Etiology

# A Snowmold Disease of Mountain Big Sagebrush

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## ABSTRACT

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A sagebrush snowmold disease, induced by an unidentified fungus, results in extensive death of mountain big sagebrush in areas of heavy snow deposition in Wyoming, Utah, and Colorado. A fungus with septate, hyaline hyphae with unique knobby wall projections has been isolated that reproduces field symptoms of snowmold in coldroom inoculation tests. It has not been induced to sporulate in culture. In temperature growth studies the isolate grew from −4 to 24 C, with an optimum near 8–12 C. In southern Wyoming, snowpack temperatures in the sagebrush crown zone ranged from −4 to −16 C in early winter; in late winter the snowpack warms and becomes isothermal at 0 C.

*Additional key words*: low-temperature fungi.

Sagebrushes, subgenus *Tridentatae* (Rydb.) McArthur (8) of *Artemisia* L., have the most extensive range of any western shrub, occurring on over one-third of the 332 million ha of western United States shrublands (2,9). These are mostly grazing lands, and because sagebrush is generally considered of low livestock forage value, it is often burned, uprooted, or sprayed with herbicides to destroy it in range conversion management. However, there are palatable, highly nutritious sagebrush varieties valuable on livestock ranges and big game winter ranges (19,20). Sagebrush is also essential habitat for small game and nongame animals that have evolved with the western sagebrush-grass ecosystem (3,21). Height of sagebrush cover can be manipulated in blowing-snow management projects to achieve a specific management objective (13,15). The ubiquitous sagebrush is an aggressive shrub with tremendous genetic diversity and site adaptation, which lends

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importance to soil stabilization, to disturbed land reclamation, and to the beauty of the expansive western landscape (6,7).

Mountain big sagebrush, *Artemisia tridentata* Nutt. ssp. *vaseyana* (Rydb.) Beetle, occurs on the more mesic areas of the sagebrush type, generally above 3,000 m, where annual precipitation exceeds 50 cm. A snowmold-type disease was first found on mountain big sagebrush at the USDA Forest Service, Stratton Sagebrush Hydrology Study Area near Saratoga, WY. The Stratton site is located on the vast sagebrush-dominated high plateaus of south-central Wyoming. In studies at the Stratton site (14), the incidence of snowmold was found to be related to snow depth. Little or no snowmold was present on plants if maximum snow accumulation was less than 40 cm. Incidence of snowmold increased rapidly until maximum accumulation reached 120 cm where 80% of plants were infected. Little or no snowmold was found on Wyoming big sagebrush, *A. tridentata* ssp. *wyomingensis* Beetle & Young, and black sagebrush, *A. nova* Nelson, which grew on windward slopes and ridges where little snow accumulates. A dense cottony mycelial growth develops on the foliage of plants under snow cover during winter and spring. Immediately after snowmelt, the mycelium appears to dry and contract, encasing killed shoots in dense weblike mats. The fungus appears to oversummer, at least in part, in the mycelial state and resumes growth under snow cover. The disease is not active during winters with little or no snow cover. The snowmold-killed zone enlarges on the crown of a plant each winter until eventually the entire plant can be killed. During 11 yr, 1969–1980, sagebrush canopy cover declined 34% at the Stratton study site, caused primarily by the snowmold disease (14). Sagebrush constituted 69% of total plant production before snowmold activity was first noticed in 1973 and declined to 52% by 1980–1981.

No fruiting structures have been positively associated with the snowmold fungus in the field. Other than the ability to grow on plants under snow, this fungus appears unique from those causing snowmolds of turfgrasses and winter cereals, such as *Typhula idahoensis* Remsberg (4), *T. ishikariensis* sensu Årsvoll & Smith (1), *Microdochium nivale* (Fr.) Samuels & Hallett, *Myriosclerotinia borealis* (Bub. & Vleug.) Kohn (11,12), and *Coprinus psychromorbidus* Redhead & Traquair (18), and snow blight of conifers, such as *Nothophacidium abietinellum* (Dearn.) Reid & Cain (10). An ultrastructural study of the sagebrush snowmold fungus (5) has revealed several unique features. Knoblike projections or tubercles cover external hyphal walls. Numerous multimembrane organelles and conspicuous deposition vesicles are present, perhaps related to thick wall and tubercle formation. Abundant glycogen is present when the fungus is grown at low temperatures and lipid bodies become more prominent at higher temperature (20 C). Striated fibers permeate the cytoplasm. The hyphal septa are typical of Ascomycetes, having a simple pore and associated conspicuous Woronin bodies. These characteristics seem to indicate adaptations of a fungus capable of growth at low temperatures and survival in the hyphal state during periods of desiccation.

Our objective in this study was to determine whether the knobby-hyphaed fungus induces a sagebrush snowmold. Our approach was to demonstrate pathogenicity using laboratory inoculation tests, to determine the possible low-temperature growth nature of the fungus using in vitro culture, and to determine the prevalent temperatures in the crown zone of sagebrush under snow cover in the field.

## MATERIALS AND METHODS

**Isolation and pathogenicity.** Specimens of snowmold were collected at the Stratton study area from plants before and just after snowmelt and stored under refrigeration. Isolations were made using 2% water agar and potato-dextrose agar (PDA) medium prepared according to the method of Toussoun and Nelson (17). Untreated tufts of host tissue and mycelium were placed on water agar in petri dishes and cultured at 20 and 1 C. Mycelial samples growing from tufts in these cultures were transferred to PDA plates. Cultures were then reared on water agar at 1 C and hyphal tipped to obtain pure cultures. The pure cultures were maintained on PDA slants at 1 C in the dark.

Sagebrush plants from two counties in Utah (Majors Flat, Deer Creek, and New Canyon sources of mountain big sagebrush) were reared from seed in the greenhouse in 750-ml "root trainer" containers (Spencer-Lemaire, Edmonton, Alberta). This hinged-type container facilitated removal of the root system and insertion into a Mason jar with minimum injury to the roots. Growing medium was heat-treated at 180 C for 2 hr. Plants were started in early May and reared for one growing season in a greenhouse where temperatures did not exceed 25–30 C. Air entering the greenhouse was passed through fiberglass filters to reduce the chance of contamination from airborne fungi. The greenhouse was fiberglass covered and, with use of evaporative cooling, no shading was necessary–thus achieving high light intensity and more normal plant growth. Plants were induced to dormancy by maintaining the greenhouse near outside temperatures and daylength at the end of the growing season.

In mid-March dormant test plants were removed from containers and the roots with soil intact were placed in 1 qt (1.14 L) Mason jars. Vacant space around the root mass was filled with washed silica sand. Water containing 1 gm/L of Plant Marvel (Plant Marvel Labs, Chicago, IL) was added until near field capacity. The mixture contained NPK (12-31-14), B (0.02), Cu (0.05), Fe (0.10), Mn (0.05), Zn (0.05), and S (0.05). Snowmold inoculum was prepared by growing the fungus on PDA at 1 C. Three blocks of agar and fungus (0.5 $cm^3$) were placed among the leaves in the central portion of each test plant crown using care not to injure plants. Agar blocks with no fungus were placed on control plants. All plants were then atomized with distilled water and covered with plastic bags. Rubber bands were used to secure bags around the mouths of the jars to reduce water loss. Thus assembled, plants were incubated in a coldroom at $1 \pm 0.5$ C under 12-hr illumination intervals using fluorescent lamps ($20\ \mu E \cdot m^{-2} \cdot s^{-1}$, Sylvania, GRO LUX). A Clear Creek (Sevier County, UT) source of mountain big sagebrush and basin big sagebrush, *A. tridenta* ssp. *tridentata,* a Manti (Sanpete County, UT) source of Wyoming big sagebrush, and a Pine Valley Ridge (Millard County, UT) source of black sagebrush were tested by the same method, except shoots were collected from plants in the field during winter when they were covered by snow. Stems of shoots were recut under water and then inserted into sand containing the Plant Marvel nutrient solution. Eight plants or shoots from each sagebrush source were inoculated. Four plants of each source were treated as controls. After 130 days bags were removed and the plants evaluated for snowmold development. Disease severity was rated on a 0 to 5 scale according to the percentage of plant foliage covered by fungus mycelium. Five samples of mycelium were removed per plant from each of five inoculated plants selected at random for reisolation and pure culture of the potential pathogen. Mycelium from plants also was examined by light microscopy.

**Temperature growth requirements, in vitro.** An isolate of the Stratton study site snowmold fungus was used to study in vitro growth at temperatures ranging from −4 to 24 C. Standard PDA medium (17) was used as the growth substrate except glycerol (0.5%) was added to medium for −4 C. Medium (pH $6.5 \pm 0.5$) was metered into 50-ml Erlenmeyer flasks in aliquots of 15 ml per flask and autoclaved. Culture flasks were seeded with 3 $mm^3$ blocks of PDA on which the snowmold fungus had been grown at 1 C. Cotton flask-plugs were covered with plastic film to reduce moisture loss. Incubation chambers were custom-built styrofoam boxes with an electronic control system that maintained desired temperatures with less than 0.1 C fluctuation. Growth intervals were in 10-day increments from 0 to 40 days. Three sample flasks were used per interval, and the experiment was repeated four times. At the end of each period, flasks were placed in a water bath at 80 C to melt the agar and vacuum filtered to remove the mycelial mat. Mats were flushed with 200 ml of distilled water at 80 C (using 50-ml increments) to remove remaining agar medium. Filter paper and mycelial mats were oven dried for 24 hr at 100 C. Mycelial dry weight was used as a measure of fungus growth.

**Temperature within the snowpack.** Temperature within the

snowpack was measured during winters 1981–1982 and 1982–1983 at the Stratton study site. Measurements were made on a north-facing hillside in a moderate snow catchment zone where snow is commonly 2 m deep by the end of winter. Thermistors were placed 0, 15, 30, 56, 81, and 114 cm above the ground surface in a vertical plane through two sagebrush plants 56 and 81 cm tall located 19 m apart. Leads from the thermistors ran to a central location about 10 m from the two plants so snow would not be disturbed during the measurement period. A portable instrument employing a modified wheatstone bridge circuit was used to read the sensors (16). Temperatures were measured at about 4-wk intervals throughtout the winter.

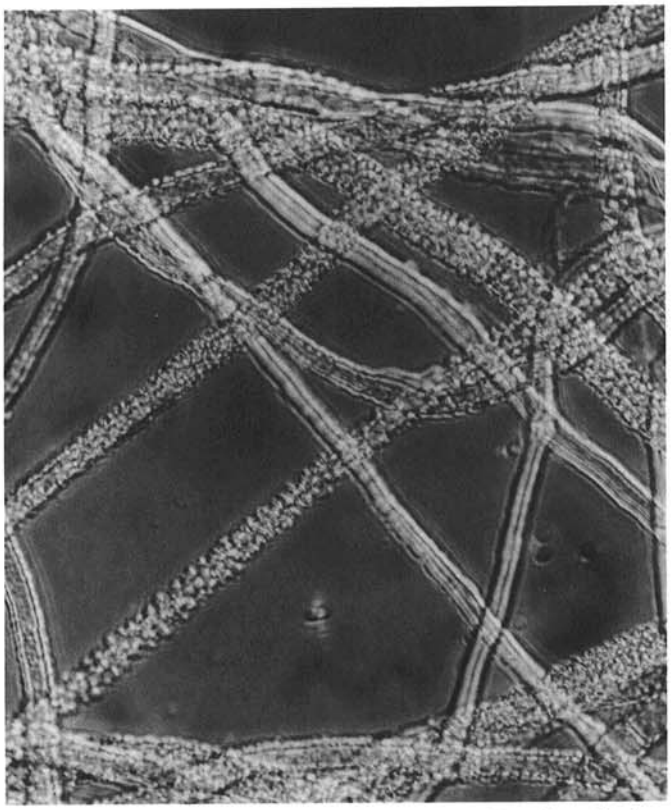

**Fig. 1.** Snowmold fungus hyphae with knobby cell wall projections (Normarski differential interference contrast optics ×1600).

## RESULTS

**Isolations.** Numerous fungi grew from the tufts of mycelium and host tissue when cultured at 20 C. Most common among these were *Cladosporium*, *Ulocladium*, and *Alternaria*. Almost pure cultures of a fungus with dense, cottony mycelium grew from tufts when cultured at 1 C. Pure cultures of this fungus grew slowly, requiring 20–30 days for a colony to reach several centimeters diameter on PDA at 1 C. Microscopic examination revealed septate, hyaline hyphae with numerous external knobby projections along the entire length except the hyphal tips (Fig. 1). This unique characteristic was identical to that found on the hyphae of the mycelial mats formed on the diseased sagebrush plants in the field. Mycelium of cultures became a light gray after several months. Mycelium on diseased plants in the field also becomes a light gray after snowmelt. Hyphae were difficult to cut when making culture or hyphal tip transfers. The fungus remained in the vegetative state on PDA and did not sporulate. The ultrastructure and morphology of this fungus has been studied and the results reported (5).

**Pathogenicity tests.** The isolate with knobby hyphae was selected for pathogenicity tests because it appeared identical to the fungus associated with the snowmold symptoms on sagebrush in the field. After inoculation, the fungus frequently formed a cottony growth that slowly extended over the surface of plant foliage, and after approximately 130 days some plants were completely covered by mycelium (Fig. 2). Leaves and stems became necrotic and turned dark gray after being enveloped by mycelium. No obvious host disease symptoms are present in the approximately 0.5–1 cm


**Fig. 2.** Comparison of control plant (left), and various degrees of snowmold development in pathogenicity tests (rating scale, plants left to right 0, 2, 5, 4).

zone of advancing aerial mycelium (Fig. 3). Plants from all collection sources became diseased after inoculation and exhibited symptoms similar to snowmold observed in the field (Table 1). Plants that were completely overgrown by the fungus mycelium were killed. Control plants developed no signs or symptoms of snowmold. All 25 reisolates from test plants made at 130 days after inoculation had the characteristic knobby hyphae.

**Temperature growth requirements, in vitro.** The snowmold fungus grew on PDA from −4 to 24 C (Fig. 4) at all temperatures tested. Initial (10 and 20 days) dry weight increase was most rapid at 12 and 16 C. After 40 days mycelial dry weight was the greatest for cultures held at 4 and 8 C. At 12 to 20 C growth appeared to be inhibited after 20 to 30 days. At 24 C growth was extremely slow

**Fig. 3.** Nature of the advancing zone of snowmold mycelium over sagebrush foliage.

TABLE 1. Pathogenicity test of the snowmold fungus isolate on *Artemisia*[a]

| Plant source | Plants infected[b] (%) | Disease rating[c] ($\bar{x}$) | ($s_{\bar{x}}$) |
|---|---|---|---|
| *A. tridentata* spp. *vaseyana* | | | |
| Majors Flat (Sanpete County, UT) | 100 | 3.1 | 0.57 |
| Deer Creek (Wasatch County, UT) | 100 | 3.1 | 0.43 |
| New Canyon (Sanpete County, UT) | 100 | 4.3 | 0.36 |
| Clear Creek (Sevier County, UT) | 100 | 4.5 | 0.29 |
| *A. tridentata* spp. *tridentata* | | | |
| Clear Creek (lower) (Sevier County, UT) | 100 | 4.3 | 0.32 |
| *A. tridentata* spp. *wyomingensis* | | | |
| Manti (Sanpete County, UT) | 100 | 1.1 | 0.14 |
| *A. nova* | | | |
| Pine Valley Ridge (Millard County, UT) | 100 | 4.4 | 0.25 |
| Controls | 0 | | |

[a] Disease status 130 days after inoculation.
[b] Percentage of eight plants or shoots tested for each sagebrush source. Controls consisted of four plants from each source.
[c] Disease rating is on a 0 to 5 scale according to percentage of foliage covered by fungus mycelium. $\bar{x}$ = mean, $s_{\bar{x}}$ = standard error of the mean.

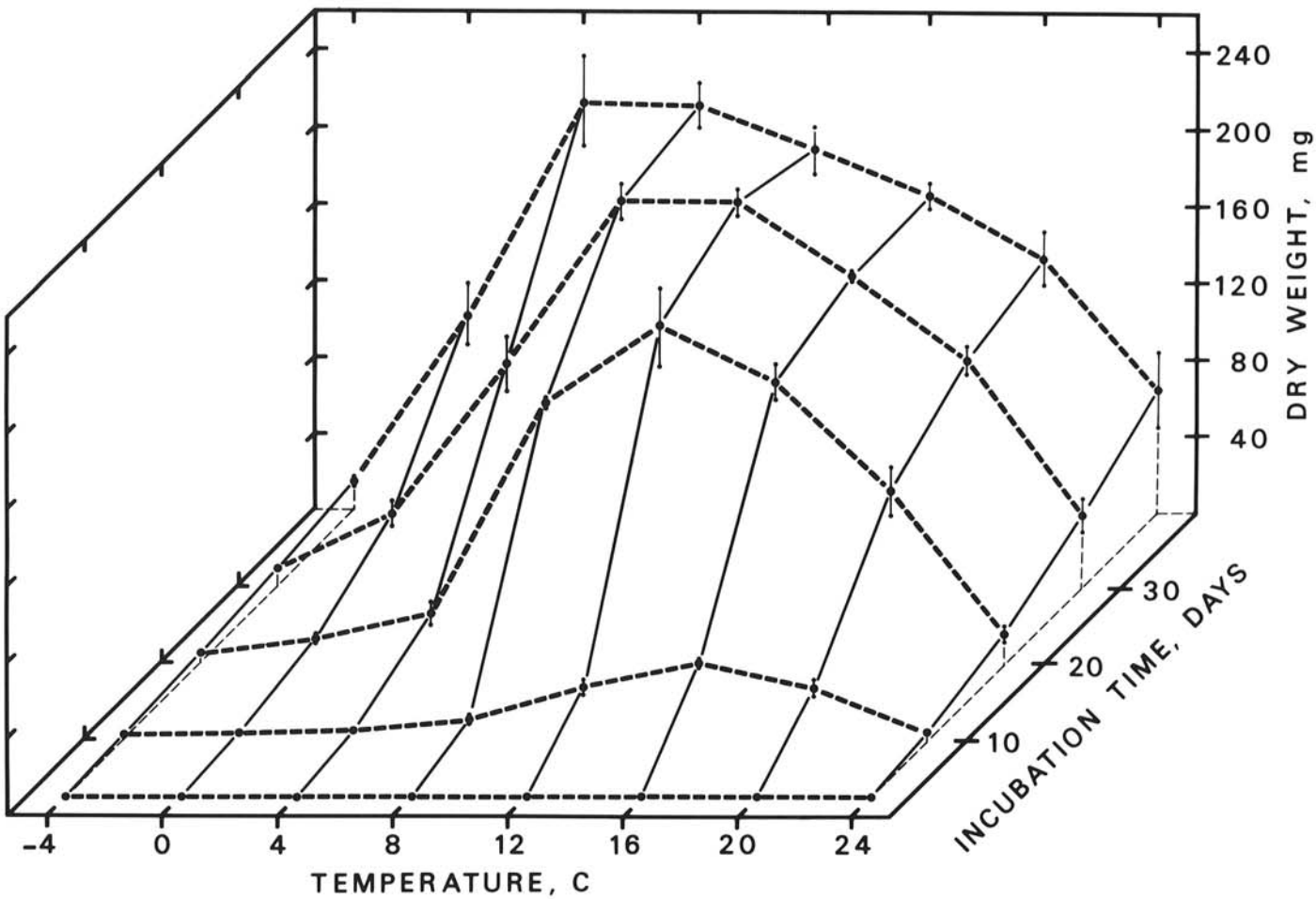


**Fig. 4.** Growth response expressed as dry weight of the snowmold fungus in PDA culture at various incubation times and temperatures. Values are means of four replications of three culture samples. Vertical bars represent ±1 standard error of the mean.

and the mycelial mat was knurled and calluslike.

**Temperature within the snowpack.** Temperature of the snowpack was the lowest and most variable near the snow surface, reflecting prevailing weather. Snow temperature increased to just below freezing at the ground surface as the winter progressed (Table 2). Temperatures were lowest in early winter and then warmed to 0 C throughout the pack during snowmelt. During early snow cover (January through February) temperatures within the sagebrush foliage zone (approximately 15–80 cm above ground level) ranged from −1 to −17 C. At approximately midcrown (40–50 cm) temperatures ranged from −4 to 0 C during the last 60–70 days of snow cover.

## DISCUSSION

The sagebrush snowmold fungus was easily isolated from tufts of mycelium and host tissue from diseased plants when cultured on PDA at 1 C. At 20 C other more rapidly growing fungi overgrew the culture and obscured the causal fungus. The unique knobby character of hyphae simplified associating the isolate with the fungus occurring and growing on sagebrush under snow cover in the field. The fungus did not sporulate under the cultural methods used during this study, indicating that a special environment may be necessary or that compatibility strains may be involved. The snowmold fungus is probably an Ascomycete because mycelium is regularly septate, having simple septal pores with associated Woronin bodies and without clamp connections (5). The widely scattered nature of infection sites on the foliage of plants in the field suggests existence of an airborne spore stage that perhaps develops on colonized duff from old diseased tissue. However, the fungus appears to oversummer, at least in part, in the mycelial state because growth resumes at the margins of killed patches where the fungus exists in the form of mycelial mats on killed foliage (14). There also remains the possibility of an unidentified microsclerotial or pycnial state existing in nature.

In the pathogenicity test, growth of the fungus was slow at 1 C and required about 130 days for mycelium to cover tests plants completely. This is similar to growth behavior observed in the field. Snowmold on the crown of plants in the field results in dead patches that enlarge several centimeters annually during snow cover (14). These patches eventually coalesce and extend to kill entire plants. The nature of this snowmold disease appears to be that of a fungus living saprophytically on host tissue prekilled by toxic exudates from advancing aerial mycelium of the fungus, although this point was not studied in detail. Variable rates of growth of snowmold mycelium over foliage of plants in the coldroom pathogenicity test suggest variable host susceptibility. Mountain big sagebrush, basin big sagebrush, and black sagebrush were affected approximately equally and severely. Wyoming big sagebrush was affected little and appears to be fairly resistant. The small number of plants used to demonstrate pathogenicity, however, limits the precision of resistance interpretation.

In culture, the snowmold fungus is a comparatively low-temperature fungus growing most rapidly at 12–16 C after 10–20 days. After 30–40 days the highest mycelial dry weight increase was at 4–8 C. Growth is slow and abnormal above 20 C. However, its ability to grow at 0 to −4 C, or perhaps lower, indicates that the isolated fungus could grow on sagebrush plants under snow cover. Both in vitro temperature tests and measurement of field snow temperature at the Stratton study site show that snow temperatures during at least the last 60 days of snow cover are favorable to growth of this fungus. Although our studies suggest the fungus has a higher optimal temperature growth potential than the temperature occurring under snow in nature, with ability to grow at 0 C or lower, the fungus could be escaping competition from other fungi. Based on our results, the disease could be most damaging during prolonged springs when snow reaches the isothermal state at 0 C and snowmelt is slow. Because mountain big sagebrush commonly occurs in areas of deep snow accumulation (40 cm or more), the snowmold fungus could be a significant element in limiting host growth and survival.

TABLE 2. Snow temperatures (C) within a sagebrush crown zone at the Stratton Sagebrush Hydology Study Area[a]

| Height (cm)[b] | Date of measurement (1981–1982 winter) | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1/6 | 1/27 | 2/24 | 3/23 | 4/20 | 4/28 | 5/5 |
| 0 | −1 | −1 | −1 | −1 | 0 | −1 | ...[c] |
| 15 | −3 | −2 | −1 | −2 | −1 | −1 | ... |
| 30 | −5 | −3 | −1 | −3 | −2 | −1 | ... |
| 56 | −11 | −4 | −3 | −5 | −4 | ... | ... |
| 81 | −17 | −9 | −5 | −4 | −6 | ... | ... |
| 114 | ... | −8 | −3 | −3 | −6 | ... | ... |

| | Date of measurement (1982–1983 winter) | | | | | |
|---|---|---|---|---|---|---|
| | 1/4 | 3/9 | 3/30 | 4/20 | 5/4 | 5/26 |
| 0 | −2 | −1 | 0 | 0 | 0 | 0 |
| 15 | −4 | −2 | −1 | 0 | 0 | 0 |
| 25 | −6 | −2 | −2 | 0 | 0 | ... |
| 58 | −5 | −3 | −2 | ... | ... | ... |
| 81 | ... | ... | ... | ... | ... | ... |

[a] Mean temperature (nearest degree) for two plants on date indicated.
[b] Height above ground extending in a vertical plane through plant crown.
[c] Thermistor was in air.

## LITERATURE CITED


1. Årsvoll, K., and Smith, J. D. 1977. *Typhula ishikariensis* and its varieties, var. *idahoensis* comb. nov. and var. *canadensis* var. nov. Can. J. Bot. 56:348-364.
2. Beetle, A. A. 1960. A study of sagebrush, section *Tridentatae* of *Artemisia*. Univ. Wyo. Agric. Exp. Stn. Bull. 368. 88 pp.
3. Braun, C. E. 1977. Guidelines for maintenance of sage grouse habitats. Wildl. Soc. Bull. 5:99-106.
4. Bruehl, G. W., and Machtmes, R. 1980. Cultural variation within *Typhula idahoensis* and *T. ishikariensis* and the species concept. Phytopathology 70:867-871.
5. Hess, W. M., Nelson, D. L., and Sturges, D. L. 1985. Morphology and ultrastructure of a snowmold fungus on sagebrush (*Artemisia tridentata*). Mycologia 77:637-645.
6. McArthur, E. D., Giunta, B. C., and Plummer, A. P. 1974. Shrubs for restoration of depleted ranges and disturbed areas. Utah Sci. 35:28-33.
7. McArthur, E. D., and Plummer, A. P. 1978. Biogeography and management of native western shrubs: A case study, section *Tridentatae* of *Artemisia*. Pages 229-243 in: Intermountain Biogeography: A Symposium. Great Basin Nat. Memoirs 2. 268 pp.
8. McArthur, E. D., Pope, C. L., and Freeman, D. C. 1981. Chromosomal studies of subgenus *Tridentatae* of *Artemisia*: evidence for autopolyploidy. Amer. J. Bot. 68:589-605.
9. Plummer, A. P. 1974. Morphogenesis and management of woody perennials in the United States. Pages 72-80 in: Plant Morphogenesis as the Basis for Scientific Management of Range Resources. K. W. Kreitlow and R. H. Hert, eds. USDA, Agric. Res. Serv. Misc. Publ. 1271. 232 pp.
10. Reid, J., and Cain, R. F. 1962. Studies of the organisms associated with "snow-blight" of conifers in North America. I. A new genus of the *Helotiales*. Mycologia 54:194-200.
11. Smith, J. D. 1974. Snow molds of turf-grasses in Saskatchewan. Pages 313-324 in: Proc. 2nd Internat. Turfgrass Res. Conf. Blacksburg, Va. Am. Soc. Agron. and Am. Crop Sci. Soc.
12. Smith, J. D. 1980. Snow molds of winter cereals: Guide for diagnosis, culture and pathogenicity. Can. J. Plant Pathol. 3:15-25.
13. Sturges, D. L. 1977. Snow accumulation and melt in sprayed and undisturbed big sagebrush vegetation. USDA For. Serv., Rocky Mount. For. and Range Exp. Stn. Res. Note RM-348, 6 pp.
14. Sturges, D. L., and Nelson, D. L. 1986. Snow depth and incidence of a snowmold disease on mountain big sagebrush. Pages 215-221 in: Proceedings–Symposium on the Biology of *Artemisia* and *Chrysothamnus*. E. D. McArthur and B. L. Welch, compilers. 9–13 July 1984, Brigham Young Univ., Provo, UT. USDA For. Serv., Intermtn. Res. Sta. Gen. Tech. Rept. INT-200. 398 pp.
15. Sturges, D. L., and Tabler, R. D. 1981. Management of blowing snow on sagebrush rangelands. J. Soil Water Conserv. 36:286-292.
16. Swanson, R. H. 1967. A low-cost instrument to measure temperature or resistance accurately. USDA For. Serv., Rocky Mount. For. and Range Exp. Stn. Res. Note RM-80. 4 pp.
17. Toussoun, T. A., and Nelson, P. E. 1968. A pictorial guide to the identification of Fusarium species according to the taxonomic system

of Snyder and Hansen. Penn. State Univ. Press, University Park and London. 51 pp.
18. Traquair, J. A., and Smith, J. D. 1981. Sclerotial strains of *Coprinus psychromorbidus*, a snow mold basidiomycete. Can. J. Plant Pathol. 4:27-36.
19. Welch, B. L., and McArthur, E. D. 1979. Feasibility of improving big sagebrush (*Artemisia tridentata*) for use on mule deer winter ranges. Pages 451-473 in: Proc. Internat. Arid Lands Conf. Plant Resour., J. R. Goodin and D. K. Northington, eds. Texas Tech. Univ., Lubbock. 724 pp.
20. Welch, B. L., and McArthur, E. D. 1981. Variation of monoterpenoid content among subspecies and accessions of *Artemisia tridentata* grown in a uniform garden. J. Range Manage. 34:308-384.
21. White, S. M., Welch, B. L., and Flinders, J. T. 1982. Monoterpenoid content of pygmy rabbit stomach ingesta. J. Range Manage. 35:107-109.