TOLERANCE TO DODINE AND INHERITANCE OF AN ASCOSPORE ABORTION FACTOR IN VENTURIA INAEQUALIS

Dissertation for the Degree of Ph. D.
MICHIGAN STATE UNIVERSITY
KEITH SEM YODER
1974

This is to certify that the

thesis entitled

TOLERANCE TO DODINE AND INHERITANCE
OF AN ASCOSPORE ABORTION FACTOR
IN VENTURIA INAEQUALIS
presented by

Keith Sem Yoder

has been accepted towards fulfillment
of the requirements for

Ph. D. degree in Plant Pathology

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Major professor

Date July 11, 1974

O-7639

ABSTRACT

TOLERANCE TO DODINE AND INHERITANCE OF AN ASCOSPORE ABORTION FACTOR IN *VENTURIA INAEQUALIS*

By

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Tolerance to dodine (Cyprex) in *Venturia inaequalis*, first reported in New York in 1969, could have serious consequences on the control of apple scab in Michigan because of similar growing conditions and considerable dodine usage.

Monoconidial isolates of *V. inaequalis*, collected in 1969 from dodine-treated and non-treated situations in Michigan, were compared with New York isolates by growth inhibition zones surrounding dodine-treated paper assay discs on potato-dextrose agar, germination of conidia in water solutions of dodine on glass slides, and growth inhibition by dodine in malt extract broth. Normal isolates were inhibited by a dodine concentration of approximately 1 µg/ml. The least sensitive Michigan isolates tolerated twice the inhibitory dodine concentration of normal isolates and were about one-half as tolerant as the least sensitive New York isolates. The tolerance levels of Michigan isolates tested did not correlate with the degree of control by dodine in orchards from which the isolates were taken.

Isolates with different levels of tolerance were crossed *in vitro* and progeny were isolated as ordered tetrads, unordered tetrads,

or random ascospore progeny. In crosses of the most tolerant isolate with normal isolates, the presence of tetratype asci having four distinct levels of tolerance to dodine suggests the additive action of at least two major independent genes conditioning the level of tolerance to dodine. This hypothesis is strengthened by the presence of highly tolerant and highly sensitive recombinant progeny from a cross of two isolates of the two intermediate tolerance levels in tetratype asci.

The presence of some progeny slightly more tolerant than either of two parents of normal sensitivity may suggest a recombination of several minor genes affecting the level of tolerance to dodine.

In crosses of highly tolerant parents and in a cross of a normal isolate with one of an intermediate tolerance level, no progeny were significantly more tolerant than the most tolerant parent, or more sensitive than the most sensitive parent.

Testing of progeny from crosses of a green color mutant with isolates representing intermediate and high levels of tolerance indicated that there was no close linkage between the genes for dodine tolerance and the green color gene of a known V. inaequalis linkage group.

An inoculation experiment with isolates of high and low sensitivities showed significant differences in control of infection on trees treated with dodine at 200 µg/ml, but symptoms produced by the two isolates were not uniform. Later experiments with isolates producing uniform symptoms showed that the tolerance levels of the isolates were factors in the control of infection on trees treated with dodine at 4 µg/ml but not at 10 µg/ml dodine.

Evidence suggests that inoculum density, temperature, and nutrient conditions may affect the tolerance levels of isolates. Such factors could explain the poor correlation of the dodine tolerance levels of isolates with the degree of control by dodine in situations where the isolates originated.

Because of its possible effect on inheritance of tolerance to dodine, an ascospore abortion factor arising from SR4, the most tolerant isolate, was further investigated. The presence of F_1 progeny as tolerant as SR4 but permitting normal development in test crosses indicated that the factors causing ascospore abortion were not the same as the genes conditioning higher levels of tolerance to dodine.

The effects of ascospore abortion in progeny of isolate SR4 are similar to some previously reported cases in other fungi. Evidence supports both genetic and environmental influences in the induction of ascospore abortion. The strongest evidence supporting a genetic influence is the recurrence of certain ratios of ascus types of crosses of SR4 X normal isolates in intercrosses of F_1 progeny and test crosses of F_1 progeny with normal isolates. The high, variable frequency of asci having odd numbers of spores is not readily explained genetically and suggests that cultural conditions may influence ascospore abortion.

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A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

DOCTOR OF PHILOSOPHY

Department of Botany and Plant Pathology

1974

TO ESTA

ACKNOWLEDGMENTS

I express my sincere gratitude to Dr. E. J. Klos who provided guidance and the opportunity to learn and grow throughout this course of study. I appreciate the advice and effort of my guidance committee, Dr. W. G. Fields, Dr. C. W. Laughlin, and Dr. J. L. Lockwood in thier review of the manuscript.

Special thanks are due to Dr. Halina Nowacka and Anna Bielenin for sharing their skills and patience in the isolation and testing of the ordered ascospore tetrads and to the technical assistance of So-Yung Jane Chai, Mary Pontoni, and Kassandra Keever.

I am indebted to Dr. D. M. Boone, University of Wisconsin for his helpful suggestions and contribution of Venturia inaequalis color mutants, to Dr. E. B. Williams, Purdue University for offering advice and sharing culture techniques, and to Dr. J. D. Gilpatrick, New York Agricultural Experiment Station for donating infected apple leaves.

The love, understanding, and patient assistance of my wife, Esta, have been most important to the completion of this project. The encouragement and forbearance of our parents and families is appreciated.

The support of the Michigan State Agricultural Experiment Station and American Cyanamid Company is gratefully acknowledged.

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PART I

TOLERANCE TO DODINE IN

VENTURIA INAEQUALIS

INTRODUCTION

With the advent of more highly selective fungicides, fungicide resistance by plant pathogens may occur more frequently (7, 11).

The tolerance of Venturia inaequalis (Cke.) Wint. to dodine is an example of this problem. Since its introduction in the mid-1950's this organic fungicide has effectively controlled a narrower range of target organisms than many of the earlier fungicides. Dodine has been remarkably effective as a fruit fungicide for the control of apple scab (V. inaequalis) and cherry leaf spot (Coccomyces hiemalis).

In 1969 Szkolnik and Gilpatrick (35) reported the failure of dodine to control apple scab in certain areas of New York State while other standard fungicides gave adequate control. This failure was attributed to fungal resistance acquired over a ten years' exposure to dodine. This report stimulated the present work in Michigan where growing conditions and practices are similar to those of New York State.

The purpose of this study was to determine the dodine tolerance levels of a group of natural isolates of Venturia inaequalis, to test the inheritance of dodine tolerance, and to observe the effects of the dodine tolerance level on control of the apple scab fungus.

LITERATURE REVIEW

Natural tolerance of V. inaequalis to dodine, first appearing in 1969 (35), has been the subject in recent reports (12, 36, 37, 38). This tolerance was shown to be inherited under the control of at least two genes (29, 30, 40). Ultraviolet-induced resistance to dodine in Nectria haematococca var. cucurbitae (15) was controlled by four mutant genes which had an additive effect on the degree of resistance when combined in progeny (16).

Natural variations have been detected in sensitivity of V. inaequalis to copper sulfate (25, 32), Paris green (25), captan, dichlone, glyodin, phenyl mercury acetate, and sulfur (32). An ultraviolet mutant grew on a medium containing a concentration of antimycin-A 5000 times greater than that required to inhibit the nonirradiated strain (19).

Attempts to develop strains of V. inaequalis resistant to captan (26), thiram, ferbam, ziram or zineb (27) by culturing the fungus in successively higher rates of the toxicants were unsuccessful. A wild type isolate developed adaptive tolerance to dodine after continuous culture on a medium containing dodine, but this tolerance was lost after brief culture on a dodine-free medium (22). The rate of spontaneous mutation to dodine tolerance of about one in 10^6 conidia was not affected by ultraviolet irradiation (22).

Fungal species differ greatly in sensitivity to dodine (2, 6, 23). In Fusarium solani f. sp. phaseoli a terminal decrease in retention of ^{14}C-labelled dodine was attributed to a modification of the dodine compound and subsequent release of a less toxic labelled compound (3). Although a slight terminal decrease in retention was also noted in V. inaequalis, evidence explaining the loss was lacking.

Studies of the mode of action of dodine have suggested that it competes for anionic binding sites (5, 33), alters membrane permeability (5, 33), and inactivates certain vital enzyme systems (5).

The inhibitory effects of dodine on natural apple scab development include the prevention of scab infection by inhibition of spore germination (1, 13, 14, 24, 31), prevention of sporulation of established lesions (1, 14, 24), and a darkening and thickening of the cell walls in the subcuticular stroma (14).

The maintenance of adequate surface residue is imperative to apple scab control with dodine (24). Inhibition of spore germination is dependent upon the amount of dodine available per spore, within the normal range of $1.7\text{-}2.6 \times 10^{-6}$ µg/conidium (23, 24). Residue disappearance studies have demonstrated a rapid loss of dodine surface residue during the first week after application, followed by a slower rate during subsequent weeks (10, 24, 34).

MATERIALS AND METHODS

Isolation from leaves

Scab-infected apple leaves were collected in Michigan from unsprayed orchards, orchards with a long history of dodine usage, orchards where scab control with dodine seemed unusually difficult and from wild crabapples. Samples were also collected from the Poray Orchard, Sodus, New York, where the tolerance problem was first discovered.

Monoconidial cultures were isolated by rubbing detached lesions across the surface of Difco potato-dextrose agar (PDA, pH 5.6) and transferring single germinating conidia to fresh PDA in petri plates. Stock cultures were maintained at 1-5 C in screw cap tubes.

Inoculum production

Inoculum for various tests was produced on cheesecloth wicks in 8 oz prescription bottles containing 30 ml 4% Difco malt extract broth, according to the methods suggested by Williams (39). Spores were collected by rinsing the nutrient medium from the bottles, adding 30 ml sterile deionized water, shaking the bottles vigorously to loosen the conidia, and straining the conidia through two layers of cheesecloth. Spore suspensions were standardized turbidimetrically by a modification of Kirkham's method (18). A standard spore suspension

contained about 4-6 X 10^5 conidia/ml when adjusted to an optical density at 0.34-0.41 in a colorimeter at 525 mμ with distilled water as a reference.

Testing of isolates

Cultures were tested for dodine tolerance using a disc assay, spore germination, and growth inhibition in liquid culture. The standard spore suspension was magnetically stirred into PDA at 42 C at the rate of 1 ml per 10 ml PDA for the disc assay. Twenty-two ml of the seeded agar was immediately poured into 9 cm plastic petri dishes and allowed to solidify. Two hours after the agar hardened a 12.7 mm assay disc (VWR Scientific, S and S No. 740E) was saturated with 0.163 ml of a 50 or 300 μg/ml dodine concentration and placed on the seeded agar. The plates were incubated at 19 C for one week before determining the zone of inhibition using indirect lighting against a light background. Two or three replicate plates were measured per test with three or four tests for each isolate.

For spore germination, 0.05 ml of the desired dodine concentration was pipetted onto ceramic-ringed glass slides and allowed to dry. An equal volume of the standard spore suspension was pipetted onto the same area of the slides. In an alternate method dodine was diluted into suspensions of conidia and the mixture was pipetted onto the slides. A minimum of 50 spores per 10x field were counted in each of the three fields of three or four replications after 24 hours incubation at 20 C.

Inhibition of growth of V. inaequalis was determined in 250 ml Erlenmeyer flasks containing 50 ml 4% malt extract broth with several rates of dodine. The cultures were inoculated with 3 ml of a standard spore suspension and incubated at 19 C for two weeks. The mycelium was filtered, oven-dried at 60 C and weighed.

The initial tests were conducted with the Cyprex 65W formulation of dodine. Subsequent tests used an equivalent rate of the technical grade of dodine (American Cyanamid Co., Princeton, N. J.) diluted with water from an ethanol stock solution with the appropriate ethanol controls.

Crossing of isolates and isolation of ascospores

Isolates were mated in 9 cm plastic petri dishes according to the methods of Keitt and Langford (17). The medium contained 0.5% malt extract and 2.5% Difco agar amended with apple leaf decoction. Aqueous spore suspensions of two single-spore isolates were mixed in the petri dish. The cooling agar was added and swirled to assure a uniform mixture. The dishes were incubated at 19 C for two weeks and then transferred to 8 C. After six months the ordered or unordered ascospore tetrads were isolated with a micromanipulator or by hand with a glass needle. Random ascospores were isolated from five or six perithecia crushed in water using sterile techniques. Single discharged spores were picked up with a small diameter capillary tube. All spores were germinated and cultured on PDA.

Greenhouse studies

Greenhouse inoculations were conducted on 2 year-old McIntosh apple trees. Actively growing trees were sprayed with Cyprex 65W applied to the runoff point with a paint sprayer (DeVilbiss Type GD3). After drying, the trees were spray-inoculated with spore suspensions of selected isolates containing approximately 2 X 10^5 conidia/ml. Immediately after inoculation the trees were placed in a moist chamber at 18-23 C for 2-4 days. Three weeks after inoculation the lesions on the one or two most heavily infected leaves of each shoot were counted and the data calculated as per cent control.

Statistical analysis

Significance of disc assays was determined by analysis of variance in a completely randomized design. Spore germination and greenhouse tests were analyzed in completely randomized or random block designs after converting percentages to per cent of control and applying the arcsine transformation as appropriate.

RESULTS

Screening of field isolates

The data in Table 1 are representative of those found in the disc assay screening of 144 isolates from apple leaves. Cultures were tested at two rates by soaking the assay discs in dodine concentrations of 50 µg/ml and 300 µg/ml. Isolate SR4, from the Poray Orchard where dodine tolerance was first noted, was the most tolerant field isolate collected. S1, from a dodine-treated commercial orchard, was the most tolerant Michigan isolate. Although zones ranging from 1 to 3 cm were noted in the 50 µg/ml test, isolates with average zone diameters of greater than 2.4 cm were considered to be quite normal.

The most tolerant isolates came from orchards where dodine was used extensively, but the tolerance level of the isolate was not always correlated with the length of dodine usage or with the apparent degree of control by dodine. S1, the most tolerant Michigan isolate, came from an orchard with excellent commercial control, while normal isolates L2, L4 and V2 were from orchards with scab control problems. Complete data on the survey of field isolates are presented in Appendix 1.

Isolates SR4, S1, and O4, representing three significantly different levels of tolerance, were chosen to determine their ability to grow in the presence of dodine. Growth of O4, a normal isolate, was almost completely inhibited by a dodine concentration of 1 µg/ml

Table 1. Inhibition of Venturia inaequalis in potato-dextrose agar by dodine diffusing from paper assay discs.

Isolate	Origin	Inhibition zone diam (cm)[a] 50 µg/ml	300 µg/ml
C2	Unsprayed tree, Michigan	3.05	3.75
L4	Commercial orchard, Michigan	3.03	3.60
V2	Commercial orchard, Michigan	3.00	3.67
B4	Unsprayed tree, Ohio	2.83	3.27
G5	Unsprayed tree, New York	2.73	3.53
04	Abandoned orchard, Michigan	2.68	3.63
CR2	Unsprayed crabapple, Michigan	2.60	3.38
S4	Commercial orchard, Michigan	2.58	3.38
H6	Unsprayed tree, Maryland	2.58	3.05
L2	Commercial orchard, Michigan	2.40	3.30
S1	Commercial orchard, Michigan	2.18	3.18
SR7	Poray Orchard, New York	1.70	2.58
SR4	Poray Orchard, New York	0.83	1.63
	LSD .05	0.48	0.34
	LSD .01	0.65	0.46

[a]Mean of four tests. Zones were measured after seven days' incubation at 19 C. Concentration of dodine with which assay disc (12.7 mm diam) was saturated.

(Table 2). S1, the most tolerant Michigan isolate, grew well at 1 µg/ml but was inhibited at 2 µg/ml. SR4, the most tolerant New York isolate collected, grew well at 2 µg/ml but was inhibited by 5 µg/ml.

Table 2. Growth of mycelia of selected Venturia inaequalis isolates after 2 wk incubation at 19 C in malt extract broth .

Isolate	Tolerance Level[b]	Dry weight (mg) at indicated dodine concn (µg/ml)[a]				
		0	0.2	1.0	2.0	5.0
04	low	42.7	40.0	5.8	NG[c]	NG
S1	intermediate	37.3	36.4	32.3	NG	NG
SR4	high	34.4	34.5	40.0	38.6	NG

[a]Mean of two replications.

[b]Tolerance levels based on inhibition zone diam in 50 and 300 µg/ml disc assays:
04 (2.68 and 3.63 cm); S1 (2.18 and 3.18); SR4 (0.83 and 1.63 cm).

[c]NG = no growth.

Crossing of isolates

Initial crosses of the most tolerant isolates with normal isolates were made to determine if the level of dodine tolerance is heritable. Test crosses of F_1 progeny with the most tolerant parent and with normal isolates, and intercrosses of F_1 progeny were set up to test the two-major-gene hypothesis for inheritance of dodine tolerance. Finally, two isolates having different levels of tolerance were crossed with 2295-2, a green color mutant from Dr. D. M. Boone, Dept. of Plant Pathology, University of Wisconsin to study possible linkage between genes for tolerance and the green color gene of a known V. inaequalis linkage group.

Progress of this study was impeded by an ascospore abortion factor arising from the most tolerant isolate, SR4. Of the asci produced by crosses of SR4 with normal isolates, only about 2% had eight spores. This greatly impaired the isolation of ascospore tetrads and further evidence was needed regarding this factor before firm conclusions about inheritance of dodine tolerance could be drawn. These studies will comprise a later portion of this dissertation.

Testing of F_1 progeny

Progeny of the initial crosses were tested for tolerance by disc assay and germination of conidia in dodine (Table 3). In both types of tests differences in results of two members of the same spore pair were insignificant but differences between members of different pairs were significant.

In these tetratype asci one pair of spores gave rise to cultures having a tolerance level similar to that of the normal parent (04), one pair was similar to the more tolerant parent (SR4), and two pairs had distinct intermediate levels of tolerance. Tetratype asci characteristic of a two gene interaction (8, 9), were found in crosses of SR4 with several unrelated normal isolates although the intermediate levels were not always significantly different. In a survey of these crosses 12 of 20 asci tested were considered to be tetratypes based on the presence of one pair of cultures having a level of tolerance similar to SR4. Usually the tolerance level of the more sensitive parent was also discernible in one pair of cultures from a tetratype ascus, although this level depended somewhat on the isolate that was

Table 3. Inhibition by dodine of germination and growth of conidia of the ordered monoascosporic cultures of a tetratype ascus of Venturia inaequalis cross 04 X SR4.

Isolate	Position in ascus	Inhibition zone diam (cm)[a] 50 µg/ml	300 µg/ml	% inhibition of germination[b]
04SR4-1-2	1	1.80	2.75	73.5 ab
04SR4-2-2	2	1.90	2.60	79.4 b
04SR4-3-2	3	1.23	2.33	65.9 a
04SR4-4-2	4	1.13	2.23	63.9 a
04SR4-5-2	5	2.30	3.15	94.7 cd
04SR4-6-2	6	2.27	3.25	94.0 c
04SR4-7-2	7	2.98	3.62	97.9 de
04SR4-8-2	8	2.70	3.30	98.1 e
04	parent	2.68	3.63	---[c]
SR4	parent	0.83	1.63	---
	LSD .05	0.41	0.35	
	LSD .01	0.56	0.48	

[a]Mean of four tests. Inhibition by dodine diffusing from 12.7 mm diam paper assay disc in potato-dextrose agar after seven days' incubation at 19 C. Dodine concentration with which paper assay disc was saturated.

[b]Inhibition in 1 µg/ml dodine in water. Generally 4 µg/ml completely inhibited germination of the most tolerant isolates. Means followed by the same letter are not significantly different (P = .05) according to Duncan's Multiple Range Test.

[c]Parent isolates not tested same as offspring.

crossed with SR4. Two of these asci were thought to be nonparental ditypes having only intermediate levels of tolerance and one was a parental ditype ascus. Five of the 20 asci did not clearly fit any

of the above tetrad types due to experimental error. Figure 1 illustrates dodine tolerance levels demonstrated by types of asci in the disc assay at 50 µg/ml.

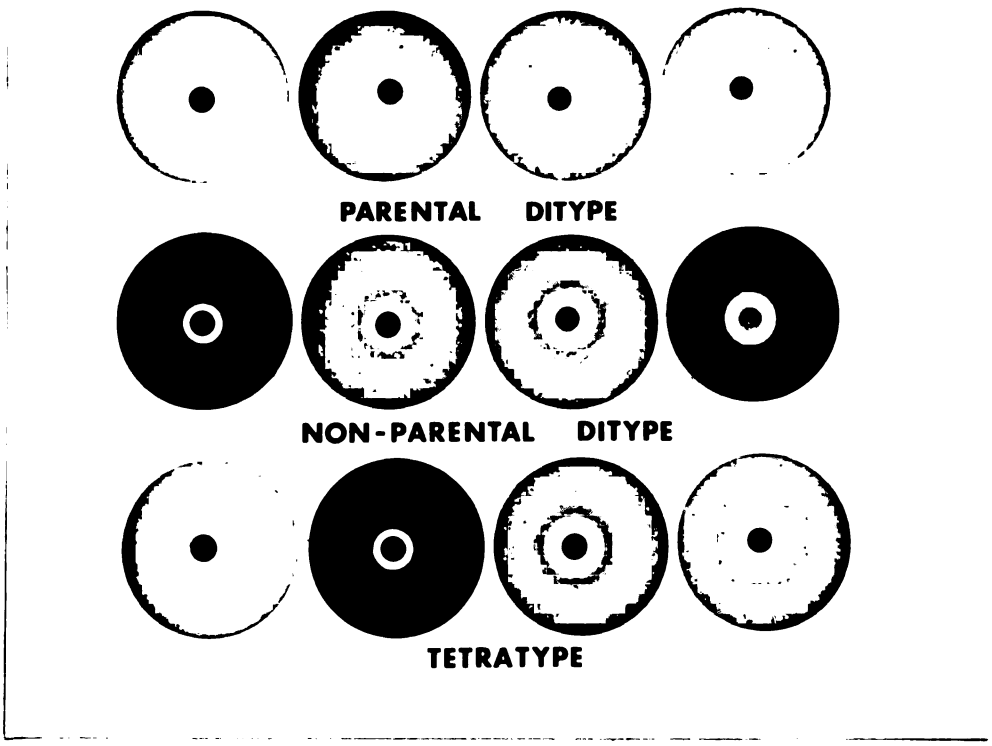

Figure 1. Inhibition of isolates from ascus tetrads of SR4 X normal crosses by dodine diffusing through potato-dextrose agar from paper assay disc.

The two most tolerant isolates, SR4 and SR7 (Table 1), were crossed to determine whether recombination of these two tolerance levels would yield progeny with dodine tolerance levels greater than SR4. None of the 39 progeny was more tolerant than SR4 (Appendix A2). Two isolates were slightly more sensitive than SR7, but not as sensitive as normal isolates. Apparently there was no recombination of major tolerance genes in this cross.

Crosses of F_1 progeny

Some progeny of a cross of isolates 04SR4-6-1 and 04SR4-1-2, representing the two intermediate tolerance levels of tetratype asci, demonstrated inheritance of the tolerance levels of 04 and SR4 in the 50 µg/ml test (Figure 2). This indicated a recombination of two major genes conditioning dodine tolerance. Fewer significant deviations from the tolerance levels of the intermediate parents were seen in the 300 µg/ml test, although eight of the 71 progeny were significantly more tolerant than the more tolerant parent, 04SR4-1-2. A complete tabulation of the data composing Figure 2 is presented in Appendix A3.

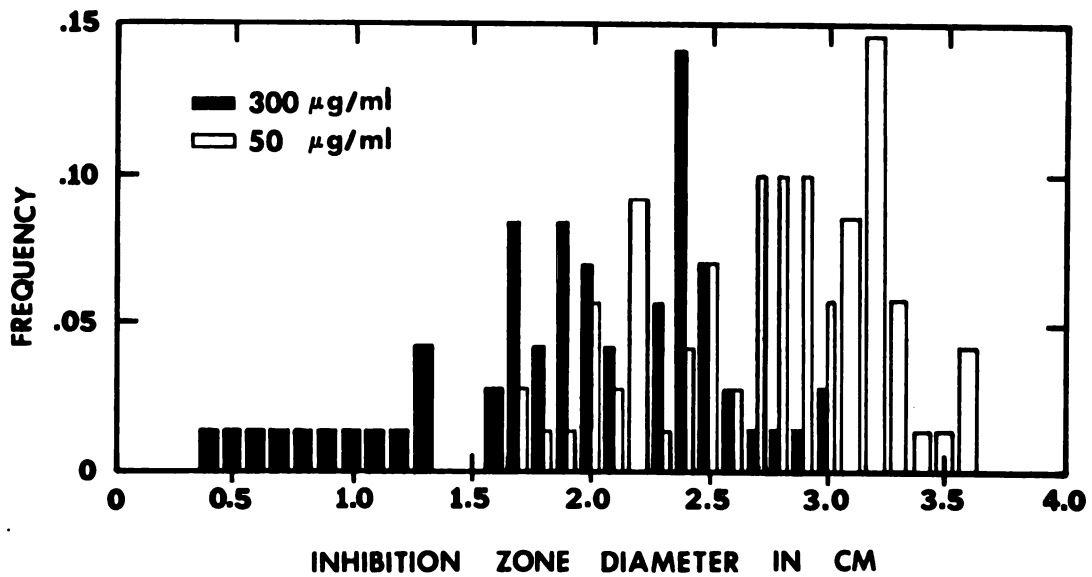

Figure 2. Frequency distribution of dodine inhibition zones of 71 progeny of cross 04SR4-1-2 X 04SR4-6-1, isolates representing the two intermediate tolerance levels in tetratype asci. Assay discs were saturated with indicated dodine concn. Four replications measured after 1 wk incubation at 19 C. LSD .05: 300 µg/ml (0.63 cm); 50 µg/ml (0.47 cm). Zone diam of parents in 50 and 300 µg/ml tests: 04SR4-1-2 (1.80 and 2.68 cm); 04SR4-6-1 (2.38 and 3.20 cm).

In a backcross of SR4 with O4SR4-3-2, representing the highest dodine tolerance level in a tetratype ascus (Table 3), none of 30 progeny were significantly more sensitive than the more sensitive parent or significantly more tolerant than the more tolerant parent (Appendix 4).

In a test cross of a normal isolate, C2, with O4SR4-5-2, intermediate in tolerance in a tetratype ascus (Table 3), none of 31 isolates were more sensitive than C2 to dodine (Appendix A5). Although seven progeny had slightly smaller zones than O4SR4-5-2 in the 300 µg/ml test, none were significantly more tolerant than this parent at 50 µg/ml.

Fourteen of 32 progeny of cross C2 X O4SR4-7-2 were significantly more tolerant at 50 µg/ml than either of their parents (Appendix A6). Seven of the isolates reacted to 300 µg/ml with significantly smaller zones than either of the parents of these progeny, although not significantly smaller than O4, normal parent of O4SR4-7-2 (Table 3).

In these three crosses, SR4 X O4SR4-3-2, C2 X O4SR4-5-2, and C2 X O4SR4-7-2, there was no apparent recombination of major tolerance genes. The presence of some progeny slightly more tolerant than either of the two parents C2 and O4SR4-7-2, but not significantly more tolerant than O4, may indicate the recombination of minor genes affecting the level of tolerance to dodine.

Significance of ascospore abortion in inheritance of dodine tolerance

The ascospore progenies whose test results are reported above were all isolated from crosses with a high frequency of ascospore abortion. Because the causal nature of this phenomenon is not known, conclusions regarding frequencies of different levels of tolerance in these crosses must be restricted. Linkage of the ascospore abortion and dodine tolerance factors cannot be excluded on the basis of present evidence. However, all tolerance levels found in tetratype asci were also found among progeny isolated from asci with less than eight spores. Also the discovery of several monoascosporic isolates similar to SR4 in dodine tolerance but not inducing ascospore abortion indicates that the two phenomena are not controlled by the same factor or factors.

Ascospore abortion was not a factor in a later cross of a normal isolate, S4, with C2SR4 7-4, an F_1 progeny as tolerant as SR4. The frequencies of isolates at different tolerance levels in the 50 µg/ml and the 300 µg/ml tests were similar in 40 progeny of this cross (Appendix A7) and in progeny of 04SR4-1-2 X 04SR4-6-1 (Figure 2). This is evidence that ascospore abortion probably did not affect the frequency distribution of dodine tolerance levels in the earlier crosses.

Non-linkage of dodine tolerance genes and green color gene

Isolates representing two significantly different levels of tolerance were crossed with 2295-2, a green color mutant with normal

dodine tolerance, to study possible linkage of the genes for dodine tolerance with the green color gene of an identified V. inaequalis linkage group (4). Distribution of the tolerance levels of 20 wild type and 24 green progeny was not significantly different throughout the tolerance range demonstrated (Appendix A8). The tolerance levels of 27 progeny of 2295-2 and C2SR4-7-3, an F_1 progeny similar to SR4 in tolerance, were also distributed throughout the tolerance range of their parents (Appendix A9). This random distribution of tolerance levels of green and wild type progeny suggests free recombination and non-linkage of the genes for dodine tolerance and the green color gene.

Inoculation studies

In an initial inoculation experiment (Table 4) significantly greater control by 200 µg/ml dodine was achieved on trees inoculated with normal isolate 04 than on trees inoculated with the most tolerant isolate, SR4. However, a qualitative difference in symptoms caused by the two isolates on the unsprayed trees was noted. 04 had normal heavily-sporulating lesions, but SR4 produced lesions which were more chlorotic with sporulation somewhat reduced.

Because of the difference in macroscopic symptoms, experiments were repeated with tolerant and normal isolates selected for uniform symptoms on the unsprayed trees (Table 5). When total lesions were counted there was no significant difference in per cent control of the two isolates by the same rate of the fungicide at any rate. However, there was significantly less suppression of sporulation of the more tolerant isolate, H6SR4 6-1, by dodine 4 µg/ml than of normal isolate H6.

Table 4. Control of infection by *Venturia inaequalis* isolates 04 (normal dodine sensitivity) and SR4 (high dodine tolerance) on trees treated with dodine (Cyprex 65W).[a]

Isolate	Dodine(μg/ml)	Lesions/leaf[b]	Percent control[c]	Percent shoots with infection
04	unsprayed	12.9	0.0 b	100.0 a
04	200	1.5	88.3 a	37.5 b
04	300	0.2	98.4 a	16.7 bc
04	600	0.0	100.0 a	0.0 cd
SR4	unsprayed	7.7	0.0 b	100.0 a
SR4	200	5.3	31.2 b	72.2 a
SR4	300	0.8	89.6 a	19.4 bc
SR4	600	0.1	98.7 a	8.3 cd

[a]Completely randomized design, three replications. Trees held in moist chamber four days at 19-22 C.

[b]Lesions per single most heavily-infected leaf per shoot.

[c]Percent control = $100 - \frac{\text{lesions/leaf, sprayed}}{\text{lesions/leaf, unsprayed}} \times 100$

Column means followed by the same letter are not significantly different (P = .05).

Table 5. Control of sporulating lesions of Venturia inaequalis isolates H6 (normal dodine sensitivity) and H6SR4 6-1 (high dodine tolerance) on trees treated with dodine (Cyprex 65W)[a]

Isolate	Dodine µg/ml	Percent control of sporulating lesions[b]
H6	4	86.7 ab
H6	10	81.9 bc
H6	200	96.9 a
H6SR4 6-1	4	66.8 c
H6SR4 6-1	10	90.3 ab
H6SR4 6-1	200	86.1 ab

[a]Randomized block design, averages of three experiments with five replications each. Trees were held in moist chamber two days at 19-22 C. Inhibition zone diameters in 50 µg/ml and 300 µg/ml disc assays: H6 (2.58 and 3.05 cm); H6SR4 6-1 (0.3 and 1.4 cm).

[b]Based on two most heavily-infected leaves per shoot. Sporulating lesions determined by macroscopic symptoms. Column means followed by the same letter are not significantly different (P = .05).

An experiment was conducted to assess the effect of inoculum density on apple scab control by dodine. After fungicide treatment trees were spray-inoculated with suspensions containing 81,000 and 312,000 conidia/ml of normal isolate, 04. At dodine concentrations of 100 µg/ml and 300 µg/ml significantly more shoots were infected on trees under the heavier inoculum conditions than under the lighter inoculum conditions (Table 6). Thus a heavy buildup of inoculum caused by negligent control measures early in the growing season could be an important factor in control by dodine throughout the entire season.

Table 6. Effect of inoculum density of Venturia inaequalis isolate 04 (normal dodine sensitivity) on apple scab control by dodine (Cyprex 65W).[a]

Dodine (µg/ml)	Percent shoots with infection at indicated inoculum density		Significance
	81,000 conidia/ml	312,000 conidia/ml	
unsprayed	100.0	100.0	N.S.
100	41.1	86.7	P = .01
200	49.0	75.3	N.S.
300	13.7	57.4	P = .05

[a]Completely randomized design, three replications.

Effect of temperature on germination of conidia in dodine

Germination tests of conidia of two isolates in dodine at controlled temperatures of 10 C, 19 C, and 26 C in water and on PDA showed that temperature had a differential effect on the germination

of the controls of the two isolates in water, but not on PDA (Table 7). Although H6SR4 6-1 (highly tolerant) had poorer germination than H6 (normal dodine sensitivity) at all three temperatures, the effect was most noticeable at 10 C and 19 C.

Temperature also had a differential effect on percent inhibition of germination by dodine on PDA (Figure 3-A). At all temperatures H6SR4 6-1 was less inhibited by dodine than H6. H6 was most inhibited by dodine at 10 C and least inhibited at 26 C. H6SR4 6-1 was most inhibited at 19 C and least inhibited at 10 C.

Table 7. Per cent germination of conidia of two Venturia inaequalis isolates on PDA and in water at indicated temperatures.[a]

	10 C		19 C		26 C	
	H6	H6SR4 6-1	H6	H6SR4 6-1	H6	H6SR4 6-1
Water	78	44	72	51	80	68
PDA	81	89	84	82	75	77

[a]Means of two three-replicate tests.

Dodine inhibition of germination in water (Figure 3-B) was not greatly affected by temperature except at 10 C, where H6 was much more inhibited at lower dodine concentrations than H6SR4 6-1.

On PDA and in water H6SR4 6-1 was least inhibited by dodine at 10 C. These data cannot be interpreted to say that the more tolerant isolates generally react to temperature in dodine inhibition in this manner, but they show that temperature may affect the relative

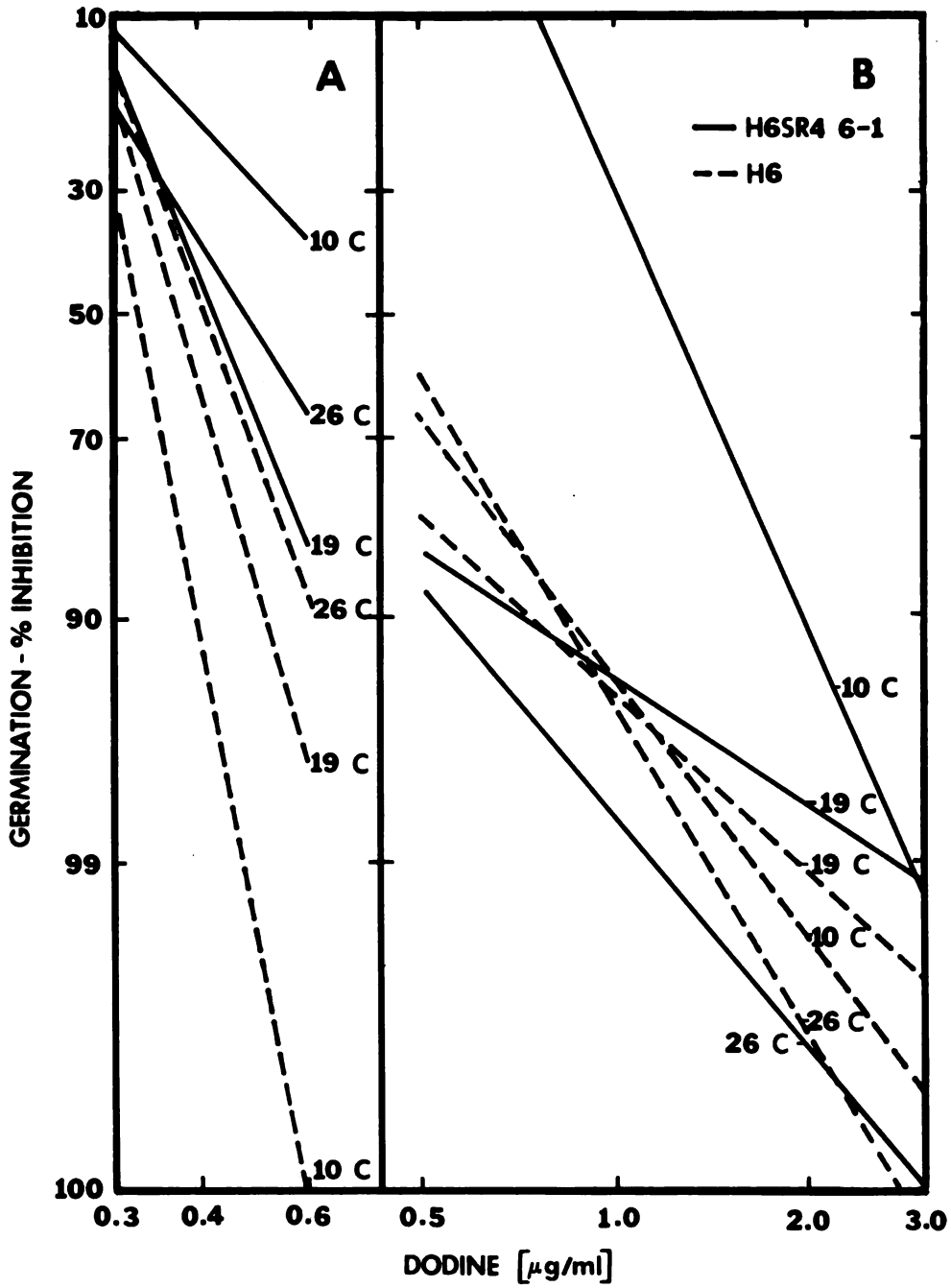

Figure 3. Effect of temperature on dodine inhibition of germination of conidia of two Venturia inaequalis isolates. A) On PDA. Both isolates tested at 0.3 and 0.6 µg/ml dodine. B) In water. Isolate H6SR4 6-1 tested at 1.0, 2.0, and 3.0 µg/ml dodine. Isolate H6 tested at 0.5, 1.0, and 2.0 µg/ml.

tolerance level of an isolate. Lower inhibition by dodine at cooler temperatures could be a significant factor in apple scab development during the earlier part of the season.

DISCUSSION

Tolerance levels of isolates

The relative dodine sensitivity levels of field isolates tested in this study varied somewhat depending on the testing method, but the most tolerant isolates were inhibited by dodine concentrations about five times the concentration inhibitory to the most sensitive isolates. These differences in dodine tolerance levels are comparable to those noted previously (12, 22, 36, 40). Actual differences between sensitivity levels reported here and those reported elsewhere may be due to differences in cultural pH and nutrient conditions or inoculum density. The relative differences in sensitivity to dodine are less than those previously reported for phenyl mercury acetate, similar to those reported for dichlone and sulfur, and about two times the natural variation in sensitivity reported for glyodin, captan, copper sulfate and Paris green (25, 32).

The degree of natural variation in sensitivity to dodine by V. inaequalis in this study is similar to, or slightly less than, that expressed by the dodine-resistant mutants in Nectria haematococca var. cucurbitae developed by Kappas and Georgopoulos (16). The variation in tolerance in V. inaequalis shown here is about 1000 times less than that of the antimycin A-resistant ultraviolet mutant developed by Leben et al (19).

Inheritance of tolerance

The pattern of inheritance of tolerance to dodine found in this work appears to follow more closely that proposed by Kappas and Georgopoulos for two Nectria mutants (16) than that suggested by Polach for V. inaequalis (30). In the system proposed for Nectria, each of two genes conditioned different levels of tolerance when inherited separately, and were additive when inherited together. In the system suggested by Polach (30) a nontolerant isolate would not grow at 0.25 µg/ml, one gene would allow growth at 0.25 µg/ml, and a second gene, effective only when the first is in dominant form, allows growth at 0.5 µg/ml dodine. In the present study disc assay of asci of crosses of the most tolerant isolate with normal isolates has shown four distinct levels of tolerance (Table 3). An explanation for this phenomenon is the independent action of two separate genes, each conditioning distinct levels of tolerance, having an additive effect when inherited together (8, 9). This has been confirmed by a cross of the two intermediate types to yield recombinant progeny more tolerant or more sensitive than either of the intermediate parents.

The differences in reaction of progenies of crosses in this work and those of Polach may be the tolerance levels of the parent isolates. SR4 was the most tolerant of 64 New York isolates in this screening (Appendix A1), including 48 from the Poray Orchard, 1969-1971, and 16 from the McQueen Orchard, 1969, another area where the tolerance problem was found (36). The tolerance level of the more sensitive parent of a cross also could contribute to a different reaction by the progeny. One difference in the testing techniques

employed in the two studies may be that in the disc assay each isolate could respond to the dodine concentration gradient to indicate a distinct level of tolerance not evident when the isolates were screened at set concentrations.

Further testing must be conducted to assure that recombinant progeny more tolerant than SR4 cannot arise from crosses of S1 with isolates having tolerance levels similar to SR4. If the gene conditioning the tolerance level of S1 is the same as one of the genes in SR4 then there should be no buildup in tolerance from this cross. However, if three separate genes are involved, then recombinant progeny should appear with no tolerance genes or with all three tolerance genes. The reaction of the progeny having three tolerance genes would depend on whether the action of the third gene is additive or whether its action is masked by the other two genes. In a cross of S1 with a highly tolerant progeny of SR4, none of 10 progeny were as tolerant as SR4, nor did they have the normal level of sensitivity which would indicate a recombination of tolerance genes.

Mechanisms for tolerance to dodine

The mechanisms for the genes for tolerance to dodine are unknown. Of the previously suggested general mechanisms (7, 11) either decreased permeability or detoxification seems to be the most likely possibility for a mechanism conferring such a comparatively low difference in sensitivity. This is in contrast to fungicides with more specific sites of action to which resistant strains have shown a 100 to 1000-fold decrease in sensitivity (7). A re-investigation of the

retention of ^{14}C by tolerant and sensitive conidia following exposure to ^{14}C-labelled dodine could shed insight on the problem of detoxification described by Bartz and Mitchell (2, 3). The interaction of response to dodine with natural variations in response to different nutrient (20, 28), pH, and temperature conditions (25) may be important to an understanding of the problem of tolerance to dodine under field conditions.

Fungicide tolerance as a factor in control of apple scab

Inheritance of tolerance to dodine in V. inaequalis has been clearly demonstrated. However, one must experimentally assess the importance of the degree of fungal sensitivity to dodine as a factor in apple scab control to place it in proper perspective with other factors such as weather factors, inoculum density, proper coverage and timing and rate of dodine application (21). A natural mutation rate to dodine tolerance as low as 1 in 10^6 (22) should be high enough to assure a constant population with this level of tolerance in the orchard if the selection pressure of dodine is the major factor inhibiting survival of the fungus.

The limited emergence of populations sufficiently tolerant to have notable economic importance in spite of a relatively high mutation frequency has been attributed to "overkill" in commercial practice (22). The ability of dodine to prevent sporulation has undoubtedly limited the population screened for dodine tolerance in nature. Additional factors limiting the success of strains with high

levels of dodine tolerance may be reduced pathogenicity or reduced sporulation. Isolate SR4 sporulated well in culture, but sporulation was limited on plants in the greenhouse.

The majority of the cases of poor scab control by dodine investigated in Michigan demand an alternate explanation because isolates collected from heavily-infected orchards have shown a normal level of tolerance to dodine while the most tolerant Michigan isolates have come from orchards with good control. Frequently poor control in mid-season can be attributed to an early buildup of inoculum due to late timing of an early application, poor coverage due to improper pruning, or careless spraying. Inoculum density has been shown to be an important factor in apple scab control by dodine (Table 6). Since inhibition of spore germination is dependent upon the amount of dodine available per spore (24), an early season buildup of inoculum means the rate of application must be increased or the spray interval reduced, or both, to maintain the level of residue essential to adequate control.

PART I

LITERATURE CITED

PART I

LITERATURE CITED

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PART II

INHERITANCE OF AN ASCOSPORE ABORTION FACTOR ARISING FROM *VENTURIA INAEQUALIS* ISOLATE SR4, AN ISOLATE HIGHLY TOLERANT TO DODINE

INTRODUCTION

The intriguing discovery of the induction of ascospore abortion in Venturia inaequalis by SR4, the isolate most tolerant to dodine, demanded further investigation. The simultaneous occurrence of both phenomena in a single isolate suggested a common genetic cause. Conclusions regarding inheritance of the dodine tolerance level of this isolate could have been erroneous without further knowledge of the relationship between the two factors because aborted meiotic products could not be examined for dodine tolerance.

Further crossing of isolates and testing of their progeny revealed that the two factors did not always occur together in the offspring. Some F_1 progeny were found to be as tolerant to dodine as SR4, but produced only normal asci in crosses with other isolates not inducing abortion. Some progeny were normal with respect to dodine tolerance, but produced abnormal asci. These two points are conclusive evidence that the two factors do not have a common genetic cause. From the present data we cannot exclude the possibility that both factors may be caused by linked genes.

This paper reports observations on the magnitude of ascospore abortion induced by isolate SR4 and by progeny of this isolate, and compares the morphological characteristics of this phenomenon with previously reported cases. The possible basis of the phenomenon and its effect on the organism's natural survival are discussed.

LITERATURE REVIEW

The ascospore abortion phenomenon has been recognized in the literature. Dodge (11) pointed out that in the Ascomycetes one usually observes gene expression in the organisms' haploid structures, and that the asci with their fusion nuclei are the only cells of structures in the entire life cycle in which one could demonstrate simple mendelian dominance. In an X-rayed line of Neurospora tetrasperma a recessive lethal factor prevented ascospore delimitation when inherited in the homozygous condition (9, 10).

Eight-sporedness appeared to be dominant in a cross of a four-spored N. tetrasperma with an eight-spored N. sitophila because all the resulting asci had eight spores (8). The first backcross of F_1 progeny with the eight-spored parent usually gave asci with eight spores, but a backcross with the four-spored parent gave asci with spore numbers ranging from one to eight, mostly three to six (11).

"Aborta", a variant strain of Hypomyces Ipomoeae (7), induced the formation of only four rather than eight spores when crossed with normal isolates. This ascospore abortion was apparently due to the inability of nuclei carrying the aborta factor to delimit spores.

Aberrant ascospore segregations occurred as a result of translocation in Neurospora crassa (3, 21). Translocations, inversions, and deletions can be distinguished by the distribution of abnormal

asci (13, 14). The effects of spindle orientation and dominance modifiers on the development of aberrant asci have been demonstrated in Neurospora (23, 25, 29).

The "sterile ascus" phenomenon in selfed monoascosporic isolates of Cochliobolus heterostrophus (22) was probably controlled by more than one factor because certain backcrosses produced perithecia with varying ratios of sterile to normal asci. If inhibition of ascospore formation were controlled only by a single dominant or a single recessive gene, either all sterile asci or all fertile asci should have resulted. The occurrence of asci containing from one to eight ascospores was also reported in C. sativus (16).

Asci of Coniochaeta malocotricha containing one to eight spores were found to arise by abortion or non-maturation or by the failure of one or more nuclei to be included into the ascospores (26). The most frequent spore numbers in asci with fewer than eight spores were four and five.

Development of perithecia, asci and ascospores of Sordaria fimicola was directly related to biotin content of the growth medium (2). The frequency of abnormal asci with one to seven ascospores increased as the biotin concentration was decreased.

Venturia inaequalis cultures of a mutant white sector, although producing abundant perithecia in crosses with normal isolates, induced partial or complete abortion of four of the eight ascospores in each ascus (30). Aborted spores were one- or two-celled, colorless and usually misshapen. Aborted spores which were viable always gave rise to white cultures.

A number of nitrogen mustard-induced color mutants of V. inaequalis (5), when crossed with normal isolates, caused partial or complete abortion of the spores carrying the mutant genes. Two white mutants gave rise to 3:1 and 1:3 ratios of normal to defective spores as well as the more common 1:1 ratio when crossed with normal isolates. Conclusive evidence explaining these aberrant segregations was not attained.

An investigation of the chromosome number of V. inaequalis encountered abnormalities among crosses of wild-type cultures (6). One cross gave rise to two types of perithecia, one type with all normal asci, and one type with all asci showing abnormal 4:4 segregations. In one perithecium, a single 4:4 ascus was suggested to have resulted from crossing-over in an inversion heterozygote. Two other cytological studies of ascus development in V. inaequalis (1, 17) did not report such abnormalities.

MATERIALS AND METHODS

Crossing and isolation of progeny

All isolates used in this study were part of the isolate collection for the dodine tolerance study. The techniques for crossing and isolation have been described in Part I. In addition to the medium of Keitt and Langford (18), a modified potato-dextrose agar (5) was also tested.

Ascospore counts

Spores were counted at 430X after placing several perithecia in water on a microscope slide and applying pressure to the coverslip to break open the perithecia and expose and spread the asci. In making the counts, only those spores with mature coloration were considered normal. Counts were not conducted until spores were fully mature in all perithecia of a cross. Plates with mature perithecia were maintained at 4 C until counted.

Perithecia on leaves

Leaves from several orchards in New York were collected during the month of May (1969-1971) by Dr. J. D. Gilpatrick, New York Agricultural Station, Geneva, New York. The leaves were dried and refrigerated until the perithecia were removed for counting.

Staining of asci

Preliminary efforts to stain nuclei in asci were only partially successful. Mature spores were quite impermeable to stain and immature spores did not stain well within the ascus. The pressure needed to flatten the asci to give good microscopic resolution frequently displaced the contents of the asci.

The most successful stains were iron haematoxylin (19, 20) and 0.5% toluidine blue dissolved in water. Both stains gave good differentiation of nuclei in ascogenous hyphae, but were less successful for nuclei inside the asci. The methods of O'Donnell, Tai and Beneke were followed for fixation and hydrolysis for iron haematoxylin staining (24). Length of hydrolysis in 1N HCl at 65 C varied from two 10 minute periods to two 2 hour periods with no appreciable difference in results.

Toluidine blue dissolved in water stained nuclei as clearly without hydrolysis as with hydrolysis, but the asci were more inclined to burst in unhydrolyzed material. Toluidine blue in 70% ethanol (15) failed to give the desired contrast and 0.5% toluidine blue in 50% propionic acid was unsatatisfactory.

RESULTS

Apparent types of abortion

Several types of ascospore abortion may be influencing the number of spores per ascus in different crosses. Because immature asci were difficult to discern from asci which had reached their maximum development without any spore delimitation, asci without spores were not included in the counts. To allow complete spore maturation before counting, perithecia were maintained at a low temperature after initial maturity was reached. This storage may have allowed some disintegration of early maturing spores. However, in most cases, asci containing less than eight spores appeared to be a result of underdevelopment rather than disintegration of spores.

Figure 4-A illustrates three developmental types of asci found in these crosses. Ascus 1 appears to be in the process of ascospore delimitation and may represent an immature form. Ascus 2 apparently failed to delimit more than four spores. The lack of mature coloration in the smaller cell of the terminal spore in this ascus may signify that this cell is inactive and could disintegrate to give rise to a one-celled spore. Ascus 3 reached its maximum development without delimiting any spores. Empty asci were found in crosses of isolates not inducing ascospore abortion as well as in crosses of isolates inducing extreme ascospore abortion.

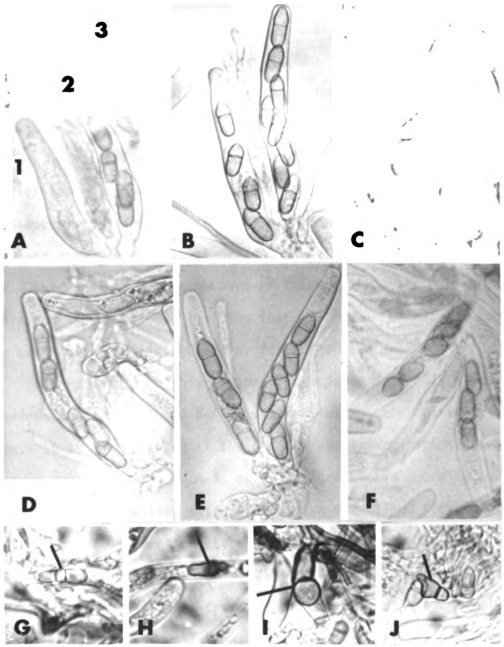

Figure 4. Ascospore abortion in progeny of Venturia inaequalis isolate, SR4. A-F) Normal and aberrant asci. G-J) Abnormal ascospores. A) 1, maturing ascus; 2, four-spored ascus, smaller cell of terminal spore is poorly pigmented; 3, aborted ascus. B) Normal eight-spored ascus.

Asci containing one to eight spores were found in crosses of SR4 with normal isolates. In most of these asci reduction of spore numbers appeared to be due to improper spore cleavage (Figure 4-A, 4-E, and 4-F) or failure of cleaved spores to mature (4-B, 4-C, and 4-D).

Occasionally odd numbers of spores in asci may have resulted from disintegration of one member of a spore pair (Figure 4-E, three-spored ascus). In one four-spored ascus a culture of one of the four spores had a much higher level of tolerance to dodine than the other three. This indicates that members of three spore pairs were present in this four-spored ascus, implying that one member of each of two spore pairs either disintegrated or failed to develop.

Very rarely, highly abnormal ascospores occurred in perithecia of various crosses. Figure 4-F shows an ascus containing single-celled spores. It is not apparent if these arose by disintegration of one cell as suggested by Figure 4-A, or by failure of the spore to undergo the final mitotic division essential to give two-celled spores, or if the two cells of a spore separated and rounded as they matured.

An abnormally developing globose ascus (Figure 4-D) contained structures similar in shape to the smaller cells of normal spores. An immature ascus (normally somewhat globose) is pictured in Figure 4-E.

Several other highly abnormal, mature-colored spores (Figure 4-G-J) occurred only rarely in crosses of SR4 or its progeny, but they may represent an unusual modification of factors contributing to the more common types of ascospore abortion. The three cells of the spore

in Figure 4-G remained intact when the ascus was broken. The bulboid protuberance from one cell of the spore in Figure 4-H suggests the possibility that one cell may undergo an additional mitotic division to yield a three-celled spore. The misshapen ascospore in Figure 4-J may be undergoing germination. An unusually large spore (Figure 4-I) maintained a fairly normal shape although it was nearly two times the normal size. Another one-celled spore nearly filled an ascus about half the size of a normal eight-spored ascus.

Frequency of abnormal asci in crosses of SR4 with normal isolates

Crosses of SR4 with normal isolates (not inducing ascospore abortion) produced a high frequency of asci containing one, three, five, or seven spores (Table 8). The ratios of these asci with odd numbers of spores to asci with even spore numbers varied considerably with the parental combination. Combined frequencies of adjacent categories (one- and two-spored asci, three- and four-spored asci, etc.) were fairly consistent regardless of the parental combination. Since abortion due to inheritance of lethal genes should occur in pairs of spores rather than single spores, the variable distribution of asci with odd spore numbers suggests a non-genetic response to some variable cultural condition causing the underdevelopment or disintegration of one member of a spore pair. The higher consistency of frequencies of asci with odd spore numbers combined with asci having even spore numbers shows that usually spores were aborted in pairs rather than singly and suggests a strong genetic influence in ascospore abortion.

Table 8. Frequency of ascospore abortion in crosses of SR4 with normal isolates.

Cross	Percent asci with indicated number of spores per ascus												Total asci
	1		2	3		4	5		6	7		8	
SR4 X T2	2	(14)[a]	12	14	(43)	29	25	(42)	17	0	(1)	1	77
SR4 X B4	1	(11)	10	28	(59)	31	20	(36)	16	1	(4)	3	110
SR4 X B6	1	(17)	16	17	(52)	35	4	(29)	25	0	(2)	2	114
SR4 X H6	5	(27)	22	16	(48)	32	10	(22)	12	0	(3)	3	116
SR4 X C2	3	(25)	22	22	(49)	27	10	(25)	15	0	(1)	1	78
SR4 X CR6	0	(25)	25	30	(50)	20	13	(25)	13	0	(0)	0	56
SR4 X L1	0	(19)	19	9	(38)	29	19	(38)	19	0	(5)	5	21
SR4 X L5	3	(12)	9	15	(52)	37	24	(36)	12	0	(0)	0	33
SR4 X 04	0	(14)	14	29	(43)	14	14	(43)	29	0	(0)	0	7
% of total	2	(19)	17	18	(48)	30	14	(31)	17	0	(2)	2	612

[a]Figures in parentheses represent totals of two adjacent categories.

Overall, in crosses of SR4 with normal isolates, only two percent of the asci had eight spores. Asci having one or two spores ranged from 11 to 28% (19% of total). Asci with three or four spores ranged from 38 to 59% (48% of total). Those having five or six spores included 31% of the total with values ranging from 23 to 43%.

Ascospore abortion in crosses of F_1 progeny of SR4

To study inheritance of the ascospore abortion factors, crosses involving F_1 progeny of SR4 were examined for abortion frequencies (Table 9). In crosses of groups of normal isolates with members of the same spore pair, frequencies of ascus types were quite

Table 9. Frequency of ascospore abortion in test crosses of F_1 progeny of 04 and SR4.

Cross	Percent asci with indicated number of spores per ascus												Total asci
	1		2	3		4	5		6	7		8	
04SR4-1-2 X N[a]	2	(17)[b]	15	9	(43)	34	12	(39)	27	1	(1)	0	146
-2-2 X N	2	(29)	27	26	(41)	15	10	(29)	19	.7	(1)	.7	135
04SR4-3-2 X N	1	(7)	6	7	(52)	45	10	(40)	30	.4	(1)	.6	627
-4-2 X N	2	(24)	22	11	(54)	43	7	(21)	14	0	(1)	1	374
04SR4-5-2 X N	0	(10)	10	7	(52)	45	2	(4)	2	8	(34)	26	97
04SR4-6-2 X N	0	(12)	12	6	(28)	22	13	(28)	15	22	(32)	10	60
04SR4-7-2 X N	0	(7)	7	2	(34)	32	2	(7)	5	8	(52)	44	108
04SR4-3-2 X SR4	2	(6)	4	17	(44)	27	15	(46)	31	4	(4)	0	48
-4-2 X SR4	1	(13)	12	5	(33)	28	9	(53)	44	0	(1)	1	102

[a]Groups of normal isolates. Tabulation of data from individual crosses is presented in Appendix B1. Members of spore pairs of a single tetrad: 04SR4-1-2 and -2-2; 04SR4-3-2 and -4-2; 04SR4-5-2 and -6-2; 04SR4-7-2.

[b]Numbers in parentheses represent totals of two adjacent categories.

consistent in two combined categories, but varied considerably in the other two. For example, the proportions of asci having three or four spores and those having seven or eight spores were similar in crosses of normal isolates with O4SR4-1-2 and O4SR4-2-2 of one spore pair and with O4SR4-3-2 and O4SR4-4-2 of another spore pair, but in these crosses there was more variation in the combined frequencies of asci having one or two spores and those having five or six spores. Crosses of O4SR4-5-2 with normal isolates and crosses of O4SR4-6-2 with normal isolates were similar in individual combined frequencies of one- or two-spored asci and combined frequencies of seven- or eight-spored asci, but were more variable in the three- or four-spore category and in the five- or six-spore category. This consistency in two classes and inconsistency in two other classes was also noted in crosses with SR4 (Table 9) and in crosses of members of one spore pair with members of another spore pair (Appendix B1).

If the ascospore abortion is entirely under genetic control then reactions in crosses of isolates with the genetically identical members of the same spore pair should be consistent in all categories. The similarity of combined frequencies in only two of the four combined categories of ascus types suggests that certain reactions were under more stable control than ones which were more variable.

The frequencies of asci in each numerical category were similar in crosses of SR4 X normal isolates, O4SR4-3-2 and -4-2 X normal isolates, O4SR4-1-2 and -2-2 X O4SR4-7-2, and O4SR4-3-2 X O4SR4-6-2. The frequencies of different ascus types in the backcrosses O4SR4-3-2 and -4-2 X SR4 were also expressed in crosses of

04SR4-1-2 and -2-2 with 04SR4-5-2 and -6-2. This similarity of the frequencies of the ascus types in crosses of SR4 X normal isolates and the frequencies in test crosses of F_1 progeny definitely indicates an inherited tendency to produce aberrant asci. The ratios of ascus types of 04SR4-1-2 and -2-2 X normal isolates, 04SR4-5-2 and -6-2 X normal isolates and 04SR4-7-2 X normal isolates did not closely approximate ratios found in other crosses, but all showed some degree of ascospore abortion (Table 10).

Several F_1 progeny of SR4 having dodine tolerance levels similar to SR4 were testcrossed with normal isolates to determine whether ascospore abortion was induced in crosses with all isolates having this level of dodine tolerance. Among this group of isolates representing nine spore pairs, isolates representing four spore pairs permitted normal development of at least 95% of the asci in combination with one or more normal isolates (Appendix B1). Thus the genetic factors conditioning the dodine tolerance level of SR4 were not responsible for the ascospore abortion of its progeny.

Ascospore abortion induced by field isolates with normal dodine sensitivity

A clearcut abortion of four spores occurred in crosses of most non-aborting isolates with three field isolates having normal dodine sensitivity. In crosses with other isolates shown to induce ascospore abortion, the mean number of spores per ascus was lower than when either of the isolates was crossed with isolates not inducing abortion.

Table 10. Frequency of ascospore abortion in crosses of SR4 X normal isolates and in test crosses of F_1 progeny of SR4.

Cross	Percent of total asci with indicated number of spores per ascus												Total asci
	1		2	3		4	5		6	7		8	
SR4 X N[a]	2	(19)[b]	17	18	(48)	30	14	(31)	17	0	(2)	2	612
04SR4													
-1-2 & -2-2 X N	2	(23)	21	17	(42)	25	11	(34)	23	1	(1)	0	281
-3-2 & -4-2 X N	1	(13)	12	8	(53)	45	9	(33)	24	0	(1)	1	1001
-5-2 & -6-2 X N	0	(11)	11	7	(43)	36	6	(13)	7	13	(33)	20	157
-7-2 X N	0	(7)	7	2	(34)	32	2	(7)	5	8	(52)	44	108
-3-2 & -4-2 X SR4	1	(10)	9	9	(37)	28	11	(51)	40	1	(2)	1	150
-1-2 & -2-2 X -5-2 & -6-2	4	(20)	16	7	(28)	21	17	(51)	34	0	(1)	1	73
-1-2 & -2-2 X -7-2	3	(18)	15	10	(48)	38	8	(32)	24	1	(2)	1	158
-3-2 X -6-2	6	(18)	12	19	(63)	44	0	(19)	19	0	(0)	0	16

[a]Groups of normal isolates. Data of individual crosses: with SR4, Table 8; with F_1 progeny, Appendix B1.

[b]Figures in parentheses represent totals of adjacent categories.

Differences in individual perithecia of a single cross

In some cases individual perithecia of single cross varied in their degree of ascospore abortion. In seven of ten perithecia examined from crosses of three normal isolates with an aborting isolate, 91% of 128 asci contained four spores and none contained more than four spores. In three perithecia, 94% of 49 asci contained eight spores and none had fewer than six spores.

Individual perithecia in crosses of normal isolates with N-156, a white color mutant producing light-colored perithecia, also varied in degree of abortion. In these cases individual perithecia contained asci having mostly four spores or mostly two spores. Such differences could not be attributed to the parental origin of the perithecium because the differences were found in both light-colored and dark-colored perithecia.

Ascospore abortion in crosses involving color mutants

A number of *Venturia inaequalis* color mutants were reported to induce ascospore abortion (5, 30) when crossed with normal or abortion-inducing isolates. Crosses of normal isolates with a white mutant (N-156) and a yellow mutant (N-150) yielded mostly four-spored asci.

When N-156 and N-150 were crossed with isolates inducing ascospore abortion, the combination further reduced the mean number of spores per ascus (Table 11) as had the three dodine-sensitive field

Table 11. Further reduction of the number of spores per ascus by crosses of aborting color mutants with aborting F_1 progeny of SR4.

Cross	Mean spores per ascus in perithecia of indicated parental combinations					
	normal isolates		04SR4-1-2 04SR4-2-2		04SR4-3-2 04SR4-4-2	
normal isolates	---	---	4.0	(281)[a]	4.3	(998)
N-289[b]	7.9	(27)	3.5	(175)	---	---
N-156	3.8	(34)	2.8	(198)	2.8	(58)
N-150	4.2	(85)	3.0	(115)	2.4	(53)

[a]Figures in parentheses indicate total asci counted.
[b]N-289 is nearly normal with respect to ascospore abortion.

isolates. Pink color mutant, N-289, and green color mutant, 2295-2, reacted quite normally in combination with normal and abortion-inducing isolates (Table 11 and Appendix B3).

Induction of ascospore abortion by New York isolates

All five Poray Orchard isolates tested were found to induce some degree of ascospore abortion (Table 12). SC8a crossed with C2 gave a ratio similar to that produced by isolates 04SR4-5-2 and -6-2 when crossed with C2 (Table 10). The degree of abortion produced in C2 X SC8b was comparable to that occurring in crosses of N-150 X 04SR4-3-2 and -4-2 (Appendix B3). The ascus ratio in cross C2 X SC12c was similar to that induced by the white color mutant N-156 in crosses with normal isolates (Appendix B3). SR7 in combination with SR4 resulted in more ascospore abortion than C2 crossed with SR4.

Table 12. Frequency of ascospore abortion in crosses involving New York isolates.

Cross	Percent asci with indicated number of spores per ascus																Total asci
	1			2	3			4	5			6	7			8	
C2[a] X SR4	3	(25)[b]		22	22	(49)		27	10	(25)		15	0	(1)		1	78
C2 X SC8a	0	(15)		15	0	(40)		40	0	(6)		6	2	(39)		37	176
C2 X SC8b	11	(75)		64	4	(25)		21	0	(0)		0	0	(0)		0	28
C2 X SC12c	4	(16)		12	0	(84)		84	0	(0)		0	0	(0)		0	80
SR4 X SR7	19	(42)		23	19	(58)		39	0	(0)		0	0	(0)		0	26

[a]Normal isolate.

[b]Figures in parentheses represent totals of adjacent categories.

Ascospore abortion in natural perithecia from New York

Examination of a few leaves collected from the Poray Orchard in 1970 showed that three of ten perithecia contained asci with ascospore numbers ranging from four to eight. The development of seven perithecia had been interrupted at a stage prior to the development of ascospores. It was difficult to determine whether the ascospore abortion was a result of the drying or some other factor, but the effect appeared similar to that occurring in the controlled crosses. On these leaves there were also perithecia with all normal asci.

DISCUSSION

Because most of these data originated from crosses made to study inheritance of tolerance to dodine rather than inheritance of the ascospore abortion factor, certain vital pieces of evidence are lacking. Despite the preliminary status of the work, some lines of evidence can be drawn to support hypotheses of both genetic and non-genetic influences on the ascospore abortion phenomenon. Crucial to a discussion of the possible causes of this phenomenon is the evaluation of the descriptive and quantitative data supporting these hypotheses.

Evidence suggesting a genetic influence on ascospore abortion

Three lines of evidence suggest that the ascospore abortion occurring in progeny of isolate SR4 is under genetic control: the recurrence of similar frequencies of abortion in crosses of SR4 and in test crosses of F_1 progeny of SR4; the tendency for ascospores to be aborted as pairs rather than singly; and an additive abortive effect in crosses combining the abortion-inducing SR4 progeny and the aborting color mutants.

Several previously demonstrated genetic bases for ascospore abortion could be active mechanisms in the abortion of SR4 progeny. Dimock concluded that the aborta factor inactivated or deleted a single

gene or gene-complex necessary for delimitation (7). Those nuclei inheriting the aborta factor were unable to delimit spores.

Genetic studies with the color mutants of V. inaequalis (4, 5, 30) have shown an abortive effect on the spore pairs inheriting the mutant factor. While mutant genes usually segregated with a 1:1 ratio of normal to aborted spores, 3:1 and 1:3 ratios also occurred. Thus several patterns of inheritance of a single gene resulted in two, four, or six spores per ascus.

Several mechanisms of chromosome segmental interchange have been shown to yield asci containing four or eight spores (13, 14, 21). Such mechanisms also may prevent the development of any spores. The frequency of empty asci could help to distinguish the type of interchange, but in this and other studies (5) empty asci were quite difficult to distinguish from immature asci.

Combinations of lethal and modifying genes, segmental interchanges, or a lethal gene and a segmental interchange could conceivably yield asci with spore numbers ranging from zero to eight. Such mechanisms would cause abortion of spore pairs unless the factors would be active only under certain marginal cultural conditions. The ratios of types of abnormal asci could reflect the linkage of genes or modifying factors or the distance of segmental interchanges from the centromere.

Evidence suggesting a non-genetic influence on ascospore abortion

Although most of the ascospore abortion of SR4 progeny appears to be under some form of genetic control, some of the effects reported here are not readily explained by genetic mechanisms.

The relatively high, variable frequency of asci containing odd numbers of spores may suggest the activity of some variable non-genetic factor such as a suboptimal cultural condition which could prevail over the genetic control of a spore's development. These asci could result from abortion of a spore after its delimitation, degeneration of a nucleus, or the cooperation of two or more nuclei in the delimitation of a single spore.

In crosses of normal isolates the presence of asci which have reached their most advanced stage of development without delimiting any spores is comparable to effects of suboptimal nutrient conditions in other fungi. In Sordaria biotin-starved cultures produced this type of ascus more frequently (2). The nutrient medium affected the degree of ascospore abortion in Neurospora (10, 12). Thiamine was essential to ascospore formation in V. inaequalis (28).

In certain crosses some perithecia contained mostly abnormal asci while other perithecia contained mostly normal asci. Such differences could reflect local differences in nutrient status of the crossing medium or a differential rate of development (5, 11).

Further cytological, cultural and genetic studies should resolve which effects are caused by genetic or non-genetic factors. Cytological studies would locate nuclei and determine the developmental stage at which abortion occurs. Cultural studies should assess the minimal requirements of normal and abortion-inducing isolates on a defined medium (27, 28) and with natural overwintering. Genetic investigations could confirm chromosome aberration by testing linkage with genetic markers.

Significance of induction of ascospore abortion by Poray Orchard isolates

The induction of ascospore abortion by all Poray Orchard isolates must have some relationship with the dodine tolerance situation in this orchard, although the two factors are not caused by the same genes. Several possible relationships exist.

Possibly mutations for dodine tolerance and for ascospore abortion were induced simultaneously by some mutagenic factor prior to 1969. Low rates of dodine application would have selected for the dodine-tolerant strain during its first growing season, allowing a buildup of the abortion-inducing population as well. It does not seem likely that the population of the self-limiting, abortion-inducing strains would increase over several growing seasons unless the genes for tolerance and the ascospore abortion factors are closely linked. Close linkage does not seem likely because of the number of highly tolerant progeny inducing very little ascospore abortion. Several of these isolates arose from partially aborted asci and this selection may have represented a dilution of abortive factors, but the partially aborted asci composed the greatest proportion of the asci in these crosses so there is only a slim chance of close linkage between the abortive factors and the genes for tolerance.

A second possible relationship could be that ascospore abortion does not occur in nature, but the factors causing abortion may be closely linked to genes for dodine tolerance. Preliminary evidence from a survey of leaves from the Poray Orchard suggests a natural occurrence of the phenomenon, but this abortion could have been caused by drying of the leaves during ascospore development. It may be significant that the two

cytological studies of ascus development of V. inaequalis not reporting ascospore abortion were conducted on perithecia produced in nature (1, 17), while the study reporting ascospore abortion examined perithecia produced in culture (6). Perhaps this again reflects cultural conditions which are slightly suboptimal for ascospore production. If ascospore abortion does not occur in nature in strains such as SR4, then the strain would not be self-limiting and its frequency in the population would increase readily with the frequency of the tolerant strains under the selection pressure of dodine.

A final possibility could be that dodine induces minor mutations causing ascospore abortion. If this situation exists, long continual usage of dodine would stimulate an increase in the abortion-inducing population while also selecting for dodine-tolerant strains. The factors causing the two phenomena would not have to be linked genetically and could easily be separated in vitro in F_1 progeny.

PART II

LITERATURE CITED

PART II

LITERATURE CITED

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APPENDICES

APPENDIX A1

Inhibition of *Venturia inaequalis* field isolates in potato-dextrose agar by dodine diffusing from paper assay discs.

Monoconidial Isolate	Date Collected[a]	Location	Treatment[b]	Inhibition zone diam (cm)[c] 50 µg/ml	300 µg/ml	Replications
AR1	Aug. 1973	Sparta, Mi.	Commercial, Red Delicious	3.37	4.10	3
AR2				3.47	4.17	3
B1	Oct. 1969	Bellefontaine, Oh.	Unsprayed seedling trees	3.00	3.53	3
B3				2.70	3.50	3
B4				2.83	3.27	4
B6				2.97	3.43	3
Ba	Sept. 1970	Beltsville, Md.	Cyprex, Experimental	2.60	3.30	3
Bb				2.73	3.50	3
Bc				2.70	3.30	2
C1	Oct. 1969	East Lansing, Mi.	Unsprayed, Experimental	4.20	4.70	1
C2				3.05	3.75	4
C3				2.77	3.27	3
C4				2.88	3.58	4
CR1	Oct. 1969	East Lansing, Mi.	Unsprayed, Crabapple "Prairie Rose"	2.90	3.57	3
CR2				2.60	3.37	4
CR3				2.77	3.47	3
CR4				2.60	3.20	1
Cy1	Aug. 1973	East Lansing, Mi.	Cyprex, Experimental	2.50	3.07	3
Cy2				2.70	3.40	1
Cy13				2.37	3.20	3
D1	Oct. 1969	East Lansing, Mi.	No Cyprex, Experimental	2.77	3.37	1
E1	Oct. 1969	East Lansing, Mi.	Ny Cyprex, Experimental	2.36	2.97	1
G1	Sept. 1969	Geneva, N.Y.	Unsprayed, Experimental	3.50	4.10	1
G2				3.30	4.20	1
G3				2.80	3.75	2
G4				2.50	3.30	1
G5				2.73	3.53	4

Appendix A1. (Cont.)

Monoconidial Isolate	Date Collected[a]	Location	Treatment[b]	Inhibition zone diam (cm)[c] 50 µg/ml	300 µg/ml	Replications
H1	Oct. 1969	Grantsville, Md.	Unsprayed	2.70	3.40	1
H2				2.83	3.57	3
H3				2.70	3.30	1
H4				3.60	4.60	1
H6				2.58	3.05	4
L2	Oct. 1969	Baroda, Mi.	Commercial	2.40	3.30	4
L3				2.80	3.40	1
L4				3.03	3.60	4
M1	Oct. 1969	Paw Paw, Mi.	Commercial	3.07	3.83	3
M2				2.47	3.37	3
M3				2.47	3.23	3
M4				3.00	3.50	1
N1	Oct. 1969	Parma, Mi.	Commercial	2.50	2.90	1
O1	Oct. 1969	East Lansing, Mi.	Unsprayed	2.25	3.20	2
O2				2.80	3.37	1
O4				2.68	3.63	4
O5				3.08	3.70	1
R4	Sept. 1969	Geneva, N. Y.	Cyprex, Experimental	2.65	3.60	2
R5				2.40	2.93	3
S1	Oct. 1969	Sparta, Mi.	Commercial	2.18	3.18	4
S3				2.07	2.83	3
S4				2.58	3.38	4
SC1	Aug. 1973	Sparta, Mi.	Commercial	3.63	4.30	3
SC2				2.37	3.00	3
SC8a	Sept. 1970	Sodus, N. Y.	Poray Orchard	1.55	2.38	4
SC8b				1.50	2.53	3
SC8c				1.38	2.43	4
SC12a	Sept. 1970	Sodus, N. Y.	Poray Orchard	1.70	2.88	4

Appendix A1. (Cont.)

Monoconidial Isolate	Date Collected[a]	Location	Treatment[b]	Inhibition zone diam (cm)[c] 50 µg/ml	300 µg/ml	Replications
SC12b				1.43	2.60	4
SC12c				1.53	2.67	4
SR4	Sept. 1969	Sodus, N. Y.	Poray Orchard	0.83	1.63	4
SR7				1.70	2.58	4
T2	Oct. 1969	East Lansing, Mi.	Experimental fungicide	3.47	3.90	3
TD1	Oct. 1969	East Lansing, Mi.	Experimental fungicide	2.57	3.17	1
TH1	Oct. 1969	East Lansing, Mi.	Experimental fungicide	2.40	2.97	1
V1	Oct. 1969	Baroda, Mi.	Commercial	2.30	3.00	1
V2				3.00	3.67	4
V3				2.30	2.90	1
V4				2.40	3.20	1
W2	Oct. 1969	Baroda, Mi.	Commercial "Jonathan"	2.55	3.50	1
W4				2.55	3.35	1
Tetrads						
CII 1	May, 1971	Paw Paw, Mi.	Commercial	2.80	3.50	3
2				2.97	3.40	3
3				3.00	3.70	2
4				2.73	3.37	3
5				2.65	3.35	2
6				2.73	3.33	3
7				2.50	3.07	3
8				2.80	3.40	3
CIII 1	May, 1971	Paw Paw, Mi.	Commercial	2.85	3.40	2
2				2.85	3.50	2
3				3.00	3.60	2

Appendix A1. (Cont.)

Tetrads	Date Collected[a]	Location	Treatment[b]	Inhibition zone diam (cm)[c] 50 µg/ml	300 µg/ml	Replications
4				2.80	3.45	2
5				2.80	3.40	2
6				2.90	3.50	2
7				3.00	3.55	2
8				2.70	3.35	2
P70 -1-1	May, 1970	Sodus, N. Y.	Poray Orchard	1.90	2.70	2
-2-1				1.90	2.50	1
-3-1				1.40	2.35	2
-4-1				1.65	2.50	2
-5-1				1.45	2.40	2
-6-1				1.85	2.60	2
-7-1				1.05	2.05	2
-8-1				1.25	2.20	2
-1-2				1.65	2.35	2
-2-2				1.55	2.40	2
-3-2				1.35	2.25	2
-4-2				1.50	2.35	2
-5-2				1.60	2.40	2
-6-2				1.45	2.30	2
-7-2				1.50	2.35	2
-8-2				1.55	2.40	2
-1-3				1.20	2.25	2
-2-3				1.35	2.30	2
-3-3				1.50	2.40	2
-4-3				1.50	2.40	2
-5-3	May, 1970	Sodus, N. Y.	Poray Orchard	1.60	2.20	1
-6-3				1.60	2.40	2
-7-3				1.50	2.30	2

Appendix A1. (Cont.)

Tetrads	Date Collected[a]	Location	Treatment[b]	Inhibition zone diam (cm)[c] 50 µg/ml	Inhibition zone diam (cm)[c] 300 µg/ml	Replications
-8-3				1.50	2.20	2
P71 -1-1	May, 1971	Sodus, N. Y.	Poray Orchard	2.20	3.05	2
-2-1				2.20	3.05	2
-3-1				2.00	2.75	2
-4-1				1.85	2.70	2
-5-1				1.70	2.55	2
-6-1				1.70	2.50	2
-7-1				1.95	2.65	2
-8-1				2.00	2.70	2
-1-2				1.90	2.80	2
-2-2				1.85	2.75	2
-3-2				1.40	2.20	1
-4-2				1.70	2.40	2
-5-2				1.45	2.45	2
-6-2				2.05	2.85	2
-7-2				1.55	2.45	2
-8-2				2.00	2.80	2
MQ 1-1	May, 1969	North Rose, N. Y.	McQueen Orchard	1.85	2.65	2
2-1				1.80	2.45	2
3-1				1.65	2.30	2
4-1				1.60	2.30	2
5-1				1.80	2.50	2
6-1				1.90	2.50	1
7-1				1.55	2.40	2
8-1				1.50	2.25	2
-1-2				1.65	2.55	2
-2-2				1.75	2.60	2

Appendix A1. (Cont.)

Tetrads	Date Collected[a]	Location	Treatment[b]	Inhibition zone diam (cm)[c] 50 µg/ml	300 µg/ml	Replications
-3-2				1.25	1.95	2
-4-2				1.30	2.00	2
-5-2				1.90	2.70	2
-6-2				1.65	2.40	2
-7-2				2.00	2.65	2
-8-2				1.65	2.45	2

[a]Date, location and treatment are listed only for the first isolate from each treatment.

[b]Isolates listed as commercial received Cyprex as part of a regular spray program. Isolates listed from experimental orchards were either unsprayed, sprayed with Cyprex or sprayed with experimental fungicides. Unless specified, all isolates were taken from "McIntosh" variety.

[c]Zones measured after seven days' incubation at 19 C. Concentration of dodine with which 12.7 mm diam assay disc was saturated.

APPENDIX A2

Inhibition by dodine of germination and growth of conidia of monoascosporic progeny of *Venturia inaequalis* cross SR4 X SR7

Isolate	Inhibition zone diam (cm)[a] 50 µg/ml	300 µg/ml	% germination[b] 1 µg/ml
Eight-spored asci			
SR4SR7-1-1	1.48	2.35	30.2
-2-1	1.35	2.43	37.4
-3-1	2.25	3.13	8.7
-4-1	2.05	2.90	12.2
-5-1	1.53	2.40	25.9
-6-1	1.33	2.35	31.4
-7-1	---	---	14.3
-8-1	1.90	2.73	13.7
-1-2	1.55	2.58	23.1
-2-2	1.50	2.50	23.1
-3-2	1.88	2.88	10.7
-4-2	1.75	2.60	11.7
-5-2	1.70	2.53	22.1
-6-2	1.68	2.55	21.5
-7-2	1.35	2.20	28.2
-8-2	1.33	2.33	28.2
Six-spored asci			
-1-1	2.05	3.05	35.0
-2-1	1.83	2.60	33.4
-3-1	1.53	2.38	41.7
-4-1	1.58	2.70	43.8
-5-1	1.75	2.80	19.1
-6-1	1.95	2.95	12.5
-1-2	1.38	2.33	38.1
-2-2	1.98	2.85	35.1
-3-2	1.58	2.55	36.2
-4-2	1.58	2.48	36.6
-5-2	1.48	2.55	37.4
-6-2	1.43	2.30	34.6

Appendix A2. (Cont.)

Isolate	Inhibition zone diam (cm)[a] 50 µg/ml	300 µg/ml	% germination[b] 1 µg/ml
Four-spored asci			
-1-1	2.03	2.83	30.7
-2-1	1.80	2.80	35.6
-3-1	1.90	2.83	17.2
-4-1	1.53	2.33	20.0
-1-2	1.53	2.48	35.9
-2-2	1.50	2.38	36.5
-3-2	2.10	2.95	17.2
-4-2	1.98	2.78	16.9
Two-spored asci			
-1-1	1.45	2.65	32.3
-2-1	1.78	3.00	28.4
-1-2	1.40	2.65	32.1
-2-2	1.45	2.53	36.00
LSD .05	0.40	0.40	
LSD .01	0.52	0.52	

[a]Means of four replications. Zones measured after seven days' incubation at 19 C. Dodine concentrations with which 12.7 mm assay disc was saturated. Inhibition zone diam in 50 µg/ml and 300 µg/ml tests: SR4 (0.83 and 1.63 cm); SR7 (1.70 and 2.58 cm).

[b]Percent of germination of control conidia in water.

APPENDIX A3

Inhibition of random progeny of Venturia inaequalis cross 04SR4-1-2 X 04SR4-6-1 in potato-dextrose agar by dodine diffusing from paper assay discs.

Isolate	Inhibition zone diam (cm)[a] 50 µg/ml	300 µg/ml	Isolate	Inhibition zone diam (cm) 50 µg/ml	300 µg/ml
-1	0.80	1.95	-40	1.70	2.45
-2	0.97	2.02	-41	2.15	2.90
-3	1.90	2.52	-42	3.00	3.57
-4	2.10	2.77	-43	2.85	3.30
-5	0.67	1.65	-44	2.60	3.05
-6	2.17	2.65	-45	1.65	2.40
-7	2.55	3.32	-46	2.40	3.22
-8	2.50	3.25	-47	2.30	2.90
-9	2.22	3.00	-48	2.02	2.87
-10	2.70	3.35	-49	2.37	3.02
-11	2.12	2.95	-50	1.30	1.97
-12	2.22	3.15	-51	1.85	2.62
-13	1.70	2.62	-52	1.65	2.40
-14	2.52	3.22	-53	2.18	2.90
-15	2.37	3.07	-54	2.50	3.15
-16	1.57	2.40	-55	2.42	3.12
-17	2.37	3.17	-56	1.25	2.07
-18	2.02	2.77	-57	1.75	2.67
-19	2.37	2.95	-58	1.30	2.30
-20	1.77	2.45	-59	3.07	3.62
-22	1.92	2.82	-60	2.15	2.90
-24	1.97	2.72	-62	1.95	2.95
-25	0.42	1.65	-63	0.87	1.95
-26	2.35	3.15	-64	2.52	3.30
-27	2.17	2.90	-65	2.35	3.15
-28	1.90	2.70	-66	1.85	2.77
-29	2.10	2.77	-67	1.90	2.70
-30	2.70	3.20	-68	1.97	2.77
-31	1.70	2.47	-69	1.82	2.55
-32	0.62	1.77	-70	3.02	3.60
-33	1.70	2.50	-73	1.55	2.67
-34	1.12	1.92	-74	2.30	2.80
-35	2.82	3.52	-75	2.27	3.05
-36	2.47	3.10	-76	1.20	2.10
-37	2.40	3.05			
-38	2.27	2.92	LSD .05	0.47	0.63
-39	2.35	3.17	LSD .01	0.62	0.83

[a]Means of four replications. Zones measured after seven days' incubation at 19 C. Dodine concentrations with which 12.7 mm assay disc was saturated. Inhibition zone diam in 50 µg/ml and 300 µg/ml tests: 04SR4-1-2 (1.80 and 2.68 cm), 04SR4-6-1 (2.38 and 3.20 cm).

APPENDIX A4

Inhibition by dodine of germination and growth of conidia of tetrad progeny of Venturia inaequalis cross SR4 X 04SR4-3-2.

Isolate	Inhibition zone diam (cm)[a] 50 µg/ml	300 µg/ml	% germination[b] dodine 1 µg/ml
1-1	.50	1.70	28.1
2-1	.73	1.68	33.8
3-1	.70	1.73	46.5
4-1	.48	1.63	61.6
5-1	1.00	1.88	58.6
6-1	.70	1.75	46.2
7-1	1.20	1.85	53.3
8-1	.63	1.73	66.8
-2-3	1.40	2.13	29.8
3-3	.43	1.43	49.9
4-3	.45	1.63	63.0
-5-3	.90	1.78	73.5
6-3	.70	1.68	74.9
7-3	1.20	1.65	72.2
8-3	.55	1.63	40.5
1-4	1.23	1.98	38.2
2-4	.83	1.65	65.1
3-4	.88	1.75	60.1
4-4	1.15	1.93	44.2
5-4	.63	1.60	60.5
6-4	1.28	2.00	25.3
7-4	.68	1.70	49.4
8-4	.53	1.65	65.9
-1-6	.45	1.63	60.9
-3-6	.75	1.70	69.7
4-6	.60	1.65	63.9
5-6	.93	1.80	61.9
6-6	1.23	2.00	34.3
7-6	.73	1.73	30.3
8-6	.68	1.65	49.6
LSD .05	0.40	0.20	
LSD .01	0.53	0.26	

[a]Means of four replications. Zones measured after seven days' incubation at 19 C. Concentrations of dodine with which paper assay disc was saturated. Inhibition zone diam in 50 µg/ml and 300 µg/ml tests: SR4 (0.83 and 1.63 cm); 04SR4-3-2 (1.23 and 2.33 cm).

[b]Percent of germination of control conidia in water.

APPENDIX A5

Inhibition by dodine of germination and growth of conidia of tetrad progeny of *Venturia inaequalis* cross C2 X 04SR4-5-2.

Isolate	Inhibition zone diam (cm)[a] 50 µg/ml	300 µg/ml	% germination dodine 1 µg/ml[b]
-1-3	2.32	2.80	13.3
-2-3	2.80	3.20	6.3
-3-3	2.70	3.23	2.4
-4-3	2.80	3.30	9.9
-5-3	2.10	2.63	23.1
-6-3	2.22	2.73	15.7
-7-3	2.42	2.78	10.8
-8-3	2.57	2.70	4.4
-1-14	2.72	3.13	4.0
-2-14	2.72	3.13	2.3
-3-14	2.24	2.85	8.6
-4-14	2.62	2.98	7.8
-5-14	2.38	2.90	7.6
-6-14	2.44	2.88	9.4
-7-14	2.36	2.80	12.5
-8-14	2.28	2.78	23.6
-1-15	2.20	2.75	11.2
-2-15	2.37	2.83	12.3
-3-15	2.75	3.18	1.2
-4-15	2.23	2.73	14.9
-5-15	2.30	2.78	19.4
-6-15	2.63	3.03	3.3
-7-15	2.62	3.05	2.9
-8-15	2.50	2.98	1.8
-1-18	2.25	2.88	19.8
-2-18	2.45	2.98	21.3
-3-18	2.32	2.90	18.0
-4-18	2.32	2.83	13.2
-5-18	2.50	2.95	8.5
-6-18	2.33	2.80	7.0
-7-18	2.85	3.23	10.6
-8-18	2.55	3.10	5.0
LSD .05	0.34	0.34	
LSD .01	0.45	0.45	

[a]Means of four replications. Zones measured after seven days' incubation at 19 C. Concentrations of dodine with which paper assay disc was saturated. Inhibition zone diam in 50 µg/ml and 300 µg/ml tests: C2 (3.05 and 3.75 cm); 04SR4-5-2 (2.30 and 3.15 cm).

[b]Percent of germination of control conidia in water.

APPENDIX A6

Inhibition by dodine of germination and growth of conidia of tetrad progeny of *Venturia inaequalis* cross C2 X 04SR4-7-2.

Isolate	Inhibition zone diam (cm)[a] 50 µg/ml	300 µg/ml	% germination dodine 1 µg/ml[b]
-1-2	2.45	3.03	2.5
-2-2	2.63	3.15	3.4
-3-2	2.68	3.05	3.3
-4-2	2.58	3.03	0.9
-5-2	2.50	2.93	4.9
-6-2	2.80	3.33	1.0
-7-2	2.88	3.40	0.1
-8-2	2.43	2.85	0.2
-1-5	3.03	3.40	3.6
-2-5	2.78	3.25	2.9
-3-5	2.85	3.33	7.1
-4-5	2.33	2.85	10.9
-5-5	2.60	2.98	1.2
-6-5	2.55	2.98	4.4
-7-5	2.43	2.90	15.4
-8-5	2.20	2.70	2.0
-1-7	2.40	2.83	15.1
-2-7	2.50	2.90	7.4
-3-7	2.75	3.08	1.6
-4-7	2.45	3.03	1.8
-5-7	2.78	2.93	0.7
-6-7	2.53	3.03	0.3
-7-7	2.45	2.90	4.5
-8-7	2.35	2.85	5.8
-1-11	2.60	3.10	1.6
-2-11	2.40	2.80	3.9
-3-11	2.50	3.03	18.0
-4-11	2.57	3.05	5.8
-5-11	2.55	3.08	11.5
-6-11	2.37	2.80	6.8
-7-11	3.02	3.45	4.8
-8-11	2.83	3.20	8.7
LSD .05	0.44	0.66	
LSD .01	0.58	0.87	

[a]Means of four replications. Zones measured after seven days' incubation at 19 C. Concentrations of dodine with which paper assay disc was saturated. Inhibition zone diam in 50 µg/ml and 300 µg/ml tests: C2 (3.05 and 3.75 cm); 04SR4-7-2 (2.98 and 3.63 cm).

[b]Percent of germination of control conidia in water.

APPENDIX A7

Inhibition of random progeny of *Venturia inaequalis* cross S4 X C2SR4 7-4 in potato-dextrose agar by dodine diffusing from paper assay discs.

Isolate	Inhibition zone diam (cm)[a] 50 µg/ml	300 µg/ml
-1	2.3	2.9
-3	1.9	2.5
-5	2.2	2.7
-6	2.3	2.9
-7	2.4	2.9
-8	2.2	2.8
-9	1.2	2.1
-10	1.0	2.0
-11	2.0	2.6
-12	2.4	2.8
-13	2.3	2.7
-15	2.0	2.7
-16	1.6	2.4
-17	1.4	2.3
-18	2.1	2.8
-19	1.5	2.3
-21	2.0	2.7
-27	2.2	2.8
-28	1.9	2.3
-31	1.6	2.4
-32	1.8	2.5
-33	2.0	2.5
-35	1.6	2.1
-37	2.5	2.8
-38	1.4	1.9
-39	1.4	2.0
-40	2.2	2.7
-41	1.3	2.1
-42	1.6	2.1
-44	2.3	2.9
-46	2.1	2.7
-47	2.0	2.6
-50	2.2	2.8
-51	2.1	2.7
-52	1.9	2.7
-54	2.4	2.9
-55	1.1	1.9
-56	1.9	2.6
-57	2.0	2.8
-58	1.8	2.3

[a]A single replication. Zones measured after seven days' incubation at 19 C. Dodine concentrations with which 12.7 mm assay disc was saturated. Inhibition zone diam in 50 µg/ml and 300 µg/ml tests: S4 (2.58 and 3.58 cm); C2SR4 7-4 (1.10 and 2.15 cm).

APPENDIX A8

Inhibition of random progeny of Venturia inaequalis cross S1 X 2295-2 (green color mutant) in potato-dextrose agar by dodine diffusing from paper assay discs.

Isolate	Inhibition zone diam (cm)[a] 50 µg/ml	300 µg/ml	Color
-1	2.3	3.0	wild type
-2	2.6	3.2	wild type
-3	2.2	2.8	wild type
-4	1.8	2.6	green
-5	1.8	2.5	wild type
-6	1.9	2.8	green
-7	2.6	3.2	green
-8	2.7	3.2	green
-9	2.4	2.9	green
-10	2.5	3.0	green
-11	2.1	2.7	green
-12	2.3	2.9	green
-13	2.1	2.8	wild type
-15	2.0	2.7	green
-16	2.6	3.3	wild type
-17	2.9	3.4	wild type
-18	2.5	3.1	wild type
-20	2.2	2.8	wild type
-24	2.1	2.7	green
-26	1.8	2.6	green
-27	2.0	2.8	wild type
-29	2.1	2.9	green
-31	2.2	2.8	wild type
-32	2.0	2.6	green
-33	2.7	3.2	wild type
-34	2.0	2.6	wild type
-36	1.9	2.5	wild type
-37	2.2	2.7	green
-38	2.0	2.7	green

Appendix A8. (Cont.)

Isolate	Inhibition zone diam (cm)[a] 50 µg/ml	300 µg/ml	Color
-42	1.8	2.5	green
-43	2.5	2.9	wild type
-44	2.8	3.2	green
-45	2.2	2.8	wild type
-46	2.4	3.0	wild type
-47	2.1	2.8	wild type
-48	2.2	2.7	green
-49	2.2	2.8	wild type
-50	2.5	3.1	wild type
-51	1.9	2.4	green
-52	2.1	2.7	green
-53	2.1	2.7	green
-54	2.0	2.6	green
-55	2.5	3.0	green
-56	2.5	3.1	wild type

[a]Results of one or two replications. Zones measured after seven days' incubation at 19 C. Dodine concentrations with which 12.7 mm assay disc was saturated. Inhibition zone diam in 50 µg/ml and 300 µg/ml tests: S1 (2.18 and 3.18 cm); 2295-2 (3.00 and 3.57 cm).

APPENDIX A9

Inhibition of random progeny of *Venturia inaequalis* cross C2SR4-7-3 X 2295-2 in potato-dextrose agar by dodine diffusing from paper assay discs.

Isolate	Inhibition zone diam (cm)[a] 50 µg/ml	300 µg/ml	Color
-1	2.5	3.0	green
-2	2.2	2.8	wild type
-4	2.2	2.5	wild type
-5	2.5	2.9	wild type
-7	1.8	2.3	wild type
-8	2.2	2.6	wild type
-10	2.2	2.7	wild type
-12	2.5	3.0	wild type
-13	2.9	3.5	green
-15	1.3	2.0	wild type
-20	1.6	2.2	wild type
-22	2.1	2.7	green
-24	1.5	2.1	wild type
-27	2.6	3.2	wild type
-28	1.4	2.0	green
-30	2.2	2.7	wild type
-31	1.2	2.0	wild type
-34	1.8	2.6	wild type
-35	2.4	2.9	wild type
-38	2.6	3.1	green
-39	2.1	2.6	wild type
-46	2.4	2.9	wild type
-48	3.1	3.4	wild type
-49	1.8	2.5	green
-53	1.5	2.1	green
-54	2.3	2.8	brown

[a]Results of one or two replications. Zones measured after seven days' incubation at 19 C. Dodine concentrations with which 12.7 mm assay disc was saturated. Inhibition zone diam in 50 µg/ml and 300 µg/ml tests: C2SR4-7-3 (1.20 and 1.90 cm); 2295-2 (3.00 and 3.57 cm).

APPENDIX B1

Frequency of ascospore abortion in crosses of F_1 progeny of 04 X SR4.

Cross	Percent asci with indicated number of spores per ascus												Total asci
	1		2	3		4	5		6	7		8	
04SR4-12 X C4	3	(24)[a]	21	7	(42)	35	15	(34)	19	0	(0)	0	74
X S1	11	(56)	45	0	(22)	22	0	(22)	22	0	(0)	0	9
X TH1	0	(3)	3	13	(46)	33	11	(49)	38	2	(2)	2	63
04SR4-22 X C4	1	(34)	33	30	(45)	15	6	(19)	13	1	(2)	1	90
X S1	0	(33)	33	34	(34)	0	0	(33)	33	0	(0)	0	3
X TH1	5	(17)	12	16	(33)	17	19	(50)	31	0	(0)	0	42
04SR4-32 X C4	1	(4)	3	8	(44)	36	15	(50)	35	0	(2)	2	124
X D4	0	(8)	8	4	(46)	42	10	(44)	34	2	(2)	0	134
X S1	0	(9)	9	12	(60)	48	14	(31)	17	0	(0)	0	58
X S4	0	(23)	23	21	(46)	25	10	(31)	21	0	(0)	0	48
X TD1	2	(9)	7	5	(49)	44	4	(39)	35	1	(3)	2	89
X TH1	0	(1)	1	2	(63)	61	8	(36)	28	0	(0)	0	174
X SR4	2	(6)	4	17	(44)	27	15	(46)	31	4	(4)	0	48
04SR4-42 X C4	3	(24)	21	11	(50)	39	3	(26)	23	0	(0)	0	74
X D4	0	(28)	28	2	(52)	50	10	(18)	8	0	(2)	2	40
X S1	1	(40)	39	6	(49)	43	5	(11)	6	0	(0)	0	67
X S4	0	(27)	27	16	(55)	39	2	(18)	16	0	(0)	0	49
X TD1	2	(15)	13	14	(56)	42	10	(26)	16	0	(3)	3	125
X TH1	0	(5)	5	6	(69)	63	21	(26)	5	0	(0)	0	19
X SR4	1	(13)	12	5	(33)	28	9	(53)	44	0	(1)	1	102

Appendix B1. (Cont.)

Cross	Percent asci with indicated number of spores per ascus												Total asci
	1		2	3		4	5		6	7		8	
04SR4-52 X C2	0	(10)	10	7	(52)	45	2	(4)	2	8	(34)	26	97
04SR4-62 X C2	0	(12)	12	6	(28)	22	13	(28)	15	22	(32)	10	60
04SR4-72 X C2	0	(7)	7	2	(34)	32	2	(7)	5	8	(52)	44	108
04SR4-12 X -62	0	(3)	3	3	(26)	23	10	(68)	58	0	(3)	3	31
-22 X -52	7	(33)	26	10	(29)	19	21	(38)	17	0	(0)	0	42
-12 X -72	4	(30)	26	19	(24)	15	9	(36)	27	0	(0)	0	53
-22 X -72	2	(11)	9	6	(55)	49	8	(31)	23	2	(3)	1	105
-32 X -62	6	(18)	12	19	(63)	44	0	(19)	19	0	(0)	0	16

[a]Figures in parentheses represent totals of adjacent categories.

APPENDIX B2

Frequency of ascospore abortion in crosses of normal isolates with selected isolates similar to SR4 in dodine tolerance.

Cross	Percent asci with indicated number of spores per ascus												Total asci
	1		2	3		4	5		6	7		8	
C2SR4-35 X D4	0	(1)[a]	1	0	(7)	7	1	(6)	5	0	(86)	86	92
X S1	0	(0)	0	0	(0)	0	0	(0)	0	0	(0)	100	11
X S4	0	(0)	0	0	(2)	2	0	(4)	4	0	(94)	94	136
X TH1	0	(1)	1	0	(1)	1	1	(7)	6	0	(91)	91	75
X TD1	0	(2)	2	0	(23)	23	0	(2)	2	0	(73)	73	52
% of total	0	(1)	1	0	(6)	6	1	(5)	4	0	(88)	88	366
C2SR4-37 X C4	0	(0)	0	0	(1)	1	0	(3)	3	0	(96)	96	114
X D4	0	(0)	0	2	(2)	0	0	(3)	3	0	(95)	95	40
X S1	0	(2)	2	0	(0)	0	2	(10)	8	7	(88)	88	87
X S4	0	(1)	1	0	(0)	0	0	(3)	3	0	(96)	96	114
S TD1	0	(0)	0	0	(20)	20	3	(20)	17	3	(60)	57	66
% of total	0	(1)	1	0	(3)	3	1	(7)	6	2	(89)	87	410
C2SR4-48 X C4	0	(0)	0	0	(1)	1	0	(4)	4	0	(95)	95	97
X S1	0	(0)	0	0	(2)	2	2	(24)	22	9	(74)	65	46
X S4	0	(0)	0	0	(0)	0	0	(2)	2	0	(98)	98	66
X TD1	0	(1)	0	0	(8)	8	2	(12)	10	1	(79)	78	160
% of total	0	(1)	1	0	(4)	4	1	(10)	9	1	(85)	84	369
C2SR4 74 X C4	0	(0)	0	0	(1)	1	0	(0)	0	0	(99)	99	184
X D4	0	(0)	0	0	(11)	11	0	(0)	0	0	(84)	84	44
X S4	0	(0)	0	0	(0)	0	0	(0)	0	0	(100)	100	34
X TH1	0	(4)	4	4	(8)	4	0	(0)	0	0	(88)	88	26
% of total	0	(0)	0	0	(3)	3	0	(1)	1	0	(96)	96	288

[a]Figures in parentheses represent totals of adjacent categories.

APPENDIX B3

Frequency of ascospore abortion in crosses involving color mutants.

Cross	Percent asci with indicated number of spores per ascus																Total asci
	1			2	3			4	5			6	7			8	
N-156 X																	
04	0	(12)[a]	12		0	(88)	88		0	(0)		1	1	(0)		0	34
2295-2	0	(10)	10		0	(90)	90		0	(0)		0	0	(0)		0	41
C2SR4-35	1	(2)	1		0	(95)	95		0	(3)		3	0	(0)		0	129
C2SR4-37	0	(5)	5		1	(95)	94		0	(0)		0	0	(0)		0	80
C2SR4-48	0	(0)	0		0	(100)	100		0	(0)		0	0	(0)		0	42
C2SR4 74	0	(18)	18		0	(78)	78		2	(2)		0	2	(2)		0	55
04SR4-12	3	(52)	49		6	(45)	39		0	(3)		3	0	(0)		0	120
04SR4-22	6	(70)	64		4	(30)	26		0	(0)		0	0	(0)		0	78
04SR4-32	0	(50)	50		10	(50)	40		0	(0)		0	0	(0)		0	52
04SR4-42	0	(50)	50		25	(50)	25		0	(0)		0	0	(0)		0	4
N-150 X																	
04	0	(10)	10		6	(68)	62		5	(20)		15	0	(2)		2	85
C2SR4-35	0	(6)	6		6	(88)	82		0	(6)		6	0	(0)		0	17
C2SR4-37	0	(0)	0		1	(100)	99		0	(0)		0	0	(0)		0	114
C2SR4 74	0	(58)	58		0	(42)	42		0	(0)		0	0	(0)		0	12
04SR4-12	10	(43)	33		9	(52)	43		3	(5)		2	0	(0)		0	58
04SR4-22	2	(49)	47		2	(51)	49		0	(0)		0	0	(0)		0	57
04SR4-32	4	(84)	80		0	(16)	16		0	(0)		0	0	(0)		0	44
04SR4-42	0	(44)	44		11	(56)	45		0	(0)		0	0	(0)		0	9
N-289 X																	
C2SR4-48	0	(0)	0		0	(0)	0		0	(7)		7	0	(93)		93	27
04SR4-12	2	(39)	37		5	(40)	35		4	(21)		17	0	(0)		0	101
04SR4-22	3	(41)	38		9	(40)	31		5	(19)		14	0	(0)		0	74
2295-2 X																	
C2SR4-51	0	(10)	10		4	(47)	43		10	(43)		33	0	(0)		0	219
C2SR4-61	1	(9)	8		8	(43)	35		11	(45)		34	1	(3)		2	127
04SR4-62	0	(4)	4		0	(6)	6		0	(0)		0	0	(90)		90	53

[a]Figures in parentheses represent totals of adjacent categories.

Typed and Printed in the U.S.A.
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