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Antoine Gazaix, Mario Klesczewski, Michel-Ange Bouchet, Manuel Cartereau, James Molina, et al.. A history of discoveries and disappearances of the rare annual plant Lythrum thesioides M.Bieb.: new insights into its ecology and biology. Botany Letters, 2020, 167 (2), pp.201-211. 10.1080/23818107.2019.1684358. hal-02465828

HAL Id: hal-02465828 https://amu.hal.science/hal-02465828

Submitted on 14 Feb 2020 $\,$

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A history of discoveries and disappearances of the rare annual plant *Lythrum thesioides* M.Bieb.: new insights into its ecology and biology

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ABSTRACT

Mediterranean temporary pools are threatened ecosystems that host a unique plant community, mostly composed of annual species with large and long-lived seed banks. The longevity of their seed bank, the scarcity of their habitat, the small size of their populations and the low frequency of above-ground vegetation result in a low probability of detection of these species. The discovery of new populations of such rare species are thus important for our understanding of the ecology of the temporary pool ecosystem. *Lythrum thesioides* M.Bieb., 1808 is a very rare annual species of temporary pools and river banks which was thought to be extinct in the South of France until 1998. Here, we review the distribution of the species and report the recent discovery of a population during a targeted search combining historical data on wetland occurrence on a particular geological substrate. We present new information on its autecology, pollen morphology and the karyotype. Only three populations are currently known worldwide for *Lythrum thesioides*, and only one of these has favorable management conditions. Thus, we outline new conservation perspectives in the context of a targeted search project and the conservation management of one population.

Introduction

The ecology of Mediterranean temporary pools is characterized by an alternation of winter flooding and summer drought with large inter-annual fluctuations in the relative duration of these phases (Grillas et al. 2004). The particular environmental conditions of these pools have resulted in the presence of a highly specialized flora, with most species having short life cycles – i.e. annual species with high fecundity (Médail et al. 1998) – and large soil propagule banks (seeds, spores) that persist in the soil during unfavorable conditions for germination (Aponte et al. 2010).

The plant communities of Mediterranean temporary pools have stimulated the curiosity and interest of botanists for decades, and are still considered as a floristic "jewel" (Braun-Blanquet 1935; Rhazi et al. 2012). However, Mediterranean temporary pools are a threatened and highly vulnerable ecosystem (Rhazi et al. 2012) and have been designated as a priority habitat of community interest at the European scale (Council of Europe 1992). Indeed, due to their small surface they are easily destroyed and have experienced a dramatic decrease in number and surface area around the Mediterranean region (e.g. Gallego-Fernández et al. 1999; Rhazi et al. 2012) due to agricultural intensification, pollution **ARTICLE HISTORY**

Received 19 February 2019 Accepted 3 October 2019

KEYWORDS

(5 à 8): Conservation; rarity; temporary pool; Mediterranean; ecological niche; historical ecology

(including eutrophication), drainage and direct destruction for urbanization (Bouahim et al. 2014).

The longevity of their seed bank, the scarcity of their habitat and their small size and low frequency in aboveground vegetation, result in a low probability of detection of many of the annual species that predominate in Mediterranean temporary pools, making it difficult to assess their abundance, dynamics and distribution (Aponte et al. 2010; Alvarez et al. 2012). A complete inventory of the plant communities of a temporary pool thus requires multiple visits during a single year and over several years that experience contrasting hydrological characteristics (Rhazi et al. 2009). This requires time, botanists and favorable conditions that may only occur one year per decade or even less (Poschlod and Rosbakh 2018). As a result, and despite the interest of botanists for the unique flora of temporary pools, the distribution of many species of this Mediterranean ecosystem remains only partially known. The conservation significance and restoration potential of Mediterranean temporary pools may thus be seriously underestimated at the current time. The discoveries of new populations of rare species are thus important to improving our

understanding of the ecology of the temporary flooded ecosystem.

Lythrum thesioides M.Bieb., 1808 (Lythraceae) is an erect annual plant (Figure 1) of temporary wetlands. It is highly variable in size, with plant height ranging from 5 to 40 cm according to hydrological conditions. The leaves are alternate, linear and elongated. The flowers are usually geminate (Figure 1, e), shortly pediculate, each node carrying axillary clusters of (1-) 2 (-4) flowers. The hypanthium is bell-shaped, with a short calyx made of four sepals. The four petals are pink with a purple line down the main vein. The four stamens are included in the hypanthium (Coste 1906). The ovary is superior and the fruit is a capsule (Tutin et al. 1968). The plant is flowering from July to September (Tison et al. 2014). This species is very rare and several elements of its description remain incomplete.

In the 1990s *Lythrum thesioides* was thought to be extinct from the South of France (Olivier et al. 1995) and in Europe (Morgan and Leon 1992). This species is a representative example of the challenges underlying the conservation of ephemeral plant species in temporary pools. There is however much piecemeal evidence of historical and more contemporary presence of this species in different sites. Therefore, the aims of this article are as follows.

- (1) Synthesize the current and historical distribution of *L. thesioides*.
- (2) Provide new elements for the botanical description of *L. thesioides*.

(3) Describe recent the 10 plants, 30 fruits were assedtargeted prospections of *L. thesioides* that have been focused on previously known populations and/or habitat suitability based on historical pool presence and geological substrate.

Material and methods

(1) Distribution of Lythrum thesioides

To produce a map of distribution of *Lythrum the*sioides observations we examined both herbarium specimens and mentions in Flora and catalogues (*Lythrum thesioides* or *Lythrum geminiflorum* Bertol. 1842). Herbarium specimens were examined using the Global Biodiversity Information Facility database (GBIF.org), and the e-Recoltnat programme (MNHN, Paris) for French collections. Other information was obtained or consulted from non-referenced herbariums (Almaty, Bologna, Budapest, Geneva, Saint Petersbourg

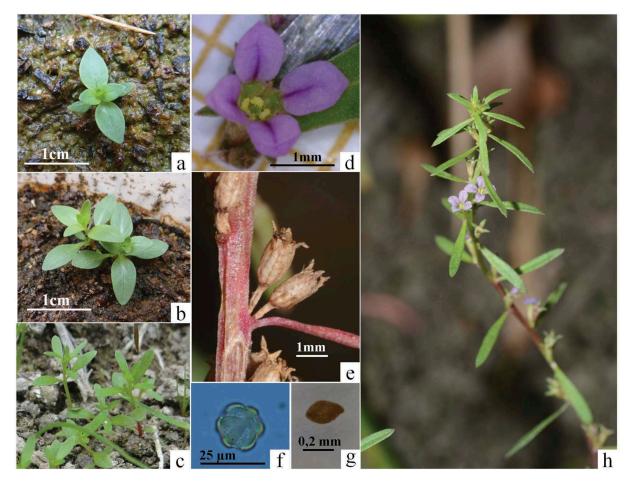


Figure 1. Pictures of *Lythrum thesioides*. (a,b) Seedlings under light, (c) seedlings under shadow conditions, (d) detailed flower, (e) fruits (geminate, © M. Charrier), (f) pollen (© S. Muller), (g) seed, (h) flowering stem (© M. Charrier).

and Vienna). Herbarium codes are later on used following Thiers (2019). Floras of the areas concerned by herbarium samples were also consulted where possible.

(2) Species description

Field and culture observations

In 2015, one individual of *Lythrum thesioides* was isolated in a greenhouse before flowering, in order to evaluate its ability to accomplish self-pollination. Seeds produced were tested for germination (Gazaix et al. in prep). Pollination was also studied in the field in August 2018 in the Campuget population. A 1-day experiment was done, with 20-min observation per flower, capturing every insect visiting the flower, for a total of 10 observed flowers.

In 2017, 10 flowering individuals cultivated in Camargue (Tour du Valat) were monitored four times a day (7 am, 12 am, 5 pm and 9 pm) during a week to evaluate the longevity of individual flowers. On each of the 10 plants, 30 fruits were assessed for the number of seeds, seed length and width (under a binocular microscope) and seed weight, by weighing 10 groups of 30 seeds (weighting scale: Mettler Toledo AG 245).

Pollen description

For the observation of pollen, 10 flowers of *Lythrum thesioides* were collected on five individuals grown in pots at the Tour du Valat Biological Station in 2018. Flowers were kept in paper bags prior to analysis. Stamens taken from flowers were submitted to an acetolysis treatment (nine parts of anhydric acetic acid and one part of sulphuric acid at 90°C for 3 min; Faegri and Iversen 1989), in order to remove the cellular content and the pectocellulosic intine from pollen grains. The remaining exines were then mounted on a slide to be observed and photographed under an optical microscope.

Chromosome count

Chromosome numbers of *Lythrum thesioides* were determined in January 2019, from flower buds of a cultivated individual (initially from the Campuget population). Young flower buds were fixed in a solution of absolute ethanol: glacial acetic acid (4:1, v/v). After 2 weeks at room temperature, the fixations were stored at -18° C. Flower buds were then stained in 45% acetocarmine stain, boiled for 3 min, and squashed between the microscope slide and cover glass. The chromosome count was performed in ovaries. Mitotic metaphases were drawn (Wild M20, 15 × 100) with a camera lucida, at high magnification, with ca 0.15 µm error per chromosome (i.e. 1 mm on the paper).

(3) Population search

The projection of both former (Pazac and Jonquières Lake) and current (Campuget) populations of Lythrum thesioides onto a geological map of the Costières area showed that all sites are located on a common geological substrate (Figure 3). This geological substrate is a recent layer of sediments corresponding to clayloam that filled-in the wetland depressions of the region. This layer may be used to identify the previous location of wetlands drained during agricultural intensification in the twentieth century and which constitute potential sites for the occurrence of populations and/or seedbanks of L. thesioides. Fourteen different previous wetlands were identified using this method, including the Vistre valley, large wetlands (such as Campuget and Jonquières) and smaller pools. Thirty points were chosen from recent aerial photographs, with at least one point per polygon. Over a period of 3 days in June 2017 every defined point was visited to search for L. thesioides (and other temporary wetland plant species).

Results

(1) Distribution of *Lythrum thesioides*

All the herbarium specimens reviewed are presented in table 1 and are also located in a map in Figure 2.

Central/Eastern Europe and Central Asia

Bieberstein (1808) first described Lythrum thesioides in Russia, from the foothills of the Caucasian mountains where a few specimens, more than 200 years old, except one from 1888, are known. In Russia, a second place of collection is the "Sarepta", near the Volga River and the city of Volgograd. There are two undated specimens from Afghanistan, one from William Griffith (1810-1845) without any precise location and another by Joharchi et al. (2007) in 1987 in the Hari River valley on the border with Iran. In Iran, there is also an undated collection from Fars. In Western Asia, there is a record from Iraq in 1957 ("Singleton on damp sandbank now exposed in ruins bed", Sarsang) that is preserved at the herbarium of Edinburgh. In Central Asia, there are two specimens from Kazakhstan, one collected in 1926 (AA herbarium) the other one in 1928 from the eastern part of the country (MW herbarium). Finally, Mallaliev and Zalibekov (2018) reported a population of L. thesioides in Dagestan (Caucasian region of Russia). However, after the discussion and exchange of photos with the authors, this observation does not correspond to L. thesioides. In Central Europe, one observation is mentioned from Hungary, with a herbarium specimen in Budapest, collected in the river banks of the Danube in 1917, a few kilometers south of Budapest.

Table 1. Herbarium specimens of *L. thesioides* in the main collections (often named as *L. geminiflorum* for French samples).Codes of herbaria: AA (Ministry of Science, Academy of Sciences, Alma-Ata, Kazakhstan), AIX (Muséum d'Histoire Naturelle d'Aix en Provence, France), ANG (Muséum des Sciences Naturelles, Angers, France), AUR (Herbier du muséum des volcans, Aurillac, France), B (Zentraleinrichtung der Freien Universität Berlin, Germany), BOLO (University of Bologna, Italy), BP (Hungarian Natural History Museum, Budapest, Hungary), CLF (Institut des Herbiers Universitaires de Clermont-Ferrand, France), E (Royal Botanic Garden, Edinburgh, UK), FUMH (Herbarium of the Ferdowsi University of Mashhad, Iran), GAP (Conservatoire Botanique National Alpin, Gap, France), K (Royal Botanic Gardens Kew, UK), L (Naturalis Biodiversity Center, Leiden, Netherlands), LE (Komarov Botanical Institute, Russia), LM (Musée vert, Le Mans, France), LYO (Université Claude Bernard, Lyon, France), MPU (Université de Montpellier, France), MW (Moscow State University, Russia), NCY (Jardins Botaniques du Grand Nancy et de l'Université de Lorraine, Nancy, France), NIME (musée d'Histoire naturelle, Nîmes, France), PC (Muséum National d'Histoire Naturelle, Paris, France), SLA (Société des Lettres de l'Aveyron, Millau, France), W (Naturhistorisches Museum Wien, Vienna, Austria).

Place of collection	Date (of collection)	Herbarium (number of parts)
France (Jonquières)	1847	MPU (2)
	1848	CLF (1), MPU (2), NCY (1), P (1)
	1849	P(1)
	1853	AUR (1), SLA (1)
	1854	LYO (1), MPU (1)
	1902	CLF (1), GAP (2), MPU (1), NIME (6)
	1911	CLF (1)
	?	MPU (1)
France (Tresques)	1853	ANG (1), AUR (1), LYO (1), MPU (1), P (2), SLA (2
France (« next to Marseille »)	1858	AIX (1)
France (Pazac)	1951	MPU (1)
France (Les Mées)	? (<1900)	LM (1)
Italie (near Mantova)	1937	BOLO (1)
Hungary	1917	BP (1)
Russia (Caucasus)	<1808	B (1, Typus)
	1800	LE (1, Typus)
	1888	MPU (1)
	?	E (1), MW (1), LE (1)
Russia (Sarepta = Volgograd)	1853	LE (1)
	?	MW (1), P (1)
Kazakhstan ("Aktobe region")	1926	AA (1)
Kazakhstan ("between the rivers First and Middle Tentek")	1928	MW (1)
Iraq (Sarsang)	1957	E (1)
Iran (Fars)	1885	K(1), W (3)
Iran (Khorassan)	1987	FUMH (1)
Afghanistan (Herat)	?	K (1)
Afghanistan (?)	? (1810–1845)	L (1)

Western Europe

The occurrence of Lythrum thesioides has mostly been documented in Western Europe. In Italy, two records were documented more than a century ago in the Po plain, one along the river, the other in a paddy field (Bertoloni 1842 and BOLO herbarium; Pignatti 1982). In the South of France, most observations have been reported near the Rhône valley, between Nimes and Orange (Figure 2(a)) from 1841 to 1951. The plant was observed from the Costières area, south and east of Nîmes, mostly at the Jonquières lake with eight observations from 1841 (Jordan 1847) to 1911 (Herbarium of d'Alleizette), at the Pazac lake (MPU herbarium, observation by Tallon in 1951), between Tresques and Connaux in 1853 (by l'Abbé Gonnet), in the valley of the Rhône River (Rouy and Camus 1901) which may be the same site as that mentioned by Charrel (1913) near Caderousse, and near Pierrelatte, in a "wet field" (Chatenier 1922). Two additional reports concern a vague observation that was made near Marseille in May 1858, i.e. very early in the season and this may be a reference to Jonquières lake (<80 km away), and in the Durance valley, near Paillerols (Les Mées, Alpes-de-Haute-Provence) but with no date (herbarium Rendu, Le Mans). A final dubious observation of *L. thesioides* was recorded from South West France near Itxassou in 1880, although there is no herbarium specimen and the description of the plant and the vegetation community suggests that this was an observation of *Lythrum hyssopifolia*, with geminate flowers (Gillot 1880).

In 1998, 47 years after the last observation in France, Michaud and Molina (1999) reported roughly 100 individuals of *Lythrum thesioides* mixed with *Lythrum tribracteatum* Salzm. ex Spreng. in a cultivated melon field at the edge of the lake of la Capelle. A few plants were also observed in 2009 in a deep ditch (3 m deep) that had been recently dug for irrigation purposes (SILENE 2019), adjacent to the previous melon fields. However, since the end of melon cultivation, the site has changed into a perennial meadow grazed by horses and *L. thesioides* has not been observed since (Girardin 2011), despite annual visits to the site.

Twelve years later, a new population was discovered in Campuget (Manduel, Gard, France), in July 2010 at Campuget (Manduel, Gard), in the context of an Environmental Impact Assessment realized for the construction of a new high-speed train line. More than 100 individuals of *Lythrum thesioides* and

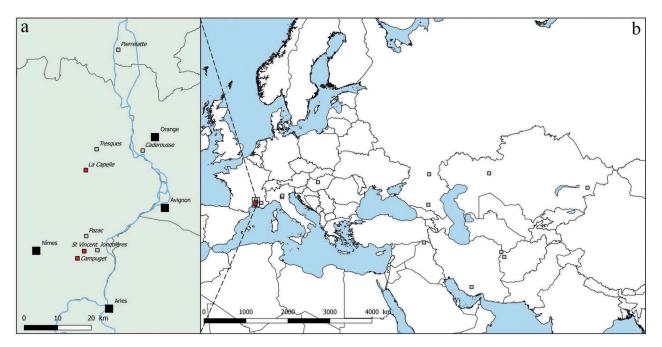


Figure 2. Distribution map of *Lythrum thesioides* based on former and current observations. (a) Low Rhone Valley zoom, (b) Eurasian distribution.

Black squares represent principal cities; red squares are recent observations of *L. thesioides* (post 2000); grey squares are historical observations of *L. thesioides* (before 2000).

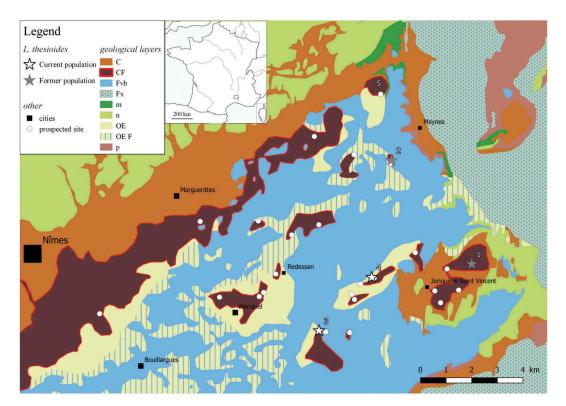


Figure 3. Geological map of the Costières area. Numbers: (1) Jonquières, (2) Pazac, (3) Campuget, (4) St Vincent, (5) Clausonne (not a population of L. thesioides). South of Nîmes: the largest polygon of CF layer represent the valley of the Vistre (small river). Geological layers, according to a simplified geological map (from BRGM, map 965, Nîmes, 1/50,000). C: colluvial deposits (Quaternary) CF: filled-basins: grey silts and limestone (Quaternary), Fvb: detrital formation (« Cailloutis villefranchien »), Fx: alluvium (Quaternary), m: marl and molasse, n: limestone, OE: loessal silts: thick layer (Quaternary), OE F: loessal silts: thin layer or discontinuous on Fvb (Quaternary), p: clay.

thousands of individuals of both *Lythrum tribracteatum* and *Lythrum hyssopifolia* L. were observed in this site. The three species grew in large ruts created by heavy machinery employed for an archaeological survey. The wetland of Campuget was originally a large temporary lake (around 120 ha), grazed by sheep. But agriculture and drainage strongly modified the landscape until the abandonment of agriculture around 2000 (Pirsoul et al. 2014). Since then, vegetation has developed spontaneously in the context of a modified hydrology with limited management to control tree encroachment after the discovery of the *L. thesioides* population.

(2) Species description

Field and culture observations

The isolated individual of *Lythrum thesioides* produced fruits and viable seeds (capable of germination). No insect were observed visiting flowers during our experiment. Flowers stayed open only during the day and closed at night (closed at 7 am and 9 pm) preventing pollination by moths and other nocturnal insects. The life-span of the flowers is very short and petals usually fall after 1 or 2 days. Capsules are 2 mm long et 1 mm wide, containing 39 ± 13 tiny light brown seeds, about $15 \pm 1.5 \ \mu g$ each (Figure 1(g)).

Pollen observations

The pollen of *Lythrum thesioides* is similar to that of *L. borysthenicum (M.Bieb.ex Schrank) Litv.*, with heterocolpate grains. There are six furrows (ectoapertures), three of them doubled with a pore (endoaperture). The size of the pollen was about 20 μ m of diameter (Figure 1(f)).

Chromosome count

A chromosome count revealed 2n=30. Considering X = 5 (Graham and Cavalcanti 2001), this indicates a hexaploid karyotype. The poor quality of the mitotic metaphase plates prevented further analysis.

(3) Population search

Site visits revealed the globally poor habitat quality and conservation status of wetlands of the Costières area that have all been drained and cultivated. Thirteen sites hosted wetland species but only five (17%) can be considered as temporary pool communities. Wetland plant communities were mostly in ditches, where the hydrology is slightly similar to temporary wetlands.

Prospections of the Pazac and Jonquières lakes where *Lythrum thesioides* was previously reported were unfruitful, no sitings were reported. Nevertheless, the plant was observed in the recently discovered population of Campuget, as in every year since its discovery in 2010. Of the 30 sites visited in the Costières territory, one new population of *L. thesioides* was detected, at Saint-Vincent (Jonquières-Saint-Vincent, Gard) in a wet depression near to the historical site of Jonquières Lake (4 km distant). It was found in a very recently enlarged ditch in an otherwise intensive agricultural context. In June 2017, this population comprised less than 30 individuals of *L. thesioides* all located along a -200 m long ditch. During the second visit in September, about 250 individuals were observed, several being very tall (50 cm high) and each of those with more than 500 fruits. Monitoring started in 2017 with the discovery of this new population, using presence absence data in order to begin a study of the population dynamics of both Saint-Vincent and Campuget populations.

Discussion

The historical description of this species is limited to the discrimination between L. thesioides and L. thymifolia L. concerning notably the presence of pedicel and the shape of the capsule for Western European samples of the former species (Bieberstein 1808), but did not mention geminate flowers, for example. The first mention of geminate flowers was made later (Bertoloni 1842), leading to the description of a new species, L. geminiflorum Bertol. 1842, which was later included within L. thesioides. However, the observation of the typus (Berlin herbarium), revealed that this sample has few geminate flowers. It can, however, be distinguished from other Lythrum species by its elongated pointed leaves, and its small, slightly bell-shaped flowers, mostly geminate. Our study completes the description of the species with the pollen and karyotype (2n = 30) description, and measures on capsule and seeds.

An ephemeral species in a disturbed environment

Lythrum thesioides has been poorly recorded during the last two centuries, since its first description. Indeed, despite a large known potential distribution, from Central Asia to Western Europe, the plant has been recorded less than 30 times. Even though a taxonomical issue with *L. linifolium* Kar. & Kir. 1841 exists, the latter has no recent and very few historical observations (Koehne 1884, Taran 1993; Mesterházy 2017). Thus, the three recent observations described in this paper from the South of France represent the only currently known populations (i.e. observed in the last 20 years) of *L. thesioides* world-wide.

It appears that this species has always been rare and erratic. Except in Jonquières Lake, where it was documented 8 times from 1841 to 1911, all other historical records were made in only a single year. In 1853, the Abbé Gonnet wrote on his herbarium specimen from Tresques (Gard), conserved in Jean Henri Fabre's herbarium (SLA herbarium) « This species has not appeared since even though there were hundreds of thousands growing plants in this place. It also disappeared from the Jonquières Lake » (translated from french). This note futher indicates the ephemeral character of this species. For recent observations, the species was seen in La Capelle only in 1998 and in 2009. Campuget is the only known place where the species has been observed annually since 2010, in and around artificial pits that were dug as part of an archaeological survey. At the Saint-Vincent site, our monitoring (unpublished data) suggests that the population appeared with a high rate of occupancy after a major physical disturbance (enlargement of the pit) that removed all vegetation, and that the species may not reappear in the absence of disturbance.

The plant only grows in late-flooded habitats. Indeed, it flowers late in the season, starting in early summer. The germination of seeds starts only at high temperatures 20-25°C (Gazaix in prep) which occur in late spring in the South of France. Ecosystems with late flooding conditions are uncommon under a Mediterranean climate regime and this germination niche is more typical of continental species (Deil 2005; Carta 2016). The most suitable habitat of Lythrum thesioides is thus in the large temporary lakes that have occurred in the Costières territory (where it occurs at the edge of fluctuating water bodies) or along the temporarily flooded edge of rivers with high spring water-flows. Plants of L. thesioides grow in the external belt of the lake in la Capelle, and on the edge of the lake in Jonquières (note in herbarium specimen: "Bord de l'étang"). In Campuget, a recent mapping of the seed bank of L. thesioides according to the topography of the wetland reveals a peripheral distribution of the seeds (Gazaix in prep.).

The other important parameter for the appearance of Lythrum thesioides appears to be a disturbance that reduces or eliminates plant competition and improves light irradiance at ground level. Indeed, the three recently discovered populations were located in highly disturbed sites with soil perturbations and a modified hydrology involving late floods in common with all the other observations. At la Capelle, the population was found within melon fields, involving soil ploughing and irrigation across the drained part of the lake. The lake of la Capelle is one of the very few large Mediterranean temporary pools that still exists in France and the only current natural body of water of the Gard department (Gayte 1991), even though it has been partially drained. At Campuget and Saint-Vincent, plants were found on recently dug or enlarged pits and in machine tracks. In some historical records, disturbance is also present, e.g. in ricefields (Pignatti 1982) or in fields next to rivers (Chatenier 1922). Finally, natural rivers provide strong disturbances and create mudflats or sandbanks where L. thesioides can grow (historical observations in the Rhone, Durance, Danube, Volga, ...). In these fluctuating habitats, the seed bank can stay buried for decades (van der Valk and Davis 1978; Brock 2011; Poschlod and Rosbakh 2018). Thus, the most probable

hypothesis is that the seed bank has remained after drainage in La Capelle, Campuget and Saint-Vincent sites, and that recent disturbances have stimulated germination of *L. thesioides* seeds.

The distribution of seeds is also explained by the localization of adult individuals (Metzner et al. 2017), and Lythrum thesioides may be excluded from deeper positions by long periods of flooding and/or the presence of other more competitive or better-adapted species, hence its position in the external ring, where only wet years allow the germination of its seeds. Very wet years may appear as a form of disturbance, by flooding of non-adapted vegetation, and be complemented by local disturbance, such as wild boars that turn the top soil (Amami et al. 2009). This pattern of distribution has been indicated for other species of temporary pools (e.g. Elatine brochonii Clavaud, Pilularia minuta Durieu), that only grow in wet years (Rhazi et al. 2009) and do not have propagules distributed in the deepest part of the wetland.

Conservation status

Temporary pools and river bank habitats are threatened by artificialisation (Brinson and Malvárez 2002). Most large rivers of Europe and Central Asia have suffered from flow regulation and human activities (Dynesius and Nilsson 1994; Ward 1998), and temporary pools have disappeared dramatically as a result of agriculture intensification and urbanization (Bouahim et al. 2014). The Costières territory has incurred and still faces these threats. In this territory, drainage started some 2,000 years ago, when the Clausonne lake (Figure 3) was drained by the Romans to build the aqueduct that was to bring water from Uzès to Nîmes, via the famous "Pont du Gard" (Fabre et al. 1997). Since that time, agricultural development has caused temporary pools to dwindle in size and number and become highly modified in their hydrological regime and poor in quality (Molinier and Tallon 1948)

Jonquières lakes, which hosted Lythrum thesioides was drained at the beginning of the XIX century (Toulemonde 2003), Pazac and Campuget were drained and cultivated later in the 1950s and 1960s. For the 14 wetlands that we visited to prospect the presence of L. thesioides (and other rare species) using the geological map of the Costières territory, all have been drained at least partially. This loss of temporary wetlands in the was reported several decades ago when Costières Molinier and Tallon prospected wetlands previously visited by de Pouzolz a century before (Molinier and Tallon 1948). In Campuget, a network of pits currently drains the wetland, although the land is partially managed by the regional Nature conservation organization (Pirsoul et al. 2014). In Saint-Vincent, changes in agricultural practices in 2017 and 2018 such as plantations of peach trees and

Populus nigra hedgerows and the pumping of groundwater represent new threats for the conservation of *L. thesioides*, not to mention ongoing use of herbicides. At La Capelle, recently dug big pits have disconnected the field from the water of the lake, probably preventing germination of *L. thesioides* seeds, which was previously stimulated by the irrigation of the melon field (Guéniot and Klesczewski 2018).

The conservation status of the species was assessed as "Endangered" by the IUCN at the Mediterranean (Diadema 2010) and European (Lansdown 2011) scales. Although a global assessment is lacking, and the fact that these evaluations were made without the knowledge of the discovery of two new populations of the species at Campuget and Saint-Vincent, the poor conservation status of the three current populations should justify the maintenance of an endangered status for this species.

Recent projects on Mediterranean temporary pools have led to the discovery of new populations of rare plants in this ecosystem (Daoud-Bouattour et al. 2009; Porto et al. 2012; Ghrabi-Gammar et al. 2017; Minissale et al. 2017; Lotterman et al. 2018). Our recent observations on *Lythrum thesioides* are another example of discovery of new populations and will contribute to improve our capacity to implement conservation management for this species.

Even though some wetlands were drained decades ago, restoration programs can still be envisaged, as has been done for pools filled more than a century before in England (Alderton et al. 2017). The search for reports of seeds and growing plants of L. thesioides in old records (like Jonquières, Tresques, Pazac for France, or elsewhere in other countries) is thus a priority in the examination of sites for restoration and the collection of seedbanks. Arguably, this may be easier in France as precise information on locations is available for several wetlands (Tresques, Pazac and Jonquières). Indeed, soil samples from Jonquières and Pazac have already been tested for the presence of a seed bank, but without any germination success for Lythrum thesioides (unpublished data). This failure may be due to a small sample size and a larger sampling programme may be necessary to maximize the chances of detecting viable seeds of L. thesioides, as was the case for the detection of the rare Centaurium favargeri Zeltner, on river banks in the South of France (Huc and Jacob 60).

Future prospections of *L. thesioides* in relation to geological substrate could help find potential populations in sites that were once temporary pools and wetlands, as we have shown by the new discovery at the Saint-Vincent site. River banks could also be surveyed. However, as the presence of such rare ephemeral species is difficult to predict (Nilsson et al. 1988), a large amount of field work, and a certain amount of luck, will be required to find new *L. thesioides* populations.

Acknowledgments

We thank Hugo Fontès & Solène Baillet for their help in the discovery of *L. thesioides* in 2018. Attila Mesterhazy and Adrew Efremov provided information and helped with the distribution of *L. thesioides*.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the SNCF Réseau and Oc'Via.

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James Molina is botanist, and regional director at the CBNMed. He is interested in the conservation of the Mediterranean Flora, particularly in Mediterranean temporary ponds ecology. He re-discovered the species in France in 1998 (La Capelle). He worked on the historical distribution of the species in France.

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