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Induction of Germination of Impermeable Cottonseed by Electrical Treatment[1]

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ABSTRACT

Electric glow-discharge and radiofrequency (RF) electric-field treatments were studied for inducing germination of impermeable cottonseed in selection 16-B-7 of *Gossypium hirsutum* L. Seed was exposed for 3 min in an evacuated glass tube chamber to the glow discharge established by application of 60-Hz and 15- and 17.5-kHz voltages to electrodes in the tube. Germination of impermeable cottonseed was increased from less than 10% to levels in the 60 to 90% range. Significant increases in germination were also achieved by exposure of seed to 40-MHz electric fields of intensities ranging from 1.3 to 3.1 kilovolts/cm, but germination was not generally increased as much as by glow-discharge treatments. RF treatments increased field emergence of a sawginned seed lot from 50 to 76%, but glow-discharge treatments gave no significant increase for this seed lot. Glow-discharge treatments offer a possible method for overcoming impermeability, but variation in cultivar response and seed storage conditions in relation to treatment needs further evaluation.

Additional index words: Seed treatment, Seed dormancy, Germination.

SEED dormancy, because of a water impermeable testa, is common in many wild species and some domestic stocks of *Gossypium*. The condition serves to preserve seed of wild cottons from season to season. Utilization of the heritable character has been proposed (2, 3, 4) and evidence given for its value in preserving domestic cottonseed from field or storage deterioration. If impermeable seeds are to be used, a method is required for rendering planting seed permeable. Walhood (12) developed an effective hot-water treatment for cottonseed. Legume seeds with impermeable coats are made permeable in several ways. Nelson and Walker (7) reported enhanced germination of alfalfa (*Medicago sativa* L.) and red clover (*Trifolium pratense* L.) by radio-frequency (RF) electrical treatments. Nelson et al. (6) found that RF, infrared, and glow-discharge electrical treatments were all about equally effective for reducing hard-seed levels in alfalfa. Davis (5) reported increased germination of impermeable cottonseed by storage at high humidity. Mechanical abrasion is commonly used with some commercial crop seed but is not effective for cottonseed. Cutting is commonly used by researchers to overcome impermeability in small quantities of cottonseed, but no commercially adaptable method is currently available for dry treatment of large lots. Thus, the objective of these experiments was to determine whether radiofrequency and glow-discharge treatments might favorably influence the germination of impermeable cottonseed.

These investigations were conducted between 1963 and 1967. This paper presents the results of several experiments with different kinds of RF and glow-discharge electrical treatments studied for improvement of germination of impermeable cottonseed.

MATERIALS AND METHODS

The cottonseed lots used were of selection 16-B-7 from the species *Gossypium hirsutum* L., which normally produces seed with an impermeable testa. Gin-run seeds, grown in 1963 at Stoneville, Miss., were selected by M. N. Christiansen for Experiments 1 and 2 conducted in 1964. In 1965, a lot of sawginned seeds grown at Stoneville during 1964 was used for field emergence tests (Experiment 3), but its impermeable seed content was relatively low (50%).

Seed for the 1967 tests (Experiments 4 and 5) was selected by Dr. R. J. Merrivalle from cotton grown in 1966 on the USDA-ARS Plant Science Research Division cotton farm near Knoxville, Tenn. The small quantity of cotton available was ginned with a small laboratory roller gin, and the seeds were stored in a desiccator with Drierite[3] (anhydrous $CaSO_4$) desiccant.

Seeds were treated in an electric glow discharge by placing them in a single-seed layer in a horizontal borosilicate glass tube with an internal diameter of 48 mm. Both ends of the tube were sealed with rubber stoppers through which tubular electrodes entered the tube. The electrodes, spaced 46 cm apart, were connected electrically to a high-voltage source. One electrode was connected through a rubber line to the vacuum system, with the other electrode connected to a needle valve used to regulate air pressure in the tube.

The tube containing the seeds was evacuated to a pressure of 3 mm Hg before voltage was applied to the electrodes, causing ionization of the gases in the tube. Potential from the voltage source was adjusted to obtain the desired current through the glow-discharge tube. Two high-voltage sources were used, one operating at a frequency of 60 Hz and another at 15 or 17.5 kHz. More detailed descriptions of the equipment have been reported (10, 11, 13).

Seeds were exposed to RF electric fields by placing them in polystyrene boxes between parallel-plate electrodes of an RF oscillator as previously described (8, 9). Dielectric properties of the cottonseed were measured and moisture content was determined by oven drying for 15 hr at 101 C. Seed dielectric properties, RF electrode voltage measurement, and geometrical data provided the basis for calculated field intensity as a treatment parameter (8). Seed mass temperatures in the boxes were determined at the end of treatment. RF treatment methods, measurements, and equipment are described elsewhere (1, 8, 9).

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[3] Mention of specific trade names is for purpose of identification and does not imply endorsement by the U. S. Government.

Germination tests for Experiments 1 and 2 were conducted at Stoneville. Miss. Twenty five seeds were placed between two 28- by 46-cm germination papers moistened with distilled water. Eight 25-seed replications were used. The germination paper was rolled with a waxed paper outer covering and incubated at 31 C. Germination counts were made after 6 days. Controls consisted of (i) nontreated seeds and (ii) seeds with a cut seedcoat. For Experiment 3, conducted in 1965, seeds were field planted at Knoxville, Tenn. in a randomized complete block design with four replications, each consisting of 30 hills with four seeds per hill. Emergence was counted at 11 and 20 days after planting.

The 1967 germination tests, Experiments 4 and 5, were also conducted at Knoxville, Tenn., using germination-grade blotting pads, and the temperature was alternated daily, 30 C for 8 hr and 20 C for 16 hr. Four replications of 50 seeds each were used for each treatment. Criterion for seed germination was emergence and continuing development of the radicle.

The entire lot of seed for each test was shipped to Lincoln, Neb., where the RF treatments were provided. The seeds were then returned to Knoxville, Tenn., for the glow-discharge treatments. The remaining untreated seeds were used for controls. Seed lots were packaged in polyethylene bags to prevent changes in moisture content during shipment. Moisture contents at time of treatment and treatment data for the three seed lots used in the experiments are summarized in Table 1.

Germination data for each test were subjected to a two-way classification analysis of variance, and Duncan's Multiple Range Test was employed for separation of means.

RESULTS AND DISCUSSION

Variation in response among seed lots and other uncontrolled variables prevent the drawing of clear-cut conclusions that might have been expected for some comparisons in these studies (Table 1) but several observations are apparent in the resulting data and some general conclusions can be drawn.

For Experiments 1 and 2, seed viability was high as evidenced by germination when seedcoats of individual seeds were cut before testing, but germination of untreated seeds was very low because of seedcoat impermeability (Tables 2 and 3). In these two experiments electrical treatments were administered to samples from the same seed lot about 2 months apart, late in February and early in May. In both experiments glow-discharge treatments provided substantial increases in germination. The best treatment in Experiment 1 (Table 2) was a 15-kHz, 120-ma glow-discharge treatment for 3 min, which resulted in 81% germination compared to 58% for the best 60-Hz treatment, which had a current level of 160 ma. This apparent superiority of the 15-kHz treatment was not borne out, however, in Experiment 2, where the two different frequencies produced the same results (Table 3).

Exposure to RF electric fields in Experiment 1 yielded significant increases in germination, with 28% germination resulting from an unmodulated or continuous-wave treatment at 43 MHz and 1.3 kV/cm (Table 2). Similar increases were achieved in Experiment 1 using pulse-modulated RF treatments. Germination increases obtained by similar continuous-wave and pulsed RF treatments in Experiment 2 (Table 3), while statistically significant, were not as great as those achieved in Experiment 1.

Effects of electrical treatment on field emergence of saw-ginned cottonseed used in 1965 are shown in Table 4. There were fewer impermeable seeds in this lot, and 50% emergence was obtained for the untreated control. Glow-discharge treatments did not increase the field emergence for this seed lot, but significant increases were observed for RF-treated samples. Shorter exposures were required for equivalent heating of the seeds compared to Experiment 2 because the seed moisture content was higher. Overexposure to either RF or glow-discharge treatment will lower seed germination.

Table 1. Summary of experiments on glow-discharge and radiofrequency electrical treatments of selection 16-B-7 impermeable cottonseed.

Seed lot	Exp. no.	Date treated	Moisture content	% germination: Control	% germination, Best treatment: Glow-discharge	% germination, Best treatment: Radio-frequency
1963 gin-run	Preliminary	1/64	6.6	5	84	29
	1	2/64	6.1	8	81	28
	2	5/64	6.5	6	64	17
1964 saw-ginned	3	4/65	8.6	50	52	76
1966 roller-ginned	4	2/67	-	0	88	-
	5	3/67	-	1	81	6

Table 2. Experiment 1. Germination of impermeable cottonseed after glow-discharge and radiofrequency electrical treatments.

Type	Frequency	Current	Field intensity	Exposure	Seed mass temperature	Germination*
		ma	kV/cm		C	Pct.
Control (seed-coat cut)						92 a
Control						6 f
Glow-discharge	60 Hz	160		3 min		58 b
		180				27 c
	15 kHz	120				81 a
		140				62 b
Radiofrequency (continuous)	43 MHz		13	15.0 sec	62	10 ef
				20.0	71	24 cd
				22.5	79	28 c
				25.0	85	26 c
				27.5	89	20 cd
				30.0	109	12 ef
(pulsed)†	43 MHz		2.6	60.0	66	9 ef
				70.0	72	10 ef
				80.0	81	12 ef
				90.0	88	21 cd
				100.0	96	18 de
				110.0	97	10 ef

* Germination after 6 days. Means followed by a common letter are not significantly different at the 5% probability level. † Pulse-modulated at 10 pulses per second with 10-millisecond pulse width.

Table 3. Experiment 2. Germination of gin-run impermeable cottonseed after glow-discharge and radiofrequency electrical treatments.

Type	Frequency	Current	Field intensity	Exposure	Seed mass temperature	Germination*
		ma	kV/cm		C	Pct.
Control (seed-coat cut)						98 a
Control						6 e
Glow-discharge	60 Hz	80		3 min		64 b
		100				62 b
		160				59 b
		180				50 c
	15 kHz	80				58 b
		120				59 b
		140				61 b
Radiofrequency (continuous)	40 MHz		1.5	12.0 sec	59	8 de
				13.5	66	17 d
				15.0	76	14 de
				16.5	73	15 d
				18.0	86	15 d
				19.5	97	11 de
(pulsed)†	40 MHz		3.1	22.0	68	7 e
				26.0	74	14 de
				30.0	82	14 de
				34.0	84	11 de
				38.0	92	8 de
				42.0	97	6 e

* Germination after 6 days. Means followed by a common letter are not significantly different at the 5% probability level. † Pulse-modulated at 10 pulses per second with 10-millisecond pulse width.

Table 4. Experiment 3. Field emergence of saw-ginned impermeable cottonseed after glow-discharge and radiofrequency electrical treatments.

Type	Treatment description: Frequency	Current	Field intensity	Exposure	Seed mass temperature	Emergence*: 11-day	20-day
		ma	kV/cm		C	Pct.	
Control						27 b	50 b
Glow-discharge	60 Hz	80		5 min		28 b	52 b
		160				18 c	28 c
	17.5 kHz	100				32 b	52 b
		120				34 b	50 b
Radiofrequency	40 MHz		1.6	8 sec	57	46 a	66 a
				10	63	46 a	71 a
				12	72	52 a	76 a
				14	82	54 a	74 a

* Averages of four replications of 120 seeds per replication. Means within a column followed by the same letter are not significantly different at the 5% probability level.

Desiccated storage of seeds harvested in 1966 maintained a high degree of impermeability in the seed lot (Tables 5 and 6). Glow-discharge treatments were highly effective for inducing germination when applied in February (Table 5) and also produced good increases when applied later in the spring for comparison with RF treatments (Table 6). Glow-discharge treatments were applied to seed samples both before and after seeds were shipped to Lincoln, Neb. Germination results for glow-discharge treatments in Table 6 are those for samples treated upon return to Knoxville, Tenn., but results of tests on samples treated before shipment agreed very well with those shown in Table 6. Seeds were in too short supply to sacrifice for oven moisture determinations, but the moisture content was very low because of the desiccated storage. Desiccant material was also enclosed when shipping the seeds. RF treatments did not provide significant increases in germination for this seed lot (Table 6).

CONCLUSIONS

Electric glow-discharge treatment was generally very effective for inducing germination in impermeable lots of gin-run and roller-ginned cottonseed of selection 16-B-7. In the same laboratory tests exposures to radiofrequency electric fields produced significant increases in germination, but to a much lesser degree than glow-discharge exposures. RF treatments increased field emergence in a moderately impermeable lot of saw-ginned cottonseed.

No differences were established in seed responses to glow discharges excited by 60 Hz and 15- or 17.5-kHz voltages that could be attributed to a difference in the frequency of the applied voltage. No advantage was obtained by pulse-modulating the RF oscillator as compared to continuous-wave or unmodulated operation in treating the cottonseed.

Electric glow-discharge treatments may provide a useful means for improving the germination of impermeable cottonseed, but the responses of additional seed lots and different cultivars need to be investigated. The influence on seed response of storage conditions between harvest and time of treatment also needs further study.

Table 5. Experiment 4. Germination of laboratory roller-ginned impermeable cottonseed after glow-discharge treatment for 3 min at 3 mm Hg pressure.

Treatment: Frequency	Current	Germination*
	ma	Pct.
Control		0
60 Hz	60	62 c
	80	73 bc
	100	88 a
	160	61 c
17.5 kHz	60	82 ab
	80	87 a
	100	86 a

* Germination after 7 days. Means followed by the same letter are not significantly different at the 5% probability level.

Table 6. Experiment 6. Germination of laboratory roller-ginned impermeable cottonseed after glow-discharge and radiofrequency electrical treatments. Seeds were stored with desiccant from harvest to time of treatment, a period of 5 months.

Type	Treatment description: Frequency	Current	Field intensity	Exposure	Seed mass temperature	Germination*
		ma	kV/cm		C	Pct.
Control (seedcoat cut)						81 a
Control						1 d
Glow-discharge	60 Hz	100		3 min		52 c
	17.5 kHz	100				62 b
Radiofrequency	39 MHz		1.6	15 sec	68	4 d
			2.2	12	86	6 d
			2.2	15	97	6 d

* Germination after 7 days. Means followed by the same letter are not significantly different at the 5% probability level.

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