ECOLOGICAL SIGNIFICANCE OF SEED COAT IMPERMEABILITY TO MOISTURE IN CRIMSON, SUBTERREANEAN AND ROSE CLOVERS IN A MEDITERRANEAN-TYPE CLIMATE

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From an evolutionary point of view, impermeability of the seed coat to moisture is a valuable characteristic for continuance of a plant species under conditions of climatic adversity. Not only do impermeable seeds remain viable for a long time, but under natural conditions increments of a seed population become permeable to water and germinate in successive intervals. Hence, seed produced in a favorable season is often capable of producing seedlings over many years, providing numerous opportunities for species survival.

This consideration is also of ecological significance in stands of winter annual clovers seeded on rangeland. A much desired objective is a permanent stand of legumes after the initial seeding effort, and the production of impermeable seeds is important for legume survival on California ranges. The timeliness and amount of precipitation received are vital factors to stand establishment on such rangeland. After sufficient moisture in autumn to germinate seeds of winter annual species, subsequent drought often causes seedling mortality. If an annual plant has produced some impermeable seeds which become permeable over a period of time, the opportunity for the establishment of a good stand is greatly enhanced. Often the favorable circumstances of seeding can not be attained again for a number of years. This makes short-term improvements of questionable value and emphasizes the desirability of permanence in the changes brought about by vegetation manipulation.

Since seed coat impermeability is important to legume survival on rangeland, information concerning its occurrence in the clover species commonly used in seeding and the way it is influenced by environmental factors is worthwhile. Consequently, research was initiated to study the following aspects: a) the amount of impermeable seed produced by the winter annual species, crimson, subterranean and rose clovers (*Trifolium incarnatum*, *T. subterraneum* and *T. hirtum*), under dryland conditions in California, b) the rate at which seeds become permeable, and c) some factors causing breakdown of impermeability of rose clover, a species with a high degree of seed coat impermeability.

LITERATURE REVIEW

Most investigators of seed coat impermeability have agreed that the thickened ends of the columnar cells(i.e. Malpighian cells) of the seed coat, immediately below the cuticle, block the intake of water by impermeable seed. The testae of both impermeable and permeable seeds show the same general organization. The functional difference is due only to permeability in various sections of the seed coat of the permeable seeds. A seed which has dried moderately, but has not become impermeable, is permeable in one or more irregular patches over the coat, generally at or near the hilum. This permeability is due to incomplete formation of the suberin layer in the Malpighian cell caps of those areas. Such seeds may become impermeable by further drying, which induces continuity of the impermeable layer (Aitken 1939). Water intake by naturally permeable seeds also may occur in an unsuberized area of the strophiole (Hamly 1932).

Probably all commercially important leguminous species in the United States produce some impermeable seeds with the exception of the peanut (*Arachis hypogea*) (Harrington 1916). At the other extreme, Stevenson (1937) collected mature seed from 10,982 single plants of white sweet clover (*Melilotus alba*) and found that more than 99% of the seed was impermeable.

Factors affecting the production of impermeable seeds by legumes have been studied by many research workers. The stage of maturity of seeds has been shown to influence impermeability in legumes. Brown (1955) found that the impermeable seed percentage of broadleaf birdsfoot trefoil (*Lotus corniculatus*) increased with increasing maturity until full size of the seed was attained. Hills (1942) reported that delaying harvest up to 6 wk after the normal stage of maturity with subterranean clover caused a considerable reduction in the percentage of hard seeds. In subterranean clover, Hills (1944) also concluded that seed coat impermeability is not a major varietal character.

Aitken (1939) found that the moisture content of seed of subterranean clover was important in the formation of impermeable seed. As the seeds became drier on the plant, the percentage of impermeable seed increased. Hard seed formation also appears to be associated with the dehydration processes which may take place independently of the plant. Hyde (1954) states that the moisture content of seeds of white clover (*Trifolium repens*), red clover (*T. pratense*), and *lupine* (*Lupinus arboreus*) fell rapidly to approximately 25% (dry-weight basis), and thereafter, more slowly until the epidermis became impermeable at 14% moisture content. Further drying of the seed took place only by diffusion of water through the hilum which acted as a hygroscopically-activated valve. When the relative humidity was low a fissure in the hilum opened, permitting the seed to dry out; when the relative humidity was high the fissure closed, obstructing the absorption of moisture. Thus, the impermeable seeds tend to have a moisture content in equilibrium with the lowest relative humidity to which they have been exposed. The duration of the impermeable condition increases with the degree of dessication brought about by loss of water through the hilum.

Impermeable seeds are capable of germination, for the most part, after a treatment that makes the seed coat permeable to water. Under field conditions extremes of temperature, soil acids, soil organisms, and animal trampling and ingestion are agencies suspected of causing permeability. Permeability can be achieved artificially by mechanical scarification, impaction, chemical treatment, freezing and thawing, boiling water, or dry heat. Mechanical scarification is commonly accomplished to a greater or lesser degree in the threshing and cleaning operations where seed is produced commercially (Lute 1928). Scarification can also be accomplished in hand harvested samples by rubbing with sandpaper or notching with a file. Mechanical scarification physically breaches the impermeable layer in the seed coat allowing water to enter.

Mechanical impaction has been used successfully as a technique for reducing impermeability (Barton 1947, Brown 1955, and Hutton and Porter 1937). Hamly (1932) working with white sweet clover and Aitken (1939) with subterranean clover found that impaction caused a cleft through the strophiole of previously impermeable seed through which water could pass.

Treatment with heat or cold may also cause a splitting of the impermeable layer. Busse (1930) found that cooling to −80°C caused most of the impermeable seeds of alfalfa (*Medicago sativa*) to become permeable, but had very little effect upon impermeable seeds of sweet clover. Martin (1945) reported that impermeable seeds of sweet clover, in natural field seedlings and when stored over-winter in unheated buildings in Iowa, became 80-100% permeable by the middle of the following April. Hutton and Porter (1937), Lute (1928), Rincker (1954), Staker (1925) and Stewart (1926) found that subjecting seeds of impermeable species to dry heat temperature of 60°-85°C for periods of 2-6 hr effectively decreased the percentage of impermeable seeds. Stone and Juhren (1951) found that the fire induced germination of *Rhus ovata*, a non-legume, was the result of high temperature exposures. They concluded that a temperature of 60°C, which is often reached at the ¼ in. soil depth on exposed sites for several hours daily during the summer in many parts of California, is adequate to rupture the seed coat and induce germination.

Scarification with chemicals has also been used to reduce impermeability in certain legumes. Barton (1947) found that the subfamilies of the Leguminosae varied in their reaction to soaking in absolute ethyl alcohol for 72 hr. Impermeable seeds of the members of the *Papilionoideae* (*Cladastris, Cytisus, Melilouts,* and *Robinia*) were unaffected by the alcohol treatment. Impermeable seeds of members of the *Caesalpinoideae* (*Cassia, Cercidium, Gleditsia, Gymnocladus, Parkinsonia,* and *Cercis*) were made permeable by the alcohol soaking. Jones (1928) found that the impermeability of vetch seed (*Vicia villosa*) was not affected by immersion in ether, but disappeared upon short exposure to concentrated sulfuric acid. Burns (1959), working with blue lupine (*Lupinus angustifolius*), found that scarification with sulfuric acid rendered impermeable seeds permeable and that water then entered either through the hilar fissure or through pits eroded through the testa. Acid scarification did not permit water entry through the strophiolar area, however.

Went (1957) and his coworkers have detected a number of other adaptive mechanisms by which non-leguminous annual species of the California deserts avoid germination until conditions are favorable to their establishment. Some examples are water soluble germination inhibitors in seeds or in seed coats, inhibition by salinity, and slow water uptake by encasing structures. The effectiveness of these mechanisms of delayed germination are dependent largely on the coincidence of appropriate temperature and intensity and duration of rain.

Procedure

Seeds of 3 introduced species of *Trifolium* were collected at monthly intervals in 1958 and early

1959 from several locations for determination of impermeable-seed and germination percentages. The crimson clover (*Trifolium incarnatum*) seeds sampled in this study were probably of the common or Dixie varieties although evidence for varietal identification was inconclusive. The subterranean clover (*T. subterraneum*) was of the Mt. Barker variety at all locations except the Yolo site which was observed to have a mixture of 45% Mt. Barker and 55% Tallarook varieties. The rose clover (*T. hirtum*) was of the strain selected and developed by Love and Sumner (1952) from an introduction from Turkey which has been used extensively in California range plantings since 1950.

Tests to compare the scarifying effects of the threshing method with hand removal of seed from the calyx were conducted on samples of rose clover. Seed used in the tests was hand harvested on July 8, 1958 from standing plants grown on the University Farm. Unhulled seed was placed in a corrugated rubber-lined box and rubbed with a block of wood which was covered with corrugated rubber. Hand threshing was accomplished by rubbing unhulled seed between the palms of the hands. Eight replications of 100 seeds each of both of the threshing methods were laboratory germinated. It was found that seed threshing by rubbing with a corrugated rubber-covered block of wood in a corrugated rubber-lined box gave impermeable seed percentages not significantly different from seed which was hand removed from the calyx (98 and 96% respectively). Thereafter, all seed was threshed in the corrugated rubber-lined box.

Preliminary germination tests were performed to determine the most effective temperature for the germination trials to be conducted on field seed collections. Four temperatures were tried: 10, 15, 20 and 25°C on commercial seed samples. Groups of 100 seeds replicated 4 times were germinated in the dark in covered petri dishes on Whatman No. 3 filter paper moistened with distilled water. The criterion of germination was the appearance of a sound radicle. Of the temperatures studied 15°C did not reduce the germination of any of the 3 species in comparison with the other favorable temperatures, and it had the advantage of inhibiting the growth of molds. All field collections were germinated soon after collection at 15°C in the manner described above. Storage prior to testing was at room temperature in envelopes. Germination counts were made on the third and seventh days for rose clover and crimson clover and on the fourth and fourteenth days for subterranean clover.

Climate, Soils and Vegetation

Seed collections were made on sites located on terraces and foothills peripheral to the Sacramento Valley of California. The climate is of the Mediterranean type or dry-summer subtropical (Köppen's Cs) and has the following characteristics: (a) precipitation occurring mainly in the winter with summers essentially rainless and (b) temperatures mild in winter and warm to hot in summer (Trewartha 1954). Collection sites in Napa and Solano Counties qualify as marine locations with cool summers (Csb) being close to San Francisco Bay. Collection sites in the 3 counties, Placer, Sacramento and Yolo, are interior locations (Csa) with hot, intensely-sunny summer days (Kesseli 1942). A monthly climatic summary for the years 1957-58 and 1958-59 when the study was in progress is in Figure 1 for 5 weather stations representing the collection sites (U. S. Department of Commerce, Weather Bureau 1957-1959).

The distance of each site from the most representative weather station, the soil type, and the age of the clover stand is indicated in Table I. All sites were between sea level and 300 ft elevation.

Table I. Location, soil type, and age of clover stand at collection sites.

Site location	Distance from weather station	Soil type	Age of stand in years
Napa Co.	6 mi. SW Napa	Denverton adobe clay	8*, 3**
Solano Co.	12 mi. SE Fairfield	Antioch fine sandy loam	4
Placer Co.	10 mi. NNW Rocklin	Placentia gravelly loam	6
Sacramento Co.	11 mi. ESE Sacramento	Redding gravelly loam	5
Yolo Co.—			
Rominger farm	14 mi. NW Davis	Hartley gravelly loam	4
Mast farm	16 mi. NW Davis	Corning complex	6
University farm	2 mi. NW Davis	Zamora clay loam	2

* Subterranean clover.
** Crimson and rose clovers.

The resident vegetation is of the annual grassland type dominated by *Avena fatua*, *Bromus mollis*, *Festuca megalura*, *Hordeum leporinum* and *Erodium botrys* with scattered individuals of *Quercus*. Relict areas of Pacific Bunchgrass typified by *Stipa pulchra* occur near the 2 sites under a marine influence (Napa and Solano). Introduction of one or more of the winter annual clovers into the resident sward has been accomplished at each of the collection sites. The planting of annual legumes for the improvement of grazing is now a widely accepted practice for the California annual-range type. Forage production is often increased 3 or more times as a result of seeding legumes, along with suitable fertilization and grazing management (Williams et al. 1956 and 1957).

Results and Discussion

The ecological significance of seed coat impermeability relative to survival of annual leguminous species involves 4 major considerations a) the production of impermeable seeds, b) the rate at which these seeds become permeable, c) the degree of confluence of environmental factors favorable to germination and breakdown of impermeability, and d) the survival and productivity of plants developing from impermeable seed after delayed germination.

Production of Impermeable Seeds

Seeds of 3 annual clovers, used for planting rangeland in California, were collected for determinations of impermeable seed and germination percentages. Crimson and subterranean clover seeds were obtained on 5 ranches representative of foothill and valley rangeland of northern California. Rose clover seed was also collected from these ranches and 2 additional ones. The seed of all 3 species had matured by June 9, 1958 when collecting was begun. Seed collections were made of the 3 species at approximately monthly intervals through the rainless summer and autumn, and for rose clover into the winter season. Crimson and rose clover seeds were collected from plants, except where noted otherwise, and subterranean clover seed was collected from the soil surface.

Crimson clover.—The impermeable seed content of crimson clover ranged from 25-90% in the seed harvested from the various sites in June (Table II). Germination varied in a converse fashion from 74-10% in the June harvested seed, the remainder being nonviable. The impermeable seed content of crimson clover declined rapidly at all locations during the summer months. By the time of last collection date, seed from one location contained 9% impermeable seed, and the remaining locations 3% or less. Thus by November, the usual month of emergence for winter annual clovers in this region, very little impermeable seed of crimson clover remained.

Germination percentage of crimson clover increased for seed harvested during the summer months, reaching a plateau between 78 and 94% at the interior sites (Placer, Sacramento and Yolo). After a similar initial increase the crimson clover germination percentage declined from 62 and 70% by the August harvest to 34 and 42%, respectively, in the autumn for the 2 sites under a marine influence (Napa and Solano). In the September and subsequent collections most of the seed collected was from the basal portions of the crimson clover heads since the seed higher on the inflorescence had fallen. It was noted that some of these seeds were a brick-red color, which is not the normal seed color in crimson clover. These seeds were not viable. The increase in the amount of nonviable seed in the September and subsequent harvests is probably due to this factor.

The varying percentages of impermeable seed from the June harvest are probably the result of varying degrees of maturity. Slow maturity in some of the seed lots was due to more effective late moisture. Precipitation received in May and June 1958 was highest at the weather station near the Napa collection site which produced the highest impermeable seed percentage (Fig. 1).

As the seed became fully mature, impermeable seed percentages were reduced to very low levels. Lack of prsistence of seed coat impermeability in crimson clover may explain why the species does not persist on the drier sites over extended periods of time. If moisture conditions do not allow for stand establishment after germination begins, there apparently is little or no reservoir of previously impermeable seed to germinate and establish new stands when moisture conditions improve.

Subterranean clover.—Samples of subterranean clover seed from the 5 locations ranged from 58-91% impereable seed for the June collection, and germination varied from 28-9% (Table II). Seed from the later collection dates decreased in impermeable seed percentage and increased in germination percentage. By October collections from 4 locations had impermeable seed contents of 0-3% and germination percentages of 96-99%, but the seed collected from the Napa site had an impermeable seed content of 30% and germination percentage of 68% as late as December. The subterranean clover at the Napa site reached a plateau of impermeable seed values early in the autumn. This is accounted for by a later date of maturity than for the other sites. Some immature burs were observed in June at the Napa site, although these were not included in the sample collected. The delayed maturity may be attributed to the greater availability of moisture during the seed maturation period at that site (Fig. 1).

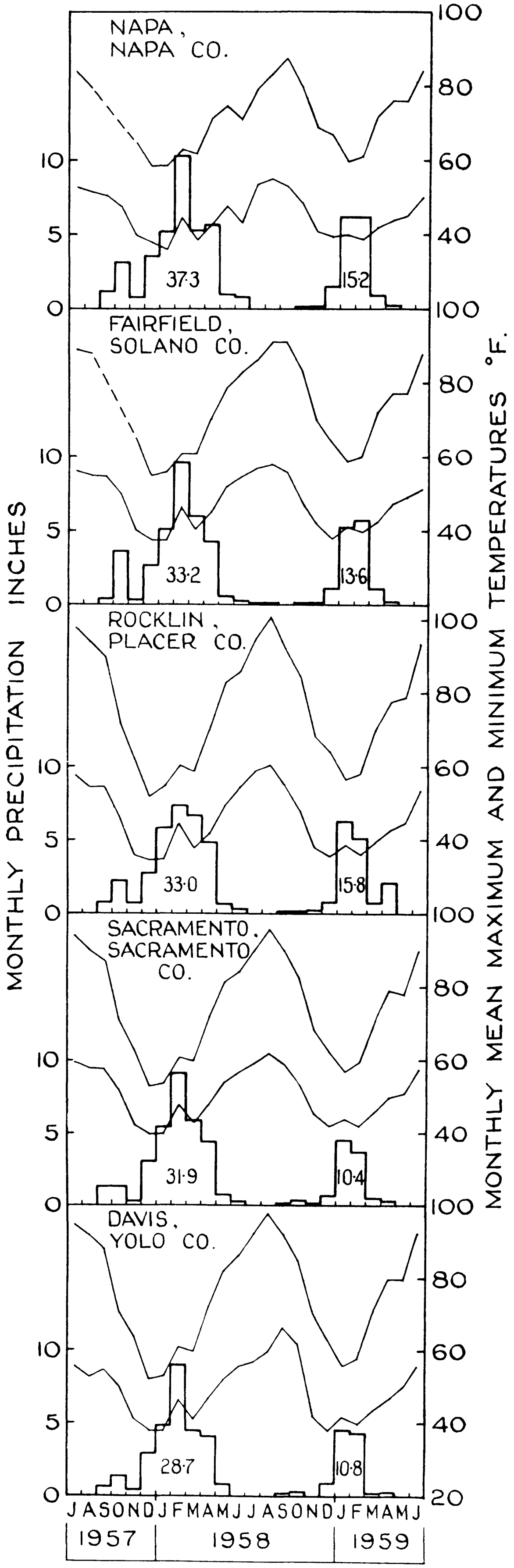

FIG. 1. Monthly precipitation and temperature data from weather stations representative of the seed collection sites.

On the whole, subterranean clover and crimson clover behaved similarly in regard to their seed coat impermeability with a rather rapid decline during the summer. However, the inception of the decline of impermeable seed content of subterranean clover was about one month later than in crimson clover. With the exception of the Napa site, the net result by October was substantially the same: a very low impermeable seed percentage.

These results are in accord with those reported for 4 varieties of subterranean clover by Donald (1959) that 90% or more of the viable seed produced in any one season germinated in the following year. He showed that the impermeable remainder was carried over and germinated in subsequent years in a logarithmically decreasing manner.

Rose clover.—Collections of rose clover seed were made for 7 to 9 months at 7 locations (Table II). The mean impermeable seed content for all times and locations was 96% with a range of 88-100%. Thus the incidence of seed coat impermeability of rose clover is markedly greater than that of the crimson and subterranean clovers. The variation in amount of impermeable seed in rose clover appeared to occur at random, and hence, no trend is evident with regard to location or time of harvest.

It is evident that rose clover seeds develop an impermeable seed coat early in the process of maturing and that impermeability is not reduced significantly during the subsequent growing season in seed remaining attached to dry standing plants.

To learn the effect on seed coat impermeability of contact with the soil surface, such as occurs following shattering of the mature inflorescences, quadrats of rose clover were hand clipped on the University Farm on July 30, 1958. Many seed-containing calyces dropped to the soil surface as a result of the plant disturbance. Subsequently, seed was collected at monthly intervals from standing plants and from the soil surface. A reduction in impermeability to 50% and a corresponding increase in germination to 49% was measured 10 wk after seed shatter occurred (Table III). In comparison, seed remaining attached to standing plants continued to have a high level of impermeable seed throughout the measurement period.

Microclimate of Mature Rose Clover Seed

Temperature is one factor known to affect the impermeability of seeds. The temperatures to

Table II. Effect of duration of field exposure on seed coat impermeability and germination of crimson, subterranean and rose clovers

Location	Sampling date	Crimson Clover		Subterranean Clover		Rose Clover	
		Impermeable %	Germination %	Impermeable %	Germination %	Impermeable %	Germination %
Napa Co.	June 13, 1958	90	10	84	15	94	5
	July 10	62	32	85	14	93	5
	Aug. 12	20	62	70	28	96	2
	Sept. 10	30	46	41	59	97	2
	Oct. 11	2	42	38	61	97	2
	Nov. 7	—	—	29	71	99	0
	Dec. 5	—	—	30	68	100	0
Solano Co.	June 10	74	23	91	9	92	6
	July 10	53	44	68	32	97	3
	Aug. 11	22	70	16	84	97	2
	Sept. 10	18	61	8	92	92	8
	Oct. 11	16	50	1	99	100	0
	Nov. 7	3	34	1	99	98	1
	Dec. 5	—	—	—	—	99	0
	Jan. 16	—	—	—	—	99	1
Placer Co.	June 9	25	74	70	28	97	3
	July 9	12	85	77	23	95	2
	Aug. 11	4	91	26	74	94	4
	Sept. 9	2	88	5	94	96	3
	Oct. 10	1	81	3	96	97	3
	Nov. 7	—	—	—	—	100	0
	Dec. 5	—	—	—	—	100	0
	Jan. 16	—	—	—	—	100	0
Sacramento Co.	June 9	40	60	58	24	88	10
	July 9	14	85	52	28	97	2
	Aug. 11	3	94	10	88	94	3
	Sept. 9	2	86	5	94	95	5
	Oct. 10	1	86	0	97	96	4
	Nov. 7	—	—	—	—	99	1
	Dec. 5	—	—	—	—	100	0
Yolo Co. Rominger	June 10	72	27	74	26	98	2
	July 10	42	58	62	37	99	1
	Aug. 12	18	78	24	75	98	2
	Sept. 9	9	78	10	88	98	1
	Oct. 10	9	78	0	99	90	9
	Nov. 6	—	—	—	—	100	0
	Dec. 4	—	—	—	—	94	6
Yolo Co. Mast	June 10	—	—	—	—	98	1
	July 10	—	—	—	—	99	1
	Aug. 12	—	—	—	—	98	1
	Sept. 9	—	—	—	—	98	2
	Oct. 10	—	—	—	—	98	2
	Nov. 6	—	—	—	—	98	2
	Dec. 4	—	—	—	—	98	2
	Jan. 16	—	—	—	—	100	0
	Feb. 25	—	—	—	—	100	0
Yolo Co. University Farm	June 11	—	—	—	—	97	2
	July 8	—	—	—	—	95	4
	Aug. 12	—	—	—	—	93	6
	Sept. 10	—	—	—	—	88	10
	Oct. 11	—	—	—	—	92	8
	Nov. 6	—	—	—	—	96	4
	Dec. 4	—	—	—	—	93	6
	Jan. 16	—	—	—	—	94	6
	Feb. 25	—	—	—	—	94	6

Table III. Effect of seed position on seed coat impermeability, in place on standing plants vs. shattered to soil surface, University Farm

Sampling date	In Place on Standing Plants		Shattered to Soil Surface on July 30	
	Impermeable %	Germination %	Impermeable %	Germination %
September 10	88	10	79*	20*
October 11	92	8	50*	49*
November 6	96	4	63*	36*
December 4	93	6	55*	44*

* Odds are greater than 99:1 that the value for shattered seed differs from the corresponding value for seed from standing plants.

which rose clover seed may be subjected on hot summer days were measured in a plot of rose clover on the University Farm. Temperature readings were obtained with a potentiometer and 20 microthermocouples. Readings were made in the calyces of rose clover placed on the soil surface, in the heads of dried clover plants approximately 6 in. above the soil surface, and in the air 12 in. above a bare soil surface in shade. All readings except air temperature readings were replicated 4 times. Air temperature readings were in duplicate and checked against a calibrated thermograph.

Readings taken on August 6, 1958 may be considered typical for a hot summer day. Temperatures as high as 140°F (60°C) were encountered inside rose clover calyces lying on the surface of the soil (Fig. 2). Temperatures approaching this value also occurred in clover heads 6 in. (15 cm) above the soil surface. The maximum temperature recorded by the Davis weather station on that day was 104°F (40°C). This temperature is not an unusual maximum at the sites in the interior valley (Csa). In 1958 temperatures of 104°F or greater occurred on 16 days at Rocklin, 3 days at Davis, 1 day at Sacramento, 0 days at Fairfield, and 0 days at Napa. Absolute maximums were 108, 107, 104, 100 and 101°F at the respective sites in 1958.

Cool Season Experiments

Field planting of impermeable rose clover seed.—Seed of rose clover collected from the University Farm in June 1958 was subjected to conditions favorable to germination in the laboratory and the impermeable fraction retained for further testing. Four plots 3 ft square were planted with 100 impermeable seeds each on October 31, 1958, prior to the first effective autumn rain. After the seeds were broadcast and raked in, the plots were enclosed with one in. poultry netting to exclude birds and rabbits. Counts of emerged seedlings were made at 15 day intervals from November 15, 1958 to April 15, 1959 when the soil moisture about the seeds was exhausted. A few seedlings

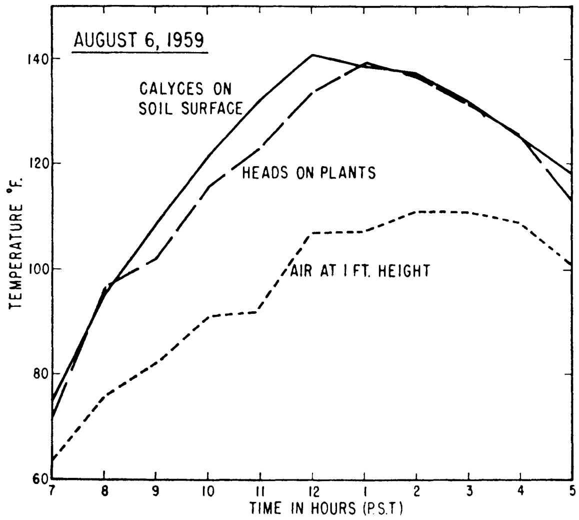

FIG. 2. Temperatures in rose clover calyces and heads on a typically hot summer day in the Sacramento Valley, California.

emerged in January and February, the total for the season being 2.4%. Thus, soil contact under a natural climatic regimen during the mild winter of 1958-59 did not result in the germination of a substantial number of impermeable seeds.

Field exposure of impermeable rose clover seed.—To observe the effect of winter climatic conditions exclusive of soil factors, impermeable rose clover seeds were placed in lots of 400 in plastic mesh bags and laid on a bare soil surface in the field, exposed to the weather, beginning October 1, 1958. Quadruplicate samples of 100 seeds each were drawn at monthly intervals and germinated in the laboratory. The percentage germination for the various periods of exposure were 1 month—1.75%, 2 months—0.75%, 3 months—2.50%, 4 months—3.50%, 5 months—2.25%, and 6 months—0.25%. The above values are net percentages and, therefore, should not be accumulated. The variation would seem to be accounted for by random sampling. The results are essentially the same as those for the field planted impermeable seeds.

Thus the climatic environment during the 1958-59 cool season at Davis, just below and above the soil surface resulted in an insignificant amount of germination of impermeable rose clover seed.

High Temperature Treatment of Impermeable Rose Clover Seed

In consequence of the partial breakdown of rose clover seed impermeability as a result of allowing ripe seed to lie on the soil surface during the summer heat, and the negative results of cool season experiments, high temperature treatments were applied to samples of the rose clover seed collected in 1958. Since temperatures of about 140°F (60°C) were measured in rose clover calyces on the soil surface, this temperature was applied for 2 hr in a forced draft electric oven to impermeable seed from the University Farm in a preliminary test. The impermeable seed content was reduced from the original 100% to 65% with a corresponding increase in germination.

Subsequently, seed of rose clover was tested similarly by heating for one hr at a series of temperatures from 100-220°F (37.8-104.4°C). Lots of 50 seeds were used from the seed obtained in the 7 monthly collections at each of 7 locations in single replication. The data for the 7 locations were averaged for presentation in Table IV.

Seed treated with temperatures from 100-150°F maintained a percentage of impermeable seed of 96% or more, averaged over the collection dates. The June collections dropped below 90% impermeable seed when treated with 160°F, the July, August, September, November and December samples dropped below 90% when treated at 180° F, and October when treated at 190°F. The decline of impermeability with increasing temperature was sharpest in the June seed collection, and sharper for July and August than for the later collections. At 220°F, the highest temperature used, 17-33% of the seed remained impermeable.

The difference between 100% and the percentage of impermeable seed is made up of 2 components, the germination and dead seed percentages. Germination largely accounts for the difference up to 180°F for June and 200°F for the later collections. The June collections exceeded 10% germination at 160°F, and the July, August, September and December collections exceeded 10% germination at 180°F. The October collections exceeded 10% germination at 190°F and September collections at 200°F. The maximum germination percentage occurred at 200°F for the August collections, although this seems to have little ecological significance. On the average the germination percentage droped sharply when temperature levels reached 30 to 40° above the level at which seed coat impermeability began to break down. Beyond these temperatures heat destroyed the viability of a large percentage of the embryos as indicated by the dead seed data in Table IV.

At the time the temperature treatments were performed, the moisture content of the seed ranged from 1.8-6.6%. The moisture in seed collected

TABLE IV. Effect of time of collection on high temperature response of impermeable rose clover seed, one hour treatment. Data are means for 7 locations

Temperature		Impermeable %							Germination %							Dead %						
°F	°C	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Jun	Jul	Aug	Sep	Oct	Nov	Dec
Untreated		94	98	95	95	95	98	99	6	1	5	4	5	2	0	0	1	0	1	0	0	1
100	37.8	93	99	95	95	95	98	98	6	1	4	5	5	1	1	1	0	1	0	0	1	1
110	43.3	94	97	97	93	96	97	98	6	1	2	6	3	2	1	0	2	1	1	1	1	1
120	48.9	97	95	99	95	97	96	98	3	4	1	4	3	3	2	0	1	0	1	0	1	0
130	54.4	94	97	97	95	93	99	99	5	2	1	3	6	1	1	1	1	2	2	1	0	0
140	60.0	93	96	96	94	99	99	98	6	3	2	5	1	1	1	1	1	2	1	0	0	1
150	65.6	90	96	97	93	97	98	98	8	3	1	5	2	1	2	2	1	2	2	1	1	0
160	71.1	89	96	97	93	95	98	98	11	3	3	5	5	1	2	0	1	0	2	0	1	0
170	76.7	80	97	93	94	96	97	96	13	2	4	3	4	2	3	7	1	3	3	0	1	1
180	82.2	45	66	61	79	90	89	86	21	30	30	15	7	9	11	34	4	9	6	3	2	3
190	87.8	26	57	52	78	87	82	84	19	36	35	16	9	16	13	55	7	13	6	4	2	3
200	93.3	21	42	33	57	69	67	62	18	42	50	30	20	28	28	61	16	17	13	11	5	10
210	98.9	19	25	29	27	36	34	30	0	0	0	26	40	20	32	81	75	71	47	24	46	38
220	104.4	17	29	26	26	33	33	28	0	0	0	10	17	8	6	83	71	74	64	50	59	66

in June ranged from 3.8-6.6%, in July 2.8-5.1%, in August 2.9-5.1%, in September 1.8-4.9%, in October 2.8-4.2%, in November 2.4-3.5% and in December 2.2-3.1%. These moisture percentages may be compared with the average moisture content of 2.4% for rose clover tested directly from the field in midsummer. Seed collected in June had the highest average moisture content and showed the greatest variability in temperature response among locations. The germination percentages of the Napa and Solano collections in June were increased by a temperature treatment lower than the remaining collections.

Ecological Significance of Seed Coat Impermeability

The soil-atmosphere interface is a harsh environment because of the rapid fluctuations between extremes of temperature and moisture. The most vulnerable period in the life cycle of an annual legume, germination and early seedling growth, is spent in the proximity of this interface. Species persistence can be materially affected by the degree to which it has the adaptive physiological characteristic of seed coat impermeability. An ample supply of impermeable seed which becomes progressively permeable over a long period of years minimizes the risk of unfavorable temperature and moisture conditions in any one germination and establishment interval, and increases the probability of persistence.

Crimson clover.—In California range plantings crimson clover does not persist more than 2 or 3 yr after planting except on sites having an unusually favorable moisture regime. After maturity there is a rapid decline in impermeable seed content of presently used varieties of this species (Fig. 3). By the advent of the first effective autumn rain, usually in November, the impermeable seed percentage of crimson clover has declined to a very low value. Hence, the lack of persistence appears to be closely associated with the low level of impermeable seed and consequent susceptibility of seedling destruction by drought following initially favorable germination conditions.

The development and use of strains with an inherited ability to maintain a satisfactory amount of impermeable seed over a more extended time would be likely to increase the persistence of this

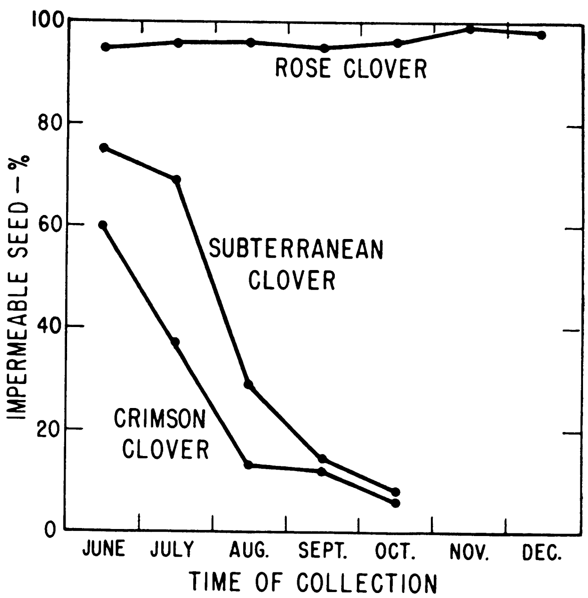

FIG. 3. Impermeable seed percentages of crimson, subterranean and rose clovers, averages of data from all collection sites.

valuable forage plant. The development of a crimson clover strain with a high content of impermeable seed was recently reported by Bennett (1959).

Subterranean clover.—Where well established initially, subterranean clover persists for many years, approaching the long term persistence of rose clover under California conditions. Subterranean clover persists particularly well under continuous close grazing. The fact that a small percentage of impermeable seed is present by the time of the first autumn rains seems to be of less importance with this species than with crimson clover. The persistence of subterranean clover under grazing is controlled more by its growth form, particularly its prostrate branching habit than by seed characteristics. Most important is the fact that as its many axillary inflorescences mature, the supporting peduncles bend towards the ground, and many seed-containing burs bury themselves just below the soil surface. The mechanism is effective in making much seed unavailable to foraging animals. Hence, large seed crops are self-planted with a minimum of consumption by domestic livestock and other fauna, and the meager impermeable seed supply is conserved.

A post-harvest dormancy in which imbibed seeds remain ungerminated has been studied in subterranean clover (Ballard 1958 and Morley 1958). Low temperature, exposure to 0.5-5.0% carbon dioxide atmosphere, exposure to moist soil, coating the seed with absorbent materials such as activated carbons, and removal of testa accelerate release from this dormancy. However, since the dormancy is relatively transitory being important only in the year following seed setting Morley (1958) concluded that long term conservation in subterranean clover is more dependent on production of impermeable seed. Post-harvest dormancy was expressed in less than 2% of the seed collected for the experiments reported here.

Rose clover.—The rose clover strain developed by Love and Sumner (1952) and used in the plantings sampled in this study produces an extremely high percentage of impermeable seed. This has posed no problem in initial establishment using commercially produced seed because the threshing process scarifies enough seed to permit a satisfactory level of germination. Commercial seed lots also contain enough impermeable seed to permit a "second chance" if the quick germinating individuals subsequently succumb to an unfavorable environmental condition (Williams et al. 1957). However, in volunteer stands the break down of seed coat impermeability in a significant portion of the seed produced by this annual species is of importance to continuance of a desirably dense plant population, particularly in the early years after establishment. This aspect of the problem was one of the primary concerns of this study.

The mild winter environment at the University Farm either immediately below or above the soil surface was not conducive to break down of impermeability. However, both field and laboratory results suggest that high temperatures of the summer microclimate, especially at the soil surface, induce a significant amount of permeability. Late summer break down of impermeability would appear to be a more useful mechanism to population continuance than winter break down since early germination provides the developing plant with a full growing season. In comparison a winter-induced germination would result in a much abbreviated growing season and much more intense competition from rapid growing resident annuals, which would have begun their growth cycle with the first effective autumn rains. The consequence of winter break down of impermeability in a Mediterranean-type climate would be a much reduced ability to produce forage and seed.

A point which this series of experiments does not resolve is whether a net accumulation of heat units causes break down of impermeability or whether there is some intrinsic effect of fluctuating temperatures per se. Although fluctuating temperatures have been shown to be effective in reducing impermeability of subterranean clover seed (Aitken 1939) no definitive tests of these hypotheses have been reported for high temperatures.

The outstanding ability of rose clover to persist under grazing and adverse soil and climatic conditions may be concluded to be due in large measure to its prolific production of impermeable seed.

Summary

Ecological aspects of seed coat impermeability were studied in 3 species of annual legumes: crimson, subterranean and rose clovers (*Trifolium incarnatum*, *T. subterraneum* and *T. hirtum*). The pattern of impermeability was explored relative to time after seed maturity in locations under the influence of the Mediterranean-type climate of California. The impermeable seed percentage for crimson clover declined to a low level during the summer months after seed maturation at all locations sampled. Subterranean clover followed a similar though slightly delayed pattern of decline with the exception of one site under a marine

influence which maintained a moderate amount of impermeable seed into the autumn period. Rose clover maintained a very high percentage of impermeable seed throughout the summer, autumn and winter months at all locations sampled.

In further studies with rose clover it was shown experimentally that a mild winter environment was not conducive to the break down of seed coat impermeability of seed placed either immediately above or below the soil surface. It is inferred from field results that the high temperatures of the summer-imposed microclimate, especially at the soil surface, induced a significant amount of permeability. Laboratory tests with elevated temperatures lend support to this thesis. In the light of the ecological significance of seed coat impermeability, it is postulated that summer break down of impermeability has a survival advantage to a winter annual species over winter break down of impermeability. The observed persistence of rose clover under adverse conditions is largely due to its prolific production of impermeable seed.

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