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ANAEROBIOSIS AS A STIMULUS TO GERMINATION IN TWO VERNAL POOL GRASSES[1]

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ABSTRACT

Tuctoria greenei and *Orcuttia californica* are two aquatic annual grasses in the small tribe Orcuttieae. They are endemic to seasonal pool habitats in California and Baja California. In years of poor rainfall their seed banks remain dormant until a season of rainfall which is adequate to fill the pool basins. This study attempts to elucidate the factors responsible for cuing germination. *Tuctoria greenei* germination is almost entirely dependent upon a combination of anaerobic conditions and light. It is suggested that in nature, such cues would be relatively specific to the uppermost soil substrate overlain by water. *Orcuttia californica* germination exhibits far less dependence upon anaerobic conditions. There is some indirect evidence that fungi may play an important role in stimulating germination of this species.

CALIFORNIA VERNAL POOLS are a unique ecosystem with many endemic plant species. Such pools are widely distributed throughout the state on relatively level sites underlain by an impervious hardpan (Holland and Jain, 1977). The mediterranean climate leads to winter and spring filling of the pools followed by summer dry-down. Due to urban expansion and agricultural development, much of the vernal pool habitat has been destroyed and it is estimated that only 10% of the pools are extant (Holland, 1978). This is a matter of some concern in that a number of the species occurring in these pools are considered to be rare and endangered.

The majority of vernal pool species are annuals, and a sizable proportion of the species are endemic to California (including Baja California) vernal pools (Holland and Jain, 1977). A remarkable example of this narrow endemism is in the grass tribe Orcuttieae. There are nine species included in the genera *Neostapfia, Tuctoria,* and *Orcuttia,* and all are endemic to pools in this region (Reeder, 1982). The species in this tribe are annuals which germinate under water in the spring and grow submerged for weeks. During this period, the five species of *Orcuttia* produce a set of leaves that float on the pool surface, but such leaves are absent in the monotypic *Neostapfia* and the three species of *Tuctoria.* After the pools have dried down, all of these grasses initiate a new set of foliage which lasts for one to two months until flowering and fruiting are completed.

Due to the unpredictable California climate, pool size varies annually. In years of very depauperate rain, pools may not fill at all. In such years, germination may be limited or in extreme years the entire seed bank may remain dormant. Griggs (1980) described one population of *Orcuttia* which was abundant one year, depauperate the next year and then completely absent for the following three years before it finally returned in abundance in the sixth year.

Such demographic patterns suggest that the seeds of these grasses possess dormancy mechanisms capable of distinguishing years with adequate precipitation from years which are unsuitable for growth. Griggs (1976) noted that germination of several *Orcuttia* species could be induced by burial in "water saturated mud" for 30 days at low temperature. He suggested that these conditions provided an anaerobic environment and that such a germination response would have selective value as a means of timing germination to years in which the pools had filled sufficiently to insure survival and reproductive success. Additionally, Griggs (1980) found that for species of *Tuctoria* and *Orcuttia,* "naked" seeds (i.e., seeds removed from the infructescence) would not germinate regardless of treatment. However, seeds which remained attached to the stalks could be induced to germinate if the infructescences were submerged and incubated for a month, during which time they became covered with a dark mycelium belonging to species of *Alternaria* and *Curvularia.* It was suggested that this germination requirement would also be adaptive as a means of cuing germination to periods

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TABLE 1. *Main effects in a multi-way ANOVA performed on arcsin-transformed data for germination of* Tuctoria greenei *and* Orcuttia californica; *light vs. dark, stratification at 5 C vs. no stratification, air vs. nitrogen, medium treatment of H_2O control, chaff extract, soil extract, or fungicide*

	Significance of *F* value	
	Tuctoria greenei	*Orcuttia californica*
Main effects		
Light	<0.001	<0.001
Stratification	<0.001	<0.001
Nitrogen	<0.001	<0.001
Medium treatment	>0.05	<0.001
Interactions significant		
@ $P < 0.01$	L × N	L × N
	L × S	S × N
	L × S × N	S × M
		N × M
		L × S × N
		L × S × M
		L × N × M
		L × S × N × M

when standing water was present in the pool basins.

The purpose of this study was to test these hypotheses. The germination of seeds of *Tuctoria greenei* (Vasey) Reeder and *Orcuttia californica* Vasey in response to the following factors was considered: anaerobic incubation, cold stratification, light, water extracts from the infructescence chaff and from the soils, and fungicide.

METHODS—Dried stalks with seeds and samples of soil were collected in late July and early August from the dried beds of vernal pools. The seed source for *Tuctoria greenei* was in northern California, south of Chico on the east side of Hwy 99, 100 m south of Pentz Rd. (Butte Co.), and the seed source for *Orcuttia californica* was in southern California, west of Murrieta, on Mesa de Colorado (Riverside Co.). These collections were maintained in paper bags at room temperature and humidity for seven months. Seeds were separated from chaff by gently blowing in a seed blower and counted out under a dissecting microscope.

Fifty seeds were sown in 60 × 15 mm polystyrene petri dishes fitted with two sheets of #42 Whatman filter paper for the control and all treatments. Control dishes were given 3.5 ml deionized H_2O. Treatments consisted of adding 3.5 ml of chaff extract, or soil extract, or fungicide (Dithane M-45) in place of H_2O. Extracts were prepared by soaking either 30 g of chaff in 400 ml of H_2O, or 200 cm^3 of soil in 600 ml of H_2O, overnight in a shaking water bath at room temperature. All treatments and the control were replicated three times.

One set of these treatments and control were incubated in air and another set under anaerobic conditions. This entire experiment was duplicated, one set received a 5 C stratification for two months and another was not stratified. One set of all of these treatments were run in the light and another set in the dark.

Anaerobic treatments were achieved by placing petri dishes in an airtight clear plastic dessicator that was evacuated and filled with nitrogen three times. To insure removal of all oxygen, a small beaker of pyrogallol (Umbreit, Burris, and Stauffer, 1964) was placed inside the dessicator. Other samples were spread out on trays and, to inhibit dessication, were covered with either clear plastic (light treatment) or three layers of black plastic (dark treatment).

Samples receiving stratification were maintained at 5 C for 2 months prior to transfer to the incubators (Percival I-35L). Incubation conditions were a 12-hr photoperiod at 23 C in the light and 13 C in the dark. Germination was scored every 2 weeks for 3 months. After this time, very little additional germination occurred. Samples in the dark were scored under indirect green light. Anaerobic treatments were scored in air but immediately returned to an anaerobic environment.

Osmolality of chaff and soil extracts and fungicide treatments was determined with a Wescor Vapor Pressure Osmometer.

Data were analyzed with a multi-way ANOVA performed on arcsin-transformed data. Multiple range tests were with the Newman-Keuls procedure.

RESULTS—The main treatments of light, stratification and nitrogen had a highly signif-

TABLE 2. *Germination of* Tuctoria greenei *in the light or dark, with or without 2 months stratification at 5 C, under air or nitrogen, on filter paper medium with H_2O control, or chaff extract, or soil extract, or fungicide (N = 3 dishes of 50 seeds each). Within a column there are not significant differences between medium treatments. Means within a row with the same superscript letter are not significantly different at $P > 0.05$*

	Percentage germination							
	Light				Dark			
	No stratification		Stratification		No stratification		Stratification	
Medium	Air	Nitrogen	Air	Nitrogen	Air	Nitrogen	Air	Nitrogen
H_2O control	3^a	70^b	16	67^b	1^a	7^a	7^a	47
Chaff extract	2^a	68^b	8^a	67^b	1^a	5^a	5^a	39
Soil extract	3^a	65^b	19	62^b	0^a	8^a	5^a	41
Fungicide	1^a	62^b	17	76^b	0^a	5^a	4^a	26

icant effect on germination of both *Tuctoria greenei* and *Orcuttia californica* (Table 1). Medium treatment had no effect on *T. greenei* but had a highly significant effect on *O. californica.*

In the light, incubation under anaerobic conditions increased germination of *Tuctoria greenei* (Table 2). Under these conditions, stratification had no effect, however, in air, stratification significantly increased germination for the H_2O control and most media treatments. In the dark, germination for *T. greenei* was quite low except for stratified seeds under nitrogen. These responses account for the highly significant interactions between the main effects of light, stratification and nitrogen (Table 1).

In the light, *Orcuttia californica* germination was markedly inhibited under nitrogen, unless the seeds were first stratified, and then nearly 50% of the seeds germinated (Table 3). In the dark, nitrogen greatly stimulated germination regardless of stratification. In most cases germination was markedly inhibited by chaff extract and fungicide. It is unlikely that this inhibition was due to the molarity of these solutions, since the molarity of these extracts was similar to the soil extract which showed no pattern of inhibition relative to the H_2O controls; chaff extract = 0.04 M, soil extract = 0.03 M, fungicide = 0.03 M. It is unknown whether or not the fungicide had inhibitory effects other than the elimination of fungi; however, it was effective in that regard and all other treatments developed fungal growth. Overall germination response was more complicated than that of *T. greenei*; this is reflected in the large number of interaction terms in Table 1.

These two species also differed in rate of germination. For *Tuctoria greenei* more than 50% of the seeds maintained under nitrogen, without stratification, and in the light, had germinated by the first examination period at 2 weeks. *Orcuttia californica* seeds germinated more slowly; after the first 2 weeks of incubation less than 3% of the seeds in air or nitrogen had germinated. *Tuctoria greenei* seeds that were stratified for 2 months, did not germinate until transferred to the incubators. In contrast, *O. californica* seeds that were stratified, germinated readily in the cold; all germination was completed by the end of the 2-month stratification period and no further germination occurred upon transfer to the incubators.

There was a marked difference in the developmental pattern of seedlings under nitrogen vs. air. For seeds under nitrogen, only the coleoptile emerged. It grew upward and, while under nitrogen, it remained achlorophyllous. For seeds that germinated in air, both the radicle and the coleoptile emerged from the seed, and the coleoptile quickly became chlorophyllous.

DISCUSSION—Germination of the vernal pool grasses *Tuctoria greenei* and *Orcuttia californica* is cued by different environmental factors. The former species exhibits a near obligate requirement for anaerobic conditions, largely in conjunction with light. In the dark, dormancy is overcome to a large degree by a combination of stratification and anaerobic conditions. The selective advantage for such germination characteristics may be to cue germination to periods when the pool basins are filled with water.

During dry years, when the soil is only moistened, the ambient environment around the seeds is unlikely to become anaerobic. Although the water in the pools is not likely to be anaerobic (Keeley and Busch, 1984), it is conceivable that the soil subsurface environment would be anaerobic or hypoxic. Seeds buried too deeply in the soil, beneath the level of light penetration, however, are less likely to germinate due to dark inhibition (Table 2). Considering the very small seed size (ca. 470 μg), it is possible that deeply buried seeds could not sustain the coleoptile long enough to reach the soil surface, a factor potentially capable of

TABLE 3. *Germination of* Orcuttia californica *in the light or dark, with or without 2 months stratification at 5 C, under air or nitrogen, on filter paper medium with H_2O control, or chaff extract, or soil extract, or fungicide (N = 3 dishes of 50 seeds each). Means within a column with the same superscript numeral are not significantly different at P > 0.05. Means within a row with the same superscript letter are not significantly different at P > 0.05*

	Percentage germination							
	Light				Dark			
	No stratification		Stratification		No stratification		Stratification	
	Air	Nitrogen	Air	Nitrogen	Air	Nitrogen	Air	Nitrogen
H_2O control	$28^{a, 1}$	$1^{c, 1}$	30^{a}	48^{b}	$0^{c, 1}$	27^{a}	$7^{c, 1}$	$36^{a, b, 1}$
Chaff extract	$9^{a, b, 2}$	$0^{a, 1}$	$5^{a, 1}$	15^{b}	$0^{a, 1}$	$13^{b, 1}$	$0^{a, 1}$	$8^{a, 2}$
Soil extract	$21^{a, 1}$	$1^{c, 1}$	$16^{a, b}$	23^{a}	$0^{c, 1}$	$8^{b, c, 1}$	$1^{c, 1}$	$33^{a, 1}$
Fungicide	$7^{a, 2}$	$0^{a, 1}$	$0^{a, 1}$	0^{a}	$0^{a, 1}$	0^{a}	$0^{a, 1}$	$1^{a, 2}$

selecting for dark inhibition. The failure of anaerobicly-germinated coleoptiles to green-up, even in the light, suggests that chloroplast differentiation is turned on when the coleoptile reaches the less anaerobic aquatic environment above the sediment. This is very similar to what has been observed in rice. Seeds germinated under anaerobic conditions produce coleoptiles that remain achlorophyllous until exposed to oxygen (Kordan, 1978).

The germination response of *Orcuttia californica* is more complicated. In the light, a quarter of the seeds germinate without further treatment but this percentage is doubled with the combination of stratification and anaerobic conditions. Thus, it appears that anaerobic conditions promote germination just as in *T. greenei,* but it is not mandatory and there are interactions with other factors. For example, there was no germination under nitrogen by unstratified seeds in the light. This is odd and not readily explicable. The contention by Griggs (1976) that fungi play a role in promoting germination seems possible considering the observed inhibition of germination by fungicide.

These germination responses act as relatively specific cues to years when the pool basins are filled with water. Although other aquatic species, e.g., rice and associated weeds, are capable of germinating under anaerobic conditions (Rumpho and Kennedy, 1981), anaerobiosis is in no sense a requirement for germination of those species. In fact, this is generally true for germination of aquatic species (Sculthorpe, 1967). Likewise, fungi and other microbes are known to inhibit germination of seeds, but only rarely have they been demonstrated to play a role in cuing germination (Bewley and Black, 1982).

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