Suspected Lesion Mimic Mutants in Mule Palms (×*Butyagrus nabonnandii*)

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We report the occurrence of spontaneous lesion formation known as "lesion mimic mutants" in mule palms, an artificial hybrid between pindo (*Butia capitata*) and queen (*Syagrus romanzoffiana*) palms. Sunlight is a trigger for lesion development in symptomatic mule palms, that eventually wither off and die. Hybridization may create novel genetic combinations that lead to lesion mimic mutants. We propose the need for additional surveys to determine the frequency of lesion mimic mutants in palms.

In summer 2021, one of the authors (BD) visited a 500-acre palm nursery in south Florida, where the palm grower expressed concern about potential health issues with mule palms (× *Butyagrus nabonnandii*). Leaf spots were observed on multiple fronds that gave the mule palms an overall yellowish appearance. These palms showed a decline in vigor as compared to adjacent healthy mule palms that had dark green fronds. The grower recorded a high cull rate of more than 20% as the symptomatic mule palms would eventually wither off and die. The decline in health and subsequent mortality was restricted only to mule palms. Over the course of next ten months, four additional farms were visited in central and south Florida with a cumulative area of over 1,300 acres, and similar decline and mortality issues were observed in mule palms, whereas no other palm species with similar symptoms were seen at these sites. No apparent spatial pattern was evident for the declining mule palms in the field. Both container- and field-grown mule palms had been treated with multiple fungicide and fertilizer applications without any improvement in the palm health suggesting that neither disease nor nutritional deficiency was responsible for causing mule palm decline. Based on their years of experience in growing

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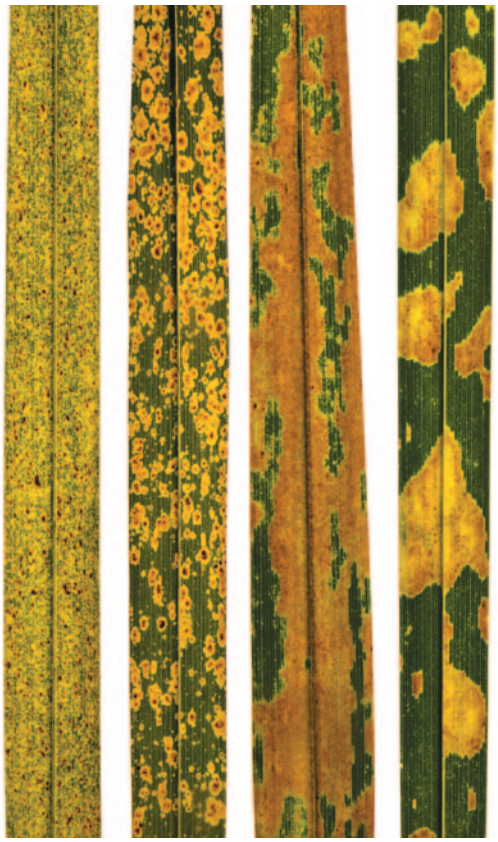


1. Lesion mimic mutant (LMM) mule palms exhibit variation in lesion size and density. Four leaflets collected from different LMM mule palms displayed lesions of various shapes and sizes including (left to right), 1) small, chlorotic and necrotic lesions, 2) lesions with necrotic center with chlorotic halo, 3) lesions that have merged to cover most of the leaflet and 4) lesions that appear as splotches.

mule palms, a consensus among growers was that the mule palms that started out as healthy seldom showed any symptoms, but the symptomatic mule palms never reverted to good health.

The symptom development seen on diseased or nutrient deficient plants is usually very consistent and characteristic of a particular pathogen or element imbalance, respectively. In the majority of cases, the symptomology is very dependable such that the disease or nutrient deficiency can be easily diagnosed based on the distinctive symptoms. However, in this case, the leaf spots or lesions on mule palms fronds were not consistent but varied by color, size, density and growth type across the different individuals (Fig. 1). This lack of uniform symptoms alludes to the absence of a pathogen as the underlying cause for the leaf spots. Moreover, the distribution of lesions across the fronds on a given individual was also non-uniform. Lesions were non-existent on the newly emerging fronds and lesion density increased as the fronds matured. Even though the older fronds were full of lesions, they did not seem to spread or affect adjacent healthy mule palms or palms of other species ruling out a pathogen as the causal agent.

Nutritional deficiency could be ruled out as the cause, as similar health decline symptoms were observed and comparable mortality rates were reported for mule palms growing across different regions, soils and environmental conditions. The majority of nutritional issues can be resolved by application of fertilizers and are rarely lethal. Even though the different palm species were subjected to a similar fertilizing regimen, mortality was still observed in mule palms. As the lesions on mule palms did not appear suddenly but rather progressed with frond maturity, it suggested that neither phytotoxic compounds nor other abiotic factors may be responsible for the lesions.

The appearance and development of leaf spots or lesions as fronds mature could be explained by a unique phenomenon, known as "lesion mimic mutant." The term "lesion mimic mutant" (LMM) implies that disease-like symptoms such as chlorotic and necrotic lesions can spontaneously form even in the absence of a pathogen, making a disease-free plant to appear like a diseased plant. The prevalence of LMMs in hybrid plants was first discovered 100 years ago in hawksbeard (*Crepis*) plants (Li & Weigel 2021) and has since been reported in several crops including maize, rice, barley, wheat, soybean, tomato, brassica, cotton, pepper, tobacco and potato (Bruggeman et al 2015, Freh et al 2022). The observations made in mule palms were similar to what has been described in LMM plants. In LMM mule palms, the appearance of lesions seemed to be developmentally regulated. A newly emerged frond did not show any lesions. On younger fronds, lesions started out as small, water-soaked flecks and as the fronds matured, the lesions increased in number as well as size (Figs. 2 & 3). Intra- or inter-specific crosses in crops like corn, wheat and rice, resulted in lesions that advance with leaf maturity leading to premature death of leaves and whole plants in a fraction of hybrids (Li & Weigel 2021). Similarly, mule palms result from an inter-generic cross where a subset of palms exhibit chlorotic and necrotic lesions as fronds mature, leading to frond death and eventually the whole palm dies.

2. Lesion mimic mutant (LMM) mule palm showing developmental progression of lesion development. The leaflets on the lower fronds are either completely necrotic or filled with chlorotic and necrotic leaf spots. The leaflets on younger fronds are green and healthy.

Lesion development in LMMs can be influenced by the genetic makeup of the plant and environmental factors, such as temperature and sunlight (Johal et al 1995). In plants, like corn and *Arabidopsis*, it was demonstrated that controlled crosses of LMM plants with different cultivars or inbred lines, can either enhance or turn off lesion formation, highlighting the importance of genetic makeup in regulating the LMM

3. Close-up of the fronds on a lesion mimic mutant (LMM) mule palm. The bottom half of the image has older fronds showing splotches on the leaflets. The upper half of the image shows younger fronds with green leaflets.

phenotype (Hoisington et al 1982). A comparable trend was found in symptomatic mule palms where lesions across multiple individuals presented in different sizes and shapes (Fig. 1).

To estimate the number of mule palms exhibiting the lesion development pattern characteristic of LMMs, we surveyed a population of approximately 450 container-grown mule palms. We found that the LMM

Table 1. Distribution of lesion mimic mutants (LMMs) in a plot of container-grown three-year-old mule palms. *Lesion mimic mutants (LMMs).

Rows	Healthy	LMMs*	Total	% LMMs
1, 2	55	14	69	20.3
3, 4	46	20	66	30.3
5, 6	43	22	65	33.8
7, 8	42	20	62	32.3
9, 10	42	18	60	30.0
11, 12	40	19	59	32.2
13, 14	40	17	57	29,8
15	22	6	28	21.4
Total	330	136	466	29.2

phenotype was present in 29% of the three-year-old mule palms growing in 15-gallon pots in full sun (Table 1). This observation matches the high cull rate for field-grown mature palms that the growers have reported.

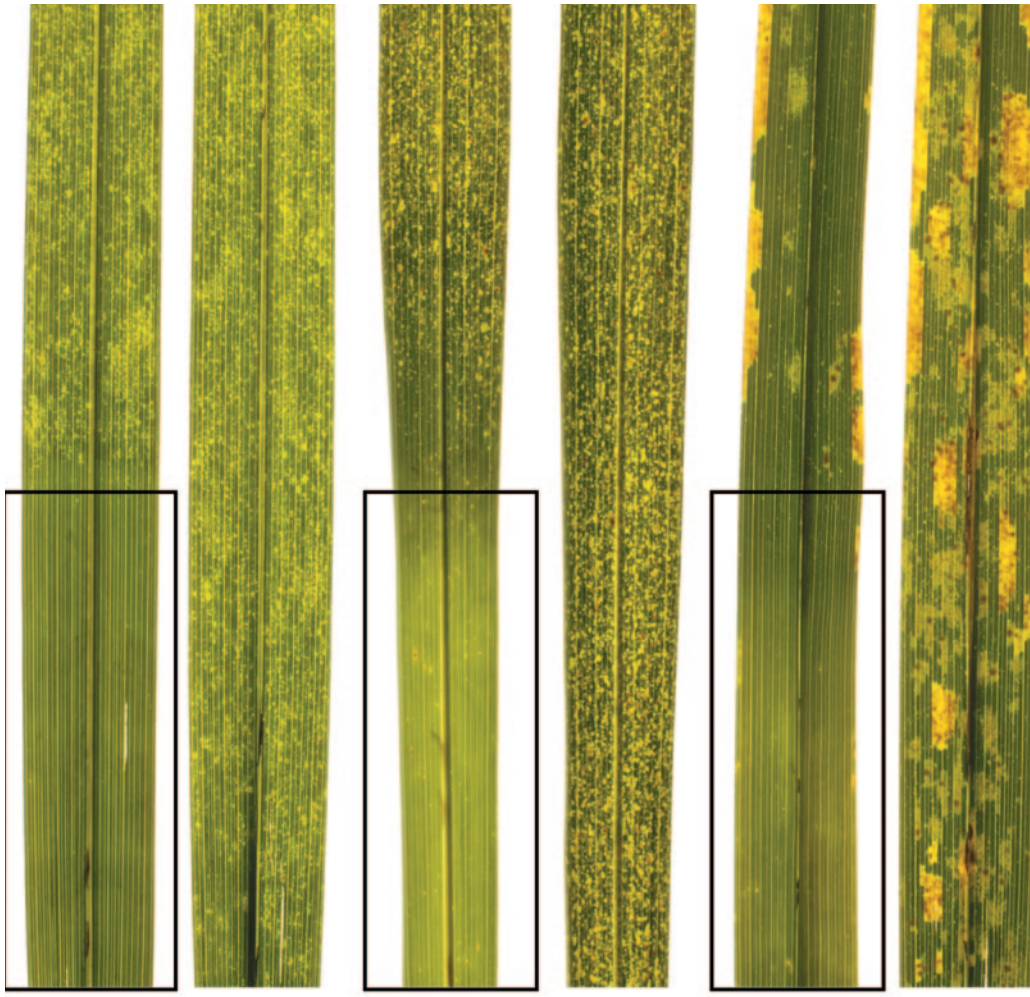
In order to confirm the LMM phenotype, leaflets on symptomatic mule palms were covered with aluminum foil, to prevent sunlight from reaching that section of the leaflet and maintain complete darkness. As sunlight is one of the triggers for lesion formation in LMMs, the expectation was that lesions will fail to develop in the leaflet section covered with the foil. Briefly, ten field-grown symptomatic mule palms were selected randomly and a 5-inch-wide piece of aluminum foil was wrapped around the base of a leaflet on the youngest fully open frond free of any lesions (Fig. 4). Only one leaflet per mule palm was covered, i.e. a total of 10 leaflets were examined. The four edges of the wrapped foil were crimped to ensure that the foil did not slide out. This experiment was started in March 2022 and the aluminum foil was removed after a period of 3 months. We found that the covered part of the leaflet was free of lesions even though it was adjacent to the section with leaf spots (Fig. 5). This observation that leaf spots failed to develop on a section of the leaflet that was wrapped with the foil and kept in the dark confirmed that these mule palms were LMMs.

The lesions observed in LMM mule palms can be compared to an 'autoimmune disorder'. The highly regulated and tightly controlled plant immune system is geared to detect and defend against pathogen attack. However, ill-timed functioning of the immune system comes with fitness costs, i.e., autoimmunity. In auto-immunity, the host fails to differentiate self from non-self and starts to attack itself leading to symptoms like leaf chlorosis and necrosis, stunted growth, followed by plant death (Freh et al 2022). Hybridization was determined to be the dominant cause for autoimmunity or hybrid necrosis (Würschum et al 2023). The Bateson-Dobzhansky-Muller model posits that hybrid necrosis may result from detrimental interactions between genetic loci that come from vigorous and fertile parents (Orr 1996).

4. Aluminum foil wrap method to detect lesion mimic mutant (LMM) mule palms. A green leaflet on a young frond lacking leaf spots was wrapped in aluminum foil to exclude sunlight. The four corners were folded so that the foil stays snug and doesn't loosen up.

Hybridization can generate novel genetic combinations that may disrupt cellular homeostasis leading to cell death in lesion mimic mutants (Li and Weigel 2021) and as cells die, lesions start to develop in LMM mule palms.

Mule palm (× *Butyagrus nabonnandii*) is an intergeneric hybrid generated from a cross between pindo palm (*Butia capitata*) and queen palm (*Syagrus romanzoffiana*), both belonging to tribe Cocoseae, subtribe Butiinae. This cross was first attempted by Paul Nabonnand, a



5. Exposure to sunlight triggers formation of leaf spots in lesion mimic mutant (LMM) mule palms. In each pair, one leaflet (on the left) was partly covered with aluminum foil while the other leaflet (on the right) was completely exposed to light. The bottom covered section was in the dark and did not develop lesion mimic phenotype, but the section exposed to sunlight shows lesion development. The black box marks the section that was covered.

French nurseryman, around 1890s (Tournay 2009) and then rediscovered almost a 100 years later in the US (Wilcox et al 1990). There are 2,600 species (Baker & Dransfield 2016) and 114 natural hybrids (Henderson 2022) known in palms, but to our knowledge this is the first documentation of occurrence of LMMs in palms. So, a targeted survey to determine the occurrence and frequency of LMMs in different palm hybrids is needed.

The appearance of lesions is usually symptomatic of a risk to plant health. Correct identification of the agent responsible for the lesions ensures that appropriate management options are utilized to restore health of the plant. When lesions are caused by external agents such as pathogens or nutrients, palms can be treated with either fungicides or nutrients to remedy the issue, but this does not hold true for LMMs. As the lesions observed in LMMs arise due to genetic anomalies, no external inputs can be applied to correct the lesions. Repeated applications of fungicides and fertilizers have not proven effective in improving the health of LMM mule palms. This underscores the need for a proper diagnosis of the agent causing the lesions and avoid unnecessary fungicide or fertilizer treatments thereby reducing input costs.

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