Discrepancies Between Direct Observation of Apple Scab Ascospore Maturation and Disease Model Forecasts in the 2014 and 2015 Growing Seasons

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Apples are one of the most heavily sprayed crops in the U.S., in part because in temperate climates the disease apple scab (caused by *Venturia inaequalis* (Cke.) Wint) requires several fungicide applications a year for adequate management, and in recent years the number of fungicide applications per season has been increasing in the Northeast (Cooley et al., *Fruit Notes*, Fall 2013). The reasons for this increase are complicated, and include scab resistance to fungicides, the loss of fungicides with significant post-infection activity, and the economic decision that a fungicide application even if scab risk is marginal is inexpensive insurance against significant losses to disease. To reduce fungicide applications, growers need confidence that there is no scab risk. One of the most important tools in estimating scab risk is the model that estimates the maturity of *V. inaequalis* ascospores

The first apple scab infections, primary infections, are initiated by these ascospores, and begin in the spring when ascospores are released from pseudothecia, the fruiting body of the *V.inaequalis* fungus. Pseudothecia develop on infected apple leaves that remain on the orchard floor through the winter (Figures 1 and 2). Once released from the pseudothecia, ascospores can land on susceptible host tissue and, if weather conditions are appropriate, cause infection (Figure 3). These primary infections typically lead to subsequent secondary infections, which can cause scab outbreaks to explode, rapidly spreading through an orchard. While scab epidemics start as primary infections on leaves, it is ultimately secondary infections on fruit that cause the most economic damage (Figure 4). If primary infec-

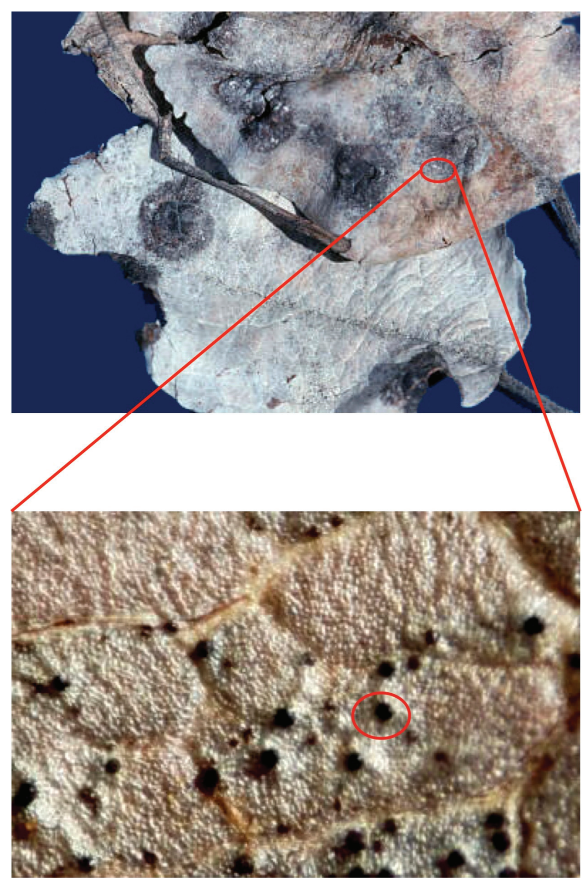

Figure 1. Upper: apple leaf from an orchard floor showing scab infections from the previous growing season (dark areas). Lower: magnified scab lesion in a leaf showing several fruiting bodies (round, dark objects), pseudothecia, with one pseudothecium circled. (Photos: upper – Ministry of Agriculture, BC, Canada; lower – North Carolina State University Extension)

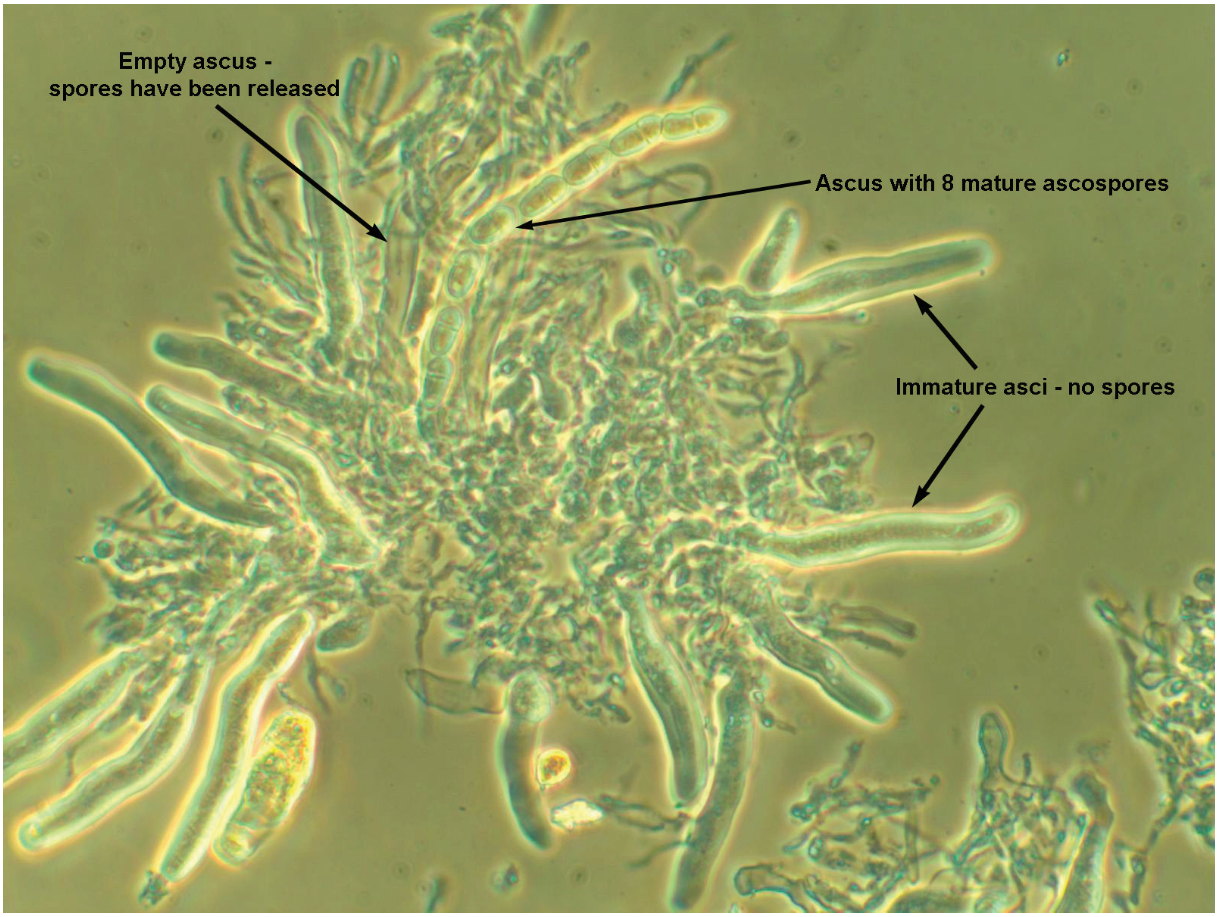

Figure 2. *Venturia inaequalis* pseudothecium magnified under a microscope (400X) in a prepared "squash mount" showing the three important stages of ascospore maturation: immature asci with no spores or immature spores; a mature ascus with mature ascospores; and an empty ascus which has discharged spores. To estimate maturity, each type of ascus must be counted for several (10) pseudothecia on each evaluation date. (D.R. Cooley)

tions are effectively managed, serious scab epidemics seldom develop.

While there are a number of key elements in effective primary scab management, it is critical that growers know when ascospores first become available, when their numbers are greatest and when their stores have been exhausted. The first release of ascospores marks the beginning of primary scab season, and the last release is the end. Generally ascospore development moves with apple tree development (phenology). Using the cultivar McIntosh as a reference, mature ascospores are generally ready for release when the first green tissue is visible, green tip, and the last mature ascospores are available during early fruit development, a week to 10 days after petal fall.

However, tree development is not always an accurate way to evaluate the chance that ascospores may be released, as tree development varies by cultivar. Additionally, in some years weather may delay or advance ascospore development relative to tree growth stage. If ascospore release starts after green tip, growers will spray fungicides unnecessarily early in the season. If the primary season lasts longer than estimated, growers may not apply fungicide against late-season primary infection events, and have to battle scab for the rest of the growing season. The early season, around green-tip, is often considered relatively low risk in "clean" orchards, because the relative amount of scab inoculum is low. However, if ascospores development is advanced, the relative amount of inoculum increases, raising risk of infection.

A much more accurate way to determine whether ascospores are mature and ready to be released, is to look for them. Because ascospores cannot be observed by the naked eye, it takes a microscope and a trained observer to run such evaluations. In the early days of apple IPM, Extension made direct ascospore maturity observations using microscopes in laboratories, a process that involved significant time and some skill.

Figure 3. Primary scab infections on an apple leaf. (Photo APSnet.org)

To eliminate this labor-intensive process, researchers developed models that relate ascospore maturity to growing degree-day (GDD) accumulations, a means of measuring heating units, often calculated by determining the mean of the high and low temperature for the day (James & Sutton, 1982; Gadoury & MacHardy 1982; Figure 5). One of these developed at the University of New Hampshire has been widely used in IPM programs. GDDs are calculated using a base of 32ºF, and accumulation is started at 50% green tip of McIntosh. The model enables users to estimate periods when a large proportion of ascospores are available, and the end of primary scab season, with somewhat better accuracy than tree growth stages. In a typical year, these times could be predicted once green tip occurred, based on historical temperature data (MacHardy & Gadoury, 1985).

Figure 4. Apple scab on an apple that was not sprayed with fungicides. (D.R. Cooley)

It was noticed, however, that ascospore maturation could deviate from the model, sometimes significantly. While the New Hampshire model used only temperature, a model developed in North Carolina had also included a moisture factor, requiring rain or high relative humidity for ascospore maturation. Based on observations of scab infections in Norway, researchers re-examined the New Hampshire model to see whether a moisture factor would improve estimates of ascospore maturity (Stensvand et al, 2005). They found that as little as four consecutive dry days, with less than 0.2 mm of precipitation and less than 12 hours of dew or

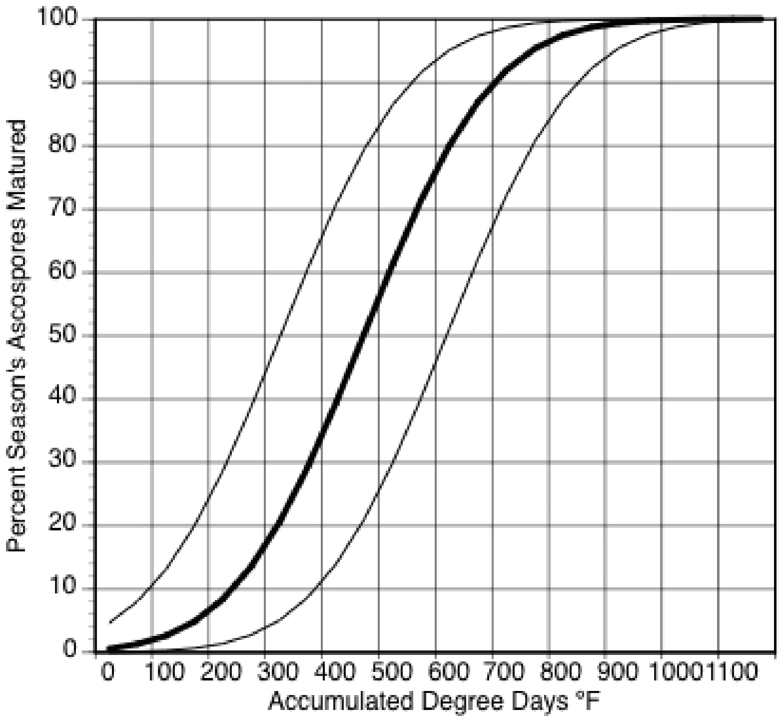

Figure 5. Graph showing the maturation of *Venturia inaequalis* ascospores in response to degree-days, base 32 ºF accumulated from green tip. The narrower lines are 90% confidence intervals indicating the range of ascospore maturation at a given number of degree-days 90% of the time.

fog, is enough to cause ascospore maturity to slow, and if these dry conditions continued for 7 days or more, maturation ceased altogether until wetting requirements have once more occured. This phenomenon is technically called a rainfall frequency threshold, and commonly referred to as the "dry switch". This led to an adjustment to the model to deal with dry periods

The New Hampshire ascospore maturity model has been incorporated into web-based Decision Support Systems (DSSs), which combine weather data with pest forecasting models to provide online advice to growers about pest risks and treatment options. There are different DSSs available in New England and New Jersey to estimate risk of scab, all of which use variations of the MacHardy/Gadoury ascospore maturity model. These include NEWA (the Network for Environment and Weather Applications) managed by the New York State IPM Program at Cornell; Ag-Radar managed by the University of Maine Extension; and the commercial product SkyBit (ZedX, Inc.). Aditionaly, RIMpro, developed and maintained by Bio Fruit Advies in the Netherlands will be available on a limited, trial basis for growers in northeast in 2016. In a typical growing season, the models in these DSSs are accurate in New England. However, when growing conditions stray from those generally experienced, failures occur. Indications are that more extreme weather events, such as prolonged dry or wet periods, as well as increasing temperatures, now occur and will continue (Kunkel et. al. 2013). This was clearly seen in 2015 when drought came to the Northeast in the spring, a typically wet time of year. The National Drought Mitigation Center's US Drought Monitor reports that 23% of the Northeast experienced a moderate drought in May, and that up to 64% of the Northeast experienced abnormally dry weather thorough April and May. The number of frost-free days is also increasing. Subsequently, timing of green-tip has become more variable, making it an even less appropriate indicator for the presence of mature ascospores.

The difference between DSS estimates and actual ascospore maturity was seen in 2012 in the Hudson Valley of New York, where the NEWA DSS estimated a much earlier end of primary ascospore production than was observed using a laboratory evaluation of ascospore release from leaves (Rosenberger, personal communication). While NEWA uses a dry switch, it apparently is not adequate for a very extended dry period with less rain and lower relative humidity levels than had been seen in that area since ascospore maturation levels had begun to be documented. Subsequent discussions with a group of researchers suggested that thresholds for "dry" and "wet" in the ascospore maturity model needed to be improved.

In an attempt to better understand how weather such as 2012 might affect the New Hampshire ascospore maturity model and DSS estimates of scab risk, in 2014 and 2015 we compared DSS estimates of ascospore maturation with different types of direct ascospore maturity observations. In the fall, scab-infected leaves from unsprayed apple trees were collected and overwintered outdoors at the University of Massachusetts Cold Spring Orchard Research and Education Center (CSOREC) in Belchertown, MA. In the spring, these leaves were used in direct observations

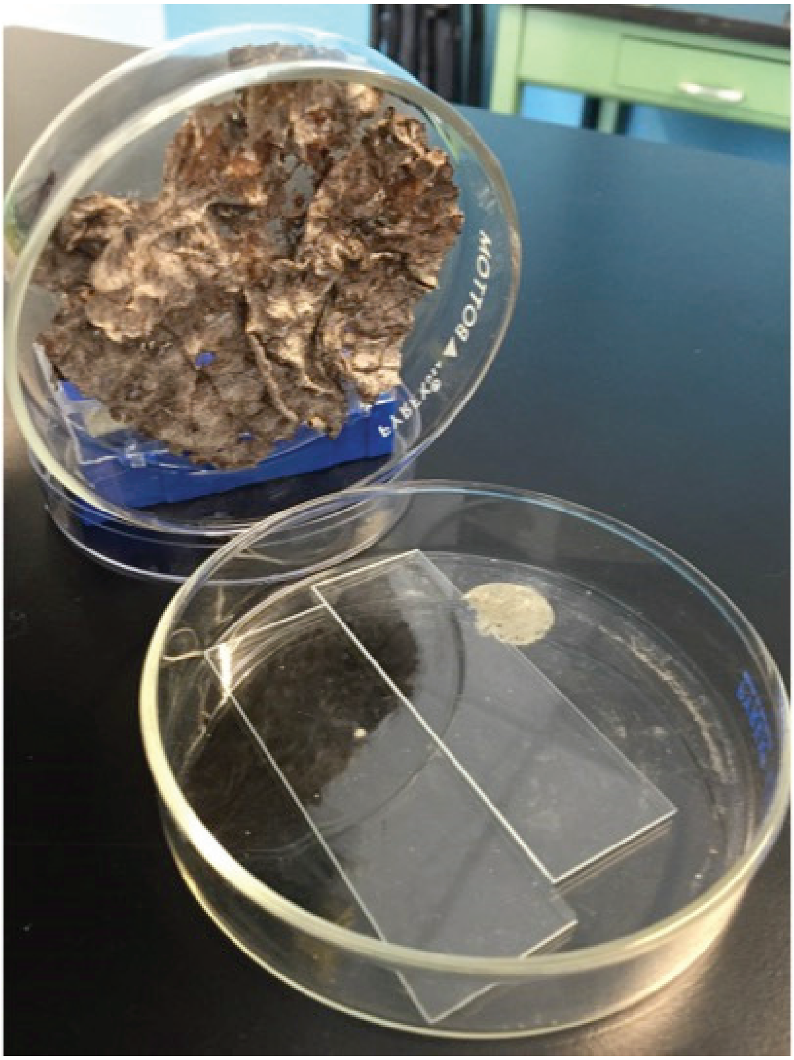

Figure 6. Petri dish with microscope slides on the bottom section, and overwintered, scab-infected apple leaves attached to the cover section. (L. Garofalo)

of ascospore maturity. In 2014, we used three assays:

1. Standard "squash mounts" of fruiting bodies of the fungus sampled from a set of leaves (Figure 2);
2. A Petri-plate assay in which, after wetting, leaf sections are put in a moist Petri plate and spores are released onto a microscope slide (Figure 6);
3. A spore trap using fans to concentrate on a microscope slide ascospores released from several leaves, the "two-fan trap" (Figure 7).
4. In 2015, we added a fourth method: A spore trap in the lab using a single fan to draw air over leaves down through a funnel to an orifice over a microscope slide, the "funnel trap" (Figure 8).

For the squash mounts, 10 pseudothecia were collected from six randomly selected leaves. The pseudothecia were placed in a drop of water on a glass microscope slide, and gentle pressure applied to a glass cover slip on top of them to break them open. The asci and any ascospores inside the pseudothecia were then evaluated for maturity.

Petri-plate assays were performed by tapping six sections of overwintered leaves containing pseudothecia into the lid of a Petri plate. Two microscope slides were placed in the bottom of the Petri plate with a small amount of distilled water, and the lid placed on top. After an hour, these slides were examined for the presence of released ascospores. These lab tests were performed approximately every week.

Ten to fifteen leaves with pseudothecia were used for the field-based spore trap. These were placed in a single layer on the ground under a large-mesh wire screen. The leaves were surrounded by a rectangular "box" with dimensions of 15 ¾" X 9 ¾" in. X 8", framed with wood strips, open sides were covered with landscape fabric and the ends were of hardboard. Within the box leaves were held in place, scabby side up, with hardware cloth. Two fans were located at opposite ends of the box, which were activated by a leaf wetness sensor when a rain event began. The fans moved an air current from one end of the box to the other, where any released spores were forced through a small opening onto a microscope slide. The slide was brought back to the lab and inspected after each rain event ended.

The funnel trap was used on leaves brought from the field to the laboratory. It consisted of a plastic funnel with a small electric fan that drew air over wet leaves placed on a screen at the wide end of the funnel down to a slot and microscope slide at the narrow end of the funnel. The fan was run for 20 minutes, after which the

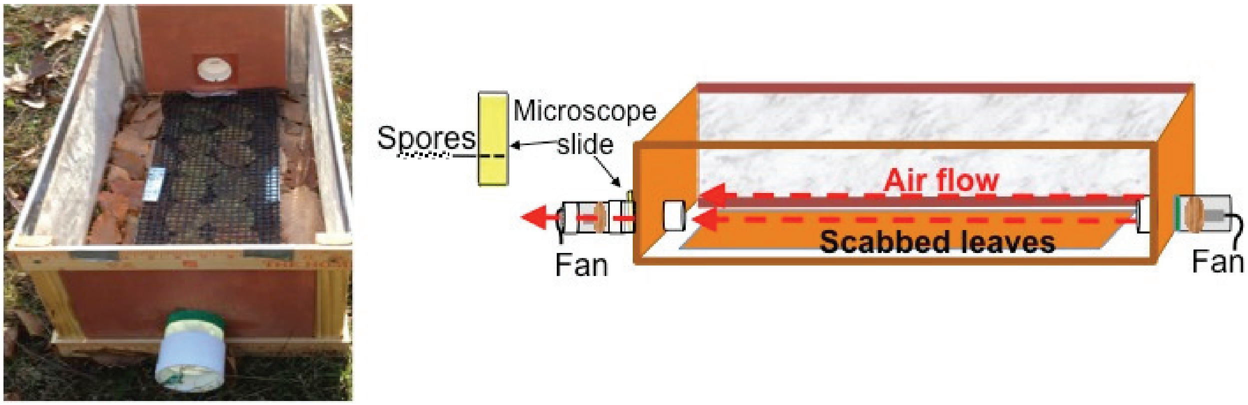

Figure 7. Two-fan trap designed by MacHardy, actual trap with leaves on the left, schematic showing design on the right. (W.E. MacHardy)

slide was inspected for spores.

We compared four DSSs: NEWA, Ag-Radar, SkyBit, and a commercial product being introduced in the U.S., RIMpro (Bio Fruit Advies, Zoelmand, Netherlands). The DSSs have rules that determine when maturation will start (the biofix), usually a simple observation of green tip as in the New Hampshire model. SkyBit estimates a green tip date, which can be corrected by supplying ZedX with the observed green tip date. RIMpro uses the first date that spores are released, or green tip, whichever comes first, as the biofix.

Weather data for the NEWA maturity estimates came from a weather station (Rainwise, Trenton, ME) at the CSOREC site. RIMpro also used data from this station. SkyBit uses virtual weather data. Virtual data are created by combining different sources of actual weather observations (e.g. National Weather Service) with proprietary mathematical techniques, which basically interpolate from the actual observations to estimate weather for locations distant from weather stations. SkyBit sells E-Weather service products and offers an "Ag-Weather IPM Apple Disease Product" that includes virtual weather data and scab risk. Ag-Radar uses the SkyBit virtual data in a somewhat different model.

In 2014, ascospore observations were not made until green tip on April 14, at which point mature ascospores were observed in all three assays (Table 1).

Figure 8. Funnel trap designed by MacHardy, showing leaves in wire mesh. Air is drawn down to an orifice over a microscope slide. (W.E. MacHardy)

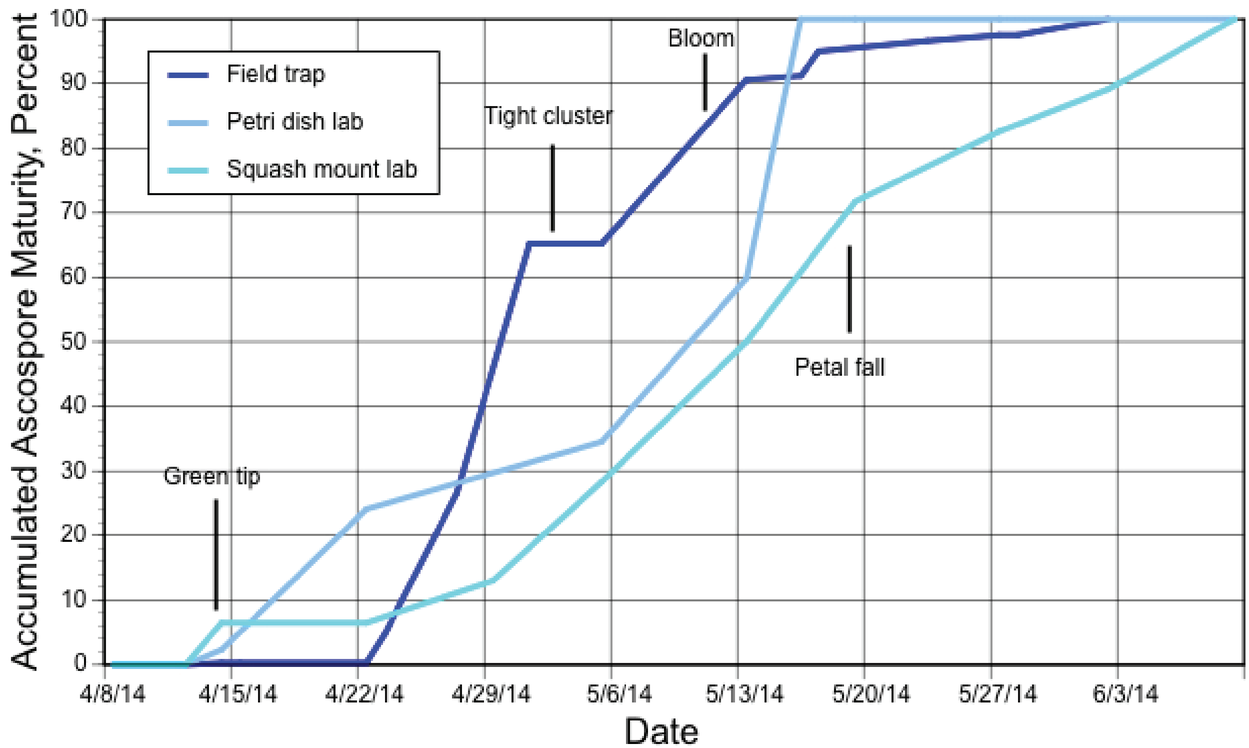

Figure 9. Observations of ascospore maturity using three methods with leaves overwintered at Belchertown, MA, 2014.

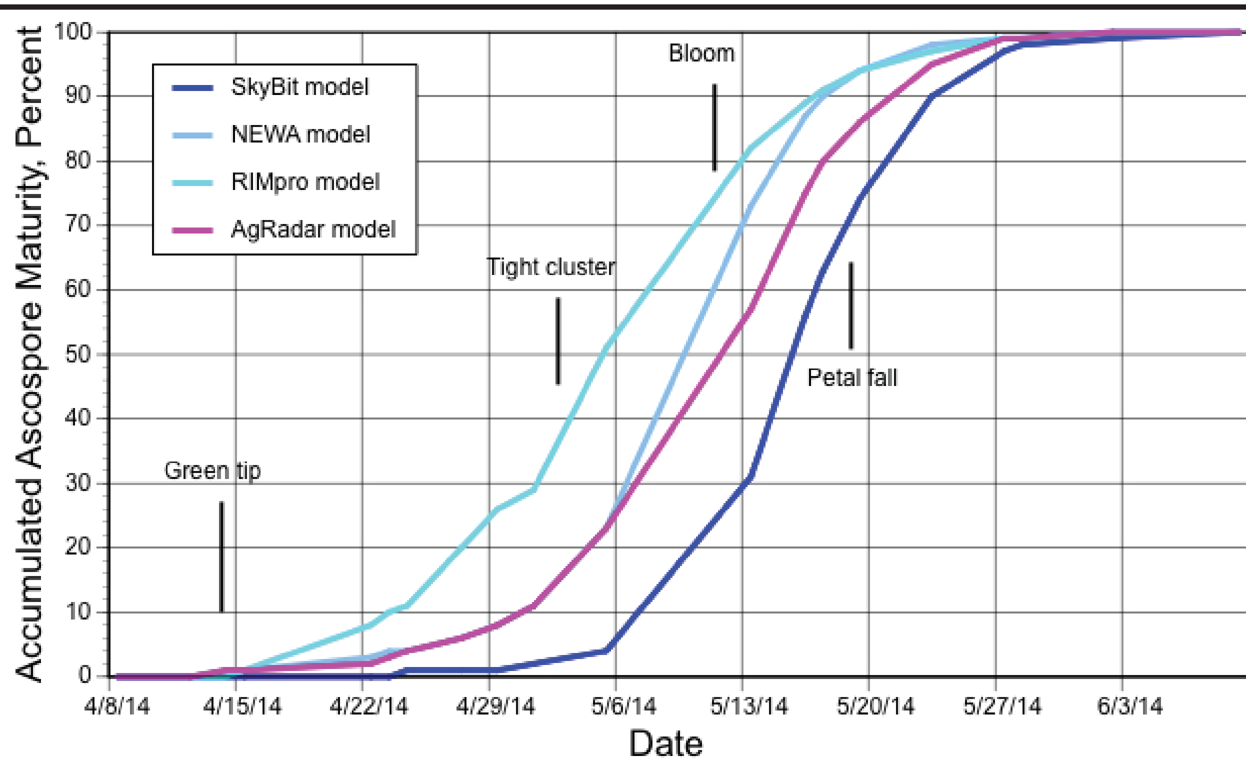

Figure 10. Ascospore maturity estimates by four different decision support systems using data from Belchertown, MA, 2014.

The Petri-dish assay and the field trap had similar patterns of ascospore maturation, while the squash mount assay had a maturation pattern that lagged behind the trapping assay (Figure 9). The direct observations of last available ascospores, indicating the end of primary seasons, were very different. For the Petri-dish assay, the last spores were seen on May 19, for the two-fan field trap on June 2, and for the squash mounts, June 9.

In 2014, the four DSSs also differed in terms of their estimates of the end of primary apple scab (Table 1). NEWA had the earliest estimate of the end of ascospore release, May 28. RIMpro, SkyBit and Ag-Radar estimated the end of the season within 3 days of each other, on June 3, June 4 and June 5, respectively. That pattern of maturation was generally similar for the DSSs, though SkyBit was consistently the latest and RIMpro the earliest (Figure 10).

In 2015, squash mounts were the first to show mature ascospores on April 6, two weeks before McIntosh green tip (Figure 11). Though the spores appeared mature in the squash mounts, no spores were released in any of the trapping assays until three weeks later on April 27 (between green tip and tight cluster), when spores were seen in the Petri plate assay. First release in the funnel trap assay and field two-fan trap were even later, at May 4 (tight cluster) and May 19 (petal fall), respectively, one to three weeks later than the first spores seen using the Petri-plate trap. Overall there was a difference of nearly six weeks in first mature spore observations between the squash mounts and the last trapping assay, the field trap (Table 2).

Similarly, squash mounts estimated the earliest ascospore development, though the Petri dish and funnel trap assays were similar to squash mounts after petal fall (Figure 11). The two-fan field trap indicated the slowest spore development; no spores were caught in it until after petal fall. The assay estimates of the end of primary season were more consistent. All were on the same day, June 22. (Table 2)

The DSS estimates of the beginning of scab season were more consistent among each other. NEWA and Ag-Radar estimated the same day, April 19 (green tip), while RIMpro and SkyBit estimated later dates, April 22 and April 27, respectively. The DSSs differed by over a period of over three weeks in their estimates of the end of primary season, from May 27 by NEWA to June 20 by RIMpro (Table 2). RIMpro had an extended period of no maturation, 22 days from April 29 to May 20. Ag-Radar showed no spore development from April 30 to May 12 (13 days), NEWA from April 29 to May 4 (6 days), and SkyBit from April 27 to May 3 (7 days) (Figure 12).

These periods reflect a prolonged period of dry weather that began on April 23 and ended May 19, when 0.13 inches of rain was recorded by the weather station. The weather station also recorded 0.02 inches of rain, the minimum recordable amount, on May 5 and again on May 12. (Figure 14). SkyBit estimated 0.02 inches of rain on May 12, and 0.42 inches on May 19.

In 2015, NEWA, SkyBit and Ag-Radar estimated the end of primary season to be two to three weeks earlier than the last date when ascospores were actually observed. The RIMpro estimate, June 20, was much closer to the last spore captures, June 22. It is clear that the different systems have different ways of estimating the impact of dry periods on spore maturation. The assays all indicated that mature ascospores were available and released from two to four weeks after petal fall, much later than the usual week to 10 days. Both NEWA and SkyBit estimated the last ascospores were available at the end of May, two to three weeks before the last spores were seen in the assays. Ag-Radar estimated June 8 as the end of ascospore production, one to two weeks earlier than the assays. RIMpro estimated an end of ascospore production to be June 20, only two days off from the June 22 assay date.

Both the DSS estimates and the assay observations matched tree phenology in 2014, a normally wet year without extended dry periods (Figure 13). In 2015, with the extended dry periods, assay observations and estimates from three of the DSSs were significantly different. From a management perspective, this means that growers depending on NEWA, SkyBit or Ag-Radar to determine the end of primary inoculum availability for apple scab would stop scab fungicide applications prematurely. For example, NEWA states "Ascospores were essentially all released on May 27" and that infections from that event should appear within 10 days by June 6. Four infection periods occurred between June 6 and June 22, when the last ascospores were trapped. If fungicides were not applied to protect against those four infection periods, there would have been a risk of primary infection. RIMpro was the one DSS that accurately estimated ascospore maturity as it was observed.

An important aspect of DSS recommendations is using weather forecasts to predict the risk of scab infections. Since fungicides should be applied as protectant materials, before infections happen, growers need to know when scab infections are likely to occur. Such forecasts use models which are based on Mills

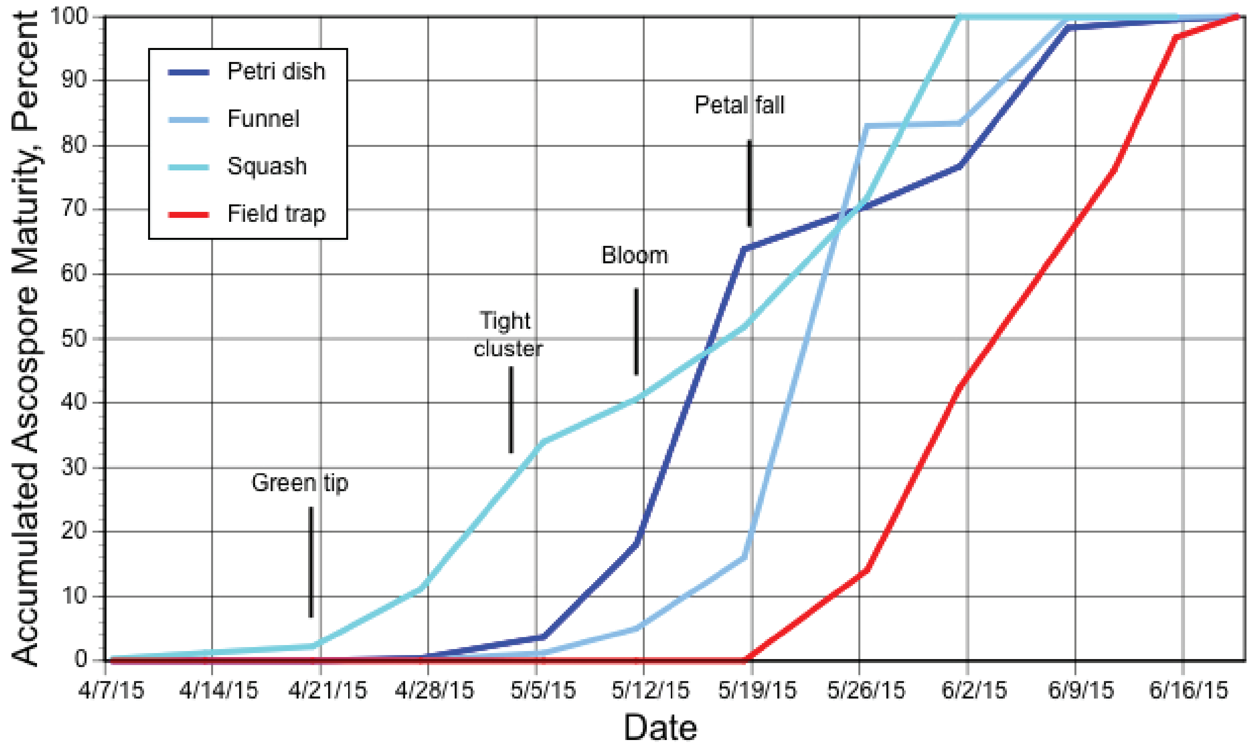

Figure 11. Observations of ascospore maturity using four methods with leaves overwintered at Belchertown, MA, 2015.

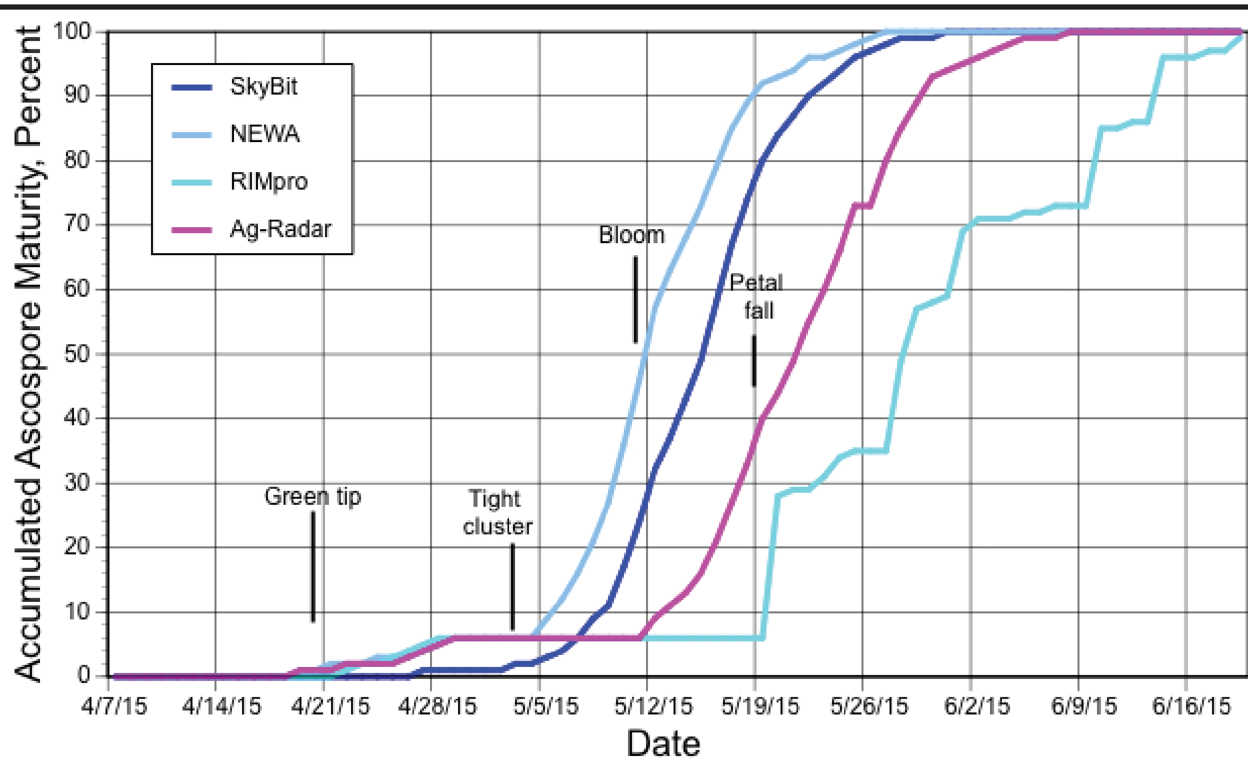

Figure 12. Ascospore maturity estimates by four different decision support systems using data from Belchertown, MA, 2015.

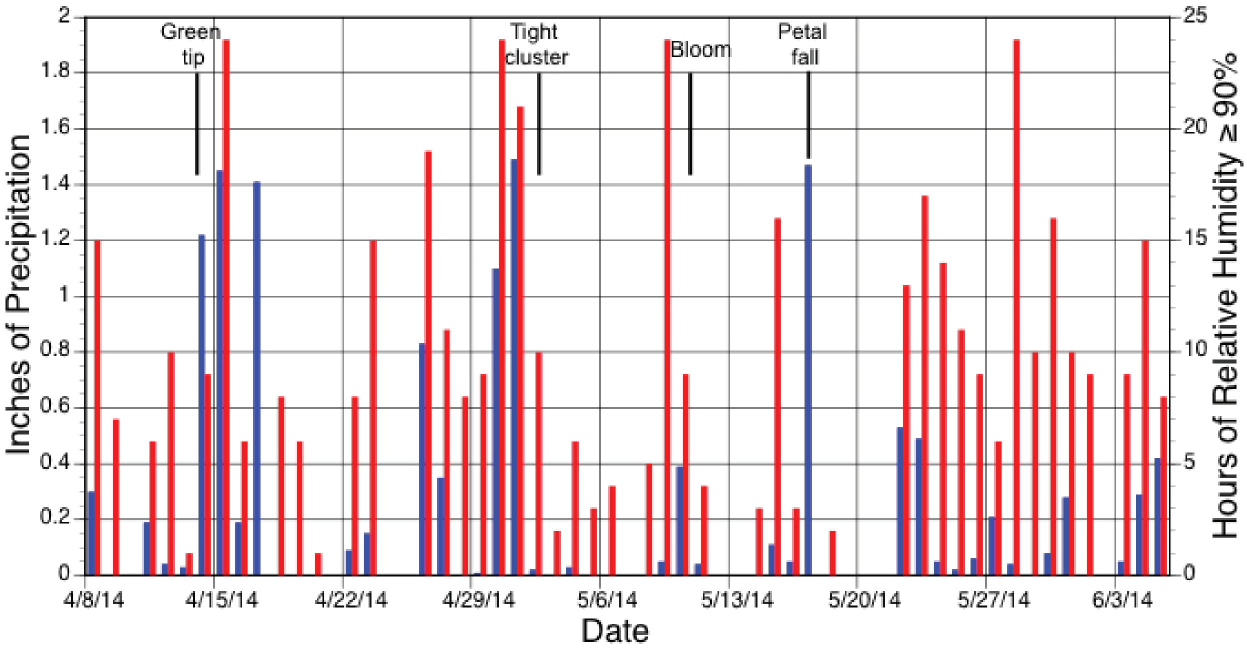

Figure 13. Daily rainfall and daily hours of relative humidity above 90% at Cold Spring Orchard, Belchertown, MA, April through June, 2014.

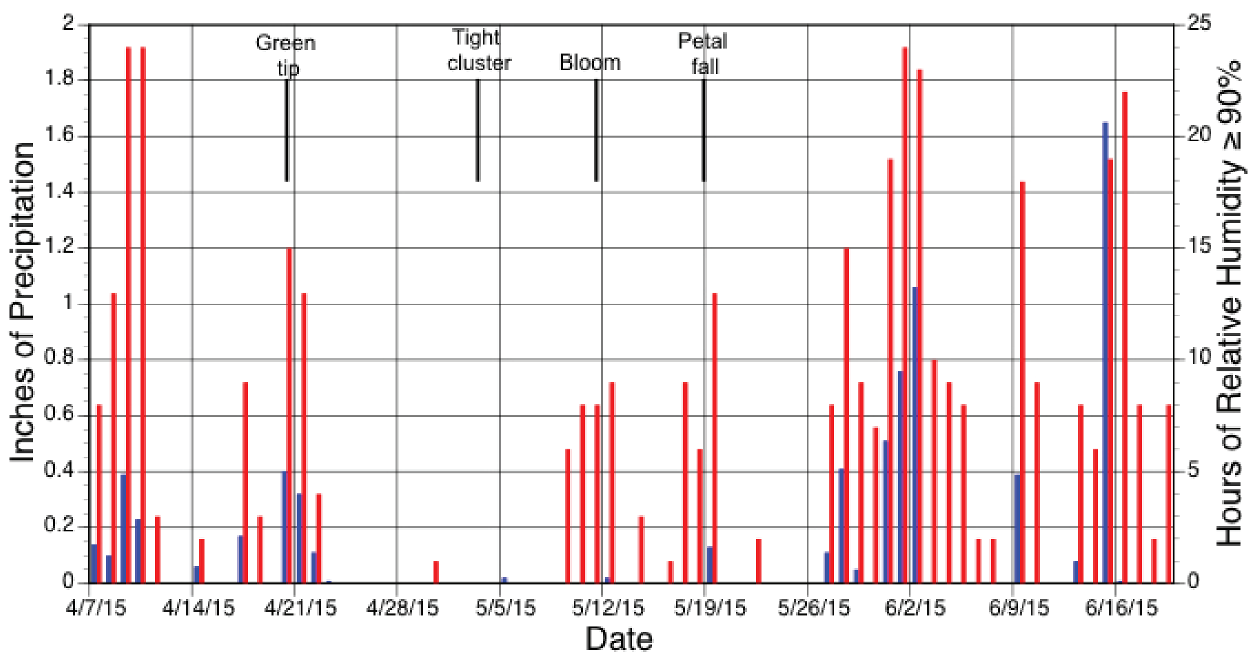

Figure 14. Daily rainfall and daily hours of relative humidity above 90% at Cold Spring Orchard, Belchertown, MA, April through June, 2015.

infection periods with different modifications, and the accuracy of the predicted risk of infection depends both on the model and the weather forecast. We do not present the details here, but generally DSSs predicted potential wetting and infection periods a few times at CSO during May, but they never developed. Growers could have decided to wait to see whether a forecast infection period in fact occurred, and then have applied a post-infection fungicide, but if preventive fungicides were applied before the predicted infection, they were

Table 1. Comparison of ascospore maturation in terms the end of primary scab at Cold Spring Orchard, Belchertown, MA, 2014

Spore Assay Method	First Mature Ascospores*	Over 95% of Ascospores Mature	Last Observed Ascospores
Squash mount	April 14	June 9	June 9
Petri plate	April 14	May 16	May 19
Two-fan trap, field	April 14	May 17	June 2
Decision Support System			
NEWA	April 14	May 20	May 28
AgRadar	April 14	May 23	June 5
RIMpro	April 14	May 21	June 3
Skybit	April 14	May 26	June 4
Tree Phenology McIntosh			
Green tip	April 14		
Petal fall plus 14 days			June 2

*First ascospore assays were performed at green tip on April 14, when all assays showed mature ascospores. DSS estimates of first spores are made prior to supplying actual green tip dates.

Table 2. Comparison of ascospore maturation in terms of first date (beginning of primary scab) and last date (end of primary scab) at Cold Spring Orchard, Belchertown, MA, 2015

Spore Assay Method	First Mature Ascospores	Over 95% of Ascospores Mature	Last Observed Ascospores
Squash mount	April 6	June 1	June 1
Petri plate	April 27	June 8	June 22
Two-fan trap, field	May 19	June 15	June 22
Funnel trap, lab	May 4	June 8	June 22
Decision Support System			
NEWA	April 19	May 22	May 27
AgRadar	April 19	June 2	June 8
RIMpro	April 22	June 14	June 20
Skybit	April 27	May 25	May 31
Tree Phenology McIntosh			
Green tip	April 19		
Petal fall plus 14 days			June 2

unnecessary. In the worst case, a grower relying on DSS information might have applied two to three unnecessary fungicides during May, and not applied fungicides when they were still needed in June.

Clearly, further modifications of some DSS models are needed in order to better estimate when fungicide applications are needed and when they are not. Information from the DSSs is useful, but needs to be tempered by grower experience and timely information from other sources. For example, during prolonged dry weather in primary scab season, growers could plan on using post-infection tactics so as not to over-apply fungicides, and be prepared to apply scab fungicides after fruit set in case observations indicate primary scab season extends beyond that point. In time, model accuracy and weather forecasts will improve, but grower experience will undoubtedly always play an important role in efficient and effective scab management.

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