REPRODUCTIVE ECOLOGY OF GIANT (*POLYGONUM SACHALINENSIS*) AND JAPANESE KNOTWEED (*POLYGONUM CUSPIDATUM*)

A. T. Niewinski, T. W. Bowersox and L. H. McCormick

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School of Forest Resources
The Pennsylvania State University
University Park, PA 16802

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Summary

Japanese knotweed (*Polygonum cuspidatum* Sieb and Zucc.) and Giant knotweed (*Polygonum sachalinensis* F. Schmidt ex Maxim) are invasive, exotic perennials. They were introduced from eastern Asia as ornamental plants. These species are found throughout Pennsylvania especially along riparian, roadway and railway corridors. At least 10 national park units in the Middle Atlantic Region have knotweed populations. Elimination of these non-native plants from national parks will require procedures to control existing populations as well as stopping the establishment of new populations. It is generally agreed that these two knotweed species are propagated asexually by rhizomes and basal buds. However, information is mixed on whether or not Giant and Japanese knotweed have the potential to develop new populations from seed. Researchers in the United Kingdom reported no viable seed set on Japanese knotweed due to the absence of male-fertile plants. Whereas in New Jersey, seedlings were found under Japanese knotweed but they did not survive. This research project was conducted to determine if Giant and Japanese knotweed populations in Pennsylvania have the potential to naturally reproduce from seed. If the potential exists, are there ways to minimize the success of new plants becoming established as perennial populations.

The reproductive ecology of Giant and Japanese knotweed seeds was studied in populations at eighteen locations from three river drainages across Pennsylvania. Field investigations showed that Giant and Japanese knotweed plants can produce millions of seed annually. Multiple-year seedlings were discovered at four locations. Viability assessments of seeds were made using seed coat and endosperm conditions. The majority of populations produced highly viable seeds. Germination requirements were determined by testing the effects of moisture (dry or moist), temperature (3°C or 18°C) and wing (natural seed or achene only) treatments on four seed sources over 30, 75 and 120 day storage periods. Any of these temperature and moisture treatment combinations meet the conditions needed to produce high numbers of seedlings. The overall mean germination value was 91%. The cold (3°C) moist conditions had the highest overall germination among the three environmental conditions. A seedbank study was conducted to determine the existence of viable knotweed seedbanks at four locations. Fifty soil cores (15-cm diameter, 10-cm deep) were collected from each location and transplanted into a greenhouse. The range of seedlings per soil core was 0 - 120. This equated to greater than 5,800 potential seedlings/m^2 in the soil for two of the four test locations.

A two year study was conducted to determine the effects of shade (0%, 30% and 63%) and seedbed condition (leaf litter absent or present) on Giant knotweed seedling germination, survival and growth. Independent of seedbed condition, the numbers of seeds that germinated were lower in the 63% shade than in either the 0 or 30% shade. Regardless of shade level, litter present condition had fewer seedlings than the litter absent condition. Seedling biomass at the end of the first growing season was not significantly affected by shade treatment but was significantly different for the two seedbed conditions. Litter absent seedlings averaged 2.25 g, while seedlings with the litter present averaged 1.17 g. Below-ground biomass accumulations in the first growing season were sufficient to produce new sprouts at the beginning of the second growing season. The majority (90%) of the 418 seedlings failed to produce perennial plants. For

the 10% of the seedlings that did resprout, there was an average of 2.6 stems per parent plant. All of the sprouting plants were from cores that had the litter absent treatment. Above-ground biomass at the end of the second growing season ranged from 1 - 432 g per stem and the stems reached a maximum height of 1.4 m.

Giant and Japanese knotweed populations sampled in this study have the potential to produce viable seeds, some more abundantly than others. Seeds were found to have no dormancy requirement, remained viable in the seedbed and were capable of establishing new, perennial populations. Greatest potential for seedlings to become established will be in non-shaded locations that are free of a well-developed leaf litter. Conversely, Giant and Japanese knotweed are unlikely to become established in forested ecosystems that contain sufficient amounts of herbaceous and woody plants to provide high amounts of shade to a seedbed that has abundant amounts of litter. Therefore, all construction and maintenance activities should be carefully planned to minimize the amount of exposed mineral soil.

Background

Giant and Japanese knotweed have been simultaneously classified in three genera (*Fallopia, Polygonum* and *Reynoutria*). Japanese knotweed was named *Polygonum cuspidatum* Siebold and Zucc., *Fallopia japonica* Ronse Decraene, *Reynoutria japonica* Houtt. (Beerling *et al.* 1994) or *Polygonum zuccarini* Small (Mitchell and Dean 1978). Giant or Sachaline knotweed (Mitchell and Dean 1978) was called *Polygonum sachalinensis* F. Schmidt ex Maxim or *Fallopia sachalinensis* and *Reynoutria sachalinensis* (F. Schmidt ex Maxim) Nakai. Naturalized, intermediate hybrids between the two specieswere known as *Fallopia* x *bohemica* (Chrtek and Chrtkova) J. Bailey (Bailey and Stace 1992). Common names include Japanese knotweed (Mitchell and Dean 1978), Mexican bamboo (Yinger 1985) and fleeceflower. There has been an ongoing dispute over the correct genus of these species. Evidence of hybridization events between *P. cuspidatum* and *Fallopia baldschuanica* (Regel) Holub (Russian vine) suggested Japanese knotweed should be in *Fallopia* section *Reynoutria* (Bailey 1988a).

An extensive search of the literature found numerous reports on Japanese knotweed in the British Isles, but only a few research reports from the United States. Japanese knotweed is native to large areas of China, Korea and Japan. Giant knotweed has a more northerly distribution from Japan to the Kurile and Sachalin islands. Japanese knotweed is considered to be a primary colonizer on volcanic sites. It can survive high concentrations of sulfur, heavy metals in the soil, a wide range of soil pH values (3 - 8.5) and many air pollutants. In its native range, it ascended to 2400 m on Mt. Fuji, Japan (Maruta 1983). Von Siebold (Beerling et al. 1994) dated official introduction into the British Isles as 1846. Three giant knotweeds were naturalized in the British Isles: *F. sachalinensis*, *F. japonica* var. *compacta* and *F. japonica* var. *japonica* (Bailey and Conolly 1985). Distribution of Japanese knotweed in Europe was primarily determined by climate and occupied broadly the same climatic range in Europe as it did in Southeast Asia (Beerling *et al.* 1995).

Japanese knotweed was introduced into North America by 1902 (Higgins 1977). In eastern North America, the range extends north from North Carolina to Newfoundland and west to Louisiana and Manitoba. The range in western North America is from California east to the Rocky Mountains and up to the Pacific northwest, especially west of the Cascades to coastal British Columbia (Beerling *et al.* 1994). Japanese knotweed has been used as animal fodder, ornamental garden plants, sand dune stabilizers, strip-mine stabilizers, and a source of medicinal and ethno-botanical products (Beerling *et al.* 1994). Recently however, Japanese knotweed has been considered uncontainable and not recommended for planting in gardens (Yinger 1985).

The sex expression of these plants may be more complicated than straight dioecy (Bailey 1994). Japanese knotweed was described as dioecious (Hooker 1880), with no mention of male fertile plants in Britain; and Giant knotweed was listed as "questionable polygamous" (Bailey 1994). Female-fertile (male-sterile) plants were characterized by a gynoecium >1.1 mm/mg, a well-developed style and stigma > 0.3 mm long with small empty anthers on short filaments (Bailey 1994). Male-fertile (female-sterile) plants had short, poorly developed gynoecia lacking stigmatal development and large, well-filled anthers born on long exserted filaments. Intermediates were morphologically hermaphrodite, possessing large ovaries, well-developed stigmas and large filled anthers borne on long filaments (Bailey 1994). Conolly (1977)

suggested that male-fertile, male-sterile and hermaphroditic in Giant knotweed individuals may be found. She also reported that Giant knotweed plants from Amroth, Wales, had male-fertile, female fertile and hermaphroditic flowers on a single inflorescence. Later cytological data revealed that what Conolly (1977) had reported to be rare male-fertile Japanese knotweed plants were hybrids with Giant knotweed (Bailey 1994). Bailey and Conolly (1985) found a positive correlation between female fertile and the octoploid chromosome level in *P. cuspidatum*. This implied that some clones were both male and female fertile.

In Britain, Giant knotweed was gynodioecious with both female-fertile and hermaphroditic flowers (Beerling *et al.* 1994). The male fertile plants sometimes produced seed. The ability of male-fertile plants to set seed suggested that ovary development was not entirely suppressed in all flowers. This was termed subdioecious (Bailey 1994). The few seeds found on hermaphroditic Giant knotweed were 2n=44, true *P. sachalinensis*.

Giant and Japanese knotweed had three different ploidy levels in Britain and the base pair chromosome number was 11. All *P. sachalinensis* plants examined in Britain were represented only at the tetraploid level (Bailey and Stace 1992). Octoploid (2n = 88) *P. cuspidatum* was found to be the most common, but it had also been published as 2n = 44 (Bailey 1994). The only variant of *P. cuspidatum* in Britain was octoploid (Bailey and Stace 1992) and female (Conolly 1977). Different specimens (2n = 66) found were morphologically intermediate suggesting a hybrid origin between Japanese (2n = 88) and Giant knotweed (2n = 44) (Bailey and Conolly 1977, Bailey 1994). A clump of hybrid seedlings was found in the field in 1987, all having 54 chromosomes (Bailey 1988a). Work on non-British Isles plants revealed Japanese knotweed had 2n = 44, 60 and 88 chromosomes and Giant knotweed had 2n = 44, 66 and 102 chromosome levels (Bailey and Conolly 1985). Hybrids with 2n = 66 had irregular meiosis and low fecundity (Bailey and Stace 1992). Polyploidy was associated with gigantism, which reached its peak in Giant knotweed. Polyploidy was also associated with asexual reproduction in these taxa.

The *Fallopia* x *bohemica* hybrid was found in the British Isles. It was often mistaken for Japanese knotweed and was very invasive. There was a chance that some plants of hybrid origin had been produced and established naturally from seed, but most examples were thought to have originated vegetatively (Beerling *et al.* 1994). The tetraploid and octoploid hybrids were important as fertile pollen sources. Male-fertile and female-fertile clones need to be adjacent to produce significant amounts of seed. Hermaphroditic plants were not generally capable of self-fertilization (Bailey 1994). There was little germination or penetration of self-fertilized stigmas. Due to the absence of male-fertile plants, there were no reports of pure Giant or Japanese knotweed seedlings in the wild in Britain (Bailey 1994). When Giant or Japanese knotweed geminates were observed in New Jersey, they didn't survive after six weeks (Locandro 1973).

Regardless of how knotweed is propagated, it quickly becomes an invasive monoculture with many undesirable attributes. Japanese knotweed was included in Britain's Wildlife and Countryside Act of 1981, making it illegal to introduce the plant into the wild (Bailey and Conolly 1985, Palmer 1994). Competition with wetland or riparian plants is a concern for ecologists and conservationists, especially in sensitive natural areas. Low plant diversities and poor habitat quality for avian, mammalian and amphibian species are effected where knotweed grows in riparian corridors. Japanese knotweed growth has resulted in the loss of wildlife habitat

and of species diversity (Seiger and Merchant 1997). Invasive exotics are generally considered to be associated with losses of native species (McIntyre 1994). Japanese knotweed needs to be controlled where it displaces native species, spoils recreation areas and intrudes on views (Scott and Marrs 1984). It is considered to be a long-term threat because it occupies woodland margins, riverbanks and verges rated high for biological and visual diversity (Scott and Marrs 1984). The dense canopy within knotweed stands has been reported to reduce native species diversity and restrict growth of ground flora (Child *et al.* 1992). The Nature Conservancy's list of invasive plant species threatening its network of reserves included over 200 species (Campbell 1993). Exotic plants are sometimes the single greatest threat to native species (Cheater 1992).

In addition to the displacement of desirable native plants in riparian area, there are concerns about the potential for stem tissues to alter waterflow. In the British Isles, knotweed was considered a potential flood hazard (Beerling 1991) and a threat to conservation (Baker 1988). Decaying shoots washed into rivers during high flows could create stream blockage and increase the risk of flooding, causing damage to urban flood protection schemes (Child *et al.* 1992). There could be an increase in sedimentation in streams and rivers after flooding events, because of soil exposure (Child *et al.* 1992). Riverbank inspection was hindered by dense stands of knotweed. New propogules become established downstream with only eight grams of fresh root (Beerling and Palmer 1994). Floating rhizomes and stems have propagated new clones down current (Beerling *et al.* 1994).

Giant and Japanese knotweed have been reported to be shade intolerant plants (Beerling 1991). Where Japanese knotweed grew under the canopy of mature trees, the height of the stems was restricted by low light levels (Beerling *et al.* 1994). Plants in woodlands exhibited suppressed growth and abundance; sites receiving low levels of solar radiation did not support dense stands of *P. cuspidatum* (Beerling 1991). Moderately dense woodlands had widespread knotweed but at low cover values, as compared to dense woodlands. This suggested that invasion and establishment of Japanese knotweed in such areas occurred readily in the absence of an established dense ground cover (Beerling 1991) and reduced floral diversity even with low percentage cover (Grime *et al.* 1988). Legitimate concerns for forest health could arise when knotweed grows in woodland perimeters. Giant and Japanese knotweed are able to rapidly colonize canopy openings by root sprouting. Once knotweed populations are established, it is very unlikely that tree species seedlings can become established under the knotweed canopy.

Long-distance expansion of these species' ranges has been primarily by human activities. Redistribution of topsoil contaminated with rhizomes during land clearance associated with building has resulted in the spread of the plant and the development of stands on most major rivers in South Wales (Beerling 1991). Roadsides with high levels of soil disturbance had the lowest densities of rare and native species and the highest number of exotics (McIntyre 1994).

Successful elimination of knotweed will require killing the extensive rhizome systems, and continued monitoring for new invasions (Child *et al.* 1992). When pockets of the species remained upstream from treated areas, the next flood brought propagules of the species back and the cycle of invasion and control was repeated (Usher 1988). Managers at Sites of Special Scientific Interest in the British Isles, nature reserves and national parks have been reluctant to use herbicides, and they have resorted to mechanical methods such as hand pulling, which were

less effective (Beerling 1991). Changes in land use towards more disturbed environments are likely to ensure knotweed would be more troublesome on sites of high conservation and amenity value (Beerling 1991). Where mechanical control means such as cutting, mowing and hand pulling have been used, it has taken multiple years to remove knotweed (Baker 1988). As the knotweed populations declined there was a rapid re-colonization by native species (Baker 1988). Cutting alone did not eliminate Japanese knotweed but was a useful adjuvant treatment to reduce the overall vigor of stands (Seiger and Merchant 1997).

Herbicides have the potential to eliminate knotweed populations faster and more completely than mechanical control methods. McCormick and Bowersox (1998) recommend foliar applications of imazapyr separately or in combination with glyphosate. Plants can absorb imazapyr through the leaves and the soil. It can not be used where there are susceptible hardwood trees and shrubs or adjacent to water. Populations can also be treated with repeated foliar applications of glyphosate (McCormick and Bowersox 1998). Glyphosate is a non-selective herbicide that can not be absorbed from the soil. It can be used selectively as directed applications as well as be used adjacent to surface water. Scott and Marrs (1984) tested herbicides including glyphosate and found that only ammonium sulphamate and picloram were effective in controlling weeds over two years. The technique of eradication will depend on the regulations governing the site and the environmental impact of the method. Surveys of flora and fauna in the habitat should be conducted prior to any control to assess what methods are safe.

General circulation models have been used to project the re-distribution of plants based on temperature and soil moisture alterations resulting from increases in greenhouse gases. A doubling of the atmospheric CO_2 concentration (Beerling 1994) resulted in predictions of the northern spread of knotweed in Europe. Experimental investigations into how the length of the growing season affects the growth of the species and the critical evaluation of the effects of low temperature on Japanese knotweed rhizome and shoot survival have been recommended (Beerling 1994).

It was not known whether the Giant and Japanese knotweed growing within or adjacent to Pennsylvania is capable of producing viable seed. It is also not known if the potential seedlings are capable of becoming established under natural conditions. To answer these questions, a set of studies were designed to examine the sexual reproductive ecology of Giant and Japanese knotweed. Specific objectives of the studies were to:

1. Determine the potential for seed reproduction from Giant and Japanese knotweed populations growing in or adjacent to Pennsylvania.
2. Evaluate the potential for the natural establishment of Giant and Japanese knotweed seedlings beneath or adjacent to seed producing populations.
3. Measure the effect of light intensity on the germination and growth of Giant and Japanese knotweed.
4. Recommend procedures for minimizing the invasion and growth of Giant and Japanese knotweed from seed.

Germination Studies

Introduction

Giant and Japanese knotweed have established populations in a variety of habitats across Pennsylvania. The plants grow especially well in riparian zones, on strip-mine spoils and on roadway and railway fill material. Long stretches of the Susquehanna River and Delaware River corridors have been colonized by both species. Large stands of Giant knotweed and Japanese knotweed existed at the Site Staple Bend Tunnel Unit of the Allegheny Portage Railroad National Historic Site and Delaware Water Gap National Recreation Area, respectively. Giant and Japanese knotweed will propagate vegetatively from extensive root systems. Researchers in the United Kingdom reported no viable seed on Japanese knotweed due to the absence of male-fertile plants (Conolly 1977, Bailey 1994). The small quantities of seed that were found were the result of hybridization with other members of *Polygonaceae*. A researcher in New Jersey reported seedlings under stands of Japanese knotweed (Locandro 1973). The observed prolific seed set on populations in the Northeastern United States warranted further investigation into the potential for reproduction from seed.

Giant and Japanese knotweed can form dense, perennial clumps which can reach heights of 4 m and 2.5 m, respectively. They have erect, bamboo-like, hollow stems that branch at the top. Perennating buds form at the base of the stems and on rhizomes between autumn and winter and emerge as vertical shoots the following spring (Beerling *et al.* 1994). Prolific, creeping rhizomes can be produced within one year. Japanese knotweed leaves are truncate at the base, 5-15 cm long and 2-10 cm wide (Mitchell and Dean 1978). Giant knotweed leaves are cordate based, up to 40 cm long and 22 cm wide. Japanese knotweed is functionally dioecious in the United States. The plants are reported to be gynodioecious (male-sterile and hermaphroditic individuals) in the United Kingdom (Beerling *et al.* 1994). Giant knotweed is dioecious, possibly polygamous when hermaphroditic flowers are produced (Bailey 1994). Achenes are dark, glossy brown, 2 - 4 mm long and 2 mm wide. The hybrid between Giant and Japanese knotweed, *Fallopia x bohemica* (Chrtek & Chrtková) J.Bailey, is intermediate in growth habit, and leaf shape and size (Bailey and Stace 1991).

Germination research on Japanese knotweed seed has been conducted in the United States. Germination values of 84 - 100 % were obtained after air storage at room temperature for five months and at 2 - 4°C in water, in moist peat and between layers of moist cotton for three months (Justice 1941). Light versus dark tests resulted in higher percentages of seeds germinating in lighted conditions (61%) as opposed to dark conditions (13%) (Seiger 1993). However, Locandro (1973) found no significant differences due to light and temperature conditions in the germination of Japanese knotweed seed.

Storage duration has produced mixed effects on germination success. Seiger (1993) had higher germination rates with two-year-old seeds (54%) than with two-week-old seeds (3%). Locandro (1973) found a decline in germination success after 270 days in storage. Significant differences were noted due to the different storage and cold treatment periods prior to germination trials. Locandro (1973) reported that germination continued to increase with longer periods of cold treatment. Germination was poor after one year of seed storage at 18°C and 32% humidity.

Cold treated seed reacted differently than non-cold treated seed after 30, 60 and 90 days of storage. Germination increased with pre-chill treatments of 60 and 90 days (Locandro 1973).

This study was designed to determine the potential for seed reproduction from Giant and Japanese knotweed populations, and to evaluate the potential for the natural establishment of knotweed seedlings beneath or adjacent to seed producing populations in Pennsylvania.

Methods

Field Locations

Eighteen locations containing populations of Giant and Japanese knotweed were chosen across Pennsylvania, in the Delaware River, Ohio River and Susquehanna River watersheds (Figure 1). Eleven of the locations were within or near the following National Park Service properties:

1. Allegheny Portage Railroad National Historic Site – Staple Bend, Lumber Road and Tunnelhill locations.
2. Delaware Water Gap National Recreation Area – Bushkill Church, Dingmans Creek, Freeman Road and Valley View locations.
3. Johnstown Flood National Memorial – Johnstown and St. Michael locations.
4. Valley Forge National Historic Park – Betzwood and Schuylkill Trail locations.

The remaining seven locations were selected from the Susquehanna River watershed which contained no national park lands reported to have knotweed populations. These locations were Archrock, Bitumen, Hyner, McElhatten, Port Trevorton, Rothrock and Sinnemahoning.

Descriptions of the habitat and morphology of the knotweed plants were recorded over two years (Fall 1996 – Summer 1998). Flower development, sex and the general location of male and female plants were recorded in July and August at each location. At that time, differences in leaf size and shape and stem height were also recorded to aid in species identification. The most reliable characteristics for the identification of the species and the hybrid were leaf shape and size. Only mature basal leaves were used because immature and upper leaves were extremely variable (Bailey 1988b). Botanical keys for the identification of *Polygonum* species differentiate between Giant and Japanese knotweeds by truncate and cordate leaf bases (Mitchell and Dean 1978, Wofford 1989). Knotweed seeds were collected in September and October of 1996 and 1997. Seed production estimates were based on the relative amount of seed present as compared to a fully developed raceme with about 1,000 seeds. The number of racemes with seed and the amount of seed produced on each raceme varied widely from location to location, and within a locations. Seed collections were random samples of whole inflorescence from different plants. Seeds were air dried and stored in paper bags at room temperature either with the wing intact (natural seed) or dewinged (achene only).

Seed Viability

Seed viability was based on seed coat and endosperm conditions. Estimates of viability were made on random samples of 100 seeds from each source collected in 1996. The seeds were inspected under a dissecting scope. The conditions of the seed coat and endosperm were evaluated. Seed coats were described as normal (hard), soft, or shrunken. The cream to white

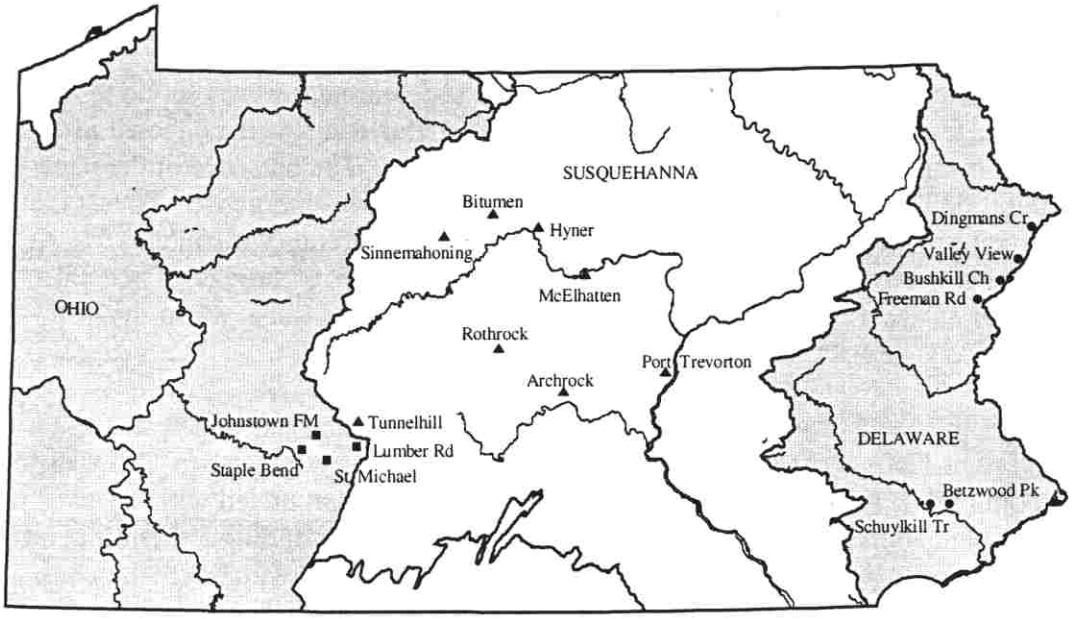


Figure 1. Locations of field sites in the Ohio River (squares), Delaware River (circles) and Susquehanna River (triangles) watersheds.

endosperm content was estimated as full, half, or absent. Similar seed viability assessments were made on 1997 seeds using 25 seeds per source. Viability was based on the number of seeds having normal seed coats and full endosperm.

Germination

Four seed sources (Rothrock, Staple Bend, Port Trevorton and Bitumen) were used to test different seed storage regimes. The seeds used in the germination trials were collected in 1996 and first stored at room temperature for 100 days after collection. The presence of the papery wing could inhibit both water absorption by the seed coat and radicle emergence. To test the effect of wing inhibition, equal numbers of seeds were either left natural (wing intact) or dewinged (achene only) by means of friction after the initial 100 days of storage. The seeds were stored for 30, 75 and 120 days under three different storage conditions: warm-dry, cold-dry and cold-moist (18°, 3° and 3°C, respectively) (Table 1).

Seeds were placed in plastic bags and all bags were then placed in glass jars and sealed (Mumford and Freire 1982). The containers were stored in either a refrigerator (3°C) for the cold condition or room (18°C) for the warm condition. The seeds were surrounded with dry sand for the dry condition or sand moistened with distilled water for the moist condition. Germination was recorded from sterile petri dishes, 9 cm in diameter, that were lined with 9 cm diameter, grade 4 filter paper. Random samples of 40 seeds were placed into 96 dishes and covered with another filter paper. Three milliliters of distilled water was poured over the filter paper to provide moisture. Each dish was placed in a quart size plastic bag with a wet paper towel as a supplemental moisture source. Twenty-four dishes were arranged in randomized block designs on four trays (four replications). The trays were placed in a growth chamber at 27°C and 50% relative humidity. Light was not provided in the growth chamber.

Knotweed seeds have epigeal germination and the basis for determining germination was radicle emergence. The number of germinates was recorded in each dish every three days over a fifteen day period. The seeds were exposed to fluorescent light during the counts. The number of seeds tested in each storage-time trial was 3,840, for a total of 11,520 for all three storage-time trials.

Analysis of variance ($\alpha = 0.05$, fixed effects model) was run to test the effects of seed source, seed condition, storage condition and all of the interactions on seed germination over time. The percent germination values were log transformed for the analyses. Tukey's Honestly Significant Difference ($\alpha = 0.05$) mean separation procedures were used to determine significant differences among the treatments (Steele *et al.* 1997).

Reliability of Viability Assessment

The reliability of using seed coat condition as an indicator of seed viability was tested on seeds collected in 1997. All seeds had been stored under dry, room temperature conditions for 60 days prior to testing. Random samples of 50 seeds were taken from each source. The seed coats were determined to be either normal or shrunken. The seeds were separated as such and put between layers of wetted filter paper in petri dishes. The growth chamber conditions were 27°C and 66% humidity. The number of germinates was recorded every four days for a sixteen day period.

Table 1. Experimental design of seed germination trials conducted in 1997 on Giant and Japanese knotweed seeds collected in 1996.

Variables	Number	Treatments
Seed sources	4	Bitumen, Port Trevorton, Rothrock, Staple Bend
Seed conditions	2	Whole seed, achene only
Storage conditions	3	Warm (18°c)-dry, cold (3°C)-dry, cold (3°C)-moist
Storage times	3	30, 75, 120 days
Replications	4	

Seeds with shrunken seed coats were believed to be those in which the endosperm had not fully developed. Chi Square, two-way test of independence (Little 1978) was used to evaluate the ability to determine seed viability based on seed coat condition ($\alpha = 0.05$, df = 1, $\chi^2 = 3.84$). The null hypothesis was that seed coat condition, normal or shrunken, made no difference in germination.

Seedbank

Soil cores from four study locations, known to have produced viable seed in the fall of 1996, were collected in the spring of 1997. Bitumen, Lumber Road, Rothrock and Staple Bend locations were used in this study. Two hundred soil cores (15-cm diameter, 10-cm deep, surface area = 182 cm^2) were transported to a greenhouse and imbedded into commercial potting media to ensure adequate moisture. The cores were collected with intact (minimally disturbed) soil profiles. The randomized block design was five replications of ten cores per soil source. The number of knotweed seedlings that emerged was recorded from May 25 to August 15. June 20 data was used for the analysis, since there were no net changes in the number of seedlings per core after that date. Analysis of variance ($\alpha = 0.05$, fixed effect model) was run to determine if there were significant differences in seedling number among the soil sources.

Results

Field Locations

Hyner, McElhatten, Port Trevorton and Sinnemahoning in the Susquehanna River drainage were within 30 m of water whereas Archrock, Rothrock and Tunnelhill were located near roads at upland sites. Bitumen was an abandoned strip-mine at an upland site. The Delaware River drainage locations were within 5 m of water, except Bushkill Church which was on abandoned fill next to a visitors center. The Ohio drainage populations were in mixed settings. Johnstown and Saint Michael were within 10 m of the South Fork of the Little Conemaugh River, while Staple Bend was approximately 150 m from the Little Conemaugh River. Lumber Road was adjacent to an abandoned strip-mine at an upland site. Litter of leaves and stems had accumulated at Archrock, Bushkill Church, Staple Bend, Rothrock and Tunnelhill but not at Bitumen or Lumber Road. The Susquehanna River and Delaware River riparian locations had extensive, impenetrable stands of Giant and Japanese knotweed. The dense litter that built up underneath the populations every fall was usually washed away by summer. The sandy soil was usually exposed underneath the plants.

The phenotypic plasticity that occurred in each knotweed community made identification of species difficult. Mature basal leaves were reliable indicators of species and were used to identify Giant and Japanese knotweed and the hybrid. There were ten Japanese knotweed locations, seven Giant knotweed locations and one hybrid location (Table 2). The Japanese knotweed populations had maximum heights of 3.5 m. The tallest Giant knotweed plants were located at Staple Bend, Sinnemahoning and Bitumen, all of which had maximum heights of four meters.

Plants at every location produced seeds in both 1996 and 1997, except for Schuylkill Trail. No seeds were found at the Schuylkill Trail location in 1997. The amount of seed produced was expressed as the relative number of seeds per raceme, with a fully developed raceme having about 1,000 seeds (Table 2). Viable seeds were not found when male-fertile plants were absent from the location. Abundant seeds were produced when both female-fertile and male-fertile plants were present at a location. Viable seeds were not found at the Valley View and Archrock locations in 1996; all of the seeds were empty.

The majority of populations in this study had two types of flowers. There were male-fertile (male) and female-fertile (female) flowers on separate plants. Male flowers were white, had 7-8 long stamens and a vestigial, small, yellow ovary. The ovaries on male flowers were presumed nonfunctional because no seeds were produced. Male inflorescence stems pointed upwards. Male flowers were fragrant, bright white and had a feathery appearance. Female flowers were yellow-green and had a larger, yellowish ovary with feathery stigmas and vestigial stamens at the base. The stamens in female flowers were presumed non-functional, due to the lack of seed set when male flowering plants were absent. The female plants were not able to fertilize each other. The inflorescences were arrayed outward or downward. Female plants drooped with the weight of abundant, mature seed. Viability tests were not conducted on the two types of flowers to determine if the vestigial sex organs were functional. Flower maturation and pollination were not simultaneous in all populations. The locations that were higher in elevation and farther to the north (Bitumen, Sinnemahoning and Lumber Road) had earlier development of flowers and seeds.

The third flower type, hermaphroditic, was found at a few locations. The pistil and stamens were proportionate in size with the stigmas and anthers equal in height. Seeds were found on male-fertile plants at Sinnemahoning and Staple Bend.

At some locations, there were instances of achene separation from the perianth prior to dispersal. It was not known if the seed naturally fell out or if it was consumed. Bees, ants, butterflies and beetles were observed on the flowers and were the likely pollinators of the flowers. Some inflorescences appeared to have had insect damage; the seed coat and the endosperm were eaten.

In May 1997 and 1998, tens to thousands of newly germinated seedlings were seen per 100 m^2 at Bitumen, Dingmans Creek, Lumber Road, Port Trevorton, Rothrock, St. Michael, Sinnemahoning and Staple Bend. Differences in the germination and the survival of seedlings were observed between the area directly under the knotweed canopy and the area beyond the perimeter of the knotweed canopy. Those seedlings that had germinated under the canopy of the parents and in the leaf litter did not survive. When seeds germinated on paths and in open areas with exposed soil, the seedlings were able to survive. Thousands of seedlings survived to the end of the first growing season. A few of the seedlings measured more than 10 cm in height by July 1998. Second-year seedlings of Giant knotweed were discovered at Bitumen and Sinnemahoning in 1997 and at Bitumen, Port Trevorton and Lumber Road in 1998. These seedlings had completely independent root systems and had remnant stems from previous years' growth.

Table 2. Species, colony height, seed crop, seed viability, sex ratio and Chi Square values for Giant and Japanese knotweed study locations in the Ohio, Susquehanna and Delaware river watersheds.

Watershed location	Knotweed species	Average height	Seed crop[a]	Viability[b] 1996	1997	Sex ratio	Chi square[c]
Ohio River		(-m-)	(----------%----------)				
Johnstown	Giant	3.0	90	95	90	F=M	---
Lumber Road	Giant	4.0	90	100	85	F=M	0.72
St. Michael	Giant	3.0	90	95	75	F=M	7.53*
Staple Bend	Giant	4.0	90	95	90	F<M	0.23
Susquehanna River							
Archrock	Japanese	3.5	1	0	40	F	14.00*
Bitumen	Giant	4.0	90	90	90	F<M	1.98
Hyner[d]	Japanese	2.7	70	60	80	F<M	7.53*
McElhatten[de]	Hybrid	4.0	90	80	-	F>M	---
Port Trevorton[e]	Japanese	4.0	90	85	95	F>M	---
Rothrock	Japanese	3.5	90	85	100	F=M	---
Sinnemahoning	Giant	4.0	90	-	95	F=M	2.62
Tunnel Hill	Giant	3.5	90	100	90	F<M	1.89
Delaware River							
Betzwood Park	Japanese	2.5	1	80	85	F>M	6.97*
Bushkill Church	Japanese	3.3	5	95	85	F>M	3.25
Dingmans Creek	Japanese	3.5	75	90	95	F>M	---
Freeman Road	Japanese	3.3	1	90	95	F	---
Schuylkill Tr.	Japanese	2.0	1	65	-	F	---
Valley View	Japanese	2.5	1	2	10	F	8.20*

[a] Based on a fully developed raceme with about 1,000 seeds. Seed crop values were the same in 1996 and 1997.

[b] Viability was based on visual assessments of seed coat and endosperm.

[c] $\chi^2 = 3.84$, df = 1. Seed coat condition was used as a predictor of viability in germination tests. Significance is designated by the * symbol.

[d] Mostly the indicated species but the other species or a hybrid of the two species was observed to be in the general vicinity.

[e] May be the hybrid of the two species, *F. bohemica*.

Seed Viability

There was little variation in color and size of seeds among populations over the two years. Giant knotweed had slightly larger seeds (approx. 1 mm longer) than Japanese knotweed seeds. Seed conditions ranged from firm seed coat with slightly convex sides and a cream endosperm to shrunken seed coats with a depleted or absent endosperm. Like the seed coat, the endosperm was generally convex trigonal. The outer cells were either green or tan. As the endosperm desiccated, there was a slight separation of the embryo from the endosperm and it turned chalky white. Some seeds were empty shells with brown tissues inside. Endosperm absence was observed to be related to times when no male-fertile plants grew near the study locations.

Viability values were determined for each location, based on the seed coat and endosperm conditions (Table 2). In 1996 and 1997, Betzwood, Bitumen, Bushkill Church, Dingmans Creek, Freeman Road, Johnstown, Lumber Road, Port Trevorton, Rothrock, Staple Bend and Tunnel Hill locations had viability values above 80%. Seed sources with the lowest viability (0% - 40%) were Archrock and Valley View. These locations produced endosperm that was only partially developed or absent.

Germination

Bitumen, Port Trevorton, Rothrock and Staple Bend were chosen to evaluate the dormancy requirements because these sources had seeds with high apparent viability. In our preliminary trials to refine our procedures we used seed stored in paper bags for 15 days at room temperature. Seed samples stored at room temperature germinated within five days of being placed in the petri dishes but no attempt was made to quantify germinate success in these trials. In the designed dormancy study, seeds from the four sources, the two seed conditions, the three storage conditions and the three storage times had an overall mean germination of 91%. The analysis of variance test indicated significant differences in germination due to seed source, seed conditions and storage conditions over time.

Overall mean germination for Bitumen (97%) was significantly different from Port Trevorton (93%), Rothrock (89%) and Staple Bend (87%); Port Trevorton was significantly different from Rothrock and Staple Bend; and there was no significant difference between Rothrock and Staple Bend. Although the ranking of mean germination values among the four seed sources did not change, significant mean germination differences among the four seed sources varied slightly for the individual time periods (Table 3).

Overall, mean germination was significantly different for the two seed condition treatments for all storage times, except the 30-day period. The dewinged seed condition had significantly different germination values after 75 and 120 days storage (93% and 94%, respectively) than the natural seed condition (89% and 90%, respectively) (Table 3). Over all storage times the mean dewinged seed condition germination value of 93% was significantly different than the natural seed condition of 90% (Table 3).

The three storage conditions tested, warm-dry, cold-dry and cold-moist, were all sufficient for germination (Table 3). Over the three storage times, mean germination percentage for seeds

Table 3. Mean[a] germination of Giant and Japanese knotweed seeds by seed source, seed condition and storage condition treatments after 30, 75 and 120 days storage.

Factors	Storage time (days) 30	75	120	Overall
	(----------%----------)			
Seed Source (SS)				
Staple Bend (SB)	86b	87b	87c	87c
Rothrock (RR)	88b	88b	90bc	89c
Port Trevorton (PT)	93a	92ab	93ab	92b
Bitumen (BT)	96a	96a	98a	97a
Seed Condition (SC)				
Natural	90a	89b	90b	90b
Dewinged	91a	93a	94a	93a
Storage Condition (StC)				
Warm-dry	90b	87b	90b	89b
Cold-dry	87b	91b	90b	89b
Cold-moist	95a	95a	96a	95a
SS * StC				
SB * warm-dry	84bc	85b	84b	84c
SB * cold-dry	83c	87b	83b	84c
SB * cold-moist	92ab	91ab	94ab	92b
RR * warm-dry	85bc	80b	84b	83c
RR * cold-dry	81c	85b	89b	85c
RR * cold-moist	98a	98ab	97ab	98a
PT * warm-dry	95ab	88b	94ab	92b
PT * cold-dry	90b	94ab	90ab	92b
PT * cold-moist	92ab	93ab	94ab	93ab
BT * warm-dry	95ab	94ab	98a	96ab
BT * cold-dry	96ab	96ab	98a	96ab
BT * cold-moist	97ab	99a	99a	98a

[a] Means within a factor and under a storage time with the same lower case letter were not significantly different at $\alpha = 0.05$. Total number of seeds was 11,520.

stored under cold-moist conditions (95%) was significantly different than for seeds stored under the warm-dry (88%) and cold-dry (90%) conditions (Table 4). Seed source and storage condition interaction factor was significant for all storage times (Table 3).

Staple Bend and Rothrock had one pattern of response to the three storage conditions, while Port Trevorton and Bitumen had another pattern (Figure 2). There were significant differences in germination among the storage conditions for Staple Bend and Rothrock seeds. The cold-moist treatment resulted in significantly higher germination, 92% and 98% for Staple Bend and Rothrock seeds, respectively. The warm-dry and cold-dry treatments resulted in 84% germination of Staple Bend seeds. For Rothrock seeds, warm-dry and cold-dry treatments resulted in 83% and 85% germination, respectively. For Port Trevorton and Bitumen seeds, no significant differences were detected among the three storage conditions.

Reliability of Viability Assessment

Chi Square analysis indicated seed coat condition resulted in significantly different germination values for these five sources: Archrock, Betzwood, Hyner, St. Michael and Valley View (Table 2). The null hypothesis was rejected for these seed sources, because seed coat condition did make a difference in germination. Seeds with shrunken seed coats had significantly lower germination values than the expected values. Germination values for Bitumen, Bushkill Church, Lumber Road, Sinnemahoning, Staple Bend and Tunnelhill were not significantly different between normal and shrunken seed coat conditions. The null hypothesis was not rejected for these six seed sources. Seed coat condition was not a consistently good descriptor of seed quality and other methods should be explored.

Seedbank Study

Soil cores were placed into a greenhouse on May 20, 1997. Germination of the Giant and Japanese knotweed seeds began May 27 and culminated on July 2. Analysis of variance test indicated the mean numbers of seedlings per core from Lumber Road (24) and Rothrock (32) soil cores were significantly different than the number per core from Staple Bend (5) and Bitumen (4). There were no significant differences between Staple Bend and Bitumen or between Rothrock and Lumber Road (Table 5). The majority of seedlings emerged from the sides of the core, where the soil profile had been slightly disturbed during field collection and placement into the greenhouse bed. Many seedlings from all four sample locations survived until the study was dismantled in October. Many germinates died in the crowded conditions but some seedlings grew well enough to produce flowers and seeds by October. Each core had a surface area of 182 cm^2, thus one seedling per core was equal to 55 seedlings / m^2. Number of potential seedlings from soil collected at four locations averaged 894 / m^2 and ranged from 0 to 6,600 seedlings / m^2.

Discussion

Giant and Japanese knotweed were found throughout three river drainages in Pennsylvania and in a variety of habitats. Many stands of these species were observed to be growing along waterways as well as in isolated upland locations. Areas of land disturbances such as flood

Table 4. Mean[a] germination percentages for storage times (30, 75 and 120 days) and environmental conditions (warm-dry, cold-dry and cold-moist) among all seed sources and seed conditions.

Environmental Conditions	Storage time (days)			
	30	75	120	Overall
	(----------pct----------)			
Warm-dry	88b	88b	90b	88b
Cold-dry	88b	90b	90b	90b
Cold-moist	95a	95a	98a	95a

[a] Values within each storage time with the same lower case letter were not significantly different at $\alpha = 0.05$. Number of seeds per storage time and storage condition was 1,280.

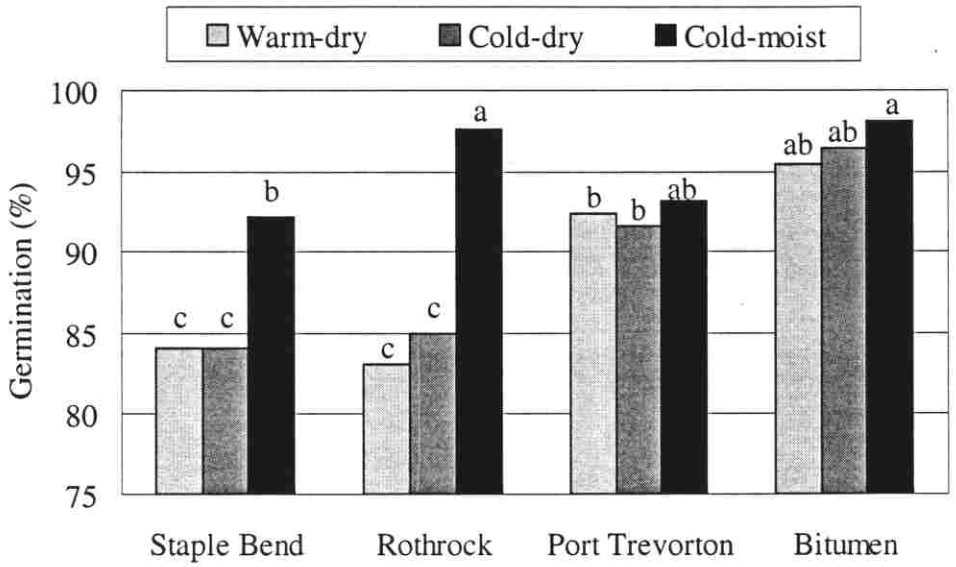


Figure 2. Overall mean germination for Giant knotweed (Staple Bend, Bitumen) and Japanese knotweed (Rothrock, Port Trevorton) seeds under the three storage conditions (warm-dry, cold-dry and cold-moist). Values among all seed sources and storage times with the same lower case letter were not significantly different at $\alpha = 0.05$.

Table 5. Mean[a] number of Giant and Japanese knotweed seedlings per core, per m^2 and the range of seedlings per core from Bitumen, Lumber Road, Rothrock and Staple Bend soil.

	Seedlings		Range
Soil Source	per core	per m^2	per core
Bitumen	4b	220	0 - 37
Lumber Road	24a	1319	0 - 120
Rothrock	32a	1758	0 - 106
Staple Bend	5b	275	0 - 28

[a] Values for mean number of seedlings per core among the soil sources with the same lower case letter were not significantly different at $\alpha = 0.05$. Number of soil cores per source was 50.

plains, railways and roadways, dump sites and strip-mines were frequent locations of knotweed communities. All populations used in this study were well established and completely dominated the site. Millions of seed were produced annually by Giant and Japanese knotweed at the majority of these locations. Seed production in a population was related to the number and proximity of female-fertile and male-fertile plants. It was observed that the intermingling of female-fertile and male-fertile plants resulted in higher quantities of seed than if the different-sex plants were spatially separated. Occasional breakdowns in dioecy must occur because hermaphroditic flowers were observed in the field.

Multiple year true seedlings were discovered at four locations in the summers of 1996 and 1997. In the summer of 1997, a few seedlings were found at the Staple Bend Tunnel location. The access road to the tunnel was reshaped by earth moving equipment in the fall of 1997. The soil disturbance resulted in thousands of Giant knotweed seedlings along the roadway the following summer.

Damage to seed and seedlings was witnessed during field visits. Racemes were found to have partially or completely consumed seeds. Predation by birds may be a factor in rarity of seedling germination and establishment in the wild (Beerling et al. 1994). At all of the locations and on most visits to the locations, birds were seen on the plants feeding on either seeds or insects. Terrestrial invertebrates in the leaf litter could also be responsible for damage done to seeds and seedlings.

Seed coat was not a consistently good indicator of seed viability. Some of the shrunken seedcoat seeds performed as expected with no germination. However, some shrunken seedcoat seeds had high germination values. The explanation was probably linked to the population structure and the relative proportion of different sex plants. A common factor among the sources that performed as expected was the ratio of female to male plants. Japanese knotweed plants at Hyner, Valley View, Archrock and Betzwood exhibited shrunken seed coats and all had mostly female or only female parent plants. Bushkill Church had more female Japanese knotweed plants than male plants. The remainder of the locations had Giant knotweed; of these, Staple Bend, Tunnelhill and Bitumen had a greater proportion of male plants. Lumber Road and Sinnemahoning had equal numbers of male and female plants. The abundance of male plants was integral to seed viability because of increased chances of pollination.

The potential of Giant and Japanese knotweed seed to germinate probably depended on the quality of the endosperm, and we were not able to non-destructively assess endosperm quality. Other non-destructive methods of assessing endosperm quality, like water floatation or X-ray analysis, may be more successful than the visual methods. Since Giant and Japanese knotweed seeds had no apparent dormancy requirement, an excellent method for determining the sexual reproduction of a population would be a simple germination test.

The germination tests on different combinations of moisture, temperature, wing conditions and storage time showed that Giant and Japanese knotweed seeds have few, if any, dormancy or storage requirements. Although significant differences were found with selected moisture and temperature conditions, the high germination success achieved with all treatments demonstrated the species' potential to reproduce sexually. The abscission zone in the pedicel may be

prematurely inactivated by the first hard frost, pre-empting dispersal by wind (Beerling et al. 1994). The seed condition of wing intact or removed was found to not be a critical factor in nature, because seed rarely dispersed without the wing. The germination trials showed that Mid-Atlantic climate was appropriate for seed germination. Warm-moist, 18°C, conditions resulted in immediate germination of seeds. The germination patterns in response to storage conditions were not attributed to species differences, because seeds collected from Staple Bend and Bitumen were Giant knotweed, while those from Rothrock and Port Trevorton were Japanese knotweed. Since collection was done in October, all seed had similar maturity and equal time to after-ripen in the laboratory.

Viable seedbanks were found at all four of the locations tested (Bitumen, Lumber Road, Rothrock and Staple Bend). The total number of seeds within the soil was not counted. Bitumen cores had lower numbers of seedlings than expected, based on the amount of seed produced and germination results of seeds from this source in the controlled conditions of the germination study. This difference may be attributed to the difficulty of collecting an intact soil core. Gravel in the strip-mine soil made it nearly impossible to extract an intact soil core close to the knotweed plants. The low number of seedlings could be due to a reduced amount of soil collected, or to increased distance from parent plants. Bitumen and Lumber Road cores had little humus and came from gravel road areas, while Staple Bend and Rothrock soil cores had thick humus layers. Rothrock had significantly different numbers of seedlings germinating from soil cores than Staple Bend. There were no significant differences in either the amount of seed produced or the germination potential for the two sources. Soil quality and abundance of seed-eating invertebrates may be contributing factors to the differences in number of seedlings emerging from the soil cores. The seedbank study indicated that Giant and Japanese knotweed seedlings from Bitumen, Lumber Road, Rothrock and Staple Bend were also capable of surviving through the first growing season. Observations during the seedbank study suggested that soil disturbance might lead to higher incidences of seedling emergence.

From this series of tests, it is clear that Giant and Japanese knotweed are capable of reproducing sexually in Pennsylvania. Efforts to eliminate these plants from national park properties must take into account the potential for new plants to become established from the seedbank. Additional information is needed on the ecology of Giant and Japanese knotweed seedling establishment and growth.

Shade and Seedbed Study

Introduction.

Given the potential for both Giant and Japanese knotweed to reproduce from seed, information on the factors controlling the establishment and growth of knotweed seedlings is needed by resource managers to develop effective management programs for eliminating knotweed from national park properties. Shade intolerance of seedlings was reported by Locandro (1973). The effects of light on Japanese knotweed rhizome fragment growth were investigated at Rock Creek Park, Washington D.C. (Seiger 1993). Compared to full light treated plants, light intensity under the forest canopy decreased the survival and growth of plants (transplanted root segments). Greenhouse experiments that tested the effects of varying light intensity (0, 51 and 80%) on growth and survival of root transplants using shade cloth indicated no significant differences in stem volume, however basal stem diameter was significantly affected by varying light intensities (Seiger 1993). Below-ground biomass was significantly affected by light; The greater the light intensity the greater the basal stem diameter and root biomass (Seiger 1993).

Survival of Japanses knotweed seedlings on Mt. Fuji, Japan was size dependent (Maruta 1983). To survive the dormant season and regrow in the second year, seedlings had to reach a critical dry weight of 40 mg in the first year (Maruta 1983). Very small seedlings did not form the characteristic perennial buds at the base of the stems. Smaller seedlings showed lower freezing resistance during winter. The development of cold hardiness depended on the sufficient accumulation of carbohydrates. Rhizomes of small seedlings had less starch than those of large seedlings (Maruta 1983). Perennial growth was directly related to the critical amount of photosynthate needed for perennating bud formation.

This study was designed to test the effects of different light intensities and the presence or absence of a leaf litter seedbed on germination, survival and growth of Gaint knotweed seedlings.

Methods

A mowed grass area (20 x 30 m) near the Forestry Resources Laboratory at the Pennsylvania State University was used for this experiment. In the fall of 1996, a 2% solution of glyphosate was applied to the entire area to eliminate the existing vegetation. Experimental plots (3.5 x 3.5 m) were installed in May 1997. There were three shade levels (0, 30 and 63%), two seedbed conditions (litter absent - LA, litter present - LP) and two seed sources (Bitumen, Staple Bend). The plots were arranged in a randomized split-split plot design of twelve plots in four replications (Figure 3). The distance between all replications and shade level plots was 1 m. Two hundred and forty soil cores (15 cm diameter, 10 cm deep, surface area of 182 cm^2) were collected from a maturing hardwood stand on Pennsylvania State University property. The collection site was at least 1.5 km from known knotweed communities and the soil type (Hagerstown silt loam, Braker 1981) was of the same origin as the soil at the Forestry Resources Laboratory. The litter was 1 to 2 cm in depth and composed of leaves from mixed oak species.

In May 1997, soil cores from the hardwood site were transplanted into same sized holes at the study site. Within each treatment plot, seed source sub-plots were randomly assigned to either Bitumen or Staple Bend seeds. Each sub-plot consisted of 10 cores arranged in 2 x 5 rows and spaced 0.5 m apart (Figure 3). Giant knotweed seeds were collected in the fall of 1996 and were stored under warm-air dry conditions at 18°C. Twenty five to thirty seeds were planted May 20 on the soil surface in each core. Cores were randomly assigned to five litter absent or five litter present treatments per seed source per sub-plot.

Shade cloth was attached to frames (3.5 x 3.5 x 1 m) that were placed over the plots prior to seed placement. Thirty percent and 63% black shade-cloth covered the top and extended 0.5 m down all sides of the frames. Plots with the 0% shade treatment did not have shade frames.

To reduce seed predation, circular PVC collars (15 cm diameter, 15 cm high) were placed around the cores, with 10 cm of the collar remaining above ground. Wire screens (1 x 1 cm openings) were attached to the top of the collars to prevent herbivory. The screens were removed from the cores when knotweed seedlings came in contract with the screens. The tallest five seedlings were allowed to grow in each core, all the others were removed. Sprinkler irrigation was used to augment rainfall so that the plants received at least 2.5 cm of water per week. Non-knotweed vegetation within the cores was manually removed. Herbaceous plants growing outside the cores were retained unless they altered the amount of sunlight reaching each core.

Slug (*Percoceras reticulatum*) foraging became a problem in May and June 1997. Metaldehyde (2,4,6,8-tetramethyl-1,3,5,7-tetraoxycyclo-octane, 2% concentration) was dispensed into the cores to reduce slug foraging. All slugs found (alive and dead) were removed from inside the collars.

A Chi square, two-way test of independence ($\alpha = 0.05$, df = 5, $\chi^2 = 11.1$) was used to determine if seedling survival was dependent on the shade and litter treatments (Steele *et al.* 1997). A contingency table was constructed with all of the shade and litter treatment combinations. There were six rows of treatments (0% Shade Litter Absent, 0% Shade Litter Present, 30% Shade Litter Absent, 30% Shade Litter Present, 63% Shade Litter Absent, 63% Shade Litter Present) and two columns of seedling observations (present, absent). The number of cores with and without surviving seedlings was tabulated. The null hypothesis tested was that the number of cores with seedlings surviving through the first growing season was independent of the shade and litter treatments.

At the end of the 1997 growing season the shade cloth was removed from the frames. All of the knotweed seedlings were harvested at ground level. The seedlings were oven dried at 90°C for seven days. The seedlings were weighed to the nearest 0.01 gm. Analysis of variance ($\alpha = 0.05$, fixed effects model) was used to test the main effects of seed source, shade and seedbed treatments and the interactions on seedling above-ground biomass. Tukey's Honestly Significant Difference mean separation procedures ($\alpha = 0.05$) were conducted on all significant effects.

The shade cloth was reattached to the frames in May of 1998. Supplemental irrigation was not applied in the second growing season. Numbers of stems from basal buds on rhizomes were

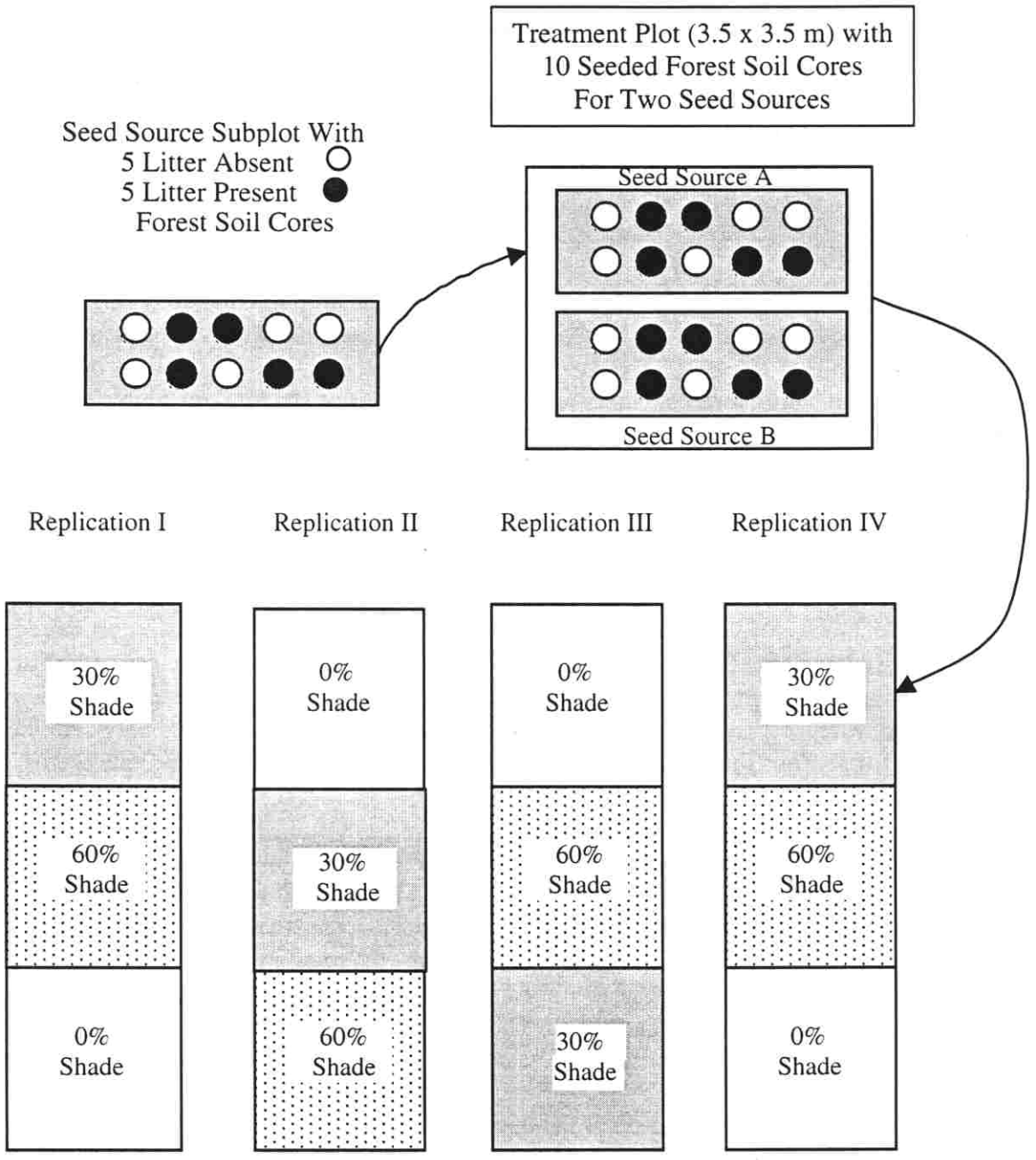


Figure 3. A diagram indicating the arrangement of shade and litter treatments for two seed sources (Bitumen and Staple Bend).

recorded. The physical conditions of the stems were recorded, including color, leaf shape and insect damage.

A Chi square, goodness-of-fit test ($\alpha = 0.05$) was conducted on the number of stems in the two seedbed treatments ($\alpha = 0.05$, df = 1, $\chi^2 = 3.84$). A Chi square, goodness-of-fit test was also conducted on the number of stems with the three shade treatments ($\alpha = 0.05$, df = 2, $\chi^2 = 5.99$). The null hypotheses tested were that the number of stems was independent of both the shade and seedbed treatments.

Final stem height (cm) and diameter (mm) of all stems were measured in August 1998. In late August, all stems were harvested, oven-dried at 105°C for 48 hours and weighed to the nearest gram. Analysis of variance ($\alpha = 0.05$, fixed effects model) was conducted on the total biomass per core and individual stem biomass data to test the main effects of shade, seed source and their interactions. Biomass data were then log transformed for analysis. Seedbed treatment was not included in the model because no stems emerged in the litter present cores.

Results

Seed germination occurred under all shade and seedbed treatments in the first growing season. Many cores had greater than five seedlings but all seedlings smaller than the five largest were removed. In September of the first growing season (1997), 418 seedlings were harvested. The number of cores with seedlings surviving through the growing season was dependent on the shade and seedbed treatment combinations. There were no significant differences between the two seed sources for all comparison and all parameters. Therefore, all seed source data were pooled.

Total numbers of Giant knotweed seedlings that survived until the end of the first growing season under 0, 30 and 63% shade with the litter absent of 140, 138 and 80, respectively. These survival values were significantly different from 35, 15 and 11, respectively, with the litter present treatments (Figure 4). Over both seedbed conditions, the total number of seedlings that survived to the end of the first growing season for the 0% shade (175) and 30% shade (153) were significantly different from the 63% shade (91). There was no significant difference between the 0 and 30% shade in the number of seedlings at the end of the first growing season.

The overall mean above-ground biomass for the one-year-old stems was 1.71 ± 0.32 g and individual stems ranged from 0.01 - 26.88 g. The mean above-ground biomass for seedlings in litter present treated cores of 1.17 ± 0.43 g was significantly different than the 2.25 ± 0.16 g for the litter absent treated cores. There was no significant difference in the mean above-ground biomass of seedlings among the shade treatments.

Four hundred one seedlings reached the critical minimum dry weight of 40 mg needed for survival into a second year (Maruta 1983). These seedlings were expected to be perennial the second year.

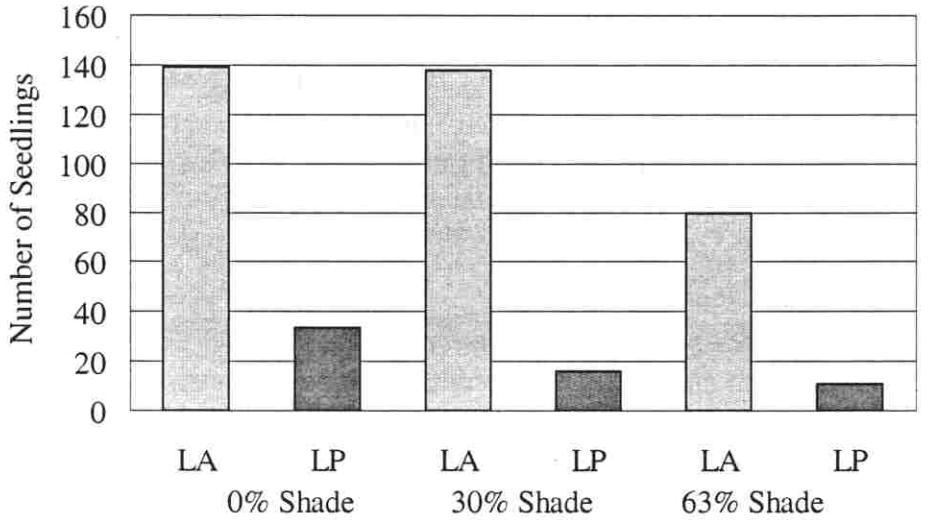


Figure 4. The number of Giant knotweed seedlings that germinated and survived until September 1997, under 0, 30 and 63% shade and litter present (LP) and litter absent (LA) treatments.

There were 125 stems from 30 cores in June 1998. In August, there were 109 stems from 41 individual rhizome crowns in 30 cores (Table 6). All stems originated inside the cores and were from the litter absent seedbed condition. Seventy nine percent of the cores had stems that originated from one rhizome crown (n = 24). Chi square analysis also indicated significant differences among the number of stems under the three shade levels (Table 6). The numbers of stems for the 0% shade (28) and 63% shade (23) were not significantly different from each other but both were significantly different from the number of stems for the 30% shade (58). The overall mean height of the 109 stems was 53.7 cm. and there were no significant differences due to shade.

The mean above-ground stem biomass produced from a single stem was 37.4 g. An analysis of variance test on stem biomass indicated no significant differences due to seed source or shade treatments (Table 7). An analysis of variance on total biomass per core indicated no significant differences among the shade and seed source treatments. The biomass ranges per origin or per core were large and resulted in too much variability to detect differences among treatments.

Four, newly germinated seedlings were found on June 9, 1988 and three survived through August. One of the seedlings was from Bitumen seed under 63% shade had an above-ground biomass of 0.5 g, one was from Bitumen seed under 30% shade had an above-ground biomass of 0.6 g and the other seedling was from Staple Bend seed under 30% shade had an above-ground biomass of 2.6 g. All seedlings were from cores that had the litter present treatment in the first year.

Flowers and a few seeds were found on the Giant knotweed stems in August 1998. Male flowers, characterized by long stamens and tiny ovaries, were found on Bitumen and Staple Bend stems under 30% and 63% shade. One Bitumen stem under 63% shade had two flower types, male and hermaphroditic. The pistil and stamens were functional size, with the stigmas and anthers at the same height. Similar flowers were found on a Staple Bend stem under 63% shade in another replication, on which seeds were produced. Since there were no flowers in the entire study area at the Forest Resources Laboratory study site that had the normal female structure, the seed that was produced came from hermaphroditic plants.

Discussion

Prior to this experiment, it was not known whether Giant or Japanese knotweed seedlings could survive through the winter into a second year. Previous researchers stated that Japanese knotweed seedlings were not capable of survival through the summer (Locandro 1978, Seiger 1993, Seiger and Merchant 1997). There have been no explanations as to why there were so few seedlings surviving in nature. This study was conducted under semi-controlled conditions and shade was created by shade cloth. Competition for water, nutrients or space that could have been present from shade producing vegetation in natural settings was not present in this study. We found evidence that perennial growth could be established from seeds produced by wild Giant knotweed communities. Shade and seedbed conditions could influence the establishment and growth of Giant knotweed. We also found that Giant knotweed seedlings from Staple Bend and Bitumen sexually matured in both the seedling and stem stages.

Table 6. The number of observed and expected Giant knotweed stems by shade and seedbed treatment and the Chi Square goodness-of-fit critical (χ^2 cr.) and calculated χ^2 ca.) values ($\alpha = 0.05$). Significant differences are denoted by the * symbol.

Factors	Cores[a]	Obs.	Exp.	χ^2 cr.	χ^2 ca.
Shade level				5.99	216.5*
0%	10	28	165		
30%	14	58	148		
63%	6	23	88		
Litter treatment				3.84	236 *
Absent	30	109	360		
Present	0	0	61		

[a] The number of cores with perennial growth from roots and below-ground buds in August 1998.

Table 7. Giant knotweed mean stem above-ground biomass per rhizome crown by shade level in 1998. The ranges of biomass per core and per individual rhizome crown are listed. Error terms are 95% confidence intervals.

Shade Level	Mean ± SE per rhizome crown	Range of Biomass per core	Range of Biomass per rhizome crown
	(----------g----------)		
0%	36 ± 33	3 - 133	3 - 133
30%	77 ± 25	1 - 433	1 - 108
63%	85 + 41	9 - 198	9 – 123

Decreased light levels were expected to reduce seedling survival and vigor, and possibly seed germination. The Giant knotweed seeds produced at Bitumen and Staple Bend were capable of germinating and surviving in a variety of natural conditions. The greater shade (63%) had lower number of seedlings than either the 0 or 30% shade but shade level was not a consistent significant factor in either the growth of seedlings or the growth of perennial stems. This was contrary to the findings of Locandro (1973) and Seiger (1993) who reported shade intolerance of Japanese knotweed.

Thick leaf litter under knotweed stands was thought to impede seedling germination and survival. We found that, compared to a seedbed with litter absent, a seedbed with litter present had substantially lower success of establishing Giant knotweed seedlings. And furthermore, if seedlings did become established with litter present, they were smaller than seedlings that developed from a litter absent seedbed. Reduced germination success and survival of Giant knotweed seedlings in the presence of the leaf litter seedbed was mainly due to physical impediment of the leaves to the germinating seedlings. The majority of seeds placed under litter germinated but there were not sufficient energy reserves for the seedlings to reach heights that surpassed the leaf litter. The cotyledons were yellow and the stems etiolated due to the lack of sunlight. The presence of herbivorous invertebrates, like slugs, using the leaf litter as habitat may also be also a factor influencing the survival of seedlings. Field location investigations showed that the thick litter beneath knotweed populations was good habitat for slugs and other terrestrial invertebrates.

None of the seedlings established in the cores with litter present survived to become perennial. Although not tested, we believe the litter-present seedlings must have used more energy for survival and were not able to allocate sufficient reserves to the below-ground tissues to become perennial. All of the second growing-season stems were from the litter absent seedbed condition. Apparently the favorable germination and initial growth conditions also enabled the seedlings to accumulate sufficient below-ground reserves for perennial growth.

All of the second growing season stems were confined to the cores. The stems originated from basal buds on the parent rhizomes. It is possible that some basal buds were damaged or included in the above-ground seedling harvest in 1997. Therefore the number of stems in the second growing season may have been lower than they could have been if the first year stems would have been permitted to die naturally.

Variation in the stem biomass data limited our ability to detect significant differences among the shade treatments. The ranges in biomass measures and standard deviations for Bitumen and Staple Bend stems under any shade treatment were large and had a high amount of overlap. The size and presence of stems in 1998 were not independent of the initial seedling growth. Whatever energy the seedlings were able to store had a direct effect on the size of any perennial growth.

Results from this study failed to provide information on how light intensity may affect the chances of developing perennial communities of Giant knotweed. It is possible that the apparent intolerance to shade is more of an intolerance to above- and below-ground competition for a variety of factors, not simply sunlight. Future studies should be directed towards gaining an

understanding of which factors may be responsible for controlling the growth of Giant and Japanese knotweed. In addition, a greater understanding of the elements affecting the contribution of roots, rhizomes and basal buds to the perennial vegetation is needed. The results from this study strongly support the importance of maintaining an intact, well developed layer of tree leaves to reduce the chances that germinating seeds will establish a perennial community. Future studies should be designed to determine which attributes of a litter layer make it a good natural control of Giant and Japanese knotweed becoming established from seed.

Conclusions and Recommendations

Conservationists and agencies concerned with controlling Giant and Japanese knotweed should be aware that the populations used in this project were capable of reproducing sexually within Pennsylvania. Millions of viable seed were produced annually in the communities examined in this study. Multiple-year seedlings with independent root systems were discovered at four of the 18 study locations. The high viability of knotweed seeds from Pennsylvanian locations was in contrast to the findings from the United Kingdom. The difference was due to the presence of both male- and female-fertile plants at the Pennsylvania locations and the absence of male-fertile plants in the United Kingdom. Giant and Japanese knotweed seeds from four communities had high germination potential, regardless of storage conditions and length of storage time. The requirements necessary for germination are easily fulfilled by Mid-Atlantic winter weather. Of the four locations tested for the presence of viable seedbanks, all were capable of producing seedlings that survived the summer growing season. The three-month old plants in this study produced flowers and seed in September. The number of potential seedlings from soil cores collected at four test locations averaged 894 / m^2 and could be a high as 6600 seedlings / m^2.

In the shade and seedbed study to determine the environmental or physical constraints that limit seedling growth, seedlings germinated and survived through two growing seasons. Increasing the shade did not result in decreased germination or seedling growth. The presence of a forest leaf litter layer was an effective means of controlling Giant knotweed seedling germination and survival. Maintenance of a leaf litter or other ground cover may effectively reduce the number of germinates and seedling survivors.

Soil disturbance was suspected to facilitate seed germination in areas with knotweed. Although soil disturbance was not directly tested, the literature strongly advised against soil disturbance in order to prevent vegetative spread. Locations with existing populations of Giant and Japanese knotweed should be labeled "unsafe" for soil transport within locations or to locations where they have not yet invaded.

A high priority must be placed on curtailing the introduction and spread of these invasive plants. This study was not designed to evaluate the degree to which Giant and Japanese knotweed can be invasive, or the potential field conditions that may influence their ability to reproduce. We found healthy, dominating populations of both Giant and Japanese knotweed at a variety of locations. And since most of these populations had the potential for producing viable seeds, the unintentional spread of knotweed seeds could be a problem in efforts to control Giant and Japanese knotweed. Water and people were known to be vectors; animals and birds were also suspected of consuming and spreading the seed. Vigilant inspections for seedlings should be conducted in the spring and summer, especially along paths, roads and waterways where soil is exposed.

Genetic work should be conducted on populations of Giant and Japanese knotweed in the United States. Such research would shed light on the relation of populations, especially within

watersheds. Genetic testing would also help with the identification of the two species and the hybrid.

The sex expression of Giant and Japanese knotweed populations within Pennsylvania was more complicated than straight dioecy. More research needs to be done on the viability of male-fertile, female-fertile and hermaphroditic flowering plants.

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