Seed-borne Phoma betae as Influenced by Area of Sugarbeet Production, Seed Processing and Fungicidal Seed Treatments

L. D. LEACH and J. D. MACDONALD[1]

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Introduction

The only important seed-borne pathogen of sugarbeet seedlings is *Phoma betae* (Oud.) Fr., [=*Pleospora bjoerlingii* Byford.]. Other seedling diseases are caused by soil inhabiting fungi such as: *Aphanomyces cochlioides* Drechs., *Pythium ultimum* Trow, *P. debaryanum* Hesse, *P. aphanidermatum* (Edson) Fitzp. or *Rhizoctonia solani* Kuhn [=*Thanatephorus cucumeris* (Frank) Donk.]

In Europe, the excellent survey by Dunning (6)[2] in 1972 showed that plant pathologists in 13 countries believed that the most important seedling pathogen of sugarbeets was *Phoma betae* and that seed treatments effective against this pathogen were indispensible. In the United States, however, our experience has been less consistent. Prior to the 1930s when most of our seed was imported from Europe, *Phoma* seedling disease was quite serious and mercury seed treatments were commonly used as the only effective means of control. With the initiation of domestic seed production in the arid southwest, sugarbeet seed was found to be essentially free from *Phoma* (8, 9), thus allowing attention to be focused on the soil-borne seedling pathogens. The use of mercury seed treatments was discontinued and newer, often selective fungicidal seed treatments were substituted to protect seedlings against the other pathogens. However, when domestic seed production was later shifted to Oregon for the production of non-bolting varieties, some seed lots were again found to carry considerable *Phoma* (10). This was cause for some concern as the protective seed treatments which had come into use were only partially effective against *Phoma* and the industry preferred not to return to the use of mercury treatments.

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[1]Professor Emeritus and Research Assistant, respectively, Department of Plant Pathology, University of California, Davis, CA 95616. The writers thank Carol Frate, research assistant, for help with part of the laboratory and greenhouse trials; A. A. Mast, Western Seed Production Company, Phoenix, Arizona, Sam Campbell, West Coast Beet Seed Co., Salem, Oregon, Frank R. Low, British Columbia Sugar Refining Company, Vancouver, B. C., and C. K. Comerford, Irish Sugar Co., Dublin, Ireland for seed samples from Oregon, Arizona, British Columbia and Europe, respectively; and the California Sugarbeet Growers and Processors for financial assistance.

[2]Numbers in parentheses refer to literature cited.

In Oregon, the seed crops are planted in August or early September and sprinkler-irrigated until the fall rains begin. *Phoma* first appears to a limited extent in the fall, as seedling or leaf spot infections, and persists through the winter as infections on leaf or crown tissue. With spring growth and bolting, leaf spots, crown infections, and later, lesions on the seed stalks appear. During periods of rainfall or high humidity, pycnidia of the fungus exude spores in gelatinous masses. These spores are readily spread by splashing rain or overhead sprinklers or, when dry, may become air-borne and by these means come into contact with developing floral parts and result in seed infection. The most important period in terms of seed infection, however, appears to occur during the harvest period. When the seed is ready to harvest, the seed stalks are cut, swathed and allowed to cure in the field for a period of 10 to 20 days before the actual threshing of the seed. If rainfall occurs during this period it further stimulates sporulation of the pycnidia and easily spreads *Phoma* spores to the seed where they may germinate and invade tissues of the seed units.

Because *Phoma* again appeared to be becoming a problem, and since the earlier work with seed-borne *Phoma* had involved European or the older domestic multigerm varieties, it was felt that a re-evaluation of the *Phoma* problem was desirable using current monogerm seed. The objectives of this study were to identify the climatic factors associated with seed infection, to determine the effects of commercial seed processing on the levels of seed transmission, and to compare the effectiveness of protective fungicidal seed treatments on seed lots with different levels of infestation.

Determination of Seed Infection

Evaluation of the amount of *Phoma* carried on beet seed was determined by both laboratory and greenhouse trials. There are three general laboratory methods for determining the percentage of seeds carrying *Phoma*.

1. *Blotter method* — Blotters of the type used in determinations of seed germination are soaked, drained and placed in petri dishes. Five seed units are placed in each dish and incubated for 7 days at 20°C with a cycle of 12 hours of darkness and 12 hours of near ultraviolet light. After incubation the seeds and seedlings are examined with enlargement of 20 to 50 times for pycnidia of *P. betae*.

2. *Potato dextrose agar method* — PDA is poured into petri dishes at approximately 15 ml per dish. Five seed units are placed in each dish, incubated for 7 days at 20° in a cycle of 12 hours darkness and 12 hours of near ultraviolet light. After incubation, all seeds, seedlings and fungal colonies are examined at 20-50× magnification for pycnidia of *P. betae*. Seed analysts usually prefer the PDA method because it is less time consuming with large numbers of samples. Unfortunately, with

some seed lots, contaminating saprophytic fungi may seriously interfere with accurate identification.

3. *Water agar method* — Water agar (1.2%) is poured into plastic petri dishes in a shallow layer. Five to 7 seed units are placed in each dish and incubated for 7 days at 20°C. The dishes are inverted and examined at the level of agar contact with the bottom of the dish for the presence of "holdfasts" as described by Mangan (11, 12). Magnification of 50-100 times is used for observation. To retard seed germination 50 ppm of 2,4-D may be added to the water agar (12). Bugbee (1) recently reported that the use of a selective medium containing boron induced the holdfasts to turn dark brown to black so they could be observed with the naked eye or a 10× hand lens.

The water agar method usually gives the highest readings, especially in the presence of contaminating fungi, and for this reason was felt the most desirable for use in this study. Additional information on seed infection can be secured with either the PDA or water agar methods by pretreating part of the seeds from each lot in NaOCl. A pretreatment consisting of a 5-minute immersion in 0.5% NaOCl was used throughout this study, although European workers favor 10-minute treatments in 1.0% solutions for more heavily contaminated seed. Examination of seeds plated without disinfestation indicates the total number contaminated by *Phoma*, while similar observations of disinfested seeds indicate the amount of deep penetration of *Phoma* into the seed tissues. In the present study, 50 seeds from each seed sample were plated on water agar following disinfestation, and 50 were plated without disinfestation.

The ability of seed-borne *Phoma* to infect seedlings was studied in a series of greenhouse trials, where 150 untreated seeds from each sample were planted in moist pasteurized soil and maintained in growth chambers at approximately 12°C for 28 days. Young seedlings which exhibited damping-off symptoms were removed from the soil, plated on water agar and observed for the characteristic holdfasts to confirm *Phoma* infection. At the end of the experiment all seedlings were lifted, washed, and examined for lesions on root or hypocotyl tissue. All seedlings suspected of infection were plated on water agar to confirm the presence of *Phoma*. In addition, approximately 20 seedlings without lesions were plated to check for incipient infections but no *Phoma* colonies were found.

Using both laboratory and greenhouse methods we have evaluated over 100 seed lots from the United States, Canada, and several European countries. Rather than classifying sugarbeet seed lots solely on the percentage of seed units carrying *P. betae*, the results have led us to propose a system of classification which recognizes four basic infection types and appears to better describe the association of *P. betae* with sugarbeet seed.

Type A. Little (<5%) or no *Phoma* present on the seeds as measured by laboratory and greenhouse trials.

Type B. *Phoma* present on a moderate percentage of the seeds (5-20%) but mostly superficial as shown by its easy removal with the NaOCl treatment. Causes little or no seedling disease in greenhouse plantings.

Type C. Moderate to high degree of contamination (30-60%) which is reduced but not eliminated by NaOCl treatment. Causes a moderate amount (20-40%) of seedling disease in soil trials.

Type D. Seeds heavily infected (>60%) with only slight reduction following NaOCl treatment. Causes fairly high amounts (>40%) of seedling disease.

Influence of Rainfall on Seed Infection Type

The relation of climatic factors to contamination or infection of sugarbeet seed by *P. betae* is illustrated by the results of laboratory and soil germination trials with seed lots from different climatic areas and with seed lots produced in the same area but in different years. Table 1 shows the results of analyses of seed lots produced in 3 areas of western North America.

Table 1. — Relation of precipitation to *Phoma* infection of sugarbeet seed — western North America.

Seed	Precipitation (mm)				Phoma Infection (%)			Inf.
	Days before harvest				Water Agar		Soil	Type
Grown	31-60	1-30	Harv.	Total	Untr.	NaOCl[a]	Sdl. Inf.	(A-D)
Arizona								
1970	8	3	2	13	0	0	–	A
Medford, Oregon								
1971	33	3	0	36	23	<1	1	B
1972	40	tr.	0	40	18	1	4	B
British Columbia								
1967	12	22	20	54	1	1	5	A
1968	42	26	53	121	68	31	37	C-D
1972	81	21	6	109	38	16	9	B-C

[a]Pretreatment — 0.5% NaOCl for 5 min.

The Phoenix area of Arizona is almost rain-free during May and June when beet seed is maturing and being harvested. Laboratory tests with 4 seed lots produced in 1970 showed no *Phoma* on any of the seeds, so they were classified as type A seeds. Because of the mild winters only beet varieties of easy or moderate bolting tendencies can be reproduced in the Phoenix area.

The Medford area of Oregon, where harder bolting varieties are produced, has considerable rainfall during the winter but very little

during July and August when beet seed is maturing and being harvested. During 1971 and 1972 the seed lots tested showed about 20% *Phoma* contamination, which was probably associated with the early rainfall. Very little penetration of the seed units occurred, as shown by inoculum reduction following NaOCl disinfestation and by a very low percentage of seedling infection. This seed was classified as having a grade B infection type.

In the Puget Sound area of British Columbia the results in 3 seasons were quite variable. In 1967 there was little rainfall before or during harvest and very little *Phoma* was found on the seed samples. In soil 5% of the seedlings showed *Phoma* infection, which was more than would be expected from the laboratory tests. In 1968 there were fairly heavy rains, especially late during the harvest period, and the seed lots showed high counts of *Phoma* on the seed and fairly high seedling infection. In 1972 most of the rain fell early during June and very little during the harvest period. The result was seed lots with a moderate amount of *Phoma* contamination on seed, which was much reduced by disinfestation treatments, and fairly low seedling infection. The British Columbia seed was classified as type A in 1967, C-D in 1968 and B-C in 1972.

Similar information was compiled for seed lots produced during 2 years in Italy, France, and Ireland, and is reported in Table 2.

Table 2. — Relation of precipitation to *Phoma* infection of sugarbeet seed — Europe.

Seed	Precipitation[a] (mm)				Phoma Infection (%)			Inf.
	Days before harvest				Water Agar		Soil	Type
Grown	31-60	1-30	Harv.	Total	Untr.	NaOCl[b]	Sdl. Inf.	(A-D)
Italy								
1969	21	57	46	124	13	1	8	B
1970	52	27	20	99	15	1	7	B
France								
1969	79	51	30	160	47	20	21	C
1970	69	95	13	177	37	15	33	C
Ireland								
1968	60	61	149	270	90	70	70	D
1970	97	58	101	256	–	62	73	D

[a]Precipitation data provided by C. Comerford, Irish Sugar Company, Dublin, Ireland.

[b]Pretreatment — 1% NaOCl for 10 min. Laboratory tests by A. Mangan, Dublin, Ireland.

In the area of seed production along the Adriatic coast in Italy, light rainfall occurred during the 60 days preceding harvest and also during the harvest period in 1969 and 1970. The seed lots produced in each year carried a low percentage of *Phoma* contamination which was apparently superficial, since it was effectively removed by a NaOCl treatment. Less than 10% of the seedlings from nontreated seed in

pasteurized soil were infected by *Phoma* and the seed was classified as type B.

In the Mediterranean region of France, however, there was considerably more rainfall than in Italy, especially during the 60 days before harvest in both 1969 and 1970. The seed lots averaged 35 to 50% of the seed infected by *Phoma*, with only about half of the inoculum removed by NaOCl treatment. Seedling infection averaged 20 to 30% and the seed was classified as type C.

In the Limerick area of Ireland there was frequent and moderately heavy rainfall during the 60 days before and quite heavy rainfall during the harvest periods in 1968 and 1970. The seed lots carried high percentages of *Phoma*, which penetrated deeply into the seed units and produced high percentages of seedling infection. In contrast to other European seed lots, those produced in Ireland in 1968 and 1970 were typical of infection type D.

A more detailed study was made of seed lots produced in the Willamette Valley of Oregon because most of the beet seed used in western United States comes from that area. Several seed lots were analyzed from each year's production from 1967 to 1973, with the seed from two years (1971 and 1973), part of which was harvested before and part after a heavy harvest rain, being reported separately.

The results in Table 3 show that in 1967 and 1970 there was very little rainfall during the 60 days before harvest or while the seed stalks were drying in the field. As a result the seed was free or nearly free of *Phoma* infection and classified as type A seed. However, in 1969, when rain fell during the period 31-60 days before harvest, and no later, the seed lots carried only superficial *Phoma* infection and seedling infection was very low, typical for seed infection type B.

In contrast, during 1968 a period of very heavy rain occurred during harvest while the seed stalks were in the swath and a high percentage of the seeds were apparently deeply infected by *Phoma* and resulted in high percentages of infected seedlings, indicating infection type D. An unusual situation occurred in both 1971 and 1973 in which a rain fell during the harvest period. A portion of several seed lots had been harvested before the rain and this permitted direct comparisons of the effect of the rain on subsamples of the same seed lots. Samples threshed prior to the rainy period showed moderate *Phoma* infection while those rained on while curing in the field showed higher infection levels.

The seed lots produced in 1972 showed a surprisingly high percentage of *Phoma* infection in view of the very low rainfall 60 days before and during harvest. This may be accounted for by the fact that all Willamette Valley seed fields are irrigated by overhead sprinklers from late spring until approximately 30 days before the cutting of seed

Table 3. — Relation of precipitation to *Phoma* infection of sugarbeet seed — Willamette Valley, Oregon.

	Precipitation (mm)				Phoma Infection (%)			Inf.
	Days before harvest				Water Agar		Soil	Type
Year	31-60	1-30	Harv.[a]	Total	Untr.	NaOCl[b]	Sdl. Inf.	(A-D)
1967	18	0	tr.	18	9	1	2	A
1968	38	10	106[e]	154	66	56	32	C-D
1969	75[e]	1	1	77	24	1	1	B
1970	22	1	tr.	23	0	0	0	A
1971[c]	78[e]	3	8	89	38	10	18	C
1971[d]	78	3	86[e]	167	48	34	25	C-D
1972	18	3	4	25	45	25	4	B-C
1973[c]	35	tr.	0	35	33	1	8	B-C
1973[d]	35	tr.	20	55	61	14	11	C

[a]Harvest period extended from cutting of the seed stalks to threshing of the dried seed and ranged from 10 days to 30 days depending on the area and season. If the harvest period could not be identified, rainfall for a 30-day period was reported.

[b]Pretreatment — 0.5% NaOCl for 5 min.

[c]Early threshing before rainfall.

[d]Late threshing after heavy rainfall during curing period.

[e]Rainfall events which are felt to have been important in the ultimate seed infection grade.

stalks. In some years this may provide enough moisture to distribute *Phoma* spores and promote infection of immature seed units.

Effect of Seed Processing on Phoma Seedling Infection

Seed lots carrying infection types A or B showed very few seeds carrying *Phoma* after disinfestation with NaOCl, indicating that most of the contamination was superficial. They also produced very few infected seedlings in greenhouse tests. Seed lots type C and D, however, carried high percentages of *Phoma*, with some penetration of the pathogen into seed tissues, and it was important to learn what effects seed processing, in which superficial tissues are milled away, could have on seedling infection.

Tests were run with processed and nonprocessed seed of the same lots to determine what effects processing has on the amount of inoculum carried on seed and the efficiency of fungicidal seed treatments. The results reported in Table 4 show whole seed produced in an area with abundant summer rainfall and having 80-90% *Phoma* infection, and classified as infection type D, compared with a seed lot produced in an area with less summer rainfall and classified as infection type C. Disinfestation of type D whole seed with NaOCl did not reduce the percentage of seed units showing *Phoma* on water agar, but

Table 4. — Effect of processing and treating of type C and D sugarbeet seed on *Phoma* infection.

	Percent Phoma			
	Type D seed		Type C seed	
	Whole	Processed	Whole	Processed
Laboratory Analysis				
Nondisinfested	84	90	66	45
Disinfested [a]	90	88	33	21
Seedling infection				
Untreated	77	73	34	18
Treated[b]	45	61	14	2

[a]Seed disinfested by immersion in 0.5% NaOCl for 5 min.

[b]Seed treatment consisted of Dexon and PCNB applied to seed at rate of 87.5 g a.i./100 Kg seed and 93.7 g a.i./100 Kg seed, respectively.

with type C whole seed, the percentage was reduced one-half by disinfestation.

In greenhouse tests, the type D whole seed showed 77% infected seedlings with no fungicidal treatment, and 45% infected seedlings after seed treatment with Dexon [p-(dimethylamino) benzenediazosodium sulfonate] at 87.5 g a.i. per 100 Kg seed (1.4 oz. a.i. per 100 lb.) + PCNB (pentachloronitrobenzene) at 93.7 g a.i. per 100 Kg seed (1.5 oz. a.i. per 100 lb.). This represents the combination treatment most commonly used in the western United States. In contrast, the type C whole seed showed 34% infected seedlings without seed treatment and only 14% with the seed treatment listed above.

After the seeds of the two lots were commercially processed by milling to remove cortical tissues, water agar trials indicated that the type D seed showed the same level of seed infection as the nonprocessed seed of that grade, but the processed type C seed showed only about 2/3 as much seed infection or carriage as the nonprocessed type C seed.

In greenhouse trials the processed type D seed showed as much seedling infection as the unprocessed seed with or without seed treatment. The untreated processed type C seed, however, showed only half as many seedling infections as whole seed. After treatment with Dexon + PCNB the seedling infection was reduced to a very low level. Similar results have been obtained with several other seed lots and it is quite clear that processing seed with type D infection does not improve the *Phoma* rating of the seed, nor incrase the protection provided by relatively mild seed treatments. This is undoubtedly due to the deep penetration of the tissues of the seed unit by the fungus and the impossibility of removing or exposing the inoculum by milling away the superficial tissues. On the other hand, the results with seed lots

carrying type C infection show that even though a high percentage of the seed units carry *Phoma*, most of the inoculum is in the superficial tissues where it can be reduced by a disinfestant and can be removed or exposed by processing so that fungistatic protectants can reduce seedling infection to a very low level.

Seed Treatments

For many years the standard control for *Phoma* seedling infection in Europe was treatment of the seed with volatile ethyl or methyl mercury fungicides, which were applied as dusts or liquids directly onto the seeds. Similar seed treatment fungicides were also used in North America when *Phoma*-infected beet seeds were imported from Europe. However, because the seed lots produced in the moist climate of Ireland were heavily and deeply infected by *Phoma betae*, even these treatments provided only partial control. In 1951, while on a Marshall Plan assignment in Ireland, the senior author demonstrated the very effective control of seed-borne *Phoma* by immersing, or steeping, the seed for 20 minutes in a 40 ppm solution of ethyl mercury phosphate (EMP) with a seed/solution ratio of 1:8 on a w/w basis (10). This treatment was first used commercially in Ireland in 1952 (5) and continued until 1972 when seed production was transferred largely to the drier Mediterranean area.

In 1954, Gates and Hull (7) also reported very good results with the EMP steep and since 1961 all sugarbeet seed in Great Britain has been steeped in ethyl mercury phosphate (4). In recent years, however, the use of ethyl and methyl mercury compounds as seed treatments has been discontinued in most northern European countries, and except for England, these countries have shifted their beet seed production to areas of lower summer rainfall such as Italy, France, Austria, Yugoslavia, and Turkey. At present over 90% of the sugarbeet seed used in western Europe is genetic monogerm or mechanical monogerm all of which is processed by rubbing and much of which in some countries is also pelleted.

The fungicides which are presently most commonly used as seed treatments in Europe are methoxyethylmercury compounds, thiram, maneb or mancozeb. In North America the most commonly used seed treatment fungicides are Dexon alone or in combination with PCNB although maneb or captan are used in some areas. These fungicides are only partially effective with seed carrying the D type of *Phoma* infection or with unprocessed seed carrying the C type of infection but in our trials gave satisfactory control with processed seed carrying the B or C type. The effects of seed processing and seed treatment with Dexon and PCNB on the infection of sugarbeet seedlings from 4 seed lots carrying the C type of *Phoma* infection are presented in Table 5. In similar trials seed treatment with thiram, maneb, or TCMTB did not differ consistently or significantly from the Dexon-PCNB seed treatments.

Table 5. — Effect of seed processing and treating on *Phoma* infection of sugarbeet seedlings.[a]

Seed	Treatment	Percent infection of seedlings per seed lot[b]			
		1	2	3	4
Whole	None	17.8a	41.1a	42.4a	24.1a
Processed	None	4.2b	30.9b	19.5b	10.3b
Processed	Dexon + PCNB	1.1b	1.8c	4.2c	3.5c

[a]All seed lots carried the C type of *Phoma* infection.

[b]Column entries followed by the same letter do not differ significantly (P = 0.05) by Duncan's Multiple Range test.

Summary and Conclusions

It is well known that contamination or infection of sugarbeet seed by *P. betae* is associated with rainfall during maturity and harvesting of the seed crop. Field observations, meterological records and laboratory analysis of seed samples suggest that rainy and cloudy periods during the 60 days preceding harvest favor the build-up of inoculum and its spread to seed stalks and flower parts. Irrigation with overhead sprinklers during this period may also contribute to the abundance of inoculum and to seed contamination or superficial infection. The deep penetration of seed units by *P. betae* appears to be associated with periods of rainfall while the cut seed stalks are curing in the field.

In the laboratory, culturing the seed units on water agar offers a convenient method of identifying the presence of *P. betae* on the seed. If part of each sample is plated without disinfestation and part after immersion in NaOCl solution, the results indicate whether the seed-borne fungus exists primarily as surface contamination, superficial infection or deep seated infection. The percentage of seedlings showing *Phoma* infection when grown in pasteurized soil is not only related to the total percentage of seed units carying *Phoma*, but also to the infection type as shown in laboratory tests. Therefore, we are suggesting with reference to seed-borne *Phoma*, that seed lots be classified as types A, B, C or D according to the percentage of seed carrying the fungus and also the type of infection as indicated by laboratory and soil tests.

Processing of sugarbeet seed by rubbing to remove cortical tissues strikingly reduced the percentage of *Phoma*-infected seedlings from B or C type of infection (Tables 4 and 5). With seed lots carrying D type of infection, however, processing did not reduce the incidence of seedling infection in soil tests.

Treating sugarbeet seed carrying D type *Phoma* infection with protective fungicides such as captan, Dexon, maneb, thiram, or TCMTB provides at best only partial protection against *Phoma* infec-

tion of seedlings. With unprocessed seed lots carrying C type of infection, treating the seed often provides unsatisfactory protection but with processed seed of the same lots, seed treatments reduce seedling infections to very low levels. Apparently processing of the seed removes the inoculum along with superficial tissues or exposes the inoculum so that fungistatic chemicals effectively protect the germinating seedlings.

The above information indicates that the primary requisite for control of *Phoma* seedling infection is the avoidance of seed lots carrying the D type of infection which are produced only in areas with abundant rainfall shortly before or during the harvest period.

In most years it appears that seed lots produced along the Pacific Coast of North America or in the Mediterranean region of Europe are unlikely to carry deep-seated *Phoma* infection, although some lots may carry fairly high percentages of contamination or superficial infection. After seed processing and treating such lots would be expected to produce only a low incidence of *Phoma* seedling infection. However, while these levels of infection may not greatly influence the ultimate stands in sugarbeet plantings, recent investigations by Bugbee (1, 2, 3) indicate that seed-borne *Phoma betae* may persist in growing plants and initiate serious losses in sugarbeet storage piles as a result of *Phoma* root rot. He also showed that the pathogen could survive in field soils or in storage areas for at least 26 months. These conclusions led him to urge seed processors to use fungicidal seed treatments more effective against *P. betae*. Whether the levels of *P. betae* described here are in fact unacceptable in those areas where beets are stored in piles is not known, but seed lots grown in areas of low summer rainfall would appear to pose little hazard as sources of seedling disease following normal commercial practices.

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