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The exotic rust fungus *Puccinia abrupta* var. *partheniicola* on the invasive alien weed *Parthenium hysterophorus* in India: rediscovery and first report of an epiphytotic

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Abstract

Puccinia abrupta var. *partheniicola*, the so-called winter rust of parthenium weed (*Parthenium hysterophorus*), an invasive alien weed in India, was rediscovered at an epiphytotic scale in February 2023 following a prolonged period of presumed absence in the field. Disease incidence was 80–90% in large tracts of *P. hysterophorus* stands along the roadsides in an area of 5 km^2 near Attibele–Sarjapura road (948A) in Anekal taluk of Bengaluru Urban district in Karnataka. Pycnial, aecial or telial stages of the rust were not found. The morphological parameters of uredinia and urediniospores completely matched those originally described, thus confirming the fungal identity. The significance of this rust epiphytotic to *P. hysterophorus* biocontrol in India is discussed together with the planned follow-up activities.

Keywords Classical biological control, Fungal pathogen, Natural enemy, Parthenium weed, Plant pathogen, Winter rust

Background

Parthenium hysterophorus L. (Asteraceae), internationally known as parthenium weed, has been accidentally introduced into at least 48 countries (Strathie and Sreerama Kumar 2018). Within a short time of its first documentation in India in 1955 (Rao 1956), this exotic weed—locally known as congress grass—had established a viable soil seed-bank to continuously flourish by the roadsides, and in pastures, wastelands and agricultural fields. Besides its encumbrance on agriculture and the landscape, its adverse effects on human well-being through airborne pollen and trichomes (Gupta and Chanda 1991; Sharma and Verma 2012), and on animal health, mainly through ingestion (Ahmed et al. 1988; Hussain et al. 2022), have brought notoriety to *P. hysterophorus*.

Despite the availability of various cultural, mechanical and chemical approaches, because of their limitations (Adkins and Shabbir 2014) as well as the sheer magnitude of the problem, classical biological control is considered the most ideal solution to suppress *P. hysterophorus* (Dhileepan et al. 2018), and thereby negate its impact on health and the environment (Sreerama Kumar and Evans 2005). The leaf-feeding beetle *Zygogramma bicolorata* Pallister (Coleoptera: Chrysomelidae), first field-released in Bengaluru (then Bangalore), Karnataka, in 1984 (Jayanth 1987), and later in several regions countrywide, is the only classical biocontrol agent deliberately introduced for the weed in India. It was, however, long felt that agents that attack flower

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parts and developing seeds should be introduced because a single plant of *P. hysterophorus* is capable of producing around 21,500 seeds (Nguyen et al. 2010), the vigorous ones of which can remain viable if buried in soil even at a depth of 15 cm (Nishanthan et al. 2018). Therefore, after a three-decade-long hiatus, in an attempt to complement the beetle and hasten biocontrol, a seed-feeding weevil, *Smicronyx lutulentus* Dietz (Coleoptera: Curculionidae), was imported from Australia in 2018 (Sreerama Kumar et al. 2018), but the weevil did not sustain itself in the quarantine. Even as additional insects could not be procured from Australia due to low field populations there in 2019, parallel endeavours to import them from South Africa too did not materialise because of the COVID-19 pandemic during 2020–2021.

Meanwhile, periodic countrywide surveys have been conducted to identify potential biocontrol agents, including accidentally introduced natural enemies of *P. hysterophorus* from its native range. Rediscovery of an exotic rust fungus for the first time in the form of an epiphytotic is reported here, and its significance to *P. hysterophorus* biocontrol in India is discussed together with the planned follow-up activities.

Materials and methods

On 3 February 2023, an intensive survey was conducted for suspected rust infection in *P. hysterophorus* stands along the roadsides in an area of 5 km^2 near Attibele–Sarjapura road (948A) in Anekal taluk of Bengaluru Urban district in Karnataka, India (Fig. 1). One hundred plants were examined for the presence of rust pustules

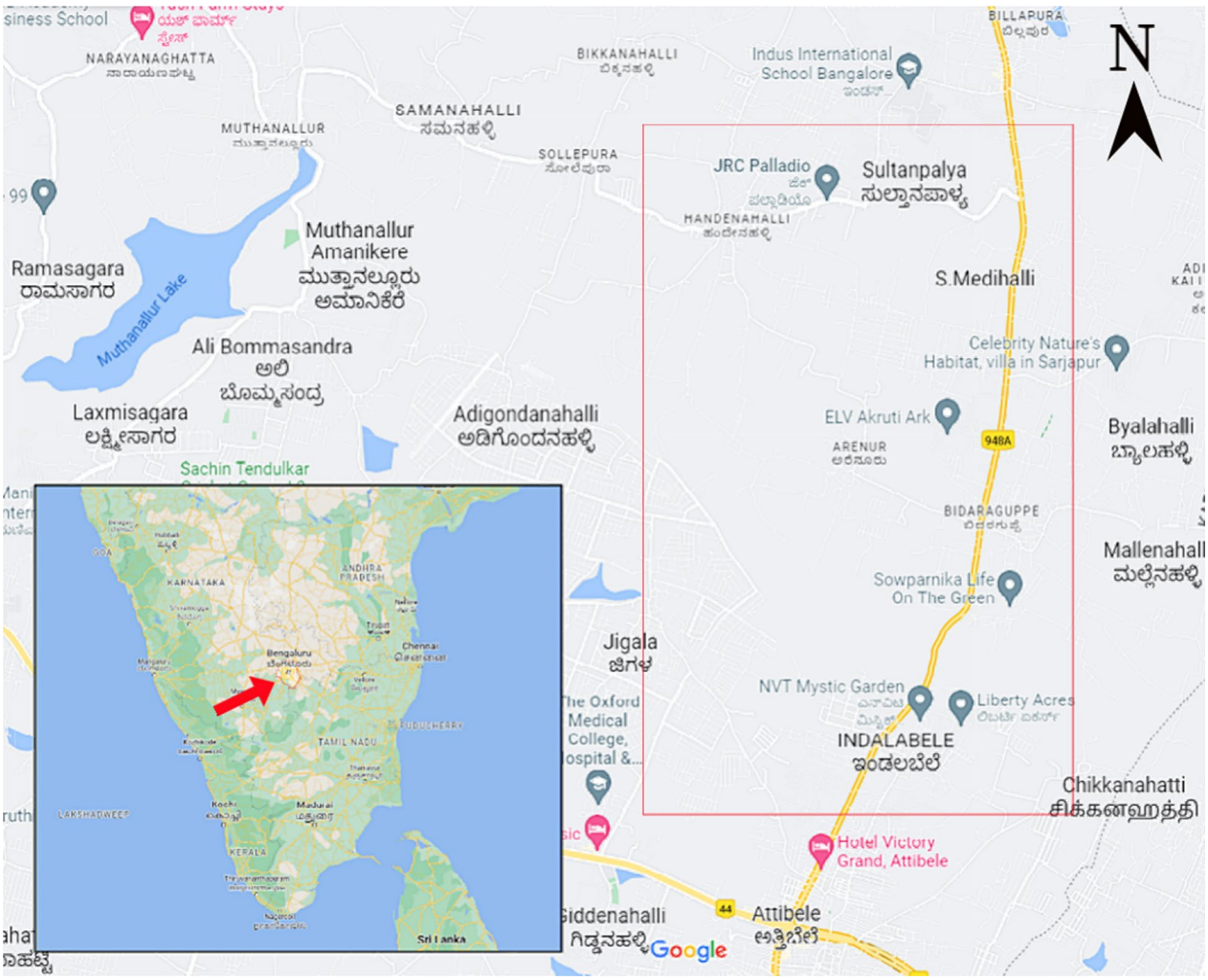

Fig. 1 The area surveyed for *Puccinia abrupta* var. *partheniicola* in Bengaluru Urban district, Karnataka, India. The route taken and the collection localities fall within the red rectangle. Inset: Location of the district in southern India pointed by the arrow

to determine the disease incidence. A plant was considered diseased even if it had a single rust pustule. Leaf and whole-plant samples were collected in paper bags for laboratory analysis. Uredinia were immediately examined in situ through the Magnifier app (version 1.3.4 by APP2U) on an Android smartphone (OnePlus A6010), and later under a stereozoom microscope (ZEISS STEREO Discovery.V12) in the laboratory for simultaneous photomicrography (MiaCam AR 6Pro). Rust spores from uredinia on the adaxial surface of infected leaves were dislodged onto a sterile petri dish before mounting them in Hoyer's fluid unstained on microscope slides. Spores were first examined through phase-contrast objectives under a research microscope (Olympus BX41), and later the dimensions of 50 randomly selected spores were measured in two perpendicular directions to obtain the values in µm. Mounted spores were re-examined under a differential interference contrast microscope (ZEISS AXIO Imager.Z2) and photomicrographed (ZEISS Axiocam 503 color) for documentation. Further samples were collected after ascertaining the continuity of rust incidence at the same collection localities on 13 and 27 February 2023, and finally on 10 March 2023. Herbarium specimens of diseased *P. hysterophorus* leaves were temporarily deposited at the Indian Council of Agricultural Research (ICAR) – National Bureau of Agricultural Insect Resources (NBAIR), Bengaluru, for further processing.

Results

Large tracts of *P. hysterophorus* stands along the roadsides in an area of 5 km^2 near Attibele–Sarjapura road (948A) in Anekal taluk of Bengaluru Urban district in Karnataka, India (Fig. 1), were found to exhibit severe rust infection on 3 February 2023. Rust infection (Fig. 2A, B) was the severest near Arenuru (12°48′51.5″N; 77°46′30.1″E) and the road leading to Handenahalli. Most of the diseased samples of *P. hysterophorus* were collected from a 100-m stretch along this road. Disease incidence was 90% there, which indicated that the level of rust infection was epiphytotic. Pycnial, aecial or telial stages of the rust were not found. Uredinia on the adaxial surface of leaves were orange-brown, subepidermal, erumpent, pulverulent and scattered (Fig. 2C, D). On leaves that exhibited advanced infections, pustules expanded, became amphigenous and turned dark brown (Fig. 2E, F). Eventually, the leaves twisted and dried up completely. Pustules also occurred on stems (Fig. 2G) and petioles of heavily infested plants. The majority of urediniospores (Fig. 3A, B) were obovoid and others deltoid with one apical and two subequatorial pores. They measured 22.50–26.25 (21.20) × 20.00–22.50 (24.15) µm. The yellow–brown wall was up to 1.7 µm thick, echinulate (Fig. 3C) with up to 0.7-µm-long spines positioned 3.0–3.5 µm apart in an indistinct pattern (Fig. 3D). The morphological parameters of uredinia and urediniospores completely matched those originally described by Parmelee (1967), thus confirming the fungal identity as *Puccinia abrupta* var. *partheniicola* (H.S. Jacks.) Parmelee (Pucciniales: Pucciniaceae).

The rust incidence was 80% at three more localities, namely, Handenahalli (near a polyhouse) (12°49′35.1″N; 77°45′35.7″E), Handenahalli (near a sericultural unit) (12°49′2.4″N; 77°46′32.2″E) and Indalabele (12°47′38.1″N; 77°46′41.8″E). The disease persisted with the same level of incidence at the all the collection localities on 13 and 27 February 2023, but during the final survey on 10 March 2023, severely diseased plants were found to have lost vigour. No young pustules could be observed on the remaining plants in the vicinity.

Discussion

Puccinia abrupta var. *partheniicola*, commonly called the winter rust, is a macrocyclic, autoecious species, but only two spore stages (uredinia and telia) have been found on *P. hysterophorus* within its native habitat in Mexico. It can reduce vegetative growth in young plants and seed production in older plants (Evans 1987). Both uredinia and telia are produced on leaves, stems and inflorescences in the semi-arid uplands of northern Mexico, but in the more humid lowland areas, only scattered uredinia are produced on the older, rosette leaves (Evans 1997). In India, in the 1980s, the rust was reported to occur at a site of 930-m elevation (Harry C. Evans and Carol A. Ellison, International Institute of Biological Control, unpublished data, 1987, cited by Parker et al. 1994) at some distance from the city of Bengaluru (then Bangalore) (Harry C. Evans, personal communication 1997). It was, however, thought to be neither widespread nor aggressive, as it was never observed on an epiphytotic scale (Sreerama Kumar and Evans 2005). The pathogen was also found in Andhra Pradesh (Bagyanarayana and Ramesh 1995; Bagyanarayana and Manoharachary 1997), but, again, not as an epiphytotic.

Under a collaborative project between CABI Bioscience (now CABI) and the ICAR, sponsored by the United Kingdom Department for International Development (DFID; now the Foreign, Commonwealth & Development Office) from 1996 to 2000, pathogens of *P. hysterophorus* were investigated as biocontrol agents (Evans et al. 2000). Although a Mexican pathotype (W1905) of *P. abrupta* var. *partheniicola* was found virulent to 12 chosen Indian ecotypes of *P. hysterophorus* when assessed under quarantine conditions at CABI, Silwood Park, United Kingdom (Seier et al. 2000), it was eventually not prioritised for importation into India at the time mainly because of increased attention towards indigenous mycoherbicides.

Fig. 2 Stands of *Parthenium hysterophorus* infected with the rust fungus *Puccinia abrupta* var. *partheniicola* (**A & B**); young pustules of the rust on the adaxial side of leaf (**C**); mature pustules (**D**); expanded, dark brown pustules (**E**); close-up of rust pustules erupting from the adaxial leaf epidermis (**F**); close-up of a rust pustule on the stem (**G**)

Meanwhile, in neighbouring Nepal, *P. abrupta* var. *partheniicola* was fortuitously found in Kathmandu Valley in 2011 (Shrestha 2012), and it is now reported to be more widespread there (Maharjan et al. 2020). The rust has also been recorded in Pakistan's Punjab and Khyber Pakhtunkhwa provinces (Iqbal et al. 2020; Weyl et al. 2021); it could, thus, be possible that *P. abrupta* var. *partheniicola* is also present in the Indian states bordering these two countries. Further, the rust is also present in Bhutan and China (Winston et al. 2023). Although it is unclear if one or more pathotypes of *P. abrupta* var. *partheniicola* are present in the Indian subcontinent, it is at least evident that the one in Karnataka is aggressive enough to spread farther. The sudden spurt in the rust incidence in this region should however be viewed from various perspectives, including climate change, which has a great impact on infectious plant diseases in natural ecosystems (Yang et al. 2022). Indeed, results from a controlled experiment in Australia suggest that the biocontrol efficacy of *P. abrupta* var. *partheniicola* could improve with an increased level of CO_2 in the atmosphere (Shabbir et al. 2014). The epiphytotic reported here could, therefore, be an indirect manifestation of a higher level of atmospheric CO_2 in the study area. At the same time, recent evidence suggests that due to the looming climatic and land use changes, there is a likelihood of *P. hysterophorus* turning into a "superweed" if the existing management strategies are not thoroughly overhauled (Mao et al. 2021). From biocontrol perspective, natural epiphytotics of the aggressive exotic agent *P. abrupta* var.

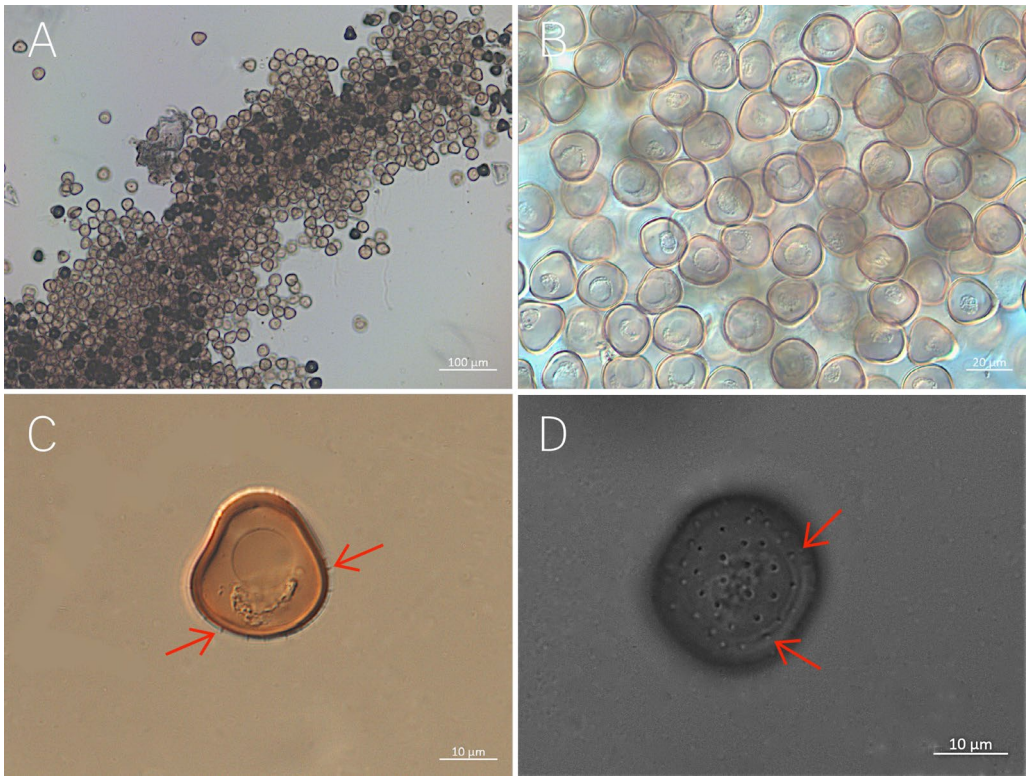

Fig. 3 Photomicrographs of unstained urediniospores of *Puccinia abrupta* var. *partheniicola*—a mass of spores, bright-field 100× (**A**); a mass of spores, differential interference contrast (DIC) 400× (**B**); single spore, DIC 1000×, arrows point at individual spines (**C**); single spore, DIC 1000×, arrows point at individual spines (**D**)

partheniicola are advantageous in winter when *Z. bicolorata* is inactive due to hibernation. Winter's long dew periods and cool temperatures favour the establishment and rapid progress of this rust (Fauzi et al. 1999). Decades-long Australian experience shows that the pathogen could be readily established in the field through releases of spores produced in the glasshouse, and that redistribution of this pathogen to the regions that have wet winters yields better results (Dhileepan et al. 2018). A similar strategy could be emulated in India to keep the parthenium weed stands suppressed during winter, probably after studying its potential natural outbreaks.

Conclusions

Amongst all invasive weeds in India, *P. hysterophorus* has attracted the maximum attention insofar as studies on the native mycobiota for mycoherbicide development (Sreerama Kumar 1998), but with little or no success. On the other hand, host-specific plant pathogens as classical biocontrol agents have received only modest attention (Sreerama Kumar et al. 2008). Given this situation, the recent resurgence of an aggressive pathotype of *P. abrupta* var. *partheniicola* in India must be exploited well. Planned follow-up activities include continuous monitoring of the rust in and around the present study area to collect further data on disease persistence across seasons. It is intended to do a sensor-based analysis of the spatial distribution of the disease beyond the originally studied 5-km^2 area during the next prospective outbreak. Also planned are a study on the disease severity and its effect on seed production in situ, and confirmatory host-specificity studies. After obtaining mandatory permissions, *P. abrupta* var. *partheniicola* could be systematically redistributed to other climate-matched areas during the short window of favourable months.

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Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The author declares that he has no competing interests.

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Publisher's Note

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