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## **Ray blight of pyrethrum in Australia: A review of the current status and future opportunities**

**M. A. H. B. Bhuiyan<sup>a</sup>, N. Vaghefi<sup>c</sup>, and P. W. J. Taylor<sup>b\*</sup>**

<sup>a</sup>*Department of Plant Pathology, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Gazipur, Bangladesh;* <sup>b</sup>*Faculty of Veterinary and Agricultural Sciences, University of Melbourne, Vic, 3010, Australia;* <sup>c</sup>*Centre for Crop Health, University of Southern Queensland, Qld, 4350, Australia.*

\*Email: paulwjt@unimelb.edu.au

### **Abstract**

Ray blight caused by *Stagonosporopsis tanacetii* is one of the most important diseases of pyrethrum (*Tanacetum cinerariifolium* (Trevir.) Sch. Bip.), a perennial herbaceous plant cultivated for the extraction of insecticidal pyrethrins in Australia. The disease is responsible for complete yield loss in severe outbreaks. Infected seed is considered as the principal source of *S. tanacetii*. Infection hyphae remain only in the seed coat and not in the embryo, resulting in pre- and post-emergence death of seedlings and latent infection. Therefore, quantification of the level of infection by *S. tanacetii* within seed using qPCR assay is important for efficient management of the disease. *Stagonosporopsis tanacetii* completes its lifecycle within 12 d after leaf inoculation through production of pycnidia and can infect every tissue of the pyrethrum plant except vascular and root tissue. Ray blight epidemics occur in pyrethrum fields through splash dispersal of pycnidiospores between adjacent plants.

Besides steam sterilization, thiabendazole/thiram and fludioxonil are effective seed-treating agents. This article has been accepted for publication and undergone full peer review but has not been through the copyediting, typesetting, pagination and proofreading process, which may lead to differences between this version and the Version of Record. Please cite this article as doi: 10.1111/ppa.13000

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chemicals in controlling *S. tanacetii* before planting begins. Ray blight is currently managed in the field through the foliar application of strobilurin fungicides in first 1-2 years of crop establishment. Later on, difenoconazole and multi-site specific fungicides in next 2-3 years during early spring successfully reduce ray blight infestation. Avoiding development of resistance to fungicides will require more sustainable management of ray blight including the development and deployment of resistant cultivars.

**Keywords** *Tanacetum cinerariifolium*, *Stagonosporopsis tanacetii*

## **Introduction**

Pyrethrum (*Tanacetum cinerariifolium* (Trevir.) Sch. Bip.), a perennial herbaceous plant belonging to the *Asteraceae* family, is commercially grown in Australia to produce insecticidal pyrethrins (Zito, 1994; Katsuda, 1999). Pyrethrins are highly effective insecticides (Andreev *et al.*, 2008; Sladonja *et al.*, 2014) used globally in food preservation and organic farming (Li *et al.*, 2011; Sladonja *et al.*, 2014). Around 94% of the pyrethrin content is produced within the secretory ducts and oil glands of achenes of pyrethrum flowers. Dried pyrethrum flowers contain about 1-2% pyrethrins by weight. The natural form of pyrethrins includes six polyacetylenes - pyrethrin I, pyrethrin II, jasmolin I, jasmolin II, cinerin I and cinerin II (Pan *et al.*, 1995). Among these, pyrethrin I and II are used for controlling insects (Elliot, 1995). Pyrethrins have low mammalian toxicity and insects have not developed resistance to these insecticides (Crombie and Elliot, 1961).

Pyrethrum originated from northern Albania and Croatia (Dalmatia) (Gnadinger, 1936). It is currently commercially grown in East Africa (Kenya, Rwanda and Tanzania), Australia (Tasmania and Victoria), China and Papua New Guinea (Pethybridge *et al.*, 2008b). In Australia, in 2017, pyrethrum was grown over approximately 3000 ha producing about 7,000

MT of flowers, which accounted for two thirds of global production (Anon, 2018). The northwest coast of Tasmania between Deloraine (41° 31' S; 146° 39' E) and Table Cape (40° 56' S; 145° 43' E) is the major pyrethrum producing area in Australia (Pethybridge *et al.*, 2008b). To increase production to meet increasing global demand, pyrethrum production has also expanded to the Ballarat region (37° 56' S; 143° 85' E) of Victoria (Suraweera *et al.*, 2014).

High-input farming system with the use of herbicides, fungicides, fertilizers and overhead irrigation is practiced for the cultivation of pyrethrum in Australia. Seeds are used as the primary planting material and fields are prepared in late winter and/or early spring (July-September). Harvest of flower heads is performed mechanically after establishment in the summer (December-January), 15-18 months after planting. After the first harvest, new shoots emerge from the crown which remain semi-dormant in winter; and flower stems develop in the spring followed by harvesting in summer (Pethybridge *et al.*, 2008b). This production cycle continues for 4-5 years (Pethybridge *et al.*, 2009). However, over the last 10 years, poor regrowth of plants has occurred after the first harvest, leading to a severe yield decline. Plants affected by yield decline have severely discoloured which may be infected by secondary pathogens such as *Fusarium oxysporum*, *F. avenaceum* and *Paraphoma vinacea* (Moslemi *et al.*, 2016, 2017b).

Commonly occurring fungal diseases of pyrethrum in Australia include tan spot (*Didymella tanacetii*/ *D. rosea*) (Pearce *et al.*, 2016; Scott *et al.*, 2017), Sclerotinia flower blight (*Sclerotinia sclerotiorum*), Botrytis flower blight (*Botrytis cinerea*) (Scott *et al.*, 2017), winter blight (*Alternaria tenuissima*) (Scott *et al.*, 2017), pink spot (*Stemphylium botryosum*) (Pethybridge *et al.*, 2008b), and anthracnose (*Colletotrichum tanacetii*) (Barimani *et al.*, 2013; Scott *et al.*, 2017). Moslemi *et al.* (2018) recently identified *Paraphoma pye* and *Pa. chlamydocopiosa*, as new foliar and crown pathogens of pyrethrum. One of the most

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significant constraints to pyrethrum production in Australia is ray blight disease, caused by the fungal pathogen *Stagonosporopsis tanacetii* (Vaghefi *et al.*, 2012). This pathogen has thus far only been detected in Australia (Vaghefi *et al.*, 2016b). Two morphologically similar and phylogenetically closely related species, *S. chrysanthemi* and *S. inoxydabilis*, cause ray blight on *Asteraceae* in the US and Europe, respectively (Vaghefi *et al.*, 2012). Despite previous reports of *S. chrysanthemi* (cause of ray blight of chrysanthemum) in Australia (Oxenham, 1963; Simmonds, 1996), our recent multi-locus analyses of historical collections in New South Wales identified the deposited pathogen as *S. caricae* (*unpublished data*). Therefore, presence of these species on cultivated or wild hosts in Australia remains unknown. Both *S. chrysanthemi* and *S. inoxydabilis* have been shown to infect pyrethrum plants and, therefore, are considered as biosecurity threats to the Australian pyrethrum industry (Vaghefi *et al.*, 2016a).

*Stagonosporopsis tanacetii* causes substantial yield reduction (Bhuiyan and Taylor 2014), and complete yield loss is possible in serious outbreaks. The biology of the host-pathogen interaction, epidemiology of *S. tanacetii*, and development of diagnostic methods for the management of ray blight of pyrethrum have been studied extensively over the last 10 years. The objective of this manuscript is to provide an overview of the current knowledge on the host-pathogen relationship ray blight management strategies to identify existing gaps and guide future research directions.

### **Ray blight of pyrethrum in Australia**

Ray blight was named after the characteristic blighting symptom of the ray florets, which resulted in discoloured heads that become straw coloured/ withered (Stevens, 1907). The disease can infect all plant organs/tissues except vascular and root tissues (Bhuiyan *et al.*, 2017a). Initial symptoms begin with necrotic lesions on leaf margins then the lesions expand and cover the whole leaf resulting in defoliation and stunted growth of the plant. Leaf lesions can spread to the petiole and flower stem, resulting in flower stem girdling. Yellowing and

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deformation of leaves also appear. The most distinct symptom of ray blight is the “Shepherd’s crook” appearance of flower buds, which is caused by infection and necrosis of one side of the upper flower stems (2 to 3 cm below the flower bud), resulting in drooping of the flower bud (Fig. 1) (Pethybridge *et al.*, 2008b).

Ray blight of pyrethrum was first reported in Australia in 1995 (Pethybridge and Wilson, 1998). The causal organism was initially identified as *Phoma ligulicola* var. *inoxydabilis* based on morphological studies (Pethybridge and Wilson, 1998) but was later re-described as *Stagonosporopsis tanacetii* based on multi-locus phylogenetic analyses (Vaghefi *et al.*, 2012). It has been hypothesized that, due to its phylogenetic affinity with *S. inoxydabilis* in Europe, *S. tanacetii* has an origin outside Australia, and was introduced to Australia either on the propagative material imported to establish the pyrethrum industry in the 1980s, or even much earlier; on the pyrethrum plants imported from Japan, UK and the U.S. in 1930s (Bhat and Menary, 1984) or Austria in 1890s (Von Mueller, 1895; Wittmann, 1976).

Ray blight commonly appears during early spring but the highest incidence of this disease is during the flowering period in late spring to early summer (November to December) (Pethybridge *et al.*, 2008b). Severe epidemics of ray blight in 1999 resulted in substantial yield losses; out of 24 sites surveyed during 1999/2000, one site had 100% yield loss, 38% of crops had below average yield, and the majority of fields had yields of 50% or less (Pethybridge and Hay, 2001). Although ray blight is the most damaging disease of pyrethrum in Australia, the exact estimation of yield loss by *S. tanacetii* is difficult to determine due to the complex of foliar, root, and flower pathogens that cause disease.

#### **Life cycle of *S. tanacetii* in pyrethrum**

Pyrethrum seeds are considered as the main source of primary inoculum of *S. tanacetii* (Pethybridge *et al.*, 2006), which results in dispersal of the pathogen and development of foliar infection in seedlings. High incidence of ray blight infection has been detected in

commercial seed lots (Pethybridge *et al.* 2006). Both spatiotemporal analyses and logistic regression modelling of ray blight epidemics supported the hypothesis that the seed is the major source of ray blight (Pethybridge *et al.*, 2005a, 2006, 2011). Population genetics studies also reported low geographical structuring of the pathogen population and widespread distribution of a few multi-locus genotypes, which, in the absence of sexual reproduction and airborne ascospores, is suggestive of human-mediated movement of seed as major means of long distance pathogen dispersal (Vaghefi *et al.* 2015b).

Infection starts by direct penetration of pycnidiospores germ tubes into the epidermal cells of leaves within 12 h, followed by pin point necrotic lesions that develop after 24 h (Bhuiyan *et al.*, 2015). Intra- and inter-cellular colonization by infection hyphae results in extensive damage and necrosis of epidermal, hypodermal and cortical tissues of pyrethrum leaves.

*Stagonosporopsis tanacetii* completes its life cycle within 12 d with the formation of fertile pycnidia.

During the growing season, water droplets (rain splash) will impact the pycnidia that develop in leaves, petioles and flower stems resulting in the release of pycnidiospores that are then dispersed to adjacent leaves (Pethybridge *et al.*, 2005a). The trichomes on the leaves and petioles of pyrethrum plants may cause water droplets containing the spores to be repelled leading to run-off and deposition of spores at the crown region. Numerous pycnidia that are formed in the infected cauline leaves, petioles, flower buds and flower stems are the source of inoculum (Bhuiyan *et al.*, 2017a). The severity of necrotic leaf lesions on pyrethrum plants is increased with an increase in overwintering frequency of *S. tanacetii* on plants (Pethybridge *et al.*, 2011, 2013).

The susceptibility of annual chrysanthemum (*Chrysanthemum carinatum*) and marigold (*Tagetes patula*) to infection by *S. tanacetii* was confirmed by Pethybridge *et al.* (2008a) but this pathogen has not yet been isolated from any of the alternative hosts in pyrethrum fields in

Australia. Population genetics studies found evidence that a genetically differentiated source of inoculum may exist outside the fields, which was introduced to pyrethrum fields in 2012 (Vaghefi *et al.*, 2015b). Sexual reproduction has yet to be ascertained in the ray blight-pyrethrum pathosystem in Australia (Vaghefi *et al.*, 2015a) since only a single *MAT* gene, *MAT1-1-1*, has been detected in the Australian *S. tanacetii* populations, indicating asexuality or heterothallism of this pathogen (Chilvers *et al.*, 2014; Vaghefi *et al.*, 2016b).

### **Infection process of *S. tanacetii* in pyrethrum**

#### *Seed and seedlings*

Within the seed, infection hyphae are confined to the infected outer layer of the seed coat and not the embryos. During the process of germination, *S. tanacetii* hyphae infect the developing embryos and, depending on level of infection, result in pre- or post-emergence damping off, or infected symptomless seedlings. The mechanism of embryo infection by *S. tanacetii* is through the direct infection from the seed coat. Disintegration of embryonic tissues results in pre-emergence death. Post-emergence death of pyrethrum seedlings results from infection of the parenchyma cell tissue (Bhuiyan *et al.*, 2017b). The infected seedlings may remain symptomless, while harbouring the pathogen in the epidermal, hypodermal, and cortical tissue of the crown region. No infection occurs in the vascular tissues of infected seedlings. The fissures and spaces within the seed surface are assumed to be the entry point of *S. tanacetii* into the seed. The empty space around the pappus appears to be the location for the buildup of infection hyphae (Bhuiyan *et al.*, 2017b). Surface sterilisation of pyrethrum seeds with sodium hypochlorite was reported to reduce the incidence of *S. tanacetii* up to 60% compared to non-surface-sterilised seeds (Pethybridge *et al.*, 2006), which only killed the *S. tanacetii* on seed surface or on seed coat and not *S. tanacetii* mycelium within the cotyledons of the seed.

### *Mature pyrethrum plants*

Infection by *S. tanacetii* begins with the attachment of the pycnidiospores on the surface of pyrethrum leaf lamina (cauline leaf). Following germination of pycnidiospores, germ tubes penetrate directly into the host without forming any specialised infection structures and without invading stomata (Bhuiyan *et al.*, 2015). Although ray blight is considered a foliar and flower disease (Hay *et al.*, 2015; Scott *et al.*, 2017), *S. tanacetii* can also infect the crown tissue of pyrethrum (Bhuiyan *et al.*, 2017a). Infection results in necrotic lesions on flower stems, flower buds (Pethybridge *et al.*, 2003), leaves, petiole bases and crown tissues (Bhuiyan *et al.*, 2017a). *Stagonosporopsis tanacetii* infects parenchyma tissue in the epidermis, hypodermis and cortex of leaf lamina, petiole, flower stem and crown. However, vascular tissues of infected plants do not become infected. Necrosis of all tissues except the vascular tissue at the distal end of flower stems results in the typical “Shepherd’s crook” symptom (Bhuiyan *et al.*, 2017a).

### **Factors affecting ray blight epidemics**

In Australia, disease incidence and severity of ray blight has been recorded to be higher in September and decrease gradually in October, when the number of consecutive days with rainfall is reduced (Pethybridge *et al.*, 2005a, 2009). A survey conducted by Pethybridge *et al.* (2003) reported that the isolation frequency of *S. tanacetii* from pyrethrum leaves was ~19.4% in early to midwinter, 37.8% in late winter and 56.9 to 82.7% over the spring. In addition, a combined effect of an abiotic stress such as waterlogging and ray blight has been shown to significantly reduce crop growth (Javid *et al.*, 2013).

Rainfall and temperature coupled with edaphic or site-specific factors such as aspect and elevation of the fields are also considered as risk factors for ray blight outbreak on pyrethrum (Pethybridge and Hay, 2001; Pethybridge *et al.*, 2009). Pyrethrum plants grown in south-



facing slopes and in valleys are at higher risk of infection by *S. tanacetii* as compared to north-facing slopes and on the crests of hills in Tasmania (Pethybridge *et al.*, 2009).

Moreover, densely populated pyrethrum fields are subjected to infection by *S. tanacetii* due to favourable microclimatic conditions (Pethybridge *et al.*, 2011). The polycyclic progression of ray blight occurs in spring when the plants grow rapidly, and the stems are more susceptible to *S. tanacetii* (Pethybridge *et al.*, 2011).

### **Diagnosis of *S. tanacetii* in pyrethrum seed and seedlings**

*Stagonosporopsis tanacetii* survives in pyrethrum seed, increasing the potential for future disease epidemics to occur. Seedborne pathogens affect risk of disease development by introducing inoculum into the growing plant (Pethybridge *et al.*, 2006). The incidence of *S. tanacetii* in pyrethrum seed varied between 0.9 and 19.5% (mean =7.7%) (Scott *et al.*, 2017).

Reliable and rapid detection methods are an integral part of disease management in seedborne diseases and will enable seed certification and use of disease-free seed. Over the last decade, traditional and molecular detection assays have been developed to assess the incidence of infection of *S. tanacetii* in commercial pyrethrum seed lots.

#### *Visual inspection*

Heavily infected pyrethrum seeds may harbour pycnidia which are readily detected by naked eye. However, visual inspection of infected pyrethrum seed and seedlings is not reliable since *S. tanacetii* may be latent in asymptomatic plant tissue (Bhuiyan *et al.*, 2017b).

#### *Agar plate incubation assay*

Culturing on biological media is used to test seeds for fungal infection (Mancini *et al.*, 2016) and has been used to identify the incidence of *S. tanacetii* infection of pyrethrum seed. The method is based on identifying the cultural characteristics of *S. tanacetii* growing on potato dextrose agar (PDA) or V8 agar media (Pethybridge and Wilson, 1998; Pethybridge *et al.*,

2008b). However, culturing seed is time-consuming, requires skills in mycology to identify fungal species, and sometimes it is not sensitive enough to detect very low levels of seed infection (Mancini *et al.*, 2016).

#### *Molecular detection assays*

A PCR-based method for detection of *S. tanacetii* in pyrethrum seed was developed based on amplification of the ITS region of the nuclear ribosomal DNA (nrDNA) of *S. tanacetii* (Pethybridge *et al.*, 2004b). However, the ITS sequences were highly similar among the three closely related ray blight pathogens *S. tanacetii*, *S. chrysanthemi* and *S. inoxydabilis*, thus, were not effective in discriminating these species. Although the presence of *S. inoxydabilis* and *S. chrysanthemi* in Australia remains unknown (Vaghefi *et al.*, 2016a), both species are capable of causing disease on pyrethrum (Vaghefi *et al.*, 2016a). Therefore, a species-specific multiplex PCR assay was developed to allow rapid and reliable differentiation of the three *Stagonosporopsis* spp. (Vaghefi *et al.*, 2016a) based on the sequence of the IGS region of the nrDNA. A TaqMan qPCR assay was further developed to enable quantification of *S. tanacetii* inoculum levels in pyrethrum seed lots prior to selecting planting material (Bhuiyan *et al.*, 2018). The assay is highly sensitive and has been validated *in planta* for quantification of *S. tanacetii* inoculum in pyrethrum seed (Bhuiyan *et al.*, 2018). Development of a multiplex real-time qPCR assay to detect and quantify multiple pathogens of pyrethrum in the same reaction would reduce both costs and labour.

### **Management of ray blight in pyrethrum**

#### *Management of ray blight using seed treatment*

As seed is the primary carrier of *S. tanacetii* therefore, use of seed treating chemicals could reduce the primary inocula present in the pyrethrum seed coat; although complete elimination may not be possible. Seedborne inoculum may be reduced significantly by treating pyrethrum

seed with thiabendazole (benzimidazole: FRAC code 1)/thiram and fludioxonil (phenylpyrrole: FRAC code 12) (Pethybridge *et al.*, 2006).

#### *Heat treatment of seed*

Heat treatment (@ 50 °C for 30 min) of pyrethrum seed can also successfully reduce *S. tanacetii* inoculum in seed, resulting in germination increases of up to 83% (Bhuiyan *et al.*, 2017b). Significant reduction in pathogen colonization following planting of steam sterilised has also been reported (Scott *et al.*, 2017). However, temperature treatment does not completely eliminate the primary inocula due to the presence of other exogenous sources of inocula (Scott *et al.*, 2017). Moreover, low level of *S. tanacetii* might be present in steam sterilised seed; therefore, complete eradication of *S. tanacetii* in fields requires application of foliar fungicides in spring (Scott *et al.*, 2017).

#### *Management of ray blight using fungicides*

Management of ray blight currently relies mostly on foliar application of fungicides. Quinone outside inhibitors (QoI: strobilurin) and demethylation inhibitors (DMI) are used frequently to control ray blight of pyrethrum. There are seven classes of strobilurins based on structural similarities. Their mode of action is binding to the Qo site of the cytochrome bc1 complex (Gisi *et al.*, 2002). As these fungicides interfere with one specific biochemical site they are called site-specific fungicides (single-site mode of action). Only a single mutation at this site is enough to develop a fungicide-resistant pathogen subpopulation (Vincelli, 2002). The risk of resistance development is generally higher in fungicides having single-site mode of action (Yamaguchi and Fujimura, 2005). However, multi-site specific fungicides affect multiple target sites in fungi; therefore, less resistance to develop against the fungicide. They are although cheaper but less effective (Brent and Hollomon, 1995; Chen *et al.*, 2013).

Fungicides such as difenoconazole (DMIs: FRAC code 3) and azoxystrobin (FRAC code 11) applied in spring were found to effectively control ray blight (Pethybridge *et al.*, 2007, 2008b). However, the Fungicide Resistance Action Committee (FRAC) classifies DMIs as medium to high risk of developing tolerance and prone to development of resistance in fungal populations (Scheinpflug, 1988; Del Sorbo *et al.*, 2000). Following detection of reduced sensitivity of *S. tanacetii* populations to difenoconazole (Jones *et al.*, 2007), DMIs were replaced with boscalid (succinate-dehydrogenase/carboxamide: FRAC code 7) (Pethybridge *et al.*, 2008c). One combined application of boscalid and pyraclostrobin (FRAC code 11) in a pyrethrum field increased yield of pyrethrin by 60% compared to the previous industry-recommended protocol (single dose of azoxystrobin [150 g ai/ha] and two additional applications of difenoconazole [125 g ai/ha] and chlorothalonil [1008 L ai/ha] at 14-21 d intervals) and non-treated control plot (Pethybridge *et al.*, 2008c). Although, boscalid is still found to be toxic to *S. tanacetii* (Hay *et al.*, 2015) urgent adoption of nonchemical methods for disease management in Australian pyrethrum fields is essential. There is evidence of boscalid tolerance in *D. tanacetii*, another important foliar pathogen of pyrethrum in Australia (Hay *et al.*, 2015). The risk of resistance to fungicides (both DMIs and strobilurins) and the mode of resistance in *S. tanacetii* against anti-fungal agents (fungicides) have yet to be determined at molecular, genetic, biochemical and physiological levels.

In 2002, Pethybridge *et al.* (2007) estimated the threshold level of defoliation severity by *S. tanacetii* in Tasmanian pyrethrum fields and found that when the defoliation severity reached 35% the severity of necrotic lesions on stems was expected to increase linearly. Meanwhile, defoliation severity less than 35% was subminimal and there was less chance to increase infection. Using this analysis, a fungicide program should keep the defoliation severity below 35% in spring (Pethybridge *et al.*, 2007). However, over time, there has been a shift in incidence of pathogens that caused foliar infection away from *S. tanacetii* to *D. tanacetii* (Hay

*et al.*, 2015), *C. tanacetii* (Barimani *et al.*, 2013), *S. botryosum* and *A. tenuissima* (Pethybridge *et al.*, 2004a); *A. infectoria* and *S. herbarum* (Moslemi *et al.*, 2017a). These changes in importance of foliar pathogens of pyrethrum may be in part due to the introduction of new fungicides as well as changes in environmental conditions that favored selected pathogen species. Therefore, current estimation of threshold level of defoliation severity only by ray blight needs to be revised to consider more complex situation of pathogen species and environments.

#### *Cultural practices*

The microclimate of densely populated pyrethrum fields is favourable for ray blight epidemics, therefore, reduction of plant density was suggested as a means for reduction of inoculum (Pethybridge *et al.*, 2011). Minimizing the use of liquid fertilizers and overhead irrigation (Fox, 1998), cultivation of disease free planting materials (Baker *et al.*, 1949, 1961; Fox, 1998), practice of good crop hygiene such as roguing of infected plants followed by burning (Fox, 1998), and implementation of deep burial of crop residues (Fox, 1998; Pethybridge *et al.*, 2008a) have been suggested as useful management practices for controlling ray blight in chrysanthemum (Pethybridge *et al.*, 2008b).

The flowers of perennial pyrethrum plants are harvested 2-3 times in a year by mechanical harvesting that separates the flower heads from the stems and then cuts the flower stems at the crown region, leaving the crop residues in the field (Moslemi *et al.*, 2017b). Surveys conducted in 2015-16 at yield-decline affected sites of pyrethrum in northern Tasmania reported necrotic leaf and crown tissues with fungal fruiting structures of different fungi including pycnidia of the ray blight pathogen in the crop residues (Moslemi *et al.*, 2017a). Infected crop residues contribute to the availability of inoculum to re infect new growth. Management of infected residues such as burying after harvest may reduce inoculum through decomposition by microbial bioagents in the soil (Keinath, 2002). In addition, crop rotation

(3-4 years) with non-host has been shown to be effective in controlling pathogens that remain in the crop residues (Keinath, 1996; Greenhill, 2007; Pethybridge *et al.*, 2008b). Although cultivation of disease resistant cultivars has been shown to be effective to manage ray blight in chrysanthemum (Strider, 1994) sources of resistance to *S. tanacetii* in pyrethrum have yet to be identified.

Reduction of ray blight severity by 18% was achieved by reducing plant density by 50-75% without affecting pyrethrin yield. However, low pyrethrum density enhanced weed density which was then minimized by the application of herbicides (Pethybridge *et al.*, 2008b). As plant debris is a rich source of pycnidia, removal from the field at harvest may reduce the inoculum level for subsequent crops, however, in a large scale this would not be cost effective.

### **Conclusions and Future challenges**

Despite recent advances in elucidating ray blight disease cycle and epidemiology, which have improved disease management strategies significantly, some aspects of the pathogen biology and life cycle are not yet fully understood. Presence of *S. tanacetii* populations outside pyrethrum fields in Australia remains unknown. Considering the recent detection of new *S. tanacetii* genotypes in Tasmanian pyrethrum fields, understanding the distribution of *S. tanacetii* in Australia is a priority. Presence of an unknown wild source of *S. tanacetii* in Australia will have severe consequences for the industry in terms of introducing new genetic diversity and/or a second mating-type into the pyrethrum fields. Moreover, investigation of *S. tanacetii* populations within and outside Australia will help elucidating the origin and reproductive strategy of the pathogen, which has significant implications for pathogen survival and dispersal. Population genetics studies on global population of *S. tanacetii* may also help elucidate its mating system through potential discovery of the second mating-type.

Major advances in understanding the infection process of *S. tanacetii* on pyrethrum has been made in the recent years. It is now well established that infected pyrethrum seed is the major source of *S. tanacetii* while secondary sources include infected foliage containing pycnidia. Crown tissues also become infected through the run-off of water droplets that carry pycnidiospores from the infected foliage. Therefore, management practices need to reduce fungal inoculum within the seed and foliage, before reaching the threshold level that will cause severe defoliation. The newly developed TaqMan PCR assay can be used to determine the amount of *S. tanacetii* infection within the seed before and after steam sterilization. The TaqMan PCR can also be used to quantify the infection level of *S. tanacetii* within the pyrethrum plant tissues remaining after harvest to enable prediction of *S. tanacetii* epidemics developing in the regrown crop. Available management practices include the recently adopted steam sterilisation and seed treatment fungicides to reduce the incidence of seed-borne pathogens, and widespread use of single-site specific foliar fungicides to reduce foliar infection. However, to rely on single-site specific fungicides is not sustainable due to the risk of resistance development in *S. tanacetii* populations. Use of multi-site specific fungicide (chlorothalonil as Bravo 720; Syngenta, Australia) is a more sustainable alternative (Pethybridge *et al.*, 2005b, 2007), although may be less effective (Brent and Hollomon, 1995; Chen *et al.*, 2013). More importantly, non-chemical management practices will provide more sustainable options for ray blight control.

Currently, ray blight is the most damaging disease of pyrethrum in Australia. No sources of resistance have been identified in ray blight-pyrethrum pathosystem. Therefore, resistant pyrethrum germplasm needs to be identified with a view to develop a resistant breeding program. Wild relatives of pyrethrum may provide sources of resistance against ray blight. Resistance breeding combined with improved knowledge of the pathogen origin, reproductive strategy, and host-pathogen interaction will lead to a more sustainable disease

control and enhanced pyrethrum production with less reliance on fungicides. Better understanding on molecular mechanisms underlying plant pathogen interactions will pave the way for potentially enhancing resistance of the high yielding pyrethrum cultivars through genome editing.

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### Figure legend

**Figure 1.** *Stagonosporopsis tanacetii* infected flower stem showing “Shepherd’s crook” symptom (white arrow) (Pethybridge *et al.*, 2008b; Vaghefi *et al.*, 2016b).



