

Californian thistle (*Cirsium arvense*): endophytes and *Puccinia punctiformis*

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Abstract

Californian thistle (*Cirsium arvense*) is a troublesome weed in pastures and cropping systems. The fungal biocontrol agent *Puccinia punctiformis*, commonly referred to as thistle rust, performs inconsistently on *C. arvense*. Problems with *P. punctiformis* establishment and control of *C. arvense* may be attributable to differing plant endophytic populations in various environments. This article provides an overview of the relationships between endophytes and their host, but also between endophytes and pathogens with a focus on rust pathogens. This review provides insights into reasons why *P. punctiformis* performs inconsistently and identifies gaps in our knowledge. Filling these gaps may help to improve performance of this classical fungal biocontrol agent.

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Keywords: Californian thistle; Canada thistle; creeping thistle; endophyte–host relationships; host–pathogen relationships; endophyte–pathogen relationships

1 INTRODUCTION

Cirsium arvense L. (Scop.), commonly referred to as Californian thistle, is one of the most troublesome weeds worldwide. It is considered a major weed in 37 countries, affecting the yield of 27 crops, including wheat, barley, oats, beans and peas.¹ It is a pioneer plant, which thrives in disturbed or bare ground. Hence, it is problematic on arable and pastoral farmland. The weed can form large patches and outcompetes more desirable vegetation.² It is extremely competitive in low-growing vegetation, where its presence leads to yield losses in crops as well as pastures.^{3,4} Despite the high nutritional value of *C. arvense*, grazing avoidance occurs primarily as a result of the spines on the leaf margin.³

Cirsium arvense is commonly controlled by herbicides with glyphosate being one of the main herbicides used.⁵ More long-lasting and sustainable ways to control *C. arvense* are desirable and biocontrol is considered as an option.

The fungus *Puccinia punctiformis* is highly host-specific and systemic infection occurs after plant root colonization.⁶ Hence, this fungus is considered as a biocontrol option.⁷ Persistent problems with achieving systemic infection have halted progress in the development of *P. punctiformis* as a classical biocontrol agent, whereby the pathogen will spread and self-perpetuate. The factors required for systemic infection are not well understood. The distance the fungus needs to travel from the local infection point to the root is thought to be an important factor, which is directly related to plant age.⁸ Endophytes, present in *C. arvense*, may be a factor mitigating the establishment of systemic *P. punctiformis* infection.⁹ The study of interactions between endophytes and their host is a growing area of research, with the effect of endophytes on pathogenic biocontrol agents generally not considered.^{10,11} To date, and to the best of our knowledge, no studies examining *C. arvense* endophyte effects on *P. punctiformis* have

been reported in the scientific literature. This article will review current literature on endophyte–host and endophyte–pathogen interactions, and evaluate how this knowledge could be used in the context of biocontrol of *C. arvense* by *P. punctiformis*.

2 CIRSIUM ARVENSE

Cirsium arvense is a perennial herb within the Asteraceae family. It is common along road and waterway verges, in woodland clearings, waste ground, cultivated paddocks and arable land, and grows well in fertile soils, with growth further stimulated by soil moisture and fertilizer applications.^{3,12} Nitrogen fertilisation increases root growth in the top 20 cm of the soil, leading to more adventitious shoots arising.¹³ Primarily *C. arvense* reproduces vegetatively with sexual reproduction also occurring. Root buds, on the lateral roots, develop into adventitious shoots with many adventitious flowering and nonflowering shoots arising from the root system.^{3,14} Most of the root buds have correlative inhibition, which results in preferential development of apical buds.¹² After fragmentation, or other adverse events such as herbicide spraying, mowing or disease, buds can be released from this inhibition leading to the development of shoots.^{3,12} As a consequence of the vegetative spread of the plant, it typically forms circular patches. A patch is clonal and consists of one plant producing multiple shoots from its rhizome-like roots. Hence, a patch

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generally has one genotype although neighbouring patches can differ genetically.^{15,16}

In most localities, *C. arvense* is dioecious, but can be gynodioecious.^{14,17} The species obligately outcrosses with pollination, apparently, solely by insects with honey bees, bumble bees and hoverflies being among the pollinators.¹⁸ Seeds are the main reproductive method of *C. arvense* to establish in new habitats. Weed seeds generally have a high genetic variability, which increases their chances of survival in new habitats.¹⁶ Seedling establishment is most successful in open areas, tending to establish in disturbed or bare soil as they are sensitive to competition from other plants.¹²

In early winter, most plant resources have been transferred to the roots, with the shoots beginning to die off. Stored sugars act as an antifreeze and protect the roots from freezing temperatures.^{12,19} Once these sugars are remobilized to the adventitious shoots in early spring, the plant becomes more susceptible to frost. A small number of enlarged root buds and subterranean shoot development occurs in winter, leading to frost-sensitive shoots. Frost damage removes apical dominance, which causes a rapid flush of new adventitious shoots in spring.¹²

New Zealand hill-country is a challenging environment with steep slopes and soils of low natural fertility.²⁰ In this environment, control of *C. arvense* is particularly problematic, as common control methods, such as mowing and spraying, are impractical on steep gradients.¹² These methods typically do not provide long-term results and are not cost-effective.² High-intensity/high-frequency grazing generally leads to an increase in thistle populations as it removes competition from neighbouring plants.²¹ Crop rotation has a similar effect as regular soil cultivation will enhance root fragmentation.³ Hence, additional control methods are desirable.

Biocontrol is being researched as a control option for *C. arvense*. Biocontrol agents as part of an integrated pest management approach, where multiple control methods are employed, are more successful than using individual control methods.² Most biocontrol agents utilized for *C. arvense*, have performed inconsistently in the field. This includes insect biocontrol agents such as *Urophora cardui* as well as fungal control agents such as *Sclerotinia sclerotiorum*.^{22–24} For insects, this is attributed to the environment, which affects their establishment in a new area. For fungal biocontrol agents, reasons for performance inconsistencies are unknown. Resident endophytes within the plant may interfere with the establishment and success of fungal biocontrol agents.^{9,25}

3 ENDOPHYTES

No naturally growing plant is free from endophytes, which inhabit plant tissues without causing disease symptoms. Some will live their entire life within the plant, whilst others are facultative and only live part of their lifecycle within plant tissue. Among endophytes, there are latent pathogens, which will only cause disease when circumstances are disease-conducive. This may be weather-related events, nutrient deficiencies or weakened plant defences such as infection by another pathogen.²⁶ Other endophytes may become saprophytic when the plant dies of other causes and will decompose plant tissues. Endophytes can actively grow through host tissues or stay localized.²⁷

Endophytes can have necrotrophic, biotrophic or hemibiotrophic interactions with their host. Biotrophic fungi such as *P. punctiformis* are obligate parasites/symbionts, accumulating

nutrients from their host through a fungal matrix. They generally have long generation times and can cause systemic infections, many displaying multiple lifecycles within the same plant host.²⁶ Despite their biotrophic nature, some biotrophic pathogens can do much damage to a plant population. For example, the rust *Puccinia myrsiphylli* is an effective classical biocontrol agent in its introduced area (New Zealand and Australia) with severe damage to populations of *Asparagus asparagoides* occurring, leading to effective control of this weed.²⁸ Biotrophs are host-specific and produce effectors such as the *pep1* effector produced by *Ustilago* spp. to suppress host defence systems.^{29,30} Upon host entry, biotrophs form a haustorium, which creates an extra-haustorial matrix. This matrix functions as an interface between the plant and fungus. The fungus uptakes nutrients such as carbohydrates and amino acids from the plant through this matrix.³¹ Both plant and fungus secrete molecules at this interface that affect each other, which allows the fungus to redirect gene expression of the host to enhance compatibility and nutrient transportation to the extra-haustorial matrix.³² Formation of haustoria is a key feature of an obligate biotrophic fungus. Necrotrophs generally have a short generation time, produce many propagules, cause nonsystemic infection in plants and have a broad host range.²⁶ The conditions conducive to necrotrophy most commonly include: leaf aging, senescence of the host plant, a nutrient imbalance or changing environmental factors.³³ They kill their host cells by a combination of cell toxins and wall-degrading enzymes. Toxins can suppress plant defence responses as well as kill host cells.³⁴ Destruction of the host's plasma membrane leads to release of nutrients that the fungus requires. Eventually, host tissues are decomposed by the fungus.³⁵ Most hemibiotrophs differentiate themselves from necrotrophs by formation of haustoria or haustoria-like structures in their biotrophic phase.³⁶ The non-haustoria-forming hemibiotrophs alter host metabolism to suit their nutritional needs and acquire these nutrients in the leaf apoplast.³⁷ When hemibiotrophs switch from biotrophy to necrotrophy depends on molecular as well as physiological factors.

Many endophytes are opportunists, which can live in the soil as saprophytes and only infect plants when opportunity arises.³⁸ This may be associated with plant stress, senescence or otherwise debilitation. Fungi can invade wounded plants and can be neutral or show weak virulence after entering the plant. Opportunistic endophytes are generalists and often act as necrotrophs towards the end of a plant's life.^{39,40} Some fungi may transition their way of interaction as well as morphology in response to their host and environment. *Colletotrichum acutatum* is such an example, whereby thick primary hyphae and haustoria are produced in the biotrophic phase followed by narrow, secondary hyphae in its necrotrophic phase. Fungal lifestyle is dependent on host type and life stage. For example in a blueberry (*Vaccinium* spp.) host, *C. acutatum* is quiescent on immature fruits and switches to necrotrophy upon fruit ripening, whereas on strawberries the fungus has no detectable biotrophic phase and acts like a necrotroph.⁴¹ On watermelons, *C. acutatum* is a commensal, whilst *C. magna* confers disease- and drought- resistance to this host.⁴² Hence, effects of endophytes on their host may be positive, negative or neutral, dependent on the host–fungus relationship.

Competition for resources between micro-organisms and herbivores might drive evolution towards mutualism, in which the endophyte protects the host and the plant provides metabolites to the endophyte.⁴³ Generally, the relationship between a mutualistic endophyte and a plant is long-term as both partners benefit.^{25,38} Endophytes can improve resistance to biotic and abiotic

stresses or increase plant growth.^{38,44} Increased growth can be accomplished by production of growth hormone(s) by endophytes, such as auxin precursors or cytokinins, or by improved uptake of soil nutrients such as phosphorus and nitrogen.⁴⁵ Endophytes can also upregulate expression of plant genes. For example, *Trichoderma hamatum* confers drought tolerance to its host *Theobroma cacao* by upregulation of the host genes *ERD1*, *CBL1*, *DREB2A*, *RD29A* and *ANAC072*, which encode transcription factors that are associated with plant stress responses.^{46–48} Upregulation of these plant genes may lead to production of enzymes, which enhance nutrient acquisition, or increase resistance against biotic stresses.^{38,45} The endophytes themselves also can produce compounds such as antioxidants, which can improve host resistance to abiotic stresses.^{33,45} In turn, host plants provide carbohydrates to endophytes. This relationship can be reversed under environmental stress, in which a previously mutualistic endophyte turns pathogenic.²⁶ When the host starts to senesce, the endophyte has immediate access to plant nutrients and thus a competitive advantage over soil-born saprophytes.³³

Some pathogens have a facultative endophytic lifestyle before they become pathogenic. *Botrytis cinerea* is one example of a fungus that was thought to be an aggressive necrotroph but can live endophytically in healthy plants.^{49,50} This highlights the important role environment plays in pathogenicity of fungi, in which disease is the result of an imbalance.⁵⁰ These endophytes become pathogenic when the plant is stressed by biotic or abiotic factors.⁴³ In the case of weed biological control, causing an imbalance between weed hosts and latent pathogens could be a possible avenue for manipulation to facilitate greater efficacy of biocontrol agents; thus far it has been underexplored. For example, the effect seen when mowing *C. arvense* before heavy rainfall is most likely a disturbance of the balance between host plant and one or more latent pathogens.⁵¹

Plants might be separated from natural enemies when introduced into a new area. This may contribute to their invasiveness as they often have an increased performance in their introduced range compared to their native range. This is the basis of the enemy release hypothesis (ERH).^{52,53} This hypothesis is a cornerstone of classical, inoculative biological control.⁵² As an extension of this hypothesis, presence or absence of certain endophytes also might be a determinant if a plant becomes invasive in its new range.^{54,55} Many endophytes can produce plant growth-enhancing substances, as well as antiherbivore and antifungal metabolites. In their natural range, plants live with co-evolved mutualistic fungi as well as with natural enemies, including pathogens.⁵³ Protection from pathogens by mutualistic fungi comes at a trade-off paid in the price of carbohydrates.⁵⁶ Plants should usually arrive with a limited number of endophytes in a new range, as many endophytes transfer horizontally. Absence of these endophytes frees up resources for growth and reproduction. As co-evolved natural enemies, including pathogens, are absent, plant fitness is increased.^{53,57} Native plants will not have this advantage, which gives the competitive advantage to newly introduced plants.⁵⁷ In classical biocontrol, often introduction of one natural enemy from the native range can restore the balance and reduce invasiveness of the introduced plant.^{54,55} Counter to ERH, is the enhanced mutualism hypothesis (EMH). The rationale is that invasive plant species enter a strong mutualistic or parasitic relationship with native mycorrhizal species. This might help the invader to displace native species as it exploits the benefits of the relationship, sometimes without paying the costs for mutualism.^{58,59} Hence, competitive interactions between invasive and

native plants may be mediated by these fungi.⁵⁸ It seems that *C. arvense* is unselective in the fungi it allows to colonize and its endophytic populations are a function of location.⁶⁰ Furthermore, a positive feedback mechanism with arbuscular mycorrhizae was found, enhancing its growth, whereas infiltration with pathogens/saprophytes had no negative effect. It may be that *C. arvense* accumulates pathogens at a slower rate than beneficial microorganisms.⁵⁹ Hence, the EMH is likely to apply to *C. arvense* although it is not excluded that presence of certain fungi from its native range could shift the balance and reduce invasiveness of this plant.

4 CALIFORNIAN THISTLE RUST (*PUCCINIA PUNCTIFORMIS* (F. STRAUSS) RÖHL)

Puccinia punctiformis is a biotrophic fungus, which is highly host-specific to *C. arvense*. If systemic infection is achieved, the fungus moves into plant roots, which may potentially kill the plant.⁷ Few *P. punctiformis*-infected *C. arvense* plants flower and seeds are rarely formed.^{13,61} Infected plants have increased respiration and lower osmotic pressure, which makes them wilt quicker than uninfected plants.^{61,62} These are qualities that make this fungus an interesting option for biocontrol. *Puccinia punctiformis* is autoecious and heterothallic.^{7,8} Only teliospores and urediniospores can cause systemic infection. Teliospores are produced in late summer. Telia containing teliospores, are observed as black circular spots on the underside of leaves. When the plant starts to senesce in autumn, leaves bearing telia with teliospores fall off onto newly emerging shoots. Spores germinate on these shoots and form a basidium with four basidiospores. These infect the rosette and produce mycelium, which moves down into the roots to overwinter. In spring, systemically infected shoots arise, and orange-coloured underside of leaves. Each shoot bears the spermatogonia of one mating type, with both mating types being equally represented in a field.^{8,63} These produce spermatia, which fuse with receptive hyphae. This results in aeciospores being produced within aecia. Aeciospores, produced once within a growing season, are wind-dispersed to nearby leaves. They subsequently form orange/red-coloured uredinia, containing urediniospores, on the underside of leaves. Wind dispersal of urediniospores occurs throughout the growing season, facilitating inoculum spread and subsequent host infection.⁶³ These infections are normally localized and will not progress into systemic disease, unless they infect a young shoot early in the growing season. Uredinia will gradually change into telia, containing teliospores, as the season progresses.^{8,63,64} There are two opportunities for establishment of systemic infection: in spring by primary urediniospores and in autumn by teliospores. This disease cycle is illustrated in Fig. 1.

There are major challenges to overcome if *P. punctiformis* is used within a biocontrol programme. Mass production is so far unachievable and the artificial establishment of systemic infection in the field is difficult. Low germination rates of teliospores have been another limiting factor.⁶⁵ When achieving systemic infection, it results in reduced flowering and root bud production as well as decreased root biomass in pot trials.⁶⁶ In an area where *P. punctiformis* is well-established, the harvest of diseased leaves that are subsequently ground and disseminated within the field, may help facilitate infection in disease-free areas. Spreading the telia-containing leaf fragments over newly emerging rosettes mid- to late-autumn in conjunction with timing to coincide with field moisture may aid infection.^{67,68} Additionally, mycelium must

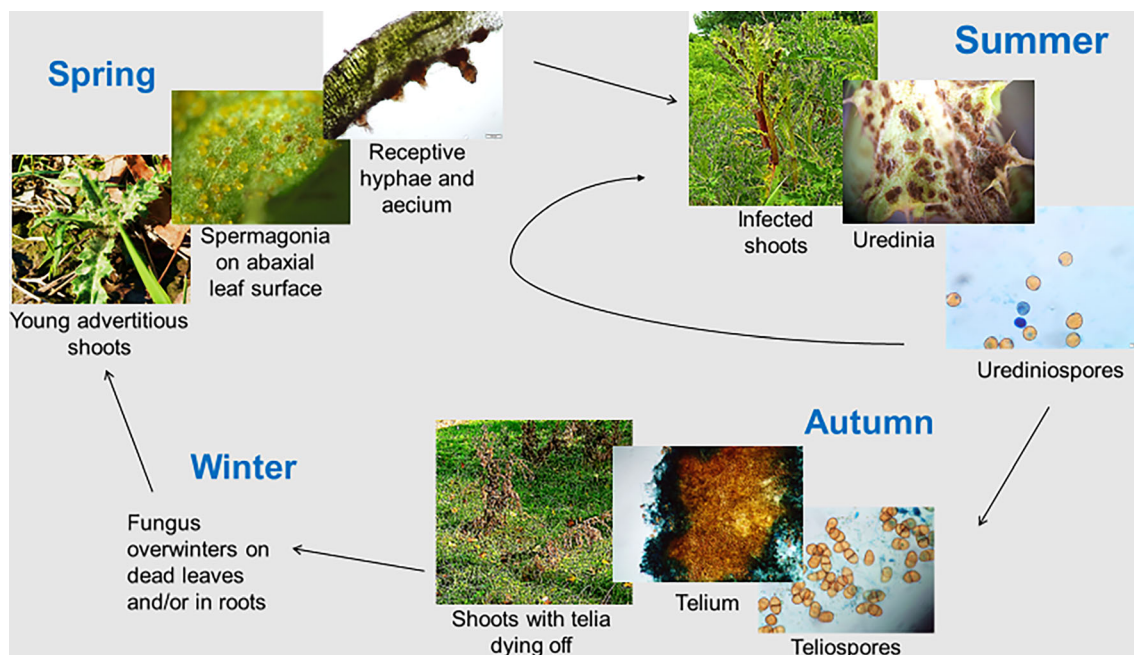


Figure 1. Disease cycle of *Puccinia punctiformis*. Teliospores infect newly emerging rosettes and then move into the roots to overwinter as mycelium. Systemically infected shoots arise in spring and produce aeciospores, which spread to new leaves. Urediniospores are produced within the season and gradually uredinia change into telia and teliospores are produced.

be present in root buds before shoots develop from buds in spring. For this to occur, the mycelium must grow down from the infected leaf to the roots.⁸

In a natural disease cycle, urediniospores are the primary source of inoculum. These spores disperse readily with inoculum increasing throughout the season. Spraying urediniospores, with spore concentrations between 0.5 and 1 mg mL⁻¹ was evaluated.^{69,70} The distance that mycelium developing from urediniospores needs to travel to the roots is thought to be important. Hence, spring application may be ideal as plants will be mostly in a rosette form and thus leaves would be only a short distance from roots, reducing the distance that the mycelium needs to travel. Infection with urediniospores in spring can lead to systemic infection in the same growing season.⁶⁴ Systemic infection percentages can be between 5% and 50% between regions. Reasons for these differences are unknown.^{67,71} Systemic infection incidence can be increased by mowing the field during summer months. This will redistribute inoculum, leading to more shoots becoming infected.^{7,72} Using an insect vector, such as the weevil *C. onopordi*, is another way that researchers have attempted to mimic natural processes. This has been successful in field trials in Europe with higher infection percentages being achieved with insect vectors than solely applying urediniospores or teliospores.^{73–75}

5 ENDOPHYTE–PATHOGEN RELATIONSHIPS

Endophytes can alter outcomes of a host–pathogen interaction. They can interact with a pathogen directly or induce resistance in the host.⁷⁶ This is a desirable effect when trying to combat pathogens on useful crop or pasture plants, but not when host plants are weeds. Effects of endophytic species on biocontrol agents are on the spectrum of antagonism to synergism. Hence, it is important to increase understanding of the effects that key

endophytic species have on the functionality of fungal biocontrol agents.⁷⁷ Little research has been done on the effects of endophytes on biocontrol. The degree and nature of endophyte interference with biocontrol depends on species and genotype of the endophyte as well as on environmental factors.⁷⁸ An antagonistic effect of endophytes on biocontrol agents seems to be most common. For example, the endophytes *Alternaria alternata*, *Cladosporium oxysporum* and *Colletotrichum acutatum* reduced the severity of infection by *Puccinia komarovii* var. *glanduliferae* on the weed *Impatiens glandulifera*, which resulted in higher biomass of the weed than in the absence of these endophytes.⁷⁹ An antagonistic effect was observed between the biocontrol agent *Puccinia polygoni-amphibii* var. *tovariae* and the endophytes *Phoma* spp. and *Alternaria* spp. on the weed *Fallopia japonica*, with fewer rust uredinia being produced in the presence of these endophytes. In the same study, a synergistic effect was exhibited between the rust and *Phomopsis* HS-SZ1J. Research showed that the effect was specific to this strain. Hence, some endophytes have the potential to be used in synergy to control weeds.⁷⁷

Some fungal endophytes produce antifungal substances that can affect pathogens, which could be deleterious for fungal biological control agents; this interaction is antibiosis. The antifungal compounds produced are generally secondary metabolites with an antagonistic, deterrent or inhibitory effect on the pathogen.²⁵ For example, the endophyte *Phomopsis cassia* in the host *Cassia spectabilis* produces the metabolite 3, 11, 12-trihydroxycadalene which has an antagonistic effect on *Cladosporium cladosporioides*.⁸⁰ Some have an indirect protective effect. For example, they can change water availability on leaf surfaces, preventing a pathogen from entering the leaf. Others can upregulate mobilization of nutrients to increase plant nutrients or move within a plant to the location of the pathogen.^{25,81} The presence of *Trichoderma harzanium* and/or *T. viride* has been shown to inhibit germination of *Puccinia graminis*. *Trichoderma harzanium* produces

the metabolite trichodermin, which inhibits protein synthesis needed for spore germination. It also produces nonanoic acid, which prevents the pH from rising, an essential step needed for spore germination. Both *T. viride* and *T. harzanium* produce several antifungal compounds, which prevent spore germination of many pathogenic fungi including rust fungi.⁸² *Trichoderma* spp. also can interact directly with pathogens by mycoparasitism. In this interaction, they grow directly towards their pathogen host and make physical contact by coiling their hyphae around hyphae of the pathogen. They use cell-wall degrading enzymes to penetrate into the pathogen's lumen, from which they directly absorb nutrients.^{11,83}

Other mycoparasites of rust species have been discovered. *Eudarluka caricis* is a mycoparasite of >300 rust species.⁸⁴ Enzymes produced by other micro-organisms also can affect pathogens. For example, chitinase isolated from *Fusarium chlamydosporum* can prevent urediniospore germination and lyse germ tubes of *Puccinia arachidis*.⁷⁶ Applying a leaf homogenate containing leaf fungal endophytes significantly reduces the pustule density of *Austropuccinia psidii*, showing that the antagonistic effect is not always on just one species, but can be the effect of a microbial community.⁸⁵ These examples show that plants have mycobiota, which may interfere with the effectiveness of a biocontrol agent.⁷⁷ Distribution patterns of endophytes are influenced by location; thus, the same plant can host different endophyte populations in different locations. Hence a biocontrol agent might work in one location, but not another.^{9,77} In some cases, the endophyte fills the same niche as the pathogen.⁸⁶

6 CONCLUDING REMARKS

There are many factors affecting the biocontrol efficacy of *C. arvense*. The plant itself is adaptable to the environment as a result of its high genetic diversity and phenological adaptations to withstand adverse conditions. Its competitiveness is enhanced in low-growing vegetation, where it can receive more sunlight. It is inhabited by many endophytes, which can differ with location. Especially outside its native range, it is extremely competitive. This might be either because it is released from co-evolved pathogens or as a consequence of new beneficial plant–pathogen relationships being formed in its invasive ranges. Although limited research on the effects of endophytes on fungal biocontrol agents has been carried out, the mycobiome of plants directly and indirectly affect pathogen establishment and it is reasonable to assume that this applies to pathogens used for biocontrol as well. The combination of a competitive plant with the protection of endophytes will most likely continue to present challenges in the biocontrol of this weed. Increasing our understanding of interactions between endophytic fungi and the biocontrol *P. punctiformis* is needed to resolve its inconsistent performance. Experiments are needed in which the *P. punctiformis* pathogenicity in plants with differing populations of endophytes is determined. Also, the differences in endophyte communities in symptomatic and asymptomatic shoots should be studied to determine which endophytes may have a synergistic relationship with *P. punctiformis*. This could be useful to augment endophyte community composition to enhance the effectiveness of *P. punctiformis*. Additionally, *P. punctiformis* strains from different regions could be introduced. As *P. punctiformis* and *C. arvense* co-evolved, using a strain of the fungus isolated from a genetically different *C. arvense* population may result in better control of this weed.

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CONFLICT OF INTEREST

No conflict of interest.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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