PHYTOPATHOLOGY.—*An evaluation of the results of treatments given narcissus bulbs for the control of the nematode* Ditylenchus dipsaci *(Kühn) Filipjev.*[1] B. G. CHITWOOD, U. S. Bureau of Plant Industry, and F. S. BLANTON, U. S. Bureau of Entomology and Plant Quarantine.

Hot-water treatment of narcissus bulbs for the control of the bulb and stem nematode, *Ditylenchus dipsaci* (Kühn) Filipjev, was inaugurated by Ramsbottom[2] and Van Slogteren.[3] In order to protect narcissus plantings in this country from this pest imported and domestic bulbs have been subjected to hot-water treatment. Originally this treatment consisted of the exposure of bulbs to hot water at 110°F. for 3 to 4 hours. Failure of such treatments to control the disease adequately was responsible for the instigation of further experimental work. As a result of this work a pre-soak of the bulbs for 2 hours in water at 70 to 80°F. was added to make the treatment more effective. Later it was suggested that the treatment bath itself might be improved by the addition of formalin. Vapor-heat treatments were also proposed as a substitute for hot-water treatments, because the latter promoted the growth of fungous diseases.

The data included herein are the result of work during the years 1931–1939 by Messrs. Spruijt, Thorne, Blanton, and Chitwood; Miss E. M. Buhrer; and Mrs. Grace S. Cobb. The writers have assembled these data according to treatment, year (except table 2), and technic of handling.

There are two possible objectives in the treatment of plants for the control of parasites: (1) Eradication of or cure from infestation; (2) reduction of infestation. Since none of the known treatments indicate probable attainment of the first objective, this paper is concerned with the reduction of infestation. This may be approached either from the standpoint of (*a*) the reduction in number of living specimens in each plant or (*b*) the reduction in number of infested plants. Treatments acceptable for one purpose may be of no great value for the other purpose. This seems to be the case in daffodil bulbs infected with the bulb and stem nematode, *Ditylenchus dipsaci*. Since a male and a female of this species could theoretically produce 200,000 off-

[1] The writers wish to acknowledge the assistance of Dr. F. M. Wadley and L. B. Reed, of the Bureau of Entomology and Plant Quarantine, who have made many helpful suggestions relative to the statistical methods. Received April 22, 1941.

[2] RAMSBOTTOM, J. K. *Investigations on the narcissus disease.* Journ. Roy. Hort. Soc. **43**: 51–64. 1918; *Experiments on the control of eelworm disease of narcissus,* ibid.: 65–78.

[3] SLOGTEREN, E. van. *De Toepassing van warmte als Bestrijdingsmiddel van eenige Bloembollensiekten.* Weekblad voor Bloembollcult. **30**: 63–66, 69–71. 1919.

spring in 90 days, it seems doubtful that mere reduction in numbers of living nemas would be of any real benefit. Moreover, reduction in numbers of nemas for each bulb would not reduce the number of foci of infestation in the field.

Inconsistent results have been a feature of all experimental work involving the determination of nemic mortality. The literature is extensive, but the particulars are not especially informative. In general, experimental work has been conducted in two manners. One method has been to treat infected stocks and make field observations the following year. This method has not been productive, since sometimes the symptoms appear to be suppressed the year following treatment and to reappear later. The other method has been to treat one or more known infected bulbs at each of a number of durations and temperatures and later to determine the percent nematode revival in them. This method does not take into consideration the natural variation in biological data. In this paper only those records showing complete mortality in a given bulb are given consideration. Much of the data was obtained prior to realization of the necessary requirements. However, since these data show that many treatments commonly thought to be of value are not satisfactory, we feel they are worthy of recording. The writers have applied, for the first time, statistical methods for the evaluation of treatments of bulbs for the control of the stem and bulb eelworm and have found that one can now predict, with reasonable assurance, the efficacy of treatments.

RESULTS FOR THE YEARS 1931–1938

The data are presented in tabular form, the hot-water and modifications of the hot-water treatments applied in the years 1932–1938 constituting table 1, and vapor heat and its modifications for the years 1931–1938 constituting table 2. In both tables each block contains two numbers, the first representing the number of examinations in which one or more living nemas (*D. dipsaci*) were found, the second representing the number of examinations in which specimens of *D. dipsaci* were found either living or dead. The number of bulbs in each examination varied from 1 to 30. A single record of "no living" *D. dipsaci* in a treatment is not evidence that this treatment is satisfactory, since sometimes the same treatment at greater duration or a similar treatment at higher temperature contains living specimens. Undoubtedly several records of no living specimens would be necessary before a treatment could be considered satisfactory, since a few records might be due to chance. Sometimes other species of nemas remain alive in bulbs in which all the *D. dipsaci* are apparently dead. Such species include *Aphelenchoides parietinus* (Bastian) Steiner, *Aphelenchus avenae* Bastian, *Cephal-*

obus spp., and *Panagrolaimus subelongatus* (Cobb) Thorne; these cases are marked by an asterisk. Often no nemas were observed in washings of chopped treated bulbs, either because they were absent or because it is often difficult to establish the presence of *D. dipsaci* in bulbs receiving satisfactory or near-satisfactory treatment even when they are present; examination of the data has shown that if it were assumed that absence of nemas indicated a kill, one would be in error nearly half the time. Consequently, such records (0–0) can not be used. A single examination based on two or more bulbs should be of more value than an examination based on one bulb. Since it is impossible to state either what proportion of the bulbs was actually infected before treatment or, if living *D. dipsaci* are present, in what proportion all the nemas were killed, each record as given in tables 1 and 2 must be interpreted as a single observation. Thus a record of 0–1 indicates one observation in which all specimens of *D. dipsaci* were found dead, regardless of number of bulbs in the given sample, while 5–10 indicates 10 observations covering 10 samples in which specimens were identified living or dead and 5 samples in which they were found living, regardless of number of bulbs in each sample.

The treatment dates of the various years were as follows:

1. *Vapor heat.*—Sept. 16–28, 1931; Sept. 7–14, 1932; Sept. 8–13, 1933; July 26 to Sept. 13 (weekly), 1934; Oct. 9–14, 1936, and Sept. 21–26, 1938. The series of vapor heat treatments in 1934 were made for 3, 4, and 5 hours, duration at three temperatures, 114, 116, and 118°F.; one sample at each temperature and duration was treated each week during the period. The total numbers of samples providing living *D. dipsaci* on these dates were 5, 6, 5, 9, 5, 9, 8, and 5 out of a possible 9 for each respective week.

2. *Hot water.*—Sept. 7–14, 1932; Sept. 20–23, 1933; Oct. 9–14, 1936; Sept. 14–16, 1938.

The average number of bulbs constituting a single sample for each of the years was as follows: 1931, vapor heat 1; 1932, vapor heat and hot water 1.4; 1933, vapor heat 1.1 and hot water 3.7; 1934, vapor heat 4.4; 1936, vapor heat 1.5 and hot water 1.9; 1938, vapor heat and hot water 12.

The results of the 1931 and 1932 vapor-heat treatments were published by Spruijt and Blanton.[4] They are included here for the sake of completeness.

Since tests on the permeability of nemic membranes[5] had indicated a relative impermeability of nemic membranes at room temperature, special tests were conducted in 1938. In these tests infected bulbs were soaked in 0.75 per cent and 0.5 per cent formalin at room temperature and served as a basis for the following records respectively (presented as in tables 1 and 2): 2 hours 1–2 and 2–2; 4 hours 2–2 and 4–4; 6 hours 2–2 and 4–4. Infected bulbs soaked in a 1 per cent solution of formalin at room temperature gave the following results: 24 hours 3–3; 48 hours 3–4; 72 hours 3–4; 96 hours 0–2. From these results, formalin at room temperature is obviously ineffec-

[4] Journ. Econ. Ent. **26**(3): 613–620, tables 1–3. 1932.

[5] Chitwood, B. G. Proc. Helm. Soc. Washington. **5**(2): 68–75. 1938.

TABLE 1.—EFFECT OF HOT-WATER AND ITS COMBINATIONS ON THE CONTROL OF THE BULB AND STEM NEMA, DITYLENCHUS DIPSACI (KÜHN) FILIPJEV

Year	Type of treatment‡	Temp.	Duration in hours†											
			1	2	3	4	5	6	7	8	9	10	11	12
		°F.												
1938	Hot water	104	3–3	13–13		13–13		6–8		7–13				
1938	Formalin (1:199)	104	6–6	12–14		5–10		2–6		2–17				
1938	Formalin (1:99)	104		2–2		2–3		2–2		1–3				
1938	Hot water	110	7–9	10–14		5–16		*0–1*		0–11				
1936	Hot water—Presoak	110			1–1	1–1	*0–1*	*0–0*	*0–1*	1–1	1–1	0–1		
1938	Hot water—Presoak	110		1–1		1–2		2–2						
1938	Formalin (1:199)	110	6–8	6–14		1–21		0–5		0–11				
1938	Formalin (1:99)	110		0–4		0–13		0–2		0–3				
1938	Formalin (1:199)—Presoak	110		0–1		0–1		0–1						
1936	Formalin (1:199)—Presoak	110			0–1	0–1	0–1	0–1	0–1	0–1	0–1	0–1		
1938	Formalin (1:132)	110		0–1		0–1		0–1						
1938	Formalin (1:132)—Presoak	110		0–1		0–1		0–1						
1933	Hot water	111.5			1–2	1–6	3–3	3–4						
1932	Hot water	112				0–1	0–1	0–1		0–1				
1936	Hot water—Presoak	112			1–1	*0–1*	1–1	0–1						
1938	Hot water—Presoak	112				1–1		0–0						
1936	Formalin (1:199)—Presoak	112			0–1	0–1	0–1	0–1						
1938	Formalin (1:199)—Presoak	112				0–1		0–1						
1932	Hot water	113			0–1	0–1	0–2	0–2	0–1	0–2	0–2	0–1	0–1	0–1
1933	Hot water	113		*0–3*	*0–3*	*0–4*	1–2							
1932	Hot water	114				0*1	0–1	0–0	0–1	0–1	0–0	0–1	0–1	0–1
1938	Hot water	114		1–1		*0–1*		1–1						
1938	Hot water—Presoak	114		*0–1*		*0–0*		1–1						
1936	Hot water—Presoak	114			0–0	0–1	0–1	0–0						
1938	Formalin (1:199)	114		0*1		0–1		0*1						
1936	Formalin (1:199)—Presoak	114			0–0	0–0	0–0	0–1						
1938	Formalin (1:199)—Presoak	114		0–1		0–0		0–1						
1938	Formalin (1:132)	114		0–1		0*1		0*1						
1938	Formalin (1:132)—Presoak	114		0*1		0*1		0*1						
1932	Hot water	115			0–1	0–0	0–1	0–1	0–1	0–0	0–0	0–0	0–1	0–1
1933	Hot water	115		3–3	3–4	1–4	1–3							
1932	Hot water	116			0–1	0–1								
1936	Hot water—Presoak	116			0–1	0–0	0–1	0–0						
1936	Formalin (1:199)—Presoak	116			0–1	0–1	0–1	0–1						
1932	Hot water	117			0–1									
1933	Hot water	117		0–4	0–3	0–3	0–3							
1932	Hot water	118		0–1	0–1	0–0	0*1							
1933	Hot water	118		1–3	0–3	0–3	0–3							
1938	Hot water	118	6–21	0–20		0–6				0–6				
1938	Formalin (1:199)	118	0–3	0–1										
1932	Hot water	119		0–1	0–1									
1932	Hot water	120		0–1										

* Other species of living nematodes also found.

† In each block two numbers are given, the first of which represents the number of examinations in which living *D. dipsaci* were observed and the second represents the number of examinations in which *D. dipsaci* were observed either living or dead. The *italic* numbers represent records invalidated by the observation of living nematodes in more severe treatments.

‡ *Presoak* means that the treatment was preceded by a presoak in water at 70–80°F.

Table 2.—Effect of Vapor Heat and its Combinations on the Control of the Bulb and Stem Nema, *Ditylenchus dipsaci* (Kühn) Filipjev

Type of treatment*	Temp.	Duration in hours†											
		1	2	3	4	5	6	7	8	9	10	11	12
	°*F.*												
Vapor heat	110			2–2	2–2	2–2	2–2						
Vapor heat—Preheat	110			1–1	1–1	1–1	1–1	1–1	1–1				
Vapor heat—Presoak	110			1–1	2–2	0–0	2–2	0–0	0–1				
Vapor heat—Formalin presoak	110			0–0	0–2	0–1	0–2	0–1	0–0				
Vapor heat	111			2–2	2–2	2–2							
Vapor heat	112		2–2	2–2	2–3	0–2	1–1		0–1				0–1
Vapor heat—Preheat	112			1–1	1–1	1–1	0–1	1–1	1–1				
Vapor heat—Presoak	112			1–1	2–2	1–1	1–2	0–1	1–1				
Vapor heat—Formalin presoak	112			1–1	0–2	0–1	0–1	0–0	0–0				
Vapor heat	113		2–2	3–3	2–3	2–2	0–1	0–1	0–2	0–1	0–2	0–1	0–1
Vapor heat	114	2–2	2–2	10–10	8–9	7–8	0–1	0–1	0–0	0–1	0–1	0–0	0–1
Vapor heat—Preheat	114			1–1	1–1	0–1	1–1	1–1	1–1				
Vapor heat—Presoak	114			1–1	0–1	0–0	0–1	0–0	0–0				
Vapor heat—Formalin presoak	114			0–0	0–0	0–0	0–1	0–1	0–1				
Vapor heat	115	2–2	2–2	0–1	0–1	0–1	0–1	0–1	0–0	0–0	0–1	0–1	0–1
Vapor heat	116			8–13	7–12	4–10	1–1						
Vapor heat—Preheat	116			1–1	1–1	0–0	0–1	1–1	0–1				
Vapor heat—Presoak	116			0–1	1–1	0–1	0–1	0–0	0–1				
Vapor heat—Formalin presoak	116			0–0	0–1	0–0	0–1	0–1	0–1				
Vapor heat	117			0–2	0–3	0–3	0–1						
Vapor heat	118	1–1	1–2	4–14	5–12	4–13	0–4						
Vapor heat—Preheat	118			1–1	0–0	0–0	0–0	0–0	0–1				
Vapor heat—Presoak	118			0–0	0–0	0–0	0–0	0–1	0–1				
Vapor heat—Formalin presoak	118			0–0	0–0	0–0	0–0	0–1	0–0				
Vapor heat	119		0–1	0–3	0–1	0–3	0–3						
Vapor heat	120	1–1	1–2	0–4	0–4	0–2	0–1						

* *Preheat* means that the treatment was preceded by 2 hours of preheating at 70–80°F.; *presoak*, treatment preceded by a presoak in water for 2 hours at 70–80°F.; *formalin presoak*, treatment preceded by a presoak in formalin (1:199) at 70–80°F.

† In each block two numbers are given, the first of which represents the number of examinations in which living *D. dipsaci* were observed and the second the number of examinations in which *D. dipsaci* were observed either living or dead.

tive as a nematocide for nemas in bulbs. Since results presented elsewhere in this paper show that formalin at higher temperature is effective, heat appears to be essential for the action of formalin on *D. dipsaci* in narcissus bulbs.

DETERMINATION OF A STANDARD OF EFFICACY

The variability in efficacy of a given treatment is emphasized by the finding of living *D. dipsaci* after treatments at higher temperature, longer duration, or greater concentration of formalin than those after which no living specimens were found. Before making a recommendation one should be able to predict the proportion of bulbs in which all specimens of *D. dipsaci* would be killed. Commercial stocks of narcissus bulbs are considered badly diseased when 5 percent are infected with nematodes. This would be 50 in 1,000. If untreated, each bulb may easily serve as a source of infection for three additional bulbs. Thus, in one year the number could increase to 200. If treated in such a manner that all the nemas are killed in 19 out of 20 infected bulbs (95 percent), then three out of 50 (6 percent) would be left infected; one might expect 12 infected bulbs in one year and 48 in two years. The treatment would then have to be repeated. If the original lot of 1,000 bulbs, 5 percent infected, were treated in such a manner as to kill all the nemas in 18 out of 20 bulbs (90 percent), then one might expect five bulbs (10 percent) to be left infected, which would increase to 20 in one year and 80 in two years. Hence it would be necessary to treat every year in order to reduce the infection. Annual treatments being impractical, the minimum standard of efficacy should be better than 90 percent, preferably 95 percent so that a treatment is necessary only in alternate years.

The problem now is to determine how many bulbs must be examined to assure a statistically sound basis for measuring efficacy. Such a basis is furnished by the binomial distribution.[6]

Let p = any assumed efficacy expressed as a proportion of 1; let q = the remainder, also expressed as a proportion of 1; and let n = the number of bulbs examined. For instance, for a desired efficacy of 90 percent, $p=0.9$, $q=0.1$.

Then expansion of $(p+q)^n$ represents the various class frequencies expected, the first term being the proportion of zeros expected, the second the number of ones, the third the number of twos, etc.

In order to predict with 19:1 probability that a treatment giving zero survival from a homogeneous lot of infected bulbs has an efficacy better than a desired efficacy p, the number must be such that $p^n=0.05$ or less. This is true because with the assumed efficacy and smaller numbers, random sampling will give zero more than 5 percent of the time and a somewhat lower efficacy will give zero 5 percent of the time. Thus with smaller numbers we will not have assurance that a zero means efficacy of p or better. With larger or equal numbers such assurance is obtained. With groups of bulbs showing heterogeneity, more variation may be expected, and somewhat larger numbers might be needed.

In order similarly to predict with 19:1 probability that a treatment giving 1 survival has an efficacy better than a desired efficacy p, the number must be such that $p^n+np^{n-1}q=0.05$ or less. With still larger numbers of survivors the formula becomes increasingly complex.

Assuming an efficacy of 90 percent, a complete kill would have to be obtained in 29 bulbs. Living nemas in one bulb would have to represent 46

[6] SNEDECOR. *Statistical methods*, rev. ed. 1938.

treated bulbs; similarly two, three and four bulbs with living nemas would have to represent 61, 76, and 89 bulbs respectively. An apparently perfect treatment would not be recommendable if based on less than 29 bulb examinations.

Assuming an efficacy of 95 percent, a complete kill in 59 bulbs would be required in order to prove the treatment better than the assumed efficacy. A treatment must be considerably better than an assumed efficacy to show its superiority with a high probability statistically. As the efficacies of treatments more closely approach 100 percent one is nearly justified in accepting the actual efficacies since the numbers of bulbs must be so large.

In order to make the greatest possible use of the data available, it would appear that one is justified in selecting any given treatment and adding to its record the records of all treatments of lesser severity until one comes to a record of living nemas. This is an approximate method leading only to tentative conclusions.

PERCENTAGE OF FORMALIN AND RATIO OF BULBS TO LIQUID

An experiment was designed to test the significance of the proportion of bulbs to quantity of liquid in the treating tank, the liquid varying in percentage of formalin. The percentages of formalin were 0.25, 0.5, 0.75, and 1.0; the proportions of bulbs to liquid (by weight) were 1:2.8, 1:4, and 1:5.3; treatment durations were 2, 3, 4, and 5 hours. All treatments were at 110°F. with no presoak. Allowance was made for actual time required for the liquid in the treating chamber to return to 110°F. after the bulbs were put in. The bulbs were medium-sized Laurens Koster. Each sample contained five supposedly infected bulbs. They were held submerged by crossed garden labels. The treatments were conducted in 1-gallon cans submerged in a standard treating tank with an agitator. No agitator was present in the individual cans. The temperature was taken for the cans and not the tank. Treatments were conducted September 26 to 29, 1939.

The data are presented in table 3.

Totals by duration show that the 2-hour treatment (13–35) was unsatisfactory; by actual record the 3-hour treatment (4–44) was better but of questionable value; the 5-hour treatment (2–36) was still better by actual record; and the 4-hour treatment (0–42) was perfect. Since both of the bulbs with living nemas in the 5-hour treatments were in 0.25 percent formalin solutions, it is apparent that this concentration is unsatisfactory.

Totals by ratio of solution weight to bulb weight show no striking differences but the 5.3:1 ratio shows a lower survival than the 2.8:1 or 4:1 ratios.

Totals by percentage of formalin are likewise inconsistent with practically identical results with the 0.25, 0.75, and 1.0 percent solutions and poorer results with the 0.5 percent solution. However, six of the seven bulbs unsatisfactorily treated in 0.5 percent formalin were in the 2-hour duration and five

TABLE 3.—EFFECT OF HOT WATER AT 110°F. ON THE CONTROL OF D. DIPSACI WITH RELATION TO THE PERCENTAGE OF FORMALIN AND THE RATIO OF SOLUTION TO BULBS, 1939

Solution weight / Bulb weight	Formalin	Duration in hours†				Total‡	
		2	3	4	5	By ratio	By percent
	Percent						
2.8:1	0.25	0–1	0–4	0–2	1–3	1–10	
4:1	0.25	1–2	0–3	0–5	0–2	1–12	
5.3:1	0.25	1–3	0–4	0–3	1–5	2–15	4–37
2.8:1	0.5	1–2	1–4	0–4	0–4	2–14	
4:1	0.5	5–5	0–2	0–4	0–4	5–15	
5.3:1	0.5	0–1	0–3	0*4	0–2	0–10	7–39
2.8:1	0.75	0–3	1–5	0–3	0–4	1–15	
4:1	0.75	1–3	0–3	0–4	0–4	1–14	
5.3:1	0.75	0*4	2–5	0–3	0–2	2–14	4–43
2.8:1	1.0	2–3	0–3	0–3	0*2	2–11	
4:1	1.0	2–5	0–3	0–2	0–3	2–13	
5.3:1	1.0	0*3	0–5	0–5	0–1	0–14	4–38
Grand total...		13–35	4–44	0–42	2–36	19–157	19–157

* Other species of nematodes.

† In each block two numbers are given, the first of which represents the number of examinations in which living specimens of *D. dipsaci* were observed and the second represents the number of examinations in which specimens of *D. dipsaci* were observed either living or dead.

‡ Totals by solution-bulb ratio are as follows: 2.8:1, 6–50; 4:1, 9–54; 5.3:1, 4–53.

were in a single treatment. This could have easily been due to error in the treatment technique.

EFFECT OF REUSING THE SAME FORMALIN SOLUTION

An experiment was designed to test the possibilities of repeated treatments in the same solution of formalin at 110°F. A constant volume of liquid and a constant weight of bulbs (5 pounds, or 2.268 kg) was maintained. The treating tank was a constant temperature bath with agitator. This bath was filled with 0.5 percent formalin solution to the 10.5-liter mark and refilled to this level with 0.5 percent formalin before each repeated treatment. The solution-bulb ratio was, therefore, 4.6:1. There were six treatments of three hours' duration and six of four hours' duration.

Ten medium-sized Laurens Koster bulbs were used for each of these tests. The treatments were conducted on October 9 to 11, 1939.

Since bulbs contain a relatively large quantity of water they would tend to dilute the formalin solution. Theoretically, for the liquid in the bulbs to contain the same amount of formalin as the external liquid contains, the percentage of formalin in the liquid would be reduced to 0.4 percent and that in the bulbs would be raised to 0.4 percent. In order to compensate for this reduction in concentration, in subsequent treatments sufficient formalin to make a 0.1 percent solution was added in tests 5 and 6.

In treatment 1 fresh formalin was used while in treatments 2–6 the formalin had previously been used one or more times. In treatments 2, 3, and 4 only sufficient formalin (0.5 per cent) was added to compensate for the loss in volume due to prior use, there being no compensation for loss in concentration.

The results of this experiment are given in table 4. The minor difference in totals by duration, 2–29 for the 3-hour and 1–42 for the 4-hour treatment, might well be due to chance. No reduction in efficacy appears to have occurred as a result of second and third use of the treating bath whether or not an attempt was made to compensate for dilution of the formalin. However, when the treating bath was used a fourth time without compensation the efficacy was reduced in both 3- and 4-hour durations. Even this difference might have been due to chance but it would not be wise to risk such treatments commercially.

TABLE 4.—EFFECT OF REPEATED TREATMENTS IN THE SAME SOLUTION OF FORMALIN AT 110°F. ON THE EFFICACY OF SUCH TREATMENTS FOR D. DIPSACI

Test no.	Character of bath*	Duration in hours†		Totals by treatment
		3	4	
1	Fresh	0–4	0–9	0–13
2	Second use	0–6	0–6	0–12
3	Third use	0–6	0–8	0–14
4	Fourth use	2–5	1–6	3–11
5	Second use, *C*	0–4	0–6	0–10
6	Third use, *C*	0–4	0–7	0–11
Totals by duration..........		2–29	1–42	

* *C* denotes that sufficient commercial formalin was added to compensate for theoretic reduction in concentration due to prior use of the bath.

† In each block two numbers are given, the first of which represents the number of examinations in which living *D. dipsaci* were observed and the second represents the number of examinations in which nemas were observed either living or dead.

EFFECT OF PRESOAK AND VARIED TEMPERATURES, DURATIONS OF TREATMENT, AND CONCENTRATIONS OF FORMALIN

This experiment was designed to test the difference in efficacy of varied treatments at varied temperatures. Treatments were made in a standard hot-water treating tank with an agitator. Ten bulbs, supposedly infected with *D. dipsaci*, were used for each test. Those in tests 1 to 8, 11 to 16, and 22 were medium-sized King Alfred bulbs rogued from the fields, while those in tests 9 to 10 and 17 to 21 were mixed varieties grown normally in the field. The size of the mixed variety bulbs naturally varied; the average size, however, was approximately the size of a small round King Alfred bulb with a diameter of about one and one-fourth inches. The experiment was conducted on September 19 to 23, 1939. Results are presented in table 5.

Using totals by treatment and temperature, the percentage of efficacy favors presoak in six cases, no presoak in two cases, and is equal in three cases. From this one might presume that presoak was advantageous. However, if one makes the same comparisons by duration with a given treatment one finds, by comparing 0.5 percent formalin treatments with and without presoak, that the percentage of efficacy favors presoak in only 2 cases, is against presoak in 3 cases and is equal in 8 cases. Presoak has no demonstrable advantage, possibly a disadvantage.

Comparing efficacy of 1 percent formalin treatments with and without presoak, 4 favor presoak, 1 is against and 4 are equal. With hot water, 4 favor presoak, 1 is against, and 7 are equal.

Using the binomial method of analysis for recommendation previously dis

TABLE 5.—EFFECT OF PRESOAK, VARIED TEMPERATURES, DURATIONS OF TREATMENT, AND CONCENTRATIONS OF FORMALIN ON THE EFFICACY OF HOT-WATER TREATMENTS FOR D. DIPSACI

No.	Type of treatment†	Temp.	Duration in hours‡						
			1	2	3	4	6	8	Total
		°F.							
1	Hot water	110				3–7	2–4	0–8	5–19
2	Hot water—Presoak	110				1–9	1–6	0–6	2–21
3	Formalin (1:99)	110	9–10	4–8	0*8		0–9		13–35
4	Formalin (1:99)—Presoak	110	3–7	7–7	0–6		0–4		10–24
5	Formalin (1:199)	110	7–9	1–7	1–6		0–9		9–31
6	Formalin (1:199)—Presoak	110	4–5	0*5	1–7		0–5		5–22
7	Hot water	114	6–7	3–9	0–9		0–7		9–32
8	Hot water—Presoak	114	6–7	0–7	1–7		0–8		7–29
9	Formalin (1:199)	114	1–2	0–2	0–4		0–3		1–11
10	Formalin (1:199)—Presoak	114	0–1	0–2	0–2		0–0		0–5
11	Hot water	116	7–8	1–7	1–10				8–25
12	Hot water—Presoak	116	2–7	0–5	0–7				2–19
13	Formalin (1:199)	116	0*8	0–9	0–8				0–25
14	Formalin (1:199)—Presoak	116	1–9	0–5	1–7				2–21
15	Formalin (1:99)	116	1–8	1–8	0–9				2–25
16	Formalin (1:99)—Presoak	116	0–10	0–3	0–8				0–21
17	Hot water	118	0–8	0–3					0–11
18	Hot water—Presoak	118	0–8	0–3					0–11
19	Formalin (1:199)	118	0–3	0–4					0–7
20	Formalin (1:199)—Presoak	118	0–5	0–6					0–11
21	Formalin (1:99)	118	0–5	0–5					0–10
22	Formalin (1:99)—Presoak	118	0–9	0–6					0–15

* Other species of living nematodes also found.

† *Presoak* means that the treatment was preceded by a presoak in water at 70–80°F.

‡ In each block two numbers are given, the first of which represents the number of examinations in which living *D. dipsaci* were observed and the second represents the number of examinations in which *D. dipsaci* were observed either living or dead.

cussed, the following treatments by addition are tentatively recommendable with probability of at least 19:1 that the efficacy is better than 90 percent, 1 percent formalin for 3 hours at 116°F. or 1 hour at 118°F. preceded by a 2-hour aqueous preosak in either case; 1 percent formalin for 2 hours at 118°F. with no presoak; and 0.5 percent formalin for 2 hours at 118°F. with a 2-hour aqueous presoak. Only one treatment is shown with probability of 19:1 to have an efficacy better than 95 percent, this being 1 percent formalin for 2 hours at 118°F. with a 2-hour aqueous presoak. Many of the other treatments may have an efficacy as high or higher if there were sufficient data.

DISCUSSION

By combining tables 1 to 5, several recommendable treatments are obtained, these being based on bulbs of various sizes and stages of disease, and treatments in different seasons. By addition of records of treatments of lesser durations, lower temperature or lesser concentration of formalin, one treatment has an efficacy significantly better than 95 per cent, this treatment being 2 hours in 1 per cent formalin at 118°F. with a 2-hour aqueous presoak at 70° to 80°F. The tolerance of bulbs to this treatment is not known.

Similarly, by addition of records one modification of the vapor heat treatment, namely 8 hours at 118°F. with a 2-hour presoak in 0.5 percent formalin at 70° to 80°F., had an efficacy significantly better than 90 percent.

The minimum hot-water treatment that, based on addition of records, had an efficacy significantly better than 90 percent was 4 hours at 118°F.

Based on addition of records other treatments with an efficacy significantly better than 90 percent are: 1 percent formalin at 118°F. for 1 hour with a 2-hour aqueous presoak; 1 percent formalin at 116°F. for 2 hours with a 2-hour aqueous presoak.

All efficacies determined by addition of records must be considered tentative. The tolerance of bulbs is not known for any one of these treatments.

The data in tables 1, 3, and 4 on the 0.5 percent formalin treatments at 110°F. for 4 hours with no presoak are adequate to demonstrate an efficacy of better than 90 percent with no addition of records from other treatments. The tolerance to this treatment, with the addition of a presoak has been determined by Blanton and Chitwood.[7] In an experiment on 40 varieties of narcissus and 41 lots during one year the controls showed a greater weight increase in every variety, while during the next year the controls showed a greater weight increase than the treated bulbs in only 23 lots and a lesser or equal weight increase in 18 lots. The only conclusion one may draw from this information is that there is great variability in tolerance to treatment.

Regarding the efficacy of a 4-hour treatment, in 0.5 percent formalin in tables 1, 3, and 4, the records for this treatment are 1–21, 0–12, and 1–42. Adding these one obtains a record of 2–75, or an observed efficacy of 97 percent. On the basis of binomial distribution this gives a predicted efficacy of better than 91 percent. Despite the rela-

[7] Proc. Helm. Soc. Washington 7(2): 91–94. 1940.

tively small number of bulbs involved, it would appear that this treatment is recommendable.

The efficacy of a 3-hour treatment in 0.5 percent formalin is indicated by records from tables 3, 4, and 5, these being 1–9, 2–29, and 0–8, respectively. Adding these one obtains the total 3–46 or an observed efficacy of 93 percent. The predicted efficacy would be considerably under 90 percent, but more extensive records might show this treatment to be recommendable.

SUMMARY

The results of narcissus-bulb treatments for *Ditylenchus dipsaci* over a period of 9 years are presented. These treatments are all modifications of the hot-water and vapor-heat treatments.

Because of the nature of the disease producing organism, *D. dipsaci*, and its mode of spread, the authors conclude that a treatment should have an efficacy of better than 90 percent, preferably 95 percent, to be recommendable in the control of this disease.

A method of evaluating the results of treatments is given. To prove a treatment efficacy of greater than 90 percent, at least 29 records of a complete kill of *D. dipsaci* are necessary. One bulb containing living specimens must represent at least 46 bulbs containing this species of nematode to substantiate an efficacy of better than 90 percent and two, three, and four bulbs with living nematodes must represent at least 61, 76, and 89 bulbs, respectively, to corroborate the same efficacy.

Hot-water treatments have been conducted at several temperatures ranging from 104° to 120°F. A 2-hour presoak in water at 70°–80°F. appears to be of some benefit from the standpoint of nemic control. However, no hot-water treatments other than those in combination with formalin are considered both practical and recommendable.

Vapor-heat treatments have shown no particular advantage over hot-water treatments from the standpoint of nemic control. No plain vapor-heat treatment is considered recommendable on the basis of the data available. A vapor-heat treatment at 118°F. for 8 hours preceded by a 2-hour presoak in 0.5 percent formalin at 70°–80°F. is recommendable from the standpoint of control, but it is considered too drastic for host tolerance. Less severe vapor-heat treatments in combination with a formalin presoak might be recommendable were sufficient data available.

It is shown that a certain degree of heat is essential to insure lethal action of formalin on *Ditylenchus dipsaci* in narcissus bulbs. A presoak

in a 0.5 percent formalin solution at 70°–80°F. followed by vapor-heat treatment probably has the same effect as a treatment in formalin solution at a higher temperature.

In hot-water formalin treatments the temperature showing the least bulb injury for the maximum efficacy appears to be 110°F. No benefit was apparent as a result of presoaks in combination with hot-water formalin treatments. The formalin used in these experiments ranged in concentration from 0.25 to 1.0 percent commercial formalin. Demonstrable differences in efficacy as a result of these various concentrations could have been due to chance but it would probably be safest to use not less than 0.5 percent formalin at the present time. Likewise, the differences as a result of varied proportions of bulbs to liquid could have been due to chance but it would be safest to use not less than 5.3 parts by weight of solution to 1 part by weight of bulbs. The treating bath may be used for two consecutive treatments providing enough formalin of the same concentration is added to bring up the volume. According to the present data the solution does not warrant further use thereafter.

On the basis of these data, a treatment in 0.5 percent formalin for 4 hours at 110°F. with no presoak is recommendable from the standpoint of nemic mortality. The tolerance of bulbs to this treatment is not known, but it is known for the same treatment with the addition of a 2 hours' aqueous presoak. According to this information narcissus varieties and lots of the same variety differ in their tolerance to the treatment. There is also a marked difference in the tolerance of the same stock of bulbs from year to year. In general, the increase in weight may be smaller in treated than untreated bulbs. Considering the damage inherent in the disease, treatment is not too drastic. Treatment for 4 hours in 0.5 percent formalin at 110°F. is the best treatment known today. It is recommended for all stocks containing a residuum of bulbs infected with *D. dipsaci.* A treatment of 3 hours in 0.5 percent formalin at 110°F. might be recommendable were sufficient data available.

In conclusion, the results of this work show that:

1. Estimates of efficacy should be based on binomial distribution formulae.
2. Considerable numbers of bulbs should be examined individually.
3. Hot-water and vapor-heat treatments require relatively high temperatures or long durations to be effective.
4. Hot-water formalin treatment at 110°F. for 4 hours is apparently a recommendable control measure for *Ditylenchus dipsaci* in narcissus bulbs