2009 Fungicide Resistance of *Monilinia fructicola* in Georgia Peach Orchards

Introduction

Control of the fungal pathogen *Monolinia fructicola*, causal agent of brown rot of peach and nectarines, is integral to the success of Georgia peach production. This pathogen can devastate orchards if left uncontrolled, and it is largely the use of systemic fungicides that allows for control. Over time, *M. fructicola* may build up genetic resistance, and resistance to existing fungicides has prompted the development of a collaborative effort between Clemson University and the University of Georgia to identify *M. fructicola* resistance prior to major economic losses. This collaboration has resulted in development of the Profile kit, a self-contained resistance management system that can be utilized by any trained individual.

Three major systemic fungicide classes are commonly used to control brown rot, and these are the benzimidazoles (BZIs), the demethylation inhibitors (DMIs) and the quinone outside inhibitors (QoIs). A lip-balm assay system was developed using agar amended with critical discriminatory concentrations of fungicides; extensive growth of *M. fructicola* on slices of the agar stored in petri dishes for 72 hours would indicate resistance. Such information is of use to producers, as being informed that resistant strains are present in their orchards, they may select alternative, active fungicides for future use. Extension pathologists can also monitor resistance trends in the area, and this information will also benefit producers by supplementing local knowledge with a more global understanding of the disease patterns, thereby educating producers of trends in their region.

In 2009, testing for brown rot resistance involved extensive fieldwork and trips to orchards across Georgia. By collecting data from numerous disparate locations, maps were assembled to better illustrate where resistance is widespread. Producers were informed of their individual resistance issues, and recommendations were made to avert destructive epidemics of brown rot throughout the state as the season progressed.

Background

Brown rot of stone fruits is caused by pathogens of the genus *Monilinia*. Separated from *Sclerotinia* in 1928, three species, ***M. fructicola***, ***M. laxa*** and ***M. fructigena*** are known to cause infestations of brown rot the world over. In the southeastern United States, *Monilinia fructicola* is the causal agent of brown rot. Brown rot of peach is described as a firm, spreading, brown decay on ripe or ripening fruit which is followed by visible fluffy conidia (spores). Mummies are known to form from small or immature fruit and are a common source of inoculum. The pathogen also infects twigs and flowers and causes blight and cankers which can also become a source of inoculum. *M. fructicola* survives the winter easily as mummies hanging in the tree branches from the last season. Peaches become infected through small wounds in the skin or at the site of a bite from an insect pest, but penetration can also be direct.

The ability to easily overwinter means that the potential of major brown rot epidemics looms every year, threatening to destroy the peach crop. Measures must be taken to ensure the safety of

the peaches, and an effective spray program is the most efficient way to accomplish this goal. Early-season (bloom/post-bloom) and cover sprays are limited to use of chemical fungicides which are generally not prone to resistance development. However, pre-harvest sprays, critical due to the increased susceptibility of the peach to infection and potential for pre- and post-harvest rot development, are largely limited to chemicals in one of three classes of systemic chemical fungicides. A number of different brands proliferate. Among the older systemic fungicides developed to control brown rot are the benzimidazoles. These chemicals operate by interfering with the formation of cell microtubules during cell division. Developed shortly after these were the demethylation inhibitors which stop the synthesis of sterols; the sterols are integral to the structure of the cell membrane, and DMIs cause breakdown and dissolution of fungal cells. A more recently developed class, the quinone outside inhibitors, halt respiration, also leading to cellular death.

All of these fungicides are very susceptible to resistance development in fungal populations, and continual use will cause localized *Monilinia* populations to develop resistance. Resistance developed to BZIs in the 1980's, which led to their replacement with DMI fungicides. Recently, it was discovered that strains of *Monilinia* in Georgia were developing reduced sensitivity to DMI fungicides. Recent data also indicates that resistance to QoI fungicides is developing in the southeast. The aforementioned field surveys were conducted to determine the extent and location of *M. fructicola* field resistance to all three classes of these fungicides in Georgia during the 2009 production season.

Materials and Methods

Orchard *M. fructicola* (brown rot) collection. An initial reconnoiter was made in each sampled orchard to determine where any obviously infected, symptomatic fruit were located; hotspots are often indicative of a resistance shift. Once brown rotted fruit were located, 10 symptomatic peaches were collected from multiple trees. Brown rot samples were only collected from peaches that were still attached by their peduncle, as fruit which had fallen to the ground were often in stages of advanced decay, and multiple other saprophytic fungi would complicate the process of determining resistance to *M. fructicola*; fruit were selected which did not show obvious signs or other rots, and early brown rot lesions were preferred over older, mummified fruit. Fruit were collected such that no two peaches came from trees within a two tree vicinity of each other; allowing two buffer trees between sample trees ensures genetic variability among collected specimens and gives a more accurate representation of the genetic population within a given orchard. Specimens were sealed in plastic containers, marked individually with a number and location designation (producer and variety for example). Additional survey information collected from the producer was then entered into the data sheet (Figure 2), along with the GPS coordinates of the site. Collected fruit were then transported in a cooler on ice to preserve the specimens.

Resistance testing using the Profile kit. As mentioned, the Profile kit has been developed collaboratively by Clemson and the University of Georgia (Figure 1). To test the pathogen for resistance, a sterile area was prepared by wiping down a bench counter with alcohol. The lip-balm kit was then organized for sample assessment. The kit contains petri dishes, toothpicks, a scalpel and replacement blades, one lighter, a box of nitrile gloves, four permanent markers,

parafilm, the lip balm tubes, and a user's manual. The lip balm tubes contain potato dextrose agar and are either impregnated with fungicide or, in the case of the control, are purely agar. White tubes contained the PDA control, red tubes contained PDA and a discriminatory dose of the DMI fungicide propiconazole, blue tubes contained PDA and a discriminatory dose of the BZI fungicide thiophanate-methyl, and the green tubes contained PDA and a discriminatory dose of the QoI fungicide azoxystrobin. Donning latex gloves and observing sterile technique, a test petri dish was prepared by first drawing a cross on the bottom of the petri dish with a black marker. The quadrants were then labeled with a color corresponding to the color coding of the tubes. Turning the disk over, the front was labeled with the name of the collection site, the date, and the number of the collected peach to which it corresponded. After labeling the petri dish, the first lip-balm tube was opened, and a scalpel (sterilized in alcohol and flamed with a lighter) was utilized to cut one disk from the tube. This disk was discarded for sterility and two more disks were cut from the top of the tube. These were placed into the quadrant on the petri dish which corresponded to the color of the tube. For each of the remaining tubes, the process proceeded in identical fashion. After the petri dish quadrants were filled with two each of each fungicide and the control, inoculations were conducted. To inoculate the disks, the peach labeled with the same number as that on the disk was furnished, and having located sporulating conidia, a sterile toothpick was utilized to gently remove a conidial mass; only conidia were carefully removed, and the peach surface was not touched. The toothpick was utilized to inoculate the agar by applying spores to the hole in the middle of the lip balm disk (Figure 3). All disks were inoculated in this manner. When all eight disks were inoculated on a petri dish, the dish was sealed and para-filmed to hold the top and bottom firmly shut and reduce moisture loss or contamination.

After 72 hours, mycelial growth was rated on each agar plug. To rate the mycelial growth, each agar plug was visually assessed as to the extent of mycelial (fungal) invasion into the agar. Contamination with other fungi had to be noted, as this was a possible confounding issue. The control disk should have had a very thick mycelium covering its surface, appearing as light gray tufts radiating out from the center of the disk. Any growth that did not fit the appearance of *M. fructicola* was considered a contaminant, and using the manual provided, the contaminant was identified and noted on the data sheet. The grading system was as follows: a disk with no growth or contamination received a grade of "-"; a disk with growth covering ≤20% of its surface received a "+" grade; a disk with growth covering between 20 and 50% of its surface received a "++" grade; and a disk with ≥50% growth covering its surface received a "+++" designation. Typically the control plate would provide an example of a "+++" rating. Examples of each grade were also included in the provided user's manual.

Analyis of risk. After the grades were recorded on the data sheet, and analysis of risk was conducted. Data was configured into a scalable form for determination of resistance. To explain, we basically reason that a +++ is disproportionately of more importance than a ++ which is disproportionately more important than a +, etc. Therefore, we utilized a power function to assess risk.

With our system, the following number of pluses is transformed to the 5^{th} power to give the value on the right.

# +'s	# generated by power of 5 for number of +'s

0	0
1	1
2	32
3	243

We first average the two values (# of +'s) for each of the peach samples. We then take this value and develop a factor of five for that value. We then average the generated values for the 10 samples. The final averaged value is then compared to the following chart to place it in a category:

0-0.5 = no resistance or no significant resistance
>0.5-4 = very low resistance or very low danger relative to resistance
>4-32 = moderate resistance
>32-243 = high resistance

An example is given below.

+++	243	243
+++	243	0
-	0	1
-	0	0
+	1	243
+	1	32
-	0	0
-	0	0
+++	243	0
+++	243	0.5
++	32	51.95
++	32	High resistance
-	0	
-	0	
-	0	
-	0	
-	0	
-	0	
+	1	
-	0	

Results

The raw data sheets are attached at the end of this document (Appendix A). The generated maps (Figures 4-6) indicate that Georgia clearly has a problem with DMI resistance, especially in the

middle Georgia peach production region. The QoI and BZI fungicides do not currently show significant resistance, but either of these classes can quickly develop resistance.

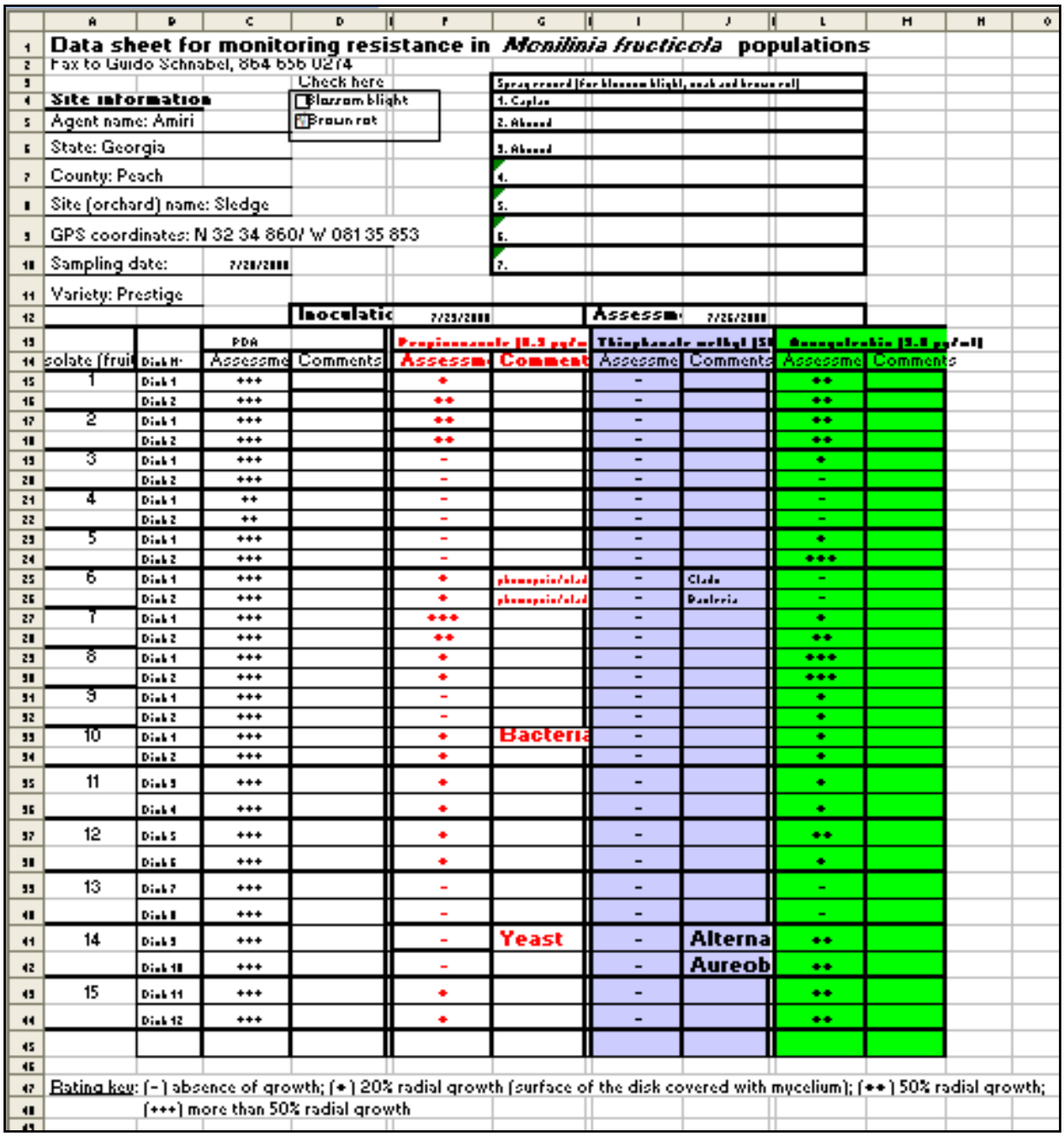
Discussion

From the generated maps, it becomes clear that the majority of DMI resistance is centered around the high density peach growing areas in proximity to Fort Valley. This area shows a high number of sites with propiconazole resistant strains of *M. fructicola*. Many producers have traditionally utilized DMI fungicides, and these are still being utilized, albeit at substantially higher rates. Six sites showed low resistance to azoxystrobin and these sites were sprayed with QoI fungicides this season; it is possible (likely) that a shift towards resistance to QoIs is occurring where they have been utilized. No site tested provided evidence of resistance to thiophanate-methyl; in the past, the use of BZIs was abandoned due to resistance issues, so this might indicate that the populations have reverted back to a background level relative this class of fungicides.

Conclusion

Propiconazole (DMI) resistant strains of *M. fructicola* are well established around the peach growing areas in the middle Georgia region. Spray records indicate that growers still utilize DMI fungicides in this region. The sites showing a shift towards resistance to azoxystrobin were also located mostly in or near this region and similarly, QoI products were used in these instances. The use of the Profile system was instrumental in preventing producer losses in 2009, a very wet year in which brown rot developed rapidly. Future use of the kit will be equally important.

Figure 1. The Profile resistance testing kit contains petri dishes, toothpicks, a scalpel and replacement blades, one lighter, a box of nitrile gloves, four permanent markers, parafilm, lip balm tubes, and a user's manual. The lip balm tubes contain potato dextrose agar and are either impregnated with fungicides at discriminatory doses, or in the case of the control, are purely agar.



Data sheet for monitoring resistance in *Monilinia fructicola* populations

Fax to Guido Schnabel, 864 656 0274

Site information	Check here: ☐ Blossom blight ☐ Brown rot
Agent name: Amiri	
State: Georgia	
County: Peach	
Site (orchard) name: Sledge	
GPS coordinates: N 32 34 860/ W 081 35 853	
Sampling date: 7/28/2008	
Variety: Prestige	

Spray record (for blossom blight, peach and brown rot)
1. Captan
2. Absent
3. Absent
4.
5.
6.
7.

Inoculatic 7/25/2008 Assessm 7/26/2008

Isolate (frui	Disk N	PDA Assessme	Comments	Propiconazole (0.3 µg/= Assessm	Comment	Thiophanate-methyl (5 Assessme	Comments	Boscalid-pyra (0.8 µg/=ml) Assessme	Comments
1	Disk 1	+++		+		-		++	
	Disk 2	+++		++		-		++	
2	Disk 1	+++		++		-		++	
	Disk 2	+++		++		-		++	
3	Disk 1	+++		-		-		+	
	Disk 2	+++		-		-		-	
4	Disk 1	++		-		-		-	
	Disk 2	++		-		-		-	
5	Disk 1	+++		-		-		+	
	Disk 2	+++		-		-		+++	
6	Disk 1	+++		+	phenopsin/alad	-	Clado	-	
	Disk 2	+++		+	phenopsin/alad	-	Rudevia	-	
7	Disk 1	+++		+++		-		+	
	Disk 2	+++		++		-		++	
8	Disk 1	+++		+		-		+++	
	Disk 2	+++		+		-		+++	
9	Disk 1	+++		-		-		+	
	Disk 2	+++		-		-		+	
10	Disk 1	+++		+	Bacteria	-		+	
	Disk 2	+++		+		-		+	
11	Disk 3	+++		+		-		+	
	Disk 4	+++		+		-		+	
12	Disk 5	+++		+		-		++	
	Disk 6	+++		+		-		+	
13	Disk 7	+++		-		-		-	
	Disk 8	+++		-		-		-	
14	Disk 9	+++		-	Yeast	-	Alterna	++	
	Disk 10	+++		-		-	Aureob	++	
15	Disk 11	+++		+		-		++	
	Disk 12	+++		+		-		++	

Rating key: (-) absence of growth; (+) 20% radial growth (surface of the disk covered with mycelium); (++) 50% radial growth; (+++) more than 50% radial growth

Figure 2. Data sheet and example data collected from the field and lab for each site.

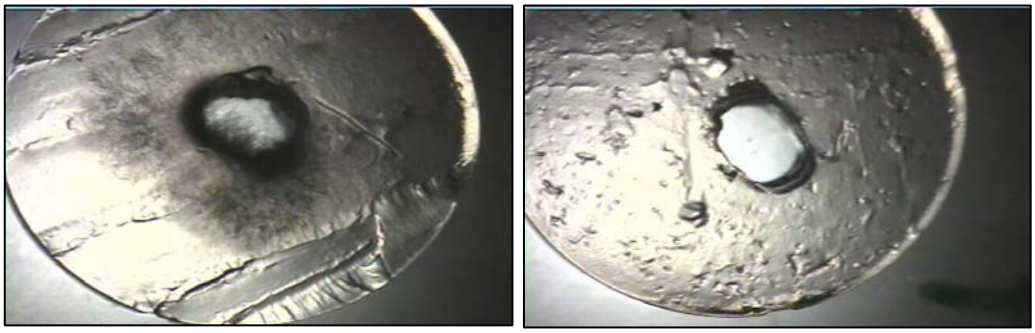


Figure 3. PDA slices showing either growth of *M. fructicola* (left photo; as expected in either the control slices or when resistance had occurred) or lack of growth (right photo; expected where resistance is not present to the specified fungicide).

Figure 4. Benzimidazole resistance risk map.

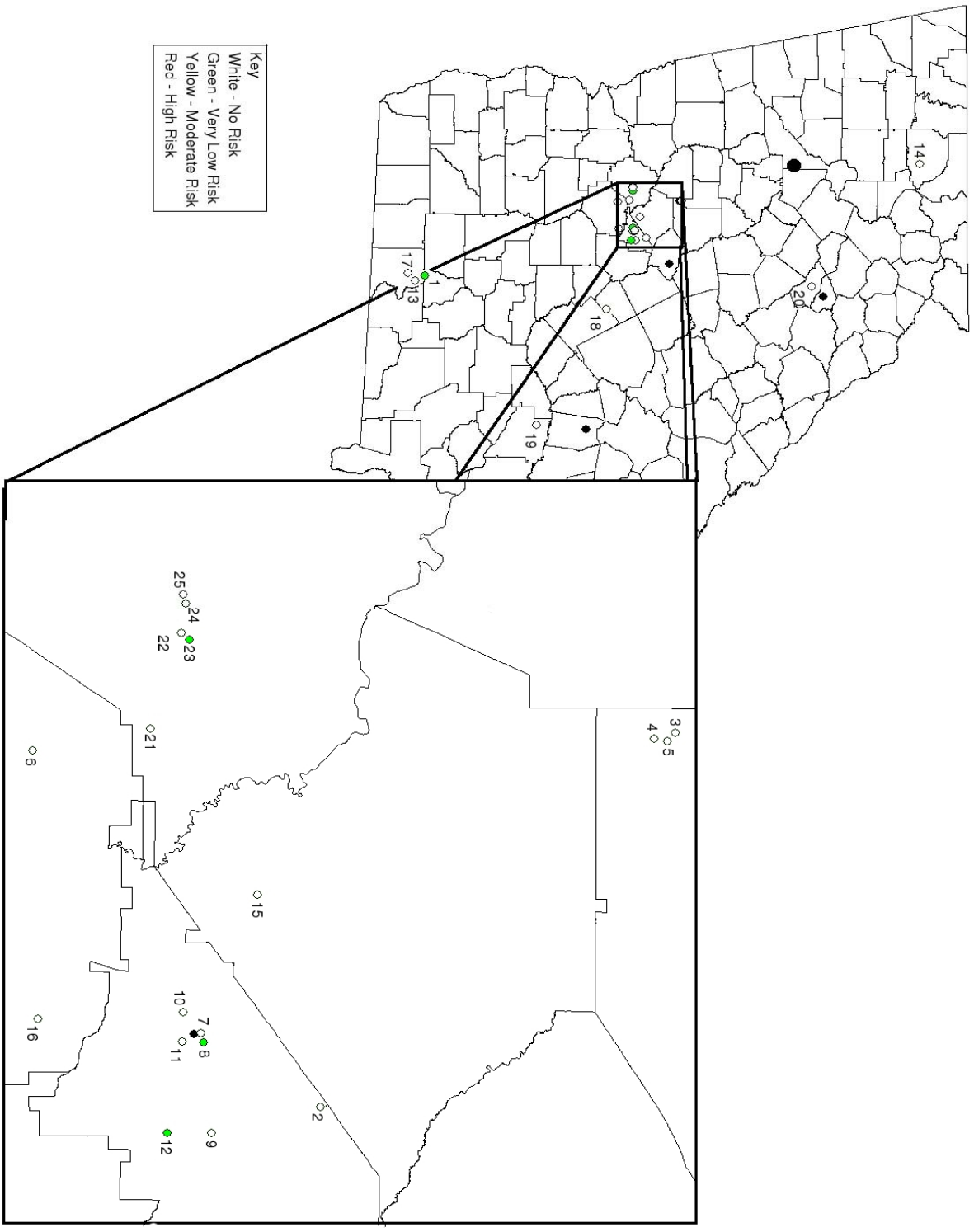


Figure 5. Demethylation inhibitor resistance risk map.

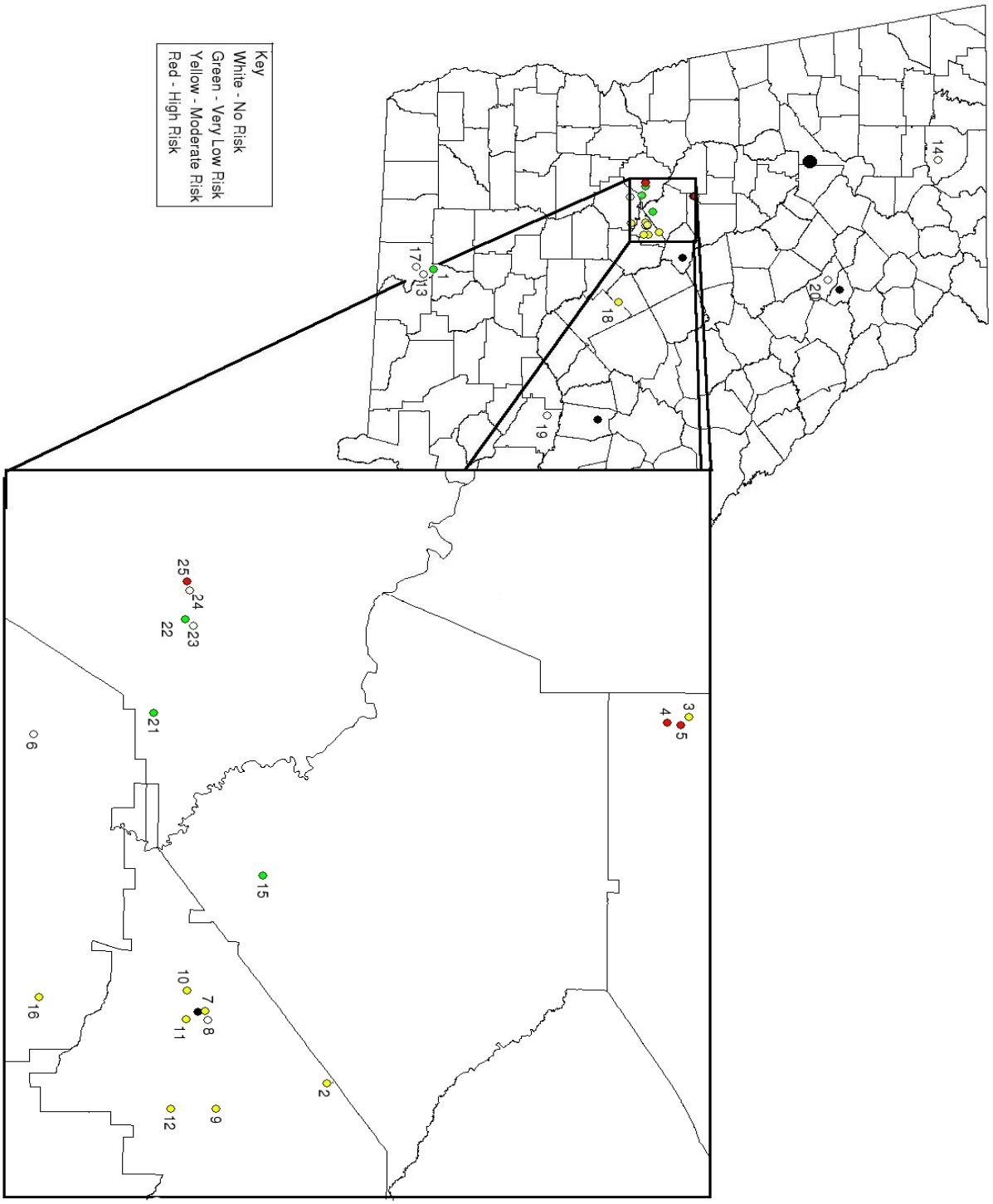


Figure 6. Quinone outside inhibitor resistance risk map.