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Developing Crapemyrtle Pollen Sampling Methods for a Neonicotinoid Pathway Study©

Asija Rice[a1] and Yan Chen[2]

[1]Southern University and A&M College, Urban Forestry Program, J.W. Fisher Hall Rm.102 Baton Rouge, Louisiana 70813, USA

[2]Louisiana State University Agricultural Center, Hammond Research Station, 21549 Old Covington Highway, Hammond, Louisiana 70403, USA

[a]Email: Asija97@gmail.com

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SUMMARY

Crapemyrtle bark scale is an exotic pest infesting crapemyrtles and has become a major concern because of its potential impact on other economically or ecologically important plant species. The systemic neonicotinoid insecticides are the most effective chemical class controlling this scale. However, pollinator safety has to be considered when developing IPM strategies, since crapemyrtle pollen is a significant food source for many pollinators. Pollen collection is the first step in analyzing for neonicotinoid concentration. In this study, the flowering phenology and the amount of pollen that can be collected from flowers in a timely manner were evaluated to determine the best timing and method for pollen collection. The first week of blooming for a total of 60 crapemyrtle cultivars were recorded to determine early-, mid-, and late-season blooming cultivars. Then, the clusters of crapemyrtle flowers of ten cultivars representing early-

and mid-season bloomers were grouped as new, full, and spent blooms based on the percentage of the cluster that showed color. Results indicated that there was a significant difference in flowering phenology among crapemyrtle cultivars, which affected pollen availability and thus sampling period for each cultivar. Three pollen collection trials were conducted to determine the amount of pollen that could be collected from 'White Natchez' crapemyrtle in a particularly timely manner. Results indicate that it is very challenging to collect the amount of pollen currently required by the laboratories for neonicotinoid concentration analysis. An improved laboratory procedure or analyzing methodology, requiring smaller amount of pollen will be critical for successful crapemyrtle pollen neonicotinoid pathway research.

INTRODUCTION

Crapemyrtles are popular ornamental shrubs or trees in the genus *Lagerstroemia* that have over 400 cultivars in the ornamental trade in the U.S. Their multiple aesthetic attributes include flower color, trunk color, fall foliage color, and long blooming time, which have made them the No. 1 summer blooming shrub or tree in the Southeastern U.S. Nursery production of crapemyrtles had a wholesale value of $66M/yr in 2014 (USDA 2015 Nursery Crop Census). Crapemyrtles have few diseases and pests. However, since 2005, an exotic scale has become a major concern (Wang et al., 2016). The crape myrtle bark scale [(CMBS), *Acanthococcus lagerstroemiae* Kuwana) infestation is now confirmed in Alabama, Arkansas, Georgia, Louisiana, Arkansas, Alabama, Mississippi, New Mexico, South Carolina, Tennessee, Texas, Virginia and Washington, D.C. In Louisiana, severe infestations were reported from major cities such as Baton Rouge, Covington, Hammond, Mandeville, New Orleans, and Shreveport. With severe infestation, the scale forms a white layer on stems and trunks, and causes unsightly black sooty mold from fungi grows on scales exudes, which covers the leaves and the ground

underneath the infested trees. Heavy infestation can cause stem dieback, reduction in flowering, stunt growth, and may kill young trees. Infestations are more severe on young and stressed trees.

Currently the most effective chemical control against CMBS is provided by foliar or drench applications of insecticides belonging to the neonicotinoid class, such as imidacloprid, dinotefuran, thiamethoxam, and clothianidin. Neonicotinoids are an important tool for an integrated pest management (IPM) strategy, however, they are systemic insecticides that may potentially be transferred to nectar and pollen, causing concerns that they may create a hazard for beneficial insects and pollinators (Stoner and Eitzer, 2012). Neonicotinoids and their metabolites also have long residual periods in plant tissue, from months up to years. There is a critical need to develop a better understanding of their pathway by analyzing their concentrations in pollen, flower, and leaf tissue of popular crapemyrtle cultivars.

Crapemyrtles do not have nectar but their pollen is an important food source to pollinators. However, current laboratory analysis methodology requires a minimum of 4 grams of pollen per sample for analyzing one neonicotinoid insecticide (i.e., imidacloprid) and two of its biologically active metabolites (i.e., imidacloprid-olefin and its 6-hydroxy acid). Pollen availability varies during the blooming period of a flowering plant, and collection methods also affect the amount and quality of pollen samples. Therefore, the objectives of this study were to: 1) determine best pollen sampling time by recording flowering phenology of selected cultivars, and 2) determine the amount of pollen that can be collected in a timely manner from ‘White Natchez’ crapemyrtles.

MATERIALS AND METHODS

This study was conducted with a new crapemyrtle collection at the Louisiana State University Agriculture Center, Hammond Research Station, that was established during 2013

and 2014. A total of 60 cultivars, three to five trees of each, were observed for their first week of blooming from Week 20 (May 14) to Week 30 (July 23rd). Observation was made on every Monday. A total of ten cultivars representing early- and mid-season bloomers were then selected for flowering phenology observations. We purposely selected certain cultivars in the Black Diamond and Delta series because of their popularity in the ornamental trade. Considering each flower cluster as a "bloom", the numbers of new bloom (less than 50% of the cluster showed color and there were no seed pods), full bloom (more than 50% of the cluster showed color), and spent bloom (less than 50% of the cluster showed color and seed pods start to form) were recorded every week for these cultivars from Week 25 (June 18) to Week 30 (July 23rd).

Three trials of pollen collection were conducted with 'White Natchez' during week 24 (June 11) to week 26 (June 25). Different flower collection methods were tested in three trials; Trial 1- individual flowers collected the day before pollen sampling and stored in small containers; Trial 2 -whole clusters of flowers collected the day of pollen sampling, and Trial 3 - the day before sampling. Pollen was collected using a compact vacuum connected to a 1.5 cm long clear tube 'chamber' (blocked with cigarette cotton filter). This was then connected to an Eppendorf pipet tip (Fig 1.). Pollen release time was observed daily from 6:30 to 15:00 over a week period of time.

RESULTS

As presented in Fig. 2, the first week of blooming was significantly different among cultivars ($p < 0.0001$). We then grouped the 60 cultivars as early-, mid-, and late-season bloomers using Week 22 (May 28) and 26 (June 25) as cut-off dates. Cultivars bloomed before Week 22 are considered early-season bloomers, and cultivars bloomed between Week 22 and 26 are considered mid-season bloomers.

Ten cultivars were selected, including five from the Black Diamond (BD) series ('Lavender Lace', 'Crimson Red', 'Pure White', 'Red Hot', and 'Shell Pink'), and three from the Delta series ('Moonlight', 'Eclipse', and 'Breeze') which are all mid-season bloomers; also included were 'Plum Magic' and 'Red Rooster' (Fig. 2). Among these cultivars, 'Lavender Lace' and 'Plum Magic' are early-season bloomers with the rest of the cultivars being mid-season bloomers.

Numbers of full blooms (flower clusters) per plant were significantly different among the ten selected cultivars ($p < 0.0001$). 'Plum Magic' had more full bloom clusters than other early-bloomers at their peak blooming weeks (Week 25 to 27, Fig.4). Mid-season bloomers generally peaked in full bloom during Week 26 to 28. From Week 29, all cultivars had less than 10 flower clusters that were in full bloom. Number of total blooms (flower clusters) is the sum of new, full and spent blooms on a tree observed each week over a period of six weeks (Fig.4). There was a significant difference among cultivars, and 'Plum Magic', 'Red Rooster', 'Delta Moonlight' and 'Delta Eclipse' had more number of total blooms than other cultivars during the majority time of the observation period. Other than data presented here, we observed that individual flowers only open for one day, and pollen release time were about 10:00 AM to 12:00 PM (Fig 1.), and visitation from pollinators (bees, bumblebees, beetles, thrips, etc.) significantly reduce pollen availability for sampling (Fig 1).

DISCUSSIONS

Based on a six-week observation, we know there is a big difference among cultivars in terms of bloom time, number of full bloom (when the pollen is most available) and total number of blooms. This significant difference in flowering phenology indicate that cultivar selection is critical in future neonicotinoid pathway research. Also, many of these popular new cultivars will

be planted in large numbers by homeowners and landscape contractors. Knowing their flowering phenology will greatly assist in the development of an IPM program that will avoid acute impact of insecticides and be friendlier to beneficial insects and pollinators.

Many challenges were encountered during the pollen collection trials. The cigarette cotton filter caught quite an amount of pollen in it, and the cotton was later covered with parafilm and tube was remade only to use duct-tape around the pipe on the vacuum and the clear tube chamber. Comparing the three trials, collecting flowers the day before pollen collection may cause mold or loss of pollen to insects (i.e., thrips) that were collected and stored in the same container as flowers. Although all six 'White Natchez' trees used in the trials were of the same age and size, we observed difference in the overall amount of pollen in full blooms among them. Prolonged rainy weather and everyday thunderstorms also significantly decreased pollen availability.

Using the compact vacuum and flowers collected on the same day, we were able to collect 0.7 gram pollen over a two hours of work time. Apparently, the compact vacuum is still the best way to collect pollen once the timing of the day and weeks of pollen availability are determined. However, it would be very difficult to collect 4 grams of pollen as currently required by contracted laboratories (personal communication). Newer methodology for pollen analysis requiring smaller amount of pollen is available (David et al., 2015 and 2016).

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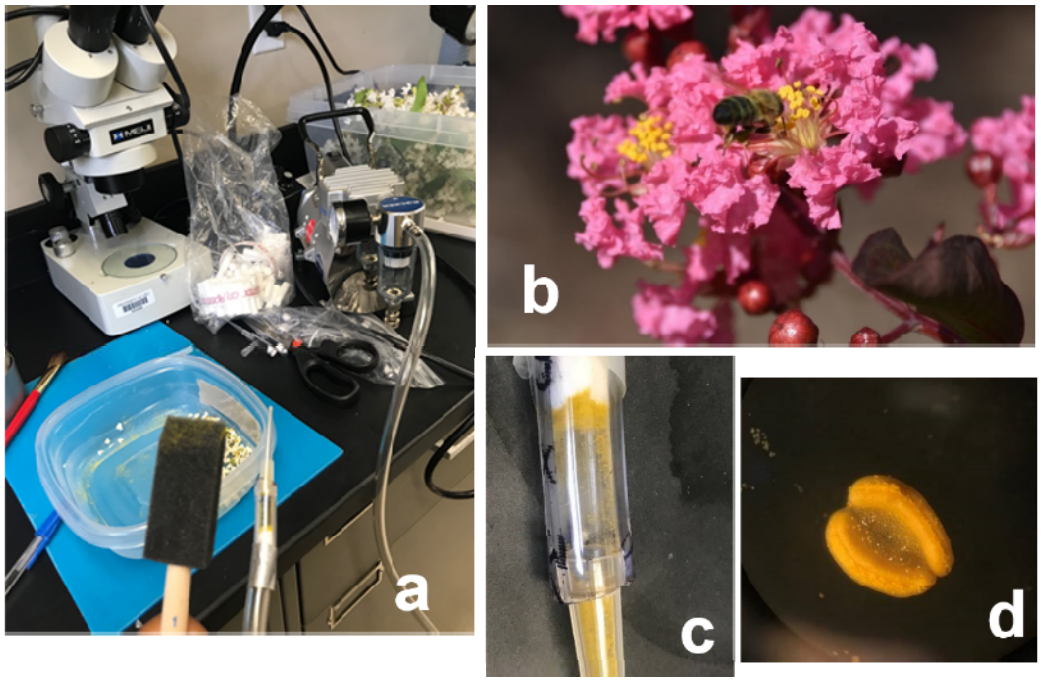


Figure 1. Pollen was collected with a mini vacuum connected to a pipet tip (a and c), and crapemyrtle pollen are only available for a short time of period after being released (d) because of pollinator activities (b).

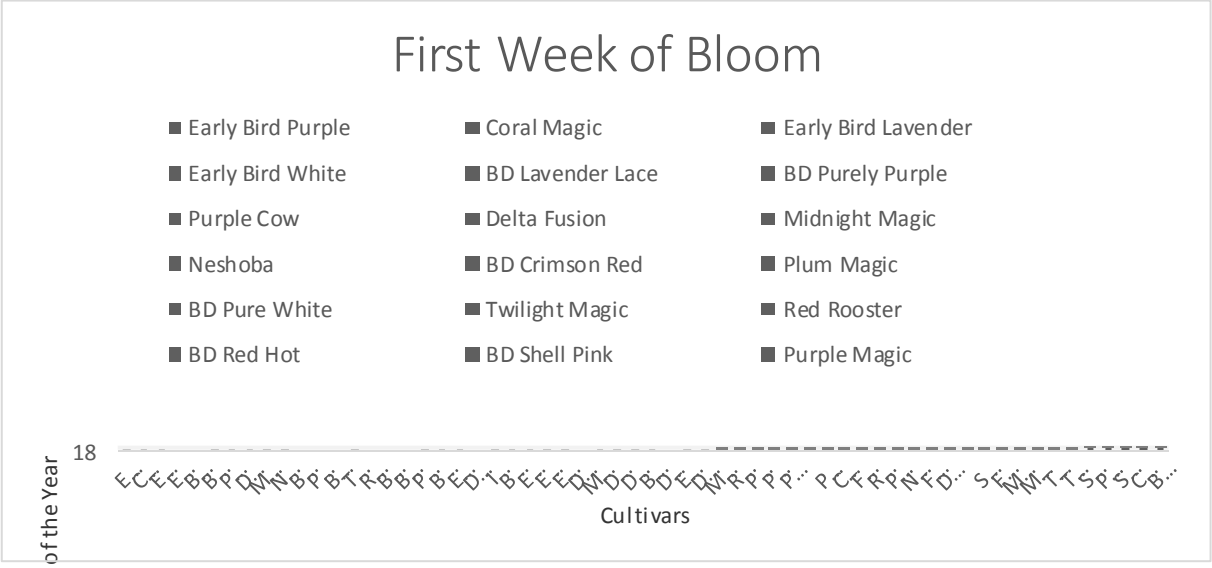


Figure 2. First week of blooming of 60 new crapemyrtle cultivars popular in the ornamental trade. Not all cultivars were listed due to limited space.

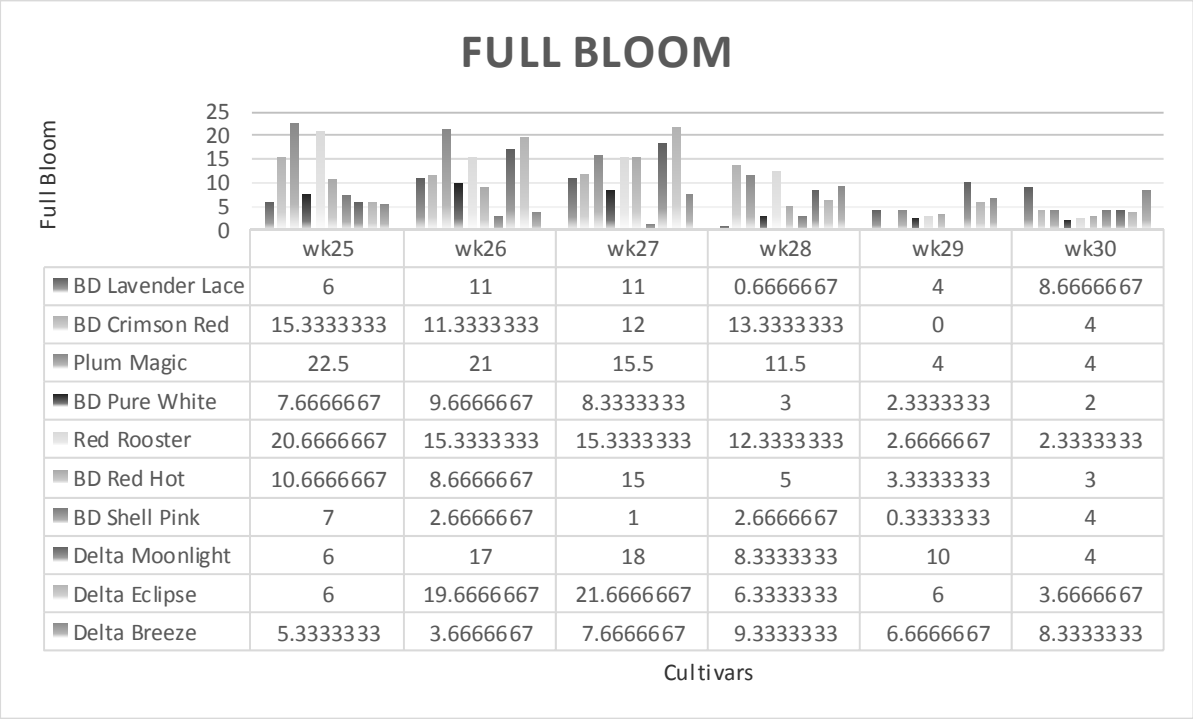
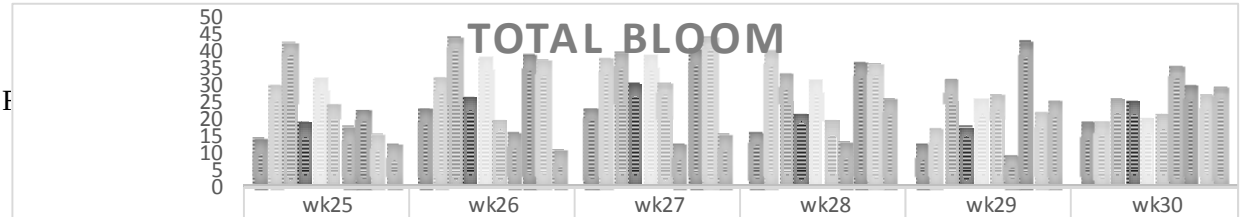


Figure 3. Number of full Blooms (flower clusters) found on ten selected crapemyrtle cultivars over a 6-week time period from week 25 to week 30 of 2018 (week of June 18 to week of July 23rd, 2018)



	wk25	wk26	wk27	wk28	wk29	wk30
BD Lavender Lace	14	23	23	16	12.6666667	18.6666667
BD Crimson Red	30	32.3333333	37.6666667	40	17	19
Plum Magic	42.5	44.5	39.5	33.5	31.5	26
BD Pure White	18.6666667	26.3333333	30.6666667	21.3333333	18	25.3333333
Red Rooster	31.6666667	37.6666667	38.3333333	31	25.3333333	20
BD Red Hot	24	19.3333333	30.3333333	19.3333333	26.6666667	21.3333333
BD Shell Pink	18	16	12.6666667	13	9	35.3333333
Delta Moonlight	22.3333333	39	41	37	43	29.6666667
Delta Eclipse	15.3333333	37.3333333	44	36	22	26.6666667
Delta Breeze	12.6666667	10.6666667	15.6666667	25.6666667	25.3333333	29.3333333

Figure 4. Total number of blooms (flower clusters) provided by ten selected crapemyrtle cultivars over a 6-week time period from week 25 to week 30 of 2018 (week of June 18 to week of July 23rd, 2018).