Growth and Sporulation of *Botryodiplodia hypodermia* in Response to Different Agar Media and Temperatures

J. M. KRUPINSKY, Plant Pathologist, ARS, USDA, Northern Great Plains Research Center, Mandan, ND 58554

ABSTRACT

Krupinsky, J. M. 1982. Growth and sporulation of *Botryodiplodia hypodermia* in response to different agar media and temperatures. Plant Disease 66:481-483.

Growth and spore production of *Botryodiplodia hypodermia* were determined on different culture media. The effects of temperature and light on fungal growth and spore production were evaluated. Three isolates of *B. hypodermia* could not be consistently differentiated from one another in these studies. There was a significant interaction between temperature and medium in three temperature studies. Growth and spore production were greatest on Difco potato-dextrose agar and "homemade" potato-dextrose agar, followed by yeast malt extract agar and V-8 juice agar. Growth and spore production were best at 25 ± 1 C on the potato-dextrose agars, but 21 ± 1 C was the optimum temperature on V-8 juice agar. Sterile wheat kernels added to the surface of these media promoted additional growth and conidial production and provided a convenient source of inoculum.

Additional key words: Siberian elm, *Ulmus pumila*

Siberian elm, *Ulmus pumila* L., has been widely planted in the northern Great Plains during the past three decades mainly because it has a rapid growth rate and is adapted to the region, and planting stock is available. A serious canker-forming pathogen on Siberian elm is *Botryodiplodia hypodermia* (Sacc.) Petr. and Syd. (2,4–6). Inoculum of *B. hypodermia* is needed to develop effective screening procedures for improved resistance to this fungus.

Mention of a trade name is solely to identify materials used and does not constitute endorsement by the U.S. Department of Agriculture.

Accepted for publication 13 August 1981.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

B. hypodermia has been grown on Difco potato-dextrose agar (PDA-D) by Riffle (5); the optimum temperature was 28 C for mean colony growth on PDA-D, 24–28 C for germination of spores on water agar, and 28 C for the germ tube growth on water agar. Ash (C. L. R. Ash, *personal communication*) found that *B. hypodermia* isolated from American elm (*U. americana* L.) sporulated when grown on V-8 juice agar at 25 C. Satour et al demonstrated changes in taxonomic criteria of *B. hypodermia* with changes in carbon sources and observed that light stimulated pycnidial formation (8).

The objective of the research reported in this paper was to determine the culture conditions and medium required for maximum mycelium and spore production of *B. hypodermia* for inoculum.

MATERIALS AND METHODS

Four isolates of *B. hypodermia* from Siberian elm cankers were used: 78-24-3 from a stem canker from Ransom County in southeastern North Dakota, 78-702-1 from a stem canker from Grant County in south central North Dakota, 79-2003-1 from a basal canker from Morton County in south central North Dakota, and 79-2632 from Lac Qui Parle County in Minnesota. These four isolates were selected because they were aggressive in causing stem cankers in greenhouse inoculations.

Standard 100 × 15 mm plastic petri dishes were used for all studies. All media plates in an individual study were inoculated by the same procedure and were sealed with Parafilm. Each combination of medium and isolate was replicated five times.

Mycelial growth was measured as colony diameter at 5, 7, 9, and 12 days of growth. Measurements on the fifth day were used for statistical analysis because mycelia were then beginning to touch the edges of the petri dishes on a few media.

Spore production was measured on the 15th day of growth by blending four plates of the same medium and isolate in 500 ml of distilled water. The suspension of spores was screened twice through four layers of cheesecloth and placed on a stirrer to keep the spores in suspension. Six samples of spore suspension were counted on a hemacytometer to determine number of spores per milliliter.

Analysis of variance was applied to colony diameter measurements and spore counts. Statistical comparisons were made with Tukey's w according to procedures described by Steel and Torrie (9).

From 15 culture media tested in four preliminary studies (Bh-1, 2, 3, and 4)

spore production was best on yeast malt extract agar (YMA) (3) and V-8 juice agar (V-8A; 177 ml of V-8 juice, 2 g of $CaCO_3$, and 20 g of agar per liter). Isolates 78-702-1 and 79-2003-1 were grown on V-8A, YMA, and PDA-D media at 16, 21, 26, and 31 C (study Bh-5) and at 21, 27, and 29 C (study Bh-7). All plates for Bh-5 and Bh-7 were inoculated in the center with cirrhi transferred from sporulating cultures of *B. hypodermia* of the same age. All cultures in these studies were grown under cool white fluorescent light with a 23-hr day length in incubators at the required temperature.

Study Bh-6 was conducted to determine if light was necessary for spore production. Plates containing media, YMA, V-8A, and PDA-D, were inoculated with all four isolates and then enclosed in a light-proof box at 21 ± 1 C for 17 days. After 17 days, the plates were rated for the presence of pycnidia. The fungal growth on plates with pycnidia or pycnidial initials was scraped and blended in a small volume of water that was examined for spores.

Study Bh-8 was conducted to compare the growth and spore production of *B. hypodermia* at different temperatures, on V-8A and on different types of potato-dextrose agar: PDA-D, PDA-BBL (PDA-B; Beeton, Dickinson and Co.), and PDA made in the laboratory (PDA-L) (10). Isolate 78-702-1 was used to inoculate the plates that were grown at 22, 25, and 29 C.

Study Bh-9 was conducted to study the effect of sterile kernels of spring wheat (*Triticum aestivum* L.) on fungal growth and spore production and the feasibility of producing inoculum on kernels. After the plates were inoculated with *B. hypodermia*, 100 sterile kernels were added to the surface of PDA-D and V-8A media plates. PDA-D and V-8A media plates without kernels were also included. Six plates of each treatment were maintained at 21 ± 1 C. Four of these plates were used to compare spore production on the total media plate. Two plates were used to check spore production on the kernels themselves. This was done by placing 20 kernels for each isolate on PDA-D in 20 ml of distilled water on a stirrer for approximately 5 min. The number of spores per milliliter was then counted on a hemacytometer.

RESULTS

There were no significant differences in colony diameters among isolates or media used in temperature studies Bh-5 and Bh-7. Overall colony diameters in Bh-5 were significantly larger at 21 and 26 C than at 16 and 31 C (Table 1). In study Bh-7, colony diameter at 27 C was larger than at 29 and 21 C (Table 1). There were significant interactions between temperature and medium and between isolate and medium in both studies.

Table 1. Growth and spore production of *Botryodiplodia hypodermia* on three media at eight temperatures (C)[a]

Media	Colony diameter (mm) on 5th day of growth[b]					Spore production (no. × 10^3 spores per ml) on 15th day of growth[c]				
	16	**21**	**26**	**31**	**Mean**	**16**	**21**	**26**	**31**	**Mean**
Study Bh-5										
PDA-D	37	69	81	47	59 a	52	106	167	120	111 a
V-8A	43	59	67	34	51 a	65	96	72	1	58 b
YMA	49	71	69	37	56 a	8	67	60	25	40 b
Mean	44 b	66 a	72 a	39 b		21 b	45 a	50 a	24 b	
		21	**27**	**29**	**Mean**		**21**	**27**	**29**	**Mean**
Study Bh-7										
PDA-D		70	83	75	77 a		76	126	126	110 a
V-8A		75	73	65	71 a		78	64	32	58 b
YMA		74	77	64	73 a		68	78	66	70 b
Mean		73 a	78 a	68 a			74 a	89 a	74 a	
		22	**25**	**29**	**Mean**		**22**	**25**	**29**	**Mean**
Study Bh-8										
PDA-D		83	85	70	79 a		88	100	96	95 a
PDA-L		84	86	63	78 a		80	102	78	86 a
V-8A		58	67	65	63 b		88	60	22	57 b
PDA-B		19	29	30	26 c		2	2	4	3 c
Mean		61 ab	67 a	57 b			64 a	66 a	50 b	

[a] Grown under cool white fluorescent light with a 23-hr day length. All colonies were started with the transfer of cirrhi. Means followed by different letters differ significantly at $P = 0.05$.
[b] Each colony diameter in Bh-5 and Bh-7 is the mean of 10 measurements (two isolates and five replications) and in study Bh-8 is the mean of five measurements (one isolate and five replications).
[c] Each spore count is the mean of 12 measurements (two isolates and six counts) for Bh-5 and Bh-7 and of six measurements (one isolate and six counts) for Bh-8.

In study Bh-6, no cirrhi were present after 17 days of darkness. Very few pycnidia were present, and only a few spores from two isolates were found on the PDA-D medium; thus, light is necessary for good spore production.

In study Bh-8, colony diameter at 25 C was significantly larger than at 29 C but not significantly different from that at 22 C (Table 1). There were significant differences in colony diameter growth on the four media at three temperatures in study Bh-8 (Table 1). Colony radial growth on PDA-L and PDA-D was significantly greater than on V-8A. All three media produced significantly greater growth than did PDA-B. On the seventh day, growth was similar on PDA-D, PDA-L, and V-8A and significantly greater than on PDA-B. Relative differences among media in colony diameter varied from one temperature to another, as indicated by a significant temperature × medium interaction.

At 21 ± 1 C, colony radial growth in study Bh-9 was significantly greater on PDA-D plus kernels (87 mm) and V-8A plus kernels (81 mm) than on PDA-D (49 mm) and V-8A (59 mm). There was a significant isolate × medium interaction.

There were no significant differences in spore production among isolates, but there were differences between media and temperatures in studies Bh-5, 7, and 8. Significantly more spores were produced on PDA-D medium than on V-8A and YMA media in study Bh-5, and overall spore production was greater at 21 and 26 than at 16 and 31 C (Table 1). The isolate × temperature and temperature × medium interactions were significant in study Bh-5. Significantly more spores were produced on PDA-D medium than on YMA and V-8A in study Bh-7 (Table 1). The isolate × medium, temperature × medium, and isolate × temperature interactions were significant.

In study Bh-8, spore production was significantly greater on PDA-D and PDA-L than on V-8A (Table 1). Isolates on PDA-B exhibited the least spore production. The overall spore production was significantly better at 22 and 25 than at 29 C (Table 1). A significant temperature × medium interaction was evident in that PDA-D and PDA-L media had highest spore production at 25 C, but spore production on V-8A was highest at 22 C.

In study Bh-9, spore production was significantly greater on PDA-D with kernels than on V-8A with kernels. Both media with kernels had higher spore production than media without kernels. The amount of spore production per kernel was not significantly different among isolates. Overall, 53×10^3 spores per milliliter were produced by *B. hypodermia* present on 20 kernels when placed in 20 ml of distilled water. Thus, 2.6×10^3 spores per milliliter could potentially be obtained when an

individual kernel was placed in 1 ml of water.

DISCUSSION

Considering all studies, PDA-D and PDA-L media ranked highest in both fungal growth and spore production. V-8A and YMA also ranked high for growth and spore production in the four preliminary studies in which 15 cultural media were compared. Although commercial preparations of PDA have been reported as inferior for culture growth of some fungi (1,7), the PDA-D preparation was comparable to the "homemade" PDA-L used in the present study. Although Satour et al (8) reported no spore production on synthetic media containing glucose or sucrose, there was good spore production on media containing these two carbon sources in the present study.

The use of sterile wheat kernels on the surface of media plates significantly increased the rate of fungal growth and spore production of *B. hypodermia*. The sterile kernels overgrown by *B. hypodermia* provided convenient inoculum for greenhouse and field inoculations. Mycelial disks from the margin of a 7-day-old culture have been used for inoculations of Siberian elm previously (2,5). Both mycelia and spores are present in the kernel inoculum, and it is more convenient to remove the kernels with forceps for inoculation than to cut and remove mycelial disks.

Isolates of *B. hypodermia* could not be significantly differentiated from one another in most studies. With two isolates and three media, fungus growth and spore production were better at 21 and 26 C than at 16 or 31 C. The same result was obtained in two other studies at slightly different temperatures. Mean colony diameters on PDA-D in three studies (81 mm at 26 C, 83 mm at 27 C, and 85 mm at 25 C) were much greater than those reported by Riffle (5) (38 mm at 24 C, 40 mm at 28 C, and 37 mm at 32 C). The three highest mean colony diameters reported by Satour et al on the 21 carbon sources tested were 53, 50, and 49 mm for cultures inoculated at 24 C for 4 days (8). Direct comparisons may not be valid because of differences in isolates, type of inoculation, lighting, and other variables.

In the three temperature studies (Bh-5, 7, and 8), the fungal response to temperature differences was not the same on all media. For example, the greatest fungal growth and spore production on PDA-D was at 26, 27, and 25 C. In contrast, the greatest fungal growth and spore production on V-8A was at 21 and 22 C.

The best media preparation of those tested for culture growth and spore production was PDA-D or PDA-L, followed by YMA and V-8A. Sterile wheat kernels added to the surface of these media provided additional growth and spore production as well as a convenient inoculum. Optimum temperatures were approximately 25 ± 1 C for PDA-D and PDA-L and 21 ± 1 C for V-8A and YMA. Since temperature × medium and isolate × medium interactions occurred, a single temperature and medium recommendation is too restrictive.

ACKNOWLEDGMENTS

Appreciation is expressed to Dawn Dunn and Virginia Monson for their technical assistance.

LITERATURE CITED

1. Harding, H. 1979. Effects of culture media containing potato extracts on colony morphology of *Bipolaris sorokiniana*. (Abstr.) Proc. Can. Phytopathol. Soc. 46:57-58.
2. Krupinsky, J. M. 1981. *Botryodiplodia hypodermia* and *Tubercularia ulmea* in cankers on Siberian elm in northern Great Plains windbreaks. Plant Dis. 65:677-678.
3. Krupinsky, J. M., Schillinger, J. A., and Scharen, A. L. 1972. Resistance in wheats to *Septoria nodorum*. Crop Sci. 12:528-530.
4. Otta, J. D., and Bode, F. L. 1972. Siberian elm canker in South Dakota. Plant Dis. Rep. 56:572-574.
5. Riffle, J. W. 1978. Development of canker on *Ulmus pumila* related to month of inoculation with *Botryodiplodia hypodermia*. Phytopathology 68:1115-1119.
6. Riffle, J. W., Wysong, D. S., and Boosalis, M. G. 1980. Canker diseases of elm. NebGuide G80-490. Univ. of Nebraska, Lincoln. 3 pp.
7. Rosenberger, D. A., and Meyer, F. W. 1979. Inhibitory effect of some potato-dextrose agar preparations on germination of pycnidiospores of *Leucostoma* species. Plant Dis. Rep. 63:793-795.
8. Satour, M. M., Webster, R. K., and Hewitt, W. B. 1969. Studies on *Diplodia* and *Diplodia*-like fungi. I. Effects of carbon sources on certain taxonomic characters and on growth in agar culture. Hilgardia 39:601-629.
9. Steel, R. G. D., and Torrie, J. H. 1960. Principles and Procedures of Statistics. McGraw-Hill Book Co., Inc., New York. 481 pp.
10. Tuite, J. 1969. Plant Pathological Methods: Fungi and Bacteria. Burgess Publishing Co., Minneapolis, MN. 239 pp.