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2,3,5-TRIPHENYLTETRAZOLIUM CHLORIDE AS AN INDICATOR OF GERMINABILITY AND DORMANCY OF GLADIOLUS CORMELS

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HOT-WATER TREATMENT OF GLADIOLUS CORMELS FOR THE ERADICATION OF *FUSARIUM OXYSPORUM* F. *GLADIOLI*[1]

CHESTER N. ROISTACHER,[2] KENNETH F. BAKER,[3] and J. G. BALD[3]

INTRODUCTION

AN OBSTACLE to the use of pathogen-free gladiolus corms as a primary disease control is the universal contamination of commercial varieties, which makes it very difficult to obtain such clean stock by simple selection. As Buxton (1955*a*)[4] has pointed out, "Once the Fusarium has become established in a stock of corms by infection from the soil, eradication is almost impossible." However, two alternative methods remain for obtaining clean stock: 1) New pathogen- and virus-free varieties may be developed from seed. This is a slow and expensive procedure, even when the factor of actual resistance is not involved. 2) Methods may be devised for commercially freeing present varieties from the major pathogens. Some studies with this promising approach are presented in this paper. Of course, with either method, it is necessary to protect the stock from reinfection.

Of the available means for completely freeing planting stock of disease organisms, treatment with chemicals has not been effective because many of the pathogens are carried internally in deep lesions or in the vascular elements. Heat therapy has been investigated by several workers with little success. Massey (1916) found that tube cultures of the hard-rot fungus (*Septoria gladioli* Pass.) and dry-rot fungus (*Stromatinia gladioli* (Drayt.) Whet.) were killed at about 122° F for 10 minutes, and that medium-size corms were not materially harmed by dry heat of 122° F for 90 minutes, or by hot water of 122° F for 30 minutes. However, when corms with dry rot and hard rot were treated as above on the day they were dug, neither pathogen

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[4] See "Literature Cited" for citations, referred to in the text by author and date.

was eliminated, and some heat injury was evident. It should be pointed out that the temperatures were too low, and that for the hot-water treatment "a half-bushel galvanized iron measure was used, the heat being supplied by an oil-stove flame." In our experience it is not possible to adequately treat plant material in so small a volume of water (slightly more than 3 gallons) heated in this way. Finally, corms have been found by several workers to be quite sensitive to heat.

Unpublished data[5] of Lucia McCulloch on hot-water treatments of corms in August to November, 1923, showed that temperatures of 95° to 140° F for 10 to 60 minutes failed to eliminate *Fusarium oxysporum* f. *gladioli* (Massey) Snyd. and Hans. in any combination which did not likewise kill the corms.

Drayton (1929) tested several hot-water and hot-fungicide treatments for elimination of the dry-rot and hard-rot pathogens from corms. Apparently hot water of 125° to 130° F for 30 minutes was ineffective. Treatment for 15 minutes in 5 or 10 per cent Semesan or Uspulun solutions heated to 122° F was more effective, but was considered impracticable because of cost and inconvenience.

Simmons (1949) found that use of high temperatures for curing corms tended to break dormancy. He found that 90 per cent of those exposed to dry heat of 135° F for 15 minutes produced shoots.

Gould (1954) tried curing corms at 110°, 120°, and 130° F for 10 hours, but produced heat injury and apparently did not reduce the incidence of *Botrytis gladiolorum* Timmermans.

Gladiolus growers in San Diego County have treated corms and cormels in hot water of 112° F for 2½ hours just before planting to control root-knot nematode. It was observed that the hot-water treatments induced a more uniform sprouting of the cormels, and treatment at 115° F for 30 minutes was, therefore, adopted for treating cormels to improve germination.

Several floricultural pathologists have also informed the writers of unsuccessful tests (unpublished) with heat treatment of corms to free them of pathogens.

There was, thus, only a suggestion that gladiolus corms and cormels would tolerate heat therapy, and no evidence that pathogens could be successfully eradicated without host injury by such treatment, when Roistacher (1951) began studies in 1950 on heat treatment of gladiolus cormels for the eradication of internal pathogens. This lack of success may have been due to the heat sensitivity of corms as compared with cormels, but the condition of the material treated must also have been involved. Indeed, it is not unlikely that some of the failure of treated stock to grow may actually have been a result of deepened dormancy, rather than death.

The control of Fusarium yellows and basal rot (*Fusarium oxysporum* f. *gladioli* (Massey) Snyd. and Hans.), of the important gladiolus diseases, probably would be most improved by the existence of an available pathogen-free stock. There are several reasons for this: 1) The losses from the disease are increasing despite careful application of present control procedures. The

[5] Courtesy of W. D. McClellan, Horticultural Crops Research Branch, U. S. Department of Agriculture, Beltsville, Maryland.

Fusarium yellows and basal-rot complex is recognized generally as the most serious disease of gladiolus. Magie (1953) reported an average annual loss in Florida of $200 an acre, and stated that nearly 200 million Picardy corms had been shipped into Florida since 1944, mainly to replace rotted corms. Magie (1954) later estimated that 40 to 50 million corms had rotted in Florida each year since 1948, about 97 per cent due to Fusarium. This was enough to plant approximately 1,000 acres or about an eighth of the total annual Florida crop. The situation in California is also serious. Growers have moved from infested to clean land, until available new areas are now almost gone. In the warmer sections of the state, Fusarium builds up very rapidly after introduction with infested planting stock. 2) Satisfactory resistant gladiolus varieties adapted to local growing areas are not available in many colors. This type of control, usually effective against Fusarium wilt diseases, is not much help against gladiolus yellows. 3) The causal Fusarium will persist in infested soil for many years, although the inoculum potential may decline somewhat after three to four years. 4) The Fusarium invades the vascular system of the plant, and because of this internal location is not effectively controlled by chemical treatment.

The cormel seemed to offer more favorable material for hot-water treatment than the corm. An investigation was undertaken to determine the heat tolerance of the gladiolus cormel, and of pathogenic fungi inside it. By increasing the margin between the exposure for thermal inactivation of cormel and of parasite, a method for the production of pathogen-free gladiolus stock was provided. Factors which would increase the thermal tolerance of the cormel were also studied.

THERMAL RELATIONS OF CORMELS

The following procedure was employed in the tests reported here. Cormels, in lots of 25 to 100, were placed in labeled cheese cloth or plastic screen sacks. All bags to be treated at the same temperature were placed in a large screen box which was submerged in the hot-water bath.

A tank designed for hot-water seed treatment (Type 7 of Baker and Roistacher, 1957) was used. The 200 to 250 gallons of water in the tank were heated by escaping steam, and the temperature held within $\pm \frac{1}{4}°$ F by manually regulating the steam inflow valve. The water was kept vigorously circulating by a pump to prevent stratification. Temperature readings were taken on two thermometers in each test.

Immediately after a specified time of submergence the bags of cormels were cooled by immersion in cool water to precisely terminate the treatment. They were then planted in pots in the greenhouse, and emergence counts taken every week.

Thermal Death Point of Cormels

Cormels of the variety Picardy were treated at 106°, 113°, 122°, 131°, and 140° F for 10, 20, and 30 minutes. Three replicates of 100 cormels each were planted in a U. C.-type soil mix in 18 × 18-inch redwood flats on benches in the glasshouse. Figure 1 shows graphically the percentage emergence of sample groups of cormels treated at 113°, 122°, and 131° F for 30 minutes, and at 140° F for 10 minutes.

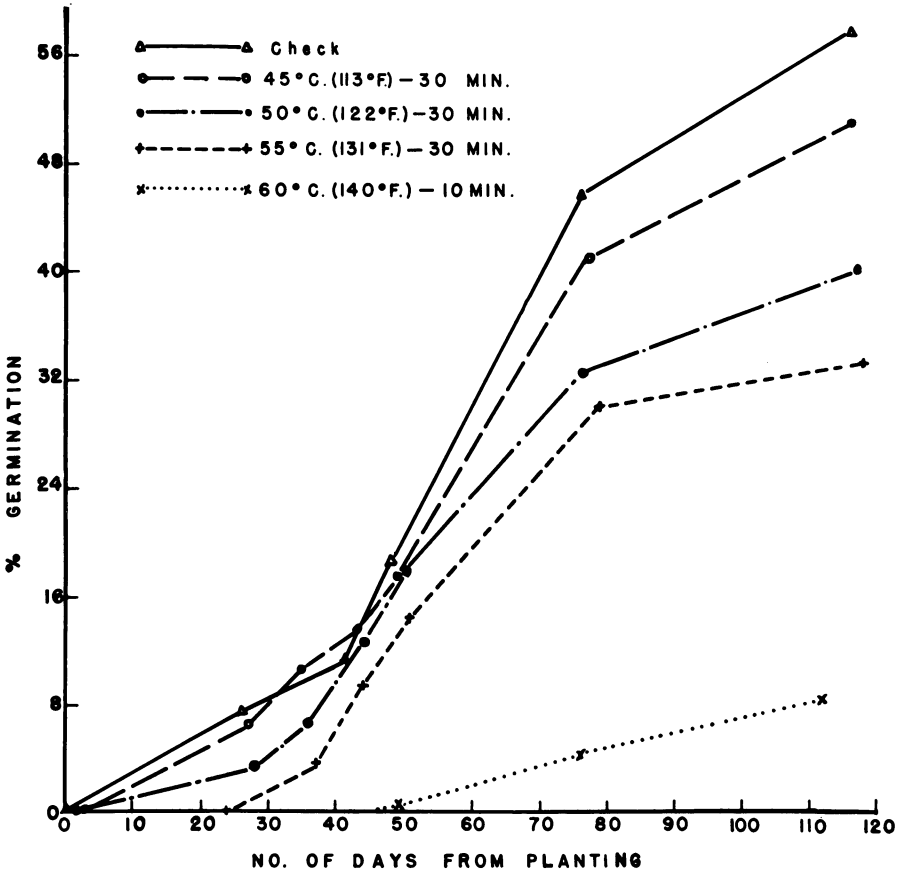

Fig. 1. The effect of four different hot-water treatments on the time and percentage of germination of Picardy cormels. Each curve represents an average of three replicates totaling 300 cormels.

An inactivation temperature between 131° and 140° F for 20 to 30 minutes was indicated by these results. The 30-minute immersion was adopted as standard for all subsequent tests.

The dormancy problem was also revealed in these tests. At the end of 110 days, cormels were still sprouting in all treated lots. It was deemed advisable to investigate some methods of breaking dormancy to see whether the germination time could be shortened.

Relationship of Hot-water Treatment to Cormel Dormancy

Test 1. An experiment was planned to study the possibility of breaking cormel dormancy in order to shorten the period of emergence after hot-water treatments. Cormels of three varieties were obtained locally in October, 1950:

1) Spotlight, dug on September 1, 1950, and soaked in water for a few days to wash away the soil. The cormels were badly diseased and a majority had cracked outer husks.

Fig. 2. Effect of heat treatment on cormels of variety Spotlight 67 days after planting. Untreated check at left; treated in hot water (128° F) for 30 minutes, in center; treated in hot 5 per cent ethyl alchohol for 30 minutes, at right. Treated lots stored at 40° F for 3 weeks prior to planting.

2) Myrna Fay, dug October 20, 1950. The cormels appeared sound and healthy, but approximately 40 per cent had cracked outer husks.
3) Miss Wisconsin, dug October 20, 1950. The cormels had a very hard, thick, and intact outer husk.

A safe hot-water bath (128° F for 30 minutes) was used, followed by various treatments to break dormancy, as follows:

Hot-water treatments

1. Check
2. Hot water at 128° F, 30 minutes
3. 5 per cent ethyl alcohol at 128° F, 30 minutes

Subsequent dormancy treatments

A. Check
B. Cold storage at 40° F for 3 weeks
C. Ethylene chlorohydrin (1.6 per cent) at 70° F, 48 hours
D. Ethylene chlorohydrin as above, followed by a 2 minute dip in 95 per cent ethyl alcohol[6]

[6] Strydom (1949) found a short dip in 95 per cent alcohol effective in breaking dormancy of corms.

All combinations of hot-water and subsequent treatment were tested, except 3C, which left 11 combinations. Since the number of cormels was limited, 25 of each variety were used per treatment, without replication.

After 96 days the soil was carefully washed from cormels of the varieties Myrna Fay and Miss Wisconsin, and the numbers dead were recorded. Ungerminated cormels were stripped of their husks, replanted, and observed after 60 days. The results are recorded in table 2.

TABLE 1

EFFECT OF VARIOUS TREATMENTS ON CORMELS OF SPOTLIGHT VARIETY 61, 88, AND 120 DAYS AFTER PLANTING IN SOIL, AS SHOWN BY EMERGENCE, DORMANCY, DEATH, AND ROOT CONDITION. FIGURES INDICATE THE NUMBER OF CORMELS OUT OF 25 IN EACH TREATMENT*

Treatment	Plant condition	Posttreatment manipulation			
		Check	Cold storage	Ethylene chlorohydrin	Ethylene chlorohydrin plus alcohol
No heat treatment (check)	Emergence, 61 days	2	11	16	4
	88 days	2	18	21	16
	120 days†	16	18	16	15
	Dormant in soil, 120 days	5	1	0	2
	Dead in soil, 120 days	9	6	17	21
	Condition of roots‡	R	W-Y	R	R
128° F for 30 min. in water	Emergence, 61 days	5	12	14	4
	88 days	22	16	17	7
	120 days†	23	20	19	12
	Dormant in soil, 120 days	0	1	1	0
	Dead in soil, 120 days	2	6	5	14
	Condition of roots‡	W	W	W-Y	W-Y
128° F for 30 min. in 5 per cent ethyl alcohol	Emergence, 61 days	16	17	..	1
	88 days	19	21	..	10
	120 days†	22	21	..	14
	Dormant in soil, 120 days	1	1	..	1
	Dead in soil, 120 days	4	3	..	10
	Condition of roots‡	W	W	..	W

* See also figure 3.
† Includes those sprouted in the soil.
‡ R = Rotted; W-Y = white roots with some yellowing; W = white roots without yellowing.

After 120 days, cormels of the variety Spotlight were carefully removed and examined for germination, rotting, and root condition. The results are presented in table 1 and figure 3. Because most of the cormels of this variety had already sprouted, they were not peeled and replanted.

The results of this experiment show a number of interesting points. Since there were no replications, higher order interactions were used as an estimate of error. Significant effects of treatment were as follows:

1) Heat-treated cormels of the variety Spotlight, in addition to germinating more rapidly, produced clean white roots. By contrast, the untreated cormels had decayed roots, and the series treated with hot water and then with ethylene chlorohydrin were somewhat yellowed. The cause of the yellowing

was not determined. It was indicated that the hot-water treatment produced clean stock in series not subsequently treated with ethylene chlorohydrin

2) In the variety Spotlight, hot-water treatment broke cormel dormancy (fig. 2). After 88 days, 22 out of 25 cormels had emerged, as against two in the controls. The results with Myrna Fay and Miss Wisconsin were less pronounced.

That hot-water treatment will break dormancy of vegetative structures has been repeatedly demonstrated. Treatment of sugar-cane stem cuttings in hot water (122° to 125.6° F) for 20 minutes causes the rapid development

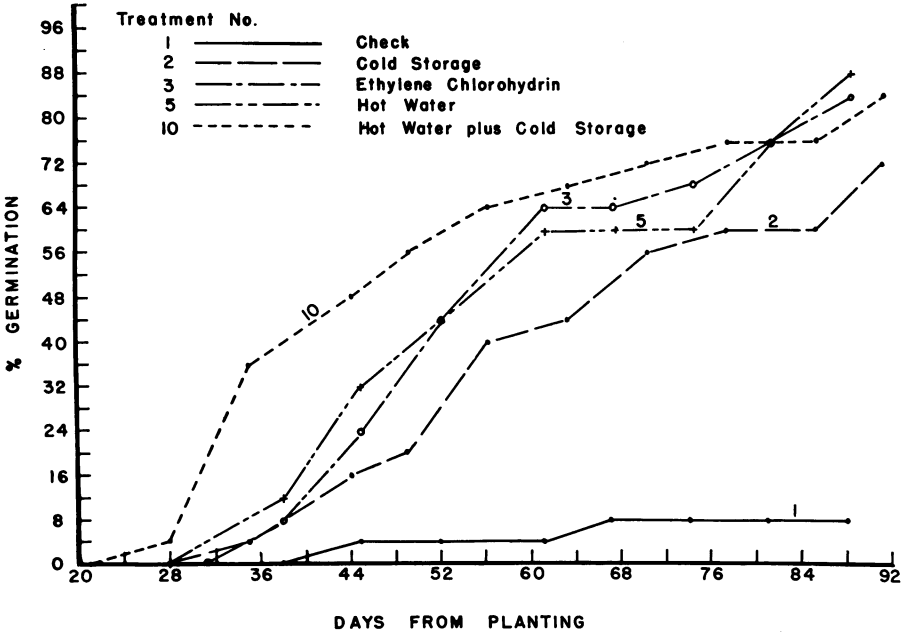

Fig. 3. Rate of sprouting of Spotlight cormels treated in four ways to break dormancy: storage at 40° F for 3 weeks; treatment with ethylene chlorohydrin; treatment with hot water at 128° F for 30 minutes; treatment with hot water at 128° F for 30 minutes, followed by 3 weeks storage at 40° F. Each curve represents 25 cormels. (See also table 1.)

of lateral buds, probably because of a lowered auxin level (Brandes and Klaphaak, 1923; Brandes and van Overbeek, 1948). Similar results have been obtained with a wide range of plants. Denny (1930) reported that sprouting of gladiolus corms was enhanced to some extent by warm storage (86° F for 3 weeks) during the later stages of dormancy. Loomis (1934) found that storage at 104° F for 1 week, at 95° F for 2 weeks, at 86° F for 4 weeks, or at 77° F for 6 weeks accelerated corm germination of all gladiolus varieties tested. As mentioned above, hot-water treatment of cormels at 115° F for 30 minutes is an accepted practice of growers in San Diego County to break dormancy and cause more uniform sprouting.

3) Although all three varieties were dug at approximately the same time, they differed in degree of dormancy. This is shown by their different reac-

TABLE 2

EFFECT OF VARIOUS TREATMENTS ON CORMELS OF VARIETIES MYRNA FAY AND MISS WISCONSIN 61, 96, AND 156 DAYS AFTER PLANTING IN SOIL, AS SHOWN BY EMERGENCE AND DEATH. FIGURES INDICATE THE NUMBER OF CORMELS OUT OF 25 IN EACH TREATMENT

Treatment	Plant condition	Posttreatment manipulation			
		Check	Cold storage	Ethylene chlorohydrin	Ethylene chlorohydrin plus alcohol
	Variety Myrna Fay				
No heat treatment (check)	Emergence, 61 days	0	8	3	3
	96 days	4	12	8	8
	Dead in soil, 96 days	2	1	1	2
	Additional emergence 60 days after peeling (156 days after planting)	19	12	14	7
128° F for 30 min. in water	Emergence, 61 days	4	10	4	1
	96 days	9	11	7	2
	Dead in soil, 96 days	11	5	5	7
	Additional emergence 60 days after peeling (156 days after planting)	5	9	7	3
128° F for 30 min. in 5 per cent ethyl alcohol	Emergence, 61 days	8	11	..	1
	96 days	10	14	..	2
	Dead in soil, 96 days	7	7	..	13
	Additional emergence 60 days after peeling (156 days after planting)	8	1	..	3
	Variety Miss Wisconsin				
No heat treatment (check)	Emergence, 61 days	1	4	1	1
	96 days	2	4	2	1
	Dead in soil, 96 days	0	1	1	5
	Additional emergence 60 days after peeling (156 days after planting)	20	12	21	19
128° F for 30 min. in water	Emergence, 61 days	0	0	1	0
	96 days	1	0	1	0
	Dead in soil, 96 days	8	6	2	6
	Additional emergence 60 days after peeling (156 days after planting)	16	13	8	9
128° F for 30 min. in 5 per cent ethyl alcohol	Emergence, 61 days	0	0	..	0
	96 days	1	0	..	0
	Dead in soil, 96 days	2	10	..	9
	Additional emergence 60 days after peeling (156 days after planting)	13	10	..	9

tions to the various dormancy-breaking treatments. The variety Miss Wisconsin would not sprout sufficiently, regardless of the treatment used. However, a sister lot of cormels of this variety planted after the removal of their outer husks gave rapid sprouting (fig. 9).

4) Cold storage of untreated cormels also enhanced the process of sprouting (tables 1 and 2). This is in accord with the results of commercial practice,

and the work of Denny and Miller (1935) Cold storage after hot-water treatment was most effective in enhancing sprouting of Myrna Fay and Spotlight cormels (fig. 3, hot water followed by cold storage).

5) Ethylene chlorohydrin was also effective in breaking dormancy, but it tended to increase discoloration and rotting of the corms and roots. Ethylene chlorohydrin plus a dip in 95 per cent alcohol appeared to be too severe a treatment.

6) Treatment in a 5 per cent ethyl alcohol bath at 128° F increased sprouting in the checks and, when followed by cold storage, produced vigorous and healthy plants (fig. 2). This was evident in top growth, the size and vigor of the new corms, and whiteness of roots.

Test 2. This experiment was designed to answer four questions: 1) What temperature between 131° and 140° F applied for 30 minutes will inactivate cormels? 2) Does this critical temperature vary for different varieties? 3) What is the comparative response of the host to a hot 5 per cent alcohol bath and a hot-water treatment? This reaction is distinct from the effect on dormancy of alcohol at a lower temperature (128° F; see above). 4) What is the effect of a period of cold storage prior to hot-water treatment of cormels?

Cormels for this experiment were harvested in late August, 1950, at Oceanside, California[7], and held in open storage sheds until late November. There were six varieties: Myrna Fay, Miss Wisconsin, True Love, Easter Parade, Beneson, and Variety X (unlabeled). The cormels were soaked in a 1:1,000 mercuric chloride solution for 2 hours, and rinsed in running water for 20 minutes. After drying, cormels of each of the six varieties were divided into two equal lots. One was placed in 40° F storage, and the other prepared for immediate hot-water treatment. The latter group was placed, in lots of 25 cormels each, in 72 small, labeled, plastic screen bags. The 24 treatments were arranged as follows for each of the six varieties:

No cold storage:
- Presoak in 5 per cent alcohol at 94° F for 20 hours
  - Untreated check
  - Treated in 5 per cent alcohol for 30 minutes at 131°, 133°, 135°, 137°, and 140° F
- No presoak
  - Untreated check
  - Treated in hot water for 30 minutes at 131°, 133°, 135°, 137°, and 140° F

Cold storage (40° F) for 3 weeks:
- Presoak in 5 per cent alcohol at 94° F for 20 hours
  - Untreated check
  - Treated in 5 per cent alcohol for 30 minutes at 131°, 133°, 135°, 137°, and 140° F
- No presoak
  - Untreated check
  - Treated in hot water for 30 minutes at 131°, 133°, 135°, 137°, and 140° F

[7] The authors are indebted to Mr. Edwin Frazee, Oceanside, for supplying cormels for many of these tests.

The cormels of the alcohol series were placed in tightly sealed glass jars of 5 per cent ethyl alcohol, and held at 94° F for 20 hours. This was to augment the effect of the subsequent treatments in hot 5 per cent alcohol.

The series treated in hot water were held dry at room temperature for 20 hours before treatment.

After 3 weeks the cormels that had been held in cold storage were removed and treated in the same ways as the above series.

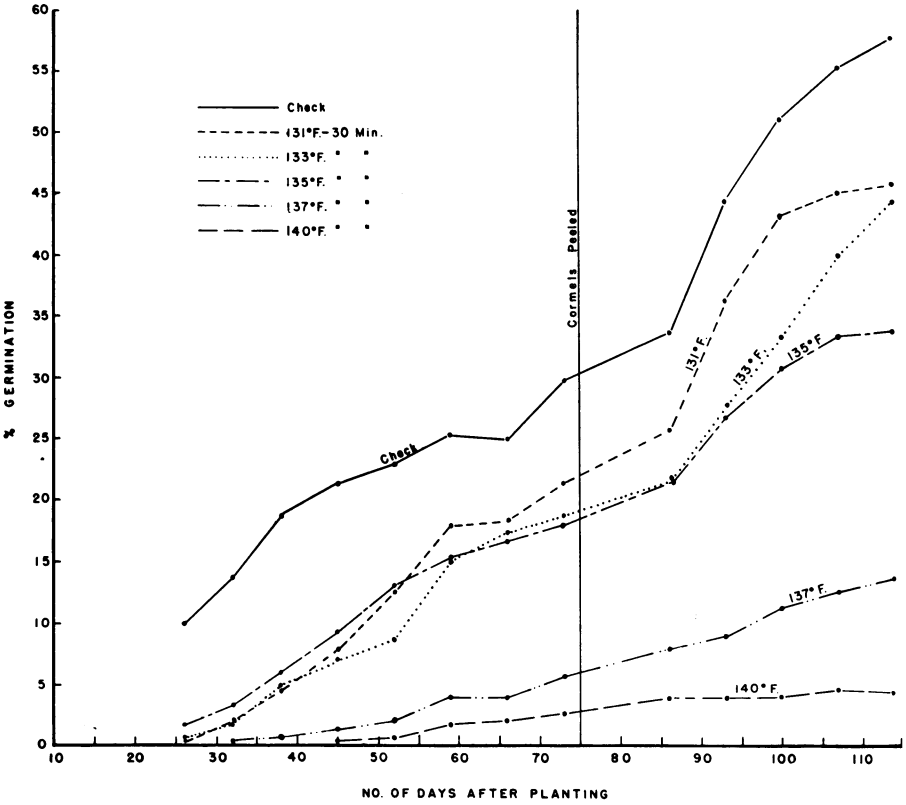

Fig. 4. The effect of heat treatment at five different temperatures on semidormant cormels. Each curve represents 300 cormels of six varieties, and includes both alcohol and water series. Cormels were dug 73 days after treatment, peeled, and replanted for 47 days.

The temperature of the water or alcohol baths had a fluctuation of ± ¼° F during treatments. The bags of cormels were placed in cold water for 3 minutes immediately after treatment. Each lot of 25 cormels was then planted in a 4-inch pot and placed in the glasshouse. Emergence counts were recorded weekly. Since the number of variety-treatments and limited number of cormels precluded replications, higher order interactions supplied an estimate of error.

The original data were arranged in several ways to evaluate the effect of the various factors on germination. The effect of heat treatment at five different temperatures on semidormant (noncold storage) cormels is pre-

sented in figure 4; the effect on nondormant (cold storage) cormels is shown in figure 5. The effect of cold storage on susceptibility of cormels to heat treatment is obtained by comparing figures 4 and 5, and by comparing figures 6 and 7. The comparison of treating semidormant cormels in hot water and in hot 5 per cent alcohol is shown in figure 6; the effect on nondormant cormels is shown in figure 7. The effect of hot-water treatments at five temperatures in inducing cormel dormancy is shown

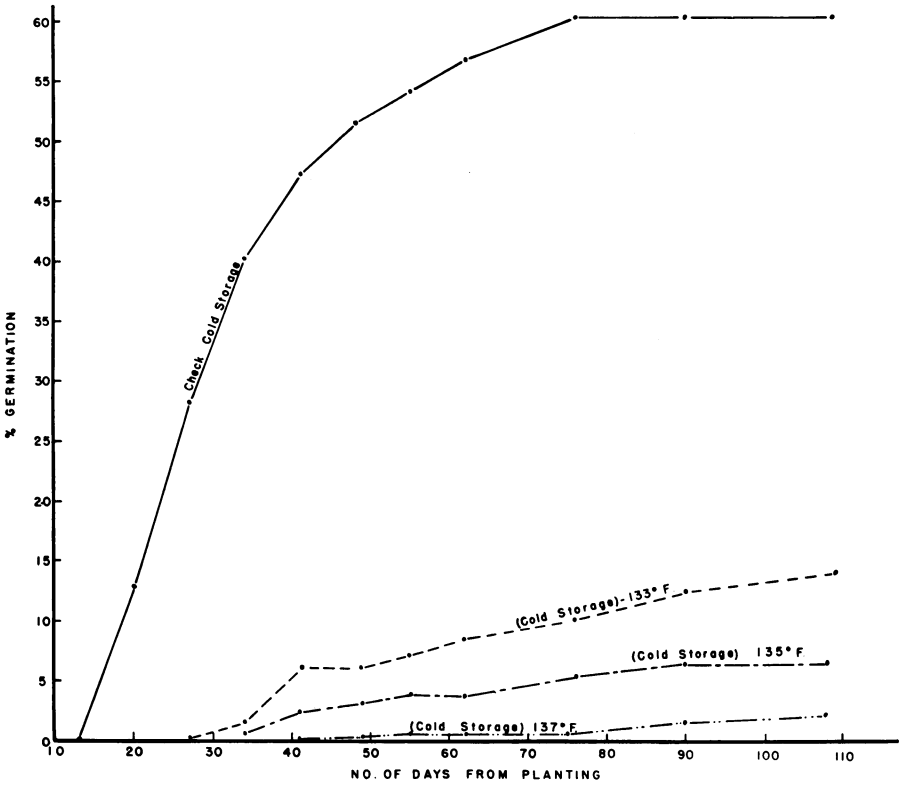

Fig. 5. The effect of heat treatment at three different temperatures on cormels whose dormancy had been broken by storage at 40° F for 3 weeks. Each curve represents 300 cormels of six varieties, and includes both alcohol and water series.

graphically in figures 4 and 5 for the combined totals of all six varieties and of the alcohol and water heat treatments, indicate that 135° F for 30 minutes was the highest temperature that could be tolerated without undue injury or delayed germination of semidormant cormels. These results are in expansion of an earlier abstract (Roistacher, 1951).

The germination of cormels held in cold storage before hot-water treatment at temperatures of 131° to 140° F was seriously depressed.

Treating in a 5 per cent alcohol bath after a 5 per cent alcohol soak impaired sprouting somewhat, but not seriously enough to prevent its use if it facilitated killing the pathogens. Actually, soaking and treating cormels in

a 5 per cent alcohol bath did not materially reduce sprouting below the levels attained by material treated in hot water. This is shown in figures 6 and 7, based on the combined totals of all six varieties and all temperatures.

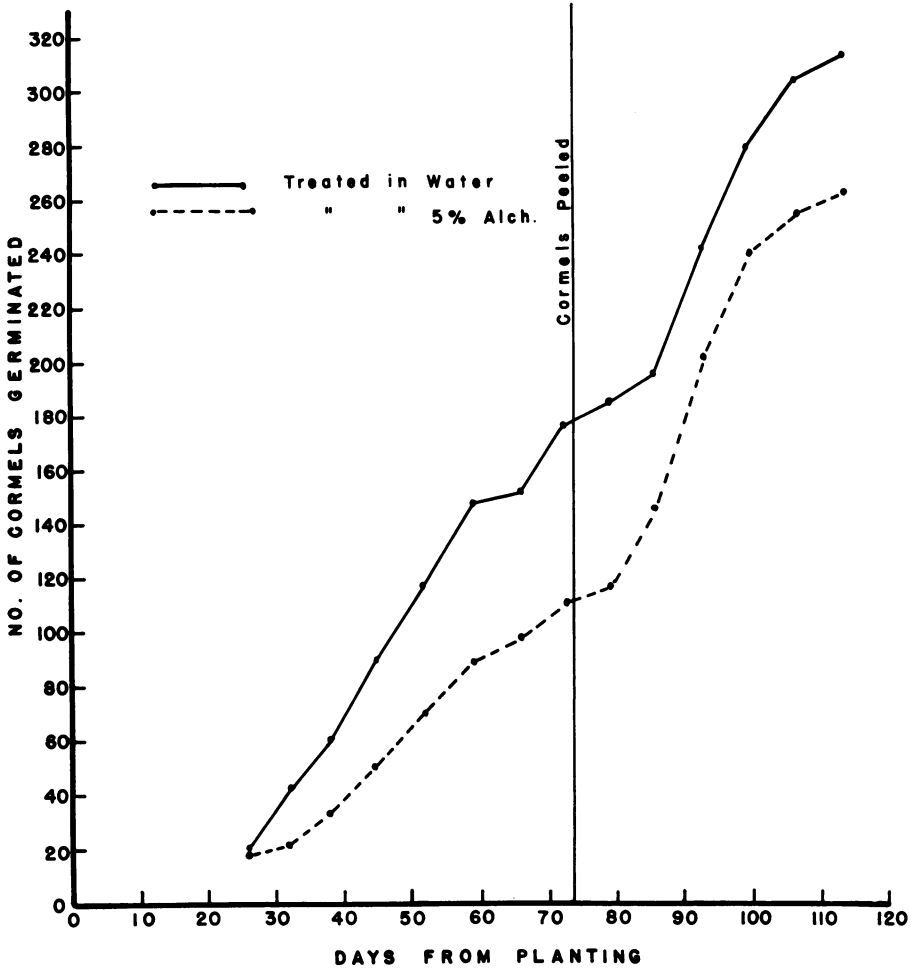

Fig. 6. The comparative effect on the germination of semidormant cormels of treatment in hot water (30 minutes at 131° to 140° F) and in hot alcohol (20-hour presoak in 5 per cent ethyl alcohol at 94° F, followed by 30 minutes at 131° to 140° F). Each curve represents 900 cormels of six varieties, and includes the untreated check and those treated at 131°, 133°, 135°, 137°, and 140° F. Cormels were dug 73 days after treatment, peeled, and replanted for 47 days.

Different varieties showed about the same general critical temperatures between 135° and 140° F. However, some of them were thrown into a state of prolonged dormancy by the high temperature, rather than killed by it. Thus, 50 per cent of the cormels treated at 140° F were found to be alive and turgid 142 days after planting. Seventy-three days after planting,

cormels in the noncold storage treated lots were carefully screened from the soil, and the number found dead was recorded (fig. 8). The unsprouted but turgid cormels were replanted after the outer husks were removed. A marked increase in sprouting occurred following removal of the husks from cormels of Myrna Fay, Easter Parade, and Variety X. It did not materially enhance sprouting of those cormels treated at 140° F. The variety Miss Wisconsin, which was relatively unaffected by all dormancy-breaking treatments in earlier experiments (test 1), sprouted readily when the outer husk was removed (fig. 9).

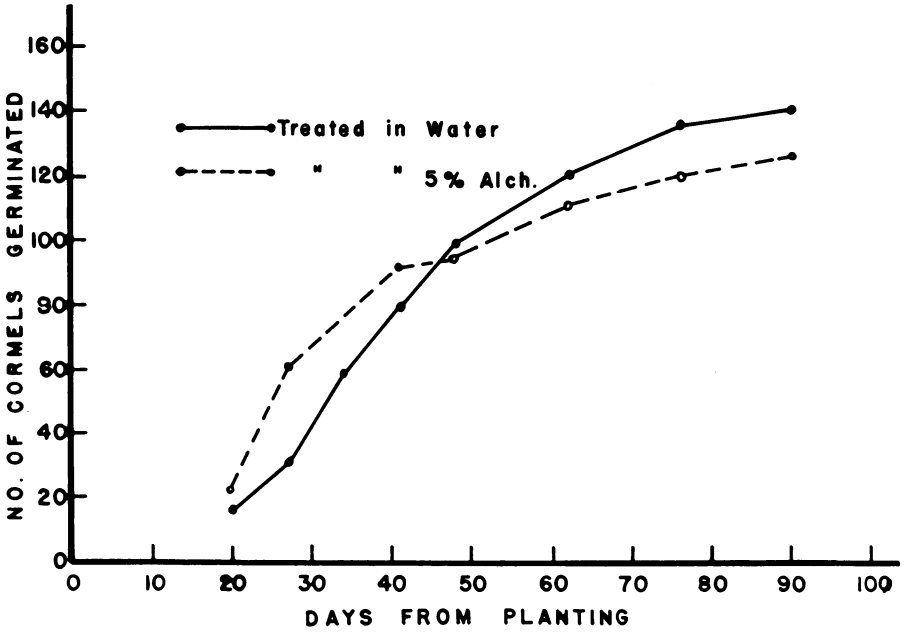

Fig. 7. The comparative effect of treatment in hot water (30 minutes at 131° to 140° F) and in hot alcohol (20-hour presoak in 5 per cent ethyl alcohol at 94° F, followed by 30 minutes at 131° to 140° F) on germination of cormels whose dormancy had been broken by storage at 40° F for 3 weeks. Each curve represents 900 cormels of six varieties, and includes the untreated check and those treated at 131°, 133°, 135°, 137°, and 140° F.

The Effect on Cormel Dormancy of Cracking or Removing the Husk

Test 3. Dormancy in cormels, as in seeds, may be affected by the thickness of the outer husk. If these are cracked or removed, dormancy may be broken (fig. 9).

An experiment was designed to test the effect on germination of cracking the outer husk of the cormels before heat treatment. The cormels used were dug at Oceanside, California, in December, and stored there in an open shed until March, when they were treated. The varieties used were Elizabeth the Queen, Leading Lady, Margaret Beaton, and Valeria. There were four

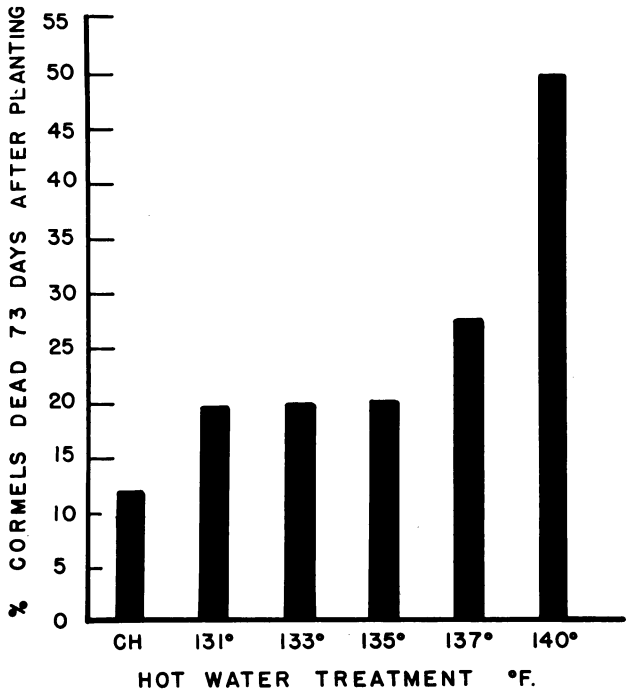

Fig. 8. The percentages of cormels found dead in the series untreated (check) and treated (at five different temperatures for 30 minutes) in hot water or alcohol, 73 days after planting. The cormels were from the semidormant group that had not been held in cold storage. Each bar represents 300 cormels of six varieties. Note the increasing number of cormels found dead in the series above 135°, but that only 50 per cent were killed, even at 140° F.

Fig. 9. The effect on germination of removing the outer husk of cormels of the variety Miss Wisconsin (left), compared with unpeeled cormels (right) 60 days from planting.

TABLE 3

MEAN PERCENTAGES OF GERMINATION OF CORMELS IN FOUR REPLICATES (200 CORMELS) OF FOUR VARIETIES 69 DAYS AFTER PLANTING IN SOIL, SHOWING THE EFFECT OF CRACKING OF THE HUSK ON GERMINATION AND SENSITIVITY TO HEAT TREATMENT

Variety	Husks not cracked (check)	Husks cracked before hot-water treatment at				
		Check	120° F	125° F	130° F	135° F
Elizabeth the Queen	22	62	54	54	44	23
Leading Lady	1	74	68	67	29	15
Margaret Beaton	8	68	73	60	26	7
Valeria	5	58	48	54	27	9

TABLE 4

MEAN PERCENTAGE OF GERMINATED, DORMANT, AND DEAD CORMELS IN FOUR REPLICATES (100 CORMELS) OF TWO VARIETIES 60 DAYS AFTER PLANTING IN SOIL, SHOWING THE EFFECTS OF HOT-WATER TREATMENT AND SUBSEQUENT CRACKING OF THE HUSKS

Treatment	Husks not cracked			Husks cracked following hot-water treatment		
	Sprouted	Dormant	Dead	Sprouted	Dormant	Dead
Variety Valeria						
Check	23	76	1	84	1	15
128° F for 30 min.	55	35	10	47	5	48
131° F for 30 min.	65	26	9	55	0	44
135° F for 30 min.	9	83	9	22	9	69
138° F for 30 min.	34	65	1	32	3	65
Variety Leading Lady						
Check	47	48	5	72	4	24
128° F for 30 min.	24	70	6	54	19	27
131° F for 30 min.	35	62	3	39	11	50
135° F for 30 min.	61	36	3	56	2	42
138° F for 30 min.	2	87	11	8	8	84

replicates of 50 cormels each in the following treatments: husk not cracked, untreated (check); husk cracked, untreated; husk cracked, treated at 120°, 125°, 130°, or 135° F for 30 minutes. Cormels were rinsed in cold water after treatment and planted in 6-inch pots. Germination counts were taken at weekly intervals. The percentage germination 69 days after planting is shown in table 3.

Cracking the husks enhanced sprouting of all four varieties, both without heat treatment and when followed by treatment at temperatures up to 130° F. Treatment at 135° F did not materially increase sprouting over the uncracked checks, and significantly reduced it compared to the cracked checks.

The results raised three questions: 1) What would be the effect of cracking the husks after hot-water treatment? 2) How would these cormels compare with uncracked heat-treated cormels of the same lots? 3) How many of the nonsprouted cormels were dead or still dormant in the soil?

Test 4. This experiment used cormels of the same lots of two varieties, Leading Lady and Valeria, used in test 3. The material had been held at room temperature. There were four replicates of 25 cormels each in the following treatments: untreated check; hot-water treatment at 128°, 131°, 135°, and 138° F for 30 minutes. Since the tetrazolium test (Roistacher, Bald, and Baker, 1953, 1957) indicated that these cormels were dormant, a series at 138° F was included to see whether they would survive treatment. An overnight soak in cool water preceded hot-water treatment. Following the heat treatment the cormels were cooled in water, dried, and the outer husks of half of each lot were cracked. The various lots were then planted in pots in a glasshouse, and emergence counts recorded weekly. The results 60 days later are recorded in table 4.

The results show that cracking the husks *after* hot-water treatment increased the lethal effect of all temperatures from 128° to 138° F but did not materially affect the percentage sprouted after 60 days. It was undetermined whether cormels dormant at 60 days were still capable of germinating. In the untreated checks, cracking of the husks materially increased sprouting, as was the case in test 3.

Dormant cormels of both varieties withstood a temperature as high as 138° F. Only 1 per cent were found to be dead in the variety Valeria, and 11 per cent in Leading Lady, in cormels with uncracked husks treated at 138° F. This indicates the value of the tetrazolium test in judging whether cormels are in a state most tolerant of hot-water treatment.

THERMAL DEATH POINTS OF FUNGI IN CORMELS

In conjunction with the above experiments on the thermal tolerance of cormels, experiments were conducted on the thermal tolerance of two important pathogens when growing in cormels.

Gassner (1933) found that the addition of 2 to 5 per cent ethyl alcohol to the water bath reduced the temperature necessary to kill the loose smut fungus (*Ustilago nuda* (Jens.) Rostr.) in wheat seed by 9° to 18° F. This effect apparently was from action on the pathogen rather than the host. Alcohol was accordingly included in some of the treatments against gladiolus pathogens. Its effect on the heat tolerance and dormancy of the cormel was presented in the preceding section of this paper.

Fusarium oxysporum f. *gladioli.*

The fungus *Fusarium oxysporum* f. *gladioli* (Massey) Snyd. and Hans. was selected for the most detailed study, for reasons outlined in the "Introduction." The primary objective was to determine the inactivation temperature of the fungus when growing in the cormel, and to compare it with that of the cormel.

Twelve separate trials were conducted with the following general procedure: The Fusarium inoculum used in trials 1 through 8 was obtained

from lesions of naturally infected corms from southern California. Single-spore isolates were made at intervals to maintain the wild type of the fungus, and these were used as inoculum. For trial 9, the isolates were obtained from four different sources. Mass-transfer cultures of these were maintained and used as inoculum. Cultures for trials 10 and 11 were obtained from an artificially inoculated cormel in trial 9, which had survived hot-water treatment at 135° F for 30 minutes. In trial 12, all available isolates were mixed for use in a spore suspension.

Procedure for wounding and inoculation in trials 1 to 9 consisted of pricking the cormels with a coarse flattened needle, and pressing fragments of an agar culture into the wounds. In trials 10 to 12, the procedure was modified by cracking the outer husks in addition to puncturing, and submerging the wounded cormels in a spore suspension of the inoculum for 10 minutes at room temperature. In either method, the inoculated cormels were placed in Petri dishes and incubated for 4 to 6 days at 80° F, which rotted the cormels approximately half way through. In trials 2, 3, and 10, the cormels were incubated for an additional 10 to 21 days at 94° F to stimulate formation of the resistant chlamydospores. Preliminary testing had indicated this was the best treatment to induce chlamydospore production.

After wounding, inoculation, and incubation the cormels were placed in small, labeled, plastic screen bags and treated in hot water or hot 5 per cent alcohol. After treatment, cormels were cooled in running tap water for a few seconds prior to plating out.

The procedure for determining the presence of living Fusarium after hot-water treatment was as follows: Each cormel was aseptically bisected through the wounded area, placed in a Clorox solution (diluted 1:12) for 1 minute, and transferred to a Petri plate containing potato-dextrose agar. Four to 13 cormels were placed in each plate, varying with the quantity used per trial. The plates were incubated for 1 week at 80° F, and then examined for Fusarium. Plates which were sterile at that time still showed no development of Fusarium when held for as long as 1 month. Results for each of the 12 trials are presented in table 5, and summarized in table 6.

In table 6 the following results are shown:

1) Presoaking is necessary for successful eradication of the fungus. The importance of presoaking before hot-water treatment to eliminate internal air pockets had been previously shown (Baker and Davis, 1950) in treating nasturtium seed for eradication of *Heterosporium tropaeoli* Bond. Unless cormels are presoaked, the Fusarium apparently can survive even at the higher temperatures. It is not unlikely that there are air pockets between the husk and the cormels, as there are between the pericarp and seed of nasturtium.

2) The addition of 5 per cent alcohol to the treatment bath may improve the effectiveness against Fusarium. It may make possible the use of lower temperatures, as suggested by Gassner (1933). Results obtained here suggest a trend favorable to this concept. Hot-water treatments at 128° to 136° F of nonpresoaked cormels gave 169 out of 342 with Fusarium, while hot 5 per cent alcohol treatments at the same temperatures showed 0 out of 30. However, in presoaked cormels the corresponding figures were 27 out of 354 and 1 out of 570.

TABLE 5

EFFECTIVENESS OF HOT WATER, PLUS VARIOUS PRETREATMENTS, IN ERADICATING *FUSARIUM OXYSPORUM* F. *GLADIOLI* FROM CORMELS OF EIGHT VARIETIES OF GLADIOLUS. UNLESS OTHERWISE NOTED, THE CORMELS NOT PRESOAKED AND THOSE PRESOAKED IN WATER WERE ALSO TREATED IN WATER, AND THOSE PRESOAKED IN ALCOHOL WERE TREATED IN ALCOHOL

Variety	Cormels per treatment	Days incubated at		Presoak treatment	Heat treatment		Per cent cormels with living Fusarium
		80° F	94° F		Temperature (° F)	Time (min.)	
Trial 1							
Picardy	30	6	0	none	check	..	100
	10	6	0	none	113	30	100
					122	20	100
					122	30	100
					131	10	100
					131	20	100
					131	30	30
					136	20	100
					136	30	0
Trial 2							
Picardy	4	4	10	water, 94° F, overnight	check	..	100
					128	60	100
					131	30	75
					131	45	50
					133	30	50
					133	45	50
					135	30	0
					135	45	0
				5 per cent alcohol, 94° F, overnight	check	..	100
					128	60	25
					131	30	0
					131	45	75
					133	30	0
					133	45	0
					135	30	0
					135	45	0
Trial 3							
Picardy	4	4	24	none	check	..	100
					131	30	100
					133	30	25
					135	30	100
				water, 94° F, overnight	check	..	75
					131	30	100
					133	30	0
					135	30	0
				5 per cent alcohol, 94° F, overnight*	check	..	100
					131	30	0
					133	30	50
					135	30	0

Table 5—*Continued*

Variety	Cormels per treatment	Days incubated at		Presoak treatment	Heat treatment		Per cent cormels with living Fusarium
		80° F	94° F		Temperature (° F)	Time (min.)	
Trial 4							
Picardy	4	4	35	water, 94° F, overnight	check	..	75
					131	30	75
					133	30	50
				5 per cent alcohol, 94° F, overnight	check	..	75
					131	30	0
					133	30	0
Trial 5							
Miss Wisconsin	22	4	0	none	134	30	32
Variety X	22	4	0	none	134	30	36
Easter Parade	21	4	0	none	134	30	48
Beneson	22	4	0	none	134	30	64
Myrna Fay	25	4	0	none	134	30	68
True Love	17	4	0	none	134	30	71
Miss Wisconsin	22	4	0	none	135	30	59
Variety X	22	4	0	none	135	30	37
Easter Parade	23	4	0	none	135	30	17
Beneson	22	4	0	none	135	30	68
Myrna Fay	25	4	0	none	135	30	72
True Love	17	4	0	none	135	30	65
Trial 6							
Miss Wisconsin	20	4	0	5 per cent alcohol, 94° F, overnight	135	30	0
Variety X	20	4	0		135	30	0
Easter Parade	20	4	0		135	30	0
Beneson	20	4	0		135	30	0
Myrna Fay	20	4	0		135	30	0
True Love	20	4	0		135	30	0
Trial 7							
Miss Wisconsin	20	4	0	5 per cent alcohol, 94° F, overnight	133	30	0
Variety X	20	4	0		133	30	0
Easter Parade	20	4	0		133	30	0
Beneson	20	4	0		133	30	0
Myrna Fay	20	4	0		133	30	0
True Love	20	4	0		133	30	0
Trial 8							
All six varieties	60	4	0	water, 94° F, overnight	check	..	98
Miss Wisconsin	10	4	0		133	30	0
Variety X	10	4	0		133	30	10
Easter Parade	10	4	0		133	30	0
Beneson	10	4	0		133	30	0
Myrna Fay	10	4	0		133	30	0
True Love	10	4	0		133	30	0

Table 5—*Continued*

Variety	Cormels per treatment	Days incubated at		Presoak treatment	Heat treatment		Per cent cormels with living Fusarium
		80° F	94° F		Temperature (° F)	Time (min.)	
Trial 8—*Continued*							
All six varieties	60	4	0	5 per cent	check	..	90
Miss Wisconsin	10	4	0	alcohol, 94° F,	133	30	0
Variety X	10	4	0	overnight	133	30	0
Easter Parade	10	4	0		133	30	0
Beneson	10	4	0		133	30	0
Myrna Fay	10	4	0		133	30	0
True Love	10	4	0		133	30	0
Trial 9							
Valeria	20†	4	0	water, 94° F,	check	..	100
	40†	4	0	overnight	131	30	2.5‡
	40†	4	0		133	30	0
	40†	4	0		135	30	0
	20†	4	0	5 per cent	check	..	100
	40†	4	0	alcohol, 94° F,	131	30	0
	40†	4	0	overnight	133	30	0
	40†	4	0		135	30	2.5‡
Trial 10§							
Valeria	10	4	24	water, 94° F,	check	..	100
				overnight	131	30	70
					133	30	10
					135	30	0
				5 per cent	check	..	100
				alcohol, 94° F,	131	30	0
				overnight	133	30	0
					135	30	0
Trial 11§							
Valeria	5	4	0	none	check	..	100
	15	4	0		131	30	0
	15	4	0		135	30	0
	15	4	0		131‖	30	0
	15	4	0		135‖	30	0
	20	4	0	water, 94° F,	check	..	100
	20	4	0	overnight	131	30	0
	20	4	0		135	30	0
	20	4	0	5 per cent	check	..	100
	20	4	0	alcohol, 94° F,	131	30	0
	20	4	0	overnight	135	30	0

Table 5—*Continued*

Variety	Cormels per treatment	Days incubated at		Presoak treatment	Heat treatment		Per cent cormels with living Fusarium
		80° F	94° F		Temperature (° F)	Time (min.)	
				Trial 12			
Valeria¶	10	5	0	water, 94° F, overnight	check	..	90
					128	30	10
					131	30	0
					135	30	0
				5 per cent alcohol, 94° F, overnight	check	..	100
					128	30	0
					131	30	0
					135	30	0
Valeria**	10	5	0	water, 94° F, overnight	check	..	100
					128	30	0
					131	30	0
					135	30	0
				5 per cent alcohol, 94° F, overnight	check	..	100
					128	30	0
					131	30	0
					135	30	0

* This alcohol presoak series was treated in hot water.
† Total for four lots of cormels infected with Fusarium from four different sources.
‡ These escapes came from a single inoculum source.
§ Cormels wounded and inoculated by dipping in a spore suspension from trial 9.
‖ Treated in 5 per cent alcohol, rather than water.
¶ Cormels had been held 14 months in storage.
** Cormels freshly dug.

3) The thermal death point of the Fusarium, following a presoak in water or 5 per cent alcohol, lies between 128° and 135° F for a 30-minute interval. Dormant cormels treated at the upper levels of this range may be expected to survive, and to be relatively free from the fungus.

The question might fairly be raised whether various strains of the gladiolus Fusarium could have different levels of thermal tolerance. For example, the known tendency of some isolates to produce large numbers of chlamydospores might provide greater heat tolerance. Some of the isolates used in these studies produced chlamydospores, and trials 2, 3, and 10 were manipulated to give maximum numbers of these structures. In addition approximately 15 to 20 different isolates were used in these tests. Trials 10 and 11 involved an isolate which had survived 135° F in trial 9; the fungus did not survive 135° F in trial 10 or 133° F in trial 11.

Also some uncertainty has existed whether there is more than one distinct type of Fusarium disease of gladiolus, and whether these might be caused by different species or forms. Forsberg (1955) has recently studied this question and concluded that the 40 isolates from three types of diseases could not be distinguished morphologically, by response to temperature or to fungicides, by various growth characteristics on artificial media, or by type of disease produced when inoculated into the host. Single isolates were found

to produce more than one type of disease. He considered that all the forms should be included in *F. oxysporum* f. *gladioli*. Buxton (1955*a*) also found that single isolates of *F. oxysporum* f. *gladioli* would cause both yellows and corm rot. The type of symptom produced was determined both by the gladiolus variety and by the given isolate of the fungus. Buxton (1955*b*) suggested that the variability in the latter might result from the recombination of existing heterocaryons. Bruhn (1955) found that his isolates caused both corm rot and vascular yellows diseases and, for this and other reasons, placed them all in *F. oxysporum* f. *gladioli*.

TABLE 6

SUMMARY OF THE 12 TRIALS FROM TABLE 5, SHOWING RATIO OF CORMELS WITH LIVING FUSARIUM TO THE TOTAL NUMBER, FOLLOWING VARIOUS HOT-WATER TREATMENTS

Treatment temperature and time	Cormels not presoaked			Cormels presoaked in water or alcohol		
	Treated in 5 per cent alcohol	Treated in water	Totals	Treated in 5 per cent alcohol	Treated in water	Totals
Untreated check			39/39			271/284
113° F—30 min.	....	10/10	10/10	....	....	....
122° F—30 min.	....	10/10	10/10	....	....	....
128° F—30 min.	....	....	....	0/20	1/20	1/40
131° F—30 min.	0/15	7/29	7/44	0/98	18/106	18/204
133° F—30 min.	....	1/4	1/4	0/238	8/126	8/364
134° F—30 min.	....	68/129	68/129	....	....	....
135° F—30 min.	0/15	73/150	73/165	1/214	0/102	1/316
136° F—30 min.	....	0/10	0/10	....	....	....
Totals	0/30	169/342	169/372	1/570	27/354	28/924

The well-known variability of Fusaria might make possible the future selection of an isolate with higher thermal tolerance if heat treatment became general, much as DDT-resistant house flies have appeared. However, this circumstance has not developed with bacteria or other microörganisms that have long been subjected to minimal lethal temperatures (*e.g.*, pasteurization of milk). Everything considered, it is unlikely that Fusaria of such thermal tolerance as to render the treatment ineffectual will appear. However, since cormels in a proper state of dormancy can often tolerate temperatures well above 135° F (*e.g.*, figs. 1, 4, and 8, and table 4), there is still some margin of safety.

Stromatinia gladioli

In March, 1951, dead gladiolus stems were collected that had large numbers of sclerotia of the dry-rot fungus, *Stromatinia gladioli*. These were held in cold storage for 2 weeks and then treated with hot water to determine the thermal death point of the sclerotia.

Basal leaf strips ¼ inch wide by ¾ inch long, heavily dotted with sclerotia, were cut from many different plants and randomly placed in five plastic screen bags. These were then treated as follows: untreated check; treated at

125°, 130°, 135°, or 140° F for 30 minutes in hot water. After treatment each sample was placed in Clorox solution (diluted 1:12) for approximately 1 minute. Five leaf strips were placed in each of three Petri dishes of potato-dextrose agar, and held at room temperature for 1 week.

The sclerotia of the fungus were killed by the hot-water bath at 125° F for 30 minutes, the lowest temperature tried, as well as at 130°, 135°, and 140° F. The untreated sclerotia germinated readily. It was of interest that nematodes noticed in the three check plates were not found in any of the plates with tissue treated at 125° F or above.

DISCUSSION

The determination of a safe temperature for hot-water treatment of gladiolus cormels involves understanding and manipulating a number of factors. Since the thermal death point of the cormel lies close to that of the most important pathogen, *Fusarium oxysporum* f. *gladioli*, treatment temperatures close to the maximum for the host must be used if the organism is to be eradicated.

Successful heat therapy presupposes a safe margin between the thermal death point of host and pathogen. Little difficulty is encountered if this difference is large. Where the margin is small it can often be increased in several ways:

1) Selecting the most favorable type of material for treatment. The use of cormels rather than the less tolerant corms, as in this study, improves chances of survival.

2) Selecting material in the state of dormancy most tolerant of treatment, or manipulation of the material to produce that state. Cormels made nondormant by cold storage (figs. 4 to 7) or by cracking their outer husks cannot stand high temperatures (table 3), and may be killed by temperatures of 131° F or above. However, if cormels are held under dry, warm conditions, and are found by a tetrazolium test to be dormant, they can withstand hot water temperatures as high as 138° F for 30 minutes without undue reduction of germination (table 4). This is in line with the results of Ryan (1955), who found that corms grown at temperatures above 59° F had a deeper rest than those grown intermittently at or below 50° F for the final few weeks before harvest. The treatment of semidormant summer-grown cormels from warm soil, rather than nondormant winter-grown cormels, similarly improves the chances of survival and germination (Bald and Markley, 1955). The lack of success from hot-water treatment of cormels grown in northern states with comparatively cool soil may be due to their lack of dormancy. The killing action of hot water on cormels is apparently associated with the metabolic rate at time of treatment. Thus, nondormant cormels are in a relatively high state of metabolic activity, and are readily killed at the lower temperatures.

3) Selecting the proper environmental conditions may:

a) Increase the thermal tolerance of the host plants (see 2 above).

b) Increase the susceptibility of the pathogen to heat treatment. The presoak treatment of the cormels may accomplish this in part by initiating fungus development, although the elimination of heat-insulating air pockets must also surely be involved. The addition of alcohol to the hot-water bath apparently decreases heat tolerance of some fungi. Gassner

(1933) found that the effect of heat on the wheat loose smut fungus in seed depended on the exclusion of oxygen, and thought that the intermediate products of host metabolism under these conditions killed the pathogen. He thought that the addition of alcohol to the hot bath augmented this action. This idea was indirectly supported by Tyner (1953) and Russell and Tyner (1954), who found that a soak in water on temperature-time schedules ranging from only 66° F for 80 hours to 86° F for 35 hours, eradicated the same fungus. Wallen and Skolko (1953) found that the incidence of *Ascochyta pisi* Lib. in pea seed was also reduced by an 18-hour soak in cool water, apparently from antibiotic substances produced by the bacterial flora, which was increased by the soak. It is not yet certain what mechanism or mechanisms may be involved in these effects.

Under our experimental conditions, by manipulation of such factors as dormancy, presoaking, and the addition of substances to the treating bath, the margin between inactivation temperatures of the pathogen and host may be increased. The pathogen may then be eradicated without significant injury to the host. The method has now moved into successful commercial application (Bald and Markley, 1955; Bald, 1956; Bald, Ferguson, and Markley, 1956; Ferguson and Markley, 1955; Magie, 1956).

SUMMARY

1. The thermal inactivation temperature of dormant gladiolus cormels treated in hot water for 30 minutes lies between 135° and 140° F. Six varieties reacted about the same toward this critical temperature range.

2. The thermal inactivation temperature by hot water of *Fusarium oxysporum* f. *gladioli* inside the cormel lies between 128° and 135° F. *Stromatinia gladioli* sclerotia are killed by 125° F for 30 minutes in basal leaf tissue.

3. Although the margin between the thermal death point of host and pathogen is small, manipulation of certain factors may so increase it that successful commercial treatment is made completely practical.

a) Since cormels appear to be much more tolerant of heat than are corms, they have been exclusively used for heat treatments.

b) The level of dormancy of the cormel strongly affected the thermal inactivation point. Nondormant cormels did not survive treatment at 131° F, whereas dormant cormels survived temperatures as high as 140° F. The use of the tetrazolium method for estimating the dormancy of cormels is, therefore, a valuable tool in reducing injury from heat treatment.

c) Presoaking the cormels before the hot-water bath increased effectiveness of treatment. Fusarium inside the cormels often survived treatment unless the overnight presoak was used.

d) The use of 5 per cent ethyl alcohol in the treatment bath increased the effectiveness in eradicating Fusarium.

4. A markedly increased sprouting was induced by removing the outer husk from the cormel 73 days after hot-water treatment, but cracking them before or immediately after treatment sharply reduced the number surviving at higher treatment temperatures.

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