Flyspeck Epidemics I: Measuring Ascospore Maturation of the Causal Fungus

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If flyspeck of apple were like apple scab and had both a primary stage and a secondary phase, it might be useful to understand how the primary stage works, so that management tactics could focus on it, the same way scab management is focused on primary scab. Theoretically, if the epidemic could be stopped early, then summer fungicides might be greatly reduced. Interestingly, the fungus that causes flyspeck, *Schizothyrium pomi* and its asexual form, *Zygophiala jamaicensis*, are ascomycetes that are similar to the apple scab pathogen *Venturia inaequalis*. That is, *S. pomi* produces ascospores and conidia; however, not much more is known about how the fungus overwinters and then ends up producing the damaging black specks on apple fruit. Of course, it grows on the waxy cuticle of fruit forming colonies of circular, black, specks called thyriothecia. These thyriothecia group in colonies of several to 50 or more, and it is these structures that eventually produce ascospores. There are a few key questions to ask. Do thyriothecia produce ascospores throughout the year, or just during a single period? Secondly, if there is just one period of ascospore production, when is it? Finally, where do the ascospores land and infect? Nearly 8 years ago, we suggested that ascospores matured only once a year, during the spring and early summer and that they might be the spores that start flyspeck epidemics each year (7). Now we have more definitive information on how ascospores of the flyspeck fungus function.

When evaluating the risk of apple scab infections in the spring, it is useful to look at the fungal structures that contain ascospores, to see how mature the spores are. The more mature spores get, the higher the risk goes. Such an approach might prove useful in both the study and, eventually, management of FS. As part of this study, we modified a technique for the preparation and interpretation of thyriothecial squash mounts of *S. pomi*.

Flyspeck can live on the waxy cuticles of many kinds of plants. We identified *S. pomi* on 26 woody plant species that commonly grow in orchard borders in Massachusetts including trees, shrubs, and vines. For these studies, we have focused on a common blackberry (*Rubus allegheniensis* Porter), which supports easily identifiable infections with abundant thyriothecia. Blackberry is an excellent indicator of the presence of *S. pomi* near orchards and is one of the most common hosts of the fungus in Massachusetts.

Thyriothecia can be picked from blackberry, using a dissecting microscope, and then squashed using a method similar to the one used for apple scab. They are examined under a high-powered microscope, and rated according to the following maturity classes: undeveloped, no asci present (0); immature asci present without ascospores (1); mature asci present containing ascospores (2); majority of asci ruptured or empty with or without released ascospores (3).

The blackberry canes can also be cut and brought into the laboratory, where they can be put into controlled environments to see how the thyriothecia develop. So, over several years, canes have been incubated in different humidity levels and at different temperatures.

Both temperature and humidity significantly affect the maturation of *S. pomi* ascospores. Thyriothecia do not produce ascospores when the air is not extremely humid, 99% relative humidity or more. It also needs to be relatively warm for ascospores to mature. At 70ºF, if the air stays nearly saturated with humidity, *S. pomi* ascospores will mature in about 48 hrs. At 57ºF, it takes 5 days, and at 50ºF it takes 6 to 9 days. Below 48º, thyriothecia never really develop in the lab, even after 18 days of incubation (Table 1).

We followed maturation of thyriothecia on canes

Table 1. Development of *Schizothyrium pomi* thyriothecia collected in the field and grown in controlled temperatures and high humidity chambers in each of three years.

Year	RH[a]	Temp°C	Days elapsed to mature spores	DD_0[b] to mature spores
1995	high	7	-[c]	-
		14	5	126
		21	2	76
1996	high	4	-	-
		6	-	-
		10	10	180
1997	99	7	-	-
		9	8	130
		21	3	113
Mean				125±33

[a]Relative humidity. In 1995 and 1996, thyriothecia were incubated in sealed glass Petri dishes containing filter paper saturated with distilled water; in 1997 incubation was in a sealed glass Petri dish containing salt-amended agar that maintained relative humidity at pre-determined levels.
[b]DD_0 are degree-days base 0°C accumulated over the incubation period.
[c]A dash indicates failure to develop at that treatment.

in borders near commercial orchards over 3 years. They developed in a consistent pattern over the 3 observation years and over the five orchard sites (Figure 1). Mature ascospores were first observed when McIntosh was in pink or bloom. In 1997 and 1999, all thyriothecia had matured 4 weeks after McIntosh petal fall, while in 1998, maturation ended 6 weeks after petal fall. When thyriothecium maturation stopped, McIntosh fruit were from 1 to 3 in diameter. Over all orchards and dates, the earliest date at which thyriothecia reached 95% maturation was June 8, while the latest was June 19.

If *S. pomi* spore development follows apple development, then like apples, the fungus maturation process may be

Table 2. Days from green tip to bloom, degree days base 0°C, and degree days base 0°C for periods at or above 95% relative humidity for orchard sites in Massachusetts, 1997, 1998 and 1999.

Year	Location	Date of gt[a]	First mature thyriothecia observed	Days gt[a] to first mature thrytiothecia	DD_0[b] gt[a] to first mature	$DD_{0;rh?95\%}$[c] gt to first mature	First Symptoms on apples
1999	Ashfield	9-Apr	26-May	47	882	-[d]	21-Sep
	Belchertown	8-Apr	24-May	46	991	82	15-Sep
	Brimfield	6-Apr	24-May	48	962	-	3-Sep
	Shelburne	8-Apr	18-May	40	757	127	16-Sep
1998	Ashfield	1-Apr	5-May	34	605	-	28-Jul
	Belchertown	28-Mar	13-May	46	956	128	27-Jul
	Brimfield	28-Mar	1-May	34	611	-	16-Jul
1997	Shelburne	23-Apr	26-May	33	638	135	11-Aug
	Sterling	16-Apr	20-May	34	655	102	7-Aug
Mean				40	763	115	

[a]Green tip, the phenological stage where 50% of 'McIntosh' fruit buds have opened and show green tissue.
[b]DD_0 are degree-days base 0°C accumulated over the specified period.
[c] $DD_{0;rh?95\%}$ are degree-days base 0°C that accumulate over the specified period when relative humidity is at or above 95%.
[d]A dash indicates that there was insufficient relative humidity data to calculate $DD_{0;rh?95\%}$.

largely driven by temperature. To evaluate this possibility, we calculated degree-days from green tip using various base temperatures from 32º to 60º. We found that 90% of the maturation could be explained by degree-days calculated from a 32º base (Figure 2). We developed a model that predicts that 5% of the thyriothecia will mature at 540 degree days, and 95% will mature by 1630 degree days. The first mature spores were actually observed at 550 degree days, and no spores were observed beyond 2100 degree days.

We needed to compare this to the degree days in the laboratory experiments. Obviously, it was taking only 2 to 9 days to go from no spores to mature spores in the lab, while it was taking weeks to see similar development in natural setting. In terms of degree days, the laboratory thyriothecia matured after a mean of 125 degree days (Table 1). The average degree days from green tip to the first mature thyriothecia in the field was 763, over six times greater than in the laboratory experiments (Table 2).

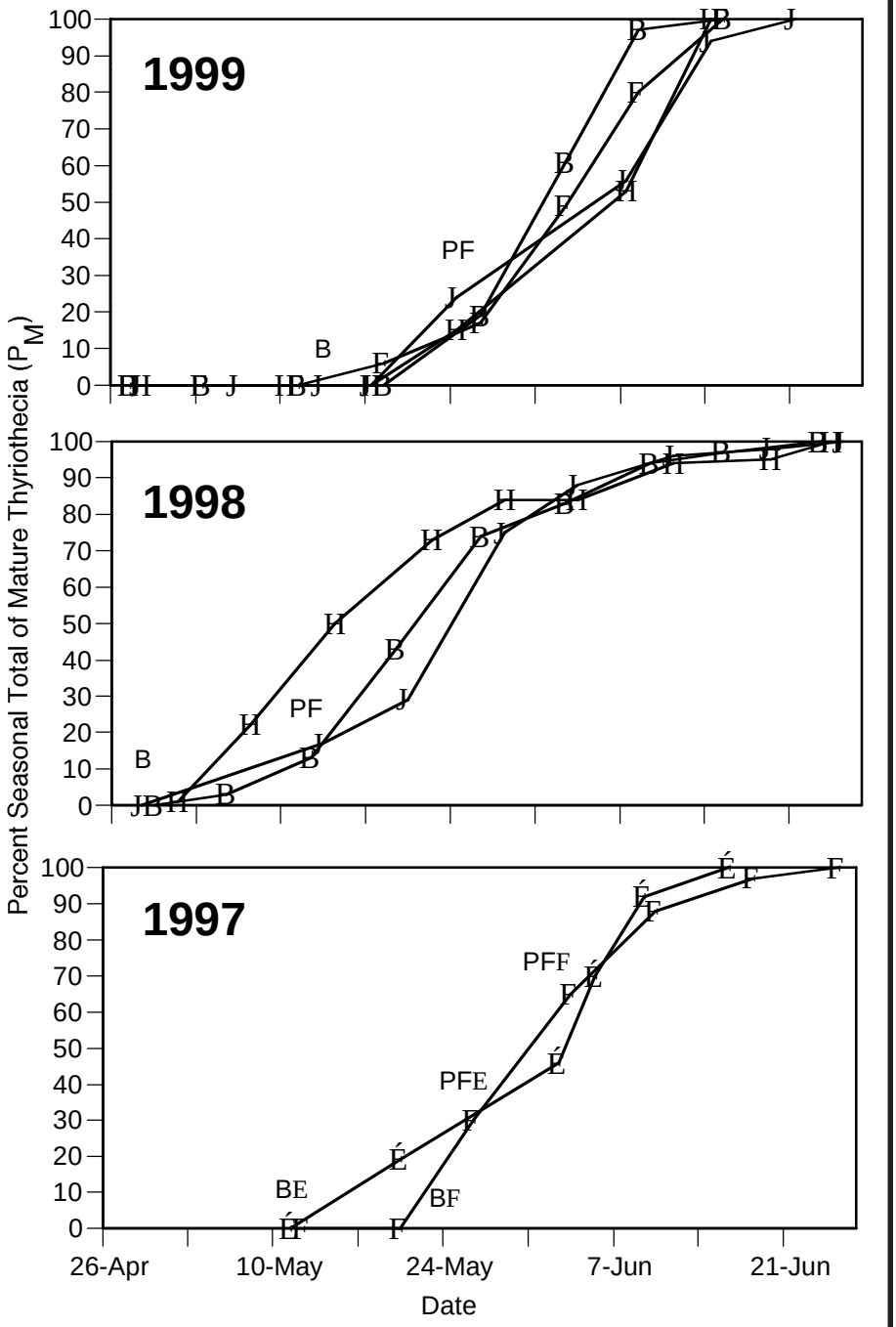

Figure 1. Percent thyriothecia on blackberry canes that matured during each year, 1997, 1998, and 1999, in five Massachusetts orchards: Ashfield (B), Belchertown (J), Brimfield (H), Shelburne (F), and Sterling (É). Times of bloom (B) and petal fall (PF) of McIntosh in 1998 and 1999 are shown, ±3 days depending on the orchard, while in 1997 the phenological stages are indicated for each site.

It is possible that the results differed because the thyriothecia in the laboratory were always exposed to high relative humidity, while those in nature have only short periods where relative humidity meets or exceeds 95%. We calculated degree-day values at the orchard sites for those periods when relative humidity was 95% or greater. Five of the nine data sets had hourly humidity data sufficient to calculate

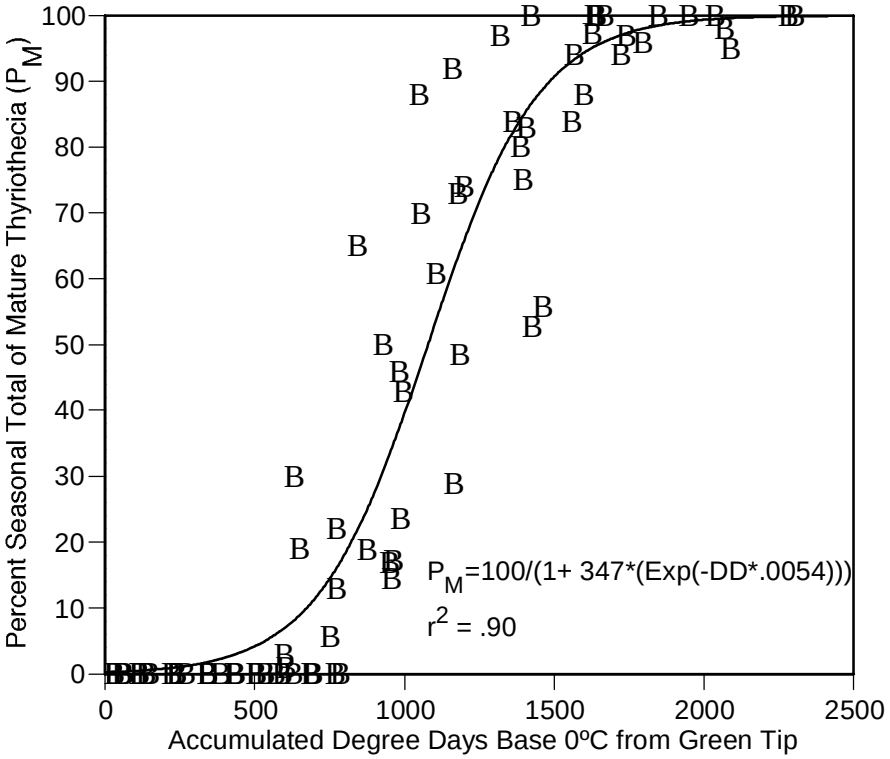

Figure 2. Percent of the season's thyriothecia matured in five Massachusetts orchard sites during 1997, 1998, and 1999 as a function of the degree days (base 0ºC) accumulated from green tip for the apple cultivar McIntosh.

degree days. The mean of these observations was 106 degree days, not significantly different from the 125 degree days observed in the laboratory. So, it appears that both high humidity and accumulation heat are required to get the maturation process started.

Surprisingly, humidity did n0t seem to play a role in spore development once it started. When we looked at high humidity degree days throughout the whole maturation process, from green tip to the end of spore development, then they were poorer predictors of what percentage of thyriothecia would mature than using total degree days.

Would a second generation of thyriothecia develop ascospores in the same growing season? Thyriothecia start to form on blackberry in mid to late summer and continue to form until late fall. The rate at which the number of thyriothecia increased depended on location (Figure 4). At two locations in the eastern Berkshires, thyriothecia counts dropped slightly in September and October, while counts increased linearly at two locations in south-central Massachusetts. The increase in the number of thyriothecia in the southwest was more rapid than that in the northwest. Examinations of these thyriothecia from July through November never found any that matured and produced new ascospores.

We feel that is because thyriothecia need to go through a winter, some sort of chilling dormancy perhaps, before they can produce ascospores. When we collected thyriothecia over the winter, they differed in their potential to mature, depending on the date they were collected and on the site where they grew. Thyriothecia collected from three sites in Massachusetts during December 1996 failed to develop after one week of incubation in high humidity at 70º (Figure 3). Of thyriothecia collected in January 1997, 4 to 13% developed mature asci after incubation, and the percent that matured in subsequent months generally increased through April, when sampling ended. In other words, with more exposure to winter temperatures, the fungus is increasingly ready to produce spores when it gets warm.

Therefore, it appears *S. pomi* has a disease cycle similar to that of *V. inaequalis*, in that ascospores get ready to grow during the winter and early spring, and then are matured and released when environmental conditions are favorable in the growing season. There is little evidence that conidia or mycelia serve as primary inoculum. While thyriothecia have been found on apple twigs and fallen fruit in orchards, it is unlikely that these serve as important sources of primary inoculum in commercial orchards where fungicides are commonly used.

Like *V. inaequalis, S. pomi* produces the season's generation of ascospores over a discrete period generally corresponding to phenological development

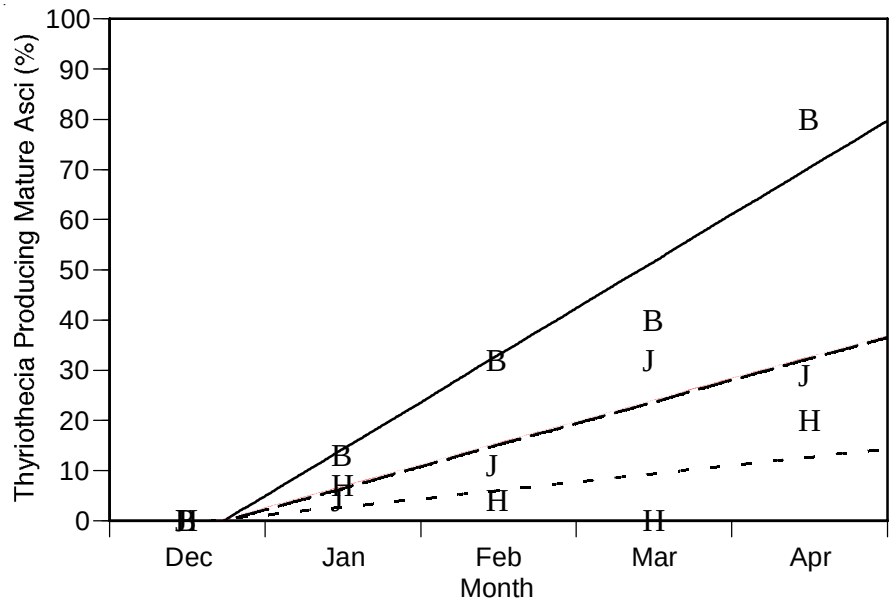

Figure 3. Percent of thyriothecia that had produced mature asci after one week incubation at 21ºC. Collections from blackberry canes at three sites in Massachusetts, Amherst (B), Wilbraham (J), and Shelburne (H), during 1996-1997. Lines indicate linear regressions for each site.

temperature.

In our study orchards, the first flyspeck infections on fruit occurred at the earliest on July 16, nearly 30 days after the production of ascospores had stopped, and as late as September 21, 90 days after the end of ascospore production. While fruit infections first appeared by mid-July at the earliest in Massachusetts, they appear earlier in warmer climates: near the end of June in West Virginia (5), by late May or early June in North Carolina (3), and by the beginning of May in Alabama (6). Again, this is not surprising given that both tree development and fungal development are in apples. In New England, the period when *S pomi* produces mature asci generally starts at late pink to bloom, and continues for 4 to 6 wks, when fruit are 0.75 to 1.75 inches in diameter. While the beginning and end of ascospore production coincided with apple growth stage, dates varied from year to year just as a growth stage such as bloom varies. For example, depending on the orchard and year, flyspeck ascospores began to mature as early as May 5 and as late as May 25, as would be expected when a process is highly dependent on

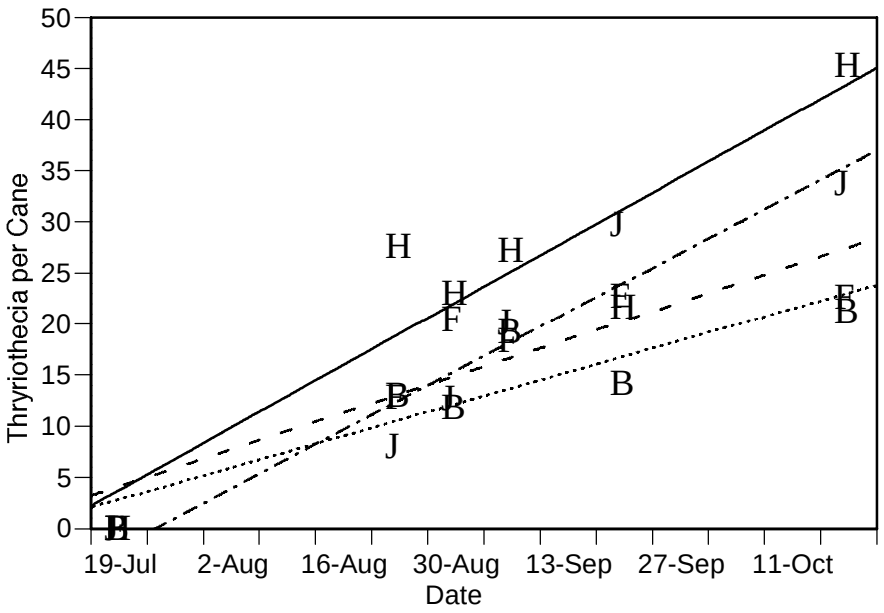

Figure 4. Thyriothecia counts per cane from blackberry canes growing in border areas of four apple orchards during 1999 in Massachusetts, Brimfield (H), Belchertown (J), Shelburne (F), and Ashfield (B). Ten canes located in a flagged area of the orchard border were counted on each date. Lines represent the linear regressions for each location.

largely temperature driven.

In our study during 1999, while apple infections first appeared between September 15 and 21, the first new thyriothecia (infections) appeared on blackberry canes in the borders of four orchards by August 17-24, about 4 weeks earlier. This may indicate that the environment in orchard hedgerows is more conducive to *S. pomi* growth, or that the infections on these canes occurred before those on adjacent apple trees. The latter possibility would support the hypothesis that epidemics originate on alternate hosts in orchard borders and spread to the orchard from there via conidia.

A significant number of thyriothecia never mature. Over the three years, from 33% to 69% of thyriothecia did not contain any signs of spores at the end of the maturation cycle. Such thyriothecia might have actually been fertile, but when examined, they had released their spores and the spore tissue had disintegrated. Possibly some thyriothecia are damaged over the winter by cold temperatures or desiccation. It takes two different mating types of the *S. pomi* fungus to produce ascospores, and perhaps some thyriothecia never come in contact with a different mating type of the fungus, so no spores can develop.

We have answered a couple of the key questions. *S. pomi* does not produce ascospores throughout the year, but only at one time, and that time is between pink and early fruit set. Unfortunately, we still have not answered the question of where these ascospores cause their infections. We do have some suggestions. A 30-day period between the appearance of inoculum and the first flyspeck symptoms in the field is typical of flyspeck (9). In one of three years in this study (1998) the end of ascospore production was 30 to 40 days before first infections were detected. Apples inoculated with ascospores from several wild hosts can cause flyspeck symptoms (1; 2). However, conidia of *Z. jamaicensis* commonly infect apple and cause flyspeck (1; 4; 8). Spore trapping and the timing of symptom development also indicated that conidia are a significant portion of the inoculum that causes flyspeck on apple (10). Conidia are common during the period of most rapid symptom development. In 2 of 3 years the latency period between the end of ascospore release and the first appearance of flyspeck on apple was 60 to 90 days, and it is unlikely that ascospores do any more than start the epidemic.

As we said at the beginning of this paper, if the initial inoculum of flyspeck epidemics is primarily ascospores, it might be possible to manage the disease by preventing these primary infections, much as apple scab is managed by targeting primary ascosporic infections. However, if most or all of the infections that blemish apples are secondary infections caused by conidia that arise on reservoir hosts in orchard borders, primary infections would have to be stopped in those borders. Given legal constraints on fungicide use off site, and the expense involved in owning and clearing border areas, such an approach is problematic. Before such treatments could be recommended, it would be necessary to know that the registration changes and expense of these approaches would be justified in terms of SBFS disease reductions. At present we have a better understanding of when primary infections occur.

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