Surveys for natural enemies of giant hogweed (*Heracleum mantegazzianum*) in the Caucasus region and assessment for their classical biological control potential in Europe

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Summary

Heracleum mantegazzianum (Apiaceae) (giant hogweed), a biennial or perennial herb indigenous to the Caucasus mountains, has become an important invasive alien weed throughout Europe as well as in parts of North America. First introduced into botanical gardens in central and northern Europe in the late 1800s, *H. mantegazzianum* was subsequently widely planted as an ornamental and has now become invasive in most of its introduced range in western Europe. Giant hogweed not only replaces the native flora and alters ecosystems along waterways, but also poses a risk to human health by causing phytophotodermatitis after contact with its sap.

In January 2002, a collaborative European Union-funded program was initiated aiming to develop an integrated strategy for management of *H. mantegazzianum* in its exotic range. Biological control forms a central theme in this program and a series of surveys for both arthropod and pathogen natural enemies are being undertaken in the Caucasus region. The initial surveys have revealed an extensive mycobiota associated with *H. mantegazzianum*, most species of which are new records for this host. At least four, purportedly co-evolved pathogens belonging to the genera *Ramulariopsis, Septoria, Phloeospora* and *Phoma* were collected, of which the first three are under evaluation regarding their potential as biological control agents. The most prominent phytophagous insect present on *H. mantegazzinanum* was found to be the stem-boring curculionid *Lixus iridis*. Other insects collected attacked different parts of the host plant: Diptera species in the roots, Lepidoptera feeding on the leaves and flowers, and thrips species sucking on leaves, stems and flower heads. Likewise, the potential of these insect agents is currently being assessed.

Keywords: biological control, Caucasus, fungal pathogens, *Heracleum mantegazzianum*, phytophagous insects.

Introduction

Heracleum mantegazzianum Somm. & Lev., commonly known as giant hogweed, is a biennial or perennial herb belonging to the Apiaceae. Native to the western part of the Caucasus, the mountain range stretching from the Black Sea to the Caspian Sea, the plant was first introduced into botanical gardens in central and northern Europe in the late 19th century (Briggs 1979, Lundström 1984). With its impressive height of up to 5 metres and its large showy leaves and flowerheads, *H. mantegazzianum* was subsequently promoted by nurseries and actively planted as an ornamental curiosity in large private gardens and parks. Since then, the plant has escaped, spreading naturally by seed propagation, with each individual potentially producing up to 120,000 seeds per year (Dodd *et al*. 1994). The initial spread occurred mainly along rivers

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and streams, affecting riparian habitats (Caffrey 1994; Dodd *et al*. 1994). However, due to human activities, *H. mantegazzianum* has colonized a variety of habitats such as agricultural land, abandoned fields, and road and railway embankments, as well as urban sites (Pyšek 1994, Tiley *et al.* 1996). It is now a widespread weed in most of its introduced European range as well as in parts of North America (Morton 1978).

Rapid growth and a large leaf area forming dense canopies makes giant hogweed highly competitive and capable of shading out native herbaceous species, thus posing a significant threat to the local biodiversity and altering whole ecosystems (Vogt Andersen 1994, Pyšek & Pyšek 1995, Otte & Franke 1998). Following its annual winter die-back the weed exposes large areas of bare ground leaving the soil vulnerable to erosion, especially along riverbanks (Williamson & Forbes 1982). The sap, which is present in all parts of *H. mantegazzianum*, contains a number of furanocumarins giving it photosensitizing properties (Hipkin 1991). Skin contact with the plant causes severe blistering and dermatitis in affected humans, making the plant a serious public health hazard, especially when growing in amenity areas (Dodd *et. al*. 1994).

Current methods of control are based on herbicide application, particularly as glyphosate, animal grazing and mechanical control, such as cutting and ploughing (Dodd *et al*., 1994, Lundström & Darby 1994). However, overall, these methods have failed to give lasting control of the weed (Sampson 1994). While biological control has been considered as an alternative or additional strategy for the management of giant hogweed, little research has been undertaken in this field to date (Sampson 1990, 1994, Fowler *et al*. 1991, Caffrey 1994).

The need to develop an integrated management strategy that comprises effective, practicable and sustainable means to control the spread of *H. mantegazzianum* in its introduced European range, led to the initiation of a collaborative multidisciplinary project funded by the European Union in January 2002. Biological control, particularly the evaluation of co-evolved natural enemies from the native range of the plant as classical biocontrol agents, forms a central theme of this project.

Materials and methods

Field surveys

Two field trips were undertaken to the Russian part of the Caucasus mountains to establish the mycobiota and herbivore fauna associated with *H. mantegazzianum* in its native range and to assess the damage caused by individual fungal and arthropod species to this host, as well as to related plant species. The surveys were undertaken at the beginning of June and July 2002, respectively, covering 11 field sites between Pyatigorsk in the east and the Krasnodar territory in the west. The altitude of these sites ranges from 510 to 1670 m.a.s.l. A map showing the location of individual field sites is given in Figure 1.

Figure 1. Map showing the sites surveyed (☆) in the Caucasus region in 2002. (ref. http://caspian.hypermart.net/caucasus.gif)

Fungal pathogens

At each field site, a representative number of *H. mantegazzianum* plants and related species was assessed, comprising all ages of plants present. Level and type of pathogen damage was recorded and samples were taken. These were brought back in a plant press into the quarantine facilities at CABI Bioscience UK Centre where all subsequent work was carried out. Suspected biotrophic pathogens were isolated immediately onto *H. mantegazzianum* plants grown from seeds ex Kew Botantical Gardens, purportedly obtained from original plant collections in the Caucasus. Facultative pathogens were isolated onto potato carrot agar. A representative range of fungal specimens was taxonomically identified and deposited either in the culture collection or the dried reference collection of the CABI Bioscience fungal herbarium (Herb. IMI).

Plant inoculations with fungal pathogens were undertaken using spores obtained either from infected plant material or from agar cultures. Spore suspensions in sterile distilled water containing 0.01% Tween 80 were applied to leaf surfaces of *H. mantegazzianum* at a concentration of 10^6 spores ml^{-1} using a fine paintbrush. Either both upper and lower leaf surfaces were treated, or exclusively one or the other. Inoculated plants were placed in a dew chamber (Mercia Scientific, Birmingham, UK) for two days at 16°C and were subsequently maintained in a controlled environment room at 20°C, with a light regime of 12h light/12h dark. Treated plants were regularly assessed for the development of macroscopic symptoms of infection, and disease development was closely monitored and recorded.

Initial host-specificity studies were conducted using the following test plant species belonging to the same subfamily (Apioideae) within the Apiaceae as *H. mantegazzianum*: *Angelica archangelica* L. (angelica), *Coriandrum sativum* L. (coriander), *Daucus carota* L. (carrot), *Ferula communis* L. (giant fennel) and *Pastinaca sativa* L. (parsnip). Three plants were tested per species and a range of different leaf stages was inoculated. Inoculations and the subsequent maintenance of treated plants were carried out as outlined above. A test run was regarded as positive once the pathogen sporulated on the three inoculated plants of *H. mantegazzianum* included as positive controls. Individual test plant species were closely monitored for the development of any disease symptoms related to the respective fungal agent for at least double the period of time required by the pathogen to sporulate on its host.

Herbivores

Plants were taken at random and dissected according to a protocol developed beforehand. Besides general data about the field site, selected parameters of the plants were recorded in datasheets: plant height, diameter of the stem, diameter of the root, number of leaves and the length of the longest leaf, number of flower heads and the diameter of the central flower head. Observations of phytophagous insects on and in these parts of the plant were also noted. Each plant was dug out completely and all stems, petioles, and roots were dissected. In addition to these randomly chosen plants, as many plants as possible were assessed for signs of insect attack.

All stages of insects found were collected together with pieces of the plant parts, where they were feeding, and kept in plastic boxes. When necessary, plant material was replaced with fresh material. The samples were transported into quarantine at the CABI Switzerland Centre, where they were transferred onto potted plants. Adults emerging out of the rearing cages are being identified.

As with fungal pathogens, initial host-specificity tests were carried out using the following closely related native and economically important plants: coriander, carrot, *Foeniculum vulgare* Miller (fennel) and *Heracleum sphondylium* L. The adult feeding and oviposition tests were carried out under single-choice as well as multiple-choice conditions, using potted plants in cages. All plants were examined for feeding traces and dissected for eggs.

Results

Fungal pathogens

An extensive mycobiota was found to be associated with *H. mantegazzianum* in its native Caucasus region, with a number of species newly recorded from this host. At least four potentially co-evolved pathogens were found during the early season survey (June), though their overall abundance was very low. These pathogens were subsequently identified and deposited as: *Septoria heracleicola* Kabát & Bubák (Herb. IMI no. 389651); *Ramulariopsis* sp. nov. (Herb. IMI nos. 389652, 389653, 389656); *Phoma* sp. (possibly *Phoma longissima* (Pers.)Westend. (Herb. IMI no. 389654)); *Phloeospora heraclei* (Lib.) Petr (Herb. IMI nos. 389658, 389659). By mid season (July 2002) some additional fungal species, identified as *Ramularia heraclei* (Oudem.) Sacc. (Herb. IMI no. 389655) and *Erysiphe heraclei* DC. (not deposited), were recorded. It was noted that the abundance of all pathogens had markedly increased both on first year and mature plants. The genera *Phloeospora* and *Ramulariopsis* were also found on related *Heracleum* species and the inter-relationships are being investigated. Further evaluations of *P. heraclei* (Herb. IMI 389658), *S. heracleicola* and *Ramulariopsis* sp. nov. (Herb. IMI 389652) have commenced under quarantine conditions in the UK.

Field observations indicated that the coelomycete fungus *P. heraclei* might have a high potential as a biocontrol agent since it occurred at all sites, causing significant damage in the form of leafspot and die-back to *H. mantegazzianum* plants of all ages, and particularly to plants at the seedling stage. Laboratory studies revealed *P. heraclei* to be a true biotroph or obligate parasite since it could be cultured only on its living host. Infection of *H. mantegazzianum* occurs through the lower leaf surface leading to chlorotic spots *ca*. 7 days after inoculation and subsequent sporulation *ca*. 2–3 days later. The pathogen forms conspicuous large pale brown acervuli bearing hyaline, curved conidia on the upper leaf surface of its host. These are associated with black crusts, considered to represent either feeding or survival structures, forming on the lower leaf surface. Under controlled environment conditions, infection of *H. mantegazzianum* with *P. heraclei* was consistently high. Initial host specificity studies showed that *P. heraclei* can sporadically infect parsnip and coriander, showing restricted sporulation with smaller pustules than those formed on *H. mantegazzianum*. To date, no symptoms have been recorded on the test plant species angelica, carrot and giant fennel.

Septoria heracleicola closely resembles *P. heraclei*, both in the disease symptoms caused as well as in macromorphology. In the field, mixed infections of both pathogens were frequently encountered on *H. mantegazzianum,* with *P. heraclei* being the dominant agent. Therefore, an accurate assessment of the impact of *S. heracleicola* on its host alone is still lacking. *Septoria heracleicola* can easily be cultured *in vitro* and produces infective conidia. Inoculation using conidia from agar culture leads to the formation of necrotic leaf spots on *H. mantegazzianum* after *ca.* 15 days. Sporulation on these necrotic lesions could be induced by incubation in a humid chamber for two days. Initial studies showed that *H. mantegazzianum* is susceptible to *S. heracleicola* under controlled environment conditions. A preliminary assessment of the host specificity of this pathogen has commenced and the results are pending.

The cercosporoid fungus *Ramulariopsis* sp. nov. is a hitherto undescribed species and is the first record of the genus *Ramulariopsis* for the family Apiaceae (J.C. David, pers. comm.). It remains to be established whether the different specimens deposited in the culture collection of the CABI Bioscience herbarium represent isolates of the same species. The pathogen causes angular lesions with a distinct cottony appearance once sporulation occurs. Its impact on *H. mantegazzianum* in the field can be variable. *Ramulariopsis* sp. nov. produces infective conidia *in vitro* and initial pathogenicity studies have shown that the first symptoms of disease, seen as necrotic lesions on infected leaves, appear *ca*. 10 days after inoculation. The pathogen sporulates predominantly on the lower leaf surface of its host.

Herbivores

While there were no feeding traces on any of the roots during the first survey in June 2002, Diptera larvae were found to mine the roots later in July. The species have not yet been identified and some of them feeding on the outer parts of the root may be saprophytic. The most obvious phytophagous insect present on the plants was *Lixus iridis* Ol. (Curculionidae), which was found on most sites, with a high abundance at some sites. It was not uncommon to find 10 larvae in one stem. The adults mate on the highest parts of the plants and, after copulation, eggs are laid inside the hollow stem. Dissections and field observation of the adults show that eggs and larvae occur mainly in the stem, but also in the larger petioles of the leaves. Adults and eggs of this weevil were also recorded on another *Heracleum* sp. (most probably *Heracleum asperum* (Hoffm.) M. Bieb.) during the survey. Some 74 adults were brought back into quarantine at the Centre. Besides these root and stem-feeding insects, some unidentified leaf-feeding larvae were found, especially in the western part of the surveyed area. Two thrips species were found in very high numbers on *H. mantegazzianum*, one feeding on the leaves and the other on the stems and flower/seed heads. The latter species had a dramatic impact on the development of flowers on single plants. Unfortunately, it was subsequently determined as the rather polyphagous *Thrips vulgatissimus* (Haliday). *Depressaria* spp. (Oecophoridae) larvae were found feeding within the flower heads.

Preliminary host-range testing with *L. iridis* showed that they did not feed or oviposit on three plants of economic importance, i.e. coriander, carrot and fennel, under single as well as multiple-choice conditions. However, during the tests only nine eggs were found on *H. mantegazzianum*, but feeding was recorded on almost all host plant replicates.

A previously undetermined noctuid larvae feeding gregariously on the leaves of *H. mantegazzianum* in the Caucasus, was also used in preliminary host-range testing. After adult emergence, the species was identified as *Mamestra brassicae* (L.) Barathra, a well known pest on *Brassica* spp. In single-choice tests the larvae fed on all the test plants offered.

Discussion

In order to develop an integrated strategy for management of *H. mantegazzianum* in Europe, biological control needs to be considered as one approach, potentially contributing to long-term control of the weed. Areas with restricted infestations of giant hogweed can be effectively managed employing mechanical and chemical control, but the only sustainable solution for large populations of the weed would be classical biological control. While a range of herbivores and fungal pathogens has been recorded from giant hogweed in its exotic distribution, these comprise mainly polyphagous insect species, which generally have little impact on the plant; and generalist pathogens, usually exhibiting a relatively wide host range (Sampson 1990, Bürki & Nentwig 1998). Hence, such insect and fungal species are considered to have little or no potential as biological control agents. In contrast, knowledge about the natural enemy complex that co-evolved with *H. mantegazzianum* in its native Caucasus range is still scarce. The recent survey work documented here has established that a diverse mycobiota and herbivore complex is associated with giant hogweed in its centre of origin, with a number of species constituting first records for this host.

Phloeospora heraclei appears to have high potential as a biological control agent due to its impact on *H. mantegazzianum*. Being particularly damaging to *H. mantegazzianum* at the seedling stage, the pathogen would affect the weed at a critical phase of its life cycle given the inability of giant hogweed to spread vegetatively (Ochsmann 1996). However, the host specificity of *P. heraclei* will need to be critically evaluated given its apparent ability to infect non-target species, such as parsnip and coriander, under controlled environment conditions. Artificial host range extension of plant pathogens in greenhouse screening is a well-documented phenomenon (Cother 1975, Evans 1995) and restricted sporulation, as seen for *P. heraclei* on these test species, is generally viewed as an expression of plant resistance (Heath 1982). However, records in the CABI Bioscience fungal herbarium revealed that *P. heraclei* has been reported from parsnip as well as from the indigenous *H. sphondylium* in the UK and other European countries. The incidence of *P. heraclei* on these hosts reported from Europe, as well as a potential presence of the pathogen on *H. mantegazzianum* in its exotic range, has to be established and pathogen strains from different hosts and regions need to be characterized and compared.

Regarding the other two fungal pathogens currently under evaluation, *S. heracleicola* and the cercosporiod fungus *Ramulariopsis* sp. nov., their impact on *H. mantegazzianum* needs to be determined and their host specificity assessed. Fungal pathogens belonging to the genus *Septoria* as well as other cercosporoid fungi have already been used, apparently with success, in biocontrol programs against invasive weeds, as for example *Septoria passiflorae* Syd. against Banana Poka (*Passiflora tripartita* (Juss.) Poir var. *tripartita* Holm-Nie. Jörg & Law) in Hawaii (Trujillo *et al*. 2001) and *Cercospora rodmanii* Conway against waterhyacinth (*Eichhornia crassipes* (Martius) Solms-Laubach) in South Africa (Morris & Cilliers 1992).

The field surveys conducted in 2002 for arthropods attacking *H. mantegazzianum* indicate that seed feeders might be the most promising insects as biocontrol agents, e.g. *Depressaria* spp. The field observations found three insects to be most damaging to the host plant, but subsequent identification of these insects revealed that they are polyphagous, and thus cannot be considered as potential biocontrol agents. Some of the insects collected as larvae have yet to emerge, whilst others have not yet been identified.

In 2003, additional surveys will be undertaken both in the invasive and the native range of *H. mantegazzianum* in order to complete the inventory of fungal pathogens and herbivores associated with this host. It is hoped that further visits in the Caucasus at different times of the year and other field sites will reveal additional agents for further studies.

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