Inching Closer to Understanding

By Jonathan Poulton, DPhil

I got into daylilies quite by chance in the summer of 2006 while on vacation in Door County, Wisconsin, after a navigation error led our family to the amazing daylily garden of Ronald Mickelson near Sister Bay. During the next few years, I began collecting and hybridizing bicolors, a subgroup of daylilies that absolutely fascinated me.

Perhaps you are like me but, each spring, I couldn't wait for the ground to thaw. I was eager to see those first green shoots emerge, heralding the start of another season. By late spring, most of my daylilies were flourishing and exhibiting normal growth patterns, but there was always a significant percentage that looked nothing short of weird!

Fig. 1. Spring foliage of *Hemerocallis* cultivar showing symptoms typical of spring sickness. Photo by Sue Bergeron.

Within these affected clumps, some of the fans appeared just fine, while others were stunted and bent sideways **(Figure 1)**. Their leaves exhibited holes and brown "saw-tooth" edges. If I pulled gently on some of the central leaves of these fans, they frequently broke off, indicative of extensive damage at their bases. Alarmed by what I saw, I turned to my mentor Barb Papenhausen (CVIDS) for advice. "Oh, don't you worry" she said. "Those lilies have what is called 'spring sickness'. They'll grow out of it!" And indeed she was right. Although some fans died, most affected plants appeared to get over the disorder. A month later, there was little or no sign of spring sickness, and I breathed a huge sigh of relief.

Wanting to know more about what causes spring sickness (SS) in daylilies, I went on line and quickly found that, although this disorder has been recognized for more than 50 years in the United States, Canada, and Europe, it is still far from being completely understood [1]. In 2010, Susan Bergeron and Keith Somers provided an excellent update on recent progress made by the Spring Sickness Task Force, a group of AHS member volunteers across the United States and Canada that is working hard to identify the cause(s) of SS [2]. What is certain is that SS is not caused by freezing and thawing after new shoots emerge in the spring. Instead, much of the damage is initiated well before spring growth emerges above the ground and the symptoms of SS start to become visible. Possible culprits include bulb mites (*Rhizoglyphus* species) and the daylily leaf streak fungus (*Aureobasidium microstictum*), both of which have been consistently found in SS fans [1-3]. For infection, this fungus must penetrate a wound in the daylily, but such damage could be inflicted by bulb mites or other pests. In research partially funded by the AHS, Hideka Kobayashi observed symptoms resembling SS after he injected spores of the leaf streak fungus into wounds made in leaves of healthy daylily plants [3]. In 2009, Keith Somers undertook garden trials to determine whether SS could be diminished by pesticide application [2]. Ninety-six daylily cultivars were subdivided into four groups that received either Senator (thiophanate-methyl, a

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fungicide) plus Orthene (acephate, an insectide), Senator alone, Orthene alone, or no treatment (i.e. untreated controls). Treatment was undertaken in April with evaluation in June. Within the control group, dormant, semi-evergreen and evergreen fans all exhibited similar levels of SS. Encouragingly, each of the three pesticide treatments reduced SS to about 50% of control levels. The fact that separate applications of fungicide and insecticide significantly diminished SS may point to multiple players being involved in this disorder. If you're wondering whether any other fungi have been linked to daylily SS, the answer is definitely "Yes". In June 2014, a collaborative team of European scientists led by Dr. Robert Grant-Downton of the University of Oxford implicated a different fungal culprit in the development of symptoms that were "typical of or closely related to SS" [4]. This work was partially funded by a grant from the British Hosta and Hemerocallis Society. Over a four-year period, these researchers investigated six different *Hemerocallis* cultivars (*H.* 'Jurassic Spider', 'Lola Branham', 'Gerda Brooker', 'Free Bird', 'Ruby Storm', and an unnamed tetraploid hybrid). After rigorously surface-sterilizing leaf samples from symptomatic plants with a strong bleach solution containing detergent, they placed the samples on a solid nutrient medium inside Petri dishes. All six daylily cultivars produced isolates (i.e. highly purified fungal samples) that contained an unidentified filamentous fungus. Its identification was made difficult by the fact that this organism rarely produced spores. However, its physical appearance, combined with a positive antibody test, suggested that this fungus belonged to the genus *Botrytis* (class Ascomycetes). To confirm their suspicion, Grant-Downton *et al.* first sequenced a tiny region of the DNA of the six isolates, comparing their findings with the same region of several well-known *Botrytis/Botryotinia* species.

EU519207 *Botrytis elliptica*	CCAAAACCCAAATTTTTCTATGGTT
EU519208 *Botryotinia squamosa*	CCAAAACCCAAATTTTTCTATGGTT
EU093077 *Botrytis aclada*	CCAAAACCCAAATTTTTCTATGGTT
FJ914712 *Botrytis byssoidea*	CCAAAACCCAAATTTTTCTATGGTT
EU519206 *Botryotinia porri*	CCAAAACCCAAATTTTTCTATGGTT
HM989942 *Botryotinia fuckeliana*	CCAAAACCCAAATTTTTCTATGGTT
Botrytis deweyae B1	CCAAAACCCAAA-TTTTCTATGGTT
Botrytis deweyae B2	CCAAAACCCAAA-TTTTCTATGGTT
Botrytis deweyae B4	CCAAAACCCAAA-TTTTCTATGGTT
Botrytis deweyae B5	CCAAAACCCAAA-TTTTCTATGGTT
Botrytis deweyae B5	CCAAAACCCAAA-TTTTCTATGGTT
Botrytis deweyae B6	CCAAAACCCAAA-TTTTCTATGGTT
Botrytis deweyae P1	CCAAAACCCAAA-TTTTCTATGGTT

Fig. 2. Comparison of the DNA sequences (within the *ITS* region) of the six *B. deweyae* isolates with corresponding regions from various *Botrytis/Botryotinia* species. Image adapted from [4].

As **Figure 2** shows, all the *Hemerocallis* isolates had the same DNA sequence in this so-called *ITS* region. Furthermore, this sequence only differed from those of characterized *Botrytis/Botryotinia* species in lacking a single thymine (T) mid-sequence. This initial analysis provided strong evidence that their *Hemerocallis* isolates belong to the genus *Botrytis* but could not distinguish whether they represented a novel species. To test this, Grant-Downton *et al.* then sequenced five key genes from each of the isolates and compared these DNA sequences with corresponding sequences from over 20 different *Botrytis* species. Taken together, their data revealed that the fungal pest of *Hemerocallis* represents a new *Botrytis* species, to which they gave the name *Botrytis deweyae* in honor of the work performed on *Botrytis* by plant pathologist Dr. Molly Dewey (University of Oxford). **Figure 3** shows the appearance of *B. deweyae* under the light microscope. Construction of a "family tree" allowed the scientists to show that *B. deweyae* was most closely related genetically to the species *B. elliptica* and *B. squamosa.* This is an exciting discovery, because *B. elliptica* not only causes leaf blight (or fire blight) in true lilies (*Lilium*) [5] but also gray mold in *H. fulva* in Korea [6]. Further support for the contention of Grant-Downton's research team that *B. deweyae* is an important contributor to SS came from additional DNA studies, in which they isolated DNA samples from both Symptomatic and asymptomatic daylily leaves. They then used a technique called PCR amplification to demonstrate the presence of fungal DNA (and therefore of the fungus) in leaves showing SS symptoms but not in asymptomatic leaves. As previously mentioned, bulb

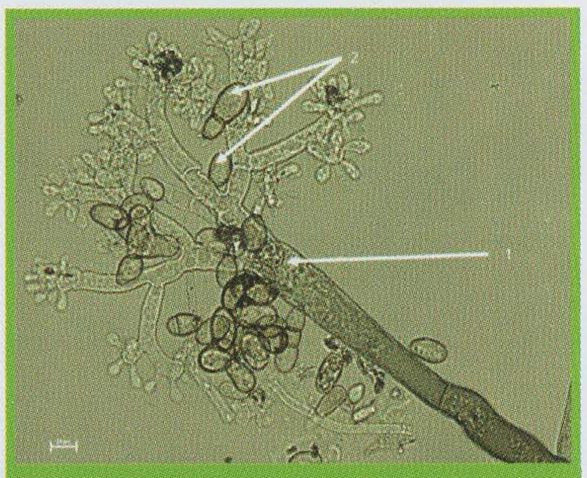

Fig. 3. *Botrytis deweyae* seen under the light microscope showing a specialized branch (1) of the fungus that produces spores (2). Scale bar indicates 10 microns. Photo adapted from [4].

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continued

mites and the leaf streak fungus have long been implicated in SS and shown to give rise to symptoms of this disorder [1-3]. After Grant-Downton *et al.* had conclusively proven that *B. deweyae* was present in infected daylilies in the United Kingdom, ideally the next step would be to challenge their daylily cultivars with this fungus under "typical infection conditions" to determine whether they develop the same SS symptoms as the plants from which the fungal pest was isolated. It is clearly difficult for scientists to replicate in the laboratory those conditions experienced by garden-grown daylilies during colder months of the year including potential attack by pests (e.g. bulb mites, fungi, and bacteria). Instead, the team investigated how *H.* 'Jurassic Spider' plantlets, generated from tissue culture, responded to inoculation with *B. deweyae*. As **Figure 4** demonstrates, inoculated plantlets showed yellowing, necrosis, collapsing and death of leaf tissue within 10-14 days after inoculation. In contrast, the roots and growing points were relatively unaffected. Control plantlets displayed no infection. To determine whether the infectivity of *B. deweyae* depended on which *Hemerocallis* cultivar was being challenged, Grant-Downton *et al.* tested another 15 cultivars using this method. These cultivars were: *H.* 'Barbara', 'Bo Knows', 'Cayenne', 'Corky', 'Dark Mosaic', 'Golden Chimes', 'Heavenly Flight of Angels', 'Jellyfish Jealousy', 'Lavender Curls', 'Miss Jessie', 'Rococo', 'Party Array', 'Persian Pattern', 'Running Late', and *H. flava* clone 3. No sign of resistance was observed; all cultivars developed similar symptoms and died within 14 days.

In summary, the authors did not observe typical SS symptoms after inoculating daylily plantlets with *B. deweyae*. Instead, infection took hold surprisingly rapidly, causing the plantlets to die within 2 weeks. To some readers, this outcome may make a role for *B. deweyae* in SS less convincing. However, it should be remembered that the infection system being studied in their laboratory is very simple; it consists only of young *Hemerocallis* plantlets and *B. deweyae.* By contrast, in our gardens, the lilies exhibiting SS symptoms are more mature plants, and their interaction with potential pests like this fungus may be influenced by other organisms in the environment. For example, Grant-Downton *et al.* did not rule out the involvement of bulb mites in spreading fungal material to new infection sites [4]. Also, many cases are known where microbial species have even acted antagonistically to *Botrytis* infections of plants including the infection of true lilies by *B. elliptica* [7, 8].

It will be extremely interesting to see what future research reveals about the interaction of *Hemerocallis* with *B. deweyae*. Although Grant-Downton *et al.* have so far examined only cultivated *Hemerocallis* material from England, they predict that *B. deweyae* is "cosmopolitan" and will be found in cultivated daylily cultivars elsewhere in

Fig. 4. Effect of inoculation of *Hemerocallis* plantlets with *Botrytis deweyae*. A. Control *H.* 'Jurassic Spider' plantlets (uninoculated with *Botrytis*) (left) and plantlets infected with *B. deweyae* (right), shown 10 days after inoculation. The plants were excised from sterile vermiculite. Scale indicates 10 cm. B. Close-up of tissue damage on the basal portion of a *H.* 'Jurassic Spider' plantlet following *B. deweyae* infection after colonization of leaf bases. Scale indicates 5 mm. Photo adapted from [4].

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the world and perhaps also in native *Hemerocallis* species [4]. Their studies should be extended to test this prediction. In this connection, it should be noted that SS has been observed in *H. fulva, H. lilioasphodelus,* and *H. thunbergii* [1]. Laboratory studies should also be expanded to include any *Hemerocallis* cultivars that purportedly show SS resistance in the field. If these cultivars also exhibited resistance in the lab, it would boost confidence that *B. deweyae* is a key player in SS development and might additionally offer some insights into the mechanism of such resistance. Other issues that certainly deserve attention are the following: At what time of the year does infection by *B. deweyae* take place? After infecting its daylily host, could this fungus remain dormant in asymptomatic plants for considerable periods of time until triggered by some as yet unknown cues to initiate SS? In an affected clump, why don't all the fans show SS symptoms? I, for one, am eagerly looking forward to learning some answers to these questions!

References

[1] Bergeron S (2001) Spring sickness. *The Daylily Journal*, Vol. 56, No. 4, 434-440

[2] Bergeron S and Somers K (2010) An update on spring sickness. *The Daylily Journal*, Vol. 65, No. 2, 16-19

[3] Kobayashi H (1998) *Aureobasidium microstictum* (Bubak) Cooke, the causal agent of leaf streak of daylily (*Hemerocallis* L.), and its involvement in spring sickness of daylily, M.Sc. thesis, Ohio State University

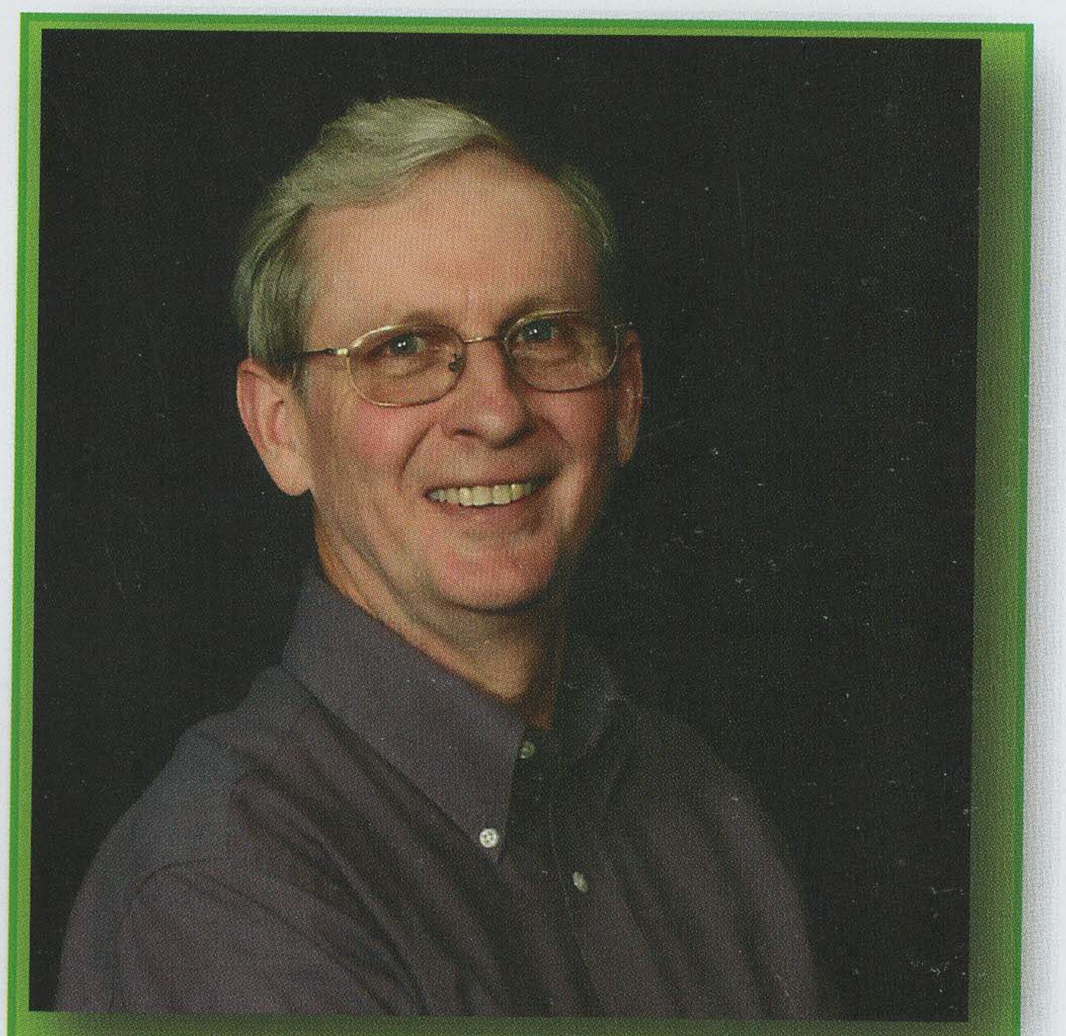
[4] Grant-Downton RT, Terhem RB, Kapralov MV, Mehdi S, Rodriguez-Enriquez MJ, Gurr SJ, van Kan JAL and Dewey FM (2014) A novel *Botrytis* species is associated with a newly emergent foliar disease in cultivated *Hemerocallis*. *PLoS ONE*, Vol. 9, Issue 6, 1-11

[5] Hsieh T-F and Huang J-W (2001) Leaf-disk method for assessment of disease severity of lily leaf blight caused by *Botrytis elliptica*. *Plant Pathology Bulletin*, Vol. 10, 37-44

[6] Chang SW, Kim SK and Hwang BK (2001) Gray mold of day lily (*Hemerocallis fulva* L.) caused by *Botrytis elliptica* in Korea. *Plant Pathology Journal*, Vol. 17, Issue **5**, 305-307

[7] Chiou AL and Wu WS (2001) Isolation, identification, and evaluation of bacterial antagonists against *Botrytis elliptica* on lily. *Journal of Phytopathology*, Vol. 149, 319-324

[8] Elad Y and Stewart A (2004) Microbial control of *Botrytis* spp. In: Elad Y, Williamson B, Tudzynski P and Delen N, eds. *Botrytis*: Biology, Pathology and Control. Dordrecht: Springer. 223-241



Jonathan Poulton received his doctorate in plant biochemistry from the University of Oxford in 1974 and, after undertaking postdoctoral research at Freiburg University (Germany) and the University of California-Davis, was a professor in the Departments of Botany and Biology at the University of Iowa in 1979-2010. In Germany, his research focused on elucidating the enzyme-catalyzed pathways by which plants make the anthocyanin pigments that confer red, purple, and blue coloration on flowers. Little did he know that 40 years later he would join the quest for a true blue daylily.