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seems, therefore, to be similar to the type of growth associated with meristematic tissue, rather than with expanding tissue. 2,4-D is in a certain way rejuvenating the stem cells, creating the characteristics of a meristematic cytoplasm.

Our results suggest that the herbicidal studies really needed are those on the biochemical relationship between 2,4-D and nuclear activity.

SUMMARY

A study was made of the subcellular distribution of the RNA synthesized in the hypocotyls of 2,4-D sprayed soybeans. During a 48 hour period the RNA content doubled, with over half of the increase appearing in the microsomal fraction and one quarter in the soluble fraction.

It is hypothesized that the herbicide renews nuclear activity in the tissue leading to a synthesis of RNA and protein and to issue proliferation, and that the biochemical basis of auxin-herbicide action lies in a reversion to a meristematic metabolism.

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Effective Burning of Rangelands Infested with Medusahead¹

CYRUS M. MCKELL, ALMA M. WILSON, and B. L. KAY²

Abstract. Medusahead (*Elymus caput-medusae* L.) matures later in the spring than most associated species, and has a seed head moisture content of above 30 per cent for approximately a month after leaves and stems begin to dry. High temperature is more injurious to seed viability when seed moisture content is high. Control burns of medusahead infested rangeland were most effective in late afternoon when burning slowly (into a mild wind) and at the soft dough stage of medusahead seed development.

FIRE has been used successfully as a tool in range improvement. Its effectiveness in reducing stands of medusahead (*Elymus caput-medusae* L.) on rangeland depends on burning conditions. Some burns have reduced medusahead stands, whereas others have produced little change in stand density. Medusahead matures 2 to 4 weeks later than most associated species and fires timed

too early either burn poorly or leave the medusahead plants to produce additional growth using soil moisture supplies left by early-maturing species (13).

Four objectives may be recognized as desirable in burning medusahead-infested rangeland: to remove old medusahead litter, to reduce the density of medusahead stands, to serve as seedbed treatment and competition-reduction before reseeding, and to minimize damage to desirable associated species.

The presence of old medusahead litter is recognized by many as a problem in the management and control of medusahead on rangelands. Plant residues from medusahead decompose slower than residues from associated species possibly because of a higher silica content (3). A dense litter mat, reported to be up to 5 inches deep in Idaho (9), which may contain a varying number of seeds that do not germinate each fall but are carried-over to following seasons, is formed. Tillage operations are impeded by a large quantity of old litter which also constitutes an extreme fire hazard.

Although recent studies have shown that medusahead is eaten during early vegetative stages the forage value

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of medusahead is reduced sharply when the seed heads emerge from the boot (11). The presence of mature seed heads and old litter appears to restrict the accessibility of dry feed during the summer and green feed early in the fall. Sheep generally concentrate their grazing in areas that have been burned before the growth of the new forage crop.

Removal of old litter by burning has been used in combination with herbicide application (12, 18). Chemicals were considered to be more effective when applied directly to seeds and forage residue on the soil surface than when diffused in a thick layer of old litter.

Various physical conditions at the time of burning will determine the degree of success in a control-burning program. Furbush (8) reported that fire had been used to reduce medusahead infestations in Mendocino County, California. He described burning conditions on a satisfactorily burned area as air temperature being 80° F., relative humidity 45 per cent, and wind 1 mile per hour. Murphy and Turner (15) concluded from burning medusahead numerous times that a reduction in seed germination was related to the intensity of the fire and amount of dry fuel present. Slow, hot burns were most effective.

Surface samples of soil from a medusahead-infested area in Idaho burned in June (17) still contained viable seeds even though scorched seeds of the current season collected from the burned area did not germinate. No burning conditions were given. Major et al. (14) recommended two seasons of burning: late spring, when associated species have matured but medusahead is not yet ripe, and summer, after maturity but before medusahead seeds drop. Slow burning into the wind or downhill was advocated. Torell et al. (18) stated that burning should be investigated further as a means of securing medusahead control.

In addition to the actual burning of seeds by fire, exposure to high temperatures may destroy the viability of moist seeds that are in the head or in the litter. Sampson (16) found that dry seeds of several annual grass species did not germinate when exposed to temperatures over 260° F for 5 minutes. Chaparral species withstood higher temperatures than grass species. Bentley and Fenner (2) used soil pyrometers to record the temperature in the surface litter and upper 2 to 3 inches of soil. In a grass burn where the litter was less than .4 inch deep an average temperature of 200° F. was recorded at the soil surface. The average temperature increased to 500° F. .4 inch above the soil surface. Incomplete burning of the litter accounted for the low temperature at the soil surface. When more litter is burned, temperatures somewhat higher than those obtained by Bentley and Fenner could be expected at the soil surface. Craddock³ measured the intensity and duration of heat during a burn with thermocouples. The results obtained indicate that maximum soil temperatures during a grass burn are held for considerably less than 5 minutes.

Barton (1) found a reduction in seed viability when both moisture content and drying temperature were high

in a seed-storage study. Crosier (5) reported differences among 24 species in their susceptibility to heat damage but did not study any relations with moisture content. Working with *Rhus laurina* Nutt. Wright (19) reported a temperature-seed moisture content interaction. Seed with 13.2 per cent moisture exposed to 104° F. for 4 hours gave 14 per cent germination. When the temperature was raised to 212° F. and the moisture content was decreased to 4.6 per cent, 30 per cent germinated.

To understand better some of the factors contributing to an effective burning operation several studies were recently conducted and are reported herein.

LABORATORY STUDIES

A preliminary study of germination response to high temperature exposure for five periods was established to determine threshold values for seed damage. Medusahead seeds from the R.E. Shellhammer Ranch, Solano County, California, that had been stored for 6 months at room temperature were used. Seeds had an average moisture content of 9.2 per cent. A randomized-block design with 4 replications consisting of 5 exposure periods (0, 15, 30, 60, and 90 seconds) was used. Fifty seeds were placed on a 1/4-inch layer of soil in a metal dish. Seeds were exposed to 392° F. in a thermostatically controlled oven. After exposure to heat the dishes of soil and seeds were removed from the oven, moistened, and placed in a seed germinator set for 66° F.

Seeds germinated 64 per cent after exposure to the 90-second treatment as compared with an average of 96 per cent for the untreated seeds. Shorter exposures caused no reduction in germination. Our observations of burning annual grasslands indicate that high temperatures do not usually last longer than 2 minutes so that these data show why a large quantity of seeds may escape injury if protected by a layer of unburned litter.

Moisture content of seeds in the field would be expected to be higher at various times than the 9.2 per cent moisture content of seeds used in the preliminary trial. Therefore, interaction of temperature and moisture content was studied. Medusahead and soft chess (*Bromus mollis* L.) seeds were compared since medusahead and soft chess are associated species in large areas of California and Western Oregon rangeland. A factorial design with 4 replications of 2 species, 3 moisture contents, and 4 temperatures was used. Samples of 50 seeds of each species were allowed to imbibe moisture in closed containers at relative humidities of 81, 52, and 20 per cent. The humidities were maintained at 20° C. over saturated solutions of ammonium sulfate, sodium bisulfate, and potassium acetate, respectively. The initial per cent moisture of the seed was determined and the changes in weight were plotted as a change in moisture content. Seeds to be included in the temperature treatments were removed after 4 days. Each sample of 50 seeds was placed on a 1/4-inch layer of soil and heat treated for 2 minutes in a thermostatically-controlled muffle furnace at the following temperatures: no heat treatment, 320°, 356°, and 392° F. Upon removal from the furnace the soil was moistened and dishes were

³Craddock, G. W. 1927. The successful influence of fire in the chaparral type. M. S. Thesis. Berkeley, U. Calif.

Table 1. Average germination percentage of medusahead and soft chess seeds of various moisture contents exposed to various temperatures for two minutes (mean of 4 replications).

Species	Moisture content %	Check	320F	356F	392F
Medusahead.....	8.1	91.5	90.5	80.0	15.5
Soft chess.....	9.0	91.0	82.0	82.0	20.5
Medusahead.....	11.0	83.0	81.0	32.5	5.0
Soft chess.....	11.5	85.5	73.5	50.5	12.5
Medusahead.....	15.4	82.0	80.0	2.5	0.0
Soft chess.....	18.6	87.5	59.0	34.0	2.0
Mean.....		86.7	77.7	46.9	9.3

placed in a germinator set for 68° F. Germination was counted after 7 days (Table 1).

Analysis of the data indicated significance at the .01 level for temperatures but not for moisture contents or species. Significance was also indicated for the interaction of temperature and moisture content and for the interaction of temperature and species. Seed germination percentage of both species was reduced to almost zero when moisture content was relatively high and seeds were exposed to 392° F. Seeds at high and medium moisture content were reduced in germination when exposed to 356° F.; seeds of low moisture content did not lose germinability until exposed to 392° F. The important point in relating these results to the field is that only a reduction of germination to near zero would be satisfactory for controlling medusahead and such a condition may be obtained in the interaction of high temperature and high seed moisture content.

The two species differed in their susceptibility to heat damage. Soft chess seeds were killed over a wider range of temperatures than were the medusahead seeds. A reduction in soft chess germination resulted from the 320 F treatment but medusahead was not affected until exposed to 356 F. Soft chess appeared to be more resistant to heat than medusahead at 356 F and 392.

FIELD STUDIES

Moisture content of seed heads and fuel.

An understanding of moisture content of seeds and of fuel in the field during and after plant maturity may be helpful in planning the best time to burn medusahead. Reports of failure in burning stands of medusahead in late spring have been frequent, possibly because of too high moisture content of medusahead plants even though the associated vegetation was sufficiently dry to burn rapidly. Medusahead often occurs in dense patches in the early stages of infestation and such areas often fail to burn completely or recover sufficiently after burning to produce some new seed heads.

The decrease in moisture content of maturing seed heads was observed during the period of maturity in 1959 and 1960. Four 5-gram samples of seed heads were collected in polyethylene bags each sampling time. Phenological observations were also recorded. Seed heads were dried in a forage drying oven and the moisture percentage was calculated.

In 1959 moisture content of seed heads remained relatively high for approximately a month after the leaves and stems had become brown and dry. In 1960 moisture loss from seed heads and other plant parts

followed the same pattern as in 1959 except that the decrease in seed head moisture content occurred about 1 month earlier because of the early onset of dry summer weather (Table 2). Between June 8 and June 15

Table 2. Average moisture contents of medusahead seed heads and litter in mid-afternoon at various stages of maturity in Solano County, California, 1960. (means of 4 replications).

Date	Seed head moisture content %	Litter moisture content %	Phenology of medusahead
June 6.....	45.9	25.0	Stems dry. Heads light green, awns green to purple. Seeds in milk to soft dough stage.
June 8.....	42.5	21.7	Awns purple, glumes beginning to stand out at angle to rachis. Awns flexible, some curled. Seeds in dough stage.
June 13.....	22.5	8.5	Tips of awns purple, mostly tan. Few awns flexible, mostly curling and stiff. Seeds nearing hard dough stage.
June 15.....	10.6	5.2	Awns tan, curled, stiff. Hard dough stage. Seed heads beginning to nod.
June 20.....	7.0	5.1	All heads nodding, seeds starting to drop. Stems breaking at first node.

the moisture content of the seed heads decreased from about 42 to 10 per cent; after June 8 the vegetative plant parts and associated species were sufficiently dry to burn.

Considering the relation between seed-moisture content and high temperatures in reducing seed viability it appears that burning would be most effective when the awns lose their purplish color and seeds are still flexible in the early hard dough stage.

Early morning change in moisture content of seed heads and litter.

Four samples each of litter and seed heads were collected at 6, 7, 8, and 9 a.m. on July 12, 1959, a clear summer day. Samples consisted of all seed heads from a square yard and all litter from a square foot. A hygrothermograph placed at ground level recorded relative humidity.

Seed heads have a higher moisture content in the early morning than litter, apparently because of the greater exposure of seed heads to the moist air (Figure 1). Soon after sunrise the relative humidity began to decrease and the seed heads began to lose moisture at a faster rate than the litter. Two hours after the humidity began to decrease, the moisture content of seed heads was 4 per cent lower than at 7 a.m. but still 2 per cent higher than that of the litter.

Burns of medusahead stands in mid-summer could be planned to take advantage of the greater moisture content in the seed heads in the morning hours for increasing the damage to the seeds. However, other considerations, such as low temperature, low humidity, and low wind velocity, could have more importance and override the small advantage of a greater seed moisture content during morning hours. Obviously, early morning is the safest time to burn fire lines and safety strips.

Burning medusahead at various times of day and speeds.

A study was conducted during August, 1959 to measure several of the factors present in fires on medusahead-infested range. A split-plot experimental design was used to test burning in morning, noon, and evening on

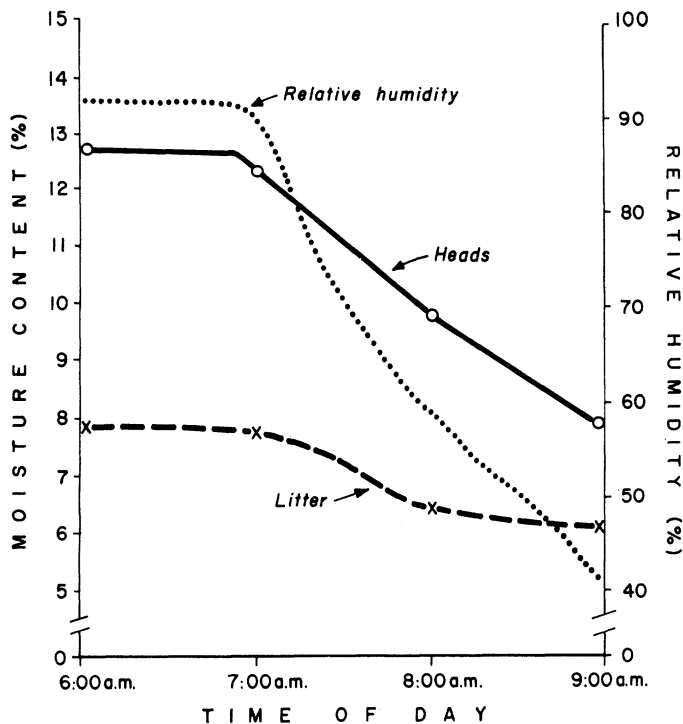


Figure 1. Changes in moisture content of medusahead seed heads and litter in the Sacramento Valley on a typical summer morning, July 12, 1959.

three different days (replications). Nine plots, each 25 by 50 feet, were split to allow slow and fast burning. Each plot was outlined by a disked fire break and the overall plot area was surrounded by a safety strip burned at daybreak when fuel was at maximum moisture content and burning was easily and safely controlled.

Temperatures within each burn were measured in a representative area of the plot from 1/2-inch below the soil surface to 10 inches above. Vertically oriented fusion pyrometers constructed and installed according to the method outlined by Bentley and Fenner (2) were used. Materials used in the construction of the pyrometers had the following melting points: 150°, 200°, 300°, 400°, 500°, 600°, and 700° F.

Fuel concentration and moisture content were measured by clipping all plant material from a 1 square-foot plot located 3 feet from each pyrometer and obtaining field weight and oven-dry weight.

Slow and fast burned sub-plots were obtained by burning the downwind end of a whole plot slowly into the wind and then with the additional safety of a burned area, the remainder of the plot was burned with the wind. Travel speed of the fire line was measured in feet per minute. The number of seconds flames were visible at the pyrometer was also recorded. Ambient temperature and relative humidity 2 inches above the soil surface within the dry vegetation was recorded. Wind speed was estimated. At the conclusion of all burning operations unburned fuel was determined by clipping and weighing all remaining litter from a square-yard area on the site where the pyrometer had been installed. A square-foot sample of litter from which to obtain seeds for germination analysis was collected from

a corner of the square-yard plot using clippers, small broom, and dust pan. In the laboratory the litter sample was thoroughly mixed and spread out on paper in a row 1 inch wide. The first 100 seeds removed from the litter were used in a germination test 3 months (10) later.

Results from the various burns indicate that a high degree of variability exists in the conditions present in the morning, noon, and evening on different days (Table 3). Morning burns were slowest, particularly when plots

Table 3. Conditions present during burns of medusahead-infested rangeland at the R. E. Shellhammer Ranch, Solano County, California, 1959.

Date of burn and time of day	Temp F	Relative humidity %	Wind velocity mph	Fuel moisture %	Type of burn	Speed of fire ft/min	Duration of fire at pyrometer sec
August 26							
7:15a.	65	75	5	8.9	slow	1.2	50
12:45p.	102	20	10	3.0	fast	20	22
8:50p.	70	57	15	5.2	slow	6	20
August 28							
7:20a.	62	92	2	16.4	fast	40	15
12:30p.	94	28	5	3.3	slow	6	25
6:45p.	84	30	15	3.1	fast	20	10
August 31							
7:10a.	53	93	3	8.0	slow	3	35
12:45p.	102	21	18	5.0	fast	18	20
6:45p.	95	20	10	2.0	slow	6	25
Mean							
7:15a.	60	87	3	11.3	slow	4	34
12:40p.	99	23	11	3.5	fast	53	18
7:10p.	83	39	13	3.5			

were burned into the wind. In the morning, humidity and fuel moisture were highest, and temperature was lowest. The best burning conditions appeared to be in the middle of the day, particularly if late afternoon winds become too strong. However, a more effective time to burn would be in the late afternoon when temperatures are lower than mid-day and just before humidity begins to increase. Fuel moisture-content was no higher in the evening than at noon and thus provided for an intense fire. The fuel averaged 5,200 pounds per acre on the experimental area. Fuel samples were variable and were influenced by an unevenly distributed carry-over of litter from the previous year. No significant differences were found in the amount of unburned fuel remaining because of the original variability.

Temperatures in the burns measured with fusion pyrometers indicated that slow burns were generally hotter than fast ones (Figure 2). The four elevations illustrated in Figure 2 represent locations where the greatest amount of medusahead seed is found. Temperatures measured at 9 to 10 inches above the soil surface would be sufficient to destroy the viability of seeds still in the head. Most seeds had dropped by the time of the study and were found in the region from the soil surface to 1 inch high, although a few seeds had fallen into cracks in the soil. Slow burning at noon or evening appeared to produce the highest temperatures in the region from soil surface to 1/2 inch above. The tempera-

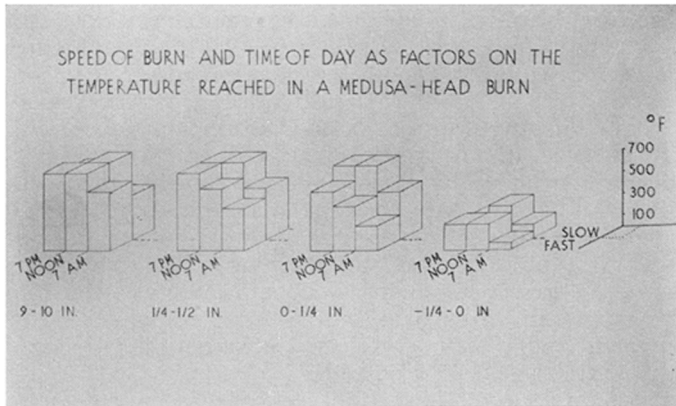


Figure 2. Temperatures measured with fusion pyrometers with a measurable range of 150° to 700° F at four heights during morning, noon and evening burns of medusahead. Various plots were burned at morning, noon and evening.

tures measured from the soil surface to 1/4 inch below the soil surface would not be lethal for seeds, particularly for the short periods of high temperature indicated in Table 3.

Germination of medusahead seeds recovered from the litter following burning may be used as an indication of the effectiveness of various burning treatments. Significance was indicated for per cent seed germination in relation to fast versus slow burning and also in relation to time of day burned. Seed germination was lowest in samples from plots burned in mid-day. Seeds from plots burned in the morning and in the evening at a slow speed were lower in germination than plots burned fast. In the middle of the day, speed of burning made no difference in seed injury. A possible reason for the difference in germination of seeds from plots burned fast and slow in morning and evening is that the heat from slow burns lasted longer and penetrated more of the protective layer of litter.

Burning medusahead at various stages of development.

The relation of seed head moisture content to various stages of medusahead maturity was used as a basis for further study. A series of medusahead-infested plots were burned at four stages of maturity: 1) seeds in soft dough, 2) seeds mature but not dropped, 3) shortly after seeds began to drop, 4) 2 months after seeds began to drop, plus unburned check plots. Seeds of associated species were mature and some had dropped by the time of the first burn. A fusion pyrometer was installed in the center of each 30-foot square plot. Pyrometers were fabricated with the same melting point materials used in the previous study plus five additional materials which had melting points at 800, 900, 1000, 1100, and 1200 F. Data on fire conditions were recorded, unburned litter was collected and weighed, and seed samples for germination trials were obtained from each plot. All plots were drill-seeded with rose clover and hardinggrass in November 1960. Species composition of the vegetation the year after the burn treatments was determined by the step-point method (7). One hundred points were analyzed on each plot. The average number of seed heads on each plot was determined at plant maturity.

Plots were burned under nearly ideal conditions for maximum damage to medusahead seeds. Fires were started in the late afternoon in the face of mild winds. Fuel moisture was low and ranged from 20 per cent in the early burn to 5 per cent in the second and third burns. Fire held in place for approximately 30 seconds in most cases. Relatively high temperatures were developed at the various levels where seeds were located (Table 4). Burns on the two early dates were generally

Table 4. Average maximum temperatures recorded at various elevations by fusion pyrometers located in controlled burns of medusahead-infested rangelands, 1960.

Height in.	Temperature* F for indicated burning date.			
	June 8	June 15	June 23	Aug. 22
-1/4-0.....	175	210	325	280
0-1/4.....	335	575	700	1100
1/4-1.....	510	960	1170	1200
1-2.....	840	1025	1200	1200
2-3.....	960	1030	1200	1200
3-4.....	960	1170	1200	1200
4-6.....	840	1170	1200	1200
6-8.....	790	1125	1200	1150
8-10.....	540	950	1150	920

*Each value is an average of 4 replications.

not as hot as the two later burns. The green heads of medusahead were not completely consumed by the fire in the first burn but the awns were burned and seed development was stopped. Although no significant differences were found in the amount of unburned litter and ashes remaining on the variously burned plots, the late-season burn appeared to be the most complete and left the least amount of unburned material.

Emergence of seedlings germinated in sand indicated a significant reduction in the number of viable seeds as a result of controlled burning (Table 5). The early burn

Table 5. Per cent emergence of burned and not burned medusahead seeds germinated in sand, 1960.*

Replication	Burning treatment				Not burned
	Early	Before seed drop	After seeds began to drop	2 months after seeds began to drop	
I.....	2	5	7	1	61
II.....	0	5	5.5	7.5	68
III.....	2	2.5	4	7	67
IV.....	1.5	6.5	7	18	49
Mean.....	1.4	4.8	5.9	8.4	61.2
Significance of means ^b					
All plots.....					
Burned.....					
Plots only.....					

*Each value is the average number of viable seeds collected from four square-foot quadrats.

^bValues included by the same line do not differ significantly at the .05 level (6).

left the fewest viable seeds and it may be that the small number of viable seeds recovered from the four plots burned early were scattered into these plots by the wind. In mid-summer, high-velocity north winds occurred and a few seed heads of medusahead were blown into the plots. In general, the number of viable seeds per plot in 1960 is lower than in 1959 and two factors could be responsible: 1) a greater accumulation of litter was present in 1959 than in 1960 which, although providing more fuel, protected the seeds on the ground from the

fire, and 2) all burns in 1960 were started in late afternoon when fuel moisture was low, relative humidity was low, and a gentle wind was present to hold the fire "in place". An average of 2500 pounds per acre of unburned fuel and ashes remained on plots burned in 1959 as compared with an average of 1130 pounds per acre on plots burned in 1960. A large proportion of the material recovered was a compact layer of partially decomposed organic matter at the soil surface.

The botanical composition of vegetation on burned plots was significantly different from the vegetation of unburned check plots (Table 6). Broadleaf filaree

Table 6. Average botanical composition on February 17, 1961, and number of mature medusahead seed heads on May 29, 1961, on plots of medusahead-infested rangeland in the first growing season after various dates of burning.

Date and stage of medusahead maturity when burned	Species composition ^a (Number of hits per 100 points examined)				Number mature medusahead seed heads ^b per sq. ft.
	Medusahead	Filaree	Legumes	Other species	
June 8. Seeds in soft dough. No seeds dropped.	1	75	13	11	3
June 15. Seeds in hard dough. No seeds dropped.	1	75	15	8	4
June 23. Seeds hard. Some seeds dropped.	1	77	13	9	7
Aug. 22. Seeds hard. Most seeds dropped.	8	69	14	9	17
Control.	53	23	4	20	82

^aEach value is an average of 4 replications.

^bMean for 40 samples per treatment.

(*Erodium botrys* (Cav.) Bertol.) increased in relative abundance from an average of 23 per cent on unburned plots to about 75 per cent on the burned plots. Legumes were about three times more abundant on burned plots than on unburned plots. In contrast to the increase in abundance of filaree and legumes on burned plots, medusahead and "other species" decreased as a result of burning. The relative abundance of medusahead was about 1 per cent on plots burned near medusahead maturity, but there was an increase in abundance to 8 per cent when medusahead was burned 2 months after maturity. These data are in agreement with the seed-germination results reported in Table 5.

A final evaluation of the effect of burning medusahead at various stages of maturity was obtained from a count of seed heads produced 1 year after burning (Table 6). The fewest seed heads were found on plots burned before seeds were completely mature. Plots burns 2 months after seeds dropped had 17 heads per square foot and check plots had an average of 82 heads per square foot.

DISCUSSION

Results of the studies described suggest that greater advantage can be taken of the interaction between seed moisture content and high temperature in reducing seed viability. Seed moisture is high in the last stages of plant maturity when other parts of the medusahead plant and associated vegetation are dry and provide a sufficient volume of fuel. Species such as filaree and wild oats (*Avena fatua* L.) mature early and drop most of their seeds before medusahead is ready to burn. Many seeds of such early-maturing species will escape dam-

age from burning. High-moisture medusahead seeds still in the head have less chance for survival than dry mature seeds located near the soil surface and protected by a layer of litter that will generally not be entirely burned.

Slow burning into a mild wind in mid-afternoon with low relative humidity provides the most favorable condition for maximum seed damage. Fast burning with the wind may be necessary in areas that have a small amount of fuel. However, fire control is more difficult with fast burns. Adequate planning and attention to safety factors are necessary if burning operations are to be successful. Advice and help from responsible government agencies and published information (4) should be obtained whenever possible.

Burning should be considered only as one phase in the management of medusahead-infested rangelands. Follow-up work should include reseeding, grazing management, and additional burning when necessary.

SUMMARY

Studies of some of the factors necessary for effective control burns of medusahead-infested rangeland indicated that—

1. In a laboratory study viability of medusahead seed exposed to 392° F. prior to germination was reduced only when the duration of exposure was at least 90 seconds.

2. The interaction of seed moisture content and exposure to high temperature under laboratory conditions reduced medusahead and soft chess seed germinability to about zero under conditions of high moisture content and 392° F. Utilization of this interaction in timing field burning operations seems advisable.

3. Moisture content of medusahead seeds remained above 30 per cent for approximately a month after leaves and stems and associated vegetation had dried.

4. Seed heads had a higher moisture content than litter of medusahead in the early morning of a typical summer day in the California Central Valley.

5. Physical conditions during various days and times of day when medusahead stands had been burned were variable. In the present study the most effective control of burns of medusahead occurred in late afternoon when fires burned slowly (into a mild wind) and at the soft-dough stage of medusahead seed development.

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Purification and Properties of the Simazine-Resistance Factor of *Zea Mays*¹

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Abstract and Summary. A procedure has been described for the partial purification of the simazine-resistance factor of *Zea*. The relative solubilities in various solvents and some of the chemical properties of this factor have been studied. Pyridine and hydroxylamine were also found to bring about the conversion of simazine-¹⁴C to the 2-hydroxy analogue. Based on these substances as models, a mechanism has been proposed for the dechlorination of simazine in *Zea*.

INTRODUCTION

CASTELFRANCO et al. (2) showed that *Zea mays* contains a non-enzymic system which converts the herbicide simazine, 2-chloro-4, 6-bis(ethylamino)-s-triazine, into the corresponding 2-hydroxytriazine. The chemical properties of this system have now been subjected to further investigation, and the active constituent has been partially purified.

A preliminary report describing the properties and a partial purification of the active constituent was presented at the Research Committee Meeting of the Western Weed Control Conference in March, 1961 (3).

MATERIALS AND METHODS

Simazine-2,4,6-¹⁴C³, specific activity 1.1 μ C per μ mole, was purified by dissolving it in chloroform and washing with water; the chloroform was then removed by evaporation and the residue was dissolved in 1 ml of methyl cellosolve per mg of crude solid.

Hydroxylamine acetate solution was prepared by stirring solid hydroxylamine sulfate with a stoichiometric

amount of 0.4 M barium acetate. The barium sulfate precipitate was removed by centrifugation and the supernatant solution of approximately 0.8 M hydroxylamine acetate was used immediately.

Glucose oxidase was obtained from Worthington Chemical Corporation, Freehold, N. J.; horseradish peroxidase, from Sigma Chemical Co., St. Louis, Mo.; and polyphenol oxidase was prepared from *Psalliotia campestri* by the method of Frieden and Ottesen (4)⁴.

All other chemicals were of the best available commercial grade and were used without further purification unless specified otherwise.

The detoxification of simazine by corn extracts was assayed by the technique previously reported (2) which depends on the conversion of simazine-¹⁴C to the 2-hydroxy analogue. The standard assay was carried out in 1.0 ml of 0.1 M sodium acetate buffer, pH 5.6. Five μ g of simazine-¹⁴C dissolved in 5 μ l of methyl cellosolve were added, and the mixture was shaken 2 hours at 22° C. The chloroform-insoluble charcoal-adsorbable radioactivity was then counted.

The paper chromatographic techniques previously described were employed.

Fractionation of the active principle. The preparation of the crude corn extract followed the previously described procedure except that fourteen day old plants were used in this study. One volume of centrifuged pressed juice was treated with 9 volumes of acetone at 0°. The mixture was filtered through a Buchner funnel with diatomaceous earth. The clear yellow filtrate was evaporated to dryness under reduced pressure, and the residue dissolved in water to half the original volume. This material was designated "90% acetone solubles."

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³¹⁴C-ring-labeled 2-chloro-4,6-bis(ethylamino)-s-triazine was synthesized by New England Nuclear, Boston, Mass. All triazine compounds were gratuitously supplied by the Geigy Chemical Corporation, Yonkers, New York.