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ORIGINAL PAPER

# *Parietaria judaica* flowering phenology, pollen production, viability and atmospheric circulation, and expansive ability in the urban environment: impacts of environmental factors

**Christina Fotiou · Athanasios Damialis · Nikolaos Krigas · John M. Halley · Despoina Vokou**

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**Abstract** *Parietaria judaica* (Urticaceae) grows abundantly in urban areas of the Mediterranean region. Its pollen is a major allergy source. We studied the species' distribution and abundance in and around Thessaloniki (Greece), pollen production and pollen season. We also examined how urban pollution affects pollen viability. Our ultimate goal was to obtain an estimate of the species' performance and ability to expand under different environmental conditions related to climate change. We mapped *P. judaica* and the other Urticaceae species. In a north- and a south-facing population, we recorded the progress of *P. judaica* flowering and estimated the pollen content per flower, shoot and surface unit. We concurrently assessed atmospheric circulation of Urticaceae pollen. We estimated *P. judaica* pollen viability and Cu, Pb and Zn concentrations in plants collected from sites differing in traffic intensity. *P. judaica* is the most abundant Urticaceae species in the area; its occurrence has increased dramatically over the last 100 years. Production of flowers is intense in spring and autumn. Flowering started 12 days earlier in the south-facing population in spring, and 3 days later in autumn. Pollen production was higher in spring and in the south-facing population. Flower and pollen production were positively correlated with the size of the plant and the flower, respectively. Copper and lead concentrations in plants were positively correlated with pollen viability, which was higher for plants collected from high-traffic sites. *P. judaica* has a high phenotypic plasticity; this is a feature that promotes success of expansive and invasive species. It is also well adapted to warm and polluted urban environments. The climatic change forecast for the Mediterranean region could provoke earlier, longer, and more pronounced flowering and, consequently, more *P. judaica* pollen in the air. In return, this would result in increased severity of *Parietaria* pollinosis.

**Keywords** Climate change · Heavy metal · Greece · Invasive species · Urticaceae

C. Fotiou · A. Damialis · D. Vokou (✉)
Department of Ecology, School of Biology, Faculty of Sciences, Aristotle University,
GR-54124 Thessaloniki, Greece
e-mail: vokou@bio.auth.gr

N. Krigas
Department of Botany, School of Biology, Aristotle University, Thessaloniki, Greece

J. M. Halley
School of Biological Applications and Technology, University of Ioannina,
GR-45110 Ioannina, Greece

## Introduction

*Parietaria judaica* (wall-pellitory) is a wind-pollinated ruderal species of the Urticaceae family growing abundantly in places extensively disturbed by man throughout urban areas of the Mediterranean region (Sukopp 2002; Woodell 1979). Urban weeds of this family are known to trigger allergies (Gadermaier et al. 2004). *Parietaria judaica* pollen, in particular, elicits severe pollinosis in Europe (Cosmes Martín et al. 2005; Geller-Bernstein et al. 2002; Loureiro et al. 2005); in some Mediterranean regions, like southern Italy and coastal Spain, it is the most important cause of allergies (D' Amato et al. 1992). In Thessaloniki, the second largest city of Greece, *Parietaria* ranks fourth after Poaceae, Oleaceae and Chenopodiaceae in inducing allergic responses by skin prick tests: in a sample of 1,311 patients with respiratory allergy symptoms, 15.3% were sensitive to its pollen (Gioulekas et al. 2004a). Ranked by pollen allergenic

properties and pollen concentration, respectively, *Parietaria* matches Urticaceae fairly well. In the air of Thessaloniki, pollen concentration of this family ranks third after Cupressaceae and *Quercus* spp.; in addition, analysis of a 20-year pollen time-series showed a significant trend towards increased Urticaceae pollen in the air of the city (Damialis et al. 2007).

Atmospheric pollen concentration is the result of pollen emission, which in turn is a function of various factors. Pollen released in a given area can be estimated on the basis of the amount of pollen produced per anther, the number of anthers per flower, the number of flowers per plant, the plant density, and the length of the flowering time. Several environmental factors affect these variables (Beri and Anand 1971; Mondal and Mandal 1998; Prieto-Baena 2003); among these are global climate change components, such as carbon dioxide concentration, temperature, and ultraviolet-B radiation, to which plant reproduction and hence pollen yield is highly vulnerable (Koti et al. 2005; Rogers et al. 2006). It is generally accepted that the production capacity of an anther is genetically fixed (Stanley and Linskens 1974; Subba-Reddi and Reddi 1986), but this is not always the case. Etterson and Galloway (2002) reported that *Campanula americana* grown under intense light produces more pollen grains per flower than when grown under low light, whereas the number of anthers per flower remains invariable. Also, de Vries (1971) remarked that if light is deficient during the stages of meiosis and subsequent formation of tetrads and mononuclear pollen grains in wheat, much less pollen is produced per anther than when deficient in other stages. At the level of the individual, production may vary considerably from year to year (Stanley and Linskens 1974).

Photoperiod, air temperature and relative humidity are regarded as the primary triggers of phenological events for temperate, boreal, and Mediterranean plants (Zhang et al. 2004). Changes in crucial abiotic factors may affect the plants' reproductive output, either directly or indirectly via affecting the reproductive season. Various factors affect pollen viability, with pollution being one of them. Heavy metal pollution is known to be high in urban areas (Aksoy et al. 1999; Harrison and Williams 1982; Sanka et al. 1995; Tomasevic et al. 2004), with the concentration of some elements, like lead, cadmium and zinc, declining with distance from road traffic (Fatoki 2003; Sanka et al. 1995). Dry hygroscopic pollen absorbs atmospheric water and pollutants dissolved in water droplets (Wolters and Martens 1987). Several studies have shown the detrimental effect of pollutants on pollen viability for both woody (Comtois and Schemenauer 1991; Gottardini et al. 2004) and herbaceous (Iannotti et al. 2000) species, but *P. judaica* pollen is found to be highly viable in polluted sites (Iannotti et al. 2000).

The ability of *P. judaica* to expand its range (Bass and Bass 1990) and its importance as a source of allergy clearly make the species deserving of careful attention in a time of changing climate. Therefore, we undertook: (1) to study *P. judaica* distribution and abundance in and around the city of Thessaloniki and to compare it with that in the past, (2) to examine the phenology of flowering and pollen production of two populations in the centre of the city that differ in exposure (north- and south-facing) so as to have a measure of the species' plasticity with respect to these features, (3) to compare the phenology of flowering of *P. judaica* with the atmospheric circulation pattern of Urticaceae pollen, and (4) to examine how urban pollution affects pollen viability. The ultimate goal of our research was to have an estimate of this species' performance and ability to expand under different environmental conditions related with climate change, thus providing a basis from which to forecast future severity of *Parietaria judaica* pollinosis.

## Materials and methods

### Site and species description

Thessaloniki, the second largest city in Greece, is located in the Aegean Sea (40°37′N, 22°57′E) to the north of Thermaikos Gulf. It has a Mediterranean-type climate, characterised by warm and dry summers and wet and rather mild winters.

*Parietaria judaica* L. is a wind-pollinated perennial herb. It can grow in shaded, ruderal communities and nitrophilous skirts, at river banks, in watered orchards, as well as in wall-fissures (Dietmar 1998). It has an extended flowering period and reproduces both sexually and vegetatively. Individual plants consist of many shoots emerging from a common rootstock. Each inflorescence consists of a central female flower surrounded by three to six hermaphrodite flowers. The stamens are bent inward in the floral bud; they spring violently upward and bend out of the flower when mature, ejecting the loose and powdery pollen as a little cloud to the ambient air (Airy Shaw and Willis 1973). Self-compatibility is reported for this species (Franchi et al. 2007).

### Distribution and abundance of Urticaceae in and around Thessaloniki

All Urticaceae species found in Thessaloniki seem to occupy similar habitats; these are man-made or influenced by human activities, and include habitats like vacant lots, roadsides, open and green areas, walls and pavement cracks, railway tracks, tree-bases and rubble (Krigas and Kokkini 2005). To estimate the distribution of *P. judaica* in relation to other members of Urticaceae in and around the

city, we made use of a detailed study on the vascular flora of the metropolitan area of Thessaloniki (Krigas 2004; Krigas and Kokkini 2004, 2005). In that study, 26 sampling sites were chosen located in four sectors, A, B, C and D (Fig. 1). Following Sukopp et al. (1980) and Wittig et al. (1993), these sectors were designated on the basis of older maps illustrating the growth of the city during past centuries (Krigas 2004). The historic centre, delimited by Byzantine Walls, is designated as sector A; it covers an area of approximately 450 ha and had ca. 90,000 inhabitants in 1880. Sectors B and C represent the urban expansion around the 1920s; at that time, the urban centre covered ca. 2,000 ha and had ca. 280,000 inhabitants. The third recent expansion of the city occurred after the 1990s, with the sprawl of sectors B and C and the development of satellite settlements in sector D; this has resulted in a rather continuous, densely urbanised area of ca. 6,100 ha and >800,000 inhabitants, roughly delimited nowadays by the ring-road of the city (Figs. 1, 2).

Four Urticaceae species are found in the metropolitan area of Thessaloniki (Krigas 2004; Krigas and Kokkini 2004, 2005): *Parietaria judaica*, *Urtica dioica* L., *U. pilulifera* L. and *U. urens* L. Data from a floristic study of the city (Krigas 2004) allowed us to create dot maps representing the frequency of occurrence and the relative abundance of the Urticaceae species in the sampling sites. However, for *P. judaica*, we conducted a further, more detailed, study. More specifically, we divided the study area into 10×11 equal squares of 2.25 km$^2$ each (Fig. 2). We visited the whole territory of all squares/cells of this grid by following the road networks within them, we made observations in each of the four quarters of every cell, thus estimating species occurrence and abundance, and scored each cell accordingly.

Depending on the species' population features, sampling sites were scored after a five-point, semi-quantitative scale that uses four criteria: (1) the effort needed to locate the species, (2) the approximate number of individuals present (defined here as clusters of spreading and/or ascending

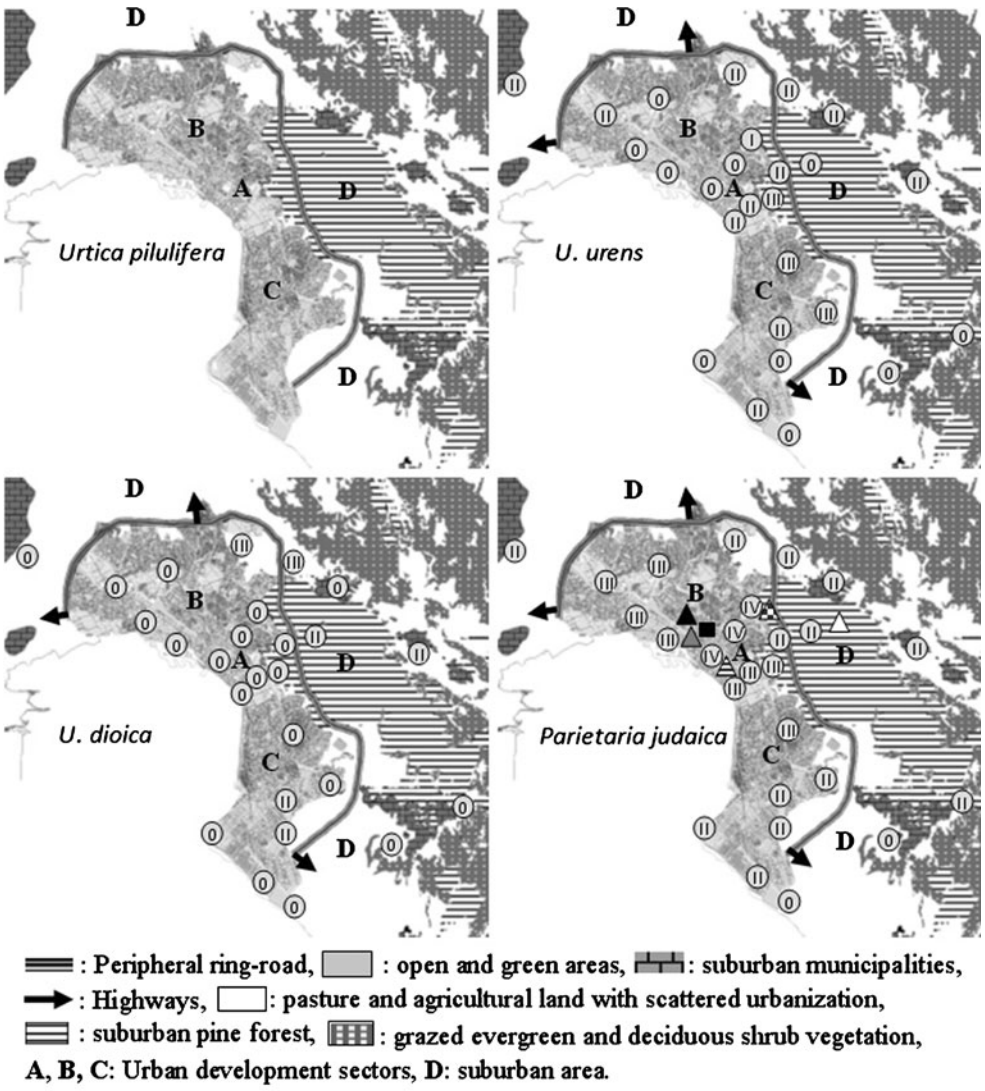


**Fig. 1** Current distribution and relative abundance of *Urtica pilulifera*, *U. urens*, *U. dioica* and *Parietaria judaica*, in the area of Thessaloniki, in northern Greece, across sectors *A*, *B*, *C*, *D*, which represent different stages of the city expansion after 1880 (from older to newer). On the *P. judaica* map, the location of the pollen trap is marked with a *black rectangle* and the sampling sites for metal concentration and pollen viability are marked with *triangles*; *black triangle* Monastiriou Street, *grey triangle* Venizelou Street, *white triangle* suburban area, the *striped triangle* Aghia Sophia Square, *tiled triangle* Zoo area. Abundance scores within circles: *0* absent, *I* rare to present, *II* occasional to frequent, *III* common to abundant, *IV* very common to dominant; for more information on scores see Table 1. For information on the sampling sites, see Table 3

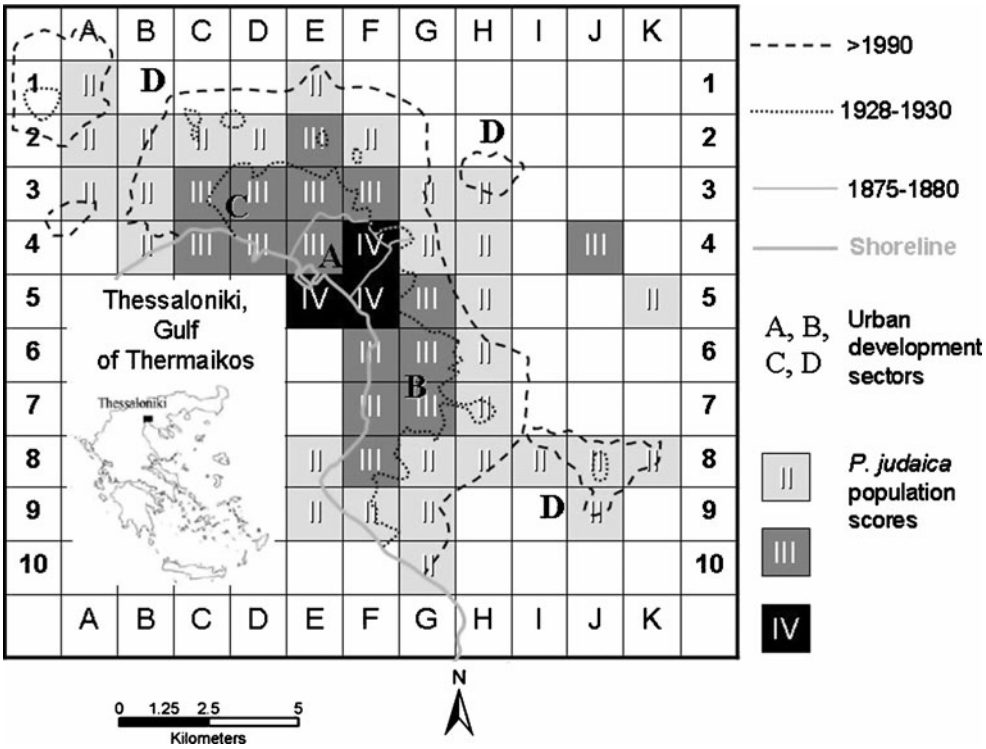


**Fig. 2** Current distribution and relative abundance of *P. judaica*, at a scale finer than that in Fig. 1, in a grid of 10×11 units of 2.25 km$^2$ each, across urban development sectors *A–D*, which represent different stages of the city expansion after 1880 (from older to newer). Abundance scores within circles: *0* absent, *I* rare to present, *II* occasional to frequent, *III* common to abundant, *IV* very common to dominant (see Table 1)

flowering shoots), (3) the relative surface occupied by the individuals, and (4) the relative abundance compared to the other co-occurring species (dominance; Table 1). This is a modification of the scoring system, which was used for floristic and/or vegetation surveys of several cities in Germany (Wittig et al. 1993), and for the floristic survey of Thessaloniki (Krigas 2004; Krigas and Kokkini 2004, 2005). This method seems able to reduce the subjectivity of empirical observations (Wittig et al. 1993), to supplement the commonly used Braun-Blanquet (1983) method that is applied in numerous phytosociological studies, and to establish an equivalence of qualitative descriptions (e.g. rare, frequent, common) with quantitative data.

Information exists on the past distribution of plant species in and around Thessaloniki (for a review, see Krigas 2004), against which the current distribution of *P. judaica* can be compared. To do so (1) former names and localities of collection sites in the study area were identified, and (2) qualitative descriptions and Braun-Blanquet scores applied by previous researchers were used to score the sampling sites after Wittig's modified scale (Table 2).

**Table 1** Semi-quantitative, modified, five-level scale (Wittig 1993; 0 = absence) for the description of Urticaceae representatives in Thessaloniki, noting equivalence with Braun-Blanquet scores as used by Oberdorfer (1954), and empirical qualitative descriptions of occurrence and/or abundance as described by Charell (1891–1892) alias Nadji (1892) and Zaganiaris (1939, 1940)

| Scale scores (equivalence to qualitative descriptions) (Braun-Blanquet scores)[a] | Criteria | | | |
|---|---|---|---|---|
| | Difficulty in locating | Approximate number of individuals | Relative surface occupied by individuals | Dominance |
| I (rare to present) (+.1, +. 2 or 1.1, 1.2) | High | A few | A few, small, scattered spots or singly growing individuals | No |
| II (occasional to frequent) (1.3, 1.4, 2.1, 2.2, 2.3) | Medium | Dozens up to a few hundred | A few, rather large patches, several medium-size patches or many small spots | Only in a few areas |
| III (common to abundant) (2.4, 3.1, 3.2, 3.3) | Low | Several hundred | Several large patches | In several areas |
| IV (very common to dominant) (3.4, 3.5, 4.3, 4.4, 4.5) | None | Many hundreds up to thousands | Extensive patches covering at least 30% of the sampled area | Almost everywhere |

[a] For scoring system, see Braun-Blanquet (1983)

**Table 2** Past and current state of *Parietaria judaica* populations in 16 repeatedly sampled sites of the metropolitan area of Thessaloniki. For definition of scores, see Table 1. For location of sites, see Fig. 1. *Rel.* Specific sampling unit defined by Oberdorfer (1954)

| Source of information for past distribution | Surveyed site (as described by the authors) | Location | Score (modified Wittig scale) | |
|---|---|---|---|---|
| | | | Past | Present |
| Charrel (1891–1892) | Salonicae | Cells E4-F4-F5 | I | III or IV |
| Nadji (1892) | 1 heure de Salonique | Sectors B-C-D | I | II or III |
| Zaganiaris (1940) | Eptapyrgion | Cell F3 | I | IV |
| Zaganiaris (1940) | Thessaloniki | Sectors B-C-D | I | II or III |
| Oberdorfer (1954, Rel. 118) | Ag. Paylos | Cell G4 | I | III |
| Oberdorfer (1954, Rel. 59) | Acropoli | Cell F3 | III | IV |
| Oberdorfer (1954, Rel. 59a) | Evraika | Cell F5 | III | IV |
| Oberdorfer (1954, Rel. 1a) | Thessaloniki | Sectors B-C-D | III | III |
| Oberdorfer (1954, Rel. 338) | Acropoli | Cell F3 | I | IV |
| Oberdorfer (1954, Rel. 344) | Pantheon | Cell F5 | I | III |
| Oberdorfer (1954, Rel. 461a) | Evraika | Cell F5 | I | III |
| Oberdorfer (1954, Rel. 125) | Bogatsikou | Cell F5 | I | III |
| Oberdorfer (1954, Rel. 385) | Ag. Sofia | Cell F5 | I | III |
| Oberdorfer (1954, Rel. 413) | Lefkos Pyrgos | Cell F5 | I | III |
| Oberdorfer (1954, Rel. 364) | Kamara | Cell F5 | I | III |
| Oberdorfer (1954, Rel. 295) | Ag. Sofia | Cell F5 | I | III |

## Phenology of flowering

In 2005, we studied the flowering phenology of a north- and a south-facing population of *P. judaica*. These populations were chosen in the centre of the city, where the species is most abundant; each occupied a narrow band adjacent to a hedgerow. The two populations were monitored throughout the year, every 2–3 weeks, and more frequently, every 5 days, in spring and autumn. On each sampling occasion and for each population, we observed 45 shoots randomly chosen across the study area; these correspond to approximately 5% of the population size. We estimated the percentage of open hermaphrodite flowers carried by these shoots using a method equivalent to the Braun-Blanquet (1983) method for estimating plant cover. To cross-validate this method, in our first spring sampling, we counted separately all hermaphrodite flowers that were in bloom or not in ten shoots. The percentage of flowers in bloom estimated by this fine, but time-consuming, method was compared with that estimated by the rapid Braun-Blanquet equivalent method; the estimated value deviated from the measured value by less than 25%. We considered this a fairly good match and, therefore, we made use of the rapid method in all subsequent samplings.

## Pollen production

We studied pollen production for the same two populations for which we studied phenology of flowering. We estimated pollen production at three stages: at full blossom, in both spring and autumn, and in the beginning of flowering in autumn. The floral unit that we used for the extraction of pollen was the flower. For each sampling event, we randomly collected 30 individual shoots from each population. Three mature and indehiscent hermaphrodite flowers were sampled from each shoot, thus making a total of 180 flowers for the two populations at each sampling time.

One of the problems encountered in pollen counting is the difficulty in bringing the pollen into uniform suspension. This is because pollen grains tend to clump due to the pollenkitt (or pollen coat) and pollen surface charges (Shivanna and Rangaswamy 1992). Despite reports that pollenkitt is absent from the pollen surface of strictly or primarily anemophilous taxa, such as those of Urticaceae, and specifically the anemophilous *Parietaria* (Pacini and Hesse 2005), pollen clumps were present in *P. judaica* samples.

To extract pollen grains, we put each flower sampled into a 10% KOH solution (300 μl) that we further boiled for 15 min to break up plant tissues and release pollen grains (Faegri and Iversen 1989; Moore et al. 1991). To eliminate pollen clumping and avoid miscalculations, we added a bipolar solvent—glycerol 70%—to a final volume of 600 μl, and stained the pollen grains with saffranine. Preliminary applications of hexane/cyclohexane, as suggested by Shivanna and Rangaswamy (1992), of ethyl alcohol (Guardia and Belmonte 2004) or other alcohols at varying concentrations, all resulted in pollen clumping;

concentrations of glycerol higher than 70% were no good either as they resulted in pollen burst. Samples for microscopic observations were taken while shaking the pollen suspension vigorously with a Vortex mixer to avoid the pollen sinking. We took three sub-samples per flower (6 μl each) and placed them on microscope slides under cover slips. All pollen grains were counted at ×100 magnification. To calculate pollen yield per flower, we multiplied the average number of pollen grains in the three sub-samples by the dilution factor (×100). To estimate pollen yield per anther, we divided the pollen yield per flower by four, which is the number of anthers carried by *P. judaica* flowers.

For the spring and autumn full blossom samplings, we measured the length of 30 of the shoots sampled as well as the corolla width of the flowers that we used for pollen extraction. By multiplying pollen production per flower by the average number of hermaphrodite flowers per shoot, we could estimate pollen production per shoot. Pollen yield at higher levels took into consideration the density of shoots and individuals in the study area and the average number of shoots per individual. For this reason, in both the spring and autumn samplings, we also counted all *P. judaica* shoots in the two populations studied and the approximate number of shoots per rootstock; this was only a rough estimate as individuals cannot be easily distinguished simply on the basis of their aboveground parts.

## Collection of airborne pollen

Airborne pollen of Urticaceae species in Thessaloniki was collected by use of a 7-day recording Burkard volumetric trap (Burkard, Rickmansworth, UK), located at about 30 m above ground level. The trap is equipped with a vacuum pump drawing 10 l $min^{-1}$ air through a thin orifice. Pollen and other particles are trapped on an adhesive-coated (Burkard gelvatol) transparent plastic tape (Melinex), supported on a clockwork-driven drum, which moves at a speed of 2 mm $h^{-1}$ and makes a complete revolution in a week. The tape is then removed and cut into seven equal sections, each representing a day of sampling. The tape sections are stained with a solution of saffranine, gelatine, glycerol and phenol, and are mounted on microscope slides. Identification and counting of pollen grains were performed under a light microscope (Krüss, Optronic) at a magnification of ×400 (British Aerobiology Federation 1995). Under the optical microscope, *P. judaica* pollen cannot be distinguished from that of other Urticaceae species.

## Heavy metal concentration and pollen viability

When *P. judaica* was in full blossom, we randomly harvested the upper shoot parts (including leaves and flowers) from populations in five sites differing in traffic intensity; in three of these sites (Table 3), sampling was performed in both spring and autumn, whereas in the other two, only in spring. *Parietaria* pollen is partially hydrated; as a result, it loses water and dies quickly if kept in a dry environment (Franchi et al. 2007). For this reason, we stored samples at 3–5°C; preliminary experiments showed a decrease in viability upon storage at lower temperatures.

To estimate pollen viability, we performed the TTC test (2,3,5-triphenyltetrazolium chloride) under high humidity conditions (>95%; Shivanna and Rangaswamy 1992). With a needle, we broke the anthers of flowers from different shoots and transferred the pollen released onto microscope slides under cover slips, after adding a few drops (3–4) of a 0.5% TTC in 10% sucrose solution, which prevents pollen grains from bursting (Shivanna and Rangaswamy 1992). The microscope slides were placed on wet cotton pieces, wrapped with aluminum foil and kept in the dark for about 2 h. Pollen grains were counted under the microscope at ×100 magnification; those stained red were considered viable.

We determined copper (Cu), zinc (Zn) and lead (Pb) in the plant material collected from the five sites. To measure the concentration of these heavy metals, the *P. judaica* upper shoots sampled were furnace-dried at 80°C for 12 h and ground to dust (Alaimo et al. 2005; Sawidis et al. 1995). For the digestion of plant material, we put 1 g dry weight of the ground tissues in an open quartz tube, to which we added 8 ml concentrated $HNO_3$ (pro analysi, Merck, Darmstadt, Germany). The mixture was left at room temperature overnight, warmed at 50°C for 2 h and heated at 180°C for 4 h. The solution was filtered through Whatman 589/2 filters and the filtrate was diluted to a volume of 25 ml with double de-ionized water. There were three replicates for each sampling site and sampling time. Copper, lead and zinc in the final solution were determined in an atomic absorption spectrometer (Perkin Elmer 2380, Boston, MA) coupled with a HGA-400 graphite furnace controller, at 324.8 nm, 283.3 nm, and 213.8 nm, respectively.

## Data analysis

### *Modeling flowering phenology*

We fitted a logistic model to the flowering phenology data. In this model, each flower is assumed to be open or not according to a time-dependent probability ($P_t$) given by the equation:

$$P_t = \frac{1}{1 + e^{-\beta(t-t_0)}} \quad (1)$$

This probability rises from zero to unity with the passage of time, $t$. In this equation, $t_0$ is the changeover time, when

**Table 3** Sampling sites for the estimation of heavy metal concentration and pollen viability. Classification of sites after their traffic load is based on Savvaidis and Lakakis (2002); for location of sites, see Fig. 1

| Location | Sampling site | Road traffic intensity |
|---|---|---|
| City centre | 1. Venizelou Street (VS) | Highest in the city |
| | 2. Aghia Sophia Square (AS) | Medium |
| Commercial-industrial area | 3. Monastiriou Street (MS) | High |
| Away from the city centre | 4. Zoo Area (ZA) | Low |
| | 5. Suburban Area (SU) | Low |

the probability of a flower having opened is 50%. The constant $\beta$ is a measure of how fast this changeover happens and is related to the maximum slope $m$ of $p_t$, which occurs at $t_0$, as follows:

$$\beta = 4m \tag{2}$$

The model can be used to describe the flowering process and to test hypotheses. Specifically, we used it to test whether there is a time difference in the flowering of the two populations (north- and south-facing). For this, we fitted Eq. 1 to the flowering phenology curves (Fig. 3) and estimated the two parameters, $\beta$ and $t_0$, separately. We estimated $\beta$ for each of the four sets (two populations, two flowering periods) by finding the maximum slope of the flowering curve and using Eq. 2 above. As this rate of change does not exhibit any compelling pattern, in subsequent calculations we used the average value from all four sets. For the flowering changeover time, $t_0$, we found the value that minimised the squared distance between the model and the data. The null hypothesis is that the flowering curves of the north- and the south-facing populations are instances of the same distribution and that, whatever differences are observed between them, they are due to chance alone. For the null hypothesis, we used a

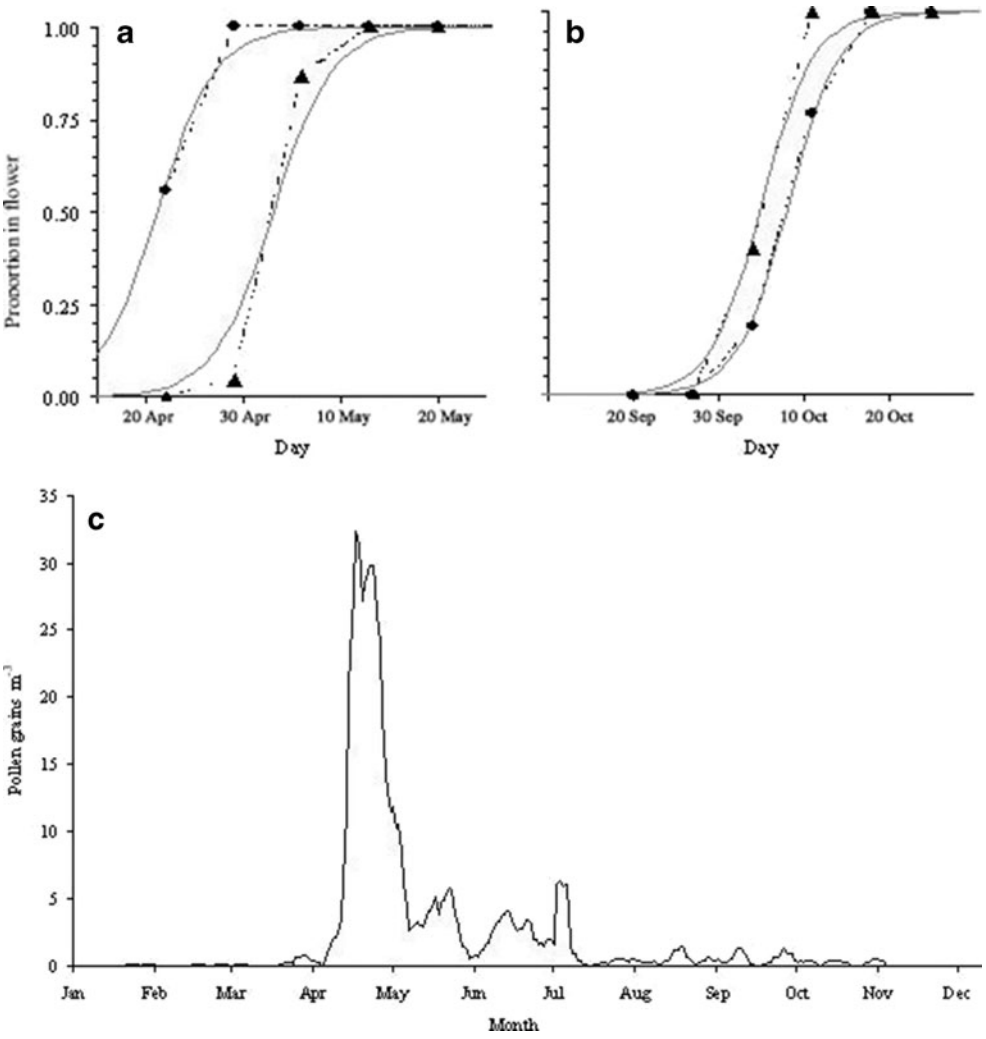


**Fig. 3** Flowering phenology patterns of two *P. judaica* populations (north- and south-facing) during the flowering periods of spring (**a**) and autumn (**b**). *Filled circles* Data associated with south-facing populations, *triangles* north-facing populations, *grey lines* corresponding fitted logistic models. (**c**) Atmospheric Urticaceae pollen concentration (5-day moving average)

logistic curve based on the average $t_0$ for a given season (spring or autumn). In order to test whether the two populations differ significantly, we used as a test statistic the maximum difference between the flowering curves of the north- and south-facing populations over the 12 weeks of the measurements. By using the model of flowering behaviour for 45 shoots, under the null hypothesis, Monte-Carlo replicates of the flowering curves for the north- and south-facing populations can be generated. A total of 10,000 pairs of replicate curves were generated and the maximum difference was found in each case. This gave us a distribution for the test statistic, $D$, under the null hypothesis. The significance of the real difference depends upon its rank in the distribution of the test statistic (Manly 1997).

### *Pollen and flower production*

To detect possible differences in pollen production per flower and also in flower and pollen production per shoot (dependent variables), we performed analysis of covariance (ANCOVA), with exposure and sampling time being the categorical predictors, and flower size and shoot length (respectively, for each analysis) being the covariates. All analyses were further checked for covariance using the Bonferroni test. We finally regressed linearly (general linear models, simple regression) pollen production per flower against corolla width. Raw data were used in all cases, after estimating the residuals for both raw values and logarithms.

### *Pollen viability—heavy metal concentration*

We checked for differences in pollen viability between sites and samplings events with analysis of covariance (ANCOVA); we further checked for covariance using the Bonferroni test. Also, we examined the relationship between pollen viability and heavy metal concentration using general linear models and simple regression analysis.

## Results

### Distribution and abundance of Urticaceae in and around Thessaloniki

As shown in Fig. 1, *P. judaica* occurs almost everywhere (24/26 sampling sites) in the metropolitan area of Thessaloniki; it is by far the most frequent and abundant Urticaceae species in the urban agglomeration (sectors A, B, C), whereas in the suburban area (sector D), it is more equally frequent and abundant with other species of the family. In contrast, *Urtica dioica* was found in only six sites, in the fringe area and in the suburban part (sector D), and thus has the lowest representation. In the city centre (sector A), *P. judaica* forms extensive patches with its populations scoring IV. In sectors B and C, it is almost twice as frequent as any of the other Urticaceae species. In the suburban area (sector D), *P. judaica* appears almost as often as *U. pilulifera* or *U. urens* (in six, seven and five sites, respectively); the three species form scattered populations of almost equivalent size.

The fine-scale mapping conducted exclusively for *P. judaica* (Fig. 2) shows that this species is present in at least 47 grid cells (of 2.25 km$^2$ each). We have estimated that 29 of these cells contain up to a few hundred individuals each (score II). Another 15 cells contain up to several hundred individuals each (score III), whereas the remaining 3 grid cells (E5, F4, F5 in Fig. 2b) contain many hundreds up to thousands of individuals (score IV). On the basis of our calculations, we can roughly estimate a total of 13,500–45,000 *P. judaica* individuals in the mapping area of 100 km$^2$, which corresponds to a density of 135–450 individuals per km$^2$. In the two populations in the centre of the city that we studied in detail (north- and south-facing), there were 170 and 130 shoots per m$^2$, in spring and autumn, respectively. As the estimated number of shoots per individual rootstock was 10–20, these correspond roughly to a range of 6–17 individuals per square metre.

### Flowering phenology and pollen season

*P. judaica* was in bloom throughout almost the entire year. Nevertheless, two main flowering periods could be distinguished when flowering was more intense: in spring (mid-April to June) and in autumn (end-September to November). The flowering phenology patterns of the two populations (north- and south-facing) during the two flowering periods are shown in Fig. 3a,b; curves produced with the data collected and the logistic models fitted to them are given. The values of parameters $t_0$ and $\beta$, being measures of the flowering time and the time required for flowers to open, respectively, for the observed data and the model, and the test statistics and the associated $P$-values are given in Table 4. We found the average value of the parameter $\beta$ to be 0.333. Given this value for $\beta$, values for $t_0$ were found by minimizing the least-square-difference between the model Eq. 1 and the data. A pronounced difference between the two populations can be seen in spring (Fig. 3a, Table 4). The flowering time for the southern population arrived fully 12 days before that for the northern population; this difference is highly significant ($P<0.001$; Table 4). For the autumn season, the difference in flowering time was only 3 days, but it was the north-facing population that started to flower first ($P<0.05$; Fig. 3b, Table 4).

In 2005, Urticaceae pollen was present in the air of Thessaloniki from end-March to early-November (Fig. 3c).

**Table 4** Parameters for the observed flowering phenology data of two *P. judaica* populations, the logistic model fitted to these data, and the associated statistics

| Flowering season | Population | Parameters | | | | Maximum difference (test statistic) | *P* |
|---|---|---|---|---|---|---|---|
| | | Observed data | | Null model | | | |
| | | $\beta$ (days$^{-1}$) | $t_0$ (days) | $\beta$ (days$^{-1}$) | $t_0$ (days) | | |
| Spring | N-facing | 0.47 | 18.00 | 0.33 | 12.05 | 0.76 | <0.001 |
| | S-facing | 0.25 | 6.10 | | | | |
| Autumn | N-facing | 0.37 | 22.03 | 0.33 | 23.60 | 0.25 | 0.013 |
| | S-facing | 0.32 | 25.17 | | | | |

Nevertheless, the main pollen season had a much shorter duration: from the beginning of April to the end of May, peaking at the end of April. The spring pattern of atmospheric pollen circulation of the family matches fairly well the phenology pattern of *P. judaica*, the most abundant Urticaceae species in Thessaloniki (Fig. 3a). However, in autumn, the amount of Urticaceae pollen in the air is low (Fig. 3c), despite the concurrent flowering and pollen production of *P. judaica* (Fig. 3b).

## Flower production

Covariance analysis showed that exposure, shoot length and the interaction of exposure and sampling period, but not the sampling period per se, had significant effects on per shoot flower production ($P<0.001$). *P. judaica* shoots were taller in spring (Fig. 4), but there was no difference among populations in either of the flowering periods. The southern population produced more flowers than the northern population in both spring and autumn mid-flowering seasons (Fig. 5).

## Pollen production

Covariance analysis showed that exposure, flower size, and the interaction of exposure and sampling period, but not the sampling period per se, had significant effects on per flower pollen production ($P<0.001$). The highest value recorded was in the south-facing population in spring (Fig. 6). Pollen production increased with flower size (Fig. 7); the latter differed between seasons, being larger in spring (Fig. 8).

At the shoot level, pollen production of the south-facing *P. judaica* population was considerably higher than that of the north-facing population. Values for both populations

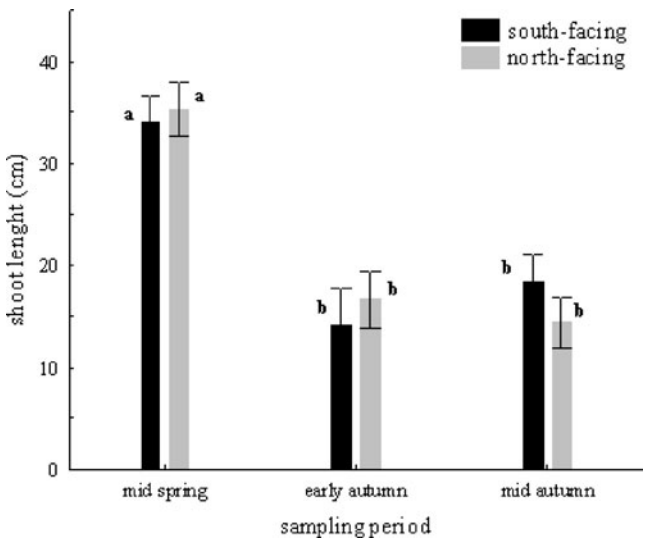


**Fig. 4** Average shoot length (± standard error) for two *P. judaica* populations (north- and south-facing) in three sampling periods (mid-flowering season in spring and autumn, early flowering season in autumn); means followed by the same letters are not significantly different at $P<0.05$

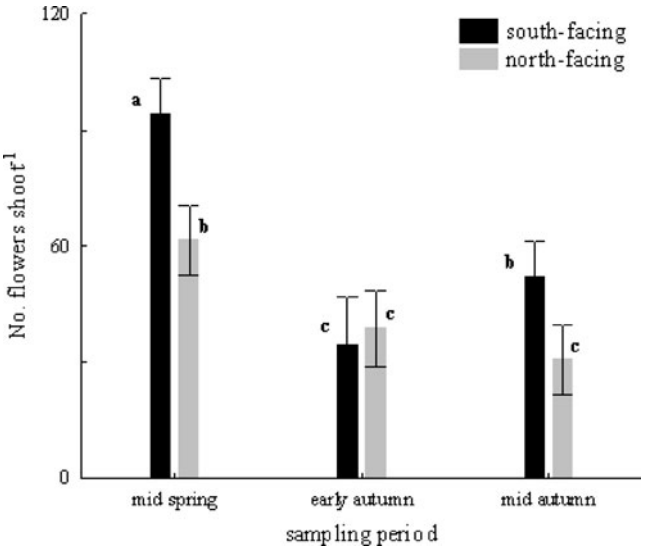


**Fig. 5** Average pollen production per flower (± standard error) for two *P. judaica* populations (north- and south-facing) in three sampling periods (mid-flowering season in spring and autumn, early flowering season in autumn); means followed by the same letters are not significantly different at $P<0.05$

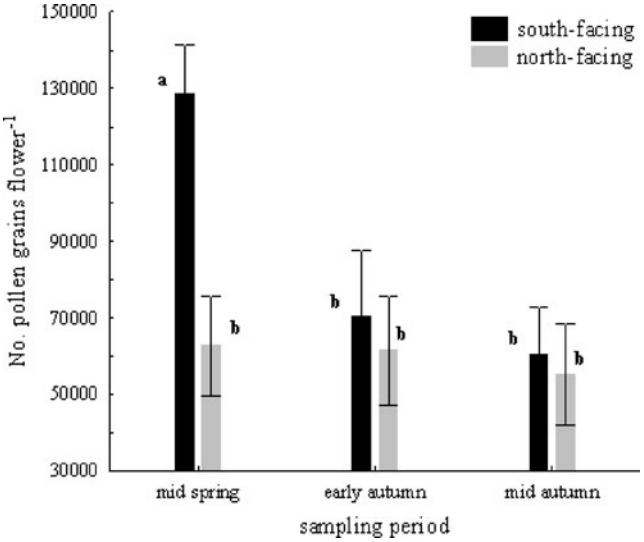


**Fig. 6** Average number of pollen grains per flower (± standard error) for two *P. judaica* populations (north- and south-facing) in three sampling periods (mid-flowering season in spring and autumn, early flowering season in autumn); means followed by the same letters are not significantly different at $P<0.05$

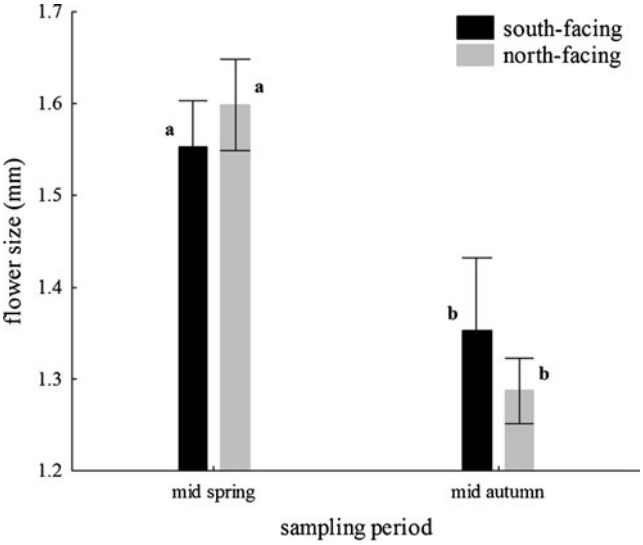


**Fig. 8** Average flower size (± standard error) for two *P. judaica* populations (north- and south-facing) in the mid-flowering seasons of spring and autumn; means followed by the same letters are not significantly different at $P<0.05$

were low in autumn—even lower than the spring value of the north-facing population (Table 5). Similarly, pollen production per surface unit ($m^2$) was maximal in the south-facing population in spring, and minimal in the north-facing population in autumn (Table 5). On the basis of the pollen yield per shoot, the number of shoots per individual, and the number of individuals per unit surface, we can make a rough estimate of total production in the whole study area. To do so, we used the following equation: pollen production per $km^2$ = (average pollen production per shoot) × (average number of shoots per $km^2$). Given the results in Table 5 and the number of shoots per $m^2$ ranging from 130 to 170, we estimated that the total pollen production ranged from $1.5\times10^{13}$ to $4.0\times10^{16}$ grains per $km^2$.

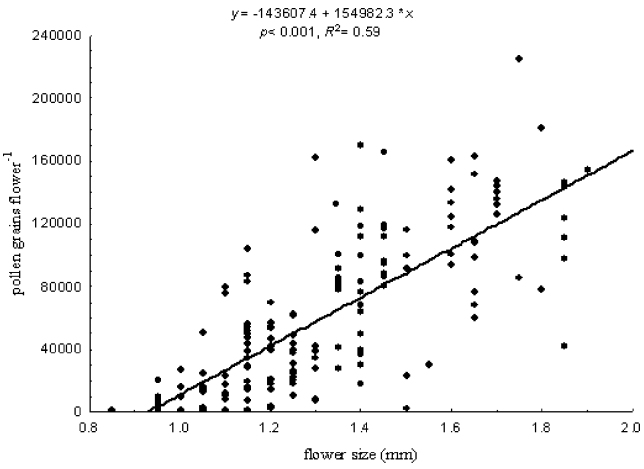


**Fig. 7** Results of linear regression analysis between the number of pollen grains per flower and flower size. The equation of the regression and $P$ and $R^2$ values are given

### Pollen viability and heavy metal concentration in plant tissues

Viability of *P. judaica* pollen differed significantly among sampling sites (Fig. 9), taking maximum values where traffic intensity, and hence road pollution, was high; it did not differ between seasons at the same site.

There were significant differences of zinc, copper, and lead concentrations in plant tissues among populations. In general, metal concentrations were high in plants collected from sites with high traffic intensity (Fig. 10), with maximum values found in plants from the Monastiriou site in spring. Pollen viability and metal concentration were positively correlated; this holds true for copper ($R^2=0.81$, $P<0.01$) and for lead ($R^2=0.55$, $P<0.05$), but not for zinc.

## Discussion

### Expansive ability of *P. judaica*

The first information on the presence of *P. judaica* in Thessaloniki was given by Charell (1891–1892) alias Nadji (1892) at the turn of the nineteenth century. He reported this species (as *P. judaica* L. var. *brevipetiolata* Boiss.) as being present both in the urban area of his time and in the suburbs. This author provides specific information on the commonness, abundance, frequency or rarity for 170 plant

**Table 5** Range of pollen production values (minima and maxima) for a north- and a south-facing population of *P. judaica* in spring and autumn at the level of flower, shoot, and area; average values are also given for number of pollen grains per shoot (± standard error) and number of shoots per square metre

| Parameter | Statistic | Spring | | Autumn | |
|---|---|---|---|---|---|
| | | South | North | South | North |
| No. pollen grains per flower | Min | 1850 | 2125 | 493 | 450 |
| | Max | $0.31\times10^6$ | $0.17\times10^6$ | $0.23\times10^6$ | $0.16\times10^6$ |
| No. flowers per shoot | Min | 44 | 28 | 12 | 13 |
| | Max | 193 | 106 | 78 | 71 |
| No. pollen grains per shoot | Min | $8.1\times10^4$ | $6.0\times10^4$ | $5.9\times10^4$ | $5.9\times10^4$ |
| | Max | $60.5\times10^6$ | $17.9\times10^6$ | $17.9\times10^6$ | $11.4\times10^6$ |
| | Average | $12.0\times10^6\pm1.2\times10^6$ | $4.1\times10^6\pm0.6\times10^6$ | $2.7\times10^6\pm0.3\times10^6$ | $1.8\times10^6\pm0.2\times10^6$ |
| No. pollen grains per $m^2$ | Min | $0.44\times10^9$ | $0.61\times10^9$ | $0.72\times10^9$ | $0.24\times10^9$ |
| | Max | $5.4\times10^9$ | $2.3\times10^9$ | $1.13\times10^9$ | $0.87\times10^9$ |

taxa corresponding to 47.5% of his floristic records; nevertheless *P. judaica* is reported as merely "present" or "occasional" (never as "dominant", "common" or "frequent"). Such descriptions correspond to scores I and occasionally II according to the five-point scoring system (see Tables 1, 2) for the sites where he sampled the species. Later, Halácsy (1906) and Turrill (1918, 1920) provided no further information on *P. judaica*, as they were interested primarily in adding new floristic records for the area (Turrill 1920). Before the start of the Second World War, Zaganiaris (1940) reported *P. judaica* as merely "present" in the urban and suburban area of Thessaloniki and did not include it in his extensive survey of weeds in the same area (Zaganiaris 1939), in which there was an indication of local abundance or rarity for almost every species assigned as a weed. Comparison of the areas sampled by the above researchers, and also by Oberdorfer (1954) and Krigas (2004), showed that the abundance of *P. judaica* has increased noticeably in 15 of the 16 commonly sampled areas; in most cases, the current scores after Wittig's modified scale are at least two orders of magnitude higher than the corresponding scores in the past (Fig. 2, Table 2).

As past and present data for *P. judaica* abundance were of different types, to compare them we had to find a common system. For this reason, we transformed the past data by making use of the approach of Wittig et al. (1993). In situations where the resolution required is very fine, such an instrument may be of limited use. However, in a situation such as ours, where we are looking for broad shifts, this is an invaluable instrument providing a crucial quantitative perspective.

Based on the above, we argue for the existence of a noticeable expansion of *P. judaica* in the study area: from relatively few score-I and occasional score-II populations in the 1880s (Charrel 1891–1892; Nadji 1892), to many score-I and occasional score-III populations, in the first half of the twentieth century (Oberdofer 1954; Zaganiaris 1939, 1940), to nowadays numerous and extensive score-II, -III and -IV populations. The expansive ability of the ruderal *P. judaica* seems to coincide with the expansion of the urban fabric of Thessaloniki during the decades following the Second World War (Fig. 2). Only then did *P. judaica* start to establish large populations in the city centre, thus becoming

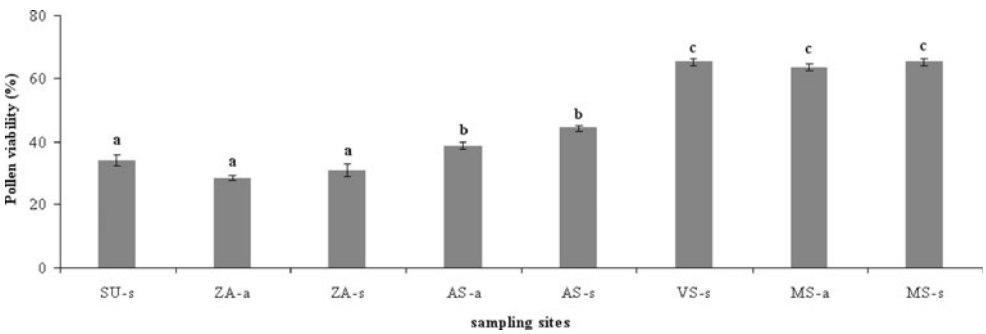


**Fig. 9** Average proportion of viable pollen (± standard error) from *P. judaica* plants collected from stations in and around the city of Thessaloniki that differ in traffic intensity; means followed by the same letters are not significantly different at $P<0.05$. See Table 3 and Fig. 1 for information on sampling sites and their locations, respectively; *-a* and *-s* in the sampling-site codes indicate autumn and spring sampling, respectively

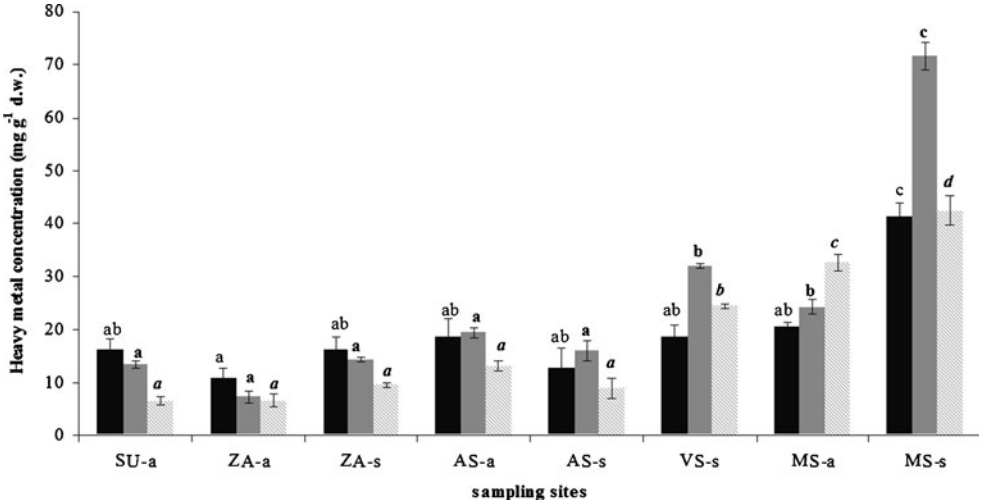


**Fig. 10** Average Cu, Zn and Pb concentrations (± standard error) in *P. judaica* upper shoots collected from sites inside and outside the city of Thessaloniki that differ in traffic intensity. Means followed by the same letters are not significantly different at $P<0.05$; regular font Zn values, bold Pb values, italic Cu values. See Table 3 and Fig. 1 for information on sampling sites and their locations, respectively; *-a* and *-s* in the sampling-site codes indicate autumn and spring sampling, respectively

an expansive man-accompanying native plant (for this terminology, see Krigas and Dardiotis 2008; Pyšek 1995; Pyšek et al. 2004) in the metropolitan area of the city.

According to Bass and Bass (1990), the rate of spread of *P. judaica* is dependent on its dispersal ability and on the availability of suitable disturbed sites. In impervious surfaces (common in urbanised areas), the discarded seeds may well be washed along cracks and walls and are easily dispersed both by wind and by human activities (Bass and Bass 1990; Davis 1951). High seed germination over a wide range of temperatures, and rapid establishment after clearance of vegetation and ground disturbance seem to favour the expansive ability of *P. judaica* (Bass and Bass 1990). Mechanical weeding is often not effective and resistance to herbicides results in regeneration and dominance of *P. judaica* (Protopapadakis 1985). Urbanisation and the consequent disturbance due to extensive man-made construction works may well trigger an increased frequency and abundance of this typical ruderal species resulting in range expansion (Sukopp 2002).

## Flowering phenology and pollen season

*P. judaica* was in flower almost throughout the entire year, with production of flowers being intense in spring and autumn. Like other anemophilous taxa, *P. judaica* releases its pollen when conditions are warm and dry to aid long-distance dispersal and transport of its pollen (Franchi et al. 2007). Extended duration of flowering may reflect a lack of seasonal differences in resource availability, as is the case for most understorey species in the tropics (Rathcke and Lacey 1985), but it may equally reflect unpredictable or sparse resources. In the urban habitat of *P. judaica*, resources are not plentiful. It seems that this ruderal species has become an urban dweller by developing strategies enabling it to take advantage of the conditions prevailing in this anthropogenic habitat.

The south-facing population started to flower about 12 days earlier than the north-facing population in spring, but 3 days later in autumn. This suggests that spring phenological events are much more sensitive to differing environmental conditions associated with exposure than are events in autumn.

The abiotic factors that differentiate the sites where the two populations grow are the daily duration of sunlight and, hence, the thermal and soil moisture properties of the environment; southern populations are characterised by longer duration of sunlight, and warmer and drier conditions. Such factors, primarily air temperature, are often related to flowering (Rathcke and Lacey 1985). Working with Graminae species, Zanotti and Puppi (2000) found differences in the onset of pollen emission between populations of southern and northern exposures at the same altitude almost equal in magnitude to the one we found for *P. judaica* in spring (13 and 12 days, respectively). Also, as early as 1966, Jackson reported flowering dates of nine species in a large gorge in Indiana (US) to be delayed by 6 days on the north-facing slope with respect to the opposing south-facing slope. Not only phenological shifts but also changes in the size of flower production are observed: more flowers are produced on plants or branches exposed in more sunlit areas (Cunningham 1997; Niesenbaum 1993), as south-facing slopes are.

Reports from the analysis of European phenological data, carried out in an attempt to track global climate change impacts, show that shifts in spring, linked to an increase in temperature, are most commonly reported, and that changes are more profound in those events that occur earlier in the year; in contrast, phenological changes are less pronounced during autumn (Menzel et al. 2001, 2006; Sparks and Menzel 2002). The smaller differences in the responses of the two *P. judaica* populations in autumn are in agreement with this lack of clear yearly differences in autumn phenological events. The earlier start of flowering of the north-facing *P. judaica* population in autumn could

be explained in terms of the thermal conditions during the preceding months. We can argue that, for autumn flowering species in the Mediterranean environment, heat-sum thresholds for flowering are meaningless, because of the hot, dry summer that precedes flowering. Hence, autumn rainfalls are more important in initiating growth and subsequent flowering than is temperature.

To explain the mismatch between the pattern of atmospheric circulation of Urticaceae pollen and the pattern of *P. judaica* flowering, particularly during autumn, and given the magnitude of *P. judaica* pollen produced then, further research is required; the priority research issues stemming from this study are the relative contribution of the other three Urticaceae species present in the area of Thessaloniki to locally produced pollen, as well as the patterns of atmospheric circulation of the pollen of these species.

## Flower and pollen production

Pollen production in spring was higher at all levels that we estimated , i.e. per flower, per shoot and per surface unit, with values being two-fold, three-fold or even higher than the corresponding ones in autumn. Differences between the two populations were rather negligible in autumn but remarkable in spring, with the south-facing population producing more pollen. Pollen production above the level of the floral unit may reflect differing allocation patterns in vegetative and reproductive structures. In spring, *P. judaica* plants were twice the size of those in autumn, bearing bigger and more plentiful flowers; among the two populations, differences were found only in the amount of flowers produced, which was higher in the south-facing population. Shoot length had a significant effect on *P. judaica* flower production, but there was a far greater production than the increase in plant size could suggest; this indicates higher biomass allocation to reproductive tissues in the south-facing population.

Flower production is often correlated with plant size, with bigger plants bearing more flowers (Dubash 1991; Cunningham 1997; Blionis and Vokou 2002). At a lower level, pollen production is correlated with flower size, with larger flowers producing more pollen; this is reported for various species like *Raphanus sativus* (Brassicaceae; Stanton and Preston 1988), *Bromus* (Poaceae; McKone 1989), *Triticum* (Poaceae; Beri and Anand 1971), *Polemonium viscosum* (Polemoniaceae; Galen and Stanton 1989). For *P. judaica*, we found a positive linear relationship between flower diameter and pollen production. Also, despite the lack of differences in flower size between the two populations, the south-facing population produced more pollen per flower than the north-facing population in spring, indicating a direct higher investment in male function.

South-facing populations are associated with longer exposure to sunlight and with warmer and drier conditions. Studying the effect of light per se on pollen production in *Campanula americana* revealed that plants grown under high light produced more pollen grains than those grown under low light (Etterson and Galloway 2002). Pollen of *P. judaica* is partially hydrated; one of the features of this type of pollen is that it loses water and dies quickly, especially if kept in dry environments (Franchi et al. 2007). Given this fact, the probability of pollen remaining viable is higher for north-facing populations. Therefore, the higher pollen production in south-facing populations can be considered as an adaptive mechanism to preserve species reproductive success under stressful, hot and dry conditions.

It seems that there is a large plasticity in the amount of pollen that *P. judaica* flowers produce; the number of pollen grains included in each flower ranged from as low as 450 to as high as 310,000. This shows that pollen production per anther is not under strict genetic control, which agrees with results reported for other species (Prieto Baena et al. 2003). Despite this, average pollen production estimated on a per flower basis (~160,000 to ~310,000) for the populations we studied in Greece matches corresponding production (~140,000 to ~350,000) in Spain, according to the measurements of Guardia and Belmonte (2004), which were calculated on a per anther basis (35,744 to 86,333 grains).

## Pollen viability and heavy metal concentration in plant tissues

Results on *P. judaica* pollen viability showed that it reached maximum values in plants collected from sites with high traffic intensity, and that it was positively correlated with copper and lead concentrations in the plant tissues. This indicates a stimulating rather than an inhibitory effect of heavy metal pollution on *P. judaica* pollen, similar to that found for the species by Iannotti et al. (2000). The positive effect of copper on pollen viability could be interpreted in terms of several findings. For instance, it was reported that copper is extremely important for the development of normal pollen (Graham 1975), that wheat pollen is non-viable under copper-deficiency conditions (Graham 1986), and that temporary copper deficiencies significantly reduce wheat pollen viability (Graham 1975). Also, Read et al. (1993) found that Cu-(II) salts play an important role in pollen-tube development in a *Nicotiana* species, and Cox (1988) reported a synergistic effect of pH and Cu on pollen function of *Populus tremuloides*; the latter author reported that lead significantly inhibits both pollen germination and tube growth of *Pinus* species, whereas zinc has little or no effect.

Large concentrations of heavy metals affect cell wall elasticity, leading to enormous tube elongation and ultimately to bursting of the tubes (Sawidis and Reiss 1995;

Sawidis 1997). At low concentrations, copper and zinc cause a distinct increase in tube length, whereas lead reduces growth rate (Sawidis 1997). Nevertheless, zinc uptake at low concentrations is negligible in pollen grains (ranging from 69 to 82 ppm; Sawidis 1997); this finding is in agreement with reports suggesting that heavy metals like lead and zinc, which have a very low mobility, accumulate mainly in roots and the lower aboveground parts of plants (Ernst and Bast-Cramer 1980), and that, even when present at high concentrations in plant tissues, they are not expected to make their way into the upper plant parts like flowers and pollen. Nevertheless, given our findings and those of previous works, this is obviously not always the case. Also, it was reported that, in highly polluted areas of Greece like Ptolemaida, respiratory allergies are more frequent and severe compared to less polluted areas like Thessaloniki (Gioulekas et al. 2004b). The Ptolemaida-Kozani-Amyntaion basin in Greece has four lignite power stations discharging around 69,500 tons year$^{-1}$ fly ash into the atmosphere (Tsikritzis et al. 2002); fines are imposed on them by the European Union for repeatedly exceeding health-mandated particle levels. The higher frequency and severity of respiratory allergies in Ptolemaida was not due to a higher sensitization to airborne pollen, except in one case: this was *Parietaria* pollen, which was associated with a higher number of allergic reactions in this polluted environment (Gioulekas et al. 2004b).

## Conclusions

*P. judaica* is an urban weed that responds to the warm and dry conditions associated with south-facing locations by flowering earlier, more abundantly and for a longer time. It also responds to heavy metal pollution with higher pollen viability; the latter feature suggests that heavy metal pollution enhances the species' reproductive ability by preserving more viable pollen. Drastic changes in *P. judaica* responses under differing environmental conditions are indicative of its high phenotypic plasticity, which is a feature considered to promote success of expansive and invasive species (Rejmánek 1996). The nature of these changes also suggests that *P. judaica* is well adapted to the conditions of warm and polluted urban environments. A climatic change, such as that forecast for the Mediterranean (Cheddadi et al. 2001), causing an increase in air temperature and unsteady rainfalls, could provoke earlier, longer and more pronounced flowering of *P. judaica* like that we recorded in the south-facing population in spring. With the number of flowers open at any one time becoming higher, the species' expansive ability could increase (Reichard 1996). With the expansive ability not hindered by urban pollution, particularly from heavy metals, the species could spread easily to new environments. The consequence of all of this would then be higher atmospheric concentrations of *P. judaica* pollen and, hence, an increase in the severity of *Parietaria* pollinosis, until some environmental factor becomes limiting to *P. judaica* growth. Urticaceae pollen has already been shown to have increased considerably over the period 1987–2005 in Thessaloniki, in parallel with an increase in temperature over the same period. (Damialis et al. 2007). Such changes are expected to occur also in other cities of the Mediterranean, and in other regions that face similar types of climate change and where the species can expand or invade.

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