Validation of the apple scab simulator RIMpro using potted trees

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In many growing areas around the world, apple scab caused by *Venturia inaequalis* (Cke.) Wint. greatly impacts production in absence of control measures. Most fungicide applications on apples target the primary infections caused by ascospores (1). Many models describe the fungus biology and help properly time these sprays. RIMpro is a dynamic apple scab simulation program (2) that encompasses many of these models and uses boxcar trains to mimic delays and dispersion (3). The software has been validated extensively in Europe and elsewhere for the past 15 years, but has received little attention in plant pathology.

The RIMpro boxcars cover all aspects of *V. inaequalis* ascospore biology. It integrates models for spore maturation, ejection and infection. Each process is described with equations obtained from the literature and the output of each process serves as the input for subsequent processes. For instance, the number of mature ascospores available influences the maximum ejection possible at any point during a given rain. Further, because the rate of discharge during rain is influenced by both temperature and availability of light, the simulated ejection is updated accordingly. The same approach is used for infection. Spore survival and infection is conditioned by availability of moisture and temperature, and their status reflects conditions encountered by each cohort of ejected spores. Technically, the season starts with a virtual inventory of 10000 virtual immature spores for every orchard. Accounting of spore cohorts of a 30-minute time step are maintained throughout the life of the spore. Spores either succeed in infection and contribute to the RIM value or die at any step during the process. Modeled spores can die either because of simulated leaf litter degradation, or insufficient leaf wetness for infection. In complete absence of leaf degradation and extended rain periods, the maximal seasonal RIM value is thus 10000. All ejected spores are presumed to land on leaf tissue that is 100% susceptible. RIMpro does not account for host effects such as ontogenic resistance and cultivar tolerance. Further, it does not account for rain scrubbing, mortality due to UV light, or any other environmental spore loss. Also, RIMpro does not account for orchards with high or low potential ascospore dose (PAD). Consequently, in the current instatement, RIMpro is said to be pathogen centric. The other components of the epidemiology triangle (host x environment interactions) must be assessed separately for the purpose of crop protection decisions.

The overall objectives of this project were to validate each component of the RIMpro simulator and determine if the final output of the relative infection risk assessment value (RIM) correctly predicts disease severity. Prediction failures will then eventually be used to identify how this simulator can be improved. This report focuses solely on the potted tree validation of the RIM value.

Potted trees were used as trap plants and exposed in orchards for single rain periods starting at budbreak and finishing after the ascospore supply was exhausted. Trees were grown outside and exposed to natural UV light to insure normal cuticle development, but were shielded when required from all other rain events. Trees surrounding the potted trees were sprayed with fungicides and scouted regularly to insure that scab was not spread from secondary infections. The ascosporic inoculum level (PAD) was very variable from year to year and reflected conditions normally observed in commercial orchards. In Italy, for each exposure event from 1989 to 2008, three potted trees of cv. Golden Delicious grafted on a commercial rootstock bearing at least 100 flower clusters or shoots were exposed at the Maso Parti farm of Istituto Agrario San Michele in Mezzolombardo. Trees were 3m tall and at least 3 years old. Date of budbreak and fruit bud phenology of the trees was in close agreement with that observed on trees in the orchard. In Québec, cv. McIntosh trees grafted on commercial rootstocks were forced to have an early budbreak in a greenhouse before trees were moved outside. At least 7 new unfurled leaves were present per shoot before the first exposure in the orchard. Potted trees were exposed from 2006 to 2008 in 4 locations (blocks) of the IRDA research orchard of the Mont-Saint-Bruno national park. There were 5 trees per block for a total of 20 trees per rain event.
For both sites, trees were returned to a shelter after the rain, but only once the trees were dry. Scab was assessed after three weeks. Shoot and cluster scab incidence was recorded for both sites. Leaf scab incidence and severity was recorded in Québec. Only the total shoot and cluster scab incidence data collapsed per exposure event is presented in this report.

A local weather file from a locality in close vicinity was used to calculate the RIMpro simulations. In Italy, the San Michele station was used from 1989 to 1994, and the Mezzecorona Pradacci station was used from 1995 to 2008. In Québec, the station was in the experimental plot. The 2009 RIMpro (version 1.1.18) was used for all simulations.
By design, parameters of the simulation have an average completion time, +/- a standard deviation. For all simulations from Italy, budbreak was used as the biofix for the first ascospore mature and no attempt was made to correct for the actual date of initial spore maturation. In Quebec, the biofix was set on April 1st. The biofix for complete leaf decay was set 3 months later in Italy and on August 1st for Québec. Maturation of ascospores was stopped when more than 5 consecutive days occurred without rain. The Tsum used for 50% maturation was 250 °C base = 0 °C. Standard sheltered air temperature was used for all calculations. Ascospore discharge patterns were left at default values suggested by the program. Specifically, nighttime discharge inhibition was from calculated sunset until 60 minutes after sunrise and was set at 5% of the uninhibited rate. Discharge was triggered when more than 0,2mm of rain (0,1mm in Québec) was accumulated in a 30mn interval. Ejections were set to stop 90mn after rain. Overhead sprinkler irrigation for frost control was recorded as normal rain and considered as such for all simulations.

Ascospore survival on dry surfaces was set at 24h +/-10 h for un-germinated spores and 12hr +/-4 h for germinated spores for which infection was not completed. Foliage was considered wet when leaf wetness sensors were saturated or when rain was recorded. In the absence of electronic leaf wetness sensors (1989 to 1994), relative humidity above 85% was considered a trigger for leaf wetness. Foliage was considered to dry when leaf wetness sensors were no longer saturated or when air relative humidity dropped below 85%. Whenever possible, manual leaf wetness observations were used to correct the wetness recordings. Spore maturation, ejection and infection (RIM values) were all derived from 30 min steps in the simulation. The individual annual simulations were run in approximately 10 seconds on an Intel™ dual core processor running at 2.4 Ghz.

Apple scab incidence was analyzed as a logistic regression in a mixed effect linear model (GLMM) using the lmer function of R (4). Year and site were set as a random intercept to account for varying inoculum levels from year to year and a random slope was used to account for year-to-year differences in tree susceptibility, observations, weather file source and other sources of systematic within year variation. Several variables were compared as predictors of apple scab incidence. The Mills' infection criteria, leaf wetness duration, exposure duration, temperature, and combinations of the above were tested. Overall model adequacy was established by residual analysis. The selection of terms was done both using conditional F-tests (ANOVA) and with the AIC information criteria.

In the 23 site-year dataset (Fig. 1), an important range of conditions was encountered. Observed scab per exposure event varied from 0% shoot scab incidence to a maximum that ranged from 0.83% (Italy, 2007) to 100% (Québec, 2006 and 2007). Thus, the total primary scab varied greatly from year to year and was most likely a reflection of the natural overwintering inoculum levels. Because of this variation, RIMpro can only provide a relative risk assessment for a given site and year. The calculated RIM index for individual events varied from 0 to a maximum that ranged from 703 (Italy, 2003) to 4754 (Italy, 1995). The total predicted risk for a given year ranged from 1330 (Italy, 2003) to 6295 (Italy, 2001). By design, this index is a reflection of how conducive the climate is for the primary infections, notwithstanding the inoculum level present.

Overall, the RIM value was the best single parameter for predicting scab incidence. Logit scab incidence = 1,68*log10(RIM+1) – 6,9. For RIM values of 10, 100, and 1000, the fixed effects components of the model predict 0.6, 2.8, and 13.5% shoot scab respectively. For 6 site-years, the predicted RIM values for individual events were in very close agreement with scab incidence observed on potted trees (Italy 1989, 1990, 1991, 1998, 2003, 2004). However, there seemed to be no relation between RIM and scab levels for 5 sites (Italy 1993, 1997, 2002, 2005, 2007). Most of the sites with poor correlation had very low scab incidence. Because of limits in the precision of the experiment, potted tree scab predictions are likely to be difficult under these circumstances. In general, all predictors tested also had very low fits for these sites. For 7 sites (Italy 1992, 1994, 1995, 1996, 1999, 2008, and Québec 2007) there are only one or two points that are not well predicted by the RIM value. The 5 remaining sites show a moderately adequate fit. There were 4 sites with at least one false negative prediction that showed more than 5% shoot scab incidence (Italy 1993, 1994, 1996, Québec 2008). In most cases, other predictors also made false negative predictions for these sites.

It is likely that some lack of fit may be due to human error while handling the potted trees over the course of this 20-year study. In the next step of this project, we will compare the expected ejection intensity to volumetric spore trap data and try to identify reasons for prediction failures where possible. We have already identified that the biofix for the first available spore is a major source of error for certain years. A related project is underway to find a better criterion for setting this biofix.

Nonetheless, the ability to predict with a reasonable level of confidence the relative scab severity of individual rain events opens the door to more tailored fungicide applications. For instance, incomplete fungicide coverage could be tolerated without consequence for rain events for which scab severity is predicted to be low, whereas events with high RIM values need perfect coverage.

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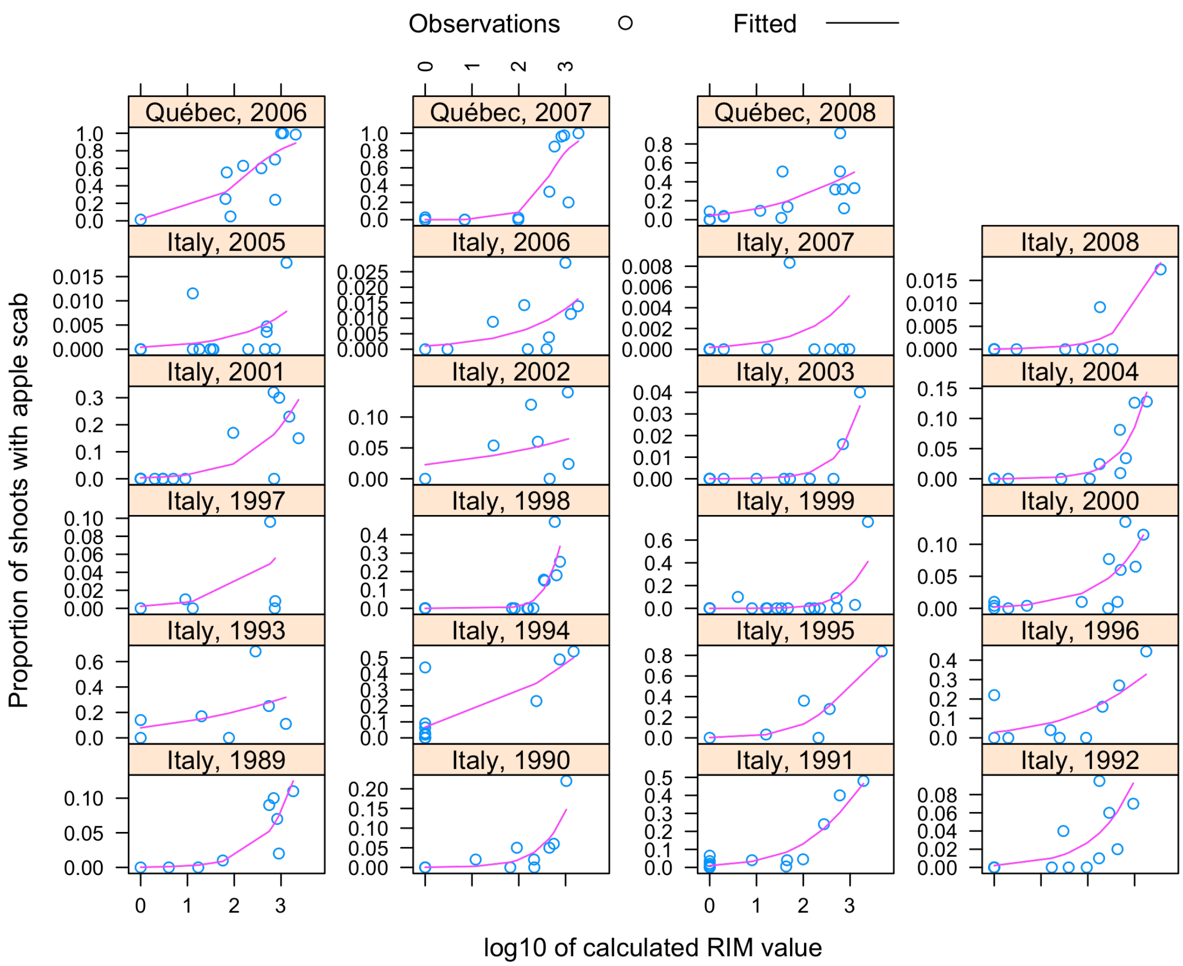

Fig. 1. Shoot scab incidence on trap plants (potted apple trees) for individual exposure events in relation to the output of the RIMpro apple scab simulator fitted with logistic regression.