

of Jackson Hole. The moraines and terraces represent two advances of ice during the Pinedale glaciation of late Wisconsin age. They may date from the interval 11,000 to 9,000 years before the present. Less than a foot of loess occurs on surfaces of Pinedale age.

A terrace 8-12 feet above river level probably correlates with the minor Temple Lake glaciation which occurred after the postglacial optimum of climate, less than 4,000 years before the present.

Alluvial surfaces with several to many feet of loess upon them in this region are of Bull Lake (early Wisconsin) age, or older. Surfaces with a few inches to a foot of loess will generally be of Pinedale (late Wisconsin) age.

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### *The Toxicity of Beta vulgaris Fruits as an Inhibitor of Germination of Grass Fruits and as an Autotoxin*

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ALTHOUGH THE presence of germination-inhibiting substance(s) in the fruits of *Beta vulgaris* (sugar beet) has been recognized for about 20 years, the ecological significance of the inhibitor is not resolved. This study is an attempt to assess the importance of the toxin as an autotoxin in the germination of sugar beet, and as an inhibitor of germination of several grass species, *Agropyron cristatum*, *Agropyron dasystachyum*, *Agropyron spicatum*, *Alopecurus arundinaceus*, *Festuca arundinacea*, *Poa bulbosa*, and *Triticum aestivum*. The supervision of this study by Dr. R. Daubenmire of Washington State University is gratefully acknowledged.

Fröschel (1939a, 1939b, 1940) with aqueous extracts of sugar beet fruits demonstrated inhibition of germination in 28 species belonging to 14 families. In greenhouse studies, it was shown that *Melandrium* and rye did not develop when planted with sugar beet in soil (Fröschel and Funke, 1941).

The toxic material has been variously ascribed to ammonia (Stout and Tolman, 1941a and 1941b; Rehm, 1953), excessively high osmotic pressure due to inorganic salts (Duym *et al.*, 1947; Rehm, 1953), unsaturated yellow oil (DeKock *et al.*, 1953), organic acids (Massart, 1957), soluble oxalates (Duym *et al.*, 1947; Miyamoto, 1957), and to unidentified volatile substances (Duym *et al.*, 1947; Fröschel, 1955). Copper, zinc, and lead, as well as gums and tannins, are not present in quantities large enough for an appreciable degree of toxicity (Stout and Tolman, 1941b); auxins are probably not involved (Duym *et al.*, 1947).

Light promotes inhibition (Duym *et al.*, 1947; Evenari, 1949; Fröschel, 1940), but high temperatures (Duym *et al.*, 1947; Stout and Tolman, 1941b) and high pH (Stout and Tolman 1941b) have no effect.

Tolman and Stout (1940) have suggested that the soil adsorbs the toxic material, which is in harmony with their results that germination is more rapid in soil than on filter paper. After filtration of sugar beet fruit extract

through diatomaceous earth or carbon black, Stout and Tolman (1941b) obtained a reduction in the toxic effect. On the other hand, Duym *et al.* (1947) and Lampe (1956) found no adsorption of the inhibitor, and Lampe found no toxic effect to other seeds at 5 mm in soil with a high humus content. According to Evenari (1951) the autotoxicity of sugar beet is manifested by a delay in germination.

### Methods

This study was not concerned with the chemical nature of the toxin(s), but with the role of the toxin, both as an autotoxin and as a possible inhibitor of germination of other seeds nearby in the soil.

Preliminary tests were carried out between sheets of moistened filter paper in petri dishes to find species with suitable germination percentages for further testing with sugar beet fruits in the soil. These tests and all subsequent tests were conducted in a Mangelsdorf germinator in which the temperature was alternated between 30°C for 7 hours and 20°C for 17 hours (optimum temperature regime for germination of sugar beets) (Cholnoky, 1948).

To study the effects of sugar beet toxicity on the germination of other seeds, the seeds of those species found to yield satisfactory germination percentages were planted in lots of 100 at carefully determined distances from the fruits of sugar beet in subsoil of Palouse silt loam having a humus content of 0.5 per cent. In those cases in which the results were not clearly established after tests of 100 pairs, an additional 300 pairs were planted.

Controls were planted singly in lots of 100 to ascertain the germination percentage without the effects of the sugar beet fruits. In addition, to determine if any of the seeds to be tested with sugar beet fruits produced an autotoxic effect, lots of these seeds were planted in pairs, 3 mm apart.

The autotoxicity of sugar beet fruits was tested in a like manner. The fruits were planted in pairs: at 0, 1.5, and 5 mm from one another; in groups of three fruits in contact; and five fruits with four touching a centrally placed one.

The disseminules were selected in such a manner as to avoid any prejudice as to size. Except for cases in which they were planted in groups of three or five, emergence from the soil was taken as germination. Owing to the difficulty in determining the origin of sprouts where three and five fruits were planted together, these were removed from the soil to determine germination.

The disseminules were planted in aluminum trays 10 x 25 x 2 cm. The

soil, passed through a 2-mm screen, was spread in the trays in a level 1-cm-thick layer. Disseminules were placed in rows so that the units in each row were 1 cm apart. The rows, including the seed to be tested and the sugar beet fruit, were spaced at least 2 cm apart with those near the edge of the tray placed at a minimum distance of 1 cm from the edge.

Using plastic strips of known thickness as spacers, the rows of disseminules were placed at the desired distance from each other and pressed lightly into the soil surface. Then, using great care to avoid disturbance of the disseminules, the tray was filled to the top with the finely sieved soil. To compact the soil, a 20-pound weight with a flat surface of 73 mm by 80 mm was gently rested on the surface of the soil. No force was applied to the weight.

The soil in the trays was brought exactly to the moisture equivalent (the percentage of water retained in a soil against a force 1000 times the force of gravity, a close approximation of field capacity) with an atomizer to achieve uniform distribution of the water without disturbing the soil. As the soil was brought to the moisture equivalent daily, only a few grams of water were added at a given time, and thus the movement of toxic material was minimized.

The trays were kept in a Mangelsdorf germinator which contained an open tray of water to maintain a saturated atmosphere. After each test the soil was discarded.

The above procedure was repeated using dune sand instead of loam to isolate the effect of clay on the movement of the toxic material. The dune sand had 96.5 per cent sand, 1.2 per cent silt, and 2.3 per cent clay. The moisture level of the sand was raised to field capacity rather than the moisture equivalent, using the equation  $F.C. = 2.62 + (M.E. + 0.865)$  (Peele and Beale, 1950).

As the origin and development of the toxin is not understood, germination trials under sterile conditions were not attempted, due to the strong possibility that sterilization of fruits and soil may lead to unknown and unpredictable alteration of the toxin and, in turn, to specious results.

Additional tests of sugar beet fruits and disseminules were performed in petri dishes. The sugar beet fruit and the disseminule to be tested with the sugar beet fruit were placed in contact between two sheets of filter paper moistened with distilled water.

In addition, 400 sugar beet fruits were germinated between two sheets of filter paper which was moistened with a leachate that was prepared by passing 100 ml of distilled water through 50 g of sugar beet fruit 10 times.

## Results

The presence of toxic material(s) in the fruits is clearly demonstrated by comparing the germination percentages of the fruits on filter paper moistened with distilled water and those on paper moistened with the sugar beer leachate. The germination of  $39 \pm 5.6$  obtained with leachate is significantly below the 98 percentage obtained with filter paper moistened with distilled water (Table 1). However, the toxin does not affect germination of sugar beer fruit in soil even if as many as five fruits are in contact in a cluster (Table 1).

No autotoxicity was shown in the disseminules to be tested with the sugar beer fruits either by reduction in germination totals or by delay in germination (Tables 2 and 3).

None of the tested species, except *Poa bulbosa*, was significantly affected when germinated with sugar beer fruits using any of the three media, as compared with the germination percentage of the isolated disseminules (Table 2). The inhibition of *Poa bulbosa* is very slight. The time required for germination of the disseminules that were paired with sugar beer was not significantly altered (Table 3).

The germination percentage or the time required for complete germination of the sugar beer fruits was not influenced by the tested disseminules (Table 4).

TABLE 1. GERMINATION PERCENTAGES OF Beta GERMINATED WITH Beta<sup>1</sup>

Media	No. of Fruits about a Point	Distance between Fruits (mm)	Percentage of Total Germination	Days for Complete Germination
Loam	1	---	95	10
	2	5	94	9
	2	1.5	89	11
	2	0	81	10
	3	0	91	8
Sand	3	0	91	8
	3	0	90	8
	5	0	85±5.8	8
Filter Paper (water)	1	---	98	8
	1	---	39±5.6	8
(Beta leachate)	1	---	39±5.6	8

<sup>1</sup> Each percentage figure is the percentage based on 100 fruits except that figures with standard errors are means based on four lots of 100 fruits each.

TABLE 2. GERMINATION PERCENTAGES OF VARIOUS SPECIES GERMINATED WITH Beta<sup>1</sup>

Species	Germination Media									
	Loam		Sand		Filter Paper					
	Not Paired	Self-Paired	Paired with Beta	Paired with Beta	Paired with Beta	Paired with Beta				
	Dis- tance (mm)	% Germ.	Dis- tance (mm)	% Germ.	Dis- tance (mm)	% Germ.	Dis- tance (mm)	% Germ.	Dis- tance (mm)	% Germ.
<i>Agropyron cristatum</i>	---	96	3	94	3	94	0	93	0	94
<i>Agropyron spicatum</i>	---	79	3	82	3	81	0	83	0	85
<i>Festuca arundinacea</i>	---	73	3	70	3	74	0	70	0	70
<i>Poa bulbosa</i>	---	69 ±2.1	3	68 ±0.8	3	62 ±1.2	0	63 ±0.9	0	63 ±2.7
<i>Agropyron dasystachyum</i>	---	83	3	87	0	81	0	83	0	87
<i>Alopecurus arundinaceus</i>	---	78 ±2.2	3	76 ±1.1	0	79 ±1.6	0	77 ±2.4	0	76 ±2.3
<i>Triticum aestivum</i> (embryo facing Beta)	---	93	3	97	0	100	0	96	0	99
(embryo away from Beta)	---	---	---	---	0	91	---	---	---	---

<sup>1</sup> Each figure is the percentage based on 100 seeds except that figures with standard errors are means based on four lots of 100 seeds each.

TABLE 3. DAYS TO COMPLETE GERMINATION OF VARIOUS SPECIES TESTED WITH *Beta*<sup>1</sup>

Species	Germination Media								
	Loam		Sand		Filter Paper		Distance (mm)		
	Not Paired	Self-Paired	Paired with <i>Beta</i>	Paired with <i>Beta</i>	Paired with <i>Beta</i>	Paired with <i>Beta</i>			
Dis- tance (mm)	time	Dis- tance (mm)	time	Dis- tance (mm)	time	Dis- tance (mm)	time		
<i>Agropyron cristatum</i>	---	10	3	10	3	0	9	0	8
<i>Agropyron spicatum</i>	---	14	3	15	3	0	13	0	11
<i>Festuca arundinacea</i>	---	15	3	13	3	0	13	0	12
<i>Poa bulbosa</i>	---	16	3	16	3	0	15	0	13
		±2.1		±2.3			±2.0		±2.4
<i>Agropyron dasystachyum</i>	---	15	3	12	0	0	11	0	8
<i>Alopecurus arundinaceus</i>	---	14	3	16	0	0	15	0	13
		±1.5		±2.1			±1.7		±2.0
<i>Triticum aestivum</i> (embryo facing <i>Beta</i> )	---	6	3	5	0	0	6	0	4
(embryo away from <i>Beta</i> )	---	---	---	---	0	7	---	---	---

<sup>1</sup> Each figure is based on 100 seeds except that figures with standard errors are means based on four lots of 100 seeds each.

TABLE 4. DAYS TO COMPLETE GERMINATION AND GERMINATION PERCENTAGE OF *Beta* GERMINATED WITH OTHER SPECIES<sup>1</sup>

Species	Germination Media								
	Loam		Sand		Filter Paper		Time in Days		
	mm. to <i>Beta</i>	% Germ.	mm. to <i>Beta</i>	% Germ.	mm. to <i>Beta</i>	% Germ.			
<i>Agropyron cristatum</i>	3	88	11	0	93	9	0	96	8
<i>Agropyron spicatum</i>	3	92	11	0	91	11	0	94	8
<i>Festuca arundinacea</i>	3	88	8	0	89	10	0	97	8
<i>Poa bulbosa</i>	3	88	8	0	86	8	0	95	9
<i>Agropyron dasystachyum</i>	0	91	11	0	88	8	0	94	9
<i>Alopecurus arundinaceus</i>	0	89	8	0	93	9	0	96	9
<i>Triticum aestivum</i> (embryo facing <i>Beta</i> )	0	91	8	0	92	6	0	93	8
(embryo away from <i>Beta</i> )	0	86	8	---	---	---	---	---	---

<sup>1</sup> Significance of figures must be evaluated in terms of results of germination of *Beta* alone (95 per cent) and *Beta* paired with itself (81-94 per cent) with a total germination time ranging from 8-11 days (see Table 1).

It is evident that with the species tested there was only one case—*Poa bulbosa*—of even feeble inhibition of germination. It is questionable whether this degree of inhibition would be important in nature, especially considering the short distances between the sugar beet fruits and the bulblets of *Poa*.

Conceivably, the loss of toxicity in soil can be attributed to colloidal adsorption, decomposition of the toxic material, leaching, and dissipation by diffusion of the toxic material away from the disseminules. Owing to the lack of a significant increase in inhibition in sand as compared with loam, colloidal adsorption is doubtful. An hypothesis favoring microbial decomposition cannot be eliminated, even though the soil is low in organic matter, because of the possibility of a rapid increase in the microbial population with

the addition of any organic matter, in this case the sugar beet fruits. The potential loss of toxic material by leaching has been largely eliminated in this study by maintaining the soils at field capacity or moisture equivalent. Diffusion and dilution of the toxic material or microbial decomposition remain as the most likely explanations for the reduced potency in the soil. Apparently, during the extraction of the solutes from the fruits of sugar beet, both in this study and in those by other investigators who reported marked sugar beet toxicity, the toxic material was concentrated and brought up to a sufficiently high level to cause inhibition.

### Summary

A number of investigators, using extracts of sugar beet (*Beta vulgaris*) fruits in nonsoil media, have demonstrated that toxic material(s) are present in the fruits of sugar beet. Many contradictory reports leave the nature of the inhibitor in doubt.

This investigation has sought to determine the possible effectiveness of the toxic material(s) with respect to the sugar beet fruits themselves and to the seeds of other species, when planted in the soil. To test the toxicity, the sugar beet fruits were planted at carefully determined distances from the disseminules of other species in loam, in sand, and on filter paper. By planting the sugar beet fruits opposite each other at carefully determined distances, the autotoxicity of the sugar beet fruits was tested.

One species, *Poa bulbosa*, seems to be slightly inhibited in sand, in loam, and on filter paper by the fruits of sugar beet. However, the degree of inhibition is so very slight that the effectiveness of the toxicity in nature is questionable. Autotoxic effects were observed only following testing with sugar beet leachate.

Considering the low humus content of the loam used and the lack of significantly increased inhibition in dune sand as compared with loam, it is highly doubtful that colloidal adsorption is a cause of the nullification of the toxic effects in the soil. Leaching was not important because the moisture status of the soil was carefully controlled. Reduction of the toxicity in the soil can best be attributed either to diffusion and dilution of the toxic material or to microbial decomposition. Strong inhibitive effects seem to have been achieved by many workers almost entirely through concentration of leachates.

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