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Seed Viability Assay for Tropical Soda Apple Treated with Weed Terminator 20 Herbicide & Boost

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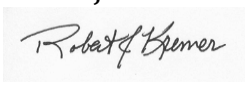

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Objective: Determine effects of Weed Terminator 20 Herbicide & Boost applied as weed management practice on seed viability parameters of tropical soda apple.

Samples of tropical soda apple (TSA) fruits from Georgia consisting of three treatments were submitted for viability assays.

Treatments:
Control - labeled as "No Spray"
One Spray - labeled as "One Spraying 10 days after"
Two Spray - labeled as "Second Spray 10 days after"

Condition of fruits and seeds:
Control: Fruits green and firm, some with light brown colored spots; dissection with scalpel revealed four chambers with numerous seeds, light brown to tan color, in a mucilaginous coating and occurring in clusters within each chamber
"One Spray": Fruits - some green to yellow, some dark brown to black and much softer than control; dissection revealed similar pattern of seed distribution; seeds dark brown coloration and little mucigel (less sticky than control)
"Two Spray": very similar to "One Spray"

Assay methods: Sterile petri plates fitted with sterile germination paper; germination paper moistened with sterile distilled water (20-25 mL per plate): One TSA fruit used per assay run; fruit dissected in half using a flame-sterilized scalpel; seeds were randomly selected from the dissected fruit using a flame-sterilized forceps; with the forceps, individual seeds were placed equidistantly on the moistened germination paper at 30 seeds per plate. For each treatment, the 30 seeds per plate was considered one replicate, 8 replicates per treatment were run for each assay.

Very little information was available on proper seed testing methods for TSA, which required several preliminary trials to determine the optimum germination conditions that yielded consistent and least variable results. We discovered that TSA seed require about 14 days incubation at ambient laboratory temperatures (65 to 72°F) for adequate germination thus preliminary, repeated testing continued during February through March time period. The final protocol used is detailed later in the report. Results shown in the Table are compiled from two complete trials in which data did not statistically differ and verifies that the protocol developed for this study yields consistent results.

Notes on measured seed viability components and comparing treatments:

Germination: TSA seed were considered germinated when the radicle (seedling root) protruded 2 mm from the seed
Hard seed: Non-germinated TSA seed that remained firm upon pressure applied when squeezed with a forceps. Hard seed may be considered dormant but viable and germination is delayed likely due to a hard seedcoat, some innate inhibitor and/or environmental condition.
Total Viable Seed: The sum of germinated seed + hard seed
Non-viable seed: Non-germinated TSA seed that were imbibed with soft seedcoats and released contents when squeezed with a forceps; often the contents were undergoing decomposition
Fungal-infected seed: Seed showing observable fungal growth on surface or from contents of seed or on germinating seed; most often associated with "non-viable seed" component.

Comparing treatments: Statistics are used to account for variability inherent in all experiments and aid in determining the *real* effect of treatment. The statistical tool used in this study is the "least significant difference" (LSD) and is used to show a significant difference between two treatments when the difference between them is greater than the LSD value; differences between two treatments that are smaller than the LSD are considered not to be significant. A significance level of 0.001 (used in this study) is the probability that the differences are real and not due to chance. Therefore, if treatment 1 differs from treatment 2 by the LSD value or greater, we are 99.9% certain that the treatments were indeed different.

Table 1. Effects of Weed Terminator 20 Herbicide & Boost spray applications on in vitro seed viability of tropical soda apple seed.

	Seed Classification (%)				
Treatment	**Germinated**	**Hard Seed**	**Total Viable**	**Non-viable**	**Fungal-infected**
Control	97.5	2.5	100.0	0.0	3.3
Spray One	4.6	7.5	12.0	87.9	88.3
Spray Two	8.0	13.3	21.3	78.8	73.3
LSD (0.001)	2.72	3.35	4.71	4.70	4.80

Summary: Weed Terminator 20 Herbicide & Boost treatments were highly significant in reducing TSA seed germination and total seed viability and increasing non-viable seed. The high increase in fungal-infected seed, which were predominantly non-viable, suggests that the herbicide increased seed vulnerability to attack and infection by opportunistic fungi that contributed to the great decrease in seed viability and germination. The predominant fungi noted on the seeds included typical opportunistic seed pathogens including *Alternaria, Curvularia, Cladosporium*, and *Fusarium*. The mechanism involved in the herbicide ability to increase seed susceptibility to fungal attack is unknown and the pathway used would require further study. The 80 to 88 % reduction in seed viability by Weed Terminator20 treatments may reduce subsequent TSA infestations in the field and continued annual treatment of TSA escapes should significantly reduce the TSA seedbank in soil, as this appears to be main source of TSA infestations.

It may be expected that the two-spray treatment would decrease TSA seed viability proportionately more than the one spray treatment. However, this was not observed based on the assays. In fact, the one spray treatment showed significantly greater effects than the two spray. These treatment differences were consistent throughout this study beginning with the preliminary bioassays. To determine the bases for the difference in treatment effects may require more investigation into application timing, sample timing, TSA physiology, etc. Examination of the seed bioassay results does not explain the apparent difference between the application treatments.

Supplementary Information.

Protocol developed for tropical soda apple (TSA) seed viability assay.

1. Aseptically dissect TSA fruit, remove seeds and place in sterile petri plate.

2. Transfer TSA seeds to moistened germination paper discs fitted into sterile petri dishes.

3. Incubate seeded plates in the dark for 3 to 5 days at ambient lab temperature (65 to 72°F).

4. After dark incubation, re-moisten germination paper as needed and incubate plates in ambient lab light and temperature for 7 days.

5. After the 7-day incubation, re-moisten germination paper, move plates to a window sill and incubate in sunlight for 2 to 4 days.

6. Terminate assay after a total of 14 days. Will yield optimum germination and all seed viability components can be measured and recorded.