JAPANESE AND GIANT KNOTWEED SEED REPRODUCTIVE ECOLOGY

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Abstract—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 were found throughout Pennsylvania especially along riparian, roadway, and railway corridors, and in waste areas. Japanese and Giant knotweed propagate asexually by rhizomes and basal buds. Researchers in the United Kingdom reported no viable seed set on Japanese knotweed due to the absence of male-fertile plants. In New Jersey, seedlings were found under Japanese knotweed. This research project was conducted to determine if the prolific seed produced within Pennsylvania was viable and capable of germinating in nature.

The reproductive ecology of Japanese and Giant knotweed seed was studied in eighteen populations from three river drainages across Pennsylvania. Field investigations showed that Japanese and Giant knotweed plants produce millions of seed annually. Two-year-old seedlings were discovered at four study sites in 1997 and 1998. Apparent viability assessments of seeds were made using seed coat and endosperm conditions. Seed coat was not a consistently good indicator of seed viability. Germination requirements were determined by testing the effects of moisture, temperature, and wing treatments on four seed sources over 30, 75, and 120 day storage periods. The temperature and moisture treatment combinations were all sufficient for germination. The overall mean germination rates were above 84 percent. Significantly different results were achieved with cold (3 degrees Celsius) moist conditions, yielding 95 percent mean germination over all storage times. Seedbank studies were conducted to determine the existence of naturally occurring knotweed seedbeds at four sites. The range of seedlings per soil core was 0-120. This equates to greater than 5,000 potential seedlings per square meter at the Rothrock and Lumber Road sites. Japanese and Giant knotweed were capable of reproducing sexually in these experiments.

INTRODUCTION

Japanese and Giant knotweed have established populations in a variety of habitats across Pennsylvania. They grew especially well in riparian zones, on strip-mine spoils, and on roadway and railway fill material. Their capabilities to out-compete and to displace other vegetation were obvious. Long stretches of the Susquehanna and Delaware River corridors were covered by both species. Large stands existed in the Allegheny Portage Railroad National Historic Site Staple Bend Tunnel Unit and Delaware Water Gap National Recreation Area. Japanese and Giant knotweed propagated vegetatively by extensive root systems. Researchers in the United Kingdom reported no viable seed on Japanese knotweed due to the absence of male-fertile plants. 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.

Japanese and Giant knotweed are rhizomatous perennials that form dense clumps and reach heights of 2.5 and 4 meters, respectively. Plants develop woody stocks with thick, bamboo-like, hollow stems that are erect and branched at the top. Perennating buds form at the base of the stem and on woody rhizomes between autumn and winter, and emerge as vertical shoots the following spring (Beerling and others 1994). Populations usually consist of parent plants called ramets. The vegetative shoots that grow from the ramets are called genets (Richards 1986). Stocks can produce prolific, creeping rhizomes within one year. Japanese knotweed leaves are truncate at the base, 5-15 centimeters long and 2-10 centimeters wide (Mitchell and Dean 1978). Giant knotweed leaves are cordate based, up to 40 centimeters long, and 22 centimeters wide. Japanese knotweed is functionally dioecious in the United States. It is gynodioecious (male-sterile and hermaphroditic individuals) in the United Kingdom (Beerling and others 1994). Giant knotweed is dioecious, possibly polygamous when hermaphroditic flowers are produced (Bailey 1994). Achenes are dark, glossy brown, 2-4 millimeters long and 2 millimeters wide. The hybrid between Japanese and Giant knotweed, *Fallopia x bohemica* (Chrtek & Chrtková)J.Bailey, is intermediate in growth habit and leaves (Bailey and Stace 1991).

Germination research on Japanese knotweed seed has been conducted in the United States. Germination of 84-100 percent was obtained after storage in air-dryness at room temperature for five months, and at 2-4 degrees Celsius 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 percent) as opposed to dark conditions (13 percent) (Seiger 1993). However, Locandro (1973) found no significant differences due to

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light and temperature conditions in Japanese knotweed seed germination. Germination trials were conducted without light and exhibited high percentages of germination.

Seiger (1993) had higher germination rates with two-year-old seeds (54 percent) than with two-week-old seeds (3 percent). Locandro (1973) found a decline in percent germination 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 degrees Celsius and 32 percent 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).

It was not known if Japanese and Giant knotweed growing within Pennsylvania was capable of producing viable seed, and if the potential seedlings were capable of becoming established under natural conditions. The specific objectives of this study were: to determine the potential for seed reproduction from Japanese and Giant 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 Sites

Eighteen field sites containing populations of Japanese and Giant knotweed were chosen across Pennsylvania. Figure 1 shows the location of the sites and the watershed designations. The majority of locations were within or near these National Park Service properties: Allegheny Portage Railroad National Historic Site, Delaware Water Gap National Recreation Area, and Valley Forge National Historic Site.

The sites were in the Delaware, Ohio, and Susquehanna River watersheds. Descriptions of the habitat and morphology of the knotweed plants were recorded over two years. Four of the seven sites in the Susquehanna River drainage were within 30 meters of water. The Delaware River drainage sites were within 5 meters of water, except Bushkill Church which was on abandoned fill. The Ohio drainage populations were in mixed settings. Johnstown and Saint Michael were within 10 meters of a stream, while Staple Bend was approximately 150 meters from a river. Bitumen and Lumber Road were in very remote forest locations on roadway material (gravel and soil mixed).

Flower development, sex, and the general location of male and female plants were recorded in July and August at each site. At that time, differences in leaf size and shape, and stem height were recorded to aid in species

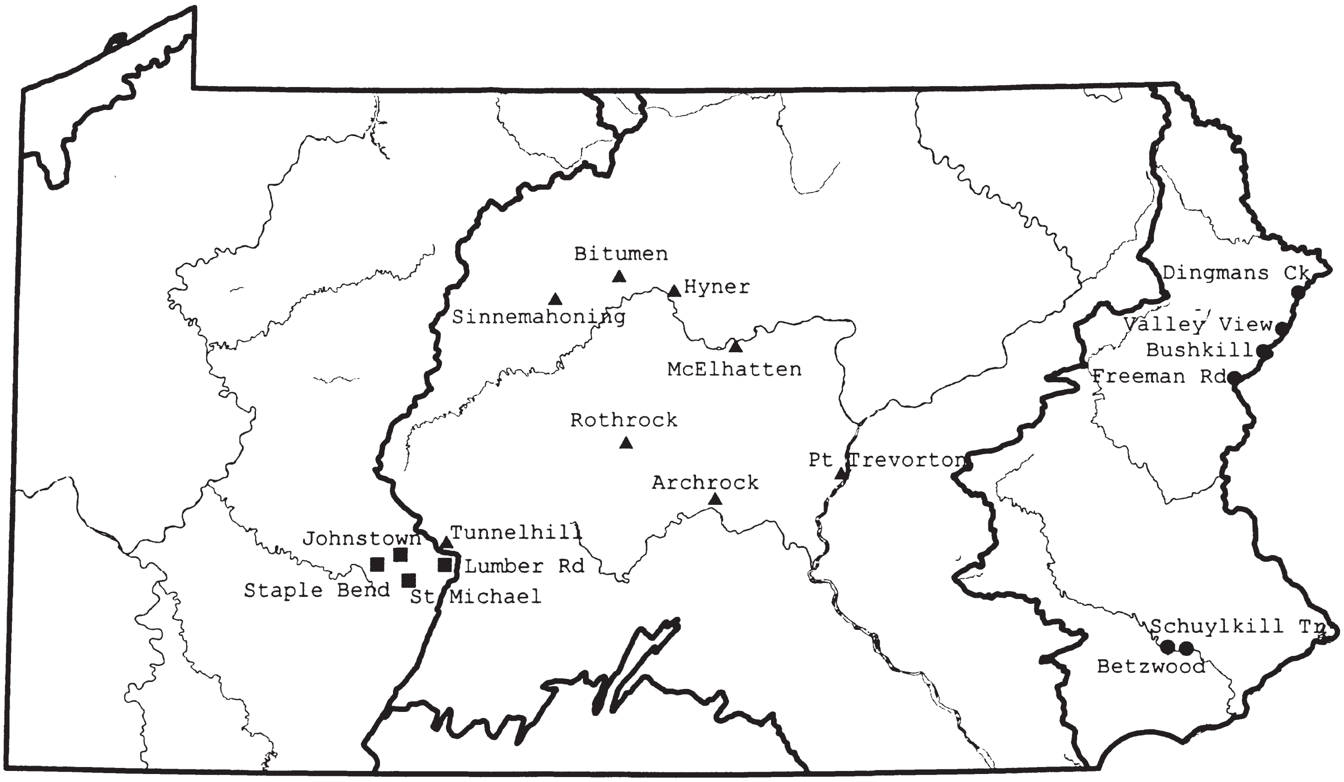

Figure 1—Locations of field sites in the Ohio River (squares), Delaware River (circles), and Susquehanna River (triangles) watersheds.

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 differentiated between Japanese and Giant knotweed by truncate and cordate leaf bases (Mitchell and Dean 1978, Wofford 1989). Knotweed seeds were collected in September and October of 1996 and 1997. The amount of seed produced at each site varied widely. Single stems produced 50,000-150,000 seeds annually. Millions of seeds were produced over a 10 square meter area at locations such as Lumber Road, Staple Bend, and Rothrock. Seed collections were random samples of whole inflorescences from different plants. Seeds were air dried, and stored with either the wing intact (natural seed) or dewinged (achene only).

Apparent Viability

Visual assessments of seed viability were 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. Endosperm content was estimated as full, half, or absent, and the color was classified as cream or white. Similar seed viability assessments were made on the 1997 seeds using 25 seeds per source. Percent viability was based on the number of seeds having normal seed coats and full endosperm. Tetrazolium chloride tests on endosperm viability were not used because Locandro (1973) reported poor results with the method.

Germination Studies

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 stored at room temperature for 100 days after collection. To test the effect of wing inhibition, seeds were either left natural (wing intact) or dewinged (achene only) by means of friction. The presence of the papery wing could inhibit both water absorption by the seed coat and radicle emergence. The seeds were stored for 30, 75, and 120 days under three different environmental conditions: warm-dry, cold-dry, and cold-moist (18, 3, and 3 degrees Celsius, respectively) (Table 1). Warm-moist, 18 degrees Celsius, conditions resulted in immediate germination of seeds. Refrigerated seeds were placed in plastic bags containing sand moistened with distilled water. All bags were then placed in glass jars and sealed. Sterile petri dishes, 9 centimeters in diameter, were lined with 9 centimeter 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 4 trays (4 replications). The trays were placed in a growth chamber at 27 degrees Celsius.

Light was not provided in the growth chamber. The basis for determining if a seed had germinated was radicle and cotyledon emergence. The number of germinates was recorded in each dish every three days over a fourteen day period. The seeds were exposed to fluorescent light during the counts. The number of seeds used in each environmental condition was 3,840, for a total of 11,520.

The percent germination values were log transformed. Analysis of Variance (alpha=.05) was run to test the effects of seed source, seed condition, environmental condition, and all of the interactions on seed germination. Tukey's HSD mean separation procedures were used to determine significant differences (alpha=.05).

Viability Germination Trials

The reliability of using seed coat condition as an indicator of seed quality 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 degrees Celsius and 66 percent humidity. The number of germinates was recorded every four days for a sixteen day period. Chi Square 2-way Test of Independence (Little 1978) was used to evaluate the ability to determine seed viability based on seed coat condition.

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

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

Soil Core Studies

Two hundred soil cores from four study sites, known to have produced viable seed in the Fall of 1996, were collected in the Spring of 1997. Staple Bend, Rothrock, Lumber Road, and Bitumen sites were used in this study. Two hundred soil cores (15 centimeters diameter, 10 centimeters deep, 182 square centimeters) were transported to a greenhouse and placed into commercial potting media to ensure adequate moisture. The cores were collected with intact (minimally disturbed) soil profiles. The randomized block design had five replications of ten cores per soil source. The number of knotweed seedlings that emerged was recorded from May 25 to August 15. The June 20 data was used for analysis, since there were no net changes in the number of seedlings per core after that date. The total number of seeds within the soil was not counted.

RESULTS AND DISCUSSION

Field Sites

The natural variation that occurs within a plant community, phenotypic plasticity, made identification of species difficult. Mature basal leaves were reliable indicators of species and were used to identify Japanese and Giant knotweed and the hybrid. Differences in stem height, leaf shape, and leaf size were recorded at each site.

Table 2 summarizes the locations and morphological characteristics of the plants found at the sites. There were 10 Japanese knotweed sites, 7 Giant knotweed sites, and 1 hybrid site. The Japanese knotweed populations had maximum heights of 3 meters. The tallest Giant knotweed plants were located at Staple Bend, Sinnemahoning, and Bitumen, all of which had maximum heights of 4 meters.

An abundance of seeds were produced when both female and male- fertile plants were present at a site. The intermingling of the two plants resulted in higher quantities of seed than if the plants were spatially separated. The amount of seed produced is expressed as seed crop values based on an average of 1,000 seeds per raceme (Table 2). Plants at every study sites produced seeds during the first year of research. No viable seeds were found when male-fertile plants were absent from the site. No viable seeds were found at the Valley View and Archrock sites in 1996, all of the seeds were empty. No seeds were found at the Schuylkill Trail site in 1997.

Table 2—Species, colony height, sex ratio, seed crop, and Chi Square values for Giant and Japanese knotweed study sites in the Ohio, Susquehanna, and Delaware River watersheds

Watershed location	Knotweed species	Colony height	Seed crop[a]	Viability[b] 1996	1997	Sex ratio	Chi square[c]
		m	---------------*Percent*---------------				
Ohio River							
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
Tunnel Hill	Giant	3.5	90	100	90	F<M	1.89
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
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] X^2 cv= 3.84, df= 1. Seed coat condition used as a predictor of viability. 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] Maybe the hybrid of the two species, *F. bohemica*.

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-fertile flowers were white, had 7-8 long stamens, and a vestigial, small, yellow ovary. The ovaries on male-fertile flowers were presumed nonfunctional because no seeds were produced. Male inflorescence stems pointed upwards. Male flowers were fragrant and had a feathery appearance. Male flowers were bright white, while female flowers were yellow-green. The female flowers had a larger, yellowish ovary with feathery stigmas, and short stamens around the base. The vestigial stamens in female-fertile flowers were presumed non-functional, due to the absence of seed set when male-fertile flowering plants were absent. The female plants were not able to fertilize each other. The inflorescences were arrayed outward or down. 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 (shorter) sex organs were functional. Flower maturation and pollination were not simultaneous in all populations. The sites that were higher in elevation 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. There were occasional breakdowns in dioecy, because seeds were found on male-fertile plants at Sinnemahoning and Staple Bend.

In the field 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. Predation by birds may be a factor in rarity of seedling germination and establishment in the wild (Beerling and others 1994). The abscission zone in the pedicel may be prematurely inactivated by the first hard frost, preempting dispersal by wind (Beerling and others 1994). For this reason, the dewinged seed condition was tested in our seed germination trials.

The flowers were pollinated by bees, ants, butterflies, and beetles. Insect damage was seen in some inflorescences where the seed coat was partially eaten and the endosperm gone. It was believed that damage was done by invertebrates. We observed slugs eating Giant knotweed seedling cotyledons in another study (Niewinski 1998).

Tens to thousands of newly germinated seedlings were seen at Bitumen, Dingmans Creek, Lumber Road, Port Trevorton, Rothrock, St. Michael, Sinnemahoning, and Staple Bend. Differences in germination and survival of seedlings were found between these two areas: the ground underneath the clone, and the area beyond the perimeter of the clone. Those seedlings that had germinated under the canopy of the parents and in the leaf litter did not survive. Thousands of seedlings survived to the end of the first growing season. Some were more than 10 centimeters tall. 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 the previous year's growth.

Apparent Viability Assessments

There was little variation in color and size of seeds among populations over the two years. Giant knotweed had slightly larger seeds, about 1 millimeter 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 it turned chalky white. Some seeds were empty shells with brown crystals inside. The cream colored endosperm may hold more favorable seed reserves than the white color. The white colored endosperm appeared to be desiccating, resulting in a slight separation of the embryo from the endosperm. We were not able to test the differences between colors. Seed production in a population was related to the number and proximity of male and female-fertile plants. Endosperm absence was found when no male-fertile plants grew near the study sites.

Viability values for each site, based on the seed coat and endosperm conditions, are given in Table 2. In 1996 and 1997, Betzwood Park, Bitumen, Bushkill Church, Dingmans Creek, Freeman Road, Johnstown, Lumber Road, Port Trevorton, Rothrock, Staple Bend, and Tunnel Hill sites had viability values above 80 percent. Seed sources with the lowest viability (0-40 percent) were Archrock and Valley View. These sites produced endosperm that was only partially developed or absent. Seed coat condition was not the most accurate method of determining potential viability. There may be better methods of assessing endosperm, like water floatation or X-ray analysis.

Germination Trials

The four seed sources chosen had shown high potential viability in the visual assessments. Cold stratification was not necessary to induce germination. Seed samples stored at room temperature germinated within five days when given water.

The overall potential of seeds to germinate was 91 percent. Analysis of Variance (alpha=.05) indicated significant differences in germination due to seed source, seed conditions, and environmental conditions over time. Tukey's HSD mean separation procedures indicated significant differences among treatments. After 30 days storage, Bitumen and Port Trevorton seeds had significantly different germination percentages (96 and 93 percent germination, respectively) than Rothrock and Staple Bend (88 and 86 percent, respectively) (Table 3). After 75 and 120 days, Bitumen seeds germinated at significantly different rates (97 and 98 percent, respectively) than Rothrock (88 and 90 percent) and Staple Bend (88 and 87 percent). Average germination among seed sources and over all seed and environmental conditions were significantly different. The results were Staple Bend 88 percent, Rothrock 89 percent, Port Trevorton 92 percent, and Bitumen 96 percent. The range

Table 3—Mean[a] germination percentages for seed source, seed condition, and environmental condition factors after 30,75, and 120 days storage on Giant and Japanese knotweed seed. N= 11,520 seeds used total

Factors	Storage time (days) 30	75	120	Overall avg.
	Percent			
Seed source (SS)				
Staple Bend (SB)	86.0b	87.4b	86.9c	86.8c
Rothrock (RR)	88.1b	87.6b	90.0bc	88.6c
Port Trevorton (PT)	92.6a	91.8ab	92.8ab	92.4b
Bitumen (BT)	95.7a	96.4a	97.9a	96.7a
Seed condition (SC)				
Natural	89.9a	88.9b	90.0b	89.6b
Dewinged	91.3a	92.7a	93.8a	92.6a
Environmental condition (EC)				
Warm-dry	89.8b	86.7b	89.9b	88.8b
Cold-dry	87.4b	90.5b	89.8b	89.2b
Cold-moist	94.6a	95.2a	96.1a	95.3a
SS * EC				
SB * warm-dry	83.6bc	84.7b	83.8b	84.1c
SB * cold-dry	82.8c	86.6b	82.8b	84.1c
SB * cold-moist	91.6ab	90.9ab	94.1ab	92.2b
RR * warm-dry	85.3bc	80.0b	84.1b	83.1c
RR * cold-dry	80.9c	85.3b	88.8b	85.0c
RR * cold-moist	98.1a	97.5ab	97.2ab	97.6a
PT * warm-dry	95.3ab	88.1b	93.8ab	92.4b
PT * cold-dry	90.3b	94.1ab	90.3ab	91.6b
PT * cold-moist	92.2ab	93.1ab	94.4ab	93.2ab
BT * warm-dry	95.0ab	94.1ab	97.5a	95.5ab
BT * cold-dry	95.6ab	95.9ab	97.5a	96.4ab
BT * cold-moist	96.6ab	99.1a	98.8a	98.1a

[a] Means within a factor (e.g. seed source) and under a storage time with the same lower case letter are not significantly different at the .05 level.

of germination inclusive of sources and different conditions was 60-100 percent.

The dewinged seed condition had significantly different germination percentages after 75 and 120 days storage (93 and 94 percent, respectively) than the natural seed condition (89 and 90 percent) (Table 3). Initially we thought that this might be important, however we found the dewinged seed condition was not a critical factor in nature, because seed rarely dispersed without the wing attached.

The three environmental conditions tested, warm-dry, cold-dry, and cold-moist, were all sufficient for germination (Table 3). Germination increased with time and reached 98 percent for the cold-moist treated seeds after 120 days storage. The cold-moist storage condition simulated Mid-Atlantic winter weather. The overall mean germination percentages for the environmental conditions over storage times were: warm-dry 88 percent, cold dry 90 percent, and cold-moist 95 percent (Table 4). Over the three storage times, germination percentages for seeds stored under cold-moist conditions were significantly different than seeds stored under the warm-dry and cold-dry conditions.

Some of the variability in seed germination was due to a significant interaction between seed source and environmental condition (Figure 2). Staple Bend and Rothrock had one pattern of response, while Port Trevorton and Bitumen had another pattern. The Staple Bend-Rothrock pattern showed statistically significant germination differences between cold-moist and warm-dry/cold-dry conditions. For the Port Trevorton-Bitumen pattern the differences among treatments could not be separated out. The patterns were not attributed to species differences because seeds collected from Staple Bend and Bitumen were Giant knotweed, while those from Rothrock

Table 4—Overall mean[a] percent seed germination for storage times and environmental conditions among all seed sources and seed conditions. N = 3,840 seeds used per storage time

	Storage time (days)			
Environmental conditions	30	75	120	Overall
	------------------*Percent*-----------------			
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 the .05 level.

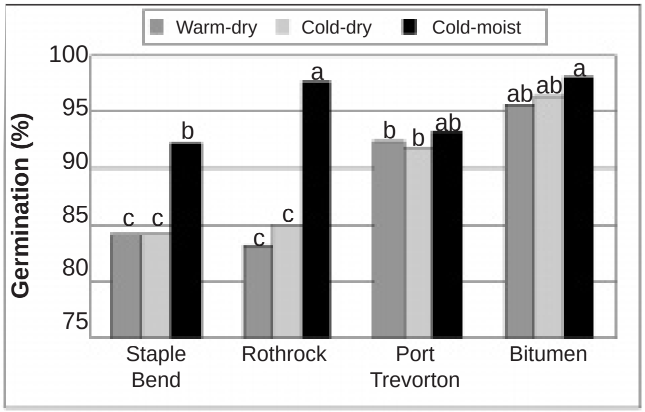

Figure 2—Overall mean percent germination for Giant and Japanese knotweed seed under three environmental conditions. N= 960 seeds per source under each environmental condition. Values among all seed sources and storage times with the same lower case letter are not significantly different at the .05 level.

and Port Trevorton were Japanese knotweed. Since collection was done in October, all seed had similar maturity. The plants growing at Bitumen produced seed with the highest percent germination under the cold-moist storage conditions.

Although germination varied due to a few factors, overall percentages were high. The different combinations we tested provided valuable evidence that Japanese and Giant knotweed were capable of reproducing sexually in the United States.

Viability Assessment Germination Trials

Seeds with shrunken seed coats were believed to be those in which the endosperm had not been fully developed. The null hypothesis was that seed coat condition, normal or shrunken, made no difference in germination. Chi Square analysis (alpha=.05, df=1, critical value = 3.84) indicated significant differences in germination percentages for these five sources: St. Michael, Archrock, Hyner, Betzwood Park, 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 percentages. Germination percentages for Lumber Road, Staple Bend, Tunnelhill, Bitumen, Sinnemahoning, and Bushkill Church were not significantly different between normal and shrunken seed coat conditions. The null hypothesis was not rejected for these six seed sources.

Half of the seeds performed as expected, in that seeds with the shrunken coat condition did not germinate. However, some shrunken seeds germinated at high percentages. The explanation is probably linked to the population structure and the relative proportion of plants of different sexes. A common factor among the sources that performed as expected was the ratio of female:male plants. Japanese knotweed plants at Hyner, Valley View, Archrock, and Betzwood Park exhibited shrunken seed coats and all had mostly female or only female parent plants. Bushkill Church had more female Japanese knotweed plants than male. The remainder of the sites 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, because of increased chances of pollination. Seed coat condition was not a consistently good descriptor of seed quality, and other methods should be explored.

Soil Core Study

Soil cores were placed into a greenhouse on May 20. Germination of the knotweed seeds began May 27 and culminated on July 2. The mean number of seedlings per core by June 21 were Bitumen 4, Lumber Road 24, Rothrock 32, and Staple Bend 5. The mean number of potential seedlings per square meter were extrapolated from this data (Figure 3). The range of seedlings in the cores was 0-120, or equivalent to greater than 6,500 seedlings per square meter). Analysis of Variance (alpha=.05) showed that the mean number of seedlings from soil samples taken at Rothrock and Lumber were significantly different than those from Staple Bend and

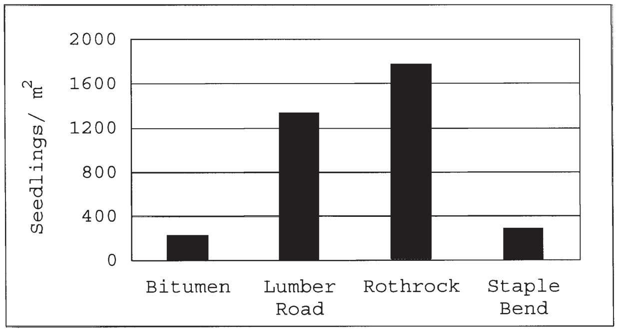

Figure 3—Mean number of potential seedlings/ m^2 at the four study sites, extrapolated from the number of seedlings per 182 cm^2 soil core. N= 50 cores per soil source.

Bitumen. 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 collection and placement. The soil was not mixed to promote germination.

The results of Bitumen cores were lower than expected, based on the viability assessments and germination data. This may be attributed to the difficulty of collecting an intact soil core. Gravel in the strip-mine spoil made it nearly impossible to get an intact soil core close to the knotweed. The low number of seedlings could be due to a reduced amount of soil collected, or to increased distance from parent plants.

Rothrock soil cores had significantly different numbers of seedlings than those from Staple Bend, although there were no significant difference in germination potentials for the two sources of seed. Soil quality and abundance of seed-eating invertebrates may be contributing factors to the differences in number of seedlings emerging from the soil cores. Staple Bend and Rothrock soil cores had thick humus layers, while Bitumen and Lumber Road cores had little humus and came from gravel road areas.

There was a naturally occurring, viable seedbank at these four locations. Seedling mortality was expected because of the reported absence of seedlings in nature. However, the soil core study showed that seedlings were capable of surviving and producing flowers and seeds within one growing season.

CONCLUSIONS

Japanese and Giant knotweed were found throughout three river drainages in Pennsylvania. The populations examined were the dominant vegetation on the sites. Millions of seed were produced annually by Japanese and Giant knotweed at these locations. Independent, multiple year seedlings were discovered at four locations in the summer of 1996 and 1997.

The use of physical characteristics to determine viability was not reliable. Seed coat condition was not a consistently good indicator of seed quality. The potential of Japanese and Giant knotweed seed to germinate depended on the viability of the endosperm. Endosperm content can be visually assessed for a rough estimate of viability. A germination test is the best method for determining the sexual reproduction of a population.

The germination trials showed that Mid-Atlantic winter weather conditions were the best for seed germination. Cold-moist storage of seeds over 30, 75, and 120 days yielded the highest percent germination, averaging 95 percent overall. The mean percent germination for the four seed sources over all storage times, seed conditions and environmental conditions was 91 percent.

Seedbanks were found to be naturally occurring at four of the study sites and had the capacity to germinate in the spring and summer. Seeds in soil can go into induced dormancy and germinate under more favorable conditions, like when exposed to air after soil disturbance. Seedlings have the capacity to survive and grow into vegetative adults in one growing season. Observations during the seedbank study suggested that soil disturbance might lead to higher incidences of seedling emergence.

Japanese and Giant knotweed pose serious threats to our natural ecosystems and should be managed carefully. Seeds germinate in the spring and summer. Areas should be monitored for seedlings, especially in riparian zones and on paths where bare soil is exposed.

Table 5—Mean[a] number of Giant and Japanese knotweed seedlings/ core, range of seedlings/core, and number of potential seedlings per m^2 from four soil sources. N = 50 cores per soil source

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

[a] Values of seedlings per core among the soil sources with the same lower case letter were not significantly different at the .05 level.

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