The publication was prepared under the LIFE III Nature project
– Preservation of alluvial forest habitats in the Morávka river basin

METHODS OF ELIMINATION OF INVASIVE KNOTWEED SPECIES

(REYNOUTRIA SPP.)

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CONTENT

1. LIFE III Nature Project – Preservation of alluvial forest habitats in the Morávka river basin

The "Preservation of alluvial forest habitats in the Morávka river basin" project has been realized thanks to financial support from the European Union under LIFE III Nature Programme–the EU's funding instrument supporting the Natura 2000 network. The project has been implemented by the Moravian-Silesian Region in cooperation with project partners during the course of four years: from January 2007 to December 2010. For detailed information see www.life-moravka.cz.

1.1. Project Targets and Results

The project targets are based on the "European Strategy on Invasive Alien Species". The project covers Niva Morávky SCI and partially Beskydy SCI. The project's main targets are:

- To develop an efficient methodology for invasive species suppression and subsequent restoration of the habitats;
- To suppress invasive knotweed species (Reynoutria spp.) in Morávka river basin;
- To create an efficient transferable model of cooperation among the interested subjects during the process of problem solving in the field of environmental protection at a regional level;
- To disseminate the project results and provide further information on Natura 2000 network and on environmental protection in general.

Expected project results:

- Development of a complex methodology for Reynoutria suppression and subsequent restoration of the habitats, as well as creation of a long-term strategy for the Niva Morávky SCI management;
- Suppression of Reynoutria spp. under 10% of its current occurrence, improvement of biodiversity through habitat restoration, shrub layer support, and reduction in the soil erosion vulnerability of selected areas;
- Development of a transferable model of cooperation between the interested subjects that shall ensure proper follow-up management of the Niva Morávky SCI as well as cooperation when dealing with similar nature protection issues in the future;
- Enhancement of the awareness of the Natura 2000 network and nature protection in general; dissemination of the project results as well as of the know-how regarding the suppression of invasive species and subsequent restoration of affected habitats (Moravian-Silesian Region, 2006).

1.2. Project Area

The project area is situated in the Moravian-Silesian Region in the northern part of the Moravian-Silesian Beskydy mountains. It covers the entire Morávka river basin extending from the spring on the side of the Sulov hill to the town of Frýdek-Místek. Its total area is approximately 17,000 ha. The project area involves territories of the following municipalities: Morávka, Pražmo, Raškovice, Vyšní Lhoty, Nižní Lhoty, Nošovice, Dobrá u Frýdku-Místku, Skalice u Frýdku-Místku, Staré Město u Frýdku-Místku, Vojkovice, Krásná pod Lysou horou, Bukovice u Dobratic, Dobratice, Dolní Tošanovice, Horní Tošanovice and Komorní Lhotka. The average altitude of the area is 350 m above the sea level. In the Morávka river basin sites of Community importance, which form a part of Natura 2000 network, are located: Niva Morávky SCI, Beskydy SCI and Beskydy SPA (Special Protection Area). The project area also includes other protected areas according to the national legislature: Beskydy Protected Landscape Area, Skalická Morávka National Nature Monument and Profil Morávky Nature Monument. The project area and original distribution of Reynoutria spp. are shown in Annex 1.

1.3. Project Management Structure

The Moravian-Silesian Region is the EU grant recipient and is responsible for the project management. It also cofinances the project from its own resources. ČSOP Salamandr, a local unit of the Czech Union for Nature Conservation (Český svaz ochránců přírody), is the project principal partner. It is responsible for most of the field work. The associate partners are: Agency for Nature Conservation and Landscape Protection of the Czech Republic (Agentura ochrany přírody a krajiny České republiky–AOPK ČR–Administration of Beskydy Protected Landscape Area and Regional Office in Ostrava); Lesy České republiky, s. p. (Správa toků–oblast povodí Odry, Frýdek-Místek); Povodí Odry, státní podnik; and Plzeňský Prazdroj a. s.–Radegast Brewery. All project partners are involved in project financing. The project budget is 1 015 000 EUR; Community support is 704 000 EUR, 69% of the project budget. The project is also supported by the Ministry of the Environment of the Czech Republic (JŮZOVÁ 2007a).

2. Overview of Application Methods for Knotweed Elimination Used in "Preservation of Alluvial Forest Habitats in the Morávka River Basin" LIFE Project

In the project area, all three knotweed species, that are invasive in the Czech Republic, can be found: Japanese knotweed *(Reynoutria japonica)*, giant knotweed *(Reynoutria sachalinensis)*, and Bohemian knotweed *(Reynoutria bohemica)*, which is the most common and the most resistant. All of these species were eliminated in the project. Knotweed plants were eliminated using **foliar spraying of herbicide** (see Section 2.2.1.) and **stem injection application of herbicide** (see Section 2.2.2.).

2.1. Knotweed Elimination Process

A survey on knotweed distribution took place before the onset of the project in 2004. In the beginning of the project in 2007, areas in which knotweed had been found were assigned to project partners responsible for its removal (AOPK ČR; ČSOP Salamandr; Lesy ČR, s. p.; and Povodí Odry, státní podnik). Great emphasis was placed on proceeding from the spring of Morávka river downstream. In the first two years the elimination of knotweed was carried out in previously determined locations. In the following years, the entire area was systematically examined and knotweed plants treated in newly discovered locations.

- Roundup Biaktiv herbicide was exclusively used for both spraying and injection treatment, as it is accounted the most environmentally friendly for use in ecologically sensitive areas. For the moment, it is the only herbicide available on the market that can be used near watercourses. Roundup Biaktiv is not classified as hazardous according to EU Directive 1999/45/EC–Dangerous Preparations. The manufacturer does not expect any significant potential impact on human health or the environment if instructions for use are followed (Roundup Biaktiv safety data sheet).
- Tall developed knotweed stands were cut first, especially in the first years, or access paths were cut out in order to reach the entire cover and apply the herbicide.
- Mist blowers were used for spraying tall stands as they were found more effective than backpack sprayers. Backpack sprayers were used for individual plants and plants not taller than 130 cm.
- All project partners used Roundup Biaktiv herbicide at the concentration of 7%, except for the area of Skalická Morávka National Nature Monument where the concentration used was 10% in accordance with the management plan.
- In order not to leave aside parts of the area untreated or not to spray againg the already treated areas as well as for better orientation, the treated areas were marked with a spray marker dye.
- In the surroundings of Morávka water reservoir (in the drinking water source protection zone), the injection application of herbicide was used to treat knotweed plants. Injectors had been ordered and purchased through the internet directly from the manufacturer in the U.S. as they were not available on the market in the Czech Republic.
- Most areas were checked and upon the occurrence of knotweed treated twice a year.

In **2007**, the area of 126 hectares was treated with herbicide, out of which 2.5 hectares by injection application. 1 500 liters of Roundup Biaktiv were applied. The area treated in 2007 is shown in Annex 2.

In **2008**, the area of 260 hectares was treated with herbicide, out of which 17 hectares by injection application. 3 100 liters of Roundup Biaktiv were applied. The area treated in 2007 is shown in Annex 3.

In **2009**, the area of 490 hectares was checked and upon the occurrence of knotweed treated, out of which 6.6 hectares by injection application. 3 180 liters of Roundup Biaktiv were applied. The area check and upon the occurrence of knotweed treated is shown in Annex 4.

In **2010**, the same procedure as in 2009 is employed, the entire project area is checked and emerging knotweed plant are treated with herbicide (spraying, injection). The same area as in 2009 is expected to be at least rechecked. The assumed amount of herbicide to be used is 2 500 liters.

2.2 Metods of Knotweed Elimination Used in the Project

2.2.1. Foliar Application of Herbicide

Foliar spraying of herbicide is a common application method of eliminating knotweed or other invasive plants. The following information applies to the use of Roundup Biaktiv herbicide (concentration), and it may vary when using a different brand of herbicide. When using herbicides, always read the label directions and follow manufacturer's instructions provided in the safety data sheet. The method involves spraying knotweed plants with the herbicide, optimally at the end of the vegetation period. This is when the knotweed plants transport assimilates from the aboveground parts of the plants to the rhizome system. The plant treated with herbicide at that period will transfer the herbicide with the assimilates into the rhizomes, causing its eventual death. The plants respond to the spraying with gradual yellowing (browning) and shedding of leaves from the stem base. The response is not immediate; it will manifest in

7–14 days. (The response time depends on the location and the weather conditions. It is shorter in shadeless locations and in sunny weather.) In some cases herbicide spraying in September produced no visible reaction; the knotweed plants, however, did not resprout in the following spring (MODRÝ in verb., KONUPKA in verb.). A single application of the herbicide is not effective to eliminate knotweed. For best results, spraying must be repeated for several years. Foliar application of herbicide–the principal method of knotweed elimination used in the project–has two modifications differing in time of onset of spraying.

2.2.1.1. Application in Late Summer

Knotweed cover is treated with Roundup Biaktiv herbicide. The concentration of herbicide used in the project was 7% and 10%. Higher herbicide concentrations will not result in higher efficacy of elimination as the plants will shed their leaves immediately and will not transport the herbicide into the rhizomes. As a result, they will resprout.

- The application of herbicide is carried out using a backpack sprayer for individual plants, or a mist blower in extensive growths. Make sure that the entire plant is equally sprayed.
- The herbicide should be applied preferably when the plants are budding and flowering (August–September).
- 4–28 days after the first application, check the treatment area and treat plants that have survived. Repeat the procedure until all growth is destroyed (approximately 2–3 times).
- Apply the herbicide with caution and with care to the surrounding flora.
- The treatment should only be performed under suitable weather conditions (windless, no precipitation during the treatment and for at least six hours after). This method cannot be used after heavy rains. The treatment should be terminated two weeks before the first frosts are to occur (the aboveground parts of the plants will freeze out, so the spraying will be ineffective).
- In protected areas, comply with relevant management plans (if they include invasive plants management).

2.2.1.2. Application in the Growing Season

- The first application of herbicide should be performed in spring (May–June) when the knotweed plants are growing, stand slightly more than one meter tall and have foliage.
- Repeat spraying in a reduced area after 2–3 months as necessary.
- For further steps see Section 2.2.1.1.

Tall stands, in which overgrowing in optimum spraying time is imminent, should be cut down before the herbicide is applied in May–June. After four to six weeks (in July at the earliest) herbicide spraying should be carried out according to the method mentioned above.

Extensive stands should be treated from the edges inwards, completing the spraying after 2–3 weeks. Cutting access paths to reach the entire cover with the sprayer is an option. In this case, it is necessary to treat the cut portions (access paths) in the following year. Establishing communication rules for the field operators, such as marking the already treated areas with a dye sprayed on the leaves, is very convenient as the noise of the machines makes verbal communication impossible.

Advantages: Foliar application of herbicide is the most effective method known. Its efficacy increases if performed in late summer (August–September).

Disadvantages: Introduction of foreign substances into the environment; undergrowth removal (the herbicide destroys all green plants); potential of accidents; potential of herbicide-resistancy in plants; limitations due to weather conditions.

2.2.2. Stem Injection Application of Herbicide

This method was used in the surroundings of Morávka water reservoir, because it serves as a source of drinking water. The following information on herbicide concentrations and rates applies to Roundup Biaktiv and JK Injection systems. For more information on this method and injection application tools see www.jkinjectiontools.com/.

- Using this method, inject 5 ml (3–7 ml) of 20–30% herbicide solution into the stem.
- The method is suitable for stems of at least 1.5 cm in diameter (commonly 1.5–5 cm).
- The herbicide should be injected into the stem low above the ground level (below the second or third node) or in 1.3 meters above the ground level in most stems in the polycormon (CROCKETT 2005).
- The method is suitable for small localities, sensitive areas or places where the density of the knotweed cover is low.

This method was used by Lesostavby a.s., the subcontractor to the project partners Lesy ČR s. p. and Povodí Odry s. p. The operators of the company made these findings:

— The knotweed plants must be at least 1.5 m tall to use this method.
— t is convenient to inject herbicide in two thirds of plants in the cluster and then about one month later revisit the site and inject the plants that have survived. To mark the already injected plants, it is convenient to break their stems.
— The injection application is a highly effective method; however, the following year new weak or retarded plants resprout. The new plants are not suitable for injection treatment, therefore it is convenient to combine the method with foliar application of herbicide using a backpack sprayer.
— The advantage is that the method can be used in worse weather conditions (wind, drizzle, light rain). The main drawback is that the method is time-consuming: it took two weeks for three people to treat 5 ha area, while the cover-abundance of knotweed in the area was not 100% (BALAŠ in verb.).

Advantages: Although it is a chemical application, it is friendly to the environment minimizing herbicide losses to water and soil. The surrounding vegetation is not affected. The method can be applied in worse weather conditions.

Disadvantages: The method is not routinely used in the Czech Republic. Foreign injection applicators can be used (US products were utilized in the project).

In very rare cases, the herbicide may be released from rhizomes in the following years. This event may be predicted to occur in large treatment areas, under unusually heavy rainfall conditions, or in low organic matter soils. (CROCKETT 2005). The method is laborious and time-consuming and cannot be used in plants with small-diameter stems, as there is no space to properly insert the application needle. Therefore, its employment is limited.

2.3. Monitoring the Impact of Roundup Biaktiv Herbicide on Water, Soil and Biota

Since the foliar application of herbicide is the principal method of knotweed elimination used in the project, periodical monitoring of Roundup Biaktiv herbicide impact on water, soil and biota is one of the project activities.

Water samples were collected monthly from June to September in nine sampling site the in first year of the project, and in five sampling sites in the following years. In the beginning of the project (before intervention) reference samples were collected.

Roundup Biaktiv degradation intermediates (glyphosphate, AMPA), total phosphorus, phosphates and total oxygen demand (since 2008) were analyzed in the samples. The analyses were carried out by an accredited laboratory. Additionally, the results of water analyses from periodical water monitoring by Povodí Odry were provided.

The results revealed that total phosphorus concentration approximated 150 µg/l–the limit of permissible pollution of surface waters according to the Government Decree No. 61/2003–only in one case in September 2008. The measured value was 130 µg/l. However, the increased concentration was proven to bear no relation to project activities, as there were no interventions in progress at that time in the area. In other cases, total phosphorus concentrations ranged between 10 and 40 µg/l. Most other measured values were below quantitation limits.

Soil samples were collected in five sampling sites three times a year (in April, August and October) in 2008, and two times a year (in April and October–i.e. before and after herbicide spraying) in 2007, 2009 and 2010.

Roundup Biaktiv degradation intermediates (glyphosphate, AMPA) were analyzed in the samples. The analyses were carried out by an accredited laboratory. Both values were largely below the quantitation limit (<0,01 mg/kg).

The impact of project activities on the biota is monitored through repeated vegetation mapping at 18 experimental plots located throughout the project area. The effects of interventions on the surrounding vegetation, its potential changes (chlorosis and deformities), changes in species composition, and other invasive plants are monitored.

3. Other Methods of Knotweed Elimination

3.1. Combined Method

This method is a combination of chemical treatment with herbicide and repetitional mechanical treatment (cutting).
- The first intervention is made with Roundup Biaktiv herbicide as foliar spray (according to Section 2.2.1.) or injected into stem (according to Section 2.2.2.).
- Further treatment in subsequent years consists in periodical cutting of plants (according to Section 3.2.) four to eight times in a season.

3.2. Cutting

Cutting is one of the mechanical methods of knotweed elimination. It is not effective unless used periodically in the long term due to fast regeneration of the plant and considerable amount of nutrient reserves in rhizomes.
- Cutting can be carried out with scythes, machetes or brush cutters. Brush cutters are the most common. The first intervention should be made in the first half of May before the plant ceases to grow and begins to translocate assimilates to the rhizomes. Besides, the shoots are not yet fully developed and cutting is therefore easier.
- Cutting frequency is to be adapted to growth. The optimum height of plants to cut is 40 cm and should always be respected. The cut should be as close to the ground as possible.
- Number of cuts in the first year may be around 8, in subsequent years about 6 cuts per year. In May–June make 4–6 cuts according to the grow rate. More cuts increase costs and do not result in higher efficiency (SOLL 2004).
- After summer break to allow other plants to flower and seed, make further cuts from the second half of August (always after the plants reach a height of 40 cm) until the end of the season. Grass underseeding is convenient, since it tolerates cutting and prevents soil from erosion. Let the cut plants dry in small piles and check for regrowing. Burn once they have dried up.

Advantages: The method is friendly to the environment. No foreign substances are used.
Disadvantages: Great time demands, time-consuming coordination, decline in number of knotweed plants after four years. The method never results in full eradication; follow-up treatments are always necessary for 4–7 years.

3.3 Grazing

Domestic animal grazing to eliminate knotweed is not commonly used; several experiments were conducted in Germany, for example. Subject to certain conditions, grazing leads to suppression of knotweed.

- Sheep are ideal knotweed grazers. They prefer ripe leaves. Grazing must be long-term or repetitive (3–4 times in a year).
- In Germany, experiments on knotweed grazing were conducted successfully with Heidschnucke sheep and with a versatile and highly adaptable Galloway breed of cattle. Other cattle breeds, sheep and horses were observed to graze knotweed as well.
- Knotweed growth should not exceed 150 cm, otherwise it must be cut. Ten to twenty animals per hectare are needed for year-round grazing. More are needed for intensive grazing; in this case supplemental feed must be provided to the animals (KRETZ 1994).

Experience with grazing in the project area varies. While some residents in Morávka river basin pointed out that their sheep would not graze knotweed, others indicated a good experience with grazing (in such cases no special breeds of sheep were involved). Knotweed in cattle and horse pens remained mostly intact.

Advantages: The method can be applied at watercourses. No foreign substance are introduced into the environment. Some sheep breeds even prefer knotweed leaves to grass and herbs.
Disadvantages: River banks may be disturbed by animal trampling. The disappearance of knotweed occurs within 4–7 years. The sites must be fenced and the animals should be provided with a shelter and access to drinking water (which is not always possible).

3.4. Digging

Digging can only be successful at an early stage of knotweed occurrence or in individual plants. It is a very laborious method, and the risk of further knotweed dispersal is high.
- Whole plants including the rhizomes are dug up. The rhizomes, however, can grow up to 2 meters deep. Digging should be carried out several times during the growing season, each time the growth reemerges after previous intervention.

A digging fork is an appropriate tool for the work.

- Great care must be taken to prevent the excavated rhizomes from transfer and their transport must be minimized. Pulled and dug biomass is dried and burned at appropriate places. After the last site treatment in a season (in autumn) sow a grass seed mixture.
- Generally, because of the high risk of propagation of plants from cuttings this method is not considered appropriate.

Advantages: Using this method, no foreign substances are introduced into the environment. It can be used in small sensitive areas where the application of herbicides is ruled out.

Disadvantages: The method requires a careful and responsible approach. There is a risk of knotweed spreading by rhizome fragment transfer. Therefore, transport of plant remnants must be minimized and rhizome transfer by water or wind must be prevented. Localities in immediate proximity to watercourses may be subject to increased soil erosion as the soil is disturbed by digging.

3.5. Biological Suppression

Biological method uses the natural enemies of the respective species (animals, molds, fungi). Knotweed, being an alien species in the Czech Republic, is not limited by its natural enemies (leaf-feeding insects, mold pathogens) as it is in its place of origin. Biological suppression methods are yet being researched on.

- The black vine weevil *(Otiorrhynchus sulcatus)* may serve as an example. Its larvae feed on roots and rhizomes, while the adults feed on knotweed leaves. According to bibliographic data, many knotweed plants have been destroyed by this pest. (BEERLING et al. 1994).
- In Japan, Japanese knotweed population growth is regulated by leaf-feeding beetle *Gallerucida nigromaculata*. The use of this species in biological control of knotweed is considered in Great Britain and U.S. (ANONYMUS 2010).
- In Great Britain, a research is in progress on biological control of knotweed using Japanese knotweed psyllid *Aphalara itadori*, a selective sap-sucking insect of Psyllidae family. The insect is highly reproductive. It weakens the affected plants by sucking the sap from the plant (like aphids), targeting only knotweed species *(Reynoutria spp.)*. Another potential biological enemy to knotweed is a *Mycosphaerella polygoni-cuspidati* ascomycetes fungus. The research is conducted by Defra's Food and Environment Research Agency; biologist Dick Shaw from University of Leicester is the project author and manager (for more information see: www.cabi.org/japaneseknotweedalliance/).

Advantages: The method is environment-friendly; no toxic substances are accumulated in the environment; neighboring organisms are usually not affected.

Disadvantages: Long-term testing must be performed in advance, which is costly, and all potential risks (biological agent outbreak, infestation of other plants) must be taken into account. Biological control process itself may take a long time (the agent needs to take hold and reproduce). There are restrictions in national legislatures to observe. Currently, no biological control of Reynoutria spp. in practice takes place; it is at the research stage.

3.6. Biotechnical Measures (Planting, Willow Mats)

Biotechnical measures are appropriate as a complementary method to ensure the stabilization of slopes and reinforcement of watercourse banks occupied by knotweed. These measures also help to eliminate flood damage. Biotechnical elements have erosion control, aesthetic and ecological function, being a supplement to the aforementioned methods of knotweed elimination.

- The willow matting is made with young narrow-leaved willow or osier twigs of about 1.5 m, then the layer is covered with soil (KRETZ 1994). Additional planting of alders and ash trees is useful.
- In "Extermination of invasive plants in Landkreis Löbau-Zittau" project suppression of Reynoutria spp. by laying willow mats was examined. The entire island where Reynoutria spp. occurs was overlaid with osier twigs (Salix viminalis), the mat was then covered with thin layer of soil. The osier took roots successfully. Emerging Reynoutria spp. shoots were cut until suppressed by osier growth. After four years, however, individual Reynoutria spp. plants were still present (MODRÝ at al. 2008).

Advantages: Biotechnical measures help to reinforce the river banks and to eliminate soil erosion caused by knotweed rhizomes. They are environment-friendly.

Disadvantages: Biotechnnical measures are long-term provisions that require time and considerable financial costs. Due to low efficiency they cannot be used alone, but only as a complement to other methods.

Comparison of Knotweed Elimination Methods

Method	Removal efficacy	Environment-friendliness	Time demands
Foliar spraying of herbicide	***	*	*
Injection application of herbicide	***	**	***
Combination of herbicide and cutting	**	**	**
Cutting	*	***	***
Grazing	*	***	***
Digging	*	**	***
Biological method	???	???	***
Biotechnical measures	*	***	***

* low   ** medium   *** high   ??? research in progress

4. Follow-up Measures Against Invasive Species

After the site affected by invasive species has been successfully treated and the invasive species removed, open niches (i.e. areas with plenty of free space, light and usually also nutrients) remain in the area, attracting many species to root. Such areas are endangered not only by the same or other invasive species recolonization, but also by undesired colonization by inadequate domestic expansive species (such as nettle, reed, etc.). Therefore, it is necessary to further stabilize the area and prevent the spread of unwanted species in the locality by an appropriate and consistently implemented management. Systematic monitoring over the next several years is inherent in these stabilization measures (MODRÝ at al. 2008).

4.1. Area Clear-up

Knotweed annually produces large quantities of biomass. Clearing away dried-up (dead) biomass contributes to faster recovery of the habitat (appropriate before sowing, for instance). It also makes the activity of operators easier when respraying the growth in subsequent years.

— Biomass (dead knotweed) removal comes after herbicide application. In large areas the dead stems can be removed in autumn of the same year, at small sites or in the undergrowth (risk of forgetting the exact location) wait until next spring in order to detect potential recovery.
— Dead biomass should be cut down, raked up or piled and burned in suitable weather safely (inform the competent authorities according to local regulations).
— Warning! Dry knotweed burns very quickly, stems burst and shoot out. The fire must be patrolled by several operators equipped with appropriate fire extinguishing equipment.

4.2. Area Maintenance in Subsequent Years

All knotweed species are very persistent; besides, the risk of reintroduction of the plants is imminent. It is therefore necessary to monitor repeatedly the treated area, look out for individual knotweed plants and encourage the habitat.

— Knotweed plants resprouting after herbicide intervention are treated the same way, i.e. by foliar spraying, best during flowering. In some cases knotweed responds to herbicide treatment with formation of a “retarded” form with deformed curly leaves. All project partners experienced that these forms of plants show almost no reaction to herbicide spraying. The experience from the Liberecký Region has shown that when these plants are left at rest, they will regress to the normal form in several years (MODRÝ in verb.).
— In extremely sensitive (valuable) areas, young shoots can be suppressed by regular mowing once every two weeks during the growing season.
— Areas, in which large growths of knotweed were removed, should be monitored for several years and new invasion subdued at the beginning.
— It is recommended to plant or seed the area with native plants, trees and shrubs in order to control potential invasions through competition for light.

4.3. Project Follow-up Measures

Two follow-up measures were taken in the project: restoration of the herb layer of selected habitats with grass-herb seed mixtures and planting of indigenous shrub species. For follow-up measures taken in 2007–2010 see map in Annex 5.

4.3.1. Restoration of the Herb Layer of Selected Habitats

Restoration of the herb layer by seeding was opted for for two reasons. In the first place, the measure together with tree planting was to serve as a biotechnical measure to prevent reinvasion of removed invasive species *(Reynoutria spp., Impatiens glandulifera)* and arrival of other invasive species, *(e.g. Solidago canadensis, Rudbeckia laciniata)*. The second reason was to encourage and accelerate restoration of selected habitats.

Before the species for seeding were chosen, vegetation mapping, as a part of biological monitoring, took place. From the identified species, those associated with forests were excluded (seeding was to be carried out in non-forest areas only), and the remaining species were taken as the basis for selecting species suitable for sowing. Oseva PRO company from Zubří was chosen as a seed supplier, being the only one to have available genetic material of local origin (collects seeds in Beskydy region).

Course of Action

In **2007**, 1.2 ha area was seeded with a grass-herb seed mixture in November. Nine species of grasses and three species of herbs were sown. Composition of the grass-herb seed mixture in 2007 (12 species): common velvetgrass *(Holcus lanatus)*, meadow fescue *(Festuca pratensis)*, red fescue *(Festuca rubra)*, crested dog's-tail *(Cynosurus cristatus)*, meadow foxtail *(Alopecurus pratensis)*, redtop *(Agrostis gigantea)*, fowl bluegrass *(Poa palustris)*, canada bluegrass *(Poa compressa)*, yellow oat grass *(Trisetum flavescens)*, rough hawkbit *(Leontodon hispidus)*, maiden pink *(Dianthus deltoides)*, peach-leaved bellflower *(Campanula persicifolia)*.

In **2008**, 5.6 ha area was seeded with a grass-herb seed mixture in October. Eight species of grasses and eight species of herbs were sown. Composition of the grass-herb seed mixture in 2008 (16 species): meadow fescue *(Festuca pratensis)*, red fescue *(Festuca rubra)*, crested dog's-tail *(Cynosurus cristatus)*, meadow foxtail *(Alopecurus pratensis)*, redtop *(Agrostis gigantea)*, fowl bluegrass *(Poa palustris)*, canada bluegrass *(Poa compressa)*, yellow oat grass *(Trisetum flavescens)*, rough hawkbit *(Leontodon hispidus)*, maiden pink *(Dianthus deltoides)*, common St. Johnswort *(Hypericum perforatum)*, wild carrot *(Daucus carota)*, bird's-foot trefoil *(Lotus corniculatus)*, oxeye daisy *(Leucanthemum vulgare)*, meadow buttercup *(Ranunculus acris)*, Nottingham catchfly *(Silene nutans)*.

In **2009**, 25 ha area was seeded with a grass-herb seed mixture in October. Eight species of grasses and twenty-two species of herbs were sown. Composition of the grass-herb seed mixture in 2009 (30 species): meadow fescue *(Festuca pratensis)*, red fescue *(Festuca rubra)*, crested dog's-tail *(Cynosurus cristatus)*, meadow foxtail *(Alopecurus pratensis)*, redtop *(Agrostis gigantea)*, fowl bluegrass *(Poa palustris)*, canada bluegrass *(Poa compressa)*, yellow oat grass *(Trisetum flavescens)*, rough hawkbit *(Leontodon hispidus)*, maiden pink *(Dianthus deltoides)*, peach-leaved bellflower *(Campanula persicifolia)*, common St. Johnswort *(Hypericum perforatum)*, wild carrot *(Daucus carota)*, oxeye daisy *(Leucanthemum vulgare)*, common agrimony *(Agrimonia eupatoria)*, common kidneyvetch *(Anthyllis vulneraria)*, common columbine *(Aquilegia vulgaris)*, bluebell bellflower *(Campanula rotundifolia)*, brown knapweed *(Centaurea jacea)*, fern-leaf dropwort *(Filipendula vulgaris)*, yellow bedstraw *(Galium verum)*, common rockrose *(Helianthemum nummularium)*, hoary plantain *(Plantago media)*, common selfheal *(Prunella vulgaris)*, meadow clary *(Salvia pratensis)*, lilac sage *(Salvia verticillata)*, small burnet *(Sanguisorba minor)*, golden larger thyme *(Thymus pulegioides)*, large speedwell *(Veronica teucrium)*, Nottingham catchfly *(Silene nutans)*. The grass-herb seed mixture in 2009 was enriched with seeds collected in the project area by students and teachers from primary school in Raškovice.

In **2010**, sowing will continue with 50 ha area seeded in autumn as planned.

4.3.2. Planting of Indigenous Shrub Species

One of the negative consequences of knotweed spreading is the biodiversity reduction due to shrub layer suppression. Due to knotweed spreading and subsequent herbicide spraying, the occurrence of shrubs was significantly reduced compared to the past, both in quantity and in the number of species. Increased vulnerability to soil erosion in the area is one of the negative consequences. Thus, it is desirable to plant certain shrub species at selected locations, especially near streams (reinforcement of the banks). According to the plan, area of 21 hectares was to be planted with at least 1500 shrubs (depending on the current price of seedlings).

In **2008**, 500 willow rods were planted. The planting material was collected in the project area during March. Planting took place in April. In June willow rods were treated with Aversol against browsing by game, which was already evident in some rods as early as in June. The rods were also shortened to adjust the ratio of aboveground and underground parts and the cut was treated with Sanatex. Introduced willow species: purple willow *(Salix purpurea)*, goat willow *(Salix caprea)*, crack willow *(Salix fragilis)*, common osier *(Salix viminalis)* and almond willow (Salix triandra). Beyond the project, Lesy ČR planted 5000 seedlings of forest trees near Morávka river.

In **2009**, another 500 willow rods of identical species composition as in 2008 were planted with the same procedure. Although the survival rate was initially very good, due to an exceptionally dry late spring and summer, deterioration of plantation health occurred and some willows died. In October 2009, the project partner AOPK ČR planted in Skalická Morávka national natural monument 780 seedlings of black poplar *(Populus nigra)*, 200 seedlings of common dogwood *(Cornus sanguinea)*, 200 seedlings of European spindletree *(Euonymus europaea)* and 600 seedlings of bird cherry *(Prunus padus)*. All the above mentioned species are indigenous.

In April **2010**, 400 willow rods (of the same species composition as in previous years), 100 seedlings of European spindletree *(Euonymus europaeus)* and of common dogwood *(Cornus sanguinea)* were planted. In May 2010, another 300 tree seedlings and 2740 shrub seedlings were planted: small-leaved lime *(Tilia cordata)*, common ash *(Fraxinus excelsior)*, sycamore maple *(Acer pseudoplatanus)* and sessile oak *(Quercus petraea)*, wild privet *(Ligustrum vulgare)*, common dogwood *(Cornus sanguinea)*, European cranberrybush *(Viburnum opulus)*, European spindletree *(Euonymus europaeus)*, blackthorn *(Prunus spinosa)* and willows *(Salix spp.)*.

As the planting of trees seems to be an effective biotechnical measure preventing knotweed from reappearing, further planting is planned to be carried out in the autumn. Planting native shrub species is also important for biodiversity improvement; for example, black poplar (Populus nigra) was not naturally present in the area anymore, although historically it had been abundant.

4.4. Mechanical Removal of Himalayan Balsam *(Impatiens glandulifera)*

As early as in summer 2008 (after the first year of spraying), massive invasion of another invasive species–Himalayan balsam *(Impatiens glandulifera)* – in river sediments released after knotweed removal was observed.

Given that this is an annual herb, successfully reproducing mainly due to overproduction of seeds, an application to the European Commission was submitted to extend the project to include mechanical removal (cutting, pulling) of Himalayan balsam *(Impatiens glandulifera)*.

The European Commission approved the application and in 2009, Himalayan balsam *(Impatiens glandulifera)* plants were cut and pulled during flowering time (June–August) to prevent seeds from maturing. Removal of Himalayan balsam *(Impatiens glandulifera)* took place in Skalická Morávka National Nature Monument and in flood debris along the river in municipalities of Pražmo and Raškovice on the area of 70 hectares.

In autumn 2009, the most affected areas were seeded with a grass-herb seed mixture. In 2010, Himalayan balsam *(Impatiens glandulifera)* plants are being removed by the same procedure.

5. Research of Optimal Method for Invasive Species Elimination and Habitat Restoration

Research of optimal method for invasive species elimination and habitat restoration has been conducted from the onset of the project. The main goal of this research is to find the optimum method of knotweed elimination. The method should be as considerate to the surroundings as possible and also be transferable within the Czech Republic and the EU countries.

5.1. Experiment Plan

In 2007, forty-eight experimental plots were established for the research. The plots were established in two basic types of habitats in the project area, in which knotweed was to be found.

Knotweed plants settled forest-free sunny habitats, such as river and stream banks, roadsides and meadows, where it was often the dominant species forming dense monocultural thickets. First twenty-four experimental plots, designated as "OUT OF FOREST", were established at such location. The second habitat type was the forest, where knotweed formed stands, sometimes fully developed. There, another twenty-four experimental plots were established, designated "FOREST". In both localities, knotweed cover was 100% almost in all experimental plots in the year the of plot establishment.

The key factor for choosing the experimental plots location was shading (or amount of light) as it is assumed that this factor plays an important role for the viability and hardiness of knotweed. Given the need to establish the plots in the same or very similar habitats, an arrangement was chosen in which plots in one type of habitat are all in the same locality. With such an arrangement, equal environment conditions for all plots is an advantage. The disadvantage is the difficulty in finding a suitable site large enough for a great number of experimental plots. Within each type of habitat, twenty-seven experimental plots were placed, out of which twenty-four with the greatest knotweed cover were selected. Each plot has surface of 3 × 3 meters. Inside each square, interior area of 1.5 × 1.5 meters is used for observations. Space around the inner square is protective and serves as a buffer. Different methods of herbicide application, its timing and different herbicide brands were subject to the research. For the experiments, Glyfogan and Dominator herbicides were chosen as an adequate replacement for Roundup Biaktiv herbicide; they both contain the same active ingredient–glyphosate–as Roundup Biaktiv. One plot in each type of location was left without intervention for reference. Operating results were evaluated after intervention and at the end of the growing season (JŮZOVÁ 2007b). For overview of experiments see Annex 6.

5.1.1. Experimental Methods

The following chemical and mechanical methods were examined in the research of optimal method for invasive species elimination. Biological methods and grazing by domestic animals were not subject to the examination. The following methods are examined:

- Foliar spraying of herbicide
- Stem injection of herbicide
- Cut stem herbicide application (with a hand-held weed wiper, brush or other tools)
- Digging plants with rhizomes
- Cutting
- Combination of cutting and foliar spraying.

5.1.2. Herbicides and Concentrations

Three brands of non-selective glyphosate-based foliar herbicides were chosen for examination of chemical methods. Glyphosate (N-phosphonomethyl glycine) is a widely used non-selective herbicide; its toxic effect on plants is caused by inhibiting the enzyme 5-enolpyruvylshikimate-3-phosphate synthase. This enzyme is used in biosythesis of aromatic amino acids in plants; if blocked, proteosynthesis is disrupted and a plant dies (WILLIAMS et al. 2000 in PATOČKA 2008). Two concentrations are tested for each herbicide.

Tested herbicides and concentrations:

- Roundup Biaktiv (concentration 5%, 10% for spraying, 50%, 100% for injection, 50% for stem application). Note: From 2008 onward, the herbicide concentration for injection application changed–from 100% to 50% and from 50% to 30%–to fit actual conditions (based on experience gained in 2007).
- Glyfogan 480 SL (concentration 5%, 10% for spraying).
- Dominator (concentration 4%, 8% for spraying).

5.1.3. Herbicide Application Timing and Habitat Restoration Methods

Different herbicide application times were examined in the research. Different herbicide brands are applied by different methods in June (in the growth phase) or in September (during flowering time). Overview of timing and habitat restoration methods:
- Herbicide application (including injection and cut stem application) in June.
- Herbicide application (including injection and cut stem application) in September.
- Biomass removal immediately when dry after treatment.
- Biomass removal in late summer and autumn (September–November) of the same year.
- Biomass removal in spring (March–April) of the following year.
- Digging throughout the growing season (as needed, depending on the recovery rate).
- Cutting throughout the growing season (as needed, depending on the recovery rate).
- Sowing herb seeds in "OUT OF FOREST" localities (grass-herb seed mixture with eight grass species and two herb species).

5.2. Experiment Results

Practical experience shows that digging whole knotweed plants including the rhizomes is very time-consuming and physically demanding, especially with knotweed growing on flood debris and regulations. In addition, there is a relatively high risk that part of the root system will remain in the ground, which is undesirable as the ability to regenerate from very small fragments of a root system is well known.

The results of injection application and cut stem application indicate that the same method cannot be used reliably after the first treatment, because the resprouts are short with thin stem, in which the herbicide cannot be injected. The same is confirmed by practical experience with injection application (see Section 2.2.2.).

The current results show that the decrease in number of knotweed plants both in "FOREST" and "OUT OF FOREST" localities occur after using any herbicide.

A decrease in the number of knotweed plants in "FOREST" localities was more pronounced in 2008, which corresponds to its lower vitality compared to plots exposed to the sun. In these localities, however, knotweed began to resprout repeatedly with smaller and weaker plants (30 cm) that can be easily overlooked in the undergrowth and cannot be injected (due to a small stem diameter). Also, herbicide efficacy is considerably lower with these weaker and smaller knotweed plants. And so in the final year of the project (2010), there are more experimental plots with knotweed in shady localities. The plants are, nevertheless, only 20–40 cm tall and weak.

Cutting (whether intensive or two times a year) and digging (plots No. 11, 17 and 22) seem to be the least effective methods in general. Rich herbaceous community was observed in all plots in spring: wood anemone *(Anemone nemorosa)*, coralroot bittercress *(Dentaria bulbifera)*, spring cress *(Dentaria glandulosa)*. In spring the undergrowth consisted almost exclusively of self-seeded saplings, such as common ash *(Fraxinus excelsior)* and sycamore maple *(Acer pseudoplatanus)*; non-native species also begin to occur, such as small balsam *(Impatiens parviflora)* and Himalayan balsam *(Impatiens glandulifera)*.

A decrease in the number of knotweed plants in "OUT OF FOREST" localities was well pronounced with any herbicide. Methods applied in plots No. 19 and 21 (digging and intensive cutting) seem to be least successful. In 2010, knotweed is not present in most experimental plots. The plots are covered with luxuriant vegetation, mostly ruderal. The most abundant species include: stinging nettle *(Urtica dioica)*, catchweed bedstraw *(Galium aparine)*, mugwort *(Artemisia vulgaris)* and common dandelion *(Taraxacum officinale)*. Other non-native species begin to appear, such as small balsam *(Impatiens parviflora)* and Himalayan balsam *(Impatiens glandulifera)*.

In general terms, a statement can be made that up to 2010, knotweed plants in most plots have been destroyed or significantly weakened. It should be noted, however, that knotweed plants communicate with one another with their underground parts, as in the reference plot in "FOREST" locality, where the plants were not treated, their occurrence is minimal. This communication between individual knotweed plants distorts the results of the experiments. To carry out similar experiments, it is necessary that the plots were at much greater distance, making such an underground communication impossible. Decrease of knotweed in "OUT OF FOREST" and "FOREST" localities is shown in charts in Annexes 7 and 8.

Biomass removal in experimental plots was only carried out in the first year of experiments. In the following years it was only done in plots where digging and cutting was examined. In other plots it was unnecessary due to the small amount of biomass.

In 2008, selected plots in localities "OUT OF FOREST" were seeded with a grass-herb seed mixture. In subsequent years, sowing was not necessary, as in all localities "OUT OF FOREST" the herb layer was fully developed, the seeded species being suppressed by ruderal ones.

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Notes: