

Germination of three selections of *Lantana* species.: *L. camara*, *L. camara* 'Pink Caprice', and *L. depressa*.

Abstract

Lantana camara is known to be naturalized in at least 60 countries worldwide and is currently listed as a Category 1 invasive species by the Florida Exotic Plant Pest Council, meaning that the species is altering native plant communities throughout the state of Florida. *L. camara* is negatively affecting the population of the native *Lantana depressa* by hybridization. This species, like many other invasive species, was thought to have been introduced as an ornamental species that escaped cultivation. *L. camara* is an important plant to the ornamental industry and cultivars are still being used as a landscape plant, many with less invasive qualities in hopes to prevent further spread of the species. Studies were conducted using two selections of *L. camara* (a locally known invasive herein after called 'Rock Road' and cultivar 'Pink Caprice') and *L. depressa* in order to determine successful germination treatments for *Lantana* spp. that can be used by growers desiring to produce less invasive cultivars. At the end of the 60-day study, viable seeds of both selections of *L. camara* had the highest germination rates when treated with either Physan, KNO₃ + Gibberellic Acid, or no treatment at all. *L. depressa* had poor germination rates with all treatments, with dormancy issues being the suspected cause. Not every selection responded similarly to all treatments or growing conditions.

Introduction

The genus *Lantana* contains over 150 species (Stirton, 1978). One of these species, *Lantana camara*, is considered quite invasive and is naturalized in numerous countries throughout the world, but is still used as an ornamental landscape plant in Florida. Many of the qualities that make this species a good choice for ornamental use, such as fast growth, high vigor, and adaptation to a wide range of environmental conditions also make this species a good candidate for the invasion of natural areas if an escape from cultivation occurs (Caley et al., 2008).

L. camara (Figure 1) is native to South America and the West Indies, from where it was originally obtained and introduced in Europe as a landscaping plant in the 1650s (Stirton, 1978). By the late nineteenth century, there were over 600 named cultivars (Stirton, 1978). Today, *L. camara* is naturalized in at least 60 countries and is considered an invasive pest plant in Australia, New Zealand, Africa, India, Asia, the Pacific Islands, and the United States (Vivian-Smith and Panetta, 2009). *L. camara* has become a problem weed in croplands worldwide. Effected crops include coffee in Indonesia, coconuts in Fiji and Trinidad, cotton in Turkey and Nicaragua, bananas in Samoa, sugarcane in Australia, India, and South Africa, tea in India and Indonesia, rubber in Malaysia, and rice in Indonesia (Nanjappa et al., 2005). The unripened fruit and foliage are extremely toxic to grazing cattle and horses, causing symptoms of weakness, loss of appetite,

bloody feces, ulcers of the mucous membranes, and eventual death from kidney failure (Stirton, 1978). There have even been reported cases of the death of several children soon after the consumption of *L. camara* fruit (Morton, 1994).



Figure 1: *Lantana camara*, Category 1 FLEPPC species. Photo courtesy of UF IRREC.

Lantana camara is listed as a Category 1 invasive plant by the Florida Exotic Plant Pest Council (FLEPPC), meaning that the species is altering native plant communities (FLEPPC, 2009). Florida's warm climate and long growing season, in addition to the presence of the related native species, *Lantana depressa* (Figure 2), have contributed to *L. camara*'s spread [Center for Aquatic and Invasive Plants (CAIP), 2009; Langeland et al., 2008]. Escaped *L. camara* has become naturalized throughout Florida, where it has become a pollen donor and hybridizes with *L. depressa*, causing it to become endangered (FLEPPC, 2009; Hammer, 2004; Southeastern Rare Plant Information Network, n.d.). Its hybridization with the native *Lantana depressa* has contaminated the gene pool of all three varieties of this endemic species (Figure 3) (Hammer, 2004; Sanders 1987). It is now extremely difficult to find unhybridized *L. depressa* in the wild, although it is still sold by native plant nurseries (Hammer, 2004).



Figure 2: *Lantana depressa*, endemic species to South Florida. Photo courtesy of UF IRREC.

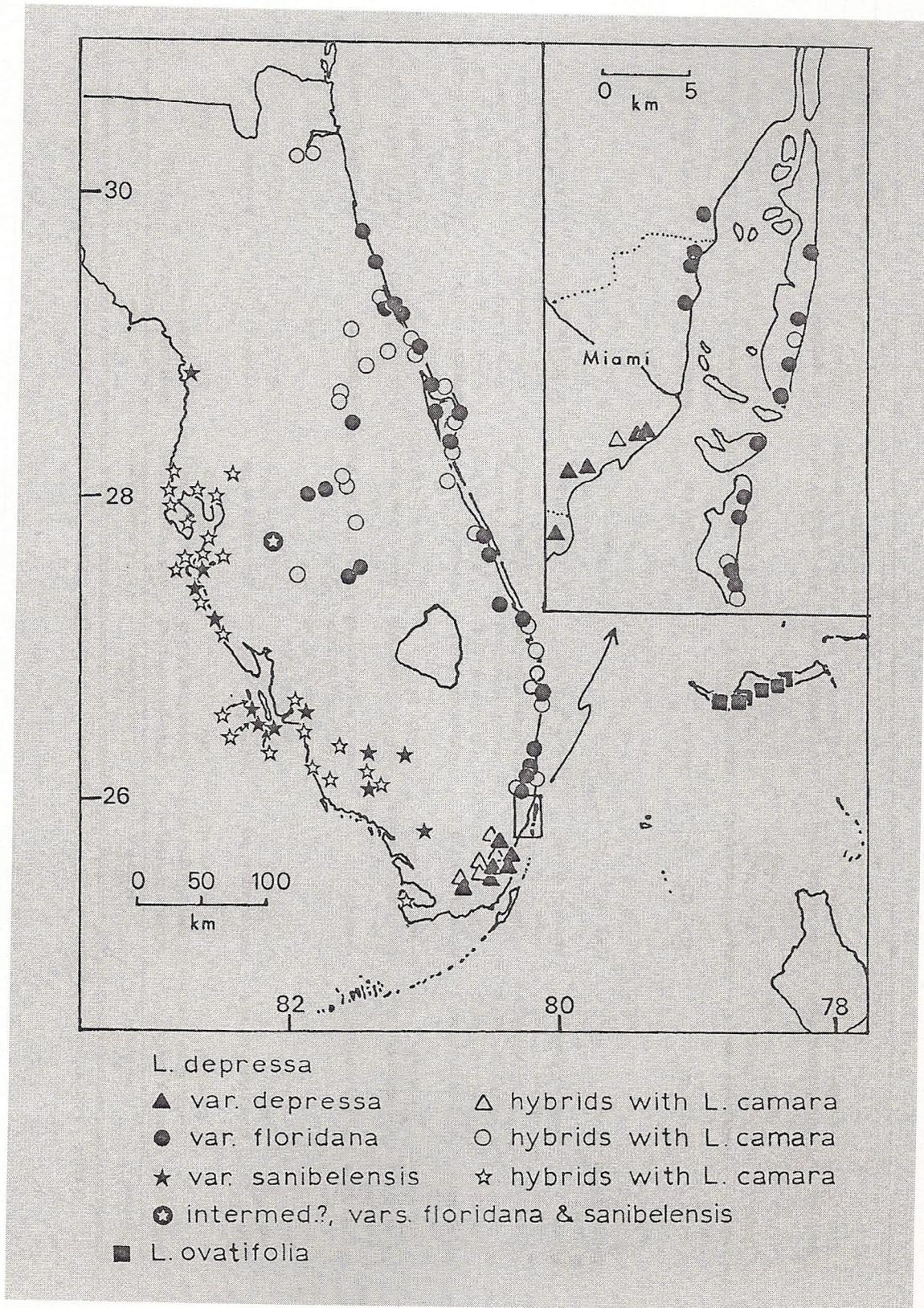


Figure 3: Map showing the extent of *L. camara* invasion and hybridization with *L. depressa* (Sanders 1987).

Lantana depressa is endemic to a limestone ridge located in Dade County that extends from the Miami River southwest into the southern Everglades (Sanders, 1987). There are three native varieties of this species, *L. depressa* var. *depressa*, *L. depressa* var. *sanibelensis*, and *L. depressa* var. *floridana* (Hammer, 2004). *L. depressa* var. *depressa*, called rockland lantana, is a low-growing shrub that is endemic to the pine rocklands of southern Dade County (Hammer, 2004). *L. depressa* var. *sanibelensis* is a large, bushy, yellow flowered shrub that is native to the west coast of Florida (Hammer, 2004). *L. depressa* var. *floridana* is also a large, bushy, yellow flowered shrub, but is found along the sandy shorelines and dunes of the east coast of Florida (Hammer, 2004). All three varieties of *L. depressa* are diploid. *L. camara* is tetraploid (Sanders, 1987). The hybridized plants between these two species are triploid (Sanders, 1987). The leaves of the hybridized plants are squared off at the base and the flowers open yellow and turn pinkish with age (Hammer, 2004). These hybridized plants combine the local adaptations of *L. depressa* with the vigor of *L. camara*, causing them to persist and spread (Levin et al., 1996).

Although extremely invasive, *L. camara* is an important and useful plant in some parts of the world, mainly due to its ease of growth and adaptability to poor environmental conditions (Morton, 1994; Nanjappa et al., 2005). In Africa, it is encouraged for use as a protective hedge to keep roaming animals out of one's property (Morton, 1994) and it is planted on rocky hillsides to prevent erosion in India (Morton, 1994). In the Himalayas, *L. camara* is formed into incense cakes that are used as mosquito repellent (Nanjappa et al., 2005). The twigs can be burned and used as fuel (Nanjappa et al., 2005) or the scabrous leaves can be used in place of sandpaper to polish wood (Morton, 1994). *L. camara* is also popular in medicinal uses (Morton, 1994). A tea made from the leaves is said to cure colds, fever, hypertension, and diarrhea (Morton, 1994). The plant can be applied externally to relieve symptoms of measles, chicken pox, snake bites, cuts, ulcers, and bruises (Morton, 1994).

Lantana camara cultivars (Figure 4) are very popular in Florida landscapes because of their ability to thrive in poor, dry soils where other plants cannot (Hammer, 2004). Because of its ability to survive under drought conditions, *L. camara* has been promoted in Florida landscapes to assist in water conservation efforts (Knox, 2001). The results of a survey conducted on the Florida nursery industry concluded that 19.0% of the responding nurseries grew some form of *L. camara* (Wirth et al., 2004). The farm-gate value of *L. camara* in Florida alone was over \$40 million (Wirth et al., 2004).



Figure 4: *Lantana camara* 'Pink Caprice', a popular landscape cultivar. Photo courtesy of UF IRREC.

Florida is the second largest producer of ornamental plants in the United States, with total sales in 2005 exceeding \$15 billion (Hodges and Haydu, 2006). Of the 124 plants listed on the 2003 FLEPPC list, more than half were introduced as ornamentals (Wilson et al., 2004). Although the majority of ornamental plants do not escape cultivation, a large number of them do, leading many to believe that standard practices within the nursery industry must change in order to prevent more ornamentals from escaping cultivation in the future (Wilson et al., 2004). Currently, there is no required protocol for nursery growers to follow regarding the sale of invasive plants, and it is unfair to assume that all cultivars of an invasive species are also invasive (Wilson et al., 2004). A select number of nurseries have chosen to adhere to voluntary codes of conduct that follow best management practices pertaining to the sale of possibly invasive plants (Wilson et al., 2004). Ornamental plants are screened for possible invasive characteristics and prior invasiveness in other parts of the world and nursery growers can choose not to sell plants deemed potentially invasive (Wilson et al., 2004).

Millions of dollars have been spent on research in hopes of discovering a successful biological control agent capable of controlling the spread of *L. camara* (Hammer, 2004). One of the more promising attempts involved the release of the seedfly *Ophiomyia lantanae* in Australia between 1914 and 1917 (Vivian-Smith and Panetta, 2006). The seedfly lays its eggs within the fruit of *L. camara* (Vivian-Smith and Panetta, 2006). The larvae then feed on the fleshy pericarp while tunneling through the seed into the area between the two embryos (Vivian-Smith and Panetta, 2006). The seedfly was hoped to have degraded the viability of the seeds, and hence reducing the germination rate and the spread of *L. camara* (Vivian-Smith and Panetta, 2006). The seedfly has shown to possibly reduce the quality of the fruit and reduce its attractiveness to birds, but has failed to control *L. camara* (Vivian-Smith and Panetta, 2006). In Florida, citrus growers tried to use biological control methods to keep *L. camara* from invading their groves, but

were met with opposition from commercial growers of *L. camara* as an ornamental (Morton, 1994). Other methods of physical, chemical, and biological control of *L. camara* have had little to no success due to high cost or inaccessibility of infestation sites for treatment (Day et al., 2003; Nanjappa et al., 2005).

Due to the fact that the spread of the majority of invasive species is caused by seed and pollen production (Anderson, 2006 Pysek and Richardson, 2007), much attention should be given to the development of genetic sterilization as a preventative control measure regarding invasive species (Anderson, 2006; Li et al, 2004; Olsen and Ranney, 2005, Ranney, 2004) Less invasive cultivars, such as the *L. camara* cultivar 'New Gold', which appears to produce no seed, are being developed to be used in place of their invasive counterparts, but in order to prevent hybridization with native species, these cultivars should be completely sterile and produce no pollen (Hammer, 2004).

The overall objective of this study was to determine successful germination treatments for *Lantana spp.* that can be used by growers desiring to produce less invasive cultivars. There is currently no germination protocol for *L. depressa*, and seed is the preferred method for propagation in order to maintain genetic diversity of the native population. Reduced germination numbers have been observed when attempting to propagate *L. camara* from seed (Graaff, 1987). The low germination is thought to be caused by the fact that many seeds do not contain embryos, are dormant or nonviable, or are possibly infected by the biological control agent *Ophiomyia lantanae* (Graaff, 1987). For this study, three types of Lantana species were selected: *L. camara*, herein after referred to as 'Rock Road' (FLEPPC Category 1 invasive), *L. camara* cultivar 'Pink Caprice' (ornamental species), and *L. depressa* (native Florida species). The intent is to develop a germination protocol that can be used to successfully germinate Lantana species from seed.

Materials and Methods

Seed germination and viability

Fruit appearing to be mature was collected from each type of Lantana by hand and cleaned. Fruit from 'Pink Caprice' was collected from plants located in the Indian River Research and Education Center (Fort Pierce, FL) field on September 25, 2009. Fruit from 'Rock Road' was collected from plants in the Indian River Research and Education Center (Fort Pierce, FL) greenhouse on September 25, 2009. Fruit from *L. depressa* was collected from plants located in the Indian River Research and Education Center (Fort Pierce, FL) on October 7, 2009. Seeds with visible indication of pathogen or insect damage were discarded. Cleaned seeds were gravity air-dried at 22 °C for 48 to 72 hours before analysis.

In accordance with the Tetrazolium Testing Handbook, Contribution No. 29 Association of Official Seed Analysis rules (Peters, 2000), pregermination viability tests were replicated twice on a subset of 100 seeds per Lantana selection. Seeds were preconditioned by allowing them to imbibe between moist

blotting paper overnight at room temperature. The distal end of the cotyledon of each seed was cut off and seeds were stained overnight at 30 °C in 1.0% Tetrazolium (2, 3, 5-triphenyl chloride). Seeds were considered viable if the entire embryo stained evenly (Mid-West Seed Service Inc., Brookings, SD).

An additional 3000 seeds per selection were subjected to germination tests of five treatments (four replications of 100 seeds per treatment in incubation conditions and two replications of 100 seeds per treatment in greenhouse conditions) for 60 days. Cleaned seeds were treated with one of five possible treatments:

1. Bleached (20% for 5 min), soaked overnight, dusted with captan (50 wp)
2. Bleached (20% for 5 min), soaked overnight, soaked 5 min in physan (1.85 ml/950 ml)
3. Bleached (20% for 5 min), soaked overnight
4. Bleached (20% for 5 min), soaked overnight, soaked 5 min in physan (1.85 ml/950 ml), treated with KNO₃ (1000 ppm) and Gibberellic Acid 3 (500 ppm)
5. Bleached (20% for 5 min), acid scarify (18M sulfuric acid), soak overnight

It is noteworthy to mention that all seeds, including the control, were bleached as preliminary research determined that bleaching yielded higher germination percentages than not bleaching. For bleaching, cleaned seeds were treated with 0.60% sodium hypochlorite for 5 min, rinsed 3 times with nanopure water, and soaked overnight in aerated water. After all treatments, floating seeds were discarded. Four replications of 100 seeds per selection were placed in a 10.9 X 10.9-cm transparent polystyrene germination boxes (Anchor Paper Company, St. Paul, MN) containing 2 sheets of germination paper (Anchor Paper Company) moistened with 15 mL nanopure water. Preliminary research determined that this substrate yielded the highest germination percentage for *Lantana* germination by incubator. Germination boxes were placed in temperature and light-controlled chambers equipped with cool-white fluorescent lamps (Model 818; Precision Scientific, Winchester, VA). Germination boxes were placed in 25/15 °C, as preliminary research determined these temperatures are most successful in *Lantana* germination. The photoperiod was administered by providing 12 h light at 25 °C (photosynthetic photon flux was 22 to 30 $\mu\text{mol m}^{-2}\text{s}^{-1}$ at shelf level followed by 12 h dark at 15 °C. Preliminary research determined that this is the most successful photoperiod for germination of *Lantana* seeds. Germination of seed was monitored every other day for a period of 60 days. An additional 5 mL to 10 mL of nanopure water was added to germination boxes as needed. A seed was considered germinated when radicle emergence was 2.0 mm or greater. Due to the polyembryonic tendencies of *Lantana spp.*, seeds were removed once germination occurred to prevent inaccurate data collection. Seed germination in the incubators was concurrent with seed germination in the greenhouse. Two replications of 100 seeds per selection were sown in propagation half flats (12 7/8" x 9 1/2") (Dillen Products, Middlefield, OH) in Fafard Superfine Germinating Mix. Half flats were placed under mist at 12 seconds/hour. Average maximum and minimum temperatures in the greenhouse were 36/19 °C (97/67 °F), respectively. A seed was considered germinated when a cotyledon was visible

above the surface of the growth medium. Due to the polyembryonic tendencies of *Lantana spp.*, germinated seeds were marked with a toothpick once germination occurred to prevent inaccurate data collection.

At the end of the germination period, final germination percentage (FGP) and T50 (days to 50% of FGP) were determined per replication. Percentage data were transformed by a sqrt arcsine prior to conducting an analysis of variance (ANOVA) within selection. Transformed means were separated by a Duncan's multiple range test, $P=0.05$. Untransformed selection means are presented in tables.

Results and Discussion

Seed viability and germination

Not every selection responded similarly to all treatments or growing conditions. Final germination percentage and T-50 was calculated for all treatment and germination methods for each selection. Table 2 below displays the results of this study. At the completion of the 60-day study period, 'Rock Road' and 'Pink Caprice' selections had significantly higher germination percentages with all treatments (excluding the acid treatment for 'Pink Caprice') than *L. depressa*. For 'Rock Road', the Physan treatment (45.5% germination) and the KNO_3 + GA treatment (31.5% germination) were most successful in the greenhouse and the control (38.75% germination) and the KNO_3 + GA treatment (36.75% germination) were most successful in the incubator. For 'Pink Caprice', the control (52.5% germination) and the KNO_3 + GA treatment (46% germination) were most successful in the greenhouse and the KNO_3 + GA treatment (49% germination) and the control (43.25% germination) were most successful in the incubator. The acid treatment had the least germination percentage for all selections, with 0.25% and 0% for *L. depressa* in the incubator and greenhouse, respectively; 13.5% and 8.5% for 'Rock Road' in the incubator and greenhouse, respectively; and 0.5% and 0% for 'Pink Caprice' in the incubator and greenhouse, respectively (Table 2). 'Rock Road' had slightly overall higher germination rates than 'Pink Caprice', but both selections had significantly higher germination rates than *L. depressa*, leading to the speculation that *L. depressa* has dormancy issues that affect germination that are not found in *L. camara*. Further germination studies are currently being conducted with *L. depressa* to overcome these dormancy issues, including cold scarification treatment and ambient germination of viable seeds.

Table 1: Percent viability and germination of Lantana selections.

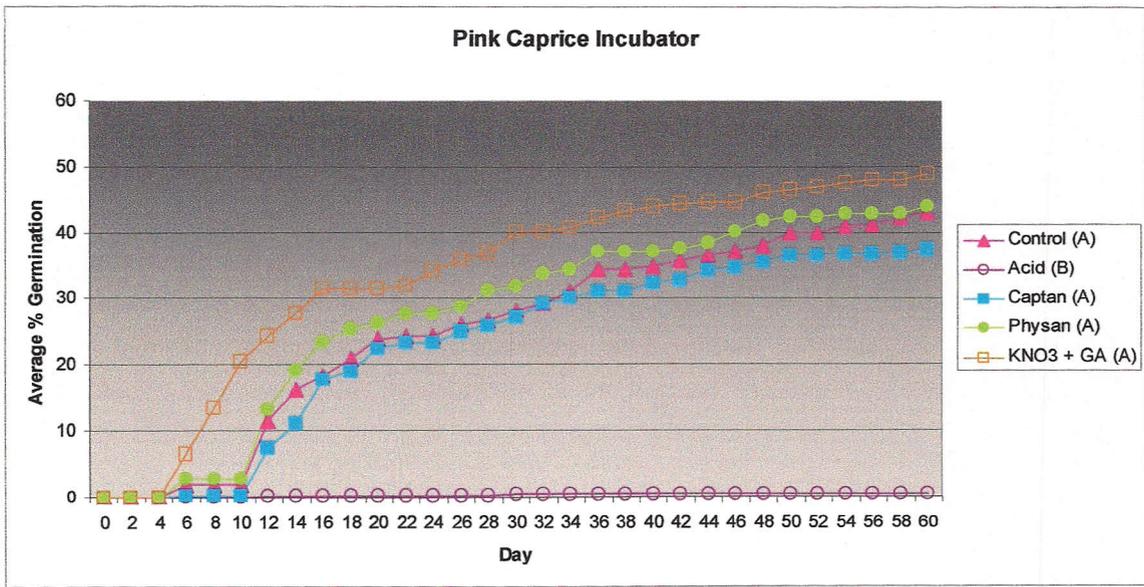
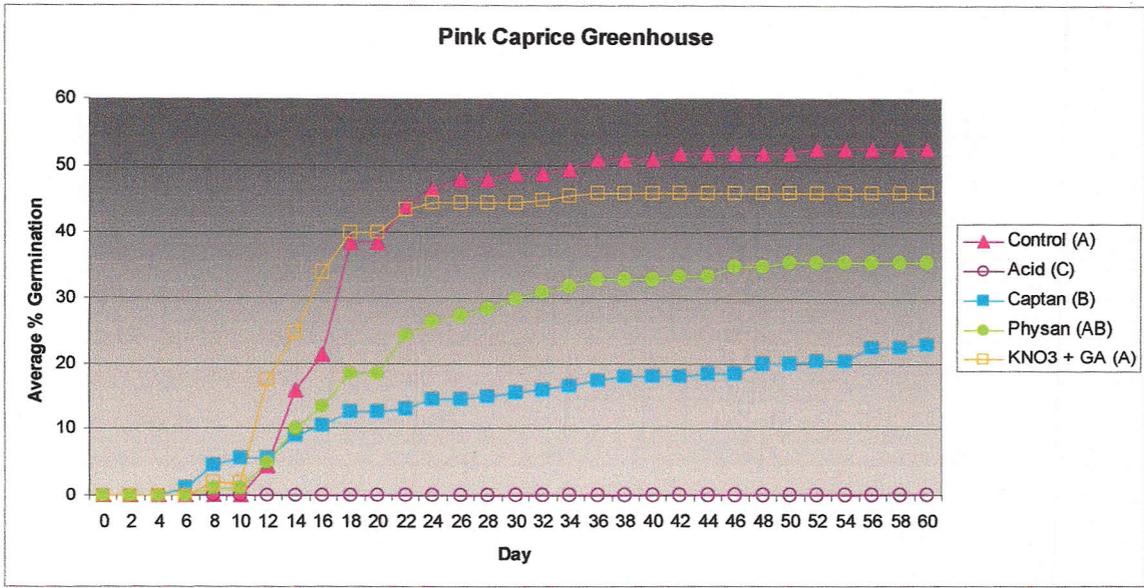
| Botanical Name | Pre-germination Viability (%) | Germination (%) | Dormant (%) | Total Viable (%) | Germination of Viable Seed (%) |
|--------------------------------------|-------------------------------|-----------------|-------------|------------------|--------------------------------|
| <i>Lantana depressa</i> | 59 | 0 | 59 | 59 | 0 |
| <i>Lantana camara</i> 'Rock Road' | 44 | 14 | 30 | 44 | 32 |
| <i>Lantana camara</i> 'Pink Caprice' | 76 | 4 | 72 | 76 | 6 |

Table 2: Percent germination and T-50 for germination treatments of Lantana selections in incubator and greenhouse.

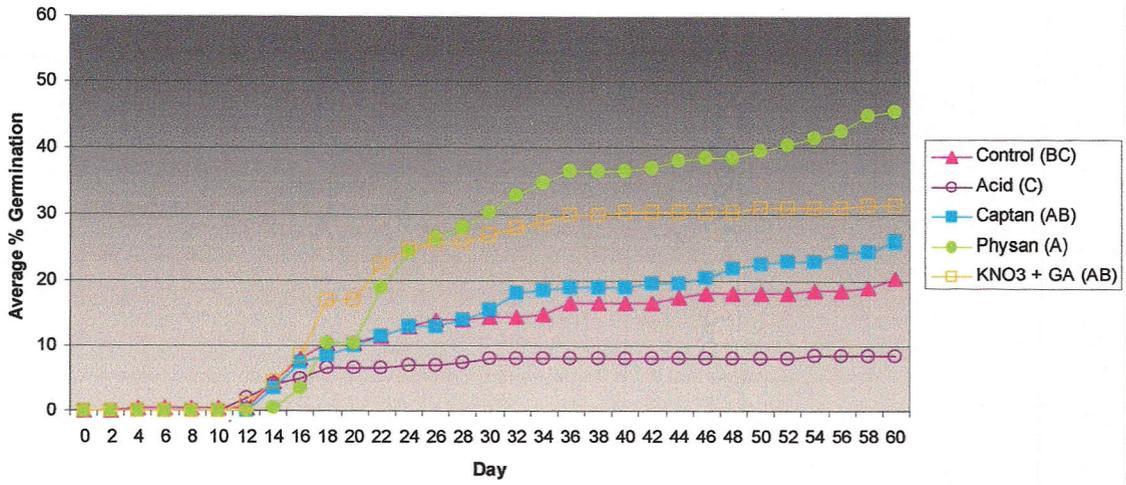
| | | INCUBATOR | | | GREENHOUSE | | |
|-------------|-----------|------------------|-----------|--------------|------------------|-----------|------------------|
| | | Depressa | Rock Road | Pink Caprice | Depressa | Rock Road | Pink Caprice |
| Germination | Control | 0.25 A | 38.75 A | 43.25 A | 5.50 A | 20.50 BC | 52.50 A |
| | Acid | 0.25 A | 13.50 C | 0.50 B | 0.00 B | 8.50 C | 0.00 C |
| | Captan | 0.25 A | 22.75 BC | 37.50 A | 0.50 B | 26.00 AB | 23.00 B |
| | Physan | 0.00 A | 30.75 AB | 44.00 A | 4.00 A | 45.50 A | 35.50 AB |
| | KNO3 + GA | 1.00 A | 36.75 A | 49.00 A | 6.00 A | 31.50 AB | 46.00 A |
| LSD (0.05) | | 0.08 | 0.13 | 0.12 | 0.14 | 0.25 | 0.21 |
| T50 | Control | 37.00 | 19.25 BC | 21.50 A | 42.00 A | 19.00 AB | 17.50 A |
| | Acid | 7.00 | 17.75 C | 21.00 A | --- ^y | 14.00 B | --- ^y |
| | Captan | 26.00 | 25.75 AB | 18.50 A | 53.00 A | 25.00 A | 21.00 A |
| | Physan | --- ^y | 25.25 AB | 18.00 A | 35.00 A | 23.50 A | 20.00 A |
| | KNO3 + GA | 41.00 | 29.25 A | 13.75 A | 56.00 A | 20.00 AB | 14.00 A |
| LSD (0.05) | | --- ^z | 6.71 | 3.33 | 41.07 | 6.45 | 14.19 |

---^y T50 could not be calculated due to lack of germination

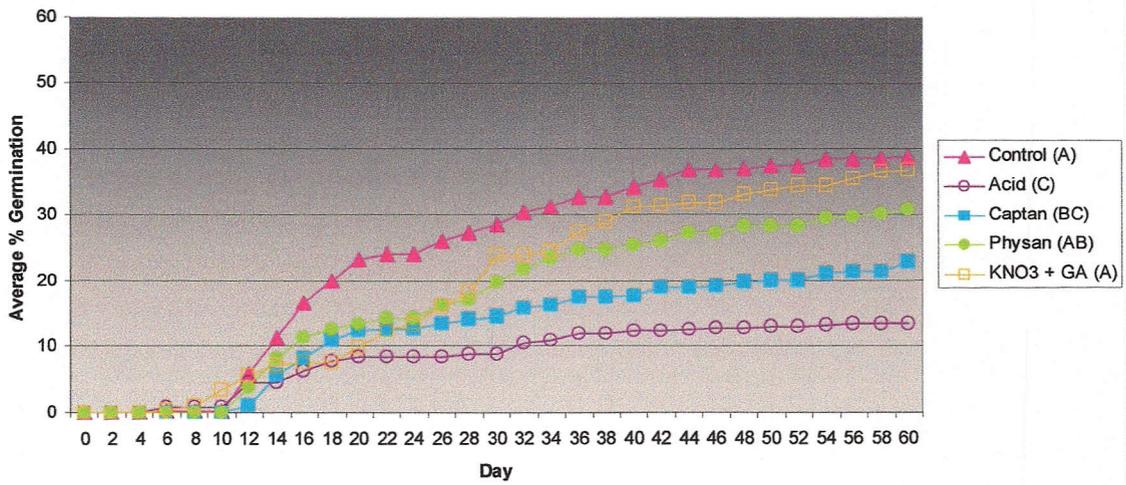
---^z LSD could not be calculated due to insufficient number of data points



Rock Road Greenhouse



Rock Road Incubator



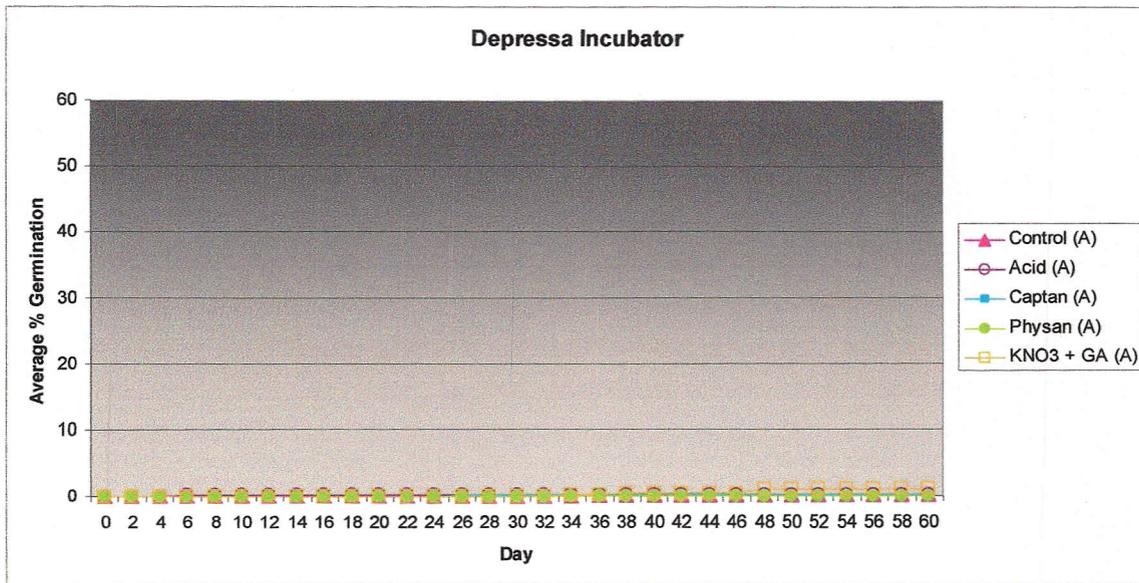
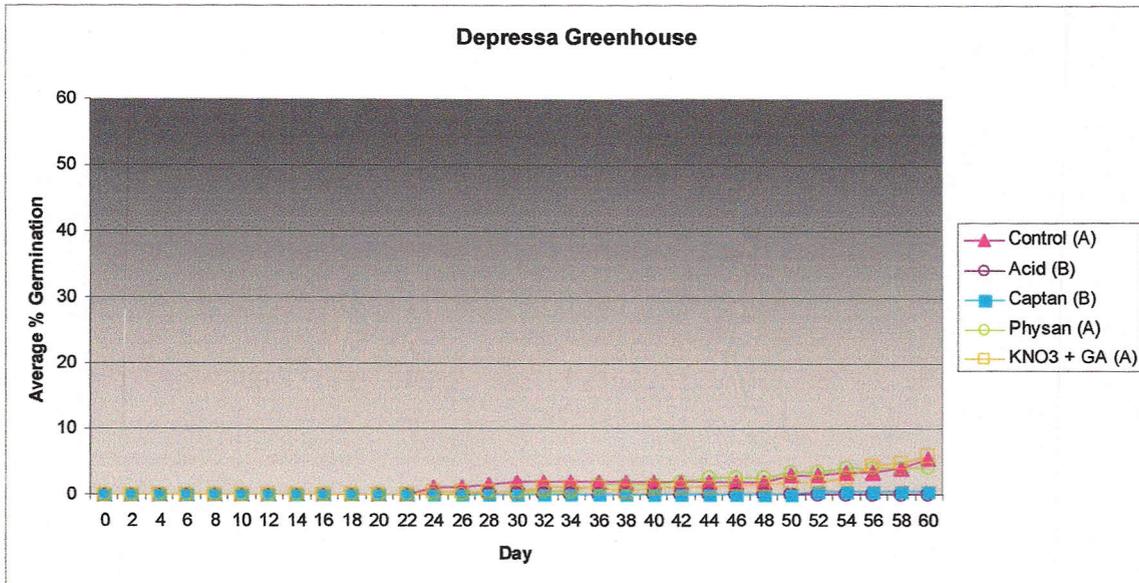


Figure 6: Daily germination of seed of three Lantana selections and five treatments in incubators with light (12 hour photoperiod) at 25/15 °C for 60 days and parallel greenhouse studies (36/19 °C max/min).

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