Establishment of Subclover in Relation to Nodulation, Time of Seeding, and Climatic Variations[1]

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Highlight

Pellet inoculated subclover (*Trifolium subterraneum*) seed planted at various autumn dates on a site where effective nodulation was known to be a problem, produced healthy plants when mean ambient air temperature in the 6 weeks following germination was between 49 and 62 F. When mean temperature for the 6-week period was about 45 F, very poor clover stands developed. Seed planted September 10, about one month before a rain, produced a good stand of vigorous clover. This indicated that sufficient viable inoculum had survived in dry soil on the pelleted seed until the rains came. It is recommended that where subclover is adapted, plantings be made in October rather than waiting until after the soil is wet. More vigorous clover grew from seed which was in the ground at the time of the first rain than from seed drilled soon after the rain.

California annual ranges are completely dry during the late spring, summer, and into the fall until the first rain of about an inch occurs. During this long dry season, protein levels are generally too low for even dry adult sheep maintenance levels. After the fall rains when pastures begin to grow they are almost universally deficient in nitrogen.

The establishment of subclover (*Trifolium subterraneum*) on California ranges is a highly beneficial method of increasing winter and spring production and summer protein levels (Love, 1952; Williams et al., 1957; Jones, 1967; Jones and Winans, 1967). However, establishment of subclover stands has not been completely successful in many instances. Williams et al. (1957) recommended seeding during October just prior to the first fall rain. But in some years the rain is late in coming, and many bacteria applied in the inoculant die in the warm, dry soil. Holland (personal communication) therefore, recommended that planting be delayed until after the soil is moist.

Hely et al. (1957) attributed the failure of subclover to establish in certain Australian soils to antagonism from native soil microorganisms. With an abundance of native clovers and their N-fixing bacteria, this antagonism is also a problem in California range soils (Holland et al., 1969). Burton (1964) pointed out that far greater numbers of rhizobia are needed to bring about effective nodulation than was formerly suspected. Thousands, rather than the often quoted 50 to 100 viable rhizobia per seed are needed. Brockwell (1962) reported that the inoculant remained viable on subclover seed stored up to 4 weeks when the inoculant was applied in a solution of gum arabic, and the seed was coated with calcium carbonate. However, such pelleted seed, sown in November into wet soil, has still failed to establish satisfactory subclover stands in some instances. These failures may have resulted from the onset of low temperatures soon after germination.

The purpose of this experiment was to determine the effect of planting date and variations in rainfall and temperature on the establishment of healthy subclover plants.

Procedure

The experimental site was located on a Sutherlin loam soil (Gowans, 1958) on the University of California's Hopland Field Station. The site was characterized by soil with poor internal drainage, a pH 6.1–6.5, a slope 10% to the southeast, and was at an elevation of 600 feet. Plants on the site were annual grasses, such as fox-tail fescue (*Festuca megalura*) and soft chess (*Bromus mollis*), filaree (*Erodium botrys*) and a number of native clovers (*Trifolium* sp.). The site was mowed, raked, disked, and ring-rolled prior to planting each year.

The plantings were made in the fall of three different years as follows: October 3, 10, 17, 24 and November 7, 1966; September 15,

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29, October 13, 27 and November 10, 1967; and September 10, October 1, 22 and November 12, 1968.

For each planting date there were paired plots, seeds inoculated and uninoculated, replicated four times. The seed pelleting method (Holland et al., 1969) was used to apply four times ("4×") the amount of inoculant recommended by the manufacturer. The inoculant was fresh "R" culture peat with 15 billion live bacteria per ounce guaranteed by the Nitragin Company. The recommended rate was 4 ounces peat inoculant per bushel (60 lb.) of subclover seed. The "4×" rate was equivalent to 4 billion bacteria per pound of seed, or about 72,000 bacteria per seed [54,000 subclover seeds per pound (Anonymous 1965)]. The uninoculated seed was also pelleted, and was planted first at each date to prevent contamination.

An individual plot consisted of five rows, 1 foot apart and 10 feet long, in a 5 by 10 foot area. Seeding was done by hand in simulated drill furrows at the rate of 10 lb. per acre. The seeds were covered with about ¼ inch of soil. All plots received single superphosphate just prior to seeding at a rate of 500 lb./acre in 1966 and 1000 lb./acre in 1967 and 1968.

Start of germination was considered as the date sufficient rain fell to start germination, or the date seed was placed into soil wet enough to start germination. Germination did not actually occur the day of rain or seeding, but the germination process began then and continued over a period of several days.

Since the number of seedlings emerging from the ground was not affected by date of planting or inoculation, the important question was whether the plants were N deficient. Healthy plants had dark green leaves, while N deficient plants were small with reddish-yellow leaves. Percent healthy subclover plants were determined by visual estimation in February and by plant counts near the end of the growing season in April or May while plants were still green. Because the two procedures gave similar results, only the result from the plant counts are reported. Temperature and rainfall data were from a weather station located about one mile north and 200 feet higher in elevation than the experimental site. Climate at the study area was similar to that at the weather station.

Table 1. Effect of seeding date, germination date, and mean air temperature (°F) during 6-week period immediately following germination on the percentage of healthy subclover plants in spring.

Planting date	Start of germination date	Mean air temp. 6-week period following germination	Percent healthy subclover
	1966–67 season		
Oct. 3	Nov. 7	49.9	94a[1]
Oct. 10	Nov. 7	49.9	82a
Oct. 17	Nov. 7	49.9	100a
Oct. 24	Nov. 7	49.9	98a
Nov. 7	Nov. 7	49.9	85a
	1967–68 season		
Sept. 15	Oct. 3	60.5	68a
Sept. 29	Oct. 3	60.5	100a
Oct. 13	Nov. 10	45.3	15b
Oct. 27	Nov. 10	45.3	30b
Nov. 10	Nov. 10	45.3	9b
	1968–69 season		
Sept. 10	Oct. 13	54.6	98a
Oct. 1	Oct. 13	54.6	100a
Oct. 22	Oct. 29	49.0	90a
Nov. 12	Nov. 12	44.5	10b

[1] Percentage values followed by the same letter are not significantly different at the 5% level using Duncan's test.

Results

There was good germination and emergence of the subclover irrespective of treatment or planting date. By January 1 each year nearly all subclover plants in the uninoculated treatments were reddish-yellow in color, an indication of N deficiency. These plants were also very small, and the few nodules present were small and light in color. As the season advanced, the plants became so deficient in N that they did not grow beyond the second trifoliate leaf stage.

The percentages of healthy plants from inoculated seed planted in the fall of 1966 varied from 82 to 100 with time of seeding (Table 1). The differences were not statistically significant at the 5 percent level. All the plantings were made in dry soil except the one on November 7. Regardless of the date of planting, germination was about on the same date, since the first effective rain (0.70 inch) fell on November 5 and 6. Apparently sufficient numbers of rhizobia lived from October 3 to November 7 in the lime pellet, under warm dry conditions (Table 2), to effectively inoculate the subclover when the rains finally came. Also, it was apparent that following the rains, temperatures were sufficiently high for nodual formation and N fixation.

High percentages of healthy inoculated plants resulted from the two earliest planting dates in the fall of 1967, when the seeds germinated after a rainfall of 1.18 inches on October 3. Temperatures were obviously warm enough for nodual formation and N fixation. After the rainfall warm temperatures caused surface soils to become too dry by mid-October to germinate

Table 2. Weather data for September–December 1966, 1967, and 1968 at Hopland Field Station, California.

Month	Year	Monthly temperature (°F) Mean Max.	Mean Min.	Mean	Monthly rainfall inches	No. of days with temperature below 32 F
Sept.	1966	86.8	48.7	67.8	0.12	0
	1967	91.1	52.0	71.6	0.01	0
	1968	87.4	51.4	69.4	0.06	0
Oct.	1966	80.6	42.0	61.3	0.00	1
	1967	77.6	44.2	60.9	1.97	0
	1968	75.1	43.5	59.3	1.80	0
Nov.	1966	62.3	40.9	51.6	9.43	4
	1967	66.2	41.3	53.8	2.91	1
	1968	59.7	40.1	49.9	3.74	5
Dec.	1966	54.0	37.4	45.7	7.55	7
	1967	52.5	30.6	41.6	5.60	22
	1968	49.4	33.3	41.4	13.81	14

seeds planted at that time. The clover seedings made October 13, 27, and November 10 resulted in a large number of relatively unhealthy plants. The seed planted at these three later dates germinated at about the same time starting with rains on November 10. Temperatures had become cool following the later date.

In 1968, seed planted September 10 and October 1 germinated about October 13, after several light rains. The seed planted October 22 germinated about October 29. The first three plantings of inoculated seeds, which had relatively mild temperatures following germination produced relatively healthy clover stands. Inoculated seed planted November 12 followed by cooler temperatures produced very unhealthy plants and resulted in a poor stand even though soil moisture was adequate.

Discussion and Conclusions

Gibson (1967a) observed that maximum constant root temperature at which nodules would form was 91 F, and the minimum was about 45 F. The most rapid initial nodulation (2–3 days after inoculation) was observed at 86 F, and plants at this temperature had the highest rate of nodule appearance. Below 72 F there was a marked increase in the time to first visible nodule and a general decline in the rate at which they appeared. At 45 F many plants took as long as 20 days to nodulate or failed to nodulate at all.

In another study Gibson (1967b) noted that nodules formed at a root temperature of 46 F, but fixed little or no N at a root temperature of 55 F, the nodules did not fix N until 21 days after inoculation. The longer the plants were kept at 72 F before transfer to a lower or higher temperature, the greater the amount of N in the plants at the start of any growth period, and the greater the amount of N fixed during that period.

Air temperature and rainfall during the fall months each year of the experiment are given in Table 2. Data by Jones et al. (1963) indicated that monthly mean soil temperatures at the 6″ depth at Hopland were from 0.1 to 5.4 F warmer than air temperatures, with the smallest differences occurring during the coolest period.

It is apparent that temperatures at Hopland were favorable for the growth of subclover during the month of October in all three years; but that during the months of November and December, mean temperatures were near the critical level for rhizobial infection and effective nodulation. The monthly mean temperatures do not tell the whole story, especially for the month of November. In each of the 3 years of the experiment, the first week or 10 days of November were considerably warmer than the latter part of the month. Therefore, the relation between mean air temperature after start of germination and percentage of healthy clover plants was studied. When mean temperatures for periods shorter than 6 weeks following germination were used there was no clear critical temperature value. When mean temperatures for periods longer than 6 weeks were used the temperature-clover vigor relationship was not improved. Mean temperature during the first 6 weeks following germination as related to clover vigor (Table 1) gave values that approached controlled temperatures reported by Gibson (1967a, 1967b) as he related them to nodulation and N fixation. When mean temperatures were near 45 F, the percentage of healthy plants was low, indicating poor N fixation. The unhealthy plants had small white nodules, which presumably were ineffective. When mean temperatures were from 49 to 62 F in the 6 weeks following start of germination, good stands of healthy plants developed.

It appears that two weeks of relatively warm weather following germination made the difference between a high and low percentage of healthy plants. For example, a planting made into dry soil October 22, 1968 began germinating about October 29, and 90% of the plants were vigorous and healthy. A planting made November 12, had only 10% healthy plants. The two mean temperatures for the six weeks following germination were 49 and 45 F, respectively. The earlier seeding not only had the benefit of the higher mean temperature six weeks following germination, but the plants were larger and hardier when frosty nights arrived, and thus better able to withstand frost heaving. In both 1967 and 1968 small seedlings were lifted out of the ground by the frost. The number of nights with frost is given in Table 2. In November and December of 1966, 1967 and 1968 the coldest nights were 25, 23 and 20 F, respectively.

The hot, dry weather that often occurs in September and October before the first fall rains, was expected to contribute to poor stands. However, the planting made September 10, 1968, 33 days before a germinating rain, produced an excellent stand. The planting made September 29, 1967, was not significantly better than a planting made September 15 with a germinating rain coming October 3. Apparently sufficient rhizobia survived to produce the effective nodules required for a healthy stand.

When using subclover seed pellet inoculated with effective N-fixing bacteria at the "4×" rate, apparently the best time to plant at the Hopland Field Station is early October, rather than after the first rain. When seed is placed in the ground early, sufficient bacteria presumably survive, and full advantage is obtained from the rain as soon as it falls. Plantings made a few days after early October rains may not germinate until more rain falls, as the top soil layer usually becomes so dry in a day or two that seeds cannot germinate.

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