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Effects of Heat and Moisture on Leguminous Seed

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THE ROLE OF FIRE in modifying ecosystems of which it is a part has been expounded by naturalists for many years. Some species of plants and animals, often referred to as fire species, apparently derive great benefit from occasional or frequent fires. The purpose of this paper is to explore possible mechanisms by which fire may benefit several species of leguminous plants through its direct effects on the seed. The work presented here is exploratory, although the effects of various treatments are quite pronounced.

SUMMARY OF PREVIOUS WORK

In general, there is little in the literature about the effect of fire or artificial sources of heat on the germination of native legume seed.

Early work on the effect of temperature on germination of seed was done by Edwards and Colin (1834). Jodin (1899) and Dixon (1902) were concerned with the resistance of seed to temperature changes and exposure to high temperatures for varying time periods. In 1893, Brown and Escombe conducted experiments to evaluate the effects of the temperature of liquid air on the germinating power of seed (Dixon 1902).

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Ewart (1908), working on the longevity of seed stored in the soil or air, listed by species seed of varying ages and the percent germination after a variety of treatments. He also cited de Candolle's work (1846) which was concerned with the germination of seed stored in dry air for 14 years. De Candolle found that 9 out of the 45 species of Leguminosae retained at least a feeble germinative power. Becquerel (1907) considerably extended de Candolle's work (Ewart 1908). He found that 18 out of 90 species of leguminous seed remained germinable for 25 to 80 years and that three species of Leguminosae were found to have germinable seed down to a depth of 1 foot in the soil. Ewart felt that the chief factor in longevity of seed was the inherent character of the seed itself, and that long-lived (macrobiotic) seed had been developed mainly among the Leguminosae. Also, that true macrobiotic seed, which were adapted for prolonged duration in the soil, always possessed a more or less impermeable seedcoat and that these seed would not germinate until the seedcoat had been softened. He cited Bergtheil and Day as having found that the hardness of seed of *Indigofera arrecta* (Leguminosae) was due to the presence of a very thin impermeable cuticle on the surface of the seed. When this cuticle was removed, the seed readily swelled. Ewart further stated that work by White, done in connection with his study, showed that in practically all cases hard seed possessed a cuticle and that the cuticle of different species varied in thickness. In addition, Ewart provided a list of macrobiotic seed of which 47 genera and 134 species belonged to the family Leguminosae. The appendix to Ewart's paper, provided by White, showed the cuticle thickness and time required to produce swelling of seed soaked in sulfuric acid. Also, White presented cuticle diagrams for a variety of species.

Whitcomb (1939) cited Nobbe (1876) as suggesting that the hard seed condition found in legumes was due to a waxy coating over the seed, agreeing with Ewart's theory.

Pammel (1899) made a detailed morphological study of seed from many genera of legumes, chiefly those covered in *Gray's Manual.* He outlined the structure throughout the seed and examined the testa or seedcoat. Pammel stated that the cuticle of many species of legume seed had the wellknown property of not allowing water to pass through or to pass only with difficulty because of its waxy or fatty nature. His data concerning the cuticle agree in general with that presented earlier by Ewart and Whitcomb.

Working with several species of *Medicago*, Schneider-Orelli (1909) found that alfalfa seed could withstand a temperature of 100° C for 17 hours, or 120° C for ½ hour; also, that a small number of these seed were able to withstand a boiling water treatment (98° C) for 7½ hours, or ½ hour in water maintained at 120° C in an autoclave.

Stoddard (1931) observed that germination of *Cassia nictitans* L. (Leguminosae) was hastened by either heating or scarifying the seed. Burning brush piles in fields where seed of this species were present usually resulted in dense stands of the plant. He also reported that Dr. E. H. Toole of the Bureau of Plant Industries heated seed of *Cassia nictitans* in boiling water for times of 5 seconds to several minutes. Germination gradually increased with time of heating up to 2 minutes, obtaining a maximum of 95 percent germination in 7 days.

Summaries of work done on seed morphology, dormancy, and germination have been published recently by Barton and Crocker (1948), Esau (1960), Barton (1961), and Mayer and Poljakoff-Mayber (1963). Levitt (1956) has summarized the work concerning the effects of temperature on plants. While all of these works provide excellent background information, none of them contain detailed information about the effects of fire or artificial heat on seed of native legumes. However, there is agreement in the literature concerning the longevity and condition of hard seed commonly found in several species of legumes which are of agronomic importance. But, because of the variation in results from germination test within a species, not to mention the variation between genera, no conclusive information covering the germination of legumes in general was found.

STATEMENT OF PROBLEM

A brief and simplified review of the field situation and possible effects of fire might be helpful. Under field conditions, seed of various species are stored in the litter and soil. Repeated fire favors repetitive abundant stands of many native legumes that are beneficial to wildlife (Stoddard 1961). Generally, burns conducted specifically for this purpose are done in open stands of slash and longleaf pine shortly after a rain, when the surface fuel will readily carry fire, and the lower fuel layers are still quite moist.

With this in mind, we might consider several possible effects of fire in bringing about regeneration of these legumes. First, the effect might be considered as a manipulation of growing conditions. This could be merely an improved seedbed, allowing the embryonic plant a better chance to germinate and to root in a favorable mineral soil. Secondly, the effect could be to change the environment of the seed or plant chemically. A third possibility could be changes in the biotic environment, either with other plants competing for light, nutrients, and water, in inhibiting factors exuded by other organisms, or in reduction of seed and seedling destruction by various organisms. A fourth possible effect could be the direct effects of fire on the seed—that is, changes in temperature and moisture conditions of the seed during the fire. In this last case, we consider the seed to have an inherent dormancy which will be directly affected by fire.

As previously mentioned, many viable legume seed do not germinate readily. This dormancy could be due to either physiological or physical factors. Generally, a physiological dormancy would be a factor within the embryo itself. A physical dormancy is most apt to be a factor within the seedcoat. A seed may have both physiological and physical dormancy, and it is possible that the embryo and seedcoat may be intermingled in dormancies.

Assuming that high temperatures during fires actually break dormancy or increase the rate of germination or overall germination, we would expect to find the type of curve illustrated in Figure 1. At low temperatures, a given species of seed would be expected to have a relatively constant germination percentage. As temperature increases, a range is finally encountered where dormancy factors are broken, resulting in an increase in germination percentage, the slope of the curve depending on the specificity of individual dormancy factors and the number of factors involved. Following this, as temperature continues to increase, a plateau of relatively constant high germination would be expected, and finally a rather sharp decrease until no germination whatsoever occurs due to lethal effects of high temperatures. Many of our laboratory experiments were designed to see if such

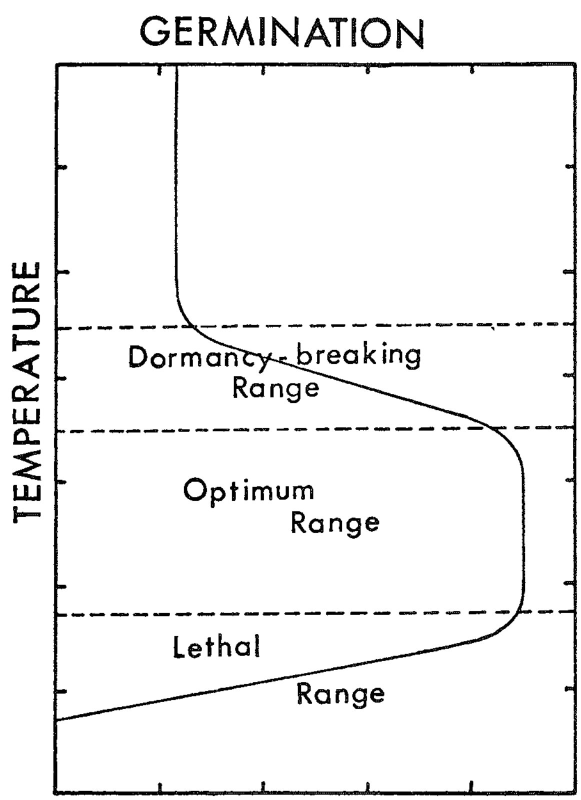


FIG. 1. Hypothetical curve of germination following exposure to high temperatures.

a curve exists for these seed. We might otherwise state our null hypothesis as—no increased germination occurs due to increasing temperature.

GERMINATION PROCEDURES

The procedures for treating seed varied throughout the study, depending on the type of experiment. Evaluation of germination, however, followed closely the specifications of the Association of Seed Analysts (1954). Their procedure is to place a batch of 100 seed between germination blotters moistened with distilled water, and to evaluate germination at 7, 14, 21, 28, and 33 days. The 33-

day evaluation has been recommended for legumes because of their general tendency toward dormancy. We varied from ASA procedures, however, in that we considered emergence of the hypocotyl from the seedcoat as germination, whereas they specify the emerging embryo shall be one that would develop into a normal seedling. Because of the very rapid germination following some treatments, we often counted seed germination at more frequent intervals.

EXPERIMENTATION AND RESULTS

Initially, we were concerned with obtaining information on germination with and without artificial scarification and on seed moisture contents. This work included lespedeza (*Lespedeza* spp.), partridge pea (*Cassia* spp.), beggarweed (*Desmodium* spp.), and milk-pea (*Glactia* spp.) seed. After preliminary high-temperature treatments of these seed, we concentrated our efforts on *Cassia nictitans* L. due to limited time and the many facets of the problem.

The first experiments evaluated germination of seed from several sources which had received no artificial treatment. These tests resulted in the following levels of germination:

Desmodium tortuosum (Commercial)	66 percent
Cassia fasciculata (Commercial)	16 percent
Cassia fasciculata (Native)	0 percent
Desmodium spp. (Native)	94 percent
Lespedeza spp. (Native)	5 percent

Following this, seed of native *Cassia fasciculata* and commercial *Cassia fasciculata*, *Desmodium tortuosum*, and *Lespedeza striata* were placed over saturated salt solutions giving relative humidities of 0, 43, and 93 percent. Those seed in the 93-percent relative humidity chamber molded rapidly. After 5 months, the seed at lower relative humidities were weighed and then ovendried. The results indicated a change in moisture content and thus at least a limited water vapor permeability of the seedcoat. In our early high temperature testing, we obtained moisture contents of partial allotments of seed prior to testing. Moisture contents ranged from 6.43 for *Glactia volubilis* to 7.83 for *Cassia nictitans*. Because little variation in moisture was evident, we did not continue this procedure.

The first testing of seed response to high temperatures was done using an oven which we modified to permit rapid insertion and removal of the seed without disturbing oven temperature. Exposures were made for 4 minutes at temperature intervals of 22° C (40° F) over the range of 38° C (100° F) to 216° C (420° F). Although the higher temperatures are rather extreme, observations from the literature indicate a high temperature tolerance of some seed. In these experiments we were looking for any increased cumulative germination or germination rate due to breaking dormancy, and for subsequent decreasing germination as the lethal temperature range of the seed was reached. The germination of all seed used in these tests indicated little response except in the temperature interval between 82° C and 104° C, where almost all germination ceased. Subsequently, we decreased the temperature interval to 5.5° C (10° F) and covered the temperature range of 82° C (180° F) to 104° C (220° F). Of the five seed sources, neither the commercial nor native *Cassia fasciculata* germinated, either with or without treatment (control). The response of *Cassia nictitans*, *Lespedeza striata*, and *Desmodium tortuosum* was varied (Fig. 2). Scarification of *Cassia nictitans* seed by standard scarification treatments of 30 minutes in water at 70° C, 25 minutes in concentrated sulfuric acid, and mechanical abrasion raised its average cumulative germination to 95, 99, and 92 percent, respectively.

From this point on we concentrated our work on *Cassia nictitans* because we felt that broader evaluation of the factors influencing germination of one species might give us a better chance to uncover some important relationships between fire and seed germination.

Because exposure of seed to high temperatures had failed to increase germination, we began looking for other possible effects of fire. The possibility of chemical effects due to the ashes or water leached through ashes presented a distinct possibility. Several beds of litter from a loblolly pine (*Pinus taeda* L.) stand were burned in the laboratory. The ashes were collected and pulverized and *Cassia nictitans* seed were germinated in the moist ashes and in water leached through the ashes. Average germinations were 27 percent and 23 percent in the ashes and leached water, respectively, compared to 58 percent for controls using distilled water.

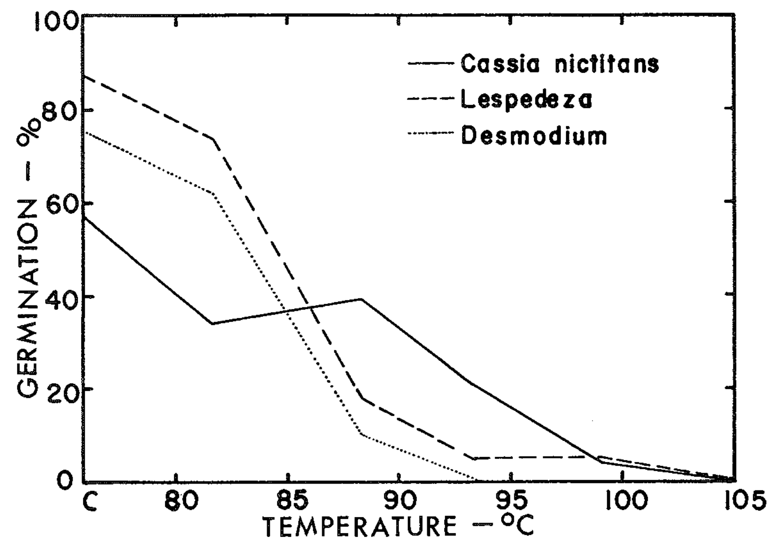


FIG. 2. Average cumulative 33-day germination of three species of seeds without treatment (C) and following 4-minute exposure to dry heat.

To this point we had failed with dry heat and with ashes to increase germination rate or cumulative germination. Hot water treatment, along with other scarification treatments, did increase germination. The next logical step was to treat seed in a moist atmosphere. One would expect that such a treatment might more nearly simulate conditions in a fire than would treatment with dry heat for two reasons: First, water is one of the major products of thermal degradation and combustion of woody fuels; and second, prescribed burning in the flatwoods is often done when the subfuels are fairly moist. Water vapor produced from either source could be expected to diffuse downward due to vapor pressure gradients and condense on cooler objects, such as seed.

Seed, contained in a wire basket, were inserted into the atmosphere above a constant temperature water bath. The atmosphere was maintained in a nearly saturated condition (100-percent relative humidity) by an insulated cover over the bath. Temperature of the atmosphere was monitored during the exposure period.

Results of the test on *Cassia nictitans* seed, stored under varying conditions in the laboratory approximately 2 years, indicated that the moist heat treatments were more effective in increasing germination than no heat (control) or the dry heat treatments (Fig. 3). *Cassia nictitans* seed collected in the fall of 1965 and treated with moist heat in January 1966 resulted in a high percentage germination (Fig. 4). Although temperature intervals varied (Figs. 3, 4), we compared results from germination test using fresh and seed 2 years old (Fig. 5). Differences in results possibly can be ascribed to population, physiological, or physical variations within the same species of seed. Note that the germination was more rapid for the older seed and also that newer seed respond to higher temperatures with more rapid and a higher percentage germination. When we treated the newer seed at a given temperature, in this case 80° C for 4 minutes, and then allowed varying times before moistening the seed, we found that germination rate increased with the length of the delay period, but that the cumulative germination was about the same (Fig. 6).

Time of exposure to 80° C in a saturated atmosphere has a pronounced effect on both rate and cumulative germination. Within the time range investigated, from 15 seconds to 16 minutes, germination increases with time to a certain degree (Fig. 7).

The experiments described have indicated a distinct difference between the effects of dry and moist heat on seed germination. This would indicate that either the particular dormancy factor involved is directly affected by moisture, or that the rate of heat transfer to the seed is quite significant. The latter possibility was considered by estimating the rate of temperature change of the seed. Calculations made by dimensional analysis indicated that the average temperature of the seed would be 99 percent of the difference between their original temperature and the temperature of the exposure medium in about 12 to 40 seconds. Exposure to the saturated atmosphere gave values for the 99-percent change of from 12 to 25 seconds. Dry heat with the estimated air circulation in the oven should have achieved the 99-percent temperature change in about 20 to 40 seconds. Although moist heat resulted in a temperature change of the seed in about one-half the time required by the dry heat, a total exposure time of 4 minutes was longer than the "heat up time" required by either moist or dry heat. Because a reduction in exposure time for

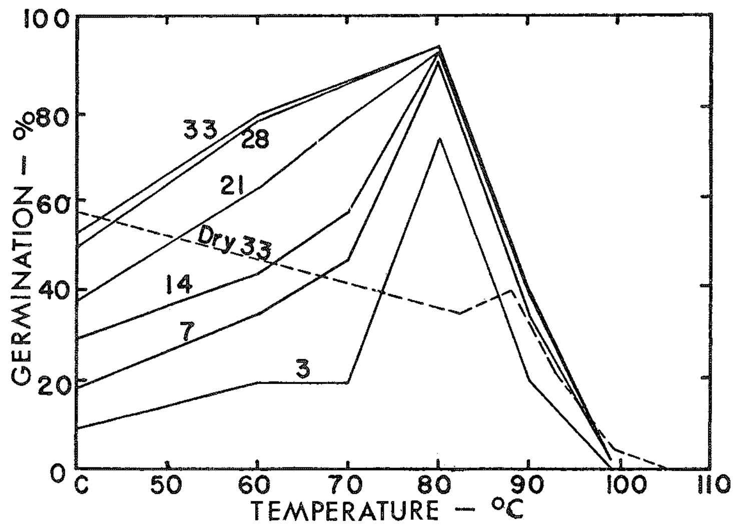


FIG. 3. Cumulative germination of 2-year-old *Cassia nictitans* seed without treatment (C) and following 4-minute exposure to dry and moist heat. Numerals on lines indicate number of days since moistened for germination.

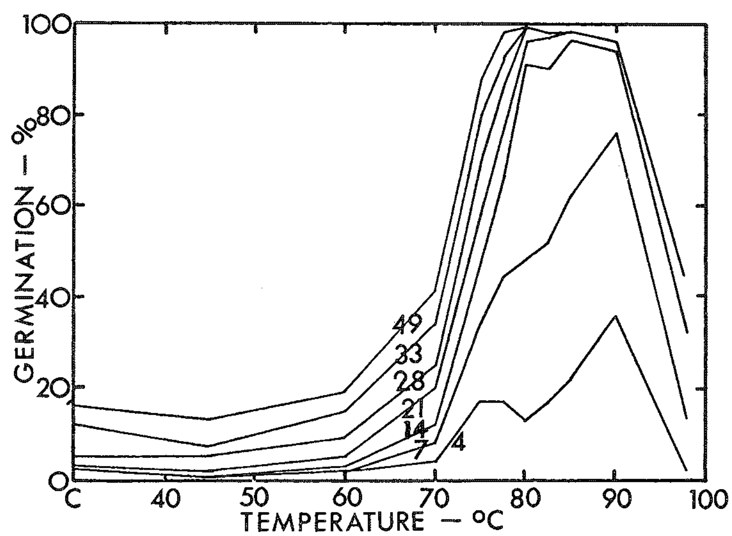


FIG. 4. Cumulative germination of newly collected seed without treatment (C) and following 4-minute exposure to moist heat. Numerals on lines indicate days since moistened for germination.

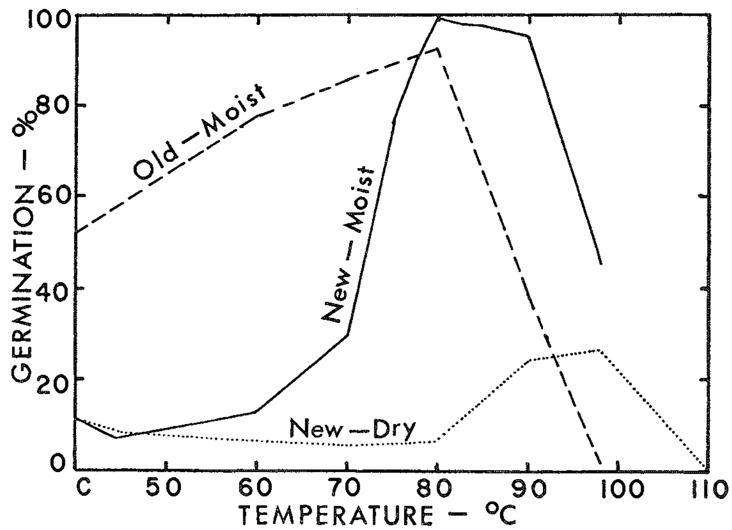


FIG. 5. Comparison of total 33-day germination of 2-year-old and new *Cassia nictitans* seed without treatment (C) and following 4-minute exposure to dry and moist heat.

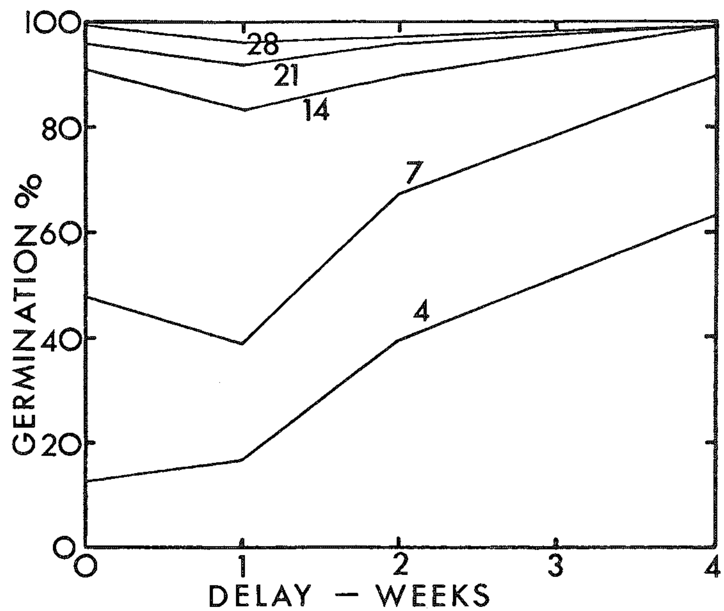


FIG. 6. Cumulative germination of new *Cassia nictitans* seed following 4-minute exposure to a moist atmosphere at 80° C and allowed to dry for varying times before germination. Numerals on lines indicate days since moistened for germination.

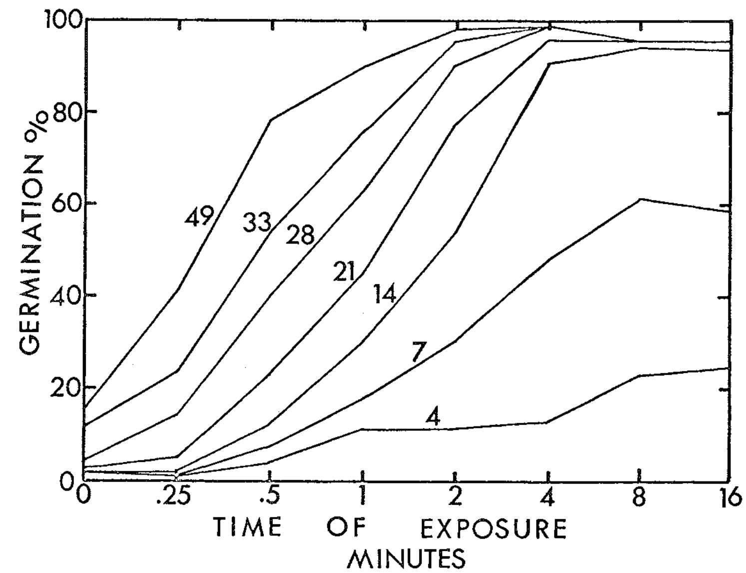


FIG. 7. Cumulative germination of new *Cassia nictitans* seed without treatment (C) and following exposure to moist heat at 80° C for varying times. Numerals on lines indicate days since moistened for germination.

moist heat to one-half minute resulted in much greater germination than seed which had not been treated (control), the difference between dry and moist heating rates would not appear to be significant.

Because these experiments indicated that the presence of water at elevated temperatures was important in affecting germination, there should be some evidence of its effect on the seedcoat. This we evaluated by reflected light microscopy and by extraction of materials from the seed with hot water.

Observations of *Cassia nictitans* seedcoats before and after immersion for 30 minutes in water at 70° C are shown in Figures 8 and 9. In Figure 8, one of the depressions or "dimples" characteristic of the seedcoat is depicted before treatment. Comparison of this with Figure 9, where the dimples now stand out above the remainder of the seedcoat, indicates that a considerable amount of material has been removed.

Further evidence of a cuticular layer soluble in hot water is presented in Figure 10. This material was extracted from the seedcoat

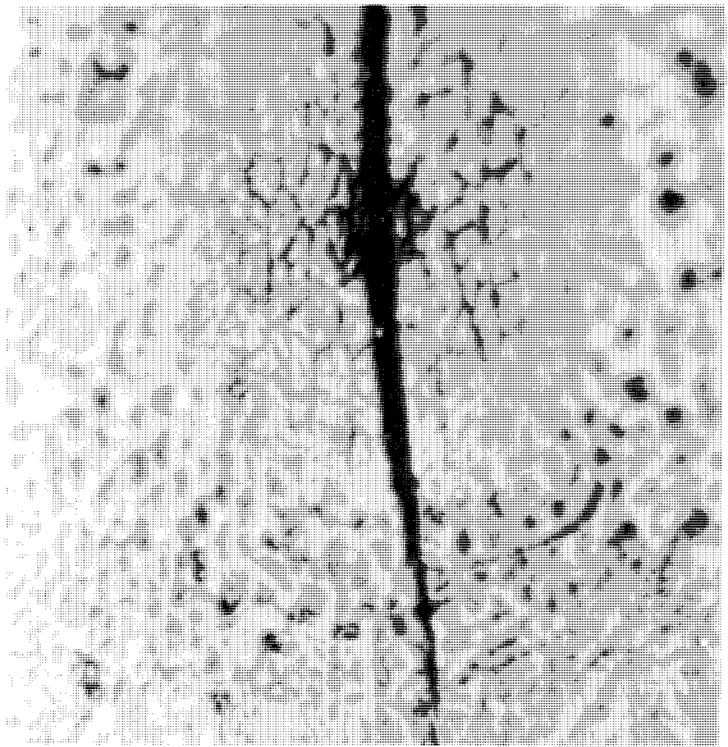
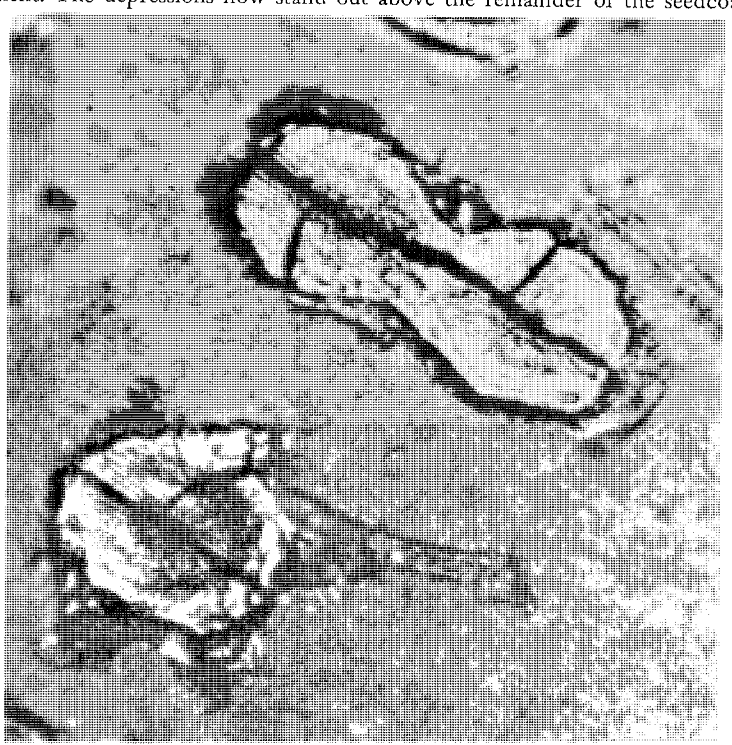


FIG. 8. Appearance of a *Cassia nictitans* seedcoat depression prior to hot water treatment.

FIG. 9. Appearance of *Cassia nictitans* seedcoat depressions following hot water treatment. The depressions now stand out above the remainder of the seedcoat.



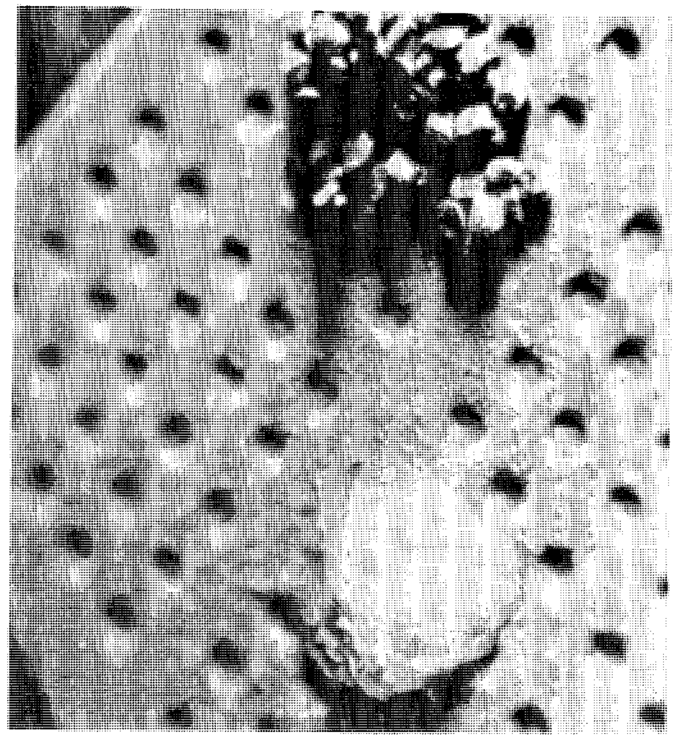


FIG. 10. *Cassia nictitans* seed and the highly hydrated material removed from them by 4 minutes treatment with water at 80° C.

by immersion in water at 80° C for 4 minutes. The water plus extract was decanted from the seed and precipitated with absolute ethyl alcohol. Although the volume of this material approximates the volume of the seed from which it was extracted, it is in a very highly hydrated form and probably represents about 1 percent by weight of the dry seed.

Additional estimates were made of the amount of material removed by hot water. Thirty minutes extraction with water at 76° C revealed an average loss of 1.5 percent of the dry weight of the seed. Four minutes extraction at 80° C indicated an average weight loss of about two-thirds of a percent.

The chemical composition of the water extracts is not known. Most literature concerning hard seedcoats ascribes the water-impervious nature to waxy or fatty acid cuticles. Such materials would not hydrate, however, nor be soluble in water. Other investigators have indicated a mucilaginous cuticle, which would be compatible with our results. In some seed these mucilages are mainly galacturonides, according to Mayer and Poljakoff-Mayber (1963). Tookey and Jones (1965) report that analysis of the entire seed of several species of *Cassia*, not including *C. nicitans*, yielded from 7 to 32 percent galactomannans, and there is a possibility the cuticle may consist of this material. Unfortunately, the physical properties of these materials are not known, and we have not yet analyzed the materials removed from the seedcoat.

If moist heat is necessary to increase germination of *Cassia nictitans*, the question then arises, are these conditions generated by fire? As mentioned previously, water vapor should be available during fires from drying subfuels and from combustion itself. A brief series of laboratory experiments was run using pine needle fuel beds to test this hypothesis, although we recognized that our laboratory conditions were a poor simulation of natural fuel beds. Seed were placed in cloth net packets within the subfuel at the soil surface and one-fourth inch below the surface. The subfuel was left dry (8- to 10-percent moisture content) on half the fuel bed and wetted on the other half. Approximate temperatures of the seed were monitored with thermocouples placed within the seed packets. The early tests resulted in overheating of many of the seed and underheating of others. No great differences in germination of seed placed in the litter soil surface and in the soil were noted. This probably was due to lack of control of the burning treatment. However, we improved the conditions under moist subfuels by first burning the fuel bed and then supplying additional heat with a propane torch and monitoring seed temperatures. Although use of the torch further distorted natural conditions, the purpose was to see if increased germination would result. Average germinations of 65 and 31 percent resulted, both of which were higher than would be expected without treatment. Also, approximately 75 percent of this germination occurred within 7 days. It was also noted that moist subfuel provided an excellent temperature buffer, preventing overheating of the seed until the excess moisture was evaporated.

SUMMARY

In general, there is little or no a priori experience in the literature about the effects of fire or artificial heat on partridge pea, lespedeza, milk-pea, or beggarweed seed.

Under laboratory conditions, we found that dry heat did not in-

crease germination of these seed, but that moist heat had a pronounced effect on total germination and germination rate of *Cassia nictitans* seed.

Test fires were conducted in the laboratory using pine needle fuel beds with *Cassia nictitans* seed placed at various depths in the litter and soil. Results indicated increased germination due to treatment. However, because of lack of control of burning treatments, results are considered inconclusive.

The data presented are preliminary; therefore, before any definite conclusions concerning the effects of fire on legume seed can be reached, more testing is necessary.

ACKNOWLEDGMENT

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