotweed infestations a. in a forest understory; b. in a mountain meadow (Credits: a Milan Zubrik, Forest Research Institute - Slovakia, bugwood.org; b Robert Emanual, bugwood.org)

Dense knotweed stands have no known value for wildlife. They typically contain fewer invertebrates compared to surrounding native vegetation (Beerling and Dawah 1993, Kappes et al. 2007, Gerber et al. 2008, McIver and Grevstad 2010). This is, in part, due to an absence of specialist herbivores (those feeding only on knotweeds) (McIver and Grevstad 2010). Compared to native plants, knotweeds are also relatively resistant to generalist herbivores (those feeding on a variety of plants) (Krebs et al. 2011). The decreased herbivore community found on knotweeds has consequences throughout the food chain. Predators of herbivores, such as spiders, are also found in reduced abundance in knotweed stands (Gerber et al. 2008). Maerz et al. (2005) found that weight gain in green frogs (*Rama clamitans* Latreille, Figure

2-11a) was greatly reduced in knotweed-invaded vs. non-invaded areas. They attribute the difference to a lack of prey availability. Similar negative food chain impacts are likely for fish and birds that rely on insects from riparian vegetation. In contrast, a few organisms that feed on decomposing organic matter (and their predators) among plant litter are relatively more abundant in knotweed stands than in surrounding native vegetation (Kappes et al. 2007, Gerber et al. 2008, Topp et al. 2008).

Knotweeds can have varying effects on soil erosion. Some stands accumulate more top soil compared to nearby native vegetation (Aguilera et al. 2010). However, along stream banks, knotweeds are less able to hold the surface soil than other plants due to a lack of fine surface roots. This can lead to increased erosion, especially during flood events (Child et al. 1992, Figure 2-11b).

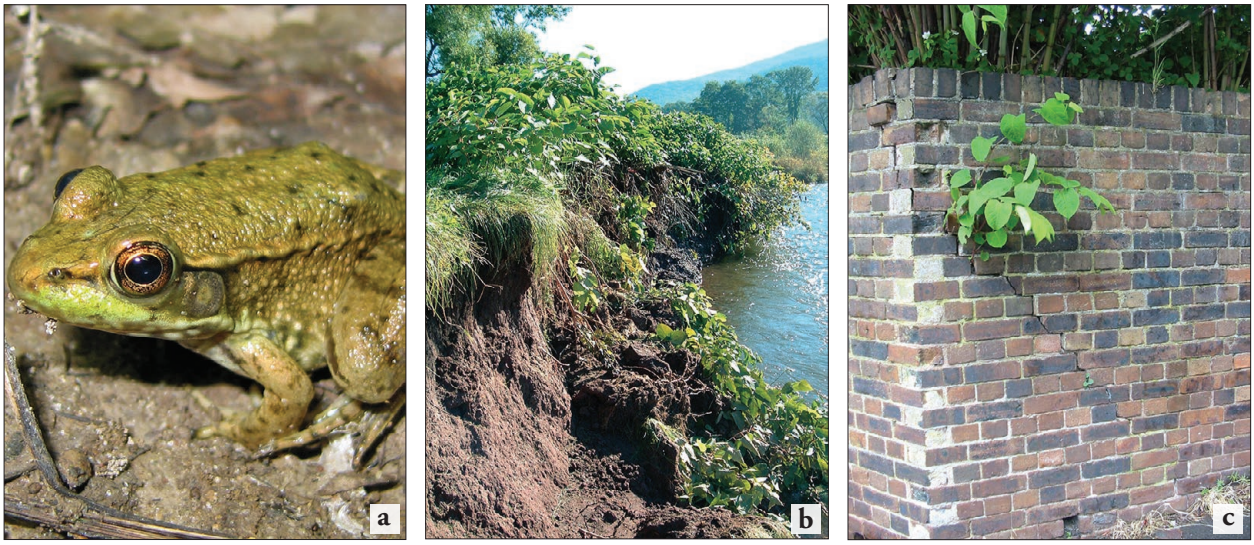


Figure 2-11. Knotweeds have negative impacts on: a. green frogs, b. soil erosion, c. infrastructure (Credits: a Greg Schechter; b Jenn Grieser, New York City Department of Environmental Protection, bugwood.org; c © Japanese Knotweed Solutions Ltd, www.jksl.com)

The soil in knotweed stands has been shown to have higher rates of nutrient cycling (Dassonville et al 2007, Aguilara et al. 2010) as compared to nearby non-invaded areas. Leaves that fall from senescing knotweed plants have low nitrogen compared to other leaf litter. The plants reabsorb much of the nitrogen into their roots before senescence. This can affect the amount of nitrogen supplied into stream ecosystems, which has subsequent effects on fish, invertebrates, and other wildlife (Urgenson et al. 2009).

Knotweeds also invade residential areas. Their forceful roots and rhizomes are capable of cracking concrete and asphalt, thus causing costly damage to roadways, parking lots, and foundations (Shaw and Seiger 2002, Figure 2-11c). In Britain, a home was reported by the BBC (2011) to have lost £250,000 (USD$352,000) in value as a result of knotweed invasion on the property. Some mortgage lenders in the UK will not finance a property if there is knotweed present. In Britain and many regions of North America, fines may be applied to homeowners that do not control knotweed. Originally planted as an ornamental, knotweed can be extremely difficult for homeowners to control or remove from their yards.

Pest Status of Knotweeds

Japanese knotweed is a regulated species in 26 states, and giant knotweed is regulated in 15 states (Table 2-2, Figure 2-13a,b). Regulated invasive plants are those whose control and/or movement are regulated by federal, state/provincial, or local law. Both Japanese and giant knotweed are regulated species in three Canadian provinces and are invasive species of concern in nine provinces. The hybrid Bohemian knotweed is specifically listed as regulated in the states of Colorado, Idaho, Illinois, Nebraska, New Hampshire, New York, Washington, and Wisconsin and the provinces of British Columbia and Alberta. However, because Bohemian knotweed has frequently been mistakenly identified in North America as Japanese knotweed (Zika and Jacobson 2003), it should be considered as included under Japanese knotweed on plant regulation lists. Knotweeds are listed among the world's worst invasive species by the World Conservation Union, having also invaded Europe, New Zealand, and Australia (Lowe et al. 2000).

Table 2-2. Regulation status of Japanese knotweed and giant knotweed in states/provinces where the plants have an official status. Because Bohemian knotweed has repeatedly been misidentified as Japanese knotweed, it should be considered regulated in the states/provinces where Japanese knotweed is regulated. Refer to state/provincial websites for current laws and definitions pertaining to each regulation category.

Species	State/Province	Status	Species	State/Province	Status
Japanese knotweed	Alabama	Class C noxious	Giant knotweed	California	Class B noxious
	California	Class B noxious		Colorado	List A noxious
	Colorado	List A noxious		Connecticut	Potentially invasive, banned
	Connecticut	Prohibited, banned		Idaho	Noxious, statewide control
	Idaho	Noxious, statewide control		Illinois	Prohibited exotic weed
	Illinois	Prohibited exotic weed		Minnesota	Specially regulated plant
	Iowa	Invasive plant species		Montana	Priority 1B
	Kentucky	Invasive plant, targeted		Nebraska	Noxious
	Maine	Prohibited invasive plant		New Hampshire	Prohibited invasive species
	Massachusetts	Prohibited		New York	Prohibited invasive species
	Michigan	Prohibited plant species		Oregon	Class B noxious
	Minnesota	Specially regulated plant		Washington	Class B noxious
	Montana	Priority 1B		Wisconsin	Prohibited species
	Nebraska	Noxious		British Columbia	Provincial noxious
	New Hampshire	Prohibited invasive species		Alberta	Prohibited noxious
	New York	Prohibited invasive species			
	Ohio	Prohibited noxious			
	Oregon	Class B noxious			
	Utah	1B noxious			
	Vermont	Class B noxious			
	Washington	Class B noxious			
	West Virginia	Noxious, invasive			
	Wisconsin	Restricted species			
	British Columbia	Provincial noxious			
	Alberta	Prohibited noxious			
	Manitoba	Tier 1 noxious			

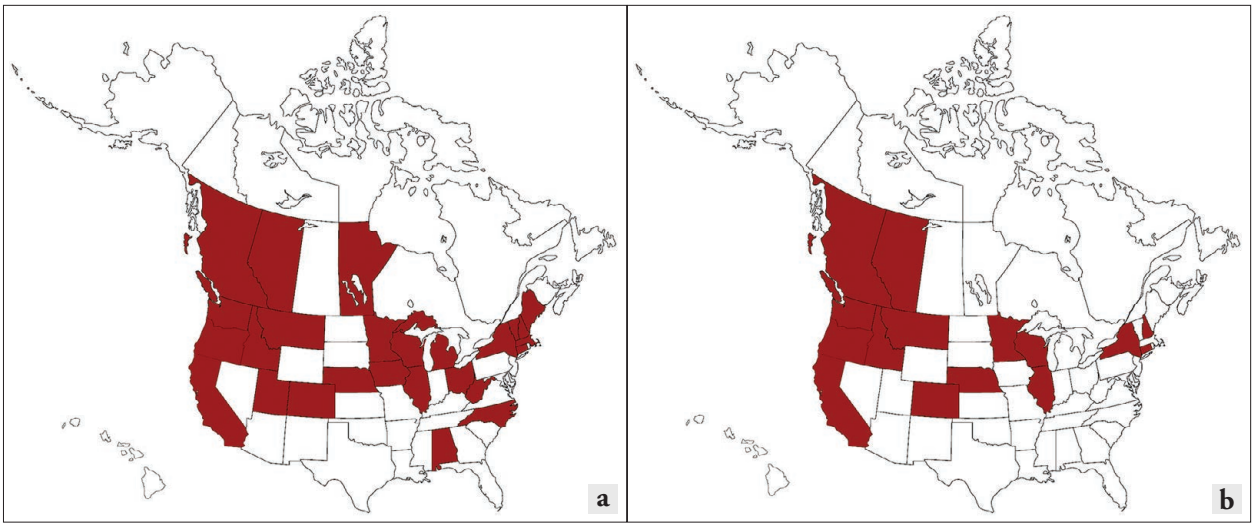


Figure 2-13. States and provinces where knotweeds are on official lists for regulation a. Japanese knotweed; b. giant knotweed (Note: because Bohemian knotweed has repeatedly been misidentified as Japanese knotweed, it should be considered regulated in the states/provinces where Japanese knotweed is regulated.)

Economic Costs of Knotweeds

Total estimates of the cost of knotweed removal are difficult to come by, in part because complete eradication from an area or river system requires repeated treatment over a very long time period. For the State of Washington, the cost of knotweed control between 2004 and 2016, including that spent by state, federal, and local agencies, was estimated to be about $30.4 million. This does not include control work carried out by private citizens or agencies not participating in the Washington Knotweed Control Program, nor does it include repairs to infrastructure damaged by knotweed (roads, foundations, etc.). In addition to the costs of control, it has been estimated that the state could lose an additional $4.5 million in annual business sales, 25 jobs, and $1.2 million in income if knotweed infestations are allowed to increase by just 1% per year (Community Attributes Inc. 2017).

Removal costs have been estimated in the United Kingdom based on the fees of private contractors carrying out 427 removal jobs. The direct costs of complete knotweed removal followed by site restoration ranges from £800-8,000 (USD$1,250-12,500) per infested 10.8 ft^2 (1 m^2), depending on the specific job. For the entire United Kingdom, the all-inclusive annual costs were estimated at over £165 million (USD$259 million). The costs include removal of knotweed for development projects, control by private landowners, devaluation of infested housing, control/restoration along riparian habitats, control along roads and railways, research on knotweed control methods, and support for local authorities to serve the public on knotweed related issues. The size of the infested range in North America is far larger than in the UK, and continuing local and regional expansions of knotweed populations are likely (Bourchier and Van Hezewijk 2010).

Additional costs of knotweed invasion include the potential environmental effects of herbicide applications. When broadcast spraying, death of adjacent or underlying non-target plants is often unavoidable. The surfactants used in some herbicide formulations are known to have detrimental effects on fish, amphibians, and aquatic invertebrates in experimental trials (Giesy et al. 2000, Relyea 2005).

CHAPTER 3: BIOLOGY AND HOST SPECIFICITY OF THE KNOTWEED BIOLOGICAL CONTROL AGENT

Introduction

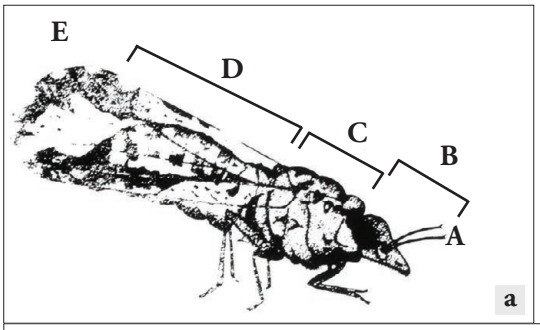
Classical biocontrol agents may be found in a number of taxonomic groups. The majority of approved biocontrol agents are invertebrates in the kingdom Animalia and the phylum Arthropoda. More specifically, most biocontrol agents are insects (class Insecta). In addition to insects, there are also mites (arthropods in the class Arachnida), nematodes (kingdom Animalia and phylum Nematoda), and fungi (kingdom Fungi) biocontrol agents. At the time of publication for this manual, only one biocontrol agent is currently approved for use in Canada and is being reviewed for use in the USA, the insect *Aphalara itadori* Shinji. This insect belongs to the order Hemiptera in the family Psyllidae.

Insects

Insects are the largest and most diverse class of animals. Basic knowledge of insect anatomy and life cycles will help in understanding insects and recognizing them in the field. All insects have an exoskeleton (a hard external skeleton) and a segmented body divided into three regions (head, thorax, and abdomen, Figure 3-1a). Adult insects have three pairs of segmented legs attached to the thorax, and a head with one pair each of compound eyes and antennae. Because insects have an external skeleton, they must shed their skeleton in order to grow. This process of shedding the exoskeleton is called molting. Larval stages between molts are called "instars." Adult insects do not grow or molt.

True Bugs, Including Psyllids (Order Hemiptera)

Most insects used in weed biocontrol have complete metamorphosis, which means they exhibit a life cycle with four distinct stages: egg, larva, pupa, and adult. The knotweed psyllid, *A. itadori*, is different. As part of the order Hemiptera, it undergoes incomplete metamorphosis with only three distinct life stages: egg, nymph, and adult. There is no true pupal stage for this order of insects. Adult Hemiptera possess two pairs of wings. The hind wings are membranous; the front wings are generally hardened at their base and membranous at their tips, but the knotweed psyllid has entirely membranous front wings.



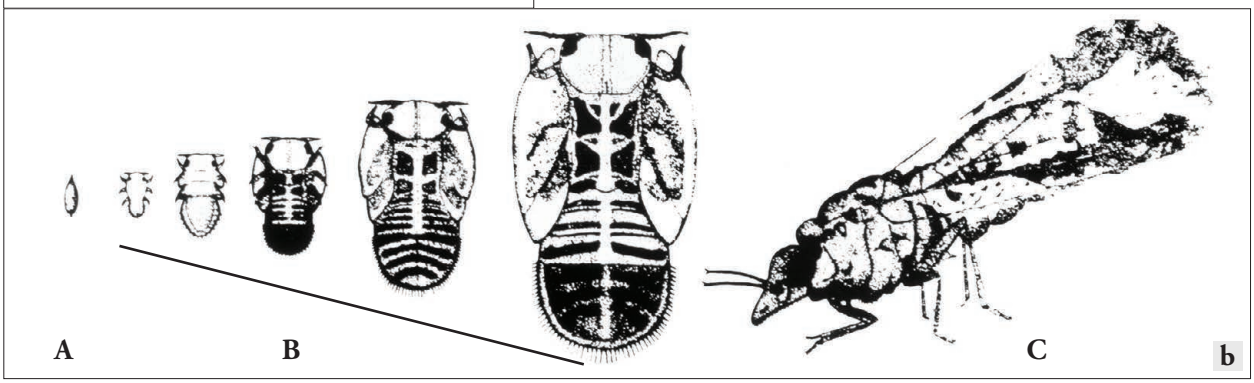


Figure 3-1. Line drawings of a. psyllid anatomy A. antennae, B. head, C. thorax, D. abdomen, E. wing; b. psyllid life cycles showing incomplete metamorphosis with A. egg, B. 5 nymphal instars, C. adult (Credits: a,b H.D. Catling, bugwood.org)

Psyllids (pronounced "sillid" with a silent p) are in a family of sap-feeding insects commonly referred to as "jumping plant lice" which contains more than 3000 species worldwide. The psyllids are part of the primitive group of true bugs called Sternorrhyncha, which also includes the aphids, white flies, and scale insects. Fossilized psyllids have been found that pre-date the appearance of flowering plants, perhaps originally feeding on conifers and club mosses. Modern psyllids are primarily associated with the flowering plants and tend to have a high degree of specialization. That is, each species uses just one or a small number of closely related plant species as its food plant.

The psyllid life cycle typically includes an egg stage, five nymph or juvenile stages, and a sexually reproducing and flight-capable adult stage (Figure 3-1b). Psyllid nymphs more closely resemble adults in each subsequent instar. Nymphs and adults feed by piercing their straw-like mouthparts into the plant and ingesting the sap. If adults or nymphs were to be manually moved while their mouthparts are "plugged in," this could result in damaged mouthparts and (later) death. Like other sap-feeding insects, psyllids excrete honeydew, which contains excess sugar from the plant that the insect doesn't need. In the case of psyllids, the honeydew crystallizes, forming a fuzzy material or distinct structure (lerp) on the back of the insect that in some cases can serve to deter predators (Figure 3-2).

Figure 3-2. Typical deposits of lerp from members of the Psyllidae family. Lerp is a structure of crystallized honeydew produced by psyllid nymphs that may serve as a protective cover. (Credit: John Jennings)

Psyllid adults tend to be highly effective dispersers, though much of their traveling is wind-assisted. Nymphs are much less mobile and typically only move short distances. It is common for psyllids to spend the summer months on their food plant, and then adults disperse to another species of sheltering plant for the winter (typically trees).

Overview: Natural Enemies of Knotweed in Japan

In their native range in Japan, knotweeds have many natural enemies. They include leaf-chewing beetles, stem-mining weevils and moths, sap-feeding insects such as aphids and psyllids, and disease-causing pathogens. Surveys for natural enemies were carried out as one of the first steps in the development of the knotweed biological control program (Figure 3-3). In total, over 180 different natural enemy species were found to use knotweed as a host (Shaw et al. 2009). Species that were found repeatedly and that were found to be damaging to knotweed were considered as possible biological control agents.

Surveys were also carried out in North America to determine if any of the Japanese natural enemies were already present and/or if any native natural enemies had potential as biological control agents (McIver and Grevstad 2010). None of the knotweed specialists from Japan were found in North America. Organisms commonly found using invasive knotweed as a food plant included slugs and snails, spittle bugs, sawflies, aphids, and caterpillars of various moth species. All of these natural enemies were determined to be generalists that feed on a variety of plant species. None were found at high enough densities to have an impact on knotweed growth or reproduction. In general, knotweed in North America is extremely robust and healthy with very little herbivory.

Figure 3-3. Surveying for natural enemies in Japan (Credit: Fritzi Grevstad, Oregon State University)

Candidate Agents *Not* Used

Four candidate biological control agents were tested in quarantine at Oregon State University. Three of these were found unsuitable due to their ability to feed and develop on native or economically important plants. These rejected natural enemies include the leaf beetle *Gallerucida bifasciata* Motschulsky (Figure 3-4a) and two leaf-tying and stem-boring moths from the family Crambidae, *Ostrinia latipennis* (Warren) (Figure 3-4b) and *Ostrinia ovalipennis* Ohno. The leaf beetle was found to be capable of feeding and developing on rhubarb (*Rheum rhabarbarum* L.) and native *Rumex* spp., and the moths could develop on native *Fallopia* spp. and buckwheat.

Other insects were similarly tested and ruled out by CABI (United Kingdom) for not being sufficiently host-specific. These included a stem-mining weevil *Lixus impressiventris* Roelofs (Figure 3-4c), a sawfly *Allantus luctifer* (Smith), an aphid *Machiatella itadori*, and a second strain of the leaf beetle *Gallerucida bifasciata* from southern Japan. The beetle *Euops chinensis* Voss (Figure 3-4d) from China was found to be host-specific in preliminary testing carried out in China (Wang et al. 2010). However, some important non-target plant species, such as the North American native *Fallopia* species, were never tested. Moreover, because this insect feeds only on the edges of leaves, it may not be very effective at controlling knotweeds.

In addition to the insect natural enemies, a pathogenic leaf-spot fungus, *Mycosphaerella polygoni-cuspidati* Hara, was extensively studied by CABI, but was found to cause restricted disease symptoms on a few key non-target plant species and was thus eliminated as a candidate for classical biological control. A non-reproductive form of this fungus is still being studied as a possible mycoherbicide that could be sprayed directly onto knotweeds plants, and patents have been applied for in the USA, Canada, UK, and the European Union.

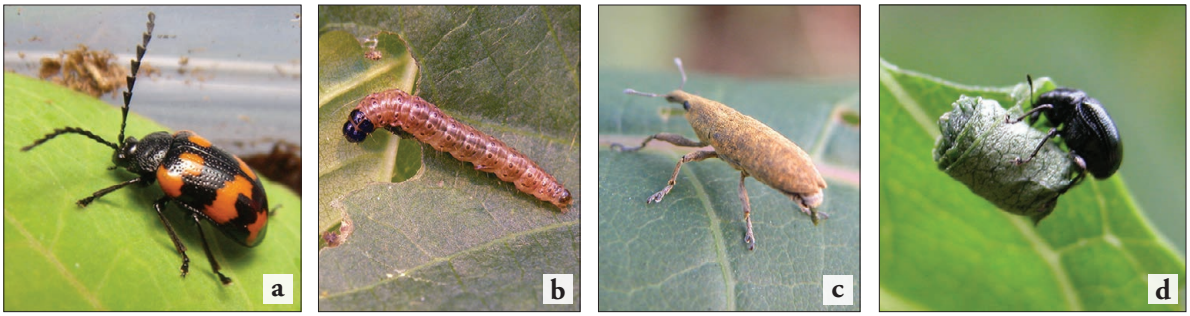


Figure 3-4. Knotweed candidate biocontrol agents rejected for use a. *Galerucella bifasciata* adult; b. *Ostrinia latipennis* larva; c. *Lixus impressiventris* adult; d. *Euops* sp. adult (Credits: a-d Fritzi Grevstad, Oregon State University)

Aphalara itadori Shinji

The Knotweed Psyllid

Quick Facts	
Order	Hemiptera
Family	Psyllidae
Native Distribution	East Asia
Original Source	Japan
First Release	2014
Non-target Effects	None reported

The most promising biocontrol agent found in Japan is the knotweed psyllid *Aphalara itadori*. All stages of this insect feed on the leaves and stems of knotweed by inserting their mouthparts and removing the sap (Figure 3-5). The psyllid is native throughout Japan where it can be found on both giant and Japanese knotweed. Initial host specificity tests for the psyllid were conducted on European plants for a release in the United Kingdom (CABI). Additional host-range testing was subsequently carried out for a North American release. It was field released into the United Kingdom in 2010 and into Canada in 2014. **As of the date of this publication, the knotweed psyllid is still under review by the USDA Animal and Plant Health Inspection Service for release into the United States.**

Figure 3-5. *Aphalara itadori* feeding on a knotweed stem (Credit: Fritzi Grevstad, Oregon State University)

General Description

Eggs are creamy-white and elongated (Figure 3-6a). Early nymphal instars are pale yellow to tan and more closely resemble adults through each subsequent instar (Figure 3-6b). Adults are typically 2 mm long, which is a little larger than a sesame seed. They are mottled tan and orange at first, turning darker brown with age. Their wings have tan veins and are translucent with mottled brown markings (Figure 3-6c). Females are slightly larger than males, and their pointed ovipositor is visible at the tip of their abdomen when viewed down-side up under a microscope.

Figure 3-6. *Aphalara itadori* a. eggs on plant stem (red arrows); b. nymphs and lerp; c. adult (Credits: a-c Fritzi Grevstad, Oregon State University)

Native Range and Habitat

The native range of *A. itadori* includes Japan, Korea, and the Kurile and Sakhalin Islands (Russia) (Burckhardt and Lauterer 1997). In surveys of Japan, it was found from sea level to 7,052 feet (2,150 m) above sea level, spanning a wide range of temperatures (Shaw et al. 2009). *Aphalara itadori* is relatively uncommon in Japan despite the abundance of its host plants. It was not found at the majority of the sites visited in a 17-day survey in Japan in 2007, but was occasionally found in high abundance. The psyllid is attacked by at least one parasitic wasp in Japan (possibly *Tamarixia* sp.) that was found in a late-stage nymph (Shaw et al. 2009). This psyllid is believed to thrive better in humid conditions than dry.

Life History

A female will lay up to 700 eggs on the surface of knotweed leaves and stems during her lifetime (Shaw et al. 2009). Eggs hatch after about 12 days, and the nymphs pass through 5 instars before becoming adults. For insects in general, the speed of development depends on the environmental temperature; higher temperatures support faster development. Development can be related to heat units (termed degree-days) that measure the daily heat above a lower developmental threshold. For the knotweed psyllid, development from egg to reproductive adult (a full generation) requires an average of 1,100 F degree-days (611 C degree-days) with development only occurring above a threshold of 44.5°F (6.9°C) (averaged among stages). This is the equivalent of 44 days at 70°F (21°C).

While feeding, nymphs excrete lerp, crystallized honeydew that is conspicuous as white strings and flakes on the plant surfaces (Figure 3-7a,b). Nymphs typically only move short distances on the plant surface, often seeking out the more sheltered locations on the plant, such as under leaf sheaths or inside of leaf curls. Feeding by nymphs causes leaves (especially of giant knotweed) to twist and curl, providing further protection from predators and the elements (Figure 3-7c). Psyllid feeding also causes Japanese knotweed to produce more, but smaller leaves, with an overall reduction in total leaf area. This can lead to a lower photosynthetic rate and slower plant growth. Adult *A. itadori* are winged, flight-capable, and quite mobile. It is not known whether there is a distinct flight season or how far the adults typically fly.

Only the adult stage overwinters. In late summer, in response to shortening day lengths, emerging adults will enter a state of dormancy (diapause) and will seek out safe overwintering sites. During this state, they neither feed nor reproduce. Adults that are entering diapause will turn distinctly darker in color with the brown patches on the wings appearing black (Figure 3-8). These are referred to as winter morph adults. In Japan, *A. itadori* adults have been found nestled into the bark of coniferous

Figure 3-7. *Aphalara itadori* a,b. deposits of lerp excreted by nymphs; c. twisting and curling damage to giant knotweed leaves (Credits: a-c Fritzi Grevstad, Oregon State University)

trees (specifically *Pinus densiflora* Zieb. & Zucc. and *Cryptomeria japonica* D. Don) (Miyatake, 2001), as is the case for other *Aphalara* species (Hodkinson 2009). However, they do not feed on these coniferous species. In field cages, they successfully overwinter on dead bark sections and even old knotweed stems, indicating that live trees are not needed.

Figure 3-8. Adult *Aphalara itadori* with overwintering coloration (Credit: Fritzi Grevstad, Oregon State University)

The number of generations of *A. itadori* in North America can be estimated based on experimentally determined temperature-dependent development rates (e.g. Myint et al. 2012) and local seasonal climate and photoperiod regimes. Experiments carried out in controlled environment chambers have shown that the Hokkaido psyllid (see next paragraph for discussion on host races) will enter diapause when daylengths are shorter than 14.9 hours, and the southern (Kyushu) population will enter diapause when daylengths are shorter than 14.1 hours (Grevstad in prep.). In most areas within the invasive range of knotweed, two generations are likely to be supported, though some southern locations could have up to three. The Kyushu host race may be more likely to go on for a 3rd generation because it takes shorter daylengths, later in summer, to induce diapause. The photoperiod response of the psyllids is likely to change with time as they adapt to local seasonal conditions.

Host Races

The psyllid populations that were imported for testing and release in North America originated from two source locations in Japan and represent two different host races. One population was collected from a site in southern Japan at 32.6° N latitude (Kyushu) and another from northern Japan at 42.5° N (Hokkaido) (see Figure 3-9). The two populations differ in their performance on the three knotweed target species (giant, Japanese, and hybrid Bohemian knotweed) (Grevstad et al. 2013). They also differ in their photoperiod response, as has been found in many other insects along the latitudinal gradient in Japan (Masaki 1999). Although both populations will be released in the USA, the majority of planned releases will be the Kyushu (southern) population because it performs better on Bohemian and Japanese knotweed. Releases of the Hokkaido (northern) psyllid, which feeds on giant knotweed,

are also planned for North America, but at far fewer locations since giant knotweed is not as widespread as Japanese and Bohemian knotweed. Select traits associated with each host race are listed in Table 3-1.

Both a lack of visible morphological differences and limited genetic differences indicate that the two psyllid host races belong to the same species. DNA sequence variation was compared between the two populations in the CO1 region (a highly variable region of mitochondrial DNA) and the small difference was found to be well within the range of variation expected within a species (E. Maw, Agriculture and Agri-food Canada, unpublished data). Additional DNA sequence analysis by Anderson et al. (2016) found that the two host races, and hybrids from crosses, can be distinguished using single nucleotide polymorphism (SNP) arrays. These SNPs will allow researchers to determine how the two host races contribute to the future populations as they spread and interbreed in the new geographic range.

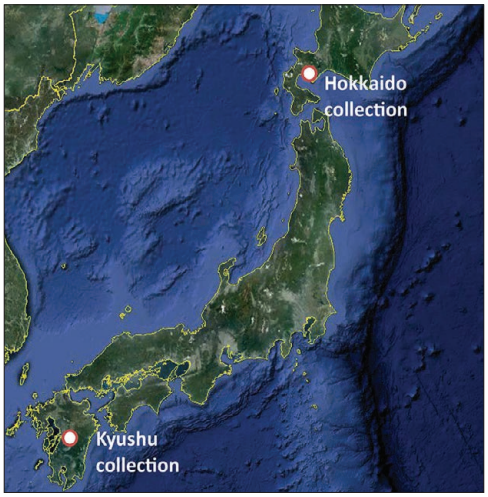


Figure 3-9. Locations in Japan where the two host races of *Aphalara itadori* originated (Credit: Fritzi Grevstad, Oregon State University, courtesy Google Earth)

Table 3-1. Traits associated with the two host races of the knotweed psyllid.

Hokkaido (Northern) Host race	Kyushu (Southern) Host race
Bigger impact on giant knotweed	Bigger impact on Japanese knotweed
Better reproduction on giant knotweed	Better reproduction on Japanese and hybrid Bohemian knotweeds
Better climate match to northern target range	Better climate match to southern target range

Host Specificity

Aphalara itadori has been reported from Japanese and giant knotweed (Burckhardt and Lauderer 1997). The genus *Aphalara* includes at least 17 species, primarily in Eurasia. The group is restricted to hosts within the Polygonaceae family including *Rumex*, *Persicaria*, *Polygonum,* and *Fallopia*. Most *Aphalara* species are restricted to just one or a few closely-related plant species (Burckhardt and Lauderer 1997).

In its native range, *Aphalara itadori* is known to feed only on knotweeds. Extensive laboratory testing of preference and performance measures on 70 different North American native and economically-important plant species confirmed this high level of specificity (Grevstad et al. 2013). The list of test plants used in the host specificity testing (Appendix I) was based on a centrifugal phylogenetic approach (Wapshere 1974) in which closely related plant species are emphasized for testing, as they are more likely to support development compared to distant species. The test plant list also included the categories recommended by the Technical Advisory Group (TAG) on Biological Control of Weeds (USDA-APHIS 2016). The TAG is an expert committee with representatives from USA federal regulatory, resource management, and environmental protection agencies, and regulatory counterparts from Canada and Mexico. All state, provincial, and federally listed threatened and endangered species in the Polygonaceae were either tested or represented using a closely-related surrogate species.

Initial Outcomes from the United Kingdom and Canada

At the time of this publication, populations of the knotweed psyllid are not yet established or permitted for release in the United States, but populations released into the United Kingdom (since 2010) and into Canada (since 2014) are becoming established.

United Kingdom

The Kyushu psyllid was mass-released between 2010 and 2013 but had limited success in establishing large populations at the eight isolated release sites. The initial focus of the work was to confirm pre-release screening data that the psyllid had no negative effects on native flora and fauna, and to date there has been no observable negative impact on native species. In 2014, a replicated caged field trial confirmed the safety of the agent for native invertebrates even when the psyllid was present in high densities. Based on these results, a release permit was granted for psyllid releases at riparian sites in 2015 and 2016, which were thought to offer better conditions for establishment. Following these releases, adults were found at all sites, though abundances were lower towards the end of the season. Early establishment (nymph stage) was observed at most sites, although there was not yet a significant impact on the knotweed. During spring 2016, overwintering was confirmed at only one southern site. For the first time, releases using winter morph adults (the overwintering generation with distinctly darker coloration) and a new psyllid strain (higher field adaptability) were carried out in autumn 2016. Surveys undertaken in spring 2017 confirmed overwintering survival at sites across the UK, but only at sites where the new stock psyllid was used, suggesting that more-recently collected Japanese adults are more robust than those that have spent 150 generations in the lab under Japanese summer conditions. There has been no detectable impact on the target knotweed to date.

Canada

Releases in Canada began four years after those in the UK, with between 500 and 1,000 overwintering adults of the Kyushu strain released into field cages on planted patches in Alberta and natural stands in British Columbia in 2014. Subsequent releases have progressed to larger numbers (between 25,000 to 30,000 per year) of both diapause (winter morph) and non-diapause adults, using open and caged sites in British Columbia, Alberta, and Ontario in 2017. The selection of release sites in multiple provinces was in order to cover large variations in climatic conditions and to assess overwintering capabilities of the psyllid. Adults have successfully overwintered in Alberta and British Columbia, but to date, no sustained psyllid populations at any single location across all release years have been confirmed. In laboratory rearing, when psyllids reach high densities and are having impact on knotweed plants, large deposits of lerp are clearly visible. Comparable lerp deposits have not been detected in the field and, similar to the UK, there has been no detectable impact on the knotweed to date.

The current preferred release method is to use adult psyllids and knotweed plants from the psyllid rearing program that are infested with all stages of the psyllids. Along with releasing adults, the infested plants are planted into the target knotweed patch. Psyllid nymphs are capable of crawling off these seeder plants onto the naturally-occurring knotweed stems.

Psyllids of the released Kyushu line were recollected from Alberta field cages after completing a generation in 2016. These “field line” psyllids were subsequently reared in the laboratory and released in summer 2017 in Ontario and Alberta. This field line persisted and completed a generation under extreme drought conditions at release sites in Alberta and under extremely high rainfall conditions in Ontario, suggesting it may be an improvement of the standard lab colony. Overwintering will be monitored in subsequent years to assess population survival and growth.

CHAPTER 4: IMPLEMENTING A KNOTWEED BIOLOGICAL CONTROL PROGRAM

Before You Begin

Biological control is one of many weed control methods available to land managers, but biological control is not appropriate for areas where knotweeds are not present or where a small number of localized populations occur. Biological control as a control method is best suited to knotweed populations in the later phases of the invasion curve, where knotweed populations are experiencing a rapid increase in distribution and abundance (containment), or where knotweeds are already widespread and abundant throughout their potential range (asset based protection, Figure 1-3 repeated here in Figure 4-1).

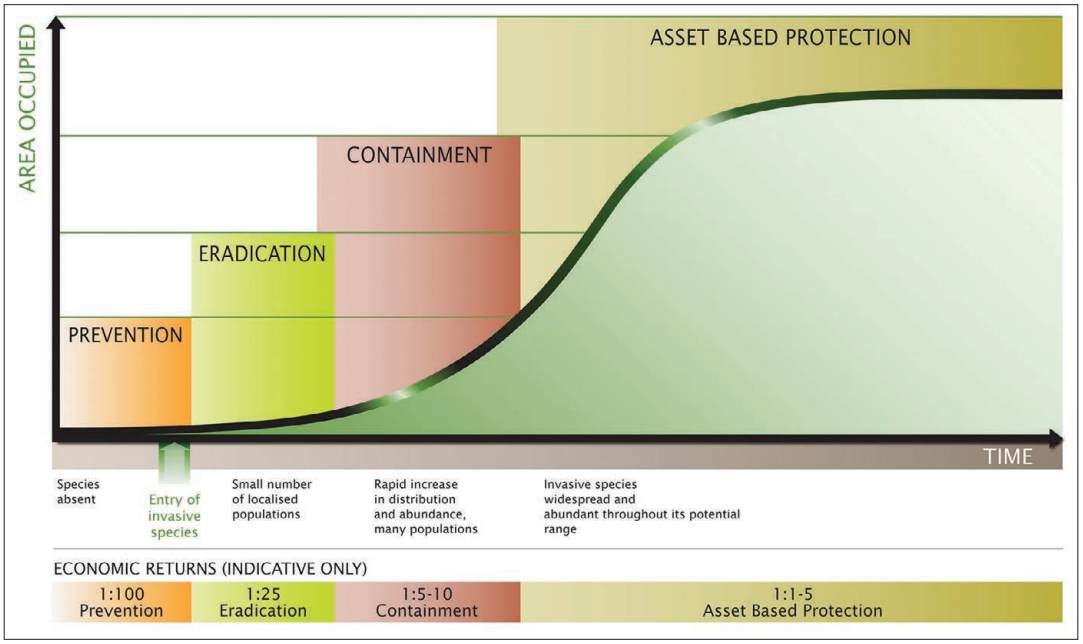


Figure 4-1. Generalized invasion curve showing actions appropriate to each stage (Credit: © State of Victoria, Department of Economic Development, Jobs, Transport and Resources, Reproduced with permission)

The knotweed biological control program is in its infancy, and proven results of significant impacts have not yet been obtained. Even if/when biocontrol proves effective against knotweeds in the field, the results of using biological control to treat knotweeds may vary greatly from site to site for a variety of reasons. Land managers should develop treatment programs that complement management activities and objectives unique to the area. This is accomplished by first understanding the scope of the knotweed problem, defining overall goals for the knotweed management program, and understanding the control methods available for accomplishing the goals.

Determining the Scope of the Problem

The first step should be to develop a distribution map of knotweeds at a scale that will allow you to address the problem in a manner consistent with your overall land management objectives and available weed management resources. The most appropriate scale may encompass a large landscape with a variety of site characteristics and land uses managed by many different land owners/managers– all of whom contribute to mapping efforts (Figure 4-2a). In large management areas with significant knotweed infestations and limited resources, aerial mapping of large patches of knotweed may be sufficient to identify priority areas for additional survey, mapping, and weed management activities. In other management areas with smaller, more discrete knotweed infestations, or where an infestation's characteristics affect your ability to meet management objectives, your weed management strategy might have to include more extensive mapping and analysis of the scope of the infestations (e.g., size, density, cover, or location in relation to roads and waterways over time) (Figure 4-2b).

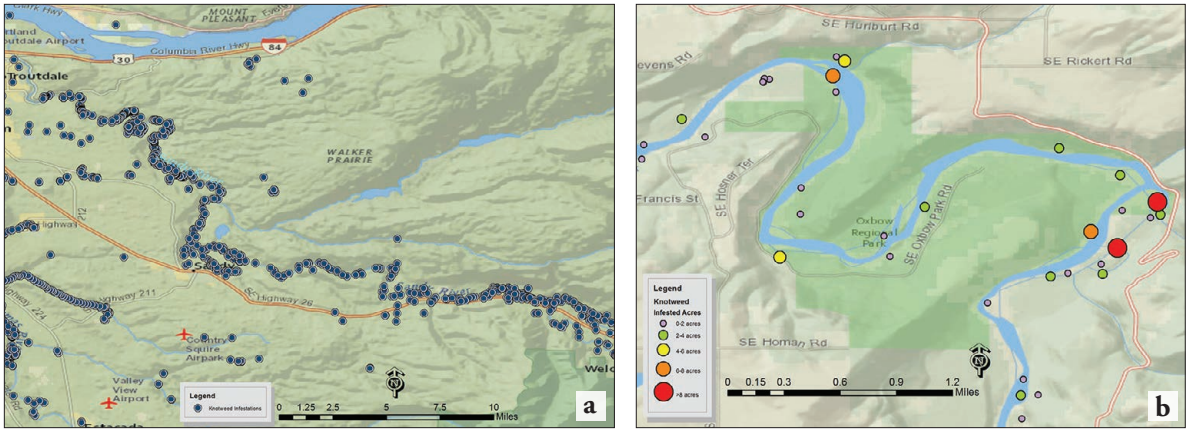


Figure 4-2. Japanese, giant, and hybrid Bohemian knotweed a. point distribution data in northern Oregon; b. patch density distribution data in northern Oregon's Oxbough Regional Park (Credits: a,b Becca Winston, MIA Consulting, EDDMapS)

In many cases, it may prove useful to check for existing knotweed distribution data before collecting your own. Several agencies and organizations maintain weed distribution databases, including state agricultural departments, provincial ministries (e.g., British Columbia IAPP Application), invasive plant/species councils, USDA PLANTS database, EDDMapS, and many others. EDDMapS can be particularly useful for land mangers interested in creating knotweed distribution maps for their area. By visiting www.eddmaps.org and creating a free account, users can view existing distribution maps for knotweeds or other weeds at the state, county, or point level. By selecting the GIS view option, users can view knotweed data on various backgrounds and zoomed into different scales, add hand drawn labels, boundaries, points and other shapes to the map, perform measurements such as perimeter estimates or distance between points, add new knotweed data from user shapefiles, edit the management status of various infestations, and print finished maps (see page 53 for more information on EDDMapS).

Defining Goals and Objectives

Goals broadly define the "what" or desired outcome of management; objectives define the "how" or specific activities through which desired outcomes can be achieved. To be effective, objectives must be SMART: specific, measurable, achievable, realistic, and timely. Defining **your** weed management goals and objectives is the crucial first step in developing a successful biological control program. By defining what you want to achieve, you will be able to determine if, when, and where you should use biological control.

As precisely as possible, you must define what will constitute a successful knotweed management program. For example, the objective of ". . . a noticeable reduction in knotweed density over the next ten years . . . " might be achievable, but it uses a subjective measurement of success that is open to observer bias. Alternatively, the objective of ". . . a 50% reduction in knotweed stems over the next three years . . ." is objectively measurable (and therefore SMART). If your goal is to reduce the abundance of knotweeds or slow their rate of spread, then biological control might be an appropriate weed management tool; however, by itself biological control will not completely and permanently remove knotweeds from the landscape. If your goal is to eradicate knotweeds, then you should plan to employ other weed control techniques instead of, or in addition to, biological control.

Understanding Knotweed Management Options

Once you determine the scope of your knotweed infestations and define your overall program goals, review all the weed control methods available (biological control, physical treatments, cultural practices, and herbicides), and determine the conditions (when, where, if, etc.) under which it might be appropriate to use each method or combination of methods. Consult state/provincial land management agencies, local extension offices, cooperative weed management area partners, or county weed coordinator/supervisors to learn about knotweed management activities underway or planned for your area, and the level and persistence of control that might be achieved by each. Because the focus of this manual is biological control, details pertaining to other control methods are summarized only briefly below. Contact the same agencies and groups mentioned above for more detailed information on other control methods.

Other Knotweed Control Methods

In the United States, several states have active control programs against knotweeds. Common control methods include herbicide foliar application and stem injection. Favored herbicide formulations contain the active ingredient glyphosate or imazapyr. In general, three years of intense herbicide management will reduce a knotweed population sufficiently for restoration to occur, including passive restoration from nearby plants or active restoration from revegetation efforts. Treated sites should be maintained and monitored for an additional 7+ years, but the site should never be disregarded under the assumption knotweed has been eradicated. Knotweed rhizomes are long-lived, and roots can extend up to 10 feet (3 m) deep. Even after knotweed patches have appeared dead for several years, they may still re-sprout.

Adding to the challenge of chemically controlling knotweeds, the use of herbicides is restricted (requiring special permits and methods) or even banned from riparian zones in some states and provinces to minimize possible ecological impacts from herbicides in these habitats. In British Columbia, the Canadian province with the most serious knotweed problem, broad spectrum herbicide use is restricted to 50 feet (15 m) above the high-water mark in riparian zones. Herbicide treatment below this level is only possible using glyphosate hand wipes or stem injection within 3.3 feet (1 m) of the high-water mark, which is extremely costly and time intensive. Knotweeds are commonly found inside this buffer zone, making long-term management difficult.

Isolated plants or small patches of knotweed may be removed by covering them for several years with sturdy tarps or by hand digging, but only if the root system is not yet well-established. Covering and digging require at least three to five years of continuous maintenance.

Identify the resources that will be available for weed management activities, and determine if they will be consistently available until you meet your weed management program objectives. If resources are not currently available, or will not be available consistently, identify what will happen at the treatment site if planned management activities are not implemented. This information will help you determine the best management activities to use as you initiate and continue your integrated knotweed management program.

With a map of knotweed infestations in your management area, an understanding of your land management goals, well-defined weed management objectives, and a list of the weed control methods available with the level of control you can realistically expect from each, you can identify sites where biological control would be a good fit, alone or in combination with other control methods.

Developing, Implementing, and Managing a Knotweed Biological Control Program

When biological control is deemed suitable for treating your knotweed infestations, there are several important factors to consider. These include selecting appropriate release sites, obtaining and releasing biocontrol agents, and monitoring the success of the program. Familiarity with all aspects of a biocontrol program before beginning will greatly facilitate its implementation and increase its chances of success. These items are discussed in their own sections in the following pages. If problems are encountered following the initiation of a biological control program, refer to the troubleshooting guide in Appendix II for potential solutions.

Selecting Biological Control Agent Release Sites

Establish Goals for Your Release Site

You must consider your overall management goals for a given site when you evaluate its suitability for the release of biological control agents. Suitability factors will differ depending on whether the release is to be a

1. general release, where biological control agents are simply released for knotweed management;
2. field insectary (nursery) release, used primarily to mass produce biological control agents for redistribution to other sites; or
3. research release, used to investigate biological control agent biology and/or the biocontrol agent's impact on the target weed and non-target plant community.

A site chosen to serve one of the roles listed above may also serve additional functions over time (e.g., biocontrol agents might eventually be collected for redistribution from a research or general release).

Determine Site Characteristics

For practical purposes, no knotweed infestation is too large for biocontrol releases; however, it might not be large enough (Figure 4-3a). Very small, isolated patches of knotweed may not be adequate for

biological control agent populations to build up and persist and are often better treated with other weed control methods, such as physical control or herbicides. An area with at least ¼ acre (0.1 ha) of knotweed is the minimum size to better ensure a successful biological control agent release site, but larger infestations are more desirable (Figure 4-3b), especially if the land manager hopes to someday use the release site as a field insectary. However, smaller infestations may be acceptable release sites in some cases, such as critical habitat zones where disturbance from physical control would be detrimental or sites where herbicides are prohibited. If the knotweed populations are extensive within a region but the individual population is below ¼ an acre (0.1 ha), biocontrol agents can be released to establish populations and encourage spread throughout the region. In addition, control of knotweed may be considered a low priority in some regions and be overlooked for intensive management. In these cases, land managers may wish to use biocontrol as a way to reduce further weed spread. Nevertheless, biocontrol agents disperse more easily in contiguous knotweed infestations than in infestations with only a few scattered plants and distant patches. However, sites with smaller patches and shorter plants facilitate the monitoring of psyllid adults. A site can be mowed in the spring and psyllids released onto the re-growth. The re-growth will not grow as tall over the remaining season as the original plants. If your biological control program goals involve evaluating the program's efficacy, establish permanent monitoring sites before you release any biocontrol agents. The monitoring sites will require regular inspections, so consider the site's ease of accessibility, terrain, and slope. See "Documenting, Monitoring, and Evaluating a Biological Control Program" on page 51 for more information on monitoring biocontrol agent release sites.

Figure 4-3. Knotweed infestations a. too small for biocontrol (single plant); b. appropriate for biocontrol (Credits: a Chris Evans, University of Illinois, bugwood.org; b Barbara Tokarska-Guzik, University of Silesia, bugwood.org)

Because knotweeds grow in a variety of habitats, potential release sites are likely to vary in their suitability for the knotweed psyllid. The suitability of a site is difficult to predict in advance. Consequently, multiple releases into separate sites will provide more opportunities for at least one population to establish in each region. More releases will also increase the likelihood that there is at least one very robust population that can serve as a nursery site to supply future releases.

Based on the knotweed psyllid's known biology, the following site characteristics may help improve establishment:

1. **Site not prone to excessive disturbance.** Sites with regular disturbances, such as a river bank that gets scoured in the winter floods, may not be the best choice because the insect mortality may be too high to allow population growth from year to year.

2. **Sunny location.** It is expected that the knotweed psyllid will develop better and have higher reproductive potential where sunshine provides extra warmth so they can develop faster.
3. **Not too close to the ocean.** Many insects cannot tolerate the cool damp weather and/or ocean salt spray.

Note Land Use and Disturbance Factors

Release sites should experience little to no regular disturbance. Abandoned fields/pastures, vacant lots, and natural areas are good choices for biological control agent releases. Sites where insecticides are used should not be used for biocontrol agent releases. Such sites include those near wetlands that are subject to mosquito abatement, rangelands that are subjected to grasshopper control, or infestations near agricultural fields or orchards where pesticide applications occur regularly. Roadside infestations along dirt or gravel roads with heavy traffic should also be avoided; extensive dust makes knotweed plants less palatable to biocontrol agents. Do not use sites where significant land use changes will take place, such as road construction, cultivation, building construction, and mineral or petroleum extraction. If supply of biocontrol agents is limited, prioritize release sites that are not regularly mowed, burned, or treated with herbicides. Knotweeds are intensively treated in many watersheds. Contact local agencies (e.g., conservation districts or county/district weed control programs) to determine whether biocontrol fits into the system-wide management plan.

Survey for Presence of Biological Control Agents

As of the writing of this publication, the knotweed psyllid is not widely established. However, over time, biocontrol agents will spread to new sites on their own, and may already be present at your target sites even if no one released them there. Always examine your prospective release sites to determine if knotweed biological control agents are already present. Look for the psyllid during any of its life stages and/or its characteristic deposits of lerp (see Figure 4-11). If the knotweed psyllid is already established at a site, you may want to consider making the release at another site where it is not yet present.

Record Ownership and Access

If you release biological control agents on private land, it is a good idea to select sites on land likely to have long-standing, stable ownership and management. Stable ownership will help you establish long-term agreements with a landowner, permitting access to the sites to sample or harvest biological control agents and collect biocontrol agent and vegetation data for the duration of the project. This is particularly important if you are establishing a field insectary site because five years or more of access may be required to complete biocontrol agent harvesting or data collection. General releases of biological control agents to control knotweed populations require less-frequent and short-term access; you may need to visit such a site only once or twice after initial release. When releasing biocontrol agents on private land, it may be a good idea to obtain the following:

- written permission from the landowner allowing use of the area as a release site
- written agreement with the landowner allowing access to the site for monitoring and collection for a period of at least six years (three years for establishment and buildup and three years for collection)
- permission to put a permanent marker at the site
- written agreement with the landowner that land management practices at the release site will not interfere with biological control agent activity

The above list can also be helpful for releases made on public land where the goal is to establish an insectary. In particular, an agreement should be reached that land management practices will not interfere with biological control agent activity (e.g., chemically spraying or physically destroying the weed infestation). It is often useful to visit the landowner or land manager at the release site annually to ensure they are reminded of the biological control endeavors and agreement. Always re-check with the landowner prior to inspecting release sites; in some cases the ownership may have changed.

You may wish to restrict access to release locations, especially research sites and insectaries, and allow only authorized project partners to visit the sites and collect biocontrol agents. The simplest approach is to select locations that are not visible to or accessible by the general public. To be practical, most if not all of your sites will be readily accessible, so in order to restrict access you should formalize arrangements with the landowner or manager. This will require you to post no-trespassing signs, install locks on gates, etc. (Figure 4-4).

Figure 4-4. "No disturbance" sign (Credit: Alan Martinson, Latah County Weed Control & Paul Brusven, Nez Perce BioControl Center)

Another consideration is physical access to a release site. You will need to drive to or near the release locations, so determine if travel on access roads might be interrupted by periodic flooding or inclement weather. You might have to accommodate occasional road closures by private landowners and public land managers for other reasons, such as wildlife protection.

Obtaining and Releasing Knotweed Biological Control Agents

Because the knotweed biological control program is in its infancy, at the time of this manual's publication the knotweed psyllid is unlikely to be widely available. Check first with your State's Department of Agriculture or Agriculture and Agri-Food Canada as they may have a rearing program for providing insects to landowners. Alternatively, they may be able to direct you to field locations where collections may be made. If available, biological control agents from local sources are best. Using local sources increases the likelihood that biocontrol agents are adapted to the climate and site conditions present and are available at appropriate times for release at your target infestation. Using locally sourced biological control agents also reduces the possibility of accidentally introducing biocontrol agent pathogens or natural enemies to your area. Local sources may include neighboring properties or locations in adjacent counties/districts. Remember that in the USA, interstate transport of biological control agents requires a USDA-APHIS-PPQ 526 Permit (see "Regulations Pertaining to the Transfer of Knotweed Biological Control Agents" on page 52). Get your permits early to avoid delays.

Field Collecting Knotweed Biological Control Agents

Planning and timing of field collections are critical. Adult psyllids will be emerging in April and May, though it is most efficient to scout the potential collection site well in advance to ensure psyllids are present at suitable densities. Ensure that all necessary collection supplies are on hand, and be sure to take the time to accurately identify the knotweed psyllid as there are many species of Psyllidae native to North America. Refer to Chapter 3 for psyllid identification features. Also be sure to collect the correct

psyllid host race for the target knotweed species. In general, giant knotweed requires the Hokkaido (northern) strain of *Aphalara itadori*, while Japanese and hybrid Bohemian knotweed require the Kyushu (southern) strain. You can be fairly certain you have the right host race if your collection is made from plants with similar morphology to the ones you are targeting (see the "Key to the Knotweed Species" in Chapter 2). Collect only on a day with good weather. Do not collect in the rain as the psyllids will likely hide and become difficult to find in rainy weather. In addition, excess moisture causes adverse effects, and biocontrol agents may drown in wet collection containers.

Field collection methods

Adult psyllids can be field collected using a beat sheet, aspirator, insect vacuum, or by collecting infested stems. **Nymphs and eggs are not easily transferred and will likely die if removed from the plant.**

Tapping (beat sheet): This method is effective for collecting psyllid adults. By using a tool such as a racket, adult psyllids can be tapped off knotweed stems and leaves and onto a beat sheet placed strategically beneath the foliage being tapped. Biocontrol agents tapped off the foliage can then be gathered directly using an aspirator (see below). Avoid disturbing the knotweed foliage before tapping because this will often cause psyllids to jump or fly away.

Aspirating: This method is effective for collecting psyllid adults. An aspirator is a device used to suck insects from a surface (such as a beat sheet) into a collection vial, though it can also be used to remove adult psyllids directly from knotweed plants. When collecting from the plant surface, it is important that the insect does not have its mouth parts "plugged in" when it is aspirated as this can result in damaged mouthparts and (later) a dead insect. To prevent this, the adult can first be tapped very lightly with the tip of the aspirator (or with a separate paint brush) which will cause it to detach. A variety of aspirators can be purchased from entomological, forestry, and biological supply companies, or you can construct them yourself. Simple aspirators are powered by mouth suction, manually by using an aspirating bulb, or mechanically using a modified hand vacuum. Mouth-powered aspirators contain rubber tubing for inhaling (Figure 4-5a) and an insect tube for collecting insects (Figure 4-5b)

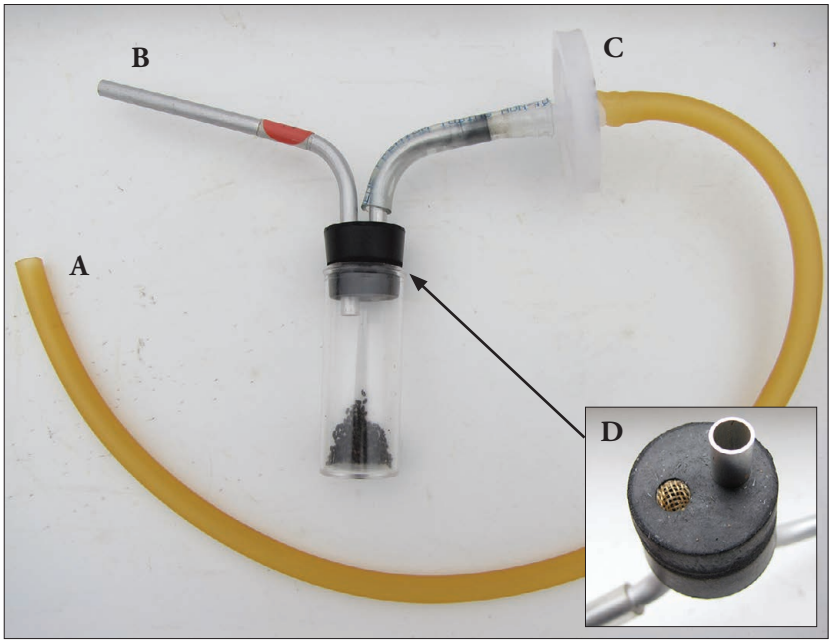


Figure 4-5. Componenents of an aspirator A. suction tube; B. insect tube; C. fine particle filter; D. larger particle screen (Credit: Jennifer Andreas, Washington State University Extension)

into a storage vial. Inline filters (e.g. HEPA filters, Figure 4-5c) are commercially available to prevent unintentional inhalation or swallowing of particles or debris during mouth aspiration. At the very least, mouth aspirators should be equipped with fine-mesh screening on the vial end of the tubing held in the mouth (Figure 4-5d) so that insects and small particles are not inhaled.

Vacuuming: A leaf blower with reverse capability, an industrial strength wet-dry vacuum cleaner, or a specialized insect vacuum sampler can be equipped with a nylon mesh net on the inside mouth of the blowing tube (held in place with a rubber band or bungee cord) to suck up adult psyllids. Retrofitting the blower/vacuum with a larger diameter tube (Figure 4-6) can help prevent insects from being crushed during the suction process. Rocks or debris vacuumed up may harm collected insects, so this method should be applied to foliage collections only. After vacuuming, net contents should be aspirated to separate psyllids from unwanted material.

Figure 4-6. Vacuum for collecting insects, using a large-diameter tube (Credit: Fritzi Grevstad, Oregon State University)

Collecting infested stems: Sometimes the easiest way to collect insects is to let them move on their own. An infested section of plant can be cut from one plant and then twist-tied so it rests close to fresh stems and leaves of a live, potted plant growing in a contained environment. This method should only be used in a laboratory/indoor setting where the knotweed stems and psyllids can be monitored. It is not recommended as a means for transferring the psyllid between field sites as the nymphs are likely to die when the shoots dry up before they find their way to the field plants.

Rearing Knotweed Biological Control Agents

Often, biocontrol agents cannot be field collected in sufficient numbers to use for new releases. In this case, it is best to rear the insects in a greenhouse or in field cages for at least one generation to build up numbers. In advance of obtaining the insects, grow knotweed plants in potting soil using pots that are at least one-gallon in size. Plants can be grown from seed (collected in October or November) or from sections of rhizomes collected in the spring. The rhizomes can usually be found a few inches underground at the edges of knotweed stands. They are easiest to dig in sandy areas or on river banks where the ground is eroding away. Place the rhizomes in trays of water until they sprout roots and shoots, then clip them into approximately 4 inch (10 cm) sections and bury them under ~ 1 inch (2.5 cm) of damp soil in the pots. When the plants are approximately 6-8 inches (15-20 cm) tall, fit the pots with a fine mesh sleeve cage (Figure 4-7) and add 30 adult psyllids to each cage. The knotweed psyllids require at least 15 hours of light per day to reproduce, so if this is not available naturally, you will need to provide artificial lighting. Keep the plants watered and at a moderate temperature (range 65-85°F or 18-29°C) until the next generation of adults emerge. Adults will begin to emerge after approximately 32 days, but it will take up to 44 days (plus or minus depending on temperature) before the majority of the next generation of adults has emerged.

Handling Knotweed Biocontrol Agents

Psyllids are delicate insects that need careful handling. As sap-feeders, they require fresh, non-wilted plant material in order to feed. They may be kept alive only for short periods of time on cut shoots or clipped leaves, requiring live plants to survive longer than a day or two. Nymphs, especially, must be

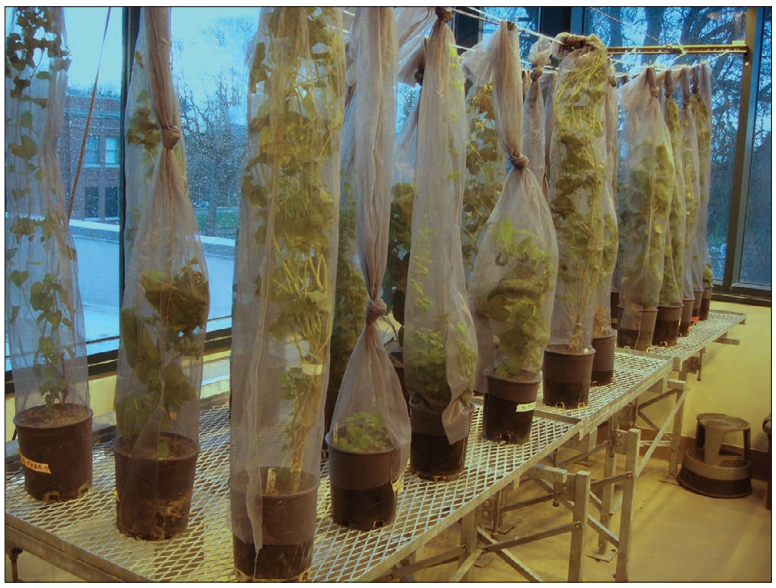


Figure 4-7. Cages for rearing the knotweed psyllid can be made from no-see-um netting (mesh with 1mm^2 holes) sewn into sleeves that fit tightly around the rim of the pots and loosely around the plants. Tie a knot in the top of the sleeve and clip it to a line above. (Credit: Fritzi Grevstad, Oregon State University)

handled with care. In a laboratory or indoor setting, a fine artist's paintbrush lightly dampened with water may be used to move individual nymphs without harming them. Dip the brush into water and wipe excess on a paper towel. First, lightly brush the insect's back to stimulate it to dislodge its mouthparts from the plant. You will know that it has dislodged when it crawls a short distance. You can then gently place the tip of the brush under the nymph to lift it and carefully set it on the leaf of another plant. For moving a larger number of psyllids, an infested section of stem or a leaf can be cut from one plant and secured with a twist-tie to another plant. This method is most useful for adults. Though it can be applied to nymphs indoors, it is not recommended as a means for transferring the psyllid between field sites as the nymphs are likely to die when the shoots dry up before they find their way to the field plants.

Storage and Release Containers for Knotweed Biological Control Agents

The manner in which biological control agents are handled during transportation to the release site will affect whether they will survive and multiply at the new site. To reduce mortality or injury, it is best to redistribute knotweed psyllids the same day they are collected.

Following collection, biocontrol agents should be transferred to storage/release containers intended to protect them (and to prevent them from escaping en route). When transferring field-collected or lab-reared adult psyllids, release containers should be rigid enough to resist crushing, but also ventilated to provide adequate airflow and reduce condensation. Good options for storage/release containers include:

1. sturdy plastic canisters with screw-top lids and screen-covered vent holes (Figure 4-8a);
2. small aspirator vials with a perforated lid snapped on over a sheet of mesh fabric, then taped on for security (Figure 4-8b);
3. unwaxed paperboard cartons (Figure 4-8c) with lids taped on for security. This option does not need vent holes because the paper is breathable;
4. tightly-sealing plastic storage containers used with screen-covered vent holes (Figure 4-8d); or
5. pop-up insect cages with fine netting (Figure 4-8e). This option provides less rigid protection from crushing, but the increased ventilation decreases problems caused by excess moisture.

Figure 4-8. Suitable knotweed psyllid storage and release containers a. plastic canister with screw-top lid and screened vent hole; b. aspirator vial with perforated and meshed lid; c. unwaxed paperboard carton; d. tightly-sealing plastic food container with mesh covered vents; e. fine mesh pop-up insect cage (Credits: a-c Fritzi Grevstad, Oregon State University; d,e Agriculture and Agri-Food Canada)

Vials can safely hold up to 30 adult insects and the canisters can hold as many as 200. Plastic storage containers and cages can hold even more. **Do not use glass or metal release containers**; they are breakable and make it difficult to regulate temperature, airflow, and humidity.

Filling less-ventilated release containers half full with crumpled paper towels or tissue paper can provide a substrate for the psyllids to rest on and hide in and helps regulate humidity. The other half should then be filled with knotweed sprigs. All plant material should be freshly collected and sized so it won't shift around inside the container (which could harm the insects). All sprigs should be free of seeds, flowers, dirt, spiders, and other insects and should not be placed in open water in the release container. Seal the release container lids with masking or label tape or with tightly fitting rubber bands. Be sure to label each container with (at least) the biological control agent(s) name, the number of biological control agents in the container, the collection date and site, and the name of the person(s) who did the collecting.

Transporting Knotweed Biological Control Agents

Keep the containers cool at all times

Once you collect and package the biocontrol agents, maintain them at temperatures between 50 and 65°F (10-18°C). If possible, place the release containers in large coolers equipped with frozen ice packs. Do not use ice cubes unless they are contained in a separate, closed, leak-proof container. Wrap the ice packs in crumpled newspaper or bubble wrap to prevent direct contact with release containers and to absorb any condensation that forms. Place extra packing material in coolers to prevent ice packs from shifting and damaging biocontrol agent containers. As an alternative to coolers with ice packs, electric car-charged coolers may be used, provided the cycle is set to cool and not warm. Always keep coolers out of direct sun, and only open them when you are ready to release the biocontrol agents. If you cannot release them immediately, place them in a refrigerator for short-term storage (no lower than 40°F or 4.4°C) until you transport or ship them (which should occur as soon as possible and preferably not longer than 24-48 hours).

Transporting short distances

If you can transport biocontrol agents to their release sites within three hours of collection, and release them the same day or early the next, you need not take any measures other than those already described.

Shipping long distances

If you will be shipping your biocontrol agents to their final destination, use a bonded carrier service with guaranteed overnight delivery (e.g., USPS, FedEx, UPS, or DHL) and send the recipient the tracking number. In such cases, the release containers should be placed in insulated shipping containers with one or more ice packs. Some specially designed foam shippers have pre-cut slots to hold small biocontrol agent containers and ice packs (Figure 4-9). This construction allows cool air to circulate but prevents direct contact between the ice and the release containers. Laboratory and medical suppliers sell foam "bioshippers" that are used to transport medical specimens or frozen foods. If neither foam product is available, you can use a heavy-duty plastic cooler. **Please note that for safety reasons, dry ice cannot be used for transporting biocontrol agents.**

Figure 4-9. Commercially made shipping container suitable for biocontrol agent transport (Credit: University of Idaho, bugwood.org)

Careful packaging is very important regardless of the shipping container you use. Ice packs need to be wrapped in crumpled newspaper, wrapping paper, or bubble wrap, and should be firmly taped to the inside walls of the shipping container to prevent them from bumping against and possibly crushing the release containers during shipping. Empty spaces in the shipping container should be loosely filled with crumbled or shredded paper, bubble wrap, packing "peanuts," or other soft, insulating material. Use enough insulation to prevent release containers and ice packs from shifting during shipment, but not so much that air movement is restricted. Enclose all paperwork accompanying the biocontrol agents (including copies of permits and release forms) before sealing the shipping container. For additional security and protection, you may place the sealed shipping containers or coolers inside cardboard boxes.

Other factors to consider

- Make your overnight shipping arrangements well before you collect your biological control agents, and make sure the carrier you select can guarantee overnight delivery.
- Plan collection and packaging schedules so that overnight shipments can be made early in the week. Avoid late-week shipments that may result in delivery on Friday through Sunday, potentially delaying release of the biocontrol agents for several days.
- Clearly label the contents of containers and specify that they contain perishable material.
- Check with a prospective courier to make sure that they can accept this type of cargo and will not treat the packages in ways that could harm the biological control agents. If the courier cannot guarantee that such treatments will not occur, choose a different carrier.
- Provide the receiver with a tracking number and verify someone will be there to accept the shipment.
- Releases should be made immediately upon receipt. If that is not possible, biocontrol agents should be checked for food depletion, excess moisture, and overcrowding and then be refrigerated.
- Have the receiver provide feedback to the shipper on the overall condition of the shipment. This can provide important guidance on packing/shipping methods.
- Note that USA interstate transport of biocontrol agents requires a USDA-APHIS-PPQ 526 Permit. Before biocontrol agents can be taken across national borders, an importation permit from the regulatory agency of the receiving country is required (USDA-APHIS in the USA and CFIA in Canada, see "Regulations Pertaining to the Transfer of Knotweed Biological Control Agents" on page 52).

Avoiding Common Shipping Mistakes

Crushing- Secure all material included in the shipping container so that blue ice, etc. does not become loose and move around in transit, thereby crushing, tearing or popping open release containers and killing or scattering the biocontrol agents inside.

Escape- Seal release containers securely with rubber bands or easily removable/resealable tape (e.g., masking tape) to prevent mobile biocontrol agents from escaping into the shipping container.

Excess heat- Do not expose release containers to direct sunlight or temperatures above 65°F (18°C). Avoid shipping delays that can expose biocontrol agents to high temperatures.

Excess moisture- Remove spilled or excess water in release and shipping containers. Do not ship weed sprigs with any type of water source (e.g., floral foam or tubes). Add crumpled paper towels to release containers to absorb incidental moisture or condensation.

Lack of ventilation- Provide adequate ventilation; use air-permeable release containers or make air holes in plastic containers with push pins or other small diameter tools, covering the holes with a fine mesh screen to prevent the escape of mobile biocontrol agents.

Starvation- Provide sufficient food. Do not store release containers with biocontrol agents more than 48 hours.

Stress- Provide root-, flower- and seed-free sprigs of the target weed (free also of other weed species' seeds, flowers, dirt, spiders, or other insects) and crumpled paper towels where biocontrol agents can shelter; avoid over-crowding.

Releasing Knotweed Biological Control Agents

Establish permanent location marker

Place a steel fence post or plastic/fiberglass pole as a marker at the release point (Figure 4-10a). Avoid wooden posts; they are vulnerable to weather and decay. Markers should be colorful and conspicuous. White, bright orange, pink, and red are preferred over yellow and green, which may blend into surrounding vegetation. In addition, white posts will not fade over time. Where conspicuous posts may encourage vandalism, mark your release sites with short, colorful plastic tent/surveyor's stakes or steel plates that can be tagged with release information and located later with a metal detector and GPS. Depending on the land ownership or management status at the release site, it may be necessary to attach a sign to the post or pole indicating a biological control release has occurred there and that the site should not be sprayed with chemicals or be mechanically disturbed (see Figure 4-4 on page 41). Where a sign is appropriate, the landowner/land manager and the local weed management authority (county, state, federal, and/or provincial) should be notified and given a map of the release location.

Record geographical coordinates at release point using GPS

Map coordinates of the site marker should be determined using a global positioning system device (GPS) or a GPS-capable tablet/smartphone. There are numerous free apps available for recording GPS coordinates on a tablet/smartphone (Figure 4-10b). Coordinates should complement but not replace a physical marker. Accurate coordinates will help re-locate release points if markers are damaged or removed. Along with the coordinates, be sure to record what coordinate system and datum you are using, e.g., latitude/longitude in WGS 84 or UTM in NAD83.

Prepare map

The map should be detailed (Figure 4-10c) and describe access to the release site, including roads, trails, and unique landmarks/terrain features that are not likely to change through time (e.g., large rocks or rocky outcrops, creeks, valleys, etc.). Avoid using ephemeral landmarks such as "red bush", "grazing cows", etc. and descriptors which may not be obvious to everyone, such as "the Miller place", or "where the old barn used to be", etc.. Use your vehicle's trip odometer to measure and record mileage between specified locations on your map, e.g., when you turn on to a new road, at cattle guards along the route, and where you park. The map should complement but not replace a physical marker and GPS coordinates. Maps are especially useful for long-term biological control programs in which more than one person will be involved or participants are likely to change. Maps are often necessary to locate release sites in remote locations or places physically difficult or confusing to access.

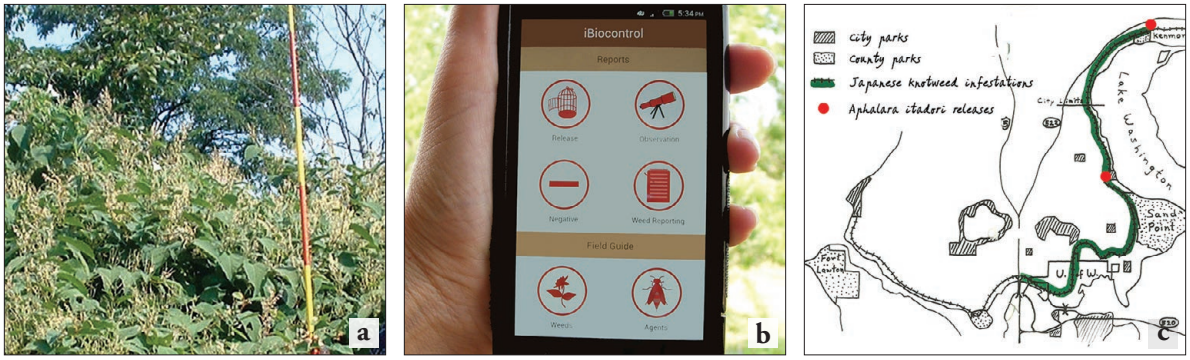


Figure 4-10. Biocontrol agent release site tools a. permanent marker; b. smartphone with free weed and biocontrol agent mapping app iBioControl; c. hand-drawn map illustrating release site (Credits: a Jenn Grieser, New York City Department of Environmental Protection, bugwood.org; b Rachel Winston, MIA Consulting; c Seattle Municipal Archives, modified)

Complete relevant paperwork at site

Your local land management agency/authority may have standard biocontrol agent release forms for you to complete. Typically, the information you provide includes a description of the site's physical location, including GPS-derived latitude, longitude, and elevation; a summary of its biological and physical characteristics and land use; the name(s) of the target weed and biocontrol agent(s) released; the number and life cycle stage of the agent(s) released; date and time of the release; weather conditions during the release; and the name(s) of the person(s) who released the biocontrol agents (see Sample Biological Control Agent Release Form in Appendix III). The best time to record this information is while you are at the field site. Consider using a smartphone and reporting app such as iBioControl. This free application uses EDDMapS (see page 53 for more information) to help county, state, and federal agencies track releases and occurrences of biological control agents of noxious weeds. Once back in the office, submit the information to your local weed control office, land management agency, or other relevant authority/database. **Always keep a copy for your own records.**

Set up photo point

A photo point is used to visually document changes in knotweed infestations and other components of the plant community over time following the release of biocontrol agents. Use a permanent feature in the background as a reference point (e.g., a mountain, large rocks, trees, or a permanent structure) and make sure each photo includes your release point marker. Pre- and post-release photographs should be taken from roughly the same place, using the same field of view, and at the same time of year. The best time of year is when the plants are in full flower. For knotweed this occurs from late August to early September. Yearly photographs can reveal a slow impact that is likely to begin with an impact on the abundance of flowers. Label all photos with the year and location; many smartphone and tablet apps such as GrassSnap or Theodolite do this automatically or with minimal input. Save all photos in a safe place (including back up) for future reference.

Choose your release method

Release in late April or May after danger of frost has passed, but when knotweed plants are still tender and growing. It is best to release the adult stage of the psyllid because they have the highest reproductive value. Nymphs may not transfer easily to new field plants and are vulnerable to predation. There are three general methods for releasing knotweed psyllids:

Method One—Setting out infested potted plants

If the recommended rearing method is used, releases can be made by simply transporting caged potted plants to the field site, nestling the potted plants among the field knotweed, and removing the sleeve cage (Figure 4-11). Releases should be made in the cool of morning or evening to avoid frenzied flight of the adult psyllids, as frequently happens at higher temperatures (they may disperse away from the site before they discover there is an adequate food source). Give the plants some extra water in the pot before leaving so any remaining nymphs complete their life cycles before the plants dry up. Using cut shoots to transfer psyllids is not recommended as the nymphs are likely to die when the shoots dry up before they find their way to the field plants. When using this

Figure 4-11. Method One—releasing the knotweed psyllid by setting out infested potted plants (Credit: Agriculture and Agri-Food Canada)

method, remove all potted plant material after the psyllids have successfully moved to adjacent plants (generally two weeks or more) to prevent the potted plants from sprouting and spreading at the site and possibly introducing new genetic material. Potted plants should not be placed in flood prone areas where they could be swept away. The use of potted plants in psyllid releases may require licensing or may be prohibited at some sites; check with local authorities.

Method Two—Caging adults directly onto field plants

If adult psyllids were obtained by field collection or in a mail shipment, they can be released into a sleeve cage placed over a knotweed shoot. This method has the advantage of establishing a cohort in a known location that can be followed throughout their development. Another advantage is that the cages can initially provide protection against predators.

Insert a tall bamboo stake into the ground next to a knotweed shoot. Place a fine mesh sleeve cage over both the stake and the shoot tip and secure it at the lower end with a wire or zip tie. Place approximately 30 adult psyllids into the sleeve and secure the top end. To avoid overloading the plant, remove the cage after a few days. The adults will disperse and lay additional eggs on other shoots. Mark the original shoot with a flag so that you can return to monitor hatching and development of the nymphs.

Method Three—Large field cages

Field cages that are large enough to walk into (Figure 4-12a) are expensive to purchase, but can be useful for keeping released insects in one location long enough to monitor their initial population growth (Figure 4-12b). The cages should be made of very durable fine mesh with a sturdy steel or pvc frame and have a zipper door for access. The frame can be attached to the ground with rebar stakes bent at the top end to hook over and pin down the lower crossbar of the cage frame. The lower edges of the cage fabric should be buried on all sides so there are no escape routes. The cages may be taken down after a generation or, if sturdy enough, kept in place during the winter to monitor emergence and winter survival of adult psyllids in the spring (Figure 4-12c).

Figure 4-12. Method Three—large field cages a. external view of cage; b. releasing knotweed psyllids on knotweed growing within the cage; c. monitoring the knotweed psyllids previously released on knotweed within the cage. Note: protective suits are not required for releases or monitoring (Credits: a-c Agriculture and Agri-Food Canada)

Additional suggestions

Whichever of the above methods is used, it can be helpful to prepare a release site in advance by cutting an area of knotweed stems back a few weeks before releasing the insects. The tender new growth that results will be especially suited for psyllid feeding, and the shorter stature of the knotweed stand will make it easier to monitor the psyllid populations.

As a general rule of thumb, it is better to release all the biocontrol agents within a release container in one spot (Figure 4-13) to ensure adequate numbers of males and females are present for reproduction and reduce the risks of inbreeding and other genetic problems. Guidelines for a minimum release size are uncertain for most biocontrol agents, but releases of at least 30 adult knotweed psyllids for sleeve cages and over 500 adults for large cages or open field releases are recommended.

Figure 4-13. Releasing all adult psyllids within a release container (Credit: CABI UK)

The only way to determine if biocontrol agents have established is to inspect release sites annually for up to 5 years (or more) after releases are made. Additional releases may be necessary if initial releases fail to establish. For locations where establishment is likely to be slow (e.g., due to high levels of overwintering mortality), planning to make releases on the same site for two or three consecutive years may increase successful establishment and reduce the time until biocontrol agent impact on target weed populations is visible. However, if it is early in the release program, it would be better to try a different site where the biocontrol agent might be more successful. If more than one release of a biocontrol agent is available in a given year, be sure to put some distance between releases; 2/3 mile (1 km) is ideal. If possible, make more than one release per drainage or in adjoining drainages; if one of your releases is wiped out by flooding, fire, herbicide application or other catastrophic disturbance, then biocontrol agents from adjoining releases can repopulate it.

Avoid making releases/transfers on rainy days. If you encounter an extended period of poor weather, it is better to release the biological control agents than wait three or more days for conditions to improve as the biocontrol agents' vitality will decline with extended storage. Avoid transferring biocontrol agents to areas with obvious ant mounds as ants may prey upon some species of biocontrol agents.

Documenting, Monitoring, and Evaluating a Biological Control Program

The Need for Documentation

The purpose of monitoring is to evaluate the success of your knotweed biological control program and to determine if you are meeting your weed management goals. Documenting outcomes (both successes and failures) of biocontrol release programs will help generate a more complete picture of biocontrol impacts, guide future management strategies, and serve education and public relations functions. Monitoring can provide critical information for other land managers by helping them predict where and when biological control might be successful, helping them avoid releasing ineffective biocontrol agents or the same biocontrol agent in an area where they were previously released, and/or helping them avoid land management activities that would harm local biocontrol agent populations or worsen the knotweed problem. (See the Code of Best Practices for Classical Biological Control of Weeds on page 8).

Monitoring activities use standardized procedures over time to assess changes in populations of the biocontrol agents, knotweeds, other plants in the community, and other components of the community. Monitoring can help determine:

- if the biological control agents have become established at the release site

- if biological control agent populations are increasing or decreasing and how far they have spread from the initial release point
- if the biological control agents are having an impact on knotweeds
- if/how the plant community or site factors have changed over time

Monitoring methods can be simple or complex. A single year of monitoring may demonstrate whether the biocontrol agents successfully overwintered, while multiple years of monitoring may allow you to identify trends in the population of the biocontrol agents, changes in the target weed population and plant community, and changes in other factors such as climate or soil.

Regulations for the Transfer of Knotweed Biological Control Agents

USA, intrastate Generally, there are few if any restrictions governing the collection and shipment of approved biological control agents within the same state; however, you should check with your state's department of agriculture or agriculture extension service about regulations governing the release and intrastate transport of your specific biological control agent. The state of California regulates release permits at the county level. It is illegal to redistribute unapproved species in the USA.

USA, interstate The interstate transportation of biological control agents is regulated by the U.S. Department of Agriculture (USDA), and a valid permit is required to transport living biological control agents across state lines. You should apply for a Plant Protection and Quarantine (PPQ) permit from the Animal and Plant Health Inspection Service (APHIS) as early as possible—but at least six months before actual delivery date of your biological control agent. You can check the current status of regulations governing intrastate shipment of weed biological control agents, PPQ Form 526 at the USDA-APHIS-PPQ website. The ePermit process can be accessed by doing an internet search for "USDA APHIS 526 permit application". This allows the complete online processing of biological control agent permit requests. It is illegal to redistribute unapproved species across state lines in the USA.

Canada Canada requires an import permit for any new biological control agent or shipment of previously-released biocontrol agents entering the country. These permit requests are reviewed and issued by the Plant Health Division of the Canadian Food Inspection Agency. Redistribution within a province (or within Canada) of weed biological control agents that have been officially approved for release in Canada is not prohibited; however, you should consult with federal and provincial authorities and specialists prior to moving any weed biological control agent, especially across ecozones (e.g., from the prairies to the interior or coast of British Columbia). Similarly, you should consult with appropriate experts when considering the movement of adventive biocontrol agents that have become established in a region, or native organisms that may feed on a weed targeted for control.

Information Databases

Many federal and state/provincial departments have electronic databases for archiving information about weed biological control releases. We have included a standardized biological control agent release form that, when completed, should provide sufficient information for inclusion in any number of databases (see Appendix III).

The USDA Forest Service (in conjunction with the University of Georgia, MIA Consulting, University of Idaho, CAB International, and the Queensland Government) also maintains a worldwide database for the Biological Control of Weeds: A World Catalogue of Agents and their Target Weeds. The database includes entries for all weed biocontrol agents released to date, including the year of first release within each country, the biocontrol agents' current overall abundance and impact in each country, and more. This database can be accessed at www.ibiocontrol.org/catalog/.

EDDMapS (Early Detection & Distribution Mapping System) is a web-based mapping system increasingly being used for documenting invasive species as well as biocontrol agent distribution in North America. EDDMapS combines data from existing sources (e.g., databases and organizations) while soliciting and verifying volunteer observations, creating an inclusive invasive species geodatabase that is shared with educators, land managers, conservation biologists, and beyond. Information can be added in online forms through home computers and/or apps created for smartphones. For more information on how to use or contribute to these tools, visit www.eddmaps.org/about/ and apps.bugwood.org/.

In addition, some states/provinces have county/district weed departments or employ weed biocontrol specialists, often affiliated with state/province departments of agriculture, county extension offices, or Animal and Plant Health Inspection Service Plant Protection and Quarantine (APHIS-PPQ) offices. Contact local entities for more information.

Monitoring Methods

There are three main components to measure in a knotweed monitoring program: biological control agent populations, knotweed populations, and the rest of the plant community (including non-target plants). More detailed monitoring might also examine effects on other biotic community components (such as other insects, birds, mammals, etc.) or abiotic factors (such as erosion, soil chemistry, etc.). Only the three main monitoring components are discussed in this manual.

Assessing biological control agent populations

Survival through the winter is a major milestone for a newly released population. In addition to surviving cold temperatures and harsh weather through the winter months, the overwintering adult stage must successfully make the transition out of dormancy, find the host plant, and begin reproduction. The timing of emergence from protective overwintering sites is important. Newly introduced populations may have high mortality if they emerge too early, exposing themselves to frosts or harsh weather, or too late, after their energy reserves are depleted. With time, it is expected that populations will adapt to the new climate and overwintering survival will improve.

If you wish to determine whether the knotweed psyllid has successfully overwintered after its initial release, you simply need to find the biocontrol agent in one or more of its life stages the following year. This is most easily done at small knotweed infestations where the psyllid populations will be more concentrated and more likely to be detected. Begin looking for psyllids where they were first released,

and then expand to the area around the release site. When searching, approach the plants slowly and avoid jostling them as adults tend to fly away when disturbed. Based on emergence timing in Japan, spring surveys to assess overwintering success should begin in early April. If psyllids are not found, repeated searches should be carried out weekly until the end of May. Because the densities may be extremely low in the early spring, a full hour of searching (1 person-hour) is recommended. A second opportunity to find the psyllid comes in autumn. When leaves start to fall there is a concentration effect on the remaining leaves, and adult psyllids that have not yet found overwintering sites can be easier to spot with fewer leaves on which to rest.

In the field, the adult stage tends to be the most visible and the easiest to distinguish from other insect species (Figure 4-14a,b). Adult psyllids can be seen on the leaves, stems, and petioles of the newly growing knotweed shoots. Nymphs are harder to spot as they are smaller and closer in color to the leaf than the adults (4-14c). They also often settle in the nodes at the base of leaf petioles and branchlets and are obscured (4-14d). However, nymphs are sometimes easily identified if they have large deposits of lerp (Figure 4-14e; see Chapter 3 for additional details). With an appropriate search image and experience, the early instar nymphs can be seen with the naked eye; however, a hand lens is recommended to search leaf node locations. Similarly, psyllid eggs can be seen through late May (depending on site location and conditions) on the tops and bottoms of leaves along leaf veins (Figure 4-14f); a hand lens is very helpful. The psyllids tend to prefer soft new leaves over harder, more mature ones. Leaf twisting (giant knotweed) and curling (Japanese/Bohemian knotweed) may also indicate the presence of psyllid populations, though these traits are likely only apparent if psyllid densities are high.

Successful overwintering is not the same as successful population establishment. One common definition for biocontrol agent population establishment is persistence into the third growing season without any additional releases being made. Revisit the site at least once annually for three years. If no evidence of biocontrol agents is found, either make additional releases at the site or select another site for release. Consult with your county extension educator or local weed biocontrol expert for assistance.

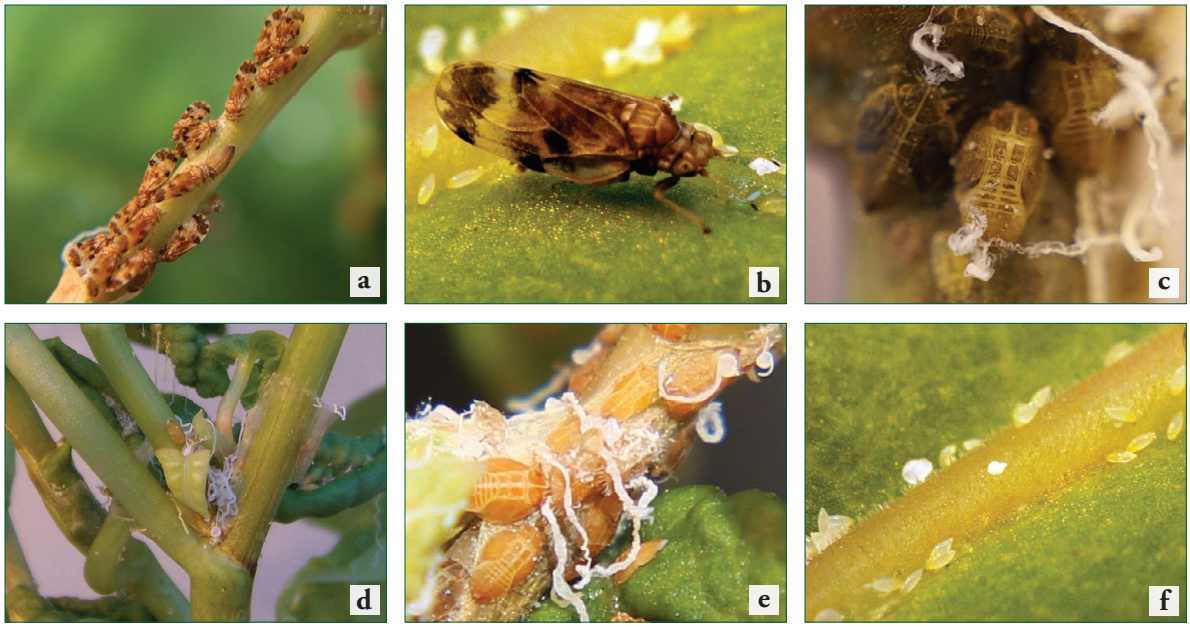


Figure 4-14. Knotweed psyllids a. adults on a knotweed stem; b. adult; c. nymphs and lerp; d. nymphs among leaf nodes; e. nymphs and lerp; f. eggs along a leaf vein (Credits: a-f Fritzi Grevstad, Oregon State University)

In order to detect changing densities of biocontrol agent populations, a systematic monitoring approach is required, and the same methodology should be used each time the site is surveyed. Sites should be monitored in sequential years. One simple approach for monitoring the knotweed psyllid is through the use of yellow sticky card traps available from nursery or agricultural supply companies. Traps can be placed at regularly spaced intervals along transect lines within a knotweed patch. The insects are attracted to the yellow color and stick when they land on the surface. Counts of trapped psyllids at regular time intervals can provide quantitative information about the distribution and abundance of the psyllids through time. However, at very low psyllid densities, this approach has the risk of killing the few psyllids that are present, or it may not provide accurate data.

Manually counting psyllids at your knotweed monitoring site will likely yield more accurate and consistent data. For year to year changes in abundance, it is recommended that the psyllids are counted once in the spring after they have fully emerged from winter diapause and are active on the plants. At this time of year, the knotweed plants are small enough to look down upon, which makes finding the insects much easier. Rather than surveying on the same date each year, it is recommended to survey using timing determined by the number of degree-days that have accumulated. When using degree-days, the timing would be earlier in a warm spring and later in a cool spring. Up to date degree-days tailored to the knotweed psyllid (and other important insects) for locations across North America can be obtained from the Oregon State University Integrated Plant Protection Center website at uspest.org.

Because the knotweed biocontrol program is still in its infancy (at the time of this publication in 2018), the most effective method for monitoring insect abundance has not been fully determined. Below are three different methods that could be used. Whichever method you choose to use in your knotweed psyllid monitoring, an accompanying monitoring form and methodology can be found in Appendix IV.

Method One—Searching the entire site

If the monitoring site is relatively small, it may be possible to search the entire patch. One advantage to this method is that it provides an estimate of the entire local population size, not just a subsample.

Method Two—Searching in permanent quadrats

Transects and permanent quadrats intended for measuring the plant response (see next section) can also be used to monitor insect abundance. A leaf blower with reverse capability, an industrial strength wet-dry vacuum cleaner, or a specialized insect vacuum sampler (Figure 4-15) can be used to vacuum psyllids from plant foliage within the quadrat. See "vacuuming" on page 43 for recommendations on using this method. The best timing for counting psyllids (early spring) is different from the end-of-season timing for measuring plants, so the transects and quadrats will be used at least twice during each growing season. Care should be taken not to damage the knotweed plants within the quadrat as this will influence the late-season plant data collection.

Figure 4-15. Collecting and counting biocontrol agents in a monitoring quadrat with a specialized insect vacuum sampler (Credit: CABI UK)

Method Three—Timed search

For large sites where transects and quadrats have not been installed/used, timed counts of adult psyllids can be used to measure their relative abundance.

Assessing the status of knotweeds and co-occurring plants

The ultimate goal of a knotweed biological control program is to permanently reduce the abundance and vigor of knotweeds and enable the recovery of more desirable vegetation on the site. To determine the efficacy of biocontrol efforts, there must be monitoring of plant community attributes, such as target weed distribution and density. Ideally, monitoring begins before biological control efforts are started (pre-release) and occurs at regular intervals after release. There are many ways to qualitatively (descriptively) or quantitatively (numerically) assess weed populations and other plant community attributes at release sites.

Qualitative (descriptive) vegetation monitoring: Qualitative monitoring uses subjective measurements to describe the knotweed infestation and the rest of the plant community at the site. Examples include listing plant species occurring at the site, estimates of density, age and distribution classes, visual infestation mapping (as opposed to mapping with GPS), and maintaining a series of photos from designated photo points over time. See "set up a photo point" on page 49 for photo point recommendations.

Qualitative monitoring provides insight into the status or change of knotweed populations; however, its descriptive nature does not generally allow for detailed statistical analyses. Data obtained in qualitative monitoring may trigger more quantitative monitoring later. See Appendix V for a sample data form useful for recording qualitative knotweed monitoring data along with information on associated vegetation.

Quantitative vegetation monitoring: Quantitative monitoring measures changes in the knotweed population as well as the vegetative community as a whole before and after a biocontrol agent release using numbers and statistics. It may be as simple as counting the number of knotweed stems in a small sample area, or as complex as measuring knotweed stem height and diameter, flower and seed production, biomass, species diversity, and species cover (Figure 4-16). Quantitative sampling data can be more readily analyzed using statistical methods to demonstrate significant plant community changes.

Figure 4-16. Measuring knotweed stem diameter as part of a monitoring program (Credit: CABI UK)

Ideally, measures of plant size and abundance should start 2-3 years before the biocontrol introduction to establish baseline estimates since these measures can vary from year to year. If this is not possible, then plant monitoring should begin the same year that the biocontrol insects are released and continue annually for up to 10 years following release. Pre- and post-release monitoring should follow the same protocol and be employed at the same time of year. See Appendix VI for a data form and methodology useful for recording quantitative knotweed monitoring data along with information on associated vegetation.

Assessing impacts on non-target plants:

Although very unlikely (given the high degree of specificity of the knotweed psyllid in laboratory tests), it is still important to actively look for possible feeding on any non-target plants species in the field. Sometimes when biocontrol agents reach high local abundance and dramatically reduce the

weed, they temporarily disperse and settle on nearby plant species. Feeding may or may not occur, but the biocontrol agents typically do not reproduce on the non-target plants. This is termed a "spillover" event. This feeding is usually inconsequential because the biocontrol agent population cannot be sustained on these alternative plants; the biocontrol agents either leave the site in search of the target weed or die from malnutrition. The target weed population is likely to recover somewhat in the next growing season and will again support the biocontrol agents (now at lower abundance).

To address possible non-target attacks on species related to or just growing adjacent to knotweeds, you must become familiar with the plant communities present at and around your release sites and be aware of species related to knotweeds. Start by compiling a list of other species in the Polygonaceae family and the genus *Fallopia* that are present at the site (see Chapter 2 and specifically Table 2-1 for more information). You may need to consult with local, state, or regional botanical experts, or review local herbarium records for guidance on areas where related non-target plants might be growing and additional information on how you can identify them. Care should be taken in the management of your knotweed biocontrol program to ensure that all closely-related native or desirable species are identified and monitored along with knotweeds.

Please be aware that there are many "look-alike" native insects that feed on related native plants. Correct identification by insect specialists is needed to confirm such records. If you observe approved biological control agents feeding on and/or developing on native species, collect samples and take them to a biocontrol specialist in your area. Alternatively, you may send the specialist the site data and/or pictures so he or she can survey the site for non-target impacts. Be sure not to ascribe any damage you observe on native species to any specific species and thus bias the confirmation of attack and the identification of the species causing the attack.

Whether or not you find non-target feeding, it is important to note your observations. Documenting a lack of non-target feeding is as important as documenting the occurrence of non-target damage. Your observations can be used as part of a continent-wide effort to improve our ability to accurately assess biocontrol agent safety.

GLOSSARY

Term	Definition
£	The British pound sterling, the official currency of the United Kingdom and its territories
abdomen	The last of the three insect body regions; usually containing the digestive and reproductive organs
adventive	A biocontrol species that arrived in the geographical area from elsewhere by any means, not through official biocontrol development processes
allelopathy	The chemical inhibition of one species by another. The "inhibitory" chemical is released into the environment where it affects the development and growth of neighboring plants
alternate	Where leaves appear singly at stem nodes, on alternate sides of the stem
annual	A plant that sprouts, flowers, and dies all in the same year
antenna (pl. antennae)	In arthropods, one of a pair of appendages on the head, normally having many joints and used in sensory
app (application)	A self-contained program or piece of software designed to fulfill a particular purpose; an application, especially as downloaded by a user to a mobile device
arthropod	An invertebrate animal having an exoskeleton, a segmented body, and jointed appendages. Arthropods form the phylum Arthropoda, which includes the insects, arachnids, myriapods, and crustaceans
back-crossing	Crossing of a hybrid with one of its parents (or an individual genetically similar to its parent), which results in offspring with a genetic identity that is closer to the parent
biological control	The reduction in the abundance of a pest through intentional use of its natural enemies (predators, parasitoids, and pathogens)
bolting	Plant stage at which the flower stalk begins to grow
centrifugal phylogenetic approach	Method used to select plants for host specificity testing for weed biocontrol agents. It focuses on the most closely related species to the target weed in the area of introduction, gradually expanding the number of species to include more distantly related plants until specificity is established
chemical weed control	Using herbicides to control weeds
clonal fragmentation	Form of asexual reproduction or cloning in which an organism is split into fragments. Each of these fragments develop into mature, fully grown individuals that are clones of the original organism
clone (in plants)	Plant that is genetically identical to the individual from which it was derived. Vegetative fragmentation is a common way to produce a plant clone
community	A naturally-occurring group of different species of organisms that live together and interact as a more or less self-contained 'unit'
complete metamorphosis	An insect life cycle with four distinct stages (egg, larva, pupa, adult)
compound eyes	Paired eyes consisting of many facets, or ommatidia, in most adult arthropods
coordinates	A set of numbers used to specify a location
cultural weed control	Manipulating the environment to suppress weed growth while promoting the development of the desired plant(s). Examples include grazing and planting more desirable species
density	Number of individuals per unit area
diapause	A suspension of development in response to regularly and recurring periods of adverse environment conditions, such as extreme temperatures, drought, or reduced food availability
dioecious plant	Male and female flowers occur on separate plants
dissemination	Dispersal. Can be applied to seeds or insects

Term	Definition
dormancy	Period in an organism's life cycle when growth, development, and physical activity are temporarily stopped. This minimizes metabolic activity and helps an organism conserve energy
emergence (insect)	Act of adult insect leaving the pupal exoskeleton, or leaving winter or summer dormancy
eradicate	To get rid of something completely
exoskeleton	Hard, external skeleton of the body of arthropods, including insects and mites
exotic	Originating in a distant foreign country; not native
field insectary	An area where host plants or animals are abundant and biological control agents are released and propagated with or without additional human manipulation
forb	Herbaceous plant (does not have solid woody stems)
fragmentation	See clonal fragmentation
generalist herbivore	Organism that eats a wide variety of plants more or less equally
genotype	The genetic makeup of an individual or taxon
genus (pl. genera)	A taxonomic category ranking below family and above species and consisting of a group of species exhibiting similar characteristics. The genus name is followed by a Latin adjective or epithet to form the name of a species
GPS	Global Positioning System; a space-based navigational system providing location and time information by using four or more satellites
growing degree-day (GDD)	A unit of development in response to daily temperatures. Measured as the sum of the daily amounts by which the temperature exceeds a species-specific lower developmental threshold. A given insect species will usually require the same number of degree-days to complete its life cycle, even though the number of calendar days required will vary in different climates
gynodioecious plant	Female and hermaphroditic flowers occur on separate plants
head	Insect segment with the mouth parts, antennae, and eyes
herbaceous plant	Plant that does not have solid woody stems
herbivory	Feeding on plants
hermaphrodite	An organism with both male and female sex organs
host	The plant or animal on which an organism feeds; the organism used by a parasitoid; a plant or animal susceptible to attack by a pathogen
host race	Populations within a species that differ (and become reproductively isolated) due to their ability to use a particular trait in a particular plant host
host specificity	The highly-evolved, often obligatory association between an insect and its host (i.e. weed). A highly host-specific insect feeds only on its host and on no other species
incomplete metamorphosis	An insect life cycle characterized by gradual changes through successive immature stages (nymph) to the adult stage
inflorescence	The flowering part of a plant
instar	The phase of an arthropod's nymphal or larval development between molts
invasive	Tending to spread prolifically and undesirably or harmfully
invertebrate	A kind of animal that does not have a spinal column or backbone, e.g. insects, spiders, crabs, mollusks
larva (pl. larvae)	Immature stage of some animals, including insects and mites. In insects with complete metamorphosis, it is the stage between the egg and pupa (examples include grubs, caterpillars, and maggots)

Term	Definition
leaky dioecy (plant)	A form of reproduction in plants where some individuals have female flowers, others have male flowers, still others have hermaphroditic flowers
lerp	Structure of crystallized honeydew produced by Psyllidae nymphs that may serve as a protective cover
litter	Dead plant material, such as leaves, bark, needles, and twigs, that has fallen to the ground
margin (of leaf)	The edge of a leaf. Margins typically fall within a handful of categories and are useful in plant identification
membranous	Thin and transparent
mitochondrian DNA	DNA found in mitochondria, structures within cells that convert the energy from food into a form that cells can use
molting	Process of arthropod development that involves shedding its exoskeleton and producing another as an arthropod grows
morphological	The size, shape, and structure of an organism or one of its parts
mycoherbicide	A fungus-based control agent for weeds
NAD 83	North American Datum, the official datum used for the UTM geographic coordinate system in North America
native	Of indigenous origin
node	Part of the stem of a plant from which a leaf, branch, or root grows
non-target effect	When control efforts affect a species other than the species they were enacted to control (can be positive or negative)
noxious weed	A weed that has been designated by an agricultural authority as one that is injurious to agricultural or horticultural crops, natural habitats or ecosystems, or humans or livestock
nymph	Immature form of invertebrates, including mites and insects, that undergoes gradual metamorphosis. Resembles adults
ocrea	Plant structure formed of stipules fused into a sheath surrounding the stem
oviposit	To lay or deposit eggs
ovipositor	An organ used by some animals for the laying of eggs. The ovipositor of the knotweed psyllid is a pointed tube attached to the tip of the female's abdomen.
parasitoid	An insect (e.g., a wasp) whose larvae live as parasites, eventually killing their hosts (typically other insects)
perennial	A plant that lives for more than two years
photoperiod	The period of time each day during which an organism receives illumination; day length
physical weed control	The removal of weeds by physical or mechanical means, such as pulling/hand digging, mowing, grazing, mulching, tilling, or covering with weed barrier
plant cover	The portion of the vegetative canopy in a fixed area attributable to an individual or a single plant species
propagule	A vegetative structure that can become detached from a plant and give rise to a new plant, e.g., a bud, sucker, or spore
pupa (pl. pupae; v. pupate)	Non-feeding, inactive stage between larva and adult for an insect with complete metamorphosis
qualitative	Measurement of descriptive elements
quantitative	Measurement of quantity; the number or amount
regulated invasive plant	Invasive plant whose control and/or movement is regulated by federal, state/provincial, or local law
rhizome	A modified stem of a plant that grows horizontally underground, often sending out roots and shoots from its nodes

Term	Definition
senescence	The final stage of a plant's life cycle characterized by color changes and die back of the foliage and stems. In the case of knotweed, senescence of the above-ground portion of the plant occurs at the end of each growing season, while the roots survive
sepals	Parts of a flower that enclose the petals; they are typically green and leaf-like
single neucleotide polymorphism (SNP)	A variation in a single base pair in a DNA sequence
specialist herbivore	Organism that relies on one plant food source (such as knotweeds), often to the exclusion of all other available plants
species	A fundamental category of taxonomic classification with a ranking below genus or subgenus and that consists of related organisms capable of interbreeding
spillover effect	When biocontrol agents reach high local abundance, successfully control the target weed, and temporarily disperse and settle on nearby plant species. Feeding that occurs on these adjacent species is typically not consequential because agent populations cannot be sustained and will die back
surfactant	A compound often applied with an herbicide mix to help bring the herbicide into closer contact with the leaf surface in order to aid absorption
synchrony	Occurring at the same time (e.g., plant flowering and insect oviposition)
taxonomy	The classification of organisms in an ordered system that indicates natural relationships. The science, laws, or principles of classification; systematics
thorax	Body region of an insect between the head and abdomen, bearing the legs and wings
transect	A straight line of varying length along which plants are periodically sampled individually or in quadrants
upright	Grows erect and vertical as opposed to prostrate (spreading on the ground)
UTM	Universal Transverse Mercator, a grid-based geographic coordinate system
vegetative fragmentation	See clonal fragmentation
weed	A plant growing where it is not wanted
WGS 84	The World Geodetic System, a datum for latitude/longitude geographic coordinate systems

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APPENDIX

Appendix I: *Aphalara itadori* Host Specificity Test Plant List

Taxon	TAG Cat.
Family Polygonaceae	
Subfamily Polygonoideae	
Tribe Polygoneae	
Target species	
Fallopia x bohemica	1
Fallopia sachalinensis	1
Fallopia japonica	1
Non-target species	
Fallopia cilinodis	2, 4
Fallopia baldshuanica	2
Fallopia scandens	2
Fallopia convolvulus	2
Fallopia dumetorum	2
Muehlenbeckia axillaris	3
Polygonum douglasii	3, 4
Polygonum aviculare	3, 4
Polygonum achoreum	3
Polygonum ramosissimum	3, 4
Polygonum paronychia	3, 4
Polygonum shastense	3
Polygonum maritimum	3, 4
Polygonella robusta	3
Polygonella articulata	3, 4
Tribe Rumiceae	
Rheum rabarbarum	3
Rheum palmatum	3
Oxyria digyna	3, 4
Rumex acetosa	3
Rumex acetosella	3
Rumex arcticus	3
Rumex britannica	3
Rumex fuegenis	3
Rumex occidentalis	3
Rumex orthoneurus	3, 4
Rumex sanguinius	3
Rumex scutatus	3
Rumex triangulivalvis	3
Tribe Fagopyreae	
Fagopyrum esculentum	3
Fagopyrum tataricum	3
Tribe Persicarieae	
Aconogonon phytolaccaefolium	3
Persicaria affinis	3
Persicaria amplexicaulis	3
Persicaria hydropiperoides	3, 4
Persicaria lapathifolia	3
Persicaria microcephala	3
Persicaria orientalis	3
Persicaria pensylvanica	3
Persicaria sagittata	3
Persicaria virginiana	3
Persicaria wallichii	3

Taxon	TAG Cat.
Family Polygonaceae	
Subfamily Polygonoideae	
Tribe Persicarieae	
Bistorta vivipara	3, 4
Bistorta bistortoides	3
Subfamily Eriogonoideae	
Tribe Brunnichieae	
Antigonon leptopus	3
Brunnichia ovata OR	3
Brunnichia ovata U.K.	3
Tribe Coccolobiae	
Coccoloba uvifera	3
Tribe Eriogoneae	
Chorizanthe membranacea	3
Eriogonum parishii	3
Eriogonum cernuum	3, 4
Eriogonum elatum	3
Eriogonum nudum	3
Eriogonum pyrolifolium	3, 4
Eriogonum umbellatum	3
Oxytheca dendroidea	3
Family Plumbaginaceae	
Armeria maritima	5
Limonium carolinianum	5
Family Brassicaceae	
Brassica oleracea	5
Family Caryophyllaceae	
Dianthus gratianopolitanus	5
Family Ericaceae	
Vaccinium macrocarpon	6
Family Poaceae	
Zea mays	6
Family Pinaceae	
Pseudotsuga mensiezii	6

TAG Category

1 Genetic types of the target weed species (varieties, races, forms, genotypes, apomicts, etc.) found in North America and the native range.

2 Species in the same genus as the target weed, divided by subgenera

3 Species in other genera in the same family as the target weed, divided by subgenera

4 Threatened and endangered species in the same family as the target weed divided by subfamily, genus, and subgenus

5 Species in other families in the same order that have some phylogenetic, morphological, or biochemical similarities to the target weed

6 Species in other orders that have some morphological or biochemical similarities to the target weed

Appendix II: Troubleshooting Guide: When Things Go Wrong

This guide is intended to assist those who encounter problems when establishing biological control agent populations. It identifies the probable cause of typical problems and offers solutions.

Problem	Probable Cause	Solution
Biological control agents unhealthy or dead when received	Physical damage to biocontrol agents in transport	Provide adequate packing material to minimize movement of containers and ice packs
	Drowning	Do not put water in containers during transport; prevent accumulation of excess moisture; too much plant material causes condensation
	Excess or prolonged heat or cold	Keep containers cool at all times; use coolers and ice packs; avoid exposure to direct sunlight while in transit
	Starvation	Put knotweed foliage (tender shoot tips and new leaves) in containers
	Release delay	Transport or ship biocontrol agents immediately after collection
		Release biocontrol agents at new site immediately upon arrival or receipt of biocontrol agent
	Parasitism and/or disease	Check source biocontrol agents. Ensure the insect population is disease-free when collecting or receiving shipment
Reproductive problems	Biocontrol agents past reproductive stage	Collect at peak activity (i.e. insects are mating and ovipositing)
	Sex ratio: not enough males or females	Collect at peak activity; observe mating among target biocontrol agents before collecting; males often emerge earlier than females
	Biocontrol agents not synchronized with the knotweed growth stage	Biological control agents require the weed to be at specific growth stage for optimal oviposition; collect biocontrol agents from sites with plants in similar stages
Few biological control agents collected	Collection at wrong time	Collect in early spring when adults are at peak activity and mating
	Collection technique	Biological control agents can be killed/damaged by sweeping so should be collected by vacuuming, aspirating, or tapping
	Conditions at time of collection wrong	Refer to the Chapter 4 section "Collecting Knotweed Biological Control Agents" for guidelines on desirable weather conditions
	Population insufficient	Only collect from well-established populations
Biocontrol agents not found after release	Site is unsuitable or too small	Refer to the Chapter 4 section "Selecting Biological Control Agent Release Sites"
	Not enough biocontrol agents released	Release as many biocontrol agents as is feasible to ensure survival and reproduction (preferably ≥ 500 individuals)
	Pesticide use/mowing in area	Select sites where land use and management practices do not interfere with biological control agent life cycles
	Released on wrong species	Ensure the correct knotweed species is targeted with the correct biocontrol agent strain (see Chapter 3)
	Biocontrol agents immediately disperse away from release point	Release only during the cool hours of the day
	Biocontrol agents not well adapted to conditions	Release field-collected biocontrol agents from local sources wherever possible rather than greenhouse-reared adults or insects collected from distant locations
	Ants or other predators preyed upon biocontrol agents	Release only at sites with no obvious ant mounds or high insect predator populations (e.g., mice, voles)
Cannot locate release site	Location marker not obvious	Use a bright-colored metal, wooden, or plastic stake
	Site destroyed	Communicate with all direct and neighboring land users
	Map poorly/incorrectly drawn	Check map; redraw with more detail or add landmarks; GPS

Appendix III: Sample Biological Control Agent Release Form

Released By:_____ **Release Date:**___/___/___ **State:**_____ **County:**_____
(mm dd yy)

Biocontrol Agent:_____ **# Released:**_____

Target Weed:_____ **Date Collected:**____/____/____
(mm dd yy)

Source of Biocontrol Agents:_____

Biocontrol Agent Life Stage (circle): Adults Nymphs Eggs

Land Ownership (circle): Private County State USFS BLM COE BOR BIA/Tribe TNC Other_____

Legal: T_____ R_____ Sec_____ Q_____ Lat: Deg____Min____Sec_____ Long: Deg___Min_____Sec_____

UTM: UTM Datum Zone_____ UTM Year_____ UTM Easting: _____ UTM Northing: _____

ENVIRONMENT

Temperature (°F):_____ **Wind:** Calm Light Moderate Strong Gusty **Wind Direction:** N S E W

Weather (circle): Clear Ptly Cloudy Cloudy Rain Snow **Release Time:**_____ AM/PM

Site Aspect (circle): N, NE, E, SE, S, SW, W, NW **Elevation:**_____

Site Slope: Flat (0-10%) _____ Gentle (10-30%) _____ Moderate (30-60%) _____ Steep (>60%) _____

Topographic Position (circle): Valley Bottom Terrace Lower Slope Mid/Upper Slope Crest

Disturbance: (check all that apply, circle most prevalent) Cultivation ___ Fire ___ Flood ___ Grazing ___
Logging___ Roads____ Mining ____ Recreation ____

SITE CHARACTERISTICS

Site Name:_____ **Size of Infestation (acres):**_____ **% Weed Cover:**_____

Est. Weed Height (cm):_____ **Weed Density (# per m^2):**_____ **Dominant Plant:**_____

Distribution of Weed: Isolated_____ Scattered_____ Sc-Patchy_____ Patchy_____ Continuous_____ Linear_____

Phenology: Seedling % ____ Rosette % ____ Bolt % ____ Bud % ____ Flowering % ____ Seed % ____ Dormant % ____

Vegetation Type (circle):
Grassland
Pasture
Dry Meadow
Moist Meadow
Shrubland Steppe
Conifer Forest
Deciduous Forest

Estimate % Cover:
Tree _____
Shrub _____
Forb _____
Grass _____
Litter _____
Bare Ground _____
Rock _____

Soil Texture: (check) Sand___ Silt___ Clay___ Gravel___ Loam___

Appendix III (cont.): Sample Biological Control Agent Release Form

CONTACT PERSON:
Name:_____
Address:_____
City:_____
State:_____
Phone: _____ - _____ - _____
e-mail:_____

LEGAL LANDOWNER:
Name: _____
Address: _____
City:_____
State:_____
Phone: _____ - _____ - _____
e-mail: _____

Road Map to Site

Site and Vegetation Map

Comments

Appendix IV: Knotweed Psyllid Monitoring Form

SITE: _____ **STATE:** _____ **DATE:** _____ _____ _____
year month day

Last name: _____ **First name:** _____

GPS: Lat N _____°_____' **Long W**_____°_____' **Elevation:** _____ **ft m**

UTM: UTM Datum Zone: _____ **UTM Year:**_____ **UTM Easting:**_____ **UTM Northing:** _____

TIME: _____ **TEMPERATURE:** _____ **WEATHER:** _____

 # OF DEGREE DAYS ACCUMULATED: _____

Knotweed psyllids can easily be quantitatively monitored using one of three different methods. Choose the method that best applies to your knotweed infestation where the psyllid has been released. For all methods, the best time for monitoring adult psyllids is in early spring. Refer to Chapters 3 and 4 of "Biology and Biological Control of Knotweeds" for images and descriptions of knotweed psyllids during the different life stages.

Method 1. Searching the Entire Site

If your knotweed biological control release/monitoring site is relatively small, take as much time as necessary to search the entire patch. Record both the patch size (this may change over time) and the number of adults observed.

# of Observers: _____ Time Spent Observing: _____

Patch Size: _____ # Adults Observed: _____

Method 2. Searching in Permanent Quadrats

If your knotweed biocontrol release/monitoring site has already had transects and permanent quadrats set up to measure plant responses (see Appendix VI), these same quadrats can be used to monitor psyllids. Count all adult psyllids within the borders of each quadrat. If psyllids appear to be absent from the site, a full person-hour of searching is recommended before deciding the psyllid is not present. Use care not to damage the knotweed plants within the quadrat since these stems will be assessed during the plant monitoring in late summer.

Quadrat	1	2	3	4	5	6	7	8	9	10	Total
Adults Observed											

Method 3. Timed Searches

If your knotweed biological control release/monitoring site is large and does not have transects and permanent quadrats set up, timed counts can be used to monitor psyllids. Count as many adult psyllids as possible in a 3-minute increment, using a tally counter if available. Move several paces away within the knotweed patch and begin a new 3-minute count. Repeat until five 3-minute counts have been made. If psyllids appear to be absent from the site, a full person-hour of searching is recommended before deciding the psyllid is not present.

3-min Count	1	2	3	4	5	Total
Adults Observed						

Appendix V: Knotweed Qualitative Monitoring Form

SITE: _____ **STATE:** _____ **DATE:** _____ _____ _____
year month day

Last name: _____ **First name:** _____

GPS: Lat N _____°_____' **Long W**_____°_____' **Elevation:** _____ **ft m**

UTM: UTM Datum Zone: _____ **UTM Year:**_____ **UTM Easting:**_____ **UTM Northing:** _____

TIME: _____ **TEMPERATURE:** _____ **WEATHER:** _____

Biocontrol Agent:_____ **Year of release:** _____

Cover class estimate by plant category (Overall infestation, ✓check one for each row, percentages may add up to more than 100% total)							
Plant Group	**0%**	**1-5%**	**6-25%**	**26-50%**	**51-75%**	**76-95%**	**96-100%**
Knotweed							
Grasses							
Forbs							
Shrubs							
Trees							

Dominant Plant Species on Site:_____
_____
_____
Other Noxious Weeds:_____
_____

Estimate knotweed density class (✓check one)			
Flower clusters/m²		**Knotweed distribution**	
0		Isolated	
1-25		Scattered	
26-50		Scattered-Patchy	
50-75		Patchy	
>75		Continuous	

Knotweed phenology class	
Knotweed stage	**Estimated percent**
Sprouting	
Bolting	
Flowering	
Seed dissemination	
Senescent	

Comments/Observations:

Appendix VI: Knotweed Quantitative Monitoring Form Instructions

General: The purpose of this activity is to record measurements of knotweed plant attributes and to estimate the abundance of other vegetation in the community. If marked with permanent stakes, the same quadrats can be measured year after year to assess long term patterns of change. This method was designed for knotweed infestations at least 50 meters long.

Materials needed:

- 2 metal fence posts (or metal plates in areas prone to vandalism or disturbance from livestock)
- 40 brightly-colored flagging stakes [**Fig. 1a**]
- 50- or 100-m measuring tape (depending on the size of the knotweed infestation)
- 1-m^2 (3.5 ft^2) pvc quadrat frame [**Fig. 1b**
- extra long measuring stick (a piece of pvc with boldly marked pre-measured increments [**Fig. 1c**] or a telescoping surveyor's rod work well)
- calipers [**Fig. 1d**]
- tally counter
- write-in-rain notebook and/or data sheets
- pencils
- clipboard
- field lens or reading glasses (even if not normally needed)
- camera
- GPS unit to relocate quadrats
- if metal plates are used for transect ends rather than metal posts, a metal detector will also be useful for locating the plates in subsequent visits

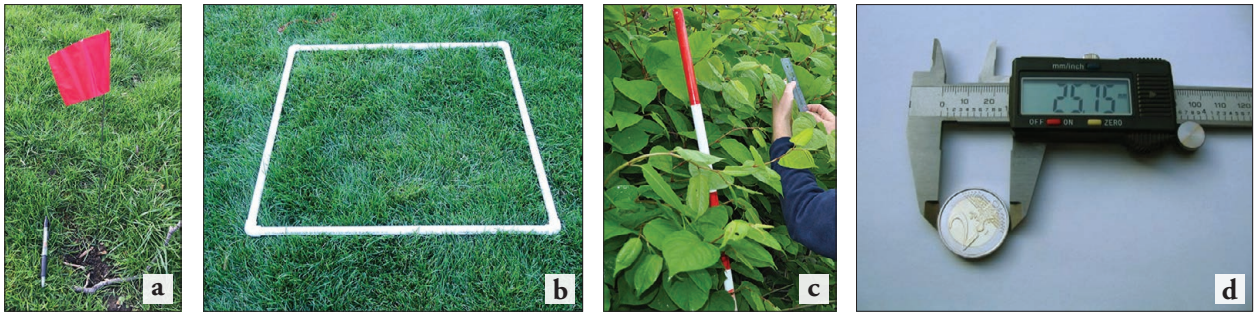

Fig. 1. Some of the materials required for applying this monitoring protocol to a knotweed biocontrol release site a. flagging stake; b. 1m^2 pvc quadrat frame; c. pvc marked in pre-measured increments; d. digital calipers (Credits: a Jennifer Andreas, Washington State University Extension; b Kojodesigns; c CABI UK; d Xofc)

Tip: Attach a piece of brightly-colored flagging tape to each of your smaller tools (calipers, tally counter, and pencils) so they can be found easily if dropped. Red and blue tape work best as yellow and orange tend to blend in with senescing leaves.

Instructions: Monitoring should be done with two people, one to make the observations and the other to hold the surveyor's rod/measuring stick and record data. Two people are preferred for health and safety reasons as well. A transect is made in year 1, with 10 permanent measurement plots (quadrats) marked along the transect. All 10 quadrats are re-measured in subsequent site visits, preferably once per year when knotweed is in full flower (typically late August or early September). Because flowering knotweed plants are often tall and dense, it is usually easiest to establish the transect in spring when knotweed is small, and return for monitoring later in the season.

1) Site selection: Select a location that is relatively easy to access with a knotweed patch at least 50 meters long. The knotweed can be either solid or patchy within this area, but it is best if the knotweed occupies at least 40% of the area.

Appendix VI (cont.): Quantitative Monitoring Form Instructions

2) Site information: Fill out the site information at the top of the monitoring form.

3) Establish the transect: Stretch the measuring tape along a straight line that roughly bisects the knotweed infestation along its long axis. Mark the ends of the transect with metal fence posts (or metal plates) hammered securely into the ground. Flagging tape is recommended to make it easier to locate the post in future visits. In addition, it is useful to draw a map of the location of the posts and record GPS coordinates in your field notes.

4) Establish/Position the quadrat: For large, continuous knotweed infestations, establish the 10 quadrats every 10 m along a 100-m measuring tape. For non-continuous knotweed infestations, establish the 10 quadrats every 5 m along a 50-m transect. Place a flagging stake in all four corners of each permanent quadrat. Don't worry if a few quadrats do not have any knotweed, you will monitor them anyway. Empty quadrats will allow you to determine if the knotweed-infested area is expanding into new locations. If at all possible, walking should be done at least a meter to one side of the transect, so as not to trample in or near the sampling quadrats. In very dense stands of knotweed, it can be difficult to move about through the knotweed stems. Intentional cutting and/or trampling of a path off to one side of the transect when knotweed first starts to grow in the spring, and then monthly thereafter, will help keep the transect accessible. Please note, this will affect the growth of the plants on the edge of the quadrat. Place the pvc quadrat frame so that the bottom left corner intersects the marker placed at the 10 m mark on a 100-m transect (5 m mark for a 50-m transect) and the top left corner intersects the marker placed at the 11 m mark on a 100-m transect (6 m mark for a 50-m transect) [**Fig. 2**]. The quadrat frame should not be permanently assembled; the four sides should be removable and put together at each quadrat to make it easier to maneuver among dense knotweed stems [**Fig. 3**].

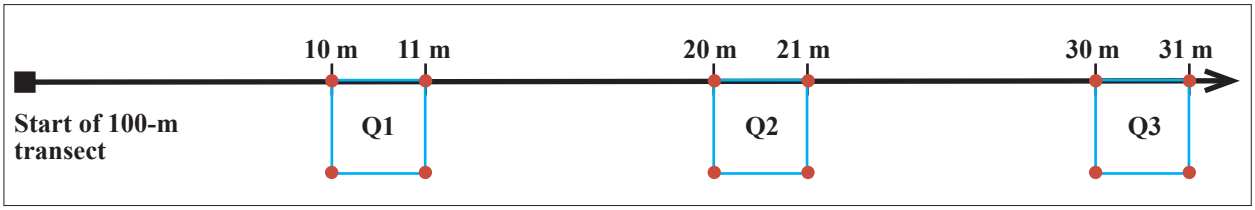

Fig. 2. 100-m transect with 10 1-m^2 quadrats (blue squares) placed every 10 m. Red circles denote flagging stakes placed in all four corners of each quadrat. For a 50-m transect, establish quadrats every 5 m. Not drawn to scale.

5) Site map: Draw a sketch of the transect relative to site landmarks and indicate the position of the quadrats relative to the marker points. Be sure to note which end of the transect corresponds with the first quadrat in your recorded data, so that you will measure the same way each year.

6) Count stems: Count the number of knotweed stems in the quadrat.

7) Measure stem diameter and height: Randomly choose 10 stems (if there are fewer than ten stems/quadrat, measure all that are present). Measure the stem diameter between the 2nd and 3rd nodes to the nearest millimeter (using calipers). Measure the height of each stem to the closest decimal meter using the surveyor rod. Note: In some knotweed infestations, knotweed stems will be growing very densely and entangled, making it impossible to get an accurate height reading for each individual corresponding stem. If this is the case for a quadrat being measured, take only one measurement of the canopy height for that quadrat. Place the surveyor rod at the center of the quadrat. While one person holds the rod, the other observer should step away from the patch (to get a better visual) and measure the approximate height of the canopy around the surveyor rod.

Fig. 3. Quadrat frame assembled around knotweed stems (Credit: Jennifer Andreas, Washington State University Extension)

8) Measure entire transect: Repeat steps 6-7 for all the quadrats.

Appendix VI (cont.): Knotweed Quantitative Monitoring Form

SITE: _____ **STATE:** _____ **DATE:** _____ _____ _____
year month day

Last name: _____ **First name:** _____

GPS: Lat N _____°_____' **Long W**_____°_____' **Elevation:** _____ **ft m**

UTM: UTM Datum Zone: _____ **UTM Year:**_____ **UTM Easting:**_____ **UTM Northing:** _____

TIME: _____ **TEMPERATURE:** _____ **WEATHER:** _____

KNOTWEED SPECIES: _____

Quad	Total Stems	Knotweed Stems (selected randomly, diameter [D] in mm, height [H] in decimal m)										
		Stem	1	2	3	4	5	6	7	8	9	10
1		D										
		H										
2		D										
		H										
3		D										
		H										
4		D										
		H										
5		D										
		H										
6		D										
		H										
7		D										
		H										
8		D										
		H										
9		D										
		H										
10		D										
		H										

Site Map: