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Production of medically valuable stilbenes and emodin in knotweed

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ABSTRACT

Most people consider knotweeds to be dangerous, invasive weeds. These plants produce useful secondary metabolites, stilbenes and emodin. We conducted a 3-yr field experiment with two parental species *Fallopia japonica* and *Fallopia sachalinensis*, and three clones of their hybrid, *F.* × *bohemica*. Knotweed biomass and resveratrol, resveratroloside, piceid and emodin contents were assessed three times per year. Their biomasses (in descending order) are as follows: the two hexaploid hybrid clones, the octoploid hybrid clone, and the two parental clones. Although more energy is needed to harvest rhizomes and roots from knotweed, these produce more resveratrol, piceid and emodin than shoots. Out of the five clones tested, *F. japonica* contained the greatest amounts of resveratrol, resveratroloside, piceid and emodin, in its belowground biomass. The optimal harvest of rhizomes and roots from productive clones was in the autumn of the second year of cultivation.

1. Introduction

Knotweeds, which belong to the genus *Fallopia* Adans. (*Polygonaceae*), are considered to be dangerous weeds in Europe (Bailey et al., 1996); efforts have been undertaken to eliminate them, especially from nature reserves (Mandak et al., 2004). These plants produce many secondary metabolites that are medically valuable, such as emodin, resveratrol and resveratrol derivatives (Kovarova et al., 2010, 2011).

Resveratrol (3,4′,5-trihydroxystilbene) is a naturally occurring plant polyphenol that is present along with piceid in various fruits and vegetables at significant levels. Resveratrol has been shown to have antibacterial (Chan, 2002; Docherty et al., 2001), antifungal (Jung et al., 2005; Schulze et al., 2005), antioxidant, antimutagenic, anti-inflammatory (Busch et al., 2012), neuroprotective (Quincozes-Santos and Gottfried, 2011), chemopreventive (Soleas et al., 1997) and anticancer properties (Jeong et al., 2010; Pezzuto, 2008; Sun et al., 2008; Ulrich et al., 2005; Wolter et al., 2004), including the inhibition of breast cancer (El-Mowafy and Alkhalaf, 2003). Resveratrol prevents cardio-vascular diseases (Wu et al., 2001) and reduces body mass (Dal-Pan et al., 2010). This plant product also inhibits α-glucosidase, which could help in the control of diabetes (Brasnyo et al., 2011; Kerem et al., 2006).

Knotweed is traditionally used for the production of resveratrol in Asia, particularly in China. In Europe, wine is the main source of this substance.

Emodin is a biologically active, naturally occurring anthraquinone derivative (1,3,8-trihydroxy-6-methylanthraquinone) produced by lichens, fungi and higher plants. This substance has been found to have purgative, anti-inflammatory and anticancer effects (Fu et al., 2007; Lu et al., 2008; Muto et al., 2007; Pecere et al., 2000) and has been shown to induce apoptosis (Shieh et al., 2004).

In the Czech Republic, knotweeds occur as two parental species – *Fallopia japonica* (Houtt.) Ronse Decr. (Japanese knotweed) and *Fallopia sachalinensis* (F. Schmidt Petrop.) Ronse Decr. (Giant knotweed) – and their hybrid, *F.* × *bohemica* (Chrtek & Chrtková) J.P. Bailey. *F. japonica* occurs in Europe as a single viable hexaploid clone. *F. sachalinensis* is present as tetraploid, hexaploid and octoploid clones. The hybrid *F.* × *bohemica* exists as a variety of clones with these three ploidy levels (Mandak et al., 2003). These clones exhibit differing growth rates, productivities, rates of establishment in plant communities, spreading potential and, presumably, organic substance content. We choose clones known for desirable production of the target substances with history of no dispersal into the surrounding landscapes. This experiment was designed to study the strictly controlled production of secondary metabolites of medicinal use by different knotweed clones. These clones

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were grown on clayish substrates reclaimed from a mine dump into arable soil in Central Europe. Our goal was to develop safe method of production of potentially invasive clones.

We formulated the objectives of the experiment in the following questions: (a) Is there any difference between the individual clones in the production of above-ground and below-ground biomass? (b) Are there any differences in the content of secondary metabolites between individual clones? (c) Is there any difference in the content of secondary metabolites between root and shoot biomass? (d) What time is the most suitable for the harvest?

2. Materials and methods

2.1. Plant material

Rhizomes for planting were collected from five clones of *Fallopia*, namely *F. sachalinensis* Nakai ($n = 88$; genome size: 2C-values DNA = 8.802 pg; FS) from a former orchard in Luštěnice, *F.* × *bohemica* Chrtek & Chrtková ($n = 88$; genome size: 2C-values DNA = 9291 pg; FB8) from a former saw-mill yard in Luštěnice, *F.* × *bohemica* ($n = 66$; genome size: 2C-values DNA = 6.945 pg; FBM) obtained from a farmer in Mošnov, *F.* × *bohemica* ($n = 66$; genome size: 2C-values DNA = 6.918 pg; FBP) and *F. japonica* Houtt. ($n = 88$; genome size: 2C-values DNA = 9.541 pg; FJ), both from a semi-natural population in an abandoned garden in Prague. Genome size was estimated using a method described previously (Suda et al., 2010). Before planting, rhizomes were hand sorted and divided into 15–20-cm segments. Rhizomes were stored before planting in a humid environment to prevent drying.

2.2. Field experiment

2.2.1. Experimental area

The experiment was conducted on one hectare of a non-irrigated arable field in Mošnov. This field was originally a clayish spoil bank that was later recultivated by organic manuring and ploughing. It is located at 50°35′N, 13°52′E. The experiment was carried out over three growing seasons, from 2006 to 2008. The rhizomes and root segments of knotweed clones were planted with a spacing of 1.0 m into ploughed rows 0.75 m apart and were immediately covered with soil. Each clone was grown in two randomly selected strips of 6 rows, which were 100-m long and 0.75 m apart. These six-row strips were separated by 5-m strips to prevent clone mixing by lateral in-growth.

2.2.2. Non-destructive measurements

Non-destructive measurements were collected as follows: within each six-row strip, four groups of four plants were chosen in two neighbouring non-marginal strips (two in each) that were 1.0 × 0.75 m apart. Each plant was permanently marked with a specific code that enabled repeated measurements of the same individual plant.

Characteristics, such as shoot, branch and leaf numbers and shoot lengths, were non-destructively measured for the 32 marked individuals from each clone three times a year, in spring, summer and autumn. In addition, 10 other plants from each clone were sampled to measure these same characteristics plus leaf area and above- and belowground biomass after thorough washing and drying. Regression relationships (equations) developed using the data from the destructive measurements from these plants were used to estimate the biomass and leaf area of plants from which only non-destructive measurements were taken. For all 32 individuals from each clone, a 3-yr growth curve was plotted. The relative growth rate (RGR) of marked plants for each growing season was calculated as RGR = LN $(W_2 - W_1)/(t_2 - t_1)$, where W_1 is the total biomass

Table 1
Macronutrient content of the soil used in the field experiment.

Element	Content (ppm)	Soil acidity	
C	1.87	pH (H_2O)	6.5
N	0.13	pH (KCl)	5.9
P	49.8		
Ca	3887		
Mg	700		
K	498		

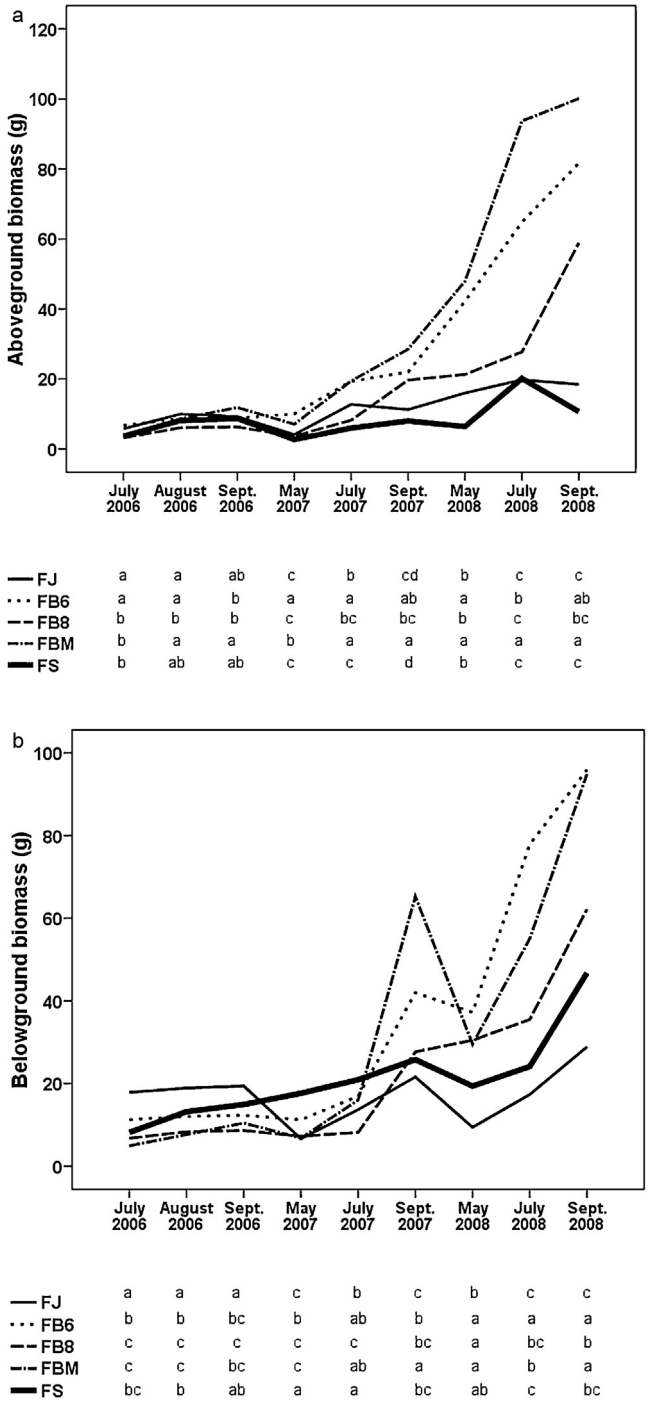

Fig. 1. (a, b) Above- and belowground dry biomasses in the two parental and the three hybrid clones in 2006–2008. FJ – *F. japonica*, FB6 – *F.* × *bohemica* clone B6, FB8 – *F.* × *bohemica* clone B8, FBM – *F.* × *bohemica* clone BM, FS – *F. sachalinensis*. Significant differences between the clones at $P \leq 0.05$ are indicated by different letters below the date; $n = 32$.