



Review Article

Endophytes of invasive weeds: pertinence to classical biological control in India

PRAKYA SREERAMA KUMAR

ICAR–National Bureau of Agricultural Insect Resources, P.O. Box 2491, H.A. Farm Post, Hebbal, Bengaluru 560 024, India.
E-mail: psreeramakumar@yahoo.co.in

ABSTRACT: Suppression of the water weed *Salvinia molesta* in India and control of the woody-perennial *Cryptostegia grandiflora* in Australia are two of the finest successes achieved through classical biological control (CBC). There is no guarantee, nevertheless, that CBC should always be successful in every situation. Though the enemy release hypothesis explains why invasive alien species thrive in exotic locations, there has never been a convincing explanation on the poor performance of some introduced natural enemies, in spite of the rigorous screening for their potential. Interestingly, Evans' endophyte–enemy release hypothesis provides enough clarification by implicating the absence of protective coevolved endophytes in weed populations in exotic locations. This review gives an introduction to endophytes and analyses the pertinence of the newly proposed hypothesis to CBC of weeds in India with examples.

KEY WORDS: Classical biological control, endophytes, natural enemies, weed management

(Article chronicle: Received: 09-09-2015; Revised: 14-09-2015; Accepted: 16-09-2015)

INTRODUCTION

Globally, 224 weeds have so far been targeted with 551 biocontrol agents (Winston *et al.*, 2014). Some of the finest successes in weed biocontrol have been achieved through classical biological control (CBC). For instance, suppression of water fern (*Salvinia molesta*) in Kerala by the introduced weevil, *Cyrtobagous salviniae*, is often considered as one of the top-notch biocontrol success stories in India. One of the most recent successes has been the suppression of the Madagascan woody-perennial, *Cryptostegia grandiflora* (rubber vine), with the release of the coevolved rust, *Maravalia cryptostegiae*, in Queensland, Australia (Tomley and Evans, 2004).

Successes of CBC, nevertheless, have neither been so common nor assured in any part of the world due to reasons both known and unknown. In several cases of CBC of weeds, out of a suite of natural enemies or biocontrol agents released, only one, or at the most a few species, would have given sustainable control of a target weed. At the same time, there is no guarantee that control would be achieved if more than one agent is released to control a single weed; none of the agents would have performed or established. Also, mere establishment of several agents in a weed population

does not always guarantee complete control of the weed species. For example, out of the nine species of insects and two rust fungi introduced into Australia to control parthenium weed (*Parthenium hysterophorus*), seven species of insects and both rusts have successfully established as biological control agents, thus offering a high establishment rate, but at the same time requiring integration of other habitat-dependant management options to totally suppress the weed (Dhileepan, 2009). In fact, Julien *et al.* (1984) estimated that up to 1980, out of 178 different species of organisms released against 101 species of target weeds across the world, 71% of the agents got established, and only 34% were effective and controlled 48% of the weeds. Since CBC of weeds relies heavily on the enemy release hypothesis, it is pertinent to understand this proposition to appreciate the reasons for success or otherwise of a CBC agent. Besides, emphasis should also be given to endophytes, which have a close association with the host plant and play an important role in CBC.

ENEMY RELEASE HYPOTHESIS

It is still a puzzling question as to why invasive alien species (IAS) thrive in exotic locations despite the availability of abundant research results. The most convincing

and widely accepted explanation implicates the freedom gained by an IAS from pests, diseases and other biotic stresses in the course of its relocation to new regions. This theory is called the ‘enemy release hypothesis’ (ERH), which in the words of Keane and Crawley (2002) asserts that ‘plant species, on introduction to an exotic region, experience a decrease in regulation by herbivores and other natural enemies, resulting in a rapid increase in distribution and abundance.’

However, the success of CBC agents may also be attributed to processes that are unrelated to enemy release (Colautti *et al.*, 2004). In this context, endophytic microorganisms or endophytes, besides natural enemies, have now achieved a greater significance than ever before with the rapidly growing number of CBC programmes against a wide range of invasive weeds.

ENDOPHYTES

Although the term ‘endophyte’ first appeared way back in 1866 itself, the work on the ecology, life cycle, phylogeny, physiology and relevance of endophytes intensified only in the last three decades (Azevedo *et al.*, 2000; Saikkonen *et al.*, 2004). Surprisingly, in spite of the continuous accumulation of information on endophytes, there has still been considerable uncertainty in the definition of an endophyte (Hyde and Soyong, 2008). Petrini (1991) proposed that endophytes should include: ‘All organisms inhabiting plant organs which, at some time in their life, can colonise internal plant tissues without causing apparent harm to their hosts.’ Thus, those endophytic organisms, which have a more or less epiphytic phase, as well as latent pathogens living asymptotically within their hosts, are also classified as endophytes (Fisher and Petrini, 1992).

Diversity

Endophytes have already been isolated from plant species belonging to many families and growing in diverse habitats (Azevedo *et al.*, 2000). They are known to form communities that are specific to a certain host and a certain environment (Petrini, 1996). Differences in endophyte communities are known to occur in the native and invaded ranges of a plant. For example, differences in the hidden diversity of endophytic fungi in the native and invaded ranges have been reported in the invasive spotted knapweed, *Centaurea stoebe* (Shipunov *et al.*, 2008).

Transmission

Endophytes are transmitted from plant to plant verti-

cally or horizontally. In vertical transmission, endophytes are transmitted from plant to offspring through seeds. Transmission of fungal endophytes such as *Neotyphodium* can happen vertically in grasses as they can synchronise their growth with the lifecycle of the host and become systemic to find entry into seeds (Saikkonen *et al.*, 2004). On the other hand, horizontal transmission of endophytes is common in large plants having a longer age of maturity (Saikkonen *et al.*, 2004). Therefore, it is likely that invasive grassy weeds would carry endophytes to their newer locations, but dicotyledonous weeds may not reach exotic locations with their native endophytes (Evans, 2008). Among the dicots, however, annual and high seed-producing weeds may also bear endophytes that are vertically transmitted.

ENDOPHYTE–ENEMY RELEASE HYPOTHESIS

Recently, Evans (2008) came up with a convincing hypothesis termed the endophyte–enemy release hypothesis (E–ERH) ‘to resolve the on-going debate on the validity of the ERH, as well as clarify inconsistencies in both the new encounter and the evolution of increased competitive ability hypothesis.’ Though Evans (2008) restricted this new hypothesis to fungi that invade living plants and colonise them without causing visible or immediate symptoms, E–ERH can encompass bacteria and other endophytic microorganisms, too. Mycorrhizal fungi, however, are excluded because of their restriction to root systems, synchronised development with the plant, as well as their involvement in nutrient transfer.

With the publication of this new hypothesis, endophytes have received the limelight that they deserved all through in the history of CBC of weeds. To strengthen the E–ERH, Evans (2008) takes the example of the rubber vine control in Queensland, Australia, where an atypically high mortality of the weed was achieved by the introduced rust pathogen. He argues that such an exceptional performance by an obligate pathogen could have happened only because of the plant leaving out the protective coevolved endophytes in its native region, and at the same time not acquiring indigenous generalist mutualists in its new home, i.e. Australia. Data on endophytic diversity of the rubber vine in Australia as well as in Madagascar may prove to be more valuable to appreciate this argument.

ROLE OF ENDOPHYTES IN WEEDS

Considering an ‘endophyte’ synonymous with mutualism is no longer valid as both ecological and functional roles of endophytic microorganisms are understood better now (Saikkonen *et al.*, 2004). In fact, Evans (2008) visu-

alises the role of specialised or coevolved mutualistic fungal endophytes as under: ‘their presence increasing plant fitness in the absence of coevolved natural enemies, especially in grass hosts with vertically transmitted endophytes; their absence coupled with release from coevolved natural enemies, contributing to increased plant fitness, especially in dicot hosts with horizontally transmitted endophytes, but leaving them highly vulnerable to classical biological control agents.’ Interestingly, endophytes also seem to have an indirect role in weed biocontrol. For example, Saikkonen *et al.* (2013) could demonstrate that endophyte-promoted competitive superiority of meadow fescue (*Scherodonus pratensis* ex. *Lolium pratense* and *Festuca pratensis*) cultivars can hinder weed invasions.

MECHANISMS INVOLVED AGAINST NATURAL ENEMIES

Pathogens

In his seminal paper on ‘Microbial penetration and immunization of uncongenial host plants,’ Matta (1971) had not used the word ‘endophyte’ though the paper revolved around ‘the “immunizing” effects that the “infections” with avirulent fungi and bacteria are capable of inducing in plants.’ Above and beyond looking at the possibility of non-pathogens penetrating and inducing both morphological and physiological responses in plants, Matta (1971) also analysed whether the “pseudo-infective process” could in any manner confer protection against virulent bacteria and fungi, as well as the mechanism involved therein. He explicitly concluded that: ‘Protection in plants by avirulent fungi and bacteria is aspecific, generally not translocated, and scarcely persistent.’

E-ERH was immediately put to practice in New Zealand by Dodd *et al.* (2010) to solve the question of inconsistent control of *Cirsium arvense* by *Sclerotinia sclerotiorum*. They assessed the endophytic microbial populations in *C. arvense* through culturing and molecular methods, and detected over 60 genetically unique endophytic microbes, with the greatest diversity in leaves than in any other part of the plant.

Several well-known plant disease antagonistic fungi have been found as endophytes in various plant species. *Trichoderma theobromicola* and *T. paucisporum*, which were found as endophytes in cocoa (*Theobroma cacao*) in South America, the centre of origin of the plant, produced secondary metabolites known to be involved in plant defence mechanisms against coevolved fungal pathogens of the host (Holmes *et al.*, 2004; Samuels *et al.*, 2006). Simi-

larly, other fungi such as *Chaetomium globosum* that are known well as antagonists have also been isolated as endophytes (Arnold, 2007). These antagonistic endophytes are therefore responsible for the resistance offered against attack by other pathogens.

Insects

Toxins produced by endophytes have been implicated in the protection offered in a plant through repelling insects, inducing unpalatability, reducing growth and development, and increasing pest mortality (Carroll, 1988; Clay, 1988 & 1996).

Phomopsis oblonga, an endophytic fungus, was reported to have protected elm trees against the Dutch elm disease (*Ceratocystis ulmi*) by controlling the breeding of the scolytid beetle *Physocnemum brevilineum*, the vector of the disease (Webber, 1981). Claydon *et al.* (1985) demonstrated that endophytic fungi belonging to the Xylariaceae family synthesise secondary metabolites in hosts of the genus *Fagus* and that these substances affect the beetle larvae. Two endophytes, *Alternaria* CID62 and *Epicoccum* CID66, were reported to protect *Centaurea stoebe* from *Larinus minutus*, a seed-feeding weevil deliberately released in North America for biological control (Newcombe *et al.*, 2009). Vega *et al.* (2008) isolated several entomopathogenic fungal genera, including *Acremonium*, *Beauveria*, *Cladosporium*, *Clonostachys* and *Paecilomyces*, from coffee plants, and showed that *B. bassiana* and *Clonostachys rosea* to be pathogenic to coffee berry borer. Recent research shows that plant–endophyte symbioses can adversely affect foraging by leaf-cutting ants (Bittleston *et al.*, 2011; Estrada *et al.*, 2015).

At this stage, therefore, it is only speculated that the fungal metabolites produced by endophytes within plant tissues induce feeding deterrence or antibiosis, thereby resisting insect attack (Cherry *et al.*, 2004; Vega *et al.*, 2008).

PERTINENCE TO CBC OF WEEDS IN INDIA

Early results

Our preliminary studies on the endophytic diversity of two invasive weeds, *viz.* *Mikania micrantha* and *P. hysterophorus*, indicate that there are both spore-forming and sterile fungal species as well as bacteria (Fig. 1) as endophytes in these hosts in India (Sreerama Kumar P, unpublished). Identification of the entire diversity of endophytes in different populations of these two weeds in India, and its comparison with the diversity in the native region, may give indica-

tions on the performance or otherwise of potential natural enemies. Though the introduced rust pathogen, *Puccinia spegazzinii*, showed initial signs of establishment on *M. micrantha* in Kerala (Sreerama Kumar *et al.*, 2008), it could not spread further. Do endophytes have a role in the *M. micrantha*–*P. spegazzinii* system? In a study on general profiling of endophytes in 12 populations of *M. micrantha* from Andaman and Nicobar Islands, the endophyte composition was found to be heterogeneous at several levels: within single organs, among organs of a single plant, and also among populations. In general, stems and roots exhibited relatively high diversity of endophytes (Sreerama Kumar P, unpublished). In a related comparative study, a mikania population (AN-9) from Andaman and Nicobar Islands harboured more endophytes than did a Karnataka population (KA-1). Similar studies on mikania samples from Kerala may give definitive leads. In its native region (Mexico), parthenium is known to harbour *Alternaria zinniae*, *A. helianthi*, *Cylindrocarpon* sp., *Curvularia brachyspora*, *Fusarium* sp., *Nigrospora oryzae*, *Penicillium funiculosum* and *Periconia* sp. as endophytes in leaf tissues (Romero *et al.* 2001). Similarly, parthenium plants in Bengaluru harbour many genera of endophytic fungi and bacteria (Velavan V and Sreerama Kumar P, unpublished). A comparison between native and exotic populations of *P. hysterophorus* is yet to be done.



Fig. 1. Endophytic bacteria isolated from *Mikania micrantha* [lamina, petiole, stem and root (anticlockwise from top right)].

How to go about?

A thorough understanding of the endophytic diversity of invasive weeds — which could be future targets for CBC in India (Sreerama Kumar *et al.*, 2008) — is necessary for zeroing in on the right candidate bioagent. For instance, if *Ambrosia psilostachya*, which has been reported only from Tumakuru district of Karnataka (Prasad *et al.*, 2013), were to spread widely, biocontrol options may have to be considered and an analysis of endophytes associated with the

plant is warranted. Similarly, silver-leaf nightshade (*Solanum elaeagnifolium*) in Tamil Nadu and Karnataka deserves attention. Also, it is important to analyse and compare the endophyte diversity in invasive weeds that occupy the same niche (Fig. 2) to understand if there has been any cross-contamination between weed species. The endophyte assemblage may affect the weed's palatability to certain insects. Therefore, where insects fail, other category of agents may offer control. In the case of *Lantana camara*, for example, the two rust species, *Prospodium tuberculatum* and *Puccinia lantanae*, may be considered (Sreerama Kumar *et al.*, 2008). Of course, desired control of lantana weed has not been achieved through insects in most of the tropics, except Hawaii (McFadyen, 1998). If the endophytes are found to interfere with the establishment of any pathogenic fungus with biocontrol potential, the latter has to be discarded (Romero *et al.*, 2001).



Fig. 2. Three invasive alien weeds (a. *Chromolaena odorata*; b. *Mikania micrantha*; c. *Mimosa diplotricha*) occupying the same niche (near Thrissur, Kerala, November 2005).

CONCLUSIONS

If the newly proposed E–ERH gains wider appreciation, better recognition and greater support through supplementary data generation, it is expected that the future programmes of CBC of weeds in India would accommodate the ‘endophyte angle’ in the project-conception stage itself. Understanding the overall constitution, individual identities, as well as general and specific functions of endophytes in a target weed may become crucial prior to the selection of the candidate bioagent in the coming years. Traditional methods may possibly be sufficient for isolating and studying culturable endophytes; but for recalcitrant endophytes, a metagenomic analysis of the DNA isolated from endophytes within the host plant sample should be useful to study their diversity. In addition to the existing criteria, future releases of biocontrol agents should also depend on the

nature of endophytes present in a population of the target weed in the country of introduction.

REFERENCES

- Arnold AE. 2007. Understanding the diversity of foliar endophytic fungi: progress, challenges, and frontiers. *Fungal Biol Rev.* **21**: 51–66.
- Azevedo JL, Maccheroni Jr W, Pereira JO, de Araújo WL. 2000. Endophytic microorganisms: a review on insect control and recent advances on tropical plants. *Electron J Biotechnol.* **3**(1): 40–65.
- Bittleston LS, Brockmann F, Wcislo WT, Van Bael SA. 2011. Endophytic fungi reduce leaf-cutting ant damage to seedlings. *Biol Lett.* **7**: 30–32.
- Carroll G. 1988. Fungal endophytes in stems and leaves: from latent pathogens to mutualistic symbionts. *Ecology* **69**: 2–9.
- Cherry AJ, Banito A, Djegui D, Lomer C. 2004. Suppression of the stem-borer *Sesamia calamistis* (Lepidoptera; Noctuidae) in maize following seed dressing, topical application and stem injection with African isolates of *Beauveria bassiana*. *Int J Pest Manage.* **50**: 67–73.
- Clay K. 1988. Fungal endophytes of grasses: a defensive mutualism between plants and fungi. *Ecology* **69**: 10–16.
- Clay K. 1996. Interactions among fungal endophytes, grasses and herbivores. *Res Popul Ecol.* **38**: 191–201.
- Claydon N, Grove JF, Pople M. 1985. Elm bark beetle boring and feeding deterrents from *Phomopsis oblonga*. *Phytochemistry* **24**: 937–943.
- Colautti RI, Ricciardi A, Grigorovich IA, MacIsaac HJ. 2004. Is invasion success explained by the enemy release hypothesis? *Ecol Lett.* **7**: 721–733.
- Dhileepan K. 2009. Managing parthenium weed across diverse landscapes: limitations and prospects. p. 227–260. In: Inderjit S (ed.). *Management of Invasive Weeds. Invading Nature—Springer Series in Invasion Ecology (Vol. 5)*, Springer Science+Business Media B.V.
- Dodd S, Ganley R, Bellgard S, Than D. 2010. Endophytes associated with *Cirsium arvense* – a step toward understanding their role in the success/failure of *Sclerotinia sclerotiorum* as a bioherbicide. p. 235–238. In: Zydenbos SM (ed.). *Proceedings of the 17th Australasian Weeds Conference. New Frontiers in New Zealand: Together We Can Beat the Weeds*, Christchurch, New Zealand, 26–30 September 2010.
- Estrada C, Degner EC, Rojas EI, Wcislo WT, Van Bael SA. 2015. The role of endophyte diversity in protecting plants from defoliation by leaf-cutting ants. *Curr Sci.* **109**(1): 55–61.
- Evans HC. 2008. The endophyte–enemy release hypothesis: implications for classical biological control and plant invasions. p. 20–25. In: Julien, MH, Sforza R, Bon MC, Evans HC, Hatcher PE, Hinz HL, Rector BG (eds). *Proceedings of the XII International Symposium on Biological Control of Weeds* [La Grande Motte (Montpellier), France, 22–27 April 2007], CAB International, Wallingford, UK.
- Fisher PJ, Petrini O. 1992. Fungal saprobes and pathogens as endophytes of rice (*Oryza sativa* L.). *New Phytol.* **120**: 137–143.
- Holmes KA, Schroers H, Thomas SE, Evans HC, Samuels GJ. 2004. Taxonomy and biocontrol potential of a new species of *Trichoderma* from the Amazon basin of South America. *Mycol Prog.* **3**: 199–210.
- Hyde KD, Soyong K. 2008. The fungal endophyte dilemma. *Fungal Divers.* **33**: 163–73.
- Julien MH, Kerr JD, Chan RR. 1984. Biological control of weeds: an evaluation. *Prot Ecol.* **7**: 3–25.
- Keane RM, Crawley MJ. 2002. Exotic plant invasions and the enemy release hypothesis. *Trends Ecol Evol.* **17**: 164–170.
- Matta A. 1971. Microbial penetration and immunization of uncongential host plants. *Annu Rev Phytopathol.* **9**: 387–410.
- McFadyen REC. 1998. Biological control of weeds. *Annu Rev Entomol.* **43**: 369–393.
- Newcombe G, Shipunov A, Eigenbrode SD, Raghavendra AKH, Ding H, Anderson CL, Menjivar R, Crawford M, Schwarzländer M. 2009. Endophytes influence protection and growth of an invasive plant. *Commun Integr Biol.* **2**(1): 1–3.
- Petrini O. 1991. Fungal endophytes of tree leaves. p. 179–197. In: Andrews JH, Hirano SS (eds). *Microbial Ecology of Leaves*, Springer Verlag, New York, USA.

- Petrini O. 1996. Ecological and physiological aspects of host-specificity in endophytic fungi. p. 87–100. In: Redlin SC, Carris LM (eds). *Endophytic Fungi in Grasses and Woody Plants: Systematics, Ecology and Evolution*, APS Press, St. Paul, USA.
- Prasad TVR, Rao RR, Sanjay MT, Sharma RA. 2013. *Ambrosia psilostachya* DC (Asteraceae) – a new record but a potential threat to Indian flora. *Curr Sci.* **104**(3): 294–296.
- Romero A, Carrión G, Rico-Gray V. 2001. Fungal latent pathogens and endophytes from leaves of *Parthenium hysterophorus* (Asteraceae). *Fungal Divers.* **7**: 81–87.
- Saikkonen K, Wäli P, Helander M, Faeth SH. 2004. Evolution of endophyte–plant symbioses. *Trends Plant Sci.* **9**(6): 275–280.
- Saikkonen K, Ruokolainen K, Huitu O, Gundel PE, Piltti T, Hamilton CE, Helander M. 2013. Fungal endophytes help prevent weed invasions. *Agric Ecosyst Environ.* **165**: 1–5.
- Samuels GJ, Suarez C, Solis K, Holmes KA, Thomas SE, Ismaiel A, Evans HC. 2006. *Trichoderma theobromicola* and *T. paucisporum*: two new species isolated from cacao in South America. *Mycol Res.* **110**: 381–392.
- Shipunov A, Newcombe G, Raghavendra AKH, Anderson CL. 2008. Hidden diversity of endophytic fungi in an invasive plant. *Am J Bot.* **95**: 1096–1108.
- Sreerama Kumar P, Rabindra RJ, Ellison CA. 2008. Expanding classical biological control of weeds with pathogens in India: the way forward. p. 165–172. In: Julien MH, Sforza R, Bon MC, Evans HC, Hatcher PE, Hinz HL, Rector BG (eds). *Proceedings of the XII International Symposium on Biological Control of Weeds* [La Grande Motte (Montpellier), France, 22–27 April 2007], CAB International, Wallingford, UK.
- Tomley AJ, Evans HC. 2004. Establishment of and preliminary impact studies on the rust, *Maravalia cryptostegiae*, of the invasive alien weed, *Cryptostegia grandiflora*, in Queensland, Australia. *Plant Pathol.* **53**: 475–484.
- Vega FE, Posada F, Aime MC, Pava-Ripoll M, Infante F, Rehner SA. 2008. Entomopathogenic fungal endophytes. *Biol Control* **46**: 72–82.
- Webber J. 1981. A natural control of Dutch elm disease. *Nature* **292**: 449–451.
- Winston RL, Schwarzländer M, Hinz HL, Day MD, Cock MJW, Julien MH (eds). 2014. *Biological Control of Weeds: A World Catalogue of Agents and Their Target Weeds*, 5th edition, USDA Forest Service, Forest Health Technology Enterprise Team, Morgantown, West Virginia, USA, 838 pp.