# Effect of Nutrients and Smoke Solutions on Seed Germination and Seedling Growth of Tropical Soda Apple (*Solanum viarum*)

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*Solanum viarum*, commonly known as tropical soda apple (TSA), is native to Brazil and Argentina but has become a harmful weed in many countries with tropical climates. This study was conducted to reassess the seed biology of TSA found in South Africa. Cold stratification (14 d), acid scarification (20% $H_2SO_4$ for 5 min), and sandpaper scarification (30 s) significantly improved percentage germination when compared to the control. The highest germination (99.5%) was achieved when seeds were germinated in 50% Hoagland's nutrient solution (HS). The lowest germination (66%) was recorded in the absence of phosphorus (P) under alternating light conditions. HS without nitrogen (N) completely inhibited seed germination of TSA under constant light conditions. These findings are useful in controlling TSA by amending the levels of N and P in soils. Seed germination of TSA was significantly enhanced by different concentrations of smoke-water and butenolide solution. Smoke-water dilution of 1:500 v/v and butenolide concentration of $10^{-8}$M showed the highest seedling vigor indices (6,688 and 6,666, respectively) in comparison to the control (1,251) and gibberellic acid ($GA_3$) concentrations ($< 5,327$). These findings suggest that germination of seeds or seedbanks of TSA might be successfully stimulated using smoke solutions. Subsequently, patches of seedlings emerging after treatment can be mechanically uprooted to reduce the infestation of TSA. However, justifying this with field trials is essential.
**Nomenclature:** Tropical Soda Apple; *Solanum viarum* Dunal SOLVI.
**Key words:** Seed germination, seedling growth, nutrient, smoke-water, butenolide.

TSA belongs to the family Solanaceae. It is a stout, highly branched, woody shrub growing up to 2 m high and 1.5 m wide. TSA is native to Brazil, Paraguay, Uruguay, and Argentina and has become a common weed in South America, North America, India, and Africa (Mullahey 1996). Over 400,000 ha of pasture land are estimated to be infested with TSA in Florida, and this has become a major concern of agriculture (Bryson et al. 1995; Medal et al. 2002). Due to its rapid and widespread occurrence in agriculture fields, TSA is placed on the federal noxious weed list in the United States (Bryson et al. 1995). It hosts several plant diseases causing economic losses to the vegetable crop industry (Duncan 2005; McGovern et al. 1994). One of the primary modes of TSA dispersal is through livestock or wildlife that eats mature fruits and scatter seeds by means of their feces (Brown et al. 1996; Coile 1993; Mullahey 1996). This weed is a prolific seed producer, with a single plant producing on average 125 fruits with more than 50,000 seeds (Mullahey et al. 1993; Weber 2003). It is reported that fruit production may increase when the levels of phosphate in soils are high (Call and Coble 1998). Seeds are small (2.2 to 2.8 mm in diameter), light red-brown to brown, and covered with a gelatinous layer. Seeds of TSA may have a short duration of dormancy. According to the seed information database of the Royal Botanic Gardens, Kew, 100% germination of TSA can be obtained at 33/19 C under 12-h light and dark cycle, respectively, using 1% agar as a medium. Germination may be stimulated by scarification and can be sensitive to temperature, light, pH, moisture, and ripening of fruits. Germination of TSA seeds is epigeal.

TSA commonly occurs in pastures, ditch banks, citrus groves, sugar cane fields, and wet areas of rangeland (Mullahey and Colvin 1996). Interestingly, TSA also infests fire-maintained communities of mesic flatwoods (Waggy

2009). It is suggested that postfire establishment of TSA may take place by vegetative regeneration or seed germination induced by heat scarification. TSA may sprout from adventitious buds after low intensity fire if the stem apex is not damaged. There are possibilities that seedlings are established from residual sources. However, to date there is no documentation on fire adaptations of TSA or its ability to establish after fire and therefore more information is needed before considering prescribed fire for its control (Waggy 2009). Recently, smoke generated from vegetation fires and biological active compounds isolated from smoke (Chiwocha et al. 2009; Flematti et al. 2004; Van Staden et al. 2004) has been widely studied and used in enhancing seed germination and seedling growth of a wide range of agricultural (Van Staden et al. 2006) and horticultural (Kulkarni et al. 2007, 2008) crops. Smoke also has stimulatory effects on germination and seedlings of a number of weed species (Daws et al. 2007, 2008). Smoke-water promoted germination of arable weed species; wild oat (*Avena fatua* L.), sterile oat (*A. sterilis* L.), common mallow (*Malva neglecta* Wallr.), common speedwell (*Veronica persica* Poir.), catchweed bedstraw (*Galium aparine* L.), prostrate knotweed (*Polygonum persicaria* L.), Pennsylvania smartweed (*P. pensylvanicum* L.), and black bindweed [*Fallopia convolvulus* (L.) A. Love] (Adkins and Peters 2001). However, the intensity of response differed with the species.

TSA is difficult to control due to its large seed production, extensive root system, and tendency of forming large patches. Cultural, mechanical, biological, and chemical methods are adopted to control this weed (Waggy 2009). A better understanding of the role of major nutrients and smoke may help to develop some strategies for control measures. Major objectives of this study were (1) to assess the effects under presence or absence of nitrogen, phosphorus, and potassium (NPK) and (2) to evaluate the effect of smoke solutions on seed germination and seedling growth of TSA. At the same time, the effects of fundamental dormancy breaking techniques such as scarification and stratification were also evaluated.

## Materials and Methods

**Seed Collection.** Dry fruits of TSA were collected between June and July 2009 from Ukulinga Farm, University of KwaZulu-Natal Pietermaritzburg, South Africa. Dried yellow fruits (2.8 to 3.2 cm in diameter) were left in trays to dehisce naturally. Seeds were collected from the trays. The mucilage covering of dried seeds were removed by wetting the seeds and gently rubbing them with coarse sand. Subsequently, the sand adhering mucilage was washed off using sieves. The seeds were dried with the help of paper toweling and stored in a plastic container for 2 mo at room temperature (25 ± 0.5 C) before using for the experiments.

**Seed Viability.** Viability was determined using 0.5% (w/v) tetrazolium salt[1] (TTC) (2, 3, 5-triphenyl tetrazolium chloride) solution. Four replicates (100 seeds) of freshly harvested seeds were counted randomly and soaked in TTC solution in the dark for 24 h at 25 C. Subsequently, each seed was dissected and a completely red stained embryo was considered a viable seed (Freeland 1976). This test was also applied to nongerminated seeds of acid scarification and cold stratification treatments.

**Seed Moisture Content and Imbibition.** Moisture content of seeds was determined by drying the seeds at 110 C in a preset incubator until the weight was stable after 7 d. Each replicate consisted of 100 seeds with four replications. In the imbibition study, four replications of 25 seeds each were placed in 9-cm disposable Petri dishes lined with two filter paper discs (Whatman No. 1) moistened with 4.5 ml distilled water and allowed to imbibe at room temperature (25 ± 0.5 C). The increase in seed weight was determined after 2, 4, 6, 8, 10, 12, and 24 h. Seeds were blotted dry, weighed, and returned to the moist filter papers. The amount of water imbibed by the seeds is represented as the percentage increase over the initial seed weight.

**Germination Protocol.** Seeds of TSA were surface decontaminated with 0.2% mercuric chloride[2] (HgCl$_2$) for 2 min, followed by rinsing with distilled water (three times). Four replications of 50 seeds were placed on two sheets of Whatman No. 1 filter paper in a 9-cm Petri dish (for all experiments mentioned below). The filter paper was moistened with 4.5 ml distilled water or test solutions. The filter paper was kept moist throughout the experiment by adding water or test solutions. The Petri dishes were placed in a plant growth chamber[3] and subjected to 16-h photoperiod with a photosynthetic photon flux density of 112 ± 5 µmol m$^{-2}$ s$^{-1}$ at 25 ± 2 C unless otherwise stated. Germination was monitored every day for 60 d. Seeds were considered germinated when the radicle had emerged 2 mm. At the end of the 60 d, the seedlings were removed from the Petri dishes and blotted with the help of filter paper before measuring and weighing. Mean germination time (MGT) was calculated by using the following equation: MGT = $\Sigma$ $(n \times d)/N$, where $n$ = number of seeds germinated on each day, $d$ = number of days from the beginning of the test, and $N$ = total number of seeds germinated at the end of the experiment (Kochankov et al. 1998). The germination rate was calculated by a modified Timson index as: Germination rate (% d$^{-1}$) = $\Sigma$ $[(G^1/t) + (G^2/t) \ldots + (G^t/t)]$ where $G$ is the percentage of seed germination at 1-d intervals, and $t$ is the total number of days of the germination period (Easton and Kleindorfer 2009). Seedling vigor index (SVI) was calculated as: SVI = [average shoot length (mm) + average root length (mm)] × percentage germination (Dhindwal et al. 1991). This study was repeated three times to confirm the results.

The seeds used in this study showed a significantly lower germination (< 17%) at normal room temperature (25 C) even though they showed high viability in the TTC test. Generally fresh seeds of TSA are viable but in some cases, they may stay dormant (Akanda et al. 1996; Mullahey 1996; Suryawanshi et al. 2001). A temperature of 25 C is generally suitable for germination of many weed species from warmer regions and was therefore selected. This study was conducted on fresh seeds after short storage with only 60 d of germination testing. Prolonged germination periods could have resulted in a higher percentage germination of control. However, to assess the exact temperature gradient and after-ripening process for seed germination of South African TSA, a separate study is required.

**Mechanical and Chemical Scarification.** Prior to the germination test, the seeds were gently scarified between two sheets of sandpaper (P120 grade). To examine the effect of chemical scarification, the seeds were soaked in 20% sulfuric acid[4] (H$_2$SO$_4$) (v/v) for 5, 10, and 15 min. The acid-treated seeds were rinsed in running tap water and subsequently with distilled water before incubating them (Rodrigues et al. 1990). Unscarified seeds were used as a control. To examine the effect of washing, the seeds were washed in running tap water for 5, 10, and 15 min. Seeds were blotted with a paper towel and air dried for 3 h before the germination test.

**Cold Stratification.** Seeds were placed between two sheets of paper toweling moistened with distilled water inside plastic bags and stored in the dark at 5 C for 7, 14, 21, and 28 d, respectively. After the desired period of cold stratification, germination tests were performed as described above. Seeds that were not subjected to cold stratification served as a control.

**Nutrient Solution.** In this study half-strength (50%) Hoagland's HS (Hoagland and Snyder 1933) was used for seed treatment. The effects of three macronutrients N, P, and K were studied by eliminating each one of these from half-strength HS. The concentration of main source of N, P, and K was 253, 20.4, and 96.4 g l$^{-1}$ respectively. Hoagland's recipe was used to eliminate the sources of N, P, and K. Stock solutions were prepared using macro- and micro-nutrient chemicals. Nitrogen source was eliminated with no addition of calcium nitrate [Ca(NO$_3$)$_2$] and potassium nitrate (KNO$_3$) replacing it with calcium chloride (CaCl$_2$) and potassium chloride (KCl) at the rate of 110.27 and 55.9 g l$^{-1}$, respectively. Phosphorus source was eliminated with no addition of potassium dihydrogen orthophosphate (KH$_2$PO$_4$) replacing it with KCl at the rate of 55.9 g l$^{-1}$. Potassium source was eliminated with no addition of KNO$_3$ and KH$_2$PO$_4$ replacing it with sodium nitrate (NaNO$_3$) and sodium dihydrogen phosphate (NaH$_2$PO$_4$) at the rate of 63.74 and 20.69 g l$^{-1}$. Each treatment was represented as

Table 1. Effect of scarification and washing treatments on seed germination of tropical soda apple under a 16-h photoperiod at 25 C for 60 d.[a]

| Treatment | Germination | Germination rate | MGT[b] |
|---|---|---|---|
| | % | % d$^{-1}$ | d |
| Control | 17 ± 8.5 d | 0.22 ± 0.13 c | 49.5 ± 2.6 bc |
| 20% sulfuric acid (min) | | | |
| 5 | 73 ± 3.7 a | 1.59 ± 0.07 a | 53.0 ± 1.5 b |
| 10 | 65 ± 15.4 ab | 1.23 ± 0.41 ab | 52.5 ± 2.9 b |
| 15 | 19 ± 10.0 d | 0.15 ± 0.03 c | 59.4 ± 0.3 a |
| Sandpaper (30 s) | 42 ± 3.8 bc | 0.77 ± 0.27 bc | 52.9 ± 2.4 b |
| Washing (min) | | | |
| 5 | 14 ± 2.0 d | 0.27 ± 0.04 c | 52.0 ± 2.6 b |
| 10 | 36 ± 3.7 cd | 0.73 ± 0.10 bc | 49.7 ± 1.8 bc |
| 15 | 30 ± 3.7 cd | 0.68 ± 0.08 bc | 45.0 ± 0.8 c |

[a] Significant differences are indicated in each column by different letter(s) according to Duncan's multiple range test ($P < 0.05$); standard error (±); ($n = 12$).
[b] Abbreviation: MGT, mean germination time.

−N, −P, and −K. This experiment was conducted under constant and alternating light (16-h photoperiod) conditions at 25 C.

**Smoke Solutions and GA₃.** GA$_3$[5] was tested at concentrations of $10^{-4}$, $10^{-5}$, and $10^{-6}$M. For preparing smoke extract, dry *Themeda triandra* Forssk. (Poaceae) leaf material (5 kg) was burnt in a 20-L metal drum and the smoke generated from it was passed through a glass column containing 500 ml of tap water for 45 min (Baxter et al. 1994). The three different concentrations of smoke-water used in this study were prepared by diluting 1 ml of smoke-extract with 250, 500, and 1,000 ml distilled water (1:250, 1:500, and 1:1,000 v/v). A pure butenolide (3-methyl-2*H*-furo[2,3-*c*]pyran-2-one) used in this experiment was isolated from plant-derived smoke-water as described by Van Staden et al. (2004). The concentrations of the butenolide tested were $10^{-7}$, $10^{-8}$, and $10^{-9}$M, respectively.

**Statistical Analysis.** The germination data of each treatment were arcsine transformed and one-way ANOVA was conducted. All data were analyzed using GENSTAT[6] statistical package (Release 11.1). Duncan's multiple range test at 5% level ($P < 0.05$) was used to separate differences between means of treatments. Values presented in tables and used for figures are untransformed.

## Results and Discussion

**Seed Viability, Moisture Content, and Imbibition.** Seeds of TSA tested with TTC solution showed 96% viability after 2 mo of storage with a mean moisture content of 9.4%. Imbibition of water by the seeds was fast between 2 and 4 h and thereafter increased gradually.

**Mechanical and Chemical Scarification.** The seeds which were subjected to acid treatment for 5 min exhibited an increase in percentage germination and germination rate in comparison to other treatments (Table 1). Mechanical scarification of seeds with sandpaper showed significantly higher percentage germination than the control. Washing treatments for 10 and 15 min showed some improvement in

Table 2. Effect of cold stratification (stored moist in the dark at 5 C) on seed germination of tropical soda apple under a 16-h photoperiod at 25 C for 60 d.[a]

| Stratification period (d) | Germination | Germination rate | MGT[b] |
|---|---|---|---|
| | % | % d$^{-1}$ | d |
| 0 | 17 ± 8.5 b | 0.22 ± 0.13 c | 49.5 ± 2.6 ab |
| 7 | 37 ± 4.3 a | 0.99 ± 0.14 b | 43.2 ± 3.8 ab |
| 14 | 40 ± 5.9 a | 0.82 ± 0.21 b | 52.0 ± 0.7 a |
| 21 | 24 ± 4.9 b | 0.71 ± 0.09 bc | 40.2 ± 3.2 b |
| 28 | 26 ± 7.3 b | 1.96 ± 0.23 a | 29.9 ± 4.3 c |

[a] Significant differences are indicated in each column by different letter(s) according to Duncan's multiple range test ($P < 0.05$); standard error (±); ($n = 12$).
[b] Abbreviation: MGT, mean germination time.

germination although this was not significantly different to the control. In this study, control seeds showed very low percentage germination (Table 1). This experiment was conducted at 25 C with 16-h photoperiod (Akanda et al. 1996), and probably this temperature was not optimal for germination of TSA found in South African climatic conditions. However, acid and mechanical scarification did improve germination at this temperature. As documented by Waggy (2009), seed germination of TSA may be stimulated by scarification and can be sensitive to changes in temperature, light, pH, moisture availability, and ripeness of fruit. Acid scarification for 15 min was detrimental to the seeds of TSA compared to the shorter duration-treated seeds (Table 1). The TTC test of nongerminated seeds from acid treatments (5, 10, and 15 min) showed loss of viability (23, 31, and 77%, respectively). On the other hand, control seeds did not show any loss of seed viability. This can be attributed to injury caused to the embryo, which may have affected percentage germination (Akanda et al. 1996).

**Cold Stratification.** The seeds of TSA exposed to cold stratification for 7 and 14 d showed a significantly greater percentage germination and rate than the nonstratified seeds (Table 2). However, MGT did not significantly change. There was no decline in viability of nongerminated seeds after 28 d of cold stratification. In South Africa, seeds of TSA get exposed to winters (natural cold stratification), which may allow them to improve germination. In black nightshade (*Solanum nigrum* L.) and hairy nightshade (*S. physalifolium* Rusby), low winter temperature helped in releasing dormancy while high temperature was responsible in imposing dormancy (Taab and Andersson 2009).

**Nutrient Study.** In comparison to the solutions of HS without N, P, or K and control (water), the maximum percentage germination, germination rate, and lowest MGT were achieved when the seeds of TSA were germinated with HS under alternating light conditions (Table 3). This result indicates that seeds of TSA may be stimulated in nutrient-rich soils. It was reported that growth and fruiting occur rapidly when nutrients are sufficient in soils (Trenholm et al. 1995). In the present study, percentage germination and germination rate were significantly decreased in the absence of P with an increase in MGT compared to HS and the absence of N and K from HS (Table 3). This clearly suggests that P plays an important role in germination of TSA. Albin (1994) and Call and Coble (1998) indicated that TSA can thrive in soils that are rich in P. In the present study, higher values were recorded

Table 3. Effect of nutrient solution (HS) in the absence of N, P, or K (i.e., −N, −P, or −K) on seed germination and seedling growth of tropical soda apple under alternating and constant light conditions (16- and 24-h photoperiod, respectively) at 25 C for 60 d.[a]

| Treatment | Germination | Germination rate | MGT[b] | Root length | Shoot length | Seedling weight |
|---|---|---|---|---|---|---|
| | % | % d$^{-1}$ | d | mm | mm | mg |
| Alternating light | | | | | | |
| Control (water) | 26 ± 0.5 c | 0.74 ± 0.5 d | 35.3 ± 6.40 a | 53.6 ± 3.3 a | 20.8 ± 1.0 b | 18.4 ± 0.7 b |
| HS | 99 ± 0.5 a | 8.7 ± 0.1 a | 11.5 ± 0.09 c | 58.4 ± 4.0 a | 27.0 ± 1.4 b | 21.5 ± 1.0 b |
| −N | 87 ± 4.4 a | 6.8 ± 0.3 b | 12.7 ± 0.11 c | 37.6 ± 2.0 b | 25.4 ± 1.8 b | 19.6 ± 0.6 b |
| −P | 66 ± 12.0 b | 3.0 ± 0.7 c | 23.5 ± 1.84 b | 60.4 ± 5.3 a | 33.8 ± 1.9 a | 32.7 ± 1.7 a |
| −K | 93 ± 1.3 a | 6.5 ± 0.5 b | 14.9 ± 1.11 c | 51.8 ± 2.2 a | 18.6 ± 1.5 b | 19.2 ± 1.3 b |
| Constant light | | | | | | |
| Control (water) | 21 ± 0.7 b | 0.63 ± 0.7 b | 37 .4 ± 2.30 a | 49.2 ± 2.2 ab | 28.4 ± 1.5 b | 17.6 ± 0.9 b |
| HS | 90 ± 1.4 a | 3.8 ± 0.2 a | 25.5 ± 1.6 b | 53.2 ± 3.5 ab | 44.6 ± 1.9 a | 25.9 ± 1.3 a |
| −N | 0 ± 0 c | 0 ± 0 b | 0 ± 0 d | 0 ± 0 c | 0 ± 0 d | 0 ± 0 c |
| −P | 87 ± 0.9 a | 3.2 ± 0.1 a | 27.9 ± 0.7 b | 45.5 ± 2.7 b | 36.7 ± 2.2 b | 19.1 ± 1.2 ab |
| −K | 79 ± 11.5 a | 4.3 ± 0.7 a | 19.1 ± 1.1 c | 62.1 ± 5.1 a | 32.7 ± 1.6 b | 22.1 ± 0.9 a |

[a] Significant differences are indicated in each column and light condition by different letter(s) according to Duncan's multiple range test ($P < 0.05$); standard error (±); ($n = 12$).

[b] Abbreviation: MGT, mean germination time.

for root/shoot length and seedling weight in the absence of P compared to other treatments. When there was no P, seed germination was suppressed but not seedling growth. It is documented that this weed species can invade on phosphate-mine reclamation sites (Albin 1994) and with increasing levels of P in soils (Waggy 2009), which may be due to its ability to promote germination. In greenhouse experiments, it was shown that increasing P levels increased plant height and fruit production (Call and Coble 1998). SVI was significantly greater for HS in comparison to either −N, −P, or −K and control (water) under alternating light conditions (Figure 1). This suggests that TSA can establish more readily in nutrient-rich soil than in soil lacking N, K, or P. In this study an interesting phenomenon was observed when the seeds were germinated without N under constant light conditions: seed germination of TSA was completely inhibited (Table 3). Finch-Savage et al. (2007) has also reported similar inhibitory effects with *Arabidopsis thaliana* (L.) Heynh. seeds with low nitrogen under constant light. This implies that interaction of N and light has a significant role that needs to be further



Figure 1. Effect of 50% Hoagland's nutrient solution (HS) and HS without N, P, or K (i.e., −N, −P, or −K) on seedling vigor index of tropical soda apple under alternating and constant light conditions (16- and 24-h photoperiod, respectively) at 25 C for 60 d. C = control (water). Standard error bars of each light condition with different letter(s) are significantly different according to Duncan's multiple range test ($P < 0.05$) ($n = 12$).

investigated. A low emergence of seed on the soil surface (long exposure of seeds to light) is attributed to dry conditions (Mullahey and Cornell 1994), but based on the findings of this study it can be also due to low levels of N.

**Smoke Solutions and GA$_3$.** All concentrations of smoke-water and butenolide and a lower concentration of GA$_3$ ($10^{-6}$M) examined showed significantly higher percentage germination compared to the control (Table 4). Smoke-water and butenolide tested at all concentrations significantly increased the germination rate and reduced MGT over the control with some exceptions (Table 4). Smoke-water (1:500 v/v) and GA$_3$ ($10^{-6}$M) significantly improved root length, whereas the same smoke-water concentration and $10^{-4}$M GA$_3$ solution showed significantly greater shoot length compared to the control and other treatments. Maximum seedling weight was achieved at $10^{-4}$M GA$_3$, $10^{-9}$M butenolide, and smoke-water dilution of 1:250 v/v, which was significantly different from the control (Table 4). The highest SVI was calculated for smoke-water dilution of 1:500 v/v followed by butenolide concentrations of $10^{-8}$ and $10^{-9}$M and these were significantly different from the control and GA$_3$ treatments (Figure 2).

It is suggested that postfire establishment of TSA may occur by vegetative regeneration or by seeds that may favor its infestation (Waggy 2009). To date there is no clarification on postfire establishment of TSA. In this study, the smoke components (smoke-water and smoke-isolated butenolide) clearly enhanced seed germination as well as seedling vigor of TSA. It is reported that smoke-water or butenolide may act like, or interact with, other plant hormones, which results in releasing dormancy and promoting seed germination of many plant species (Kulkarni et al. 2006; Light et al. 2009; Van Staden et al. 2000). A plant hormone GA$_3$ was therefore tested to compare the effects of smoke components. The results showed that smoke-water and butenolide solutions in most cases were more effective than GA$_3$ in promoting germination and seedling vigor. Daws et al. (2007) reported that butenolide was effective in enhancing percentage germination, rate, and seedling mass of many arable weed species. Adkins and Peters (2001) tested a wide range of arable weed species from northern Europe and subtropical regions where germination responses to smoke differed. Some species positively responded to smoke, while
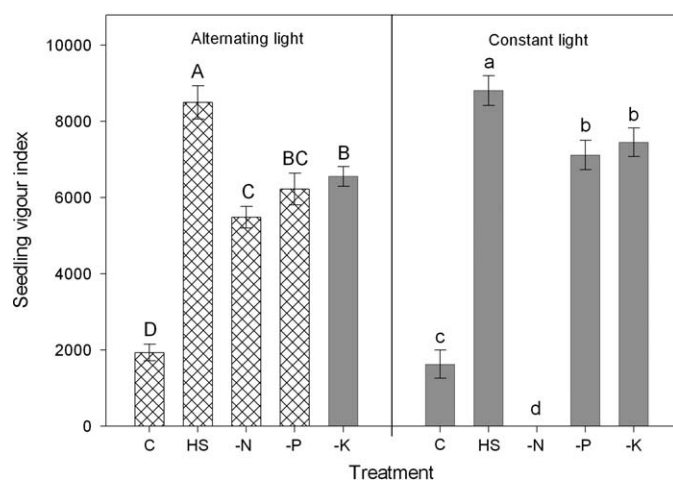
Table 4. Effect of smoke and gibberellic acid (GA$_3$) solutions on seed germination and seedling growth of tropical soda apple under a 16-h photoperiod at 25 C for 60 d.[a]

| Treatment | Germination | Germination rate | MGT[b] | Root length | Shoot length | Seedling weight |
|---|---|---|---|---|---|---|
| | % | % d$^{-1}$ | d | mm | mm | mg |
| Control | 17 ± 8.5 c | 0.22 ± 0.13 e | 49.5 ± 2.6 a | 50.6 ± 5.5 c | 16.8 ± 1.0 b | 13.4 ± 0.5 b |
| Smoke-water (1:250 v/v) | 63 ± 13.5 ab | 1.78 ± 0.42 abc | 38.7 ± 2.20 b | 54.8 ± 5.1 bc | 17.4 ± 1.3 b | 16.5 ± 3.0 a |
| Smoke-water (1:500 v/v) | 71 ± 5.5 ab | 1.59 ± 0.11 abcd | 48.2 ± 1.97 a | 71.5 ± 4.7 a | 25.7 ± 0.9 a | 13.1 ± 0.5 b |
| Smoke-water 1:1,000 v/v) | 84 ± 8.2 a | 2.06 ± 0.26 ab | 42.3 ± 1.72 b | 57.0 ± 6.0 abc | 17.4 ± 1.0 b | 12.6 ± 0.4 b |
| Butenolide (10$^{-7}$M) | 73 ± 17.4 ab | 1.90 ± 0.43 ab | 40.9 ± 1.93 b | 50.4 ± 4.2 c | 17.8 ± 1.0 b | 15.5 ± 0.6 ab |
| Butenolide (10$^{-8}$M) | 91 ± 4.1 a | 2.28 ± 0.16 a | 40.9 ± 1.72 b | 53.6 ± 3.7 c | 20.4 ± 1.4 b | 12.7 ± 0.5 b |
| Butenolide (10$^{-9}$M) | 86 ± 12.5 a | 2.05 ± 0.37 ab | 40.1 ± 1.63 b | 56.2 ± 6.6 abc | 19.7 ± 0.6 b | 17.0 ± 0.4 a |
| GA$_3$ (10$^{-4}$M) | 43 ± 16.8 bc | 0.88 ± 0.37 cde | 51.2 ± 1.11 a | 58.2 ± 2.9 abc | 24.9 ± 2.5 a | 17.7 ± 0.7 a |
| GA$_3$ (10$^{-5}$M) | 41 ± 2.5 bc | 0.78 ± 0.08 de | 52.2 ± 2.30 a | 66.0 ± 5.1 abc | 19.7 ± 1.2 b | 13.5 ± 0.6 b |
| GA$_3$ (10$^{-6}$M) | 60 ± 16.8 b | 1.13 ± 0.33 bcde | 50.5 ± 2.67 a | 70.2 ± 5.5 ab | 19.2 ± 1.0 b | 12.3 ± 0.7 b |

[a] Significant differences are indicated in each column by different letter(s) according to Duncan's multiple range test ($P < 0.05$); standard error (±); ($n = 12$).
[b] Abbreviation: MGT, mean germination time.

other species had no or even an inhibitory effect. In their study, the members of Poaceae were more smoke-responsive than other tested families. Plants from Solanaceae investigated so far have shown positive responses to smoke (Ahmed et al. 2006; Kulkarni et al. 2008). The results of this study show that TSA, a member of the Solanaceae family, responded positively to smoke.

This study has shown that even though there was lower germination at 25 C, when these seeds were treated with either nutrients or smoke components, germination was promoted. These treatments can therefore be very useful for management practices. TSA is a serious weed and many control measures such as mechanical, chemical, and biological are used to curb its infestation. In effective weed control strategies, eliminating seeds and soil seed banks of TSA is one of the main priorities (Mullahey 1996). The findings of this study suggest that proper soil amendment with N, P, and K can be useful in promoting or suppressing seed germination of TSA. More importantly, application of smoke-water and butenolide solutions can be a useful strategy to stimulate seed germination and seedling growth of TSA. The patches of seedlings that emerge after smoke treatments can be mechanically uprooted during their early developmental stages, which can certainly help in reducing the spread of TSA. The application of smoke liquids is easy, economical, and nontoxic to soils and crops unlike commercial herbicides. Field trials using these techniques are now required.

## Sources of Materials

## Acknowledgments

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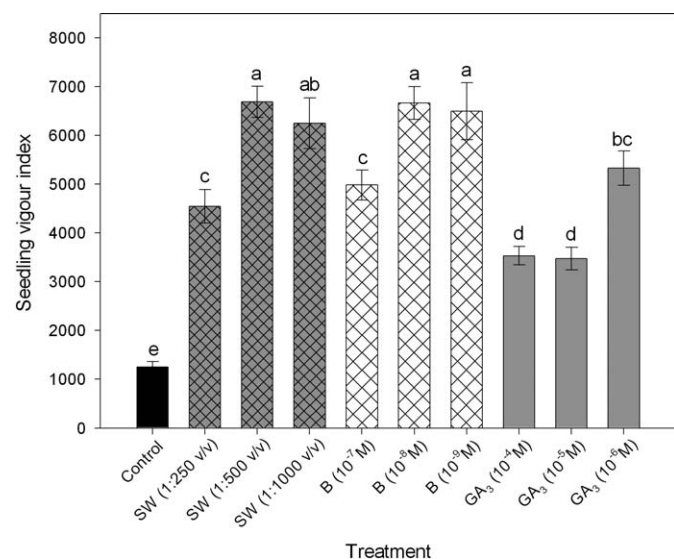
Figure 2. Effect of smoke (SW = smoke-water; B = butenolide) and gibberellic acid solutions on seedling vigor index of tropical soda apple under a 16-h photoperiod at 25 C for 60 d. Standard error bars of each light condition with different letter(s) are significantly different according to Duncan's multiple range test ($P < 0.05$) ($n = 12$).

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