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Germplasm Evaluation for the Incidence of Fungal Spot and Cracking Prevalence in the Fruit of Pawpaw [*Asimina triloba* (L.) Dunal]

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Abstract

Fungal lesions have been observed on the fruit surface of many pawpaw [*Asimina triloba* (L.) Dunal] cultivars and consist of a complex of three fungi, *Mycocentrospora asiminae* Ellis & Kellerm., *Rhopaloconidium asiminae* Ellis and Morg, and *Phyllosticta asiminae* Ellis and Kellerm. Pawpaw fruit occasionally exhibit cracking, and field observations suggest cracking may vary by cultivar and be related to infection by this fungal complex. The objective of this study was to determine if fungal lesions and cracking incidence varied by cultivar or advanced selection, and if there was a correlation between fruit lesion occurrence and fruit cracking. Ripe fruit were harvested three times per week from a mature pawpaw planting consisting of ten pawpaw cultivars and advanced selections ['Sunflower' (control), G4-25, G5-23, G6-120, G9-111, Haz-1, Haz-2, Hi 4-1, Hi 7-5, and NC-1]. Percent coverage by fungal lesion was visually estimated, and fruit cracking was evaluated for each fruit harvested. Fruit weight, size of disease lesion, and cracking all varied significantly by cultivar. Overall, the genotypes G4-25, G5-23, G6-120, and Haz-2 displayed less than 10% fungal lesion of fruit in 2012 and 2013. A positive correlation was found between cracking and fungal lesion as well as fruit weight and cracking. However, other factors could also be related to fruit cracking, such as genotype and environment. Future studies are needed to determine the relationship between fruit lesions and fruit cracking in pawpaw, and to determine varietal resistance to these fungi and possible control measures.

The North American pawpaw [*Asimina triloba* (L.) Dunal] is a tree fruit native to the eastern and midwestern United States and is under small-scale commercial production across the United States. This tree produces the largest edible fruit native to the United States and it may reach up to 1 kg in size (Darrow, 1975). Pawpaw fruit is mainly sold fresh at farmers' markets, directly on the farm, or as part of a Community Supported Agriculture (CSA) (Pomper and Layne, 2005); however, there is potential for expansion of the processing market (Templeton et al., 2003; Duffrin and Pomper, 2006). The fruit is very nutritious (Peterson et al., 1982); it has an almost tropical aroma, smooth custard-like texture, and flavors reminiscent of a combination of banana (*Musa* x *paradisiaca* L.), mango (*Mangifera indica* L.), and pineapple (*Ananas comosus* [L.] Merr.) (Layne, 1996; Shiota, 1991; Duffrin and Pomper, 2006). In addition to the pawpaw's utility as a fruit crop, there are natural compounds in the leaf, bark, and twig tissues that possess insecticidal and anticancer properties (McLaughlin, 2008).

Pawpaws produce a ripe fruit with a soft creamy yellow-orange flesh which is high in antioxidants, vitamins, minerals, amino acids, and essential fatty acids (Peterson et al., 1982; Kobayashi et al., 2008). Ripe pawpaw flesh firmness ranges from avocado-like to custard-like in texture. The fruit ripen during a 4-6 week period of mid-August through early October in Kentucky, varying by cultivar and growing location. The pawpaw fruit bruises easily and has a short shelf life of 2-3

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days at room temperature, or 2-3 weeks under refrigeration at 4°C (McGrath and Karahadian, 1994; Layne, 1996, Archbold et al., 2003). Fruit can be picked slightly under-ripe, or firm-ripe, to increase shelf life, but fruit that is picked prematurely will not ripen properly with favorable flavor profiles or soluble solids.

Pawpaw trees are considered to have few disease problems; however, monoculture production in orchards and the buildup of fungal inoculum over time may lead to higher disease incidence and pest pressure in pawpaw orchards. In some areas and years, pawpaw leaves and fruit can exhibit fungal lesions that reduce the fresh market appeal of fruit and that possibly results in fruit injury.

Phyllosticta sp., according to Agrios, is a "common mitosporic fungi causing primarily foliar but also other symptoms on a large variety of host plants" (Alexopoulos and Mims, 1979; Agrios, 2005). Mitosporic fungi is another name for the imperfect fungi and this fungus produces asexual spores called conidia. Pawpaw leaves can exhibit leaf spot, principally a complex of *Mycocentrospora asiminae* Ellis & Kellerm., *Rhopaloconidium asiminae* Ellis & Morg., and *Phyllosticta asiminae* Ellis & Kellerm. (Anderson, 1919; Martin, 1886; Farr et al. 1989; Peterson 1991) and trees in plantings in Frankfort, Kentucky have exhibited signs of these foliar diseases. Symptoms include tan spots with dark brown borders on leaves, and dark brown to black superficial spots on the fruit epidermis. Leaves displaying these symptoms were collected from plantings at the Kentucky State University Research Farm and positively identified as *Phyllosticta* leaf spot by the University of Kentucky Plant Diagnostic Lab. *P. asiminae* can occur on the foliage and surface of the fruit and may cause the fruit to crack when lesions expand, and lead to fruit rot. If the fruit lesions are not severe, the lesions can be removed from the peel and the fruit can still be consumed. Pawpaw fruit cracking may vary by cultivar and be related to infection by *P. asiminae* (Fig. 1). *Phyllosticta asimi-*

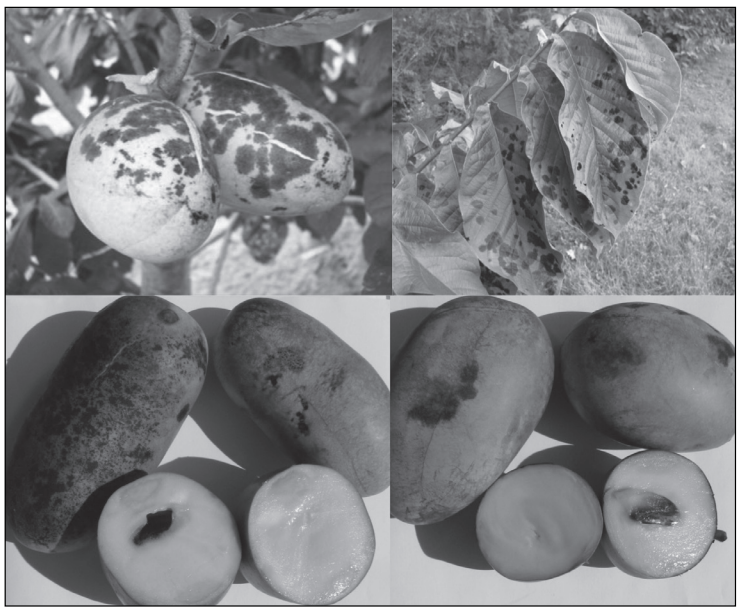

Fig. 1: Pawpaw fruit with fungal spots and cracking (left, top); pawpaw leaves with *Phyllosticta* spots (right, top); pawpaw fruit from the cultivar 'Sunflower' with some fungal spotting and small cracks (left, bottom; pawpaw fruit from the cultivar NC-1 with little fungal spotting (right, bottom).

nae infection may be enhanced during periods of high humidity and frequent rainfall. Dense foliage and lack of air circulation may contribute to the development of this fungus. The susceptibility of fruit from pawpaw cultivars to *Phyllosticta* damage, in terms of appearance for fresh market sale and cracking, represents a significant challenge to pawpaw growers.

In 1994, KSU was designated as a satellite repository for *Asimina* preservation in the U.S. Department of Agriculture (USDA), National Plant Germplasm System (NPGS). As a result, germplasm evaluation, preservation, and dissemination are a high priority. The repository orchards currently contain over 1700 accessions collected from the wild in 17 states and more than 40 cultivars. One of the goals of the repository is to assess levels of genetic diversity in native populations, in the repository collection, and in commercially available cultivars. Another goal is to acquire unique germplasm to add to the Repository collection for preservation and for use in future pawpaw breeding efforts.

The objectives of this study were to determine if fungal fruit spot and cracking incidence varied by a range of pawpaw germplasm, and if there was a positive correlation between the size of lesion and fruit cracking.

Materials and Methods

In 2012 and 2013, ripe fruit were harvested three times per week from a mature pawpaw planting at the KSU Research and Demonstration Farm consisting of pawpaw cultivars and advanced selections: 'Sunflower' (control), NC-1, Hi4-1, Hi7-5, G4-25, G5-23, G6-120, G9-111, Haz-1, and Haz-2. Trees in the orchard, seven to eight years in age, were at a spacing of 2.4 m between trees in row and 4.6 m between rows. Lesions had been noted on fruit for at least 5 years prior to this study and although fruit drops were routinely removed, apparently inoculum remains in the orchards and surrounding area. No thinning of pawpaw fruit is conducted and crop loads were similar for all genotypes for each individual year of the study. Lesions continue to develop and expand as the fruit develops and expands; therefore, the number of fruit lesions and the percent for lesion coverage of fruit, as well as cracking, were recorded for ripe fruit. The cultivar 'Sunflower' was used as a control since it is a widely planted commercial pawpaw cultivar that is susceptible to *Phyllosticta* attack on the leaves and fruit. Weather conditions were measured using a weather station located at the KSU Research and Demonstration Farm near the pawpaw orchards that is part of the Kentucky Mesonet system (http://www.kymesonet.org). Due to spring frosts that resulted in crop losses in 2012, there were very few fruit on the KSU advanced selections, therefore 15 fruit per selection or cultivar was used. Fruit were randomly selected. In 2012, size of fungal lesion in percent of the total fruit surface area was visually estimated, and fruit cracking was evaluated for 15 ripe fruit, they were soft and easily pulled from trees, harvested per cultivar and advanced selection. In 2013, the size of fungal lesion was visually estimated, and fruit cracking was evaluated for 25 fruit harvested per cultivar and advanced selection. Mean separation and correlation were calculated using the statistical program CoStat (CoHort Software, Monterey, Calif.). Data from each year were analyzed separately. Analysis of variance was performed using LSD means separation at a significance level of $P \leq 0.05$. Correlation was calculated for fruit weight and percentage fungal coverage.

Results and Discussion

In the 2012 growing season, conditions had high rainfall amounts with 59.9 cm of rainfall from April until September corresponding to the period from flowering to fruit harvest (Table 1). In 2013, weather conditions during the growing season also had high rainfall amounts with 73.9 cm of rainfall from April until September corresponding to flowering to fruit harvest (Table 1). Weather conditions in 2012 and 2013 were both conducive to *Phyllosticta* infection.

Table 1. Weather conditions in 2012 and 2013 measured from pawpaw flowering until the end of fruit harvest using a weather station located at the KSU Research and Demonstration Farm near the pawpaw orchards using the Kentucky Mesonet system.

	2012			
	Avg. Temperatures (°C)			Precipitation
	Max	Min	Daily	(cm)
April	19.8	6.3	13.1	5.28
May	26.5	13.7	20.1	12.19
Jun	28.7	14.7	21.7	5.77
July	32.8	19.7	26.3	15.24
Aug	29.7	16.3	23.0	2.23
Sept	24.7	13.0	18.9	19.03
Total				59.74
	2013			
	Avg. Temperatures (°C)			Precipitation
	Max	Min	Daily	(cm)
April	18.9	6.1	12.5	10.97
May	23.7	12.8	18.2	11.30
Jun	27.9	16.6	22.3	17.90
July	27.7	18.1	22.8	20.98
Aug	28.6	17.5	23.0	8.30
Sept	26.3	13.7	20.0	4.47
Total				73.93

In 2012 and 2013, fruit weights for the various cultivars and advanced selections were significantly different indicating genetic and environmental factors contribute to pawpaw fruit weight (Table 2). Fruit on trees was not thinned. Both sampling years were conducive to *Phyllosticta* infection and subsequent progression with warm rainy periods with high humidity. The control cultivar 'Sunflower', which usually shows moderate fungal lesion, displayed 10% lesion coverage in both 2012 and 2013. In 2012, Hi4-1 displayed the largest fungal lesion surface at 19%, while G4-25 displayed the least size at 1% of the total fruit surface. In 2013, both Haz-1 and NC-1, displayed the biggest fungal lesion at 13%, while Hi7-5 displayed the smallest lesion at 1%. Overall, G4-25, G5-23, G6-120, and Haz-2 displayed less than 10% surface area covered by fungal lesion for fruit of these genotypes in both 2012 and 2013. In terms of fruit cracking, Hi4-1 displayed the greatest cracking in 2012 with 52% of the fruit of this variety displaying cracking. In 2013, both Hi4-1 (16%) and NC-1 (20%) displayed the greatest fruit cracking. There were positive correlations between lesions and fruit cracking in five of the 10 pawpaw cultivars and advanced selections examined in 2012 and 2013 (Table 3). There was a significant positive linear correlation between increasing fungal lesion and fruit cracking across all cultivars in 2012 (P= 0.00001 ***; r = 0.18) and in 2013 (P= 0.0077 **; r = 0.17). In 2013, NC-1 did not display any fungal coverage, and yet 20%

Table 2. Average fruit weights, average percent area of fruit surface covered with fungal spot, number of fruit exhibiting cracking in 10 pawpaw cultivars and advanced selections in 2012 and 2013.

	Fruit weight (g)		Average size of lesions on fruit (%)		Fruit with cracking (%)	
Selection	2012	2013	2012	2013	2012	2013
Sunflower	181 c[z]	120 f	10% b	10% ab	5% bc	0% c
NC-1	305 ab	129 ef	3% bcd	13% a	0% c	20% a
Hi4-1	244 b	210 a	19% a	8% bcd	52% a	16% ab
Hi7-5	327 a	187 ab	11% b	1% e	11% b	0% c
G4-25	156 d	155 cde	1% d	5% de	6% bc	0% c
G5-23	173 cd	120 f	2% cd	8% bcd	0% c	8% abc
G6-120	150 d	143 def	5% bc	9% abc	0% c	4% bc
G9-111	150 d	174 bcd	3% bcd	10% ab	2% bc	4% bc
Haz-1	170 cd	139 ef	6% bc	13% a	0% c	0% c
Haz-2	277 ab	180 abc	3% bcd	6% cd	0% c	4% bc
Significance	***	***	***	***	***	**

[z] Statistical analysis performed using CoStat statistical software, LSD means separation at a significance level of $P \leq 0.05$.

Table 3. Correlations between *Phyllosticta* lesions and fruit cracking in 10 pawpaw cultivars and advanced selections in 2012 and 2013.

Selection	2012	2013
Sunflower	r = 0.12 ***	NA
NC-1	NA[z]	r = 0.29 NS
Hi4-1	r = 0.53 ***	r = -0.05 NS
Hi7-5	r = 0.01 NS	NA
G4-25	r = 0.53 ***	NA
G5-23	NA	r = 0.52 **
G6-120	NA	r = -0.11 NS
G9-111	r = 0.19 **	r = 0.28 NS
Haz-1	NA	NA
Haz-2	NA	r = -0.05 NS

[z]This comparison was not applicable (NA) since no cracking was observed in fruit of this selection in that year.

of the fruit sampled showed cracking, indicating that fruit cracking can also be related to the pawpaw genotype. In drought years, pawpaw fruit cracking can occur after a large rainfall event; however, the two years sample were years of ample and fairly consistent rainfall. Cracks can occur on lesions throughout the summer and fall as the fruit develop.

Lesions on the fruit surface consisting of a complex of *Mycocentrospora asiminae*, *Rhopaloconidium asiminae*, and *Phyllosticta asiminae* have been observed in many pawpaw cultivars and accessions (unpublished observations). Pawpaw fruit occasionally exhibit cracking, and field observations suggest cracking may vary by variety and be related to infection by this fungal complex. It was hypothesized that there may be a correlation between incidence of fungal fruit spot on pawpaw and fruit cracking, due to epidermal damage by the fungus complex. Both 2012 and 2013 were conducive to *Phyllosticta* infection with warm rainy periods with high humidity. While there was wide variation in fungal lesion from year to year, some selections did display fewer lesions on consecutive years, indicating lower susceptibility of the fruit of some genotypes to fungal pathogens. Lesions had been noted on fruit for at least 5 years prior to this study in this orchard indicating inoculum remains in the orchards and surrounding area to challenge trees. Most fruit across the selections examined displayed lesions in both years on the study. In 2012, fruit of 'Sunflower' (87% of fruit had lesions), NC-1 (67%), Hi4-1 (87%), Hi7-5 (53%), G4-25 (20%), G5-23 (67%), G6-120 (80%), G9-111 (13%), Haz-1 (80%), and Haz-2 (47%) had lesions. In 2013, fruit of 'Sunflower' (88%), NC-1 (92%), Hi4-1 (92%), Hi7-5 (28%), G4-25 (80%), G5-23 (92%), G6-120 (92%), G9-111 (88%), Haz-1 (100%), and Haz-2 (96%) had lesions. The genotypes G4-25, G5-23, G6-120, and Haz-2 displayed less than 10% lesions covering the fruit in both years of the study. These selections could represent parents for future breeding efforts toward developing future pawpaw cultivars; however, there was no evidence of total fungal spot resistance in any of the germplasm examined. There was a significant positive linear correlation between increasing fungal lesion and fruit cracking in 2012 and 2013; however, both 'NC-1' and 'Wabash' showed little or no lesion of fruit and still displayed fruit cracking. Fruit cracking can apparently be dependent on lesion, genotype, and environment.

In conclusion, fruit weight, fungal lesion, and cracking all varied significantly by variety. A positive correlation was found between cracking and disease lesion as well as fruit weight and cracking. However, other factors could also be related to fruit cracking, such as firmness and thickness of the epidermis. Future studies are needed to determine the relationship between fungal fruit spot and fruit cracking in pawpaw, and to determine varietal resistance to this fungus and possible control measures.

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Pre-harvest fruit bagging: a useful approach for plant protection and improved post-harvest fruit quality - a review

Abstract:

Several good agricultural practices (GAP) are becoming popular throughout the World for the production of high quality fruit with less dependence on man-made chemicals. Among such practices, pre-harvest fruit bagging has emerged as an effective method. Bagging is a physical protection method which not only improves the visual quality of fruit by promoting skin colouration and reducing blemishes, but can also change the micro-environment for fruit development, which can have several beneficial effects on internal fruit quality. Pre-harvest bagging of fruit can also reduce the incidence of disease, insect pests and/or mechanical damage, sunburn of the skin, fruit cracking, agrochemical residues on the fruit, and bird damage. Due to its many beneficial effects, fruit bagging has become an integral part of peach, apple, pear, grape, and loquat cultivation in Japan, Australia, China, and the USA. Moreover, countries such as Mexico, Chile, and Argentina do not import apples unless they are bagged. Several studies have been conducted to identify the desirable effects of pre-harvest fruit bagging on skin colour development and quality, but contradictory results have been reported. These may be due to differences in the type of bag used, the stage of fruit development when bagged, the duration of fruit exposure to natural light following bag removal, and/or fruit- and cultivar-specific responses. Bagging is laborious and its cost:benefit ratio must be investigated in order to promote adoption of the method in much of the world. The aim of this review is to improve our understanding of the beneficial effects of bagging in different fruit by collecting otherwise scattered information so that more growers could consider using this method on a commercial scale.

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