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Yong Sup Cho
University of Minnesota, St. Paul

T. H. King
University of Minnesota, St. Paul

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Factors Affecting Infection and Oospore Formation of Aphanomyces euteiches Drech. In Excised Root Tip of Pisum sativum [1]

YONG SUP CHO and T. H. KING[2]
University of Minnesota, St. Paul

Common root rot of canning peas incited by *Aphanomyces euteiches* Drech. is one of the most serious root diseases of this host. It is the limiting factor in the production of peas in the Midwest at the present time, and little resistance is available in any commercial varieties now in use.

It is suspected that the organism is a free-living saprophyte in the soil and continually cropping an area to peas results in a build-up of the fungus to such a level that severe losses occur. It has been reported to remain dormant in the soil for as long as 10-20 years (14).

Hyphae from zoospores grow through cell walls into the root and the fungus causes a severe cortical rot (10). The fungus produces oospores in the host tissue and it has been suggested that the oospores remaining in plant debris are the principal means of survival. If an adequate and inexpensive means of control of this important disease is to be developed, more information is needed on the factors that affect infection, the sporulation of the organism and the relationships of the host and other micro-organisms in the development of the disease.

The causal organism, *A. euteiches*, was first fully described by Jones and Drechsler in 1925 (10). Their observations established that the fungus enters cortex tissue of roots at the base of stems, where it produces a rapid decay, leaving the vascular elements exposed to attack by other organisms. Thick-walled oospores, considered to be the sexual and dormant stage of the fungus, could be observed in large numbers in the cortical tissue.

Cunningham and Hagedorn (4) exposed pea roots to zoospore suspensions of *A. euteiches* and within 2 hours penetration and infection had occurred. They also observed that germ tubes of zoospores enter through root hairs, and between epidermal cells.

A method for culturing the organism and obtaining sufficient zoospores for use as inoculum was first developed by Schneider and Johnson (15). The fungus was grown on corn meal agar and then transferred to a sterilized decoction of maize kernels in distilled water. After 5-7 days, the decoction was poured off and the mycelium rinsed in three changes of sterilized tap water and allowed to remain in the last rinse. Twelve hours later more than 100,000 zoospores per ml were obtained. In all instances the zoospores were produced asexually and the optimum temperature for production was between 15° and 20° C. Carmen and Lockwood (1, 2) modified this technique slightly and reported maximum production occurred at 24° C, the range being 20° to 28° C.

Various methods have been established for inoculation of peas with zoospores of *A. euteiches* (8, 9, 11, 13). Johnson and Bissonnette (9, 11) inoculated soil in which peas were growing, with a known concentration of zoospores. Lockwood and Ballard (13) followed the same procedure, using silica sand instead of soil. At present the technique followed at the University of Minnesota is that developed by Haglund and King (8). Plants are germinated in sterilized vermiculite for 7 days at 21° C, removed, and the roots washed with running tap water. The plants are then suspended in a zoospore concentration of 100,000 zoospores per plant root for 24 hours at room temperature. After that time they are planted in 5-inch pots containing steamed soil and placed in the greenhouse.

With this method of inoculation, a uniform infection and destruction of seedling roots is obtained with pathogenic isolates of the organism, and is effective in screening varieties and lines of peas for resistance to the disease. The disadvantage of the method is the time required and the greenhouse space necessary.

Very little information is available on the factors affecting infection on the roots of pea plants, the factors that affect oospore formation and germination and the relation of the roots of susceptible and resistant pea plants to sporulation of the fungus. Therefore, studies were made to determine the relationship that exists between the roots of the host, the organism and the factors that influence the host-parasite relationship.

MATERIALS AND METHODS: The four isolates of *A.*

[1] Paper No. 4935, Scientific Journal Series, Minnesota Agricultural Experiment Station, University of Minnesota, St. Paul 1, Minnesota.

[2] Graduate student and Professor, respectively, Department of Plant Pathology and Botany, University of Minnesota.

euteiches used (isolates 125, 304 and 572) were originally obtained from diseased pea plants growing in Minnesota and have been maintained in the Department of Plant Pathology and Botany since 1951. Isolate NY 4 was made available by Dr. W. T. Schroder, New York Experiment Station, Geneva, New York. The isolates have not varied in pathogenicity and isolates 125, 572 and NY 4 are more pathogenic than isolate 304.

Seed of the pea varieties New Era, Alaska, and Perfected Wales were obtained from Green Giant Company, Le Sueur, Minnesota. Seed of the Minnesota selections 494A-1 and 494A-9 were available from the pea improvement project in the Department of Plant Pathology and Botany. These lines and varieties were used because previous work indicated that New Era and Perfected Wales were susceptible to *A. euteiches*. Alaska was moderately resistant and 494A-1 and 494A-9 were the most resistant.

The procedure for obtaining zoospores was that developed by Schneider and Johnson (15). The fungus was grown on a corn-water medium for 7 days at 20° C after which time the mycelial mats were removed, washed in sterile tap water and incubated at 20° C for 24 hours. A hemocytometer was used to determine the concentration of zoospores.

To obtain root tissue for inoculation, seed was surface sterilized with 1% sodium hypochlorite for one minute and allowed to germinate on water agar. After 7 days, root tips measuring 3 to 5 mm in length were removed from lateral roots of plants free of microorganisms. The excised root tips were placed in a zoospore suspension adjusted to a concentration of 30,000 per ml. Excised root tips in sterile tap water without zoospores served as checks.

Each experiment was replicated three times and observations were made every 12-14 hours for a four-day period after inoculation unless otherwise indicated.

RESULTS: Effect of temperature on infection of excised root tips by zoospores. — To determine the most favorable temperature for infection, excised root tips of Perfected Wales peas were placed in a zoospore suspension and incubated at 5°, 10°, 15°, 20°, 25°, 30°, and 40° C for 24 hours. Since it was previously demonstrated that 2 hours was sufficient time for infection (4) it was thought that 24 hours would be ample time to insure that infection occurred. After the incubation period, the root tips were removed, rinsed in sterile distilled water to remove any non-germinated zoospores or mycelial fragments and placed in petri dishes containing sterile tap water and kept at room temperature. The number of oospores that developed in the root tips were counted and were considered an indication of the degree of infection.

At 5° and 40° C no oospores were observed to develop in the tissue. When inoculations were made at 10° C and 15° C, oospores were not observed until 48 hours after the tissue had been removed from the inoculation temperature. The most rapid development occurred at temperatures of 20° C, 25° C and 30° C. From 3 to 10 times as many zoospores formed at 20° and 25° C as at 10°, 15°, and 30° C (Table 1).

TABLE 1. The effect of a 24-hour period of temperature on the infection of excised root tips of Perfected Wales peas by zoospores of *A. euteiches* as shown by the formation of oospores [1]

Temperature (° C)	Hours after removal from inoculation temperature	Isolate			
		NY 4	125	572	304
5	—	—	—	—	—[2]
10	24	0	0	0	0
	48	57	0	0	0
	72	59	19	44	0
	96	59	19	44	0
15	24	0	0	0	0
	48	96	58	55	0
	72	101	106	79	0
	96	102	107	80	0
20	24	173	102	100	20
	48	179	106	111	27
	72	237	108	111	27
	96	251	113	113	27
25	24	197	182	106	27
	48	221	226	113	30
	72	235	226	113	30
	96	234	231	113	30
30	24	33	23	46	6
	48	72	51	46	13
	72	81	52	46	13
	96	89	53	46	13
40	—	—	—	—	—

[1] Average number of oospores formed in 3 replications.
[2] No oospore development.

Differences in oospore formation among the isolates was also found. The NY 4 isolate produced a greater number of oospores than did isolates 125 and 572 and confirms the results of other studies that the isolate is the most virulent of those studied on Perfected Wales peas. The few oospores formed by isolate 304 and the limited temperature range in which they formed is probably an indication of the weak pathogenicity of this isolate.

The results indicate that a temperature of 20° C and 25° C is optimum for infection of excised root tips of Perfected Wales peas.

Effect of temperature on the formation of oospores. — Inoculations were made in the manner described in the preceding study, but the root tips were incubated at 20° C for 24 hours and then transferred to the following temperatures: 5°, 10°, 15°, 20°, 25°, 30°, and 40° C.

Oospores did not develop at 5° and 40° C. At 10° and 15° C the number of oospores was less than the number that developed at 20° C and 25° C. The largest number developed at 20° C and 25° C for each of the four isolates. Isolate NY 4 produced the largest number of oospores. Isolate 304 formed oospores at 20° and 25° C but not at 5°, 10° or 30° C (Table 2). A temperature of 20° C to 25° C evidently is optimum for both infection and oospore formation in the excised root tips of Perfected Wales peas.

Comparison of oospore formation in different sections of the root. — There existed the possibility that cutting of the tissue had an influence on infection and that possibly

TABLE 2. The effect of temperature on the formation of oospores of *A. euteiches* in excised root tips of Perfected Wales peas [1]

Temperature (° C)	Incubation Period (° C)[2]	Isolate NY 4	125	572	304
5	—	—	—	—	—[3]
10	24	0	0	0	0
	48	23	5	0	0
	72	23	5	14	0
	96	23	5	14	0
15	24	3	0	0	0
	48	68	25	35	0
	72	76	60	89	4
	96	75	61	93	4
20	24	145	97	133	0
	48	148	116	137	0
	72	149	114	138	34
	96	151	117	139	34
25	24	190	163	164	0
	48	196	186	173	25
	72	200	186	175	76
	96	203	189	176	74
30	24	73	30	46	0
	48	77	37	61	0
	72	86	37	62	0
	96	84	37	62	0
40	—	—	—	—	—

[1] Average number of oospores formed in 3 replications.
[2] Hours the tissue was at the indicated temperature following a 24-hour inoculation period at 20°C.
[3] No oospore development.

TABLE 3. The effect of temperature on the formation of oospores in the tissue of the root above the root tip of Perfected Wales peas inoculated with zoospores of *A. euteiches*.[1]

Temperature (°C)	Incubation Period (°C)[2]	Isolate NY 4	125	572	304
5	—	—	—	—	—[3]
10	24	0	0	0	0
	48	4	0	0	0
	72	32	0	0	0
	96	32	0	0	0
15	24	0	6	0	0
	48	47	21	20	0
	72	47	27	20	0
	96	47	27	20	0
20	24	34	37	19	6
	48	64	37	28	9
	72	66	37	28	9
	96	66	37	28	9
25	24	49	36	21	8
	48	59	41	37	13
	72	59	41	37	13
	96	59	41	37	13
30	24	46	28	16	0
	48	51	31	22	0
	72	51	31	22	0
	96	51	31	22	0
40	—	—	—	—	—

[1] Average number of oospores formed in 3 replications.
[2] Hours the tissue was at the indicated temperatures following a 24-hour inoculation period at 20°C.
[3] No oospore development.

other portions of the root would react similarly. It was shown by Dukes and Apple (5) that there is an attraction of motile zoospores of *Phytophthora parasitica* (Dast.) var *nicotianae* (B. de Hand) Tucker, to cut and wounded areas of excised roots of tobacco, potato, pepper and egg plant and it is in these areas that infection primarily takes place. Zentmyer (17) demonstrated chemotaxis occurred between excised root tips of avocado and zoospores of *Phytophthora cinnamoni* Randal, the area of elongation being where the majority of the zoospores encysted, germinated and penetrated the host. It was thought desirable to determine if the same type of response occurred in relation to infection and oospore formation in the root tips of pea.

The variety Perfected Wales was again used as the test plant and sections were removed from the area of root hair development. Inoculation and incubation with the four isolates were as previously described.

Fewer oospores formed in the root hair region than in the root tip region (Tables 2 and 3). Isolates 125 and 572 did not form oospores at 10° C. Temperature may have an effect on oospore formation of some isolates as the tissue of the pea matures. Figures 1 and 2 illustrate the differences in oospore formation in the root tip compared with the region of root hair development. It appears possible that morphological and physiological changes that have occurred in this region of the plant may restrict infection and oospore formation.

Effect of root age on the formation of oospores. — Three commercial varieties and two Minnesota breeding lines were used in the study. Root tips were removed from plants 7, 15 and 23 days old. Inoculations were made with isolate NY 4 and incubated at 25° C as previously described. After 24 hours the tissue was removed, rinsed in sterile, distilled water and returned to 25° C. The number of oospores formed were determined after 5 days.

With the exception of the variety Alaska, twice as many oospores formed on root tips 7 days old as in those 15 days old (Table 4). Figures 3 and 4 illustrate the differences in oospore development in excised root tips of 7 and 23-day-old Perfected Wales peas, inoculated with NY 4 and incubated at 25° C.

TABLE 4. Average number of oospores formed in excised root tips of different ages of varieties and breeding lines inoculated with isolate NY 4 and incubated at 25°C.[1]

Plant lines	Ages of Plants (days) 7	5	23
Perfected Wales	221	96	63
New Era	144	79	65
Alaska	85	66	71
494A-1	22	8	0
494A-9	55	19	0

[1] Average number from 3 replications.

Fewer oospores developed in the breeding lines 494A-1 and 494A-9 than in the three commercial varieties. Oospores were not formed in the 23-day-old root tips of these lines.

As the root tip of a pea plant matures, it becomes resistant to the formation of oospores of *A. euteiches*. In addition, the amount of oospore formation in the root

tissue of a plant may be a means of determining the degree of resistance of the plant to common root rot.

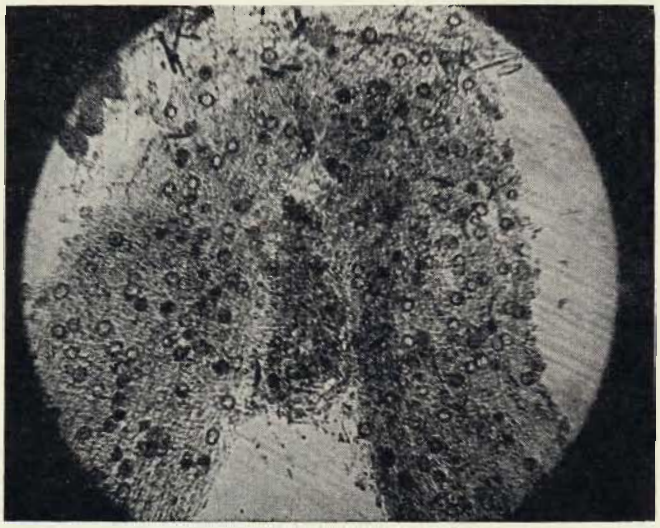

FIGURE 1. Oospore formation in the root tip of Perfected Wales inoculated with isolate 125 and incubated at 20°C.

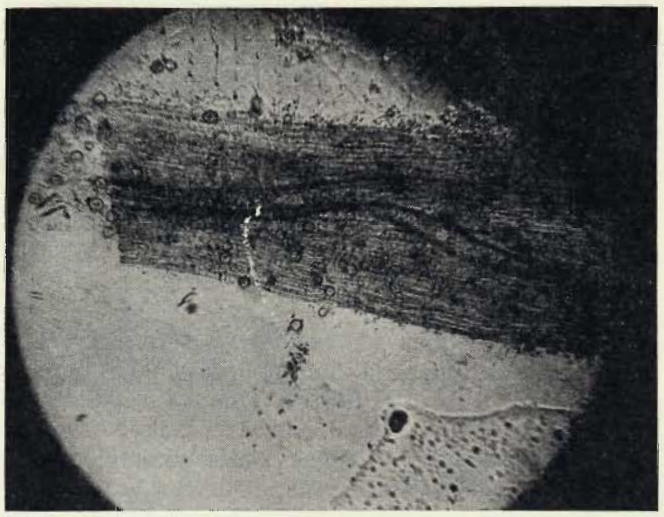

FIGURE 2. Oospore formation in the tissue of the root above the root tip of Perfected Wales peas inoculated with isolate 125 and incubated at 20°C.

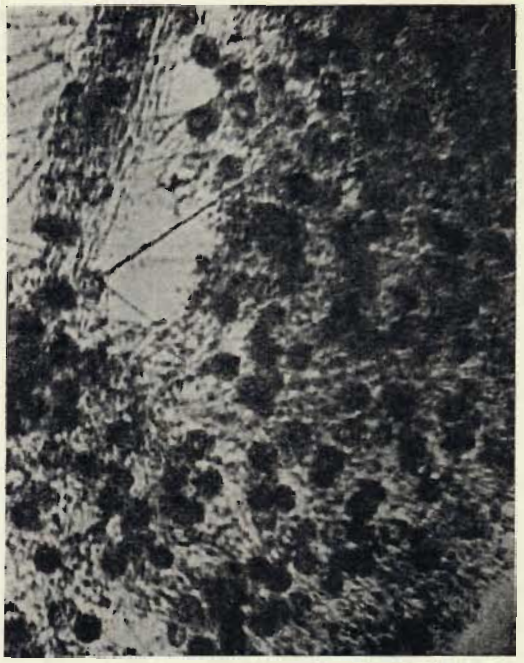

FIGURE 3. Oospore formation in excised root tips taken from 23-day old Perfected Wales plants inoculated with isolate NY 4 and incubated for 96 hours at 25°C.

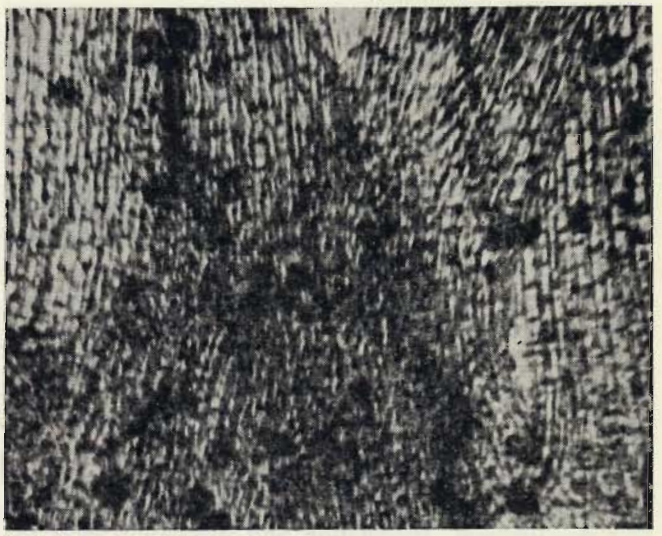

FIGURE 4. Oospore formation in excised root tips taken from 7-day old Perfected Wales plants inoculated with isolate NY 4 and incubated for 96 hours at 25°C.

SUMMARY: Temperatures of 20° C and 25° C were optimum for infection and oospore formation by *Aphanomyces euteiches* in excised root tips of Perfected Wales peas. Both infection and oospore formation were greater in the root tip region when compared to the region of root hair development. It appears that as the regions of the root tip mature there is a reduction in the amount of infection and sporulation by *A. euteiches*. Oospore formation also was greater in root tips excised from 7-day-old pea plants than in root tips from 15- and 23-day-old plants. In addition oospore formation was greatest in the excised root tips of varieties known from previous studies as susceptible to *A. euteiches* than in root tips of Minnesota Selections 494A-1 and 494A-9. The relative quantities of oospores in root tips of pea plants may indicate relative resistance to common root rot.

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