Report for 2003-2004 FNGA Award on

Controlling Lantana's Invasiveness through Genetic Sterilization

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Abstract

The objectives of this project were to find the breeding techniques or genetic mechanisms that can be used to effectively sterilize lantanas. We identified two diploids and three tetraploids, evaluated their pollen viability and seed production, and investigated the genetic cause of the sterility in "New Gold". The three tetraploids had pollen viability as good as the two diploids, and produced more seeds than diploids. The high level of sterility in "New Gold" was very likely due to aneuploidy. These results indicated that chromosome doubling may not be a viable option to sterilize lantanas, but aneuploidy (and triploidy) may lead to high levels of sterility in lantana. One of the approaches to sterilize lantanas and develop new sterile varieties seems to be to cross available tetraploids with diploids to produce aneuploids and triploids, followed by screening for seed production and pollen viability. The information gained and the materials characterized will play critical roles in achieving a longer-term goal to produce sterile *Lantana camara*.

Objectives

Lantana camara can hybridize with Florida's native lantana species, and produce numerous seeds that may be dispersed to neighboring areas where they produce new plants. These habits have led to its being classified as a Category I species by the State's Department of Environmental Protection (DEP), and the Florida Exotic Pest Plant Council (FEPPC). It was assigned "Caution" in north Florida and received "Do not use" in central and south Florida. It was indicated that sterile cultivars of *L. camara* would likely receive more favorable acceptance by the State's DEP and the FEPPC (Fox et al., 2003).

A number of genetic mechanisms can lead to male and/or female sterility in plants: gene mutations, tetraploidy, triploidy, aneuploidy, and chromosome rearrangements. In a plant species, the specific mechanism for the sterility varies greatly from one variety to another.

A number of breeding techniques have been developed to induce male or female sterility in plants. These techniques include chromosome doubling to produce tetraploids, tetraploid x diploid crossing to produce triploids or aneuploids, irradiation to induce chromosome rearrangements or gene mutations, etc. Some of these techniques have been successfully used in a number of horticultural crops, such as tetraploid grapes with few seeds, seedless citrus or seedless watermelons. The effectiveness of these techniques is dependent on species to some extent and the level of sterility required.

The goals of this project were to identify the breeding techniques or genetic mechanisms that can effectively induce sterility in lantana and to apply these techniques to control lantana's invasiveness and develop new sterile varieties. The specific objectives of this project were (1) to

identify or induce tetraploid lantanas and evaluate their male and female sterility levels; (2) to identify sterile lantanas and find out the genetic cause(s) of the sterility; and (3) to develop procedures that can be easily adopted for quick evaluation of male sterility in lantana. Thus the completion of this project could provide the necessary platform for achieving the longer-term project goals.

Materials and Methods

Plant materials

"Cream", "Pink Caprice", "Prof. Raux", and "Radiation" were from Dr. Dehgan's lantana collection in Gainesville, Fla. CV7 might be "Gold", but its identity needs to be determined. "New Gold" was donated by the Myers Nurseries as potted plants. Cuttings were made from semi-woody shoots, dusted with rooting powder, and rooted in a mist house at the GCREC, Bradenton.

Flow cytometry for ploidy level analysis

Tender leaves were collected from potted plants grown in a greenhouse at the GCREC, Bradenton, and kept fresh in plastic bags with moist paper towels. Ploidy level analysis was conducted in Dr. Grosser's laboratory at the Citrus Research and Education Center, Lake Alfred, Fla, using the Partec PA-II. The protocol developed for citrus ploidy level analysis was followed, but with modified PA-II settings.

Chromosome squashing and counting

Chromosome counting was conducted using root tip cells. Root tips were collected from rooted cuttings that had been exposed to 8-9°C overnight, treated in 0.05% colchicine solution in the dark for 4 hours at room temperature, and fixed in Carnoy's solution (acetic acid:ethanol). Root tip cells were hydrolyzed in 1 N hydrochloric acid for 30-45 minutes, stained in 2% acetocarmine solution (Jude Grosser; Carolina Biochemical Supplies), and squashed onto glass slides. Chromosomes were observed under a microscope (Olympus BH-2), and recorded with a digital camera. For confirmation, images of chromosomes were further examined on a computer in Microsoft Photo Editor.

Pollen viability staining and seed counting

A randomized complete block design was used with 5 blocks (replication) and one plant in a block. Plants were grown in 6" azalea pots, placed on a metal bench in a fan-cooled greenhouse, and watered using a drip irrigation system. Pollen viability staining and seed counting were performed on 3 1/2 month-old plants.

Twenty newly opened flowered were collected from each plant (5-10 flower clusters x 2-4 flowers per cluster), and their anthers were collected onto a glass slide using forceps. Pollen grains (and microspores) were squashed in acetocarmine solution and stained for 2-5 hours before they were observed under a microscope. Pollen grains and microspores were counted into 3 categories: well stained, red grains (viable), poorly stained, gray grains (non-viable), and aborted grains (deformed, empty microspores). Five or more fields on the slide and >200 grains were counted for each plant. The percentage of well stained, red grains was used as an indication of viable pollen production.

Twenty flower (or berry) clusters on each plant were examined, and berries on these clusters were counted. The total number of seeds from 20 berry clusters was used as an indication of seed production of the plant. Mature berries from these clusters were collected; seeds were extracted, soaked in water overnight, and germinated in 5" pots containing Verlite container mix A. One month later, seedlings were counted as an indication of seed emergence.

Results and Discussion

1. Identification of tetraploid lantanas and evaluation of chromosome doubling on male and female sterility.

We surveyed 14 lantana varieties or clones for their ploidy levels using the flow cytometrical analysis and identified two diploids ("Cream" and CV7) and three tetraploids ("Pink Caprice", "Prof. Raux", and "Radiation") (Table 1). Their somatic chromosome numbers were further confirmed by chromosome counting. The two diploids had an average of 67.1% pollen viability (Fig. 1A), while the three tetraploids had an average of 64.3% pollen viability (Fig. 1B and 1C). These data revealed that the tetraploid lantanas have pollen viability as good as diploids, and they may indicate that increasing chromosome numbers simply from a diploid level (2x) to a tetraploid level (4x) may not be an option to sterilize lantana's pollen.

All three tetraploids produced more seeds (an average of 66.3 seeds produced on 20 flower clusters) than the two diploids. Again, this indicates that chromosome doubling may not result in any reduction in seed production in lantana. It was noticed that the two diploids produced few seeds. Whether or not this is a common phenomenon remains to be confirmed with more diploid varieties.

No triploid lantana varieties were identified in the materials examined so far. In citrus, watermelon, and some other plant species, triploids show high levels of pollen sterility and produce few seeds (referred as to being commercially seedless). It will be very interesting to identify or produce triploids and examine the effect of triploidy on lantana pollen viability and seed production.

2. Identification of sterile cultivars and their genetic mechanisms for sterility.

"New Gold" has been marketed as a sterile variety. Pollen viability and seed counting data show that it produced a very small amount of viable pollen (0.5-5.3%, average 3.3%; Fig. 1D) and few seeds (1.2 – 11.2 seeds per 20 flower clusters, average 6.2 seeds) (Table 1). Compared to the three tetraploids, "New Gold" showed 20 fold reduction in viable pollen production and 10 fold reduction in seed production. The data also indicate the existence of differences in pollen viability and seed production among the five pots or "clones" of "New Gold" initially obtained from the nursery. It is particularly interesting that one of the clones had extremely high levels of pollen sterility (0.5% pollen viability) and low seed counts (approximately one seed on 20 flower clusters).

In chromosome counting, we found that "New Gold" seems to be an aneuploid, i.e. plants have fewer chromosomes compared to the typical tetraploids. It is known in other plants that aneuploids have much reduced male and/or female fertility. It is very likely that this aneuploidy is the genetic cause of the high levels of sterility observed in "New Gold". This finding will provide a very important guideline for future work on sterilizing other invasive lantana varieties.

3. Development of a procedure for quick examination of lantana pollen viability.

Compared to seed counting, pollen viability examination is much more difficult for county-extension personnel to perform. After a quick survey of several methods including pollen germination, pollination tests, we have found that acetocarmine staining is a convenient way to check lantana's pollen viability. The procedure, as described in the materials and methods section, involves a microscope and a few other items that can be easily obtained (Fig. 1E and 1F).

Table 1. Ploidy levels, chromosome counts, pollen viability and seed counts of lantana cultivars

Variety	Flow cytometry analysis	Chromosome counting	Pollen viability (%)	Seed counts	Seed emergence (%) (n=no. seeds sown)
Cream	2x	2x	44.6	0.4	-[z]
CV7	2x	2x	89.6	0.2	-[z]
Pink Caprice	4x	4x	62.5	92.8	39 (n=150)
Prof. Raux	4x	4x	68.4	38.2	71 (n=7)
Radiation	4x	4x	62.0	68.0	21 (n=29)
New Gold, clone 1		Aneuploid	4.8	8.2	0 (n=8)
New Gold, clone 2		Aneuploid	5.3	11.2	0 (n=5)
New Gold, clone 3		-[y]	3.0	7.2	0 (n=8)
New Gold, clone 4		Aneuploid	2.8	3	-[z]
New Gold, clone 5		Aneuploid	0.5	1.2	0 (n=3)

Notes: [z] no seeds available at the sowing time; [y] chromosome counting not performed.

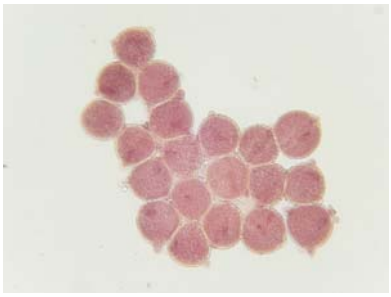

Fig. 1A. CV7 pollen grains

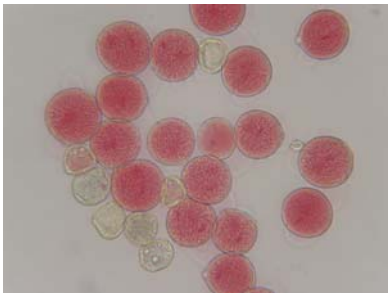

Fig. 1B. Pink Caprice pollen grains

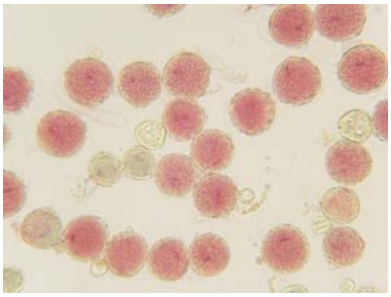

Fig. 1C. Radiation pollen grains

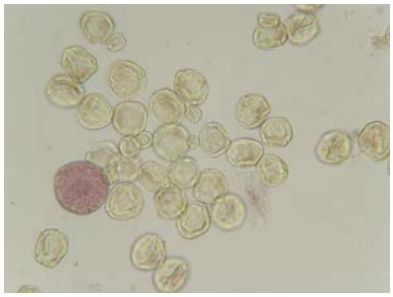

Fig. 1D. New Gold pollen grains

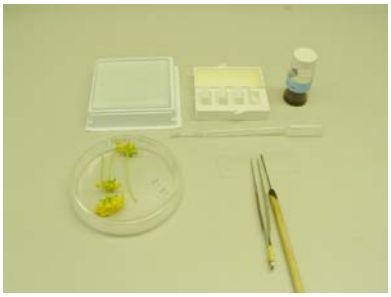

Fig. 1E. Items for pollen viability examination

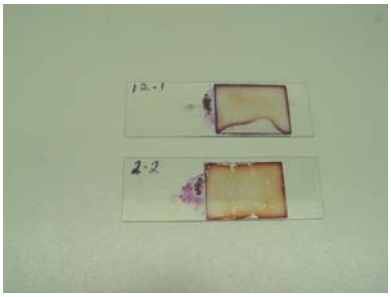

Fig. 1F. Pollen grains stained on glass slides

Conclusions

1. Chromosome doubling itself and resulting tetraploids may not be an effective option to sterilize lantanas.
2. Aneuploid lantanas seem to be highly sterile in both pollen and seed production. Likely, triploid lantanas might be highly sterile as well, based on the genetic mechanisms they share.
3. Lantana pollen sterility can be examined using the acetocarmine staining procedure.
4. The information on chromosome counts and pollen viability will serve as guidelines and the characterized materials will provide the platform that will be critical for future work on sterilizing lantanas.

Recommendations

1. To sterilize lantanas, the appropriate approach may be through production of aneuploids (and triploids), screening progeny based on seed production, followed by examining pollen viability via acetocarmine staining.
2. Chromosome doubling itself can not sterilize lantanas, but the resulting tetraploids can provide important materials for subsequent production of aneuploids (and triploids).
3. Genetic approaches may be the only way to sterilize lantanas, but as always, genetic manipulations and subsequent screening, tests and confirmation take a relatively long time (at least 4-5 years), compared to other types of research. It is critical for a project on genetic sterilization to receive continued support during the process.
4. Some of the varieties in the trade have 20 fold reduction in pollen viability tests. Then the question is “What is the level of pollen sterility for a lantana variety to be considered to be non-invasive?”.

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