Dodine Sensitivity in *Venturia inaequalis* Populations and The Possibility for Renewed Use Against Apple Scab in The Northeastern US

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The apple scab pathogen, *Venturia inaequalis*, causes extensive crop loss in all production regions in the northeastern US. In the absence of durable host resistance in all commercially desirable cultivars, producers are forced to undertake intensive chemical management programs to avoid fresh market losses. Indeed, apple scab is managed with as many as 10 fungicide applications per season. The demethylation inhibitor fungicides (DMI) (e.g. Rally 40 WSP, Vintage SC, Indar 2F, and Inspire Super MP) and quinone outside inhibitor fungicides (QoI) (Flint WG and Sovran) fungicides are some of the safest and most effective fungicides for use against apple scab. However, because of their specific mode of action, these fungicides have the propensity for selecting resistant *V. inaequalis* populations. Indeed, many apple scab populations throughout NY and the northeastern United States have developed some level of resistance to both the demethylation inhibitor (DMI) and quinone outside inhibitor (QoI) fungicides. Fungicide resistance concerns have left apple producers with few fungicide options to manage apple scab, and as a result, producers are becoming increasingly reliant on calendar-based applications of multi-site protectant fungicides (e.g. Captan mixed with mancozeb) to manage the disease.

"Recent evaluations of dodine (Syllit) resistance in NY State have shown that the resistance level to Syllit has declined and that dodine could potentially be used for control of early season apple scab. In such orchards Syllit should be used with caution until the changes in population sensitivity following dodine use are better understood. Tank mixing Syllit with mancozeb might prove useful in early-season applications if resistance testing has shown that the population is sensitive to Syllit, or if Syllit has not been applied at the site for more than 15-20 years."

The fungicide, dodine, was a highly effective tool for managing apple scab during the 1950s and 1960s. Dodine has excellent protectant activity and can inhibit conidia development in active lesions, which affords it a level of post-infection activity. In the late 1960s dodine resistance began to develop in western NY. This resistance was first reported in orchards that had more than 10 years of exclusive dodine use. Later, dodine resistance was reported in other regions of the US and Canada. By the mid 1970s, dodine use had diminished in NY as a result of resistance along with the introduction of new fungicide chemistries capable of managing several diseases in addition to apple scab. By the 1990s, dodine use for apple scab management in the region had all but ceased. During this time, scientists at the New York State Agricultural Experiment Station (NYSAES) reassessed the stability of dodine resistance by investigating several orchards in NY and Michigan. They had found that although the frequencies of resistant isolates had declined, they had not returned to baseline sensitivity levels.

Although, it has been only been a little over a decade since the last investigation into dodine stability, increasing concerns over QoI and DMI resistance warrant a more in-depth investigation into the prevalence of dodine resistance in the region and the possibility for renewed dodine use in apple orchards. The objectives of our investigations were to: 1) determine the prevalence of dodine resistance in commercial, research, and baseline apple orchards in the northeastern United States from 2007-2009 using microscopy-aided mycelial relative growth assays, and 2) monitor changes in dodine sensitivity following use in a research orchard with a *V. inaequalis* population formerly resistant to dodine. Having dodine as an additional tool for apple scab would improve resistance management strategies for regional apple producers and prolong the usefulness of other site-specific fungicide chemistries at risk for fungicide resistance.

Materials and Methods

In Vitro Evaluation of Dodine Sensitivity. From 2007 to 2009 apple scab populations from 93 commercial, research, and baseline apple orchards from several states across the northeastern US were evaluated for sensitivity to dodine (guanidine). Specifically, the survey included 69 commercial, 18 research, and 6 baseline orchards from NY, VT, WV, NH, MA, ME, RI, MI, OH, IN, PA, & CT. From each location, a minimum of 25 single-leaf lesion isolates were collected. Dodine sensitivity was determined by conducting microscopy-aided mycelial relative growth assays as described by Olaya and Köller (1999) using a SPOT Idea digital camera attached to an Olympus SZX12 stereo scope. Sensitivity to dodine was tested at a discriminatory dose (0.2 µg/mL). This discriminatory dose was used by several prior studies for the evaluation of dodine sensitivity in apple scab populations. For each single-leaf lesion isolate, five germ tubes or "microcolonies" were analyzed. Mean isolate sensitivity, expressed as percent relative growth, was determined for each isolate (n = 5) and orchard (n >25). Means of individual isolates were used to generate a population mean, which was used to create frequency distributions of population responses for region. Thresholds representing population mean relative growths for collections of baseline orchards and those with confirmed practical resistance

to dodine were also established. Data from the 2007-2009 data set was compared to an identically generated data set compiled from 78 apple orchards in the same region collected previously by Köller et al. from 2004 to 2006.

Field Evaluation of Dodine. To evaluate the potential for renewed dodine use, fungicide efficacy field trials were conducted in 2009 and 2010 at the NYSAES in Geneva, NY. The orchard site was a mature (~4m tall, ~6m canopy width, ~31cm trunk diameter, >30 years of age) planting of 34-yr-old 'McIntosh' and 'Cortland' trees on MM.106 rootstocks. The orchard had been used for fungicide evaluations for the entirety of its history, and has a *V. inaequalis* population formerly resistant to dodine, but currently composed primarily of isolates with reduced sensitivity. Prior to the study, applications of dodine (Syllit) were made in the orchard in 2006, but were not made for many years prior to 2006.

In each year, fungicide treatment programs consisting of a dodine, a mancozeb, and an untreated program were evaluated for impacts on the development of primary apple scab symptoms. Fungicide applications were made at 7-10 day intervals beginning at green tip (early April) and continuing through second cover (mid-June). From green tip to tight cluster, trees were either left untreated (negative control), received an application of dodine (Syllit, 48 fl. oz/A), or received an application of mancozeb (Manzate 75DF, 6 lb/A). All treatment programs received at total of three applications of myclobutanil (Rally 40WSP, 5 oz/A) mixed with mancozeb (Manzate 75DF, 3 lb/A) from pink to petal fall. Captan 80WDG (5 lb/A) was applied at 1st and 2nd cover to manage secondary scab infections. All applications were made dilute (~300 gal/A tree row volume) using a handgun at 300 psi. The incidence of apple scab symptoms on 'McIntosh' and 'Cortland' was assessed for cluster leaves and young fruit in late May or early June of each year. Later evaluations of apple scab on terminal leaves and mature fruit were made, but not considered relevant as infections at the later stages likely occurred after tight cluster when all fungicide treatment programs were under the same cover spray fungicide regime. The incidence of apple scab on cluster leaves and fruit was expressed as the number of cluster leaves or fruit with scab lesions out of the total number of cluster leaves and fruit with 20 clusters assessed for four replicate trees per treatment. Disease incidence data was subject to analysis of variance (ANOVA) for a randomized block design using the Mixed Models procedure (PROC MIXED) of SAS). Percent incidence data was subject to arcsine square root transformation prior to analysis.

Changes in dodine sensitivity for the orchards' *V. inaequalis* population in relation to dodine use from 2005 to 2010 were also monitored. In each year, a minimum of 25 single-leaf lesion isolates were collected. Dodine sensitivity was determined by conducting microscopy-aided mycelial relative growth assays as described in the first objective. From 2007-2010, primary apple scab lesions for *in vitro* sensitivity evaluation were collected from untreated (negative) control trees with an equivalent number of leaves selected from each of the untreated trees in each block. Although only untreated trees were sampled in each year, we've found in previous studies that the population sensitivity profiles are nearly the same regardless of whether we sampled trees only from specific in-season fungicide treatments, uniformly from the block, or from a few corner trees. In 2005-2006, the sampling was also conducted on untreated trees, but the sampling pattern could not be verified.

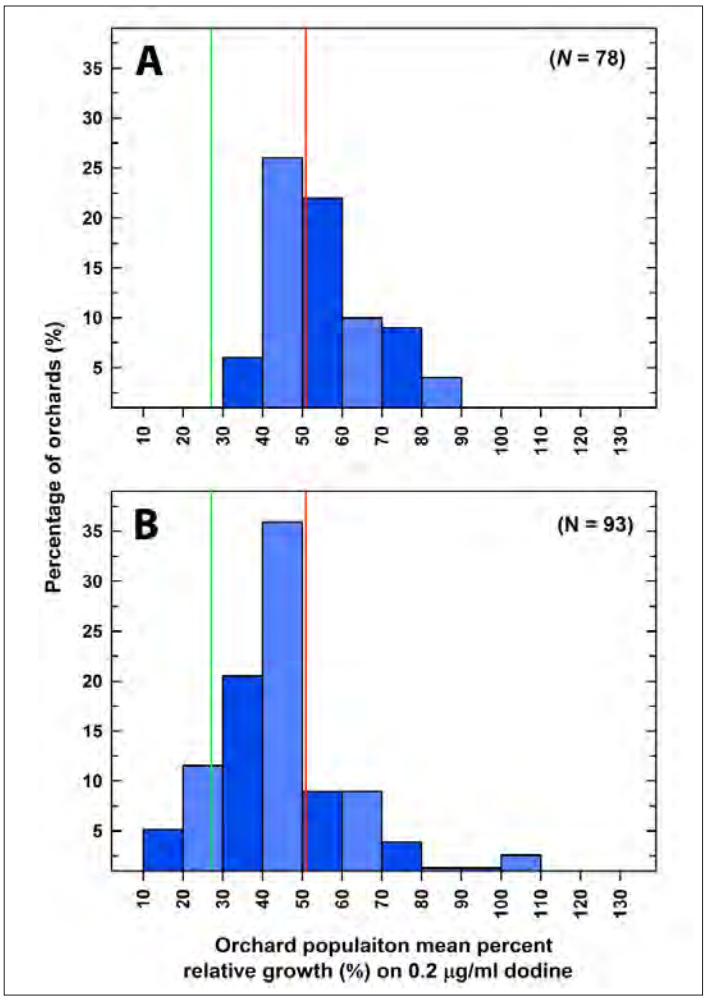

Figure 1. Frequency distributions of dodine sensitivity for *V. inaequalis* populations from (A) 78 orchards surveyed between 2004-2006 and (B) 93 orchards surveyed from 2007-2009. Values are mean relative growths for individual orchards determined from more than 25 *V. inaequalis* single lesion isolates with 5 conidial growth measurements for each isolate. The vertical green line represents the mean percent relative growth (28%) of a historical compilation of baseline *V. inaequalis* populations, while the vertical red line represents the mean percent relative growth of a historical compilation of *V. inaequalis* populations with practical resistance to dodine.

Results and Discussion

In Vitro Evaluation of Dodine Sensitivity. The majority of *V. inaequalis* populations surveyed from 2004-2006 demonstrated reduced sensitivity to dodine and 45% of the orchards exceeded the practical resistance threshold (population mean %RG > 50%) (Fig. 1A). No orchards from 2004-2006 demonstrated baseline levels of dodine sensitivity (mean %RG < 28%). The prevalence of dodine resistance in 2004 to 2006 was not unexpected given that the underlying assumption is that dodine resistance was widespread in the region. However, there was still considerable decline in the prevalence of dodine resistance since the study by Köller et al. in the 1990s. Although only 8 orchards were surveyed in the 1990's, the authors found that historically resistant orchards were still resistant and none of the orchards had returned to baseline levels of sensitivity. However, the authors did observe that the frequency of resistant isolates in orchards confirmed to be practically resistant to dodine had declined by

10-14%. From 2004-2006, Köller et al. surveyed a considerably larger number of orchards, and still no orchards were found to be of baseline sensitivity.

Our current evaluation of sensitivity to dodine (2007-2009) has shown that the frequency of sensitivity to dodine observed in *V. inaequalis* populations is now even higher than in 2004-2006 (Fig. 1B). While the majority of orchards tested still exhibited a population phenotype of reduced sensitivity, 17% of the orchards demonstrated baseline sensitivity. Overall, the percentage of dodine resistant orchards in the region decreased from 45% (2004-2006) to 27% (2007-2009). Overall the number and location of orchards surveyed in the two collection periods and the distribution of orchard classifications (research, baseline, commercial) surveyed was fairly comparable between collections. As expected, the level of dodine use (data not shown)

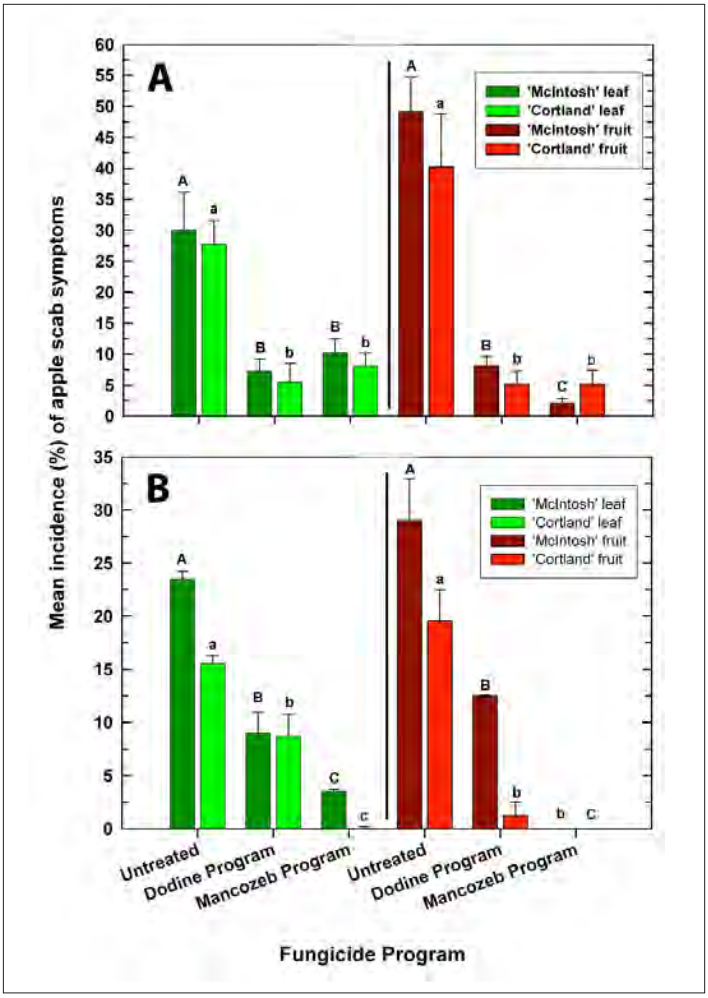

Figure 2. Management of apple scab in a 'McIntosh'/'Cortland' orchard with a *V. inaequalis* population formerly resistant to dodine. Values are the mean incidence and standard error of primary apple scab symptoms on cluster leaves and fruit. The incidence of apple scab symptoms on cluster leaves and fruit was expressed as the number of cluster leaves or fruit with scab lesions out of the total number of cluster leaves or fruit with 20 clusters assessed for each of four replicate trees. Incidence of primary apple scab symptoms following dodine, mancozeb, or untreated (control) programs are depicted in A) 2009 and B) 2010. Bars representing treatments denoted by the same letter are not significantly different from one another. Bars representing treatments denoted by capital letters illustrate treatment difference for the cultivar 'McIntosh', while lower case letters illustrates treatment differences for the cultivar 'Cortland'.

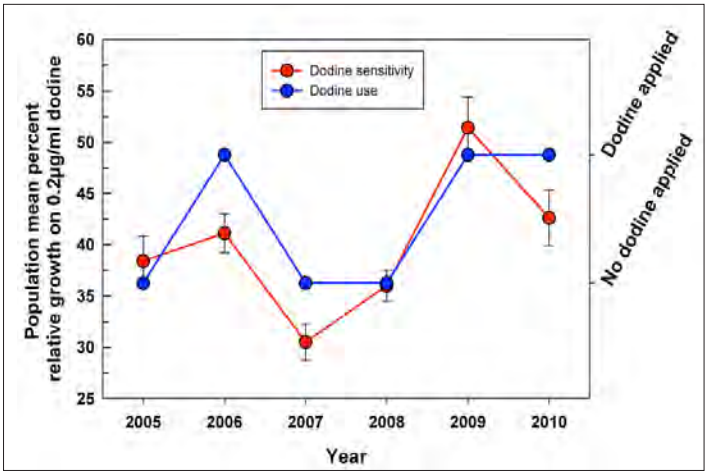

Figure 3. Effect of dodine applications on *V. inaequalis* population sensitivity to dodine in a NYSAES research orchard from 2005-2010. The points and line in red represents the mean relative growth values for the NYSAES field trial population sampled from untreated control trees. In each year, the mean relative growth for the population was determined from more than 25 *V. inaequalis* single lesion isolates with five conidial growth measurements per isolate. The points and line in blue indicates Syllit use or the lack thereof for the last 5 years in the orchard.

as indicated in grower records included with orchard sample data was higher in the 2004-2006 data set than the 2007-2009 data set. Although it appeared that dodine resistance was fairly stable within individual orchard populations in the 1990s, the frequency of dodine resistant isolates in regional populations has likely continued to steadily decline with lack of use. With nearly a decade between the work in the 1990s and efforts in this study, the observed decline is less surprising. We hypothesize that the observed decline in dodine sensitivity over the last two decades is not due to individual isolates losing acquired resistance to dodine, but due to a lack of selective pressure for isolates with dodine resistance resulting from the curtailment of dodine use since the 1970s. Similarly, selective pressures for DMI and QoI resistance over the last two decades has likely further served to dilute the frequency of dodine resistant isolates. In short, the absence of selective pressures for dodine resistance combined with constant selection for DMI and QoI resistance may have effectively eliminated the majority of dodine resistant individuals in orchard populations in the region.

Field Evaluation of Dodine. Despite a previous history of practical resistance to dodine in the NY State Agricultural Experiment Station research orchard, dodine consistently demonstrated a high level of efficacy for controlling apple scab for two consecutive years. In 2009 (Fig. 2A) and 2010 (Fig. 2B), the incidence of apple scab resulting from primary infections of leaves and fruit on both apple varieties treated with dodine or mancozeb was significantly lower than the untreated control. However, in 2010 the dodine program was slightly less effective than the mancozeb program, especially on 'McIntosh' fruit. This is likely attributed to the presence of isolates with reduced sensitivity to dodine. Moreover, the dodine program was not improved over the mancozeb program in any given year. However, the incidence of apple scab following fungicide programs was so low that only complete control would be the only possibility for improved performance. Aside from apple scab symptom development on 'McIntosh', there

was no indication of practical resistance following two seasons of dodine use. Evaluation of dodine performance in the orchard in 2011 (year 3) will provide a more definitive indication of the potential for renewed dodine use in orchards with reduced sensitivity.

Interestingly, there were changes in population sensitivity to dodine in response to field use. During seasons in which Syllit was applied, *in vitro* dodine sensitivity (mean percent relative growth, %RG) for the orchard population of *V. inaequalis* was lower (i.e. high mean %RG) than years without Syllit applications (Fig. 3). However, the reduced dodine sensitivity observed during application years may be transient given that mean sensitivity was higher (i.e. low mean %RG) in later seasons without dodine applications (Fig. 3). These reductions in sensitivity in years following Syllit applications are similar to what we have observed with late fall applications with QoI fungicides, which also results in population sensitivity reductions in the spring.

Overall, field applications of Syllit two years in a row did not result in the reemergence of a resistant population, and the trial in 2006 also had only a transitory effect on population sensitivity to dodine. The research block is typically exposed to selection pressure for DMI and QoI resistance, which could contribute to transitory dodine insensitivity. Hence, it stands to reason that if growers alternate site-specific chemistries, a few Syllit applications each year should not permanently affect overall population sensitivity to dodine. Indeed, dodine resistance originally arose due to sole reliance on a single-specific chemistry. In 2011, we have several effective site-specific fungicide chemistries with which growers can manipulate natural selection to prevent the emergence of resistance to any specific chemistry.

In summary, consistent performance of Syllit in an orchard formerly resistant to dodine combined with transitory changes in population sensitivity, suggests that the dodine could potentially be used for control of early season apple scab. Syllit should be used with caution in orchards with *V. inaequalis* populations formerly resistant to dodine until the changes in population sensitivity following dodine use are better understood. Tank mixing Syllit with mancozeb might prove useful in early-season applications if resistance testing has shown that the population is sensitive to Syllit, or if Syllit has not been applied at the site for more than 15-20 years.

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