INHERITANCE OF RESISTANCE TO ROOT ROT OF TOBACCO CAUSED BY *THIELAVIOPSIS BASICOLA* (BERK AND BR) FERR.

by

Shamshad Akhtar Khan

Thesis submitted to the Graduate Faculty of the

Virginia Polytechnic Institute

in candidacy for the degree of

MASTER OF SCIENCE

in

Agronomy

APPROVED:	APPROVED:
Director of Graduate Studies	Head of Department
Dean of Agriculture	Supervisor

February, 1953

Blacksburg, Virginia.

ACKNOWLEDGEMENTS

The writer sincerely wishes to express his gratitude to Mr. T. M. Starling, Associate Agronomist, for the guidance and help rendered throughout the course of study. He is equally grateful to Dr. R. G. Henderson, Plant Pathologist, for his advice relative to various aspects of this problem.

He is obliged to Dr. Max Levitan, Associate Professor of Genetics, for his helpful suggustions and criticism. His thanks are also due to Mr. Luben Spasoff, Assistant Plant Pathologist, for his assistance in the greenhouse and to the staff members of the Agricultural Library and others with whom the problem was discussed.

TABLE OF CONTENTS

INTRODUCTION

Tobacco is a major cash crop in Southern Virginia, North Carolina, South Carolina and other southeastern states. The economic production of tobacco requires a high yield of good quality leaf. Of the several diseases contributing to a reduced yield and lowered quality, root rot is one of the most important. Root rot and root knot is estimated by Clayton, et al (4), to cause damage to some 500,000 acres of tobacco in Virginia, North Carolina, Georgia, and Florida. Thus, several millions of pounds of leaf and thousands of dollars are lost annually by growers because of these root diseases.

There are five major factors that largely determine the occurance and development of black root rot (Thielaviopsis basicola): (1) the degree of infestation of the soil by the causal organism, (2) the soil reaction, (3) the soil moisture, (4) the soil temperature and (5) the degree of susceptibility of the tobacco to black root rot. The latter one is a factor of major importance, (although it is affected in turn by the other aforesaid factors). The development of varieties tolerant to black root rot has greatly reduced the prevalence of the disease. The tolerance or resistance of varieties to root rot, however, is complicated by the presence of races or strains of the pathogen which differ in pathogenicity on different varieties of tobacco (1).

In this study an attempt has been made to determine the nature of inheritance of resistance in tobacco to root rot caused by the fungus Thielaviopsis basicola (Berk and Br.) Ferr, with the view of evaluating the possibility of developing higher resistance to root rot by combining sources of resistance from different varieties.

Because of the complicated interaction of the fungus with its environment, the variability in different cultures of the organism (14,27), and the peculiar manner in which varieties of tobacco respond to it, definite conclusions regarding inheritance were not expected to be drawn. However, it was expected that data obtained in the study would give some idea regarding the breeding behavior and nature of resistance to root rot in the varieties involved in the study.

REVIEW OF LITERATURE

Black root rot is caused by the fungus *Thielaviopsis basicola* (Berk and Br.) Ferr, which was first discovered in England on peas in the year 1850 by Berkeley and Brome and later described by Farraris in 1912 (according to McCormick (20)).

History in United States: In the United States, *Thielaviopsis basicola* was first observed by Thaxter (28) in 1891, in Connecticut. Six years later Selby (25) found it on the roots of begonias which were growing in the greenhouse. In 1899, he (26) gave a short account of the disease on tobacco seedlings. Shortly afterwards, in 1909, Gilbert (7) warned against the seriousness of the disease on tobacco, although at that time it was only recognized in Connecticut, and even there in only very limited areas. But, wherever the plants were attacked the crop was a total failure. Since then, black root rot has been considered to be a major disease of tobacco in the cooler regions on soils that are not very acid (3), (6).

Considerable work has been done to breed strains of tobacco which are resistant to black root rot. Many varieties with different degrees of resistance have been developed, but no variety with an immune reaction has been reported. Henderson et al (19) selected what appeared to be two highly resistant varieties in 1943, but later considered them to be only tolerant or partially resistant (10).

Efforts to breed resistant varieties have been handicapped, apart from other factors, by differences in pathogenecity of various races of the pathogen.

The existance of strains of _Thielaviopsis basicola_ in different areas was suggested in a test reported by MacRae (18) in 1935. He obtained seed of white burley tobacco, which was reported to be resistant to root rot, from the Kentucky Agricultural Experiment Station. In trials at Harrow, Canada, this variety proved to be no better than other susceptible varieties already in use. Later, Allison (1) recognized four races of black root rot fungus on the basis of their pathogenecity on different varieties of tobacco. Stover (27) also described four races, which, in his opinion were probably the same as those recognized earlier by Allison (1) and Johnson et al (19). Stover (27) used four "so-called" resistant varieties in his studies. One of the varieties was found to be moderately susceptible to susceptible to all races; another was resistant to one race and susceptible to three; a third variety was resistant to two races, moderately susceptible to a third and susceptible to the fourth race; and the fourth variety was resistant to three races and moderately susceptible to one.

Description and nature of the fungus and symptoms of the disease.

Thielaviopsis basicola (Berk and Br.) Ferr, is generally recognized as the cause of black root rot disease of tobacco. This fungus is frequently found in association with an ascomycete, _Thielavia basicola_, which at first was thought to be the perfect stage of _Thielaviopsis basicola_. However, McCormick (20) demonstrated that it was a distinct and separate fungus from _Thielaviopsis basicola._ Stover (27) observed _Thielavia basicola_ in an on the roots of tobacco only if they were infected with

Thielaviopsis basicola. He found that *Thielavia basicola* neither assists nor hinders an attack of *Thielaviopsis basicola.* He concluded that differences in pathogenicity were not due to the two species but to the presence of races of *Thielaviopsis basicola.*

Several studies (6,13,15) have shown that the fungus *Thielaviopsis basicola* is capable of living in decaying organic matter in the soil for several years in the absence of a major host plant. Many suspects are found in the families Solonacae, Leguminosae and Cucurbitacae (11). For these reasons, it appears that inoculum is almost always present in tobacco soil, and whenever factors, such as temperature and pH, are favorable the disease develops.

The fungus first attacks the cortical tissue of the roots either throughout their length or only in segments (16,24). It may extend to the underground portions of the stem and cause a roughening and a black charcoal-like discoloration which sometimes extends above the ground. Within the roots the diseased tissues are tinted red or pink. The smaller roots may be entirely rotted away while the larger roots are only moderately affected. In severe cases, most of the root system may be rotted away, leaving only stubs of roots or a whisk composed of root stubs and short fibrous roots which have grown from above the older lesions. These, in turn, may have been attacked, killed and decayed.

Kightlinger (16) and Ramsey et al (24) state that the disease disrupts the uptake of water and materials by the roots, causing a stunted growth of the plant. The first above ground symptom usually is a greater than normal wilting of the plants. Later the leaves become narrow, thick and tough. Badly infested plants "top out" or produce their inflorescence prematurily. Stover (27) stated that in young seedlings of susceptible varieties which were 10 mm. or less in diameter, hyphae were frequently observed to pass from the roots into the stem and colonize the vascular region of the stem up to, and occasionally into, the basal portions of the first leaves.

Factors affecting development of the disease.

The most important factors which influence the development of the disease, were enumerated by Kightlinger (16) as follows:

1) Degree of infestation of soil
2) Soil reaction
3) Soil moisture
4) Soil temperature
5) Susceptibility of the variety

One of the most important of these factors undoubtedly is the temperature, (6,13,15). Jones, et al (15) and Doran (6) observed that root rot failed to develop on tobacco at 32°C. but developed readily at 17°C.. Root rot was most marked at 17°C. to 23°C. and was reduced at temperatures below 15°C. and above 26°C.. It is of interest to note that in culture *Thielaviopsis basicola* grows best from 28°C. to 30°C. and poorly at 17° to 23°C. (14). Conent (5) determined that at high temperatures the advance of the fungus in the tissue was limited by a corky layer of cells. It has been suggested also that differences in cell composition may account for resistence of the plants at higher temperatures. The action of temperature is interrelated with other variable factors. High soil moisture, especially near the saturation point, favors the disease. This development of the disease in moist soil may be explained by the fact that the fungus thrives better under moist conditions and, also, that the soil temperature is lowered by a high moisture content (13,15).

The development of black root rot is checked by a very alkaline reaction or a very high acidity (2). Injury from black root rot may occur when the pH is about 5.6, but the disease develops more rapidly at a higher pH. As a general rule, the higher the soil reaction, the worse the root rot becomes and the poorer the growth of tobacco (2). Doran (6) studied the joint effect of temperature and soil reaction and concluded, "The critical point for black root rot of tobacco on the pH scale was affected somewhat by soil temperature. In the soil used there was no black root rot or only a trace at any temperature in soil with a pH value of 5.6 or lower, but marked injury occurred with a reaction of pH 5.7 and a temperature of 15°C.. As the temperature was increased, a slightly higher reaction was required: 18°C., pH 5.7 or 5.8; 21° and 24°, pH 5.8; 27°, pH 5.8 or 5.9. There was little or no injury at 30°C. even in soil with pH values of 6.0 to 6.9". He further observed that black root rot in the less acid soils, (pH 5.9 to 6.5), with a constant temperature, retarded the growth of tobacco plants more when they were young than when they were old. Also, at higher temperatures root rot was more severe in seedlings than on older plants in soil at a given reaction.

MacRae (17) found a direct relationship between the degree of infection and the amount of the pathogen in the soil, but he further observed that a few diseased plants left in the soil from a previous year provide sufficient inoculum to cause a complete crop failure the next year.

The last and the most important factor in the development of the disease is the degree of susceptibility of the variety of tobacco. Johnson (12) and Henderson (9), have observed that there are a number of degrees of resistance shown by different varieties in a given infested soil. One variety may be a complete failure, while another variety may not show perceptible signs of injury from root rot even under adverse conditions. Other varieties may be moderately resistant or moderately susceptible.

Nature of resistance in tobacco.

Conent's (5) investigations show that resistance in tobacco to root rot caused by Thielaviopsis basicola is "definitely" correlated with the ability of the host to develop a cork layer beneath the point of infection. The cork formation in tobacco roots is hastened by a rise in soil temperature up to 26° to 30° C. which might explain why plants become resistant to attack by the root rot organism at high temperatures. He also observed that the protective corking occured in the plants of resistant varieties at all temperatures, whereas, in plants of the susceptible varieties the "corking off" of the fungus occured only at higher temperatures. This information is thought to explain why the fungus cannot attack plants at temperatures of 28° to 30° C., which is optimum for the growth of the fungus in culture (14), but does attack them readily at 17° to 23° C., which is relatively unfavorable for the fungus in culture.

It cannot be assumed, however, that resistance in tobacco or the entry of fungal hyphae into the roots is entirely mechanical, because some races of the fungus may attack supposedly resistant varieties. The available information appears to indicate that resistance in tobacco plants may be both mechanical and chemical.

Breeding for root rot resistance.

Through breeding and selecting, fairly satisfactory progress has been made in developing root rot resistant varieties of tobacco, although these varieties have not been as resistant as desired. Resistant varieties have been produced in Virginia (10), North Carolina (22), Tennessee (8), Massachusetts (16), and Canada (18,30). In a recent paper Valleau (29), reviewed in detail the history of the development of various resistant varieties. He states, "These details are mentioned to emphasize the fact that chance plays a great part in the development of a satisfactory variety." He also pointed out that the origin of some resistant varieties is obscure and that in some cases, a variety "has evidently resulted from accidental mixture."

Although Valleau (29) has emphasized the importance of chance in the development of a variety, it can be easily seen that lack of knowledge concerning the inheritance of resistance to root rot and the biology of the pathogen has delayed the development of more resistant varieties.

Inheritance Studies.

Wingard (31) has reviewed the literature on inheritance of disease resistance. Many workers have shown that resistance or susceptibility to some diseases is controlled by one or more Mendelian factors. Many diseases, especially root rots, vary in the degree of injury caused to the host. With these diseases, in order to measure resistance, it is necessary to set up arbitrary divisions to indicate the degree of injury. For example, Johnson (12), while studying the inheritance of resistance to black root rot, used the height of the plants as an index of the amount of disease. While it is true that the height of the plant is much affected by the amount of root rot, height also may be influenced by genetic factors as well as by the environment. Limited attempts have been made to study the inheritance of root rot resistance in tobacco (12,23,30). In most instances, however, these studies were not planned to obtain specific information on genetic behavior but were observations made on breeding material being used in other experiments. The data obtained on inheritance are, therefore, not complete.

White (30), working in Canada thought that resistance was dependent on two pairs of dominant genes and that the degree of resistance was influenced by the number of genes present. Similar conclusions were drawn by Nelson (23), but neither worker presented data to support these conclusions. Earlier, Johnson (12) found that the F_1 progeny of a cross between root rot resistant and root rot susceptible types were intermediate in resistance and that the F_2 generation yielded individuals of all grades of resistance from those with greater resistance than the resistant parent to those with as much susceptibility as the susceptible parent. He did not draw any definite conclusions about the manner of inheritance, except to suggest that it could only be explained by a multiple factor hypothesis. His studies were made on breeding stocks in varietal trials where he measured the heights of plants to indicate the amount of disease. In discussing the findings, Johnson pointed out that, "It is evident that in a disease such as "Thielavia" root rot no practical method of determining the actual amount of infection on the roots is available." The need for a better method of measuring the degree of susceptibility of plants in inheritance studies is readily recognized.

MATERIALS AND METHODS

Seed of five parental lines which were thought to have different degrees of root rot resistance were obtained from Dr. Thurston Mann, Agronomy Department, N. C. State College, Raleigh, together with seed of the ten F_1 populations of all possible crosses among them (excluding reciprocal crosses) and also of five F_2 populations. A list of the parental lines and the F_2 seed lots received from Dr. Mann and used in these studies follows:

1. Virginia Gold
2. Vamorr 48
3. Gold Dollar
4. Kentucky 16
5. 402

F_2 seed lots:

1. Gold Dollar x Vamorr 48
2. 402 x Gold Dollar
3. Vamorr 48 x Kentucky 16
4. Kentucky 16 x 402
5. 402 x Vamorr 48

All the parental lines were presumed to be homozygous. Seed of each of the parents, the ten F_1's and five F_2's was planted in six inch pots in the greenhouse in late October, 1951, in sterilized soil. When the plants were three to four inches in height they were transplanted to three inch pots, one plant per pot. The soil used in transplanting was taken from a plot on the farm at Virginia Polytechnic Institute, where tobacco had been grown for several years and where the damage from root rot had been rather severe.

The plants were allowed to grow for a week after transplanting at which time twenty-five plants of uniform size of each parental line and of each F_1 population and fifty plants of each F_2 population (except for Gold Dollar x Vamorr 48 of which only twenty-five plants were available), were selected and arranged in five randomized blocks. Each block contained five plants of each parental line and of each F_1 and ten plants of each F_2 except for Gold Dollar x Vamorr 48 of which there were only five plants in each block. The plants were allowed to grow in the root rot infested soil for five weeks from the time of transplanting. (Normally it takes about two to three weeks for development of root rot under favorable conditions). At the end of that period there was good development of root rot as indicated by the wilting of most plants. The plants were then removed from the pots, the soil washed from the roots and the plants individually scored on a zero to ten basis for severity of root rot. The plants that had no visible root rot damage were scored zero while those that were heavily damaged, that is, all the roots rotted away, were scored ten. The plants that had only a part of the roots rotted were scored in between these extremes according to the amount of damage.

An additional group of five plants of each parent and of each F_1 was transplanted to a bed in the greenhouse and allowed to grow to maturity in order to make backcrosses and to obtain F_2 seed for further studies.

Some of the floral shoots of the F_1 plants were bagged and allowed to self pollinate to give F_2 seed while other shoots were backcrossed, to each of the parents involved in the cross, to give backcross seed. This gave a total of ten F_2 and twenty backcross populations along with five parental lines and ten F_1 populations saved from the seed obtained from Dr. Mann. A second experiment was conducted in the same manner as the previous one using twenty plants of each parental line, twenty of each F_1, forty-five of each F_2 and forty of each backcross, making a total of 1550 plants. However, the actual number of plants examined was a few less, because some of the plants died and in one or two cases the labels were mixed while washing, making it necessary to discard those plants. The plants again were arranged in five randomized blocks of 310 plants, made up of four plants of each parent, four of each F_1, nine of each F_2 and eight of each backcross.

They were then studied for reaction to root rot, using the same method of scoring as described above

It may be pointed out that in both tests the plants were not transplanted the same day nor washed on the same day. It took two to three days to transplant and two to three days to make the readings on the degree of infection of the roots.

No measures were taken to assure that only one race of the root rot organism was being used in this study. A homogeneous mixture of soil from a root rot infested field was used for all studies. If more than one race existed in the soil, it was assumed that they would be present in all pots.

The first test was conducted during the winter months when the temperature of the greenhouse was between 65° and $70^{\circ}F$. The second test was conducted during the summer and the temperature fluctuated widely, often reaching a high of 90° to $100^{\circ}F$.

EXPERIMENTAL RESULTS

The summer results, although not typical of those expected if conducted under cooler temperature, are presented as a supplement to the winter results, and to indicate the type of results obtained under conditions of high temperature.

The data from the two studies conducted are given in table 1. All data presented are original observations but because of large accumulation of readings at the higher end of the root rot scale and the small number of individuals in the populations studied, the statistical analyses of the data were made on transformed values. The square-root transformation, accomplished by adding 0.5 to each value and extracting the square-root, was used.

The statistical analyses of the data, given in table 2, indicated a significant difference among population means. The data for each cross are presented graphically in figures 1 through 10. (The root rot score has mistakenly been called fungus score in these figures. Any place where fungus score has been used refers to the root rot score based on the amount of visible damage caused to the roots by the fungus.) The mean of each variety and means of all progeny from the several crosses in which they were involved are given in table 3.

Parental lines. The parental lines differed greatly in their reaction to root rot as shown by the root rot score (table 1). There was also a wide range shown in the root rot score of individual plants within

Table - 1 - Mean root rot score, range, and number of plants of parent, F_1, F_2 and BC_1 populations studied in the winter and summer tests.

Varieties or crosses	Winter Study Root Rot score Mean	S.D*	Range	No. of Plants	Summer Study Root Rot score Mean	S.D*	Range	No. of Plants
Parents								
Ky 16	2.44	0.77	1 to 4	25	1.70	0.74	1 to 3	20
Va. Gold	6.16	2.12	2 to 9	25	3.30	0.83	2 to 5	20
Vamorr 48	7.60	1.50	4 to 10	25	2.60	1.70	1 to 9	20
Gold Dollar	8.92	0.81	8 to 10	25	2.50	1.40	1 to 6	20
402	9.54	0.75	7 to 10	24	3.65	1.60	1 to 7	20
F_1 populations								
Ky 16 x 402	7.44	1.44	5 to 10	25	2.50	0.52	2 to 3	12
Vamorr 48 x Ky 16	4.60	1.89	2 to 9	25	2.35	0.83	1 to 4	20
402 x Vamorr 48	8.45	1.18	6 to 10	24	2.80	1.10	1 to 5	20
Gold Dollar x Vamorr 48	8.28	1.60	5 to 10	25	3.05	0.96	1 to 4	17
402 x Gold Dollar	9.84	0.12	8 to 10	25	3.85	0.87	2 to 5	20
Ky 16 x Va. Gold	5.92	1.84	3 to 9	25	2.45	0.95	1 to 5	20
Gold Dollar x Ky 16	4.24	1.90	1 to 8	25	3.20	1.06	1 to 5	20
Va. Gold x 402	7.63	1.70	3 to 10	25	2.45	0.62	1 to 3	20
Va. Gold x Gold Dollar	6.33	1.57	3 to 9	24	3.18	1.17	1 to 5	16
Va. Gold x Vamorr 48	7.52	1.55	5 to 9	25	3.75	1.39	2 to 6	16
F_2 populations								
Ky 16 x 402	6.72	3.15	1 to 10	50	2.40	0.96	1 to 4	44
Vamorr 48 x Ky 16	7.02	2.53	1 to 10	50	2.09	0.91	1 to 4	44
402 x Vamorr 48	8.60	1.34	5 to 10	50	2.57	1.05	1 to 4	45
Gold Dollar x Vamorr 48	8.80	1.22	6 to 10	25	2.73	1.23	1 to 9	45
402 x Gold Dollar	9.26	1.01	6 to 10	50	3.82	1.12	2 to 7	45
Ky 16 x Va. Gold	—	—	—	—	2.38	0.92	1 to 4	44
Gold Dollar x Ky 16	—	—	—	—	2.43	1.03	1 to 5	44
Va. Gold x 402	—	—	—	—	2.90	1.43	1 to 5	45
Va. Gold x Gold Dollar	—	—	—	—	2.93	1.23	1 to 5	45
Va. Gold x Vamorr 48	—	—	—	—	2.67	1.13	1 to 6	43
BC_1 populations								
(Ky 16 x 402) x 402					3.25	0.74	2 to 4	40
" " x Ky 16					2.43	1.43	1 to 4	39
(Vamorr 48 x Ky 16) x Vamorr 48					1.92	0.80	1 to 4	40
" " x Ky 16					2.24	0.75	1 to 4	37
(402 x Vamorr 48) x Vamorr 48					2.32	1.18	1 to 6	40
" " x 402					2.42	1.20	1 to 5	40
(Gold Dollar x Vamorr 48) x Vamorr 48					2.10	0.93	1 to 4	40
" " x Gold Dollar					3.33	1.20	2 to 7	40
(402 x Gold Dollar) x 402					3.62	1.05	1 to 5	39
" " x Gold Dollar					3.10	1.25	1 to 5	39
(Ky 16 x Va. Gold) x Ky 16					2.40	0.78	1 to 4	40
" " x Va. Gold					3.23	0.94	1 to 5	38
(Gold Dollar x Ky 16) x Ky 16					2.82	0.98	1 to 7	39
" " x Gold Dollar					3.07	1.11	1 to 5	40
(Va. Gold x 402) x Va. Gold					2.52	0.95	1 to 5	40
" " x 402					2.97	0.81	1 to 4	39
(Va. Gold x Gold Dollar) x Va. Gold					2.81	0.92	1 to 5	37
" " x Gold Dollar					4.17	1.21	2 to 6	40
(Va. Gold x Vamorr 48) x Va. Gold					2.64	0.92	1 to 4	39
" " x Vamorr 48					2.23	0.81	1 to 4	40

* Standard deviation

Table - 2 - Analyses of variance of parental F_1, F_2 and backcross populations based on root rot score of individual plants.

	Winter Study				Summer Study			
	Degrees of Freedom	Sum of Squares	Mean Square	F.	Degrees of Freedom	Sum of Squares	Mean Square	F.
<u>Parents</u>								
Among parents	4	34.881	8.220	26.182**	4	3.616	0.911	7.528*
Within parents	119	6.170	0.333		95	11.531	0.121	
Total	123	41.051	——		99	15.177	——	
<u>F_1 populations</u>								
Among F_1	9	23.655	2.628	71.270**	9	3.463	0.380	9.142**
Within F_1	238	8.978	0.037		171	7.274	0.042	
Total	247	32.633	——		180	10.737	——	
<u>F_2 populations</u>								
Among F_2	4	9.817	2.454	14.022**	9	7.357	0.817	7.306**
Within F_2	220	38.343	0.175		433	48.467	0.111	
Total	224	48.160	——		442	55.824	——	
<u>BC_1 populations</u>								
Among BC	-	——	——	——	19	16.238	0.854	9.308**
Within BC	——	——	——	——	768	62.839	0.081	
Total	——	——	——	——	787	79.077	——	

* Significant at 5% level.

** Significant at 1% level.

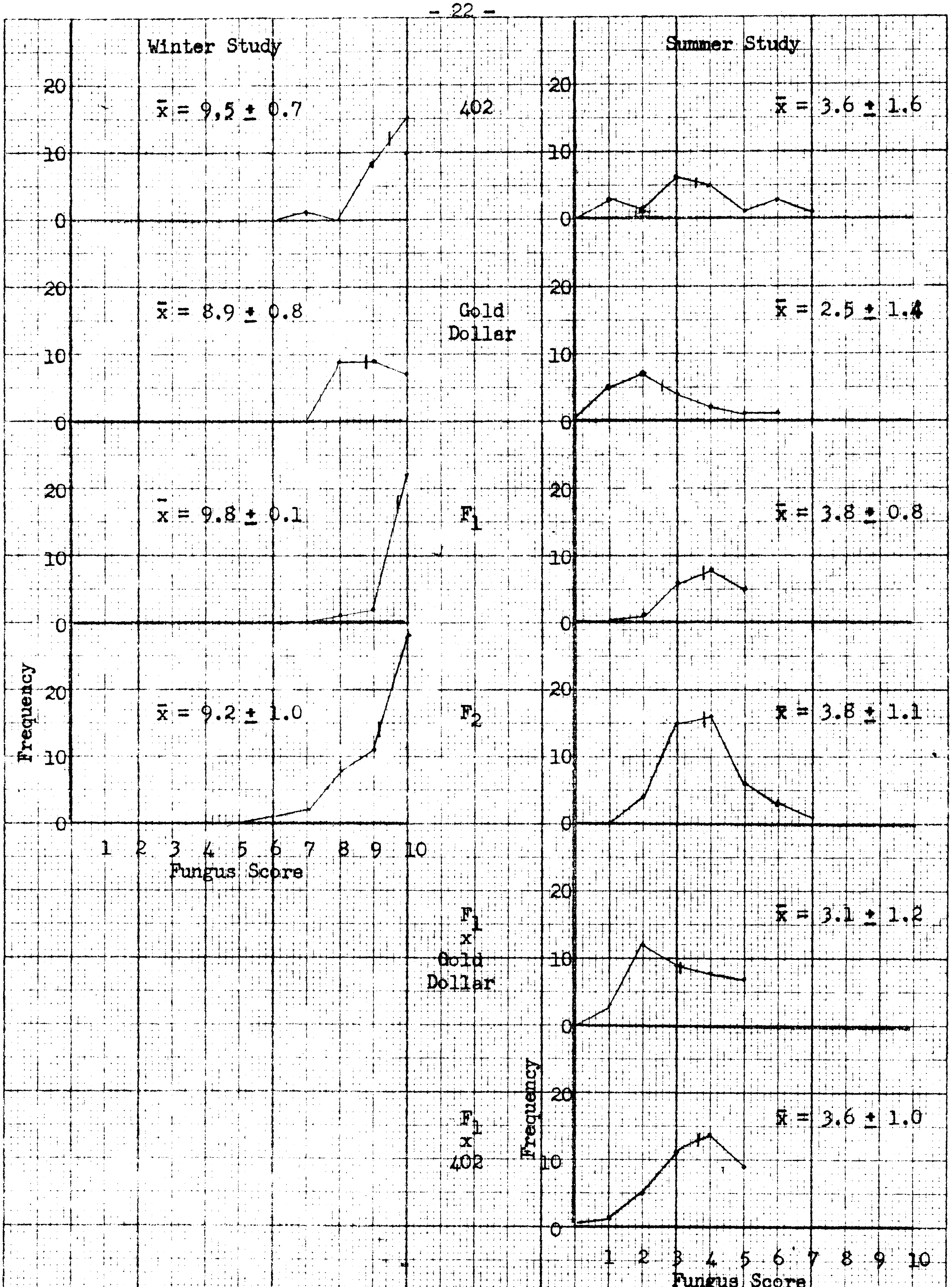

Figure 1. Frequency distribution of plants in various fungus score classes for parental lines, F_1, F_2 and backcross populations of the cross 402 x Gold Dollar.

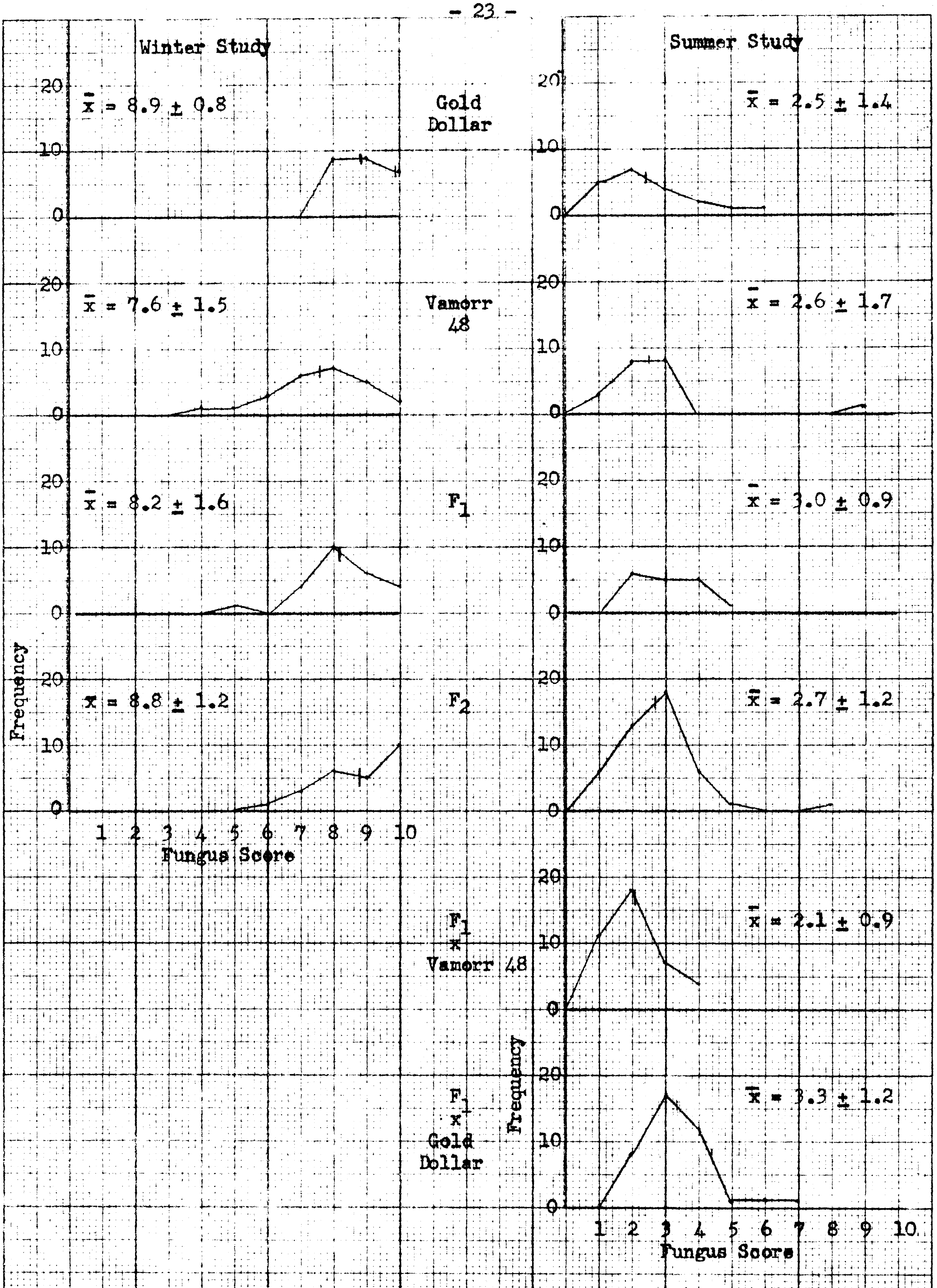

Figure 2. Frequency distribution of plants in various fungus score classes for parental lines, F_1, F_2 and backcross populations of the cross Gold Dollar x Vamorr 48.

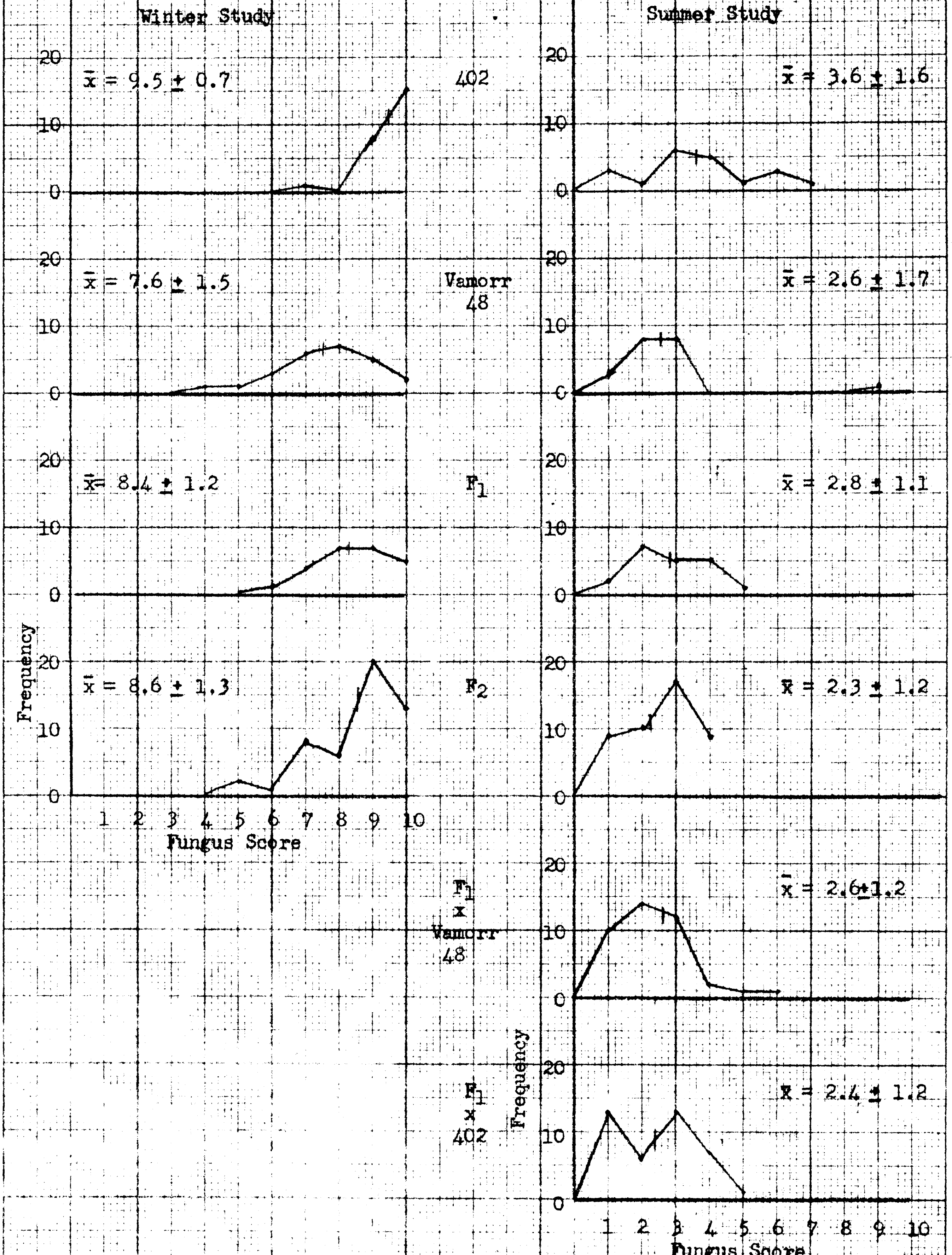

Figure 3. Frequency distribution of plants in various fungus score classes for parental lines, F_1, F_2 and backcross populations of the cross 402 x Vamorr 48.

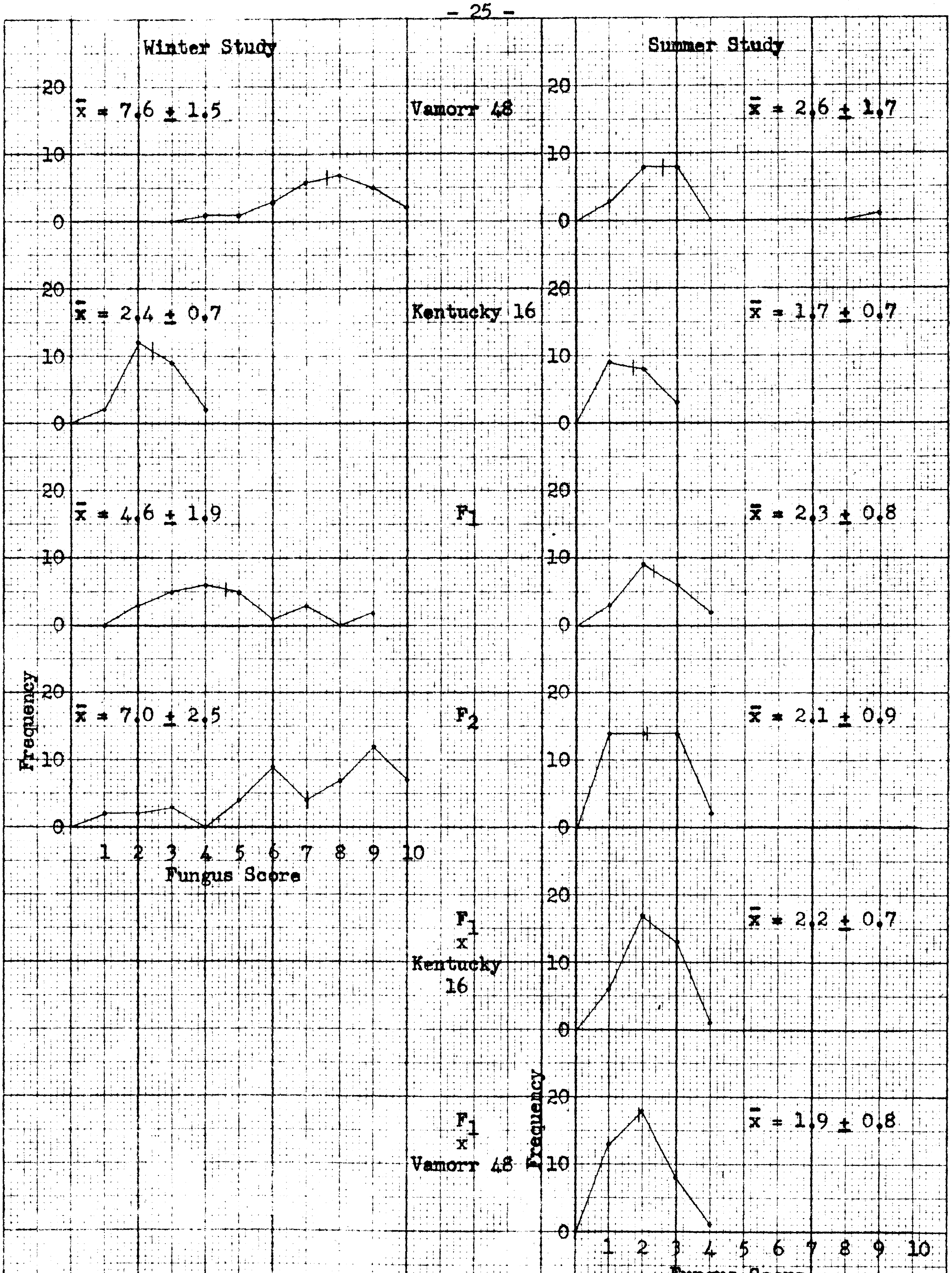

Figure 4. Frequency distribution of plants in various fungus score classes for parental lines, F_1, F_2 and backcross populations of the cross Vamorr 48 x Kentucky 16.

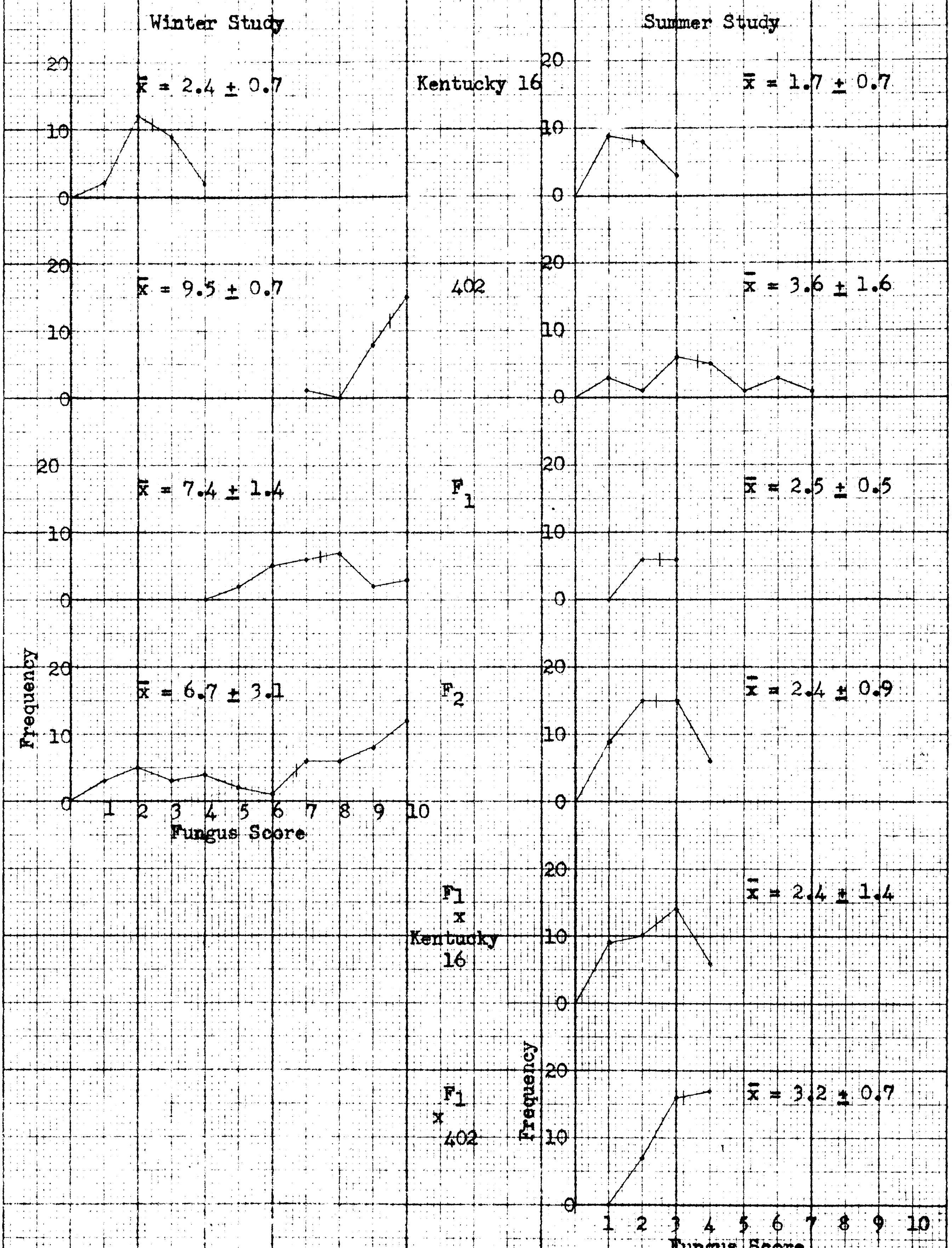

Figure 5. Frequency distribution of plants in various fungus score classes for parental lines, F_1, F_2 and backcross populations of the cross Kentucky 16 x 402.

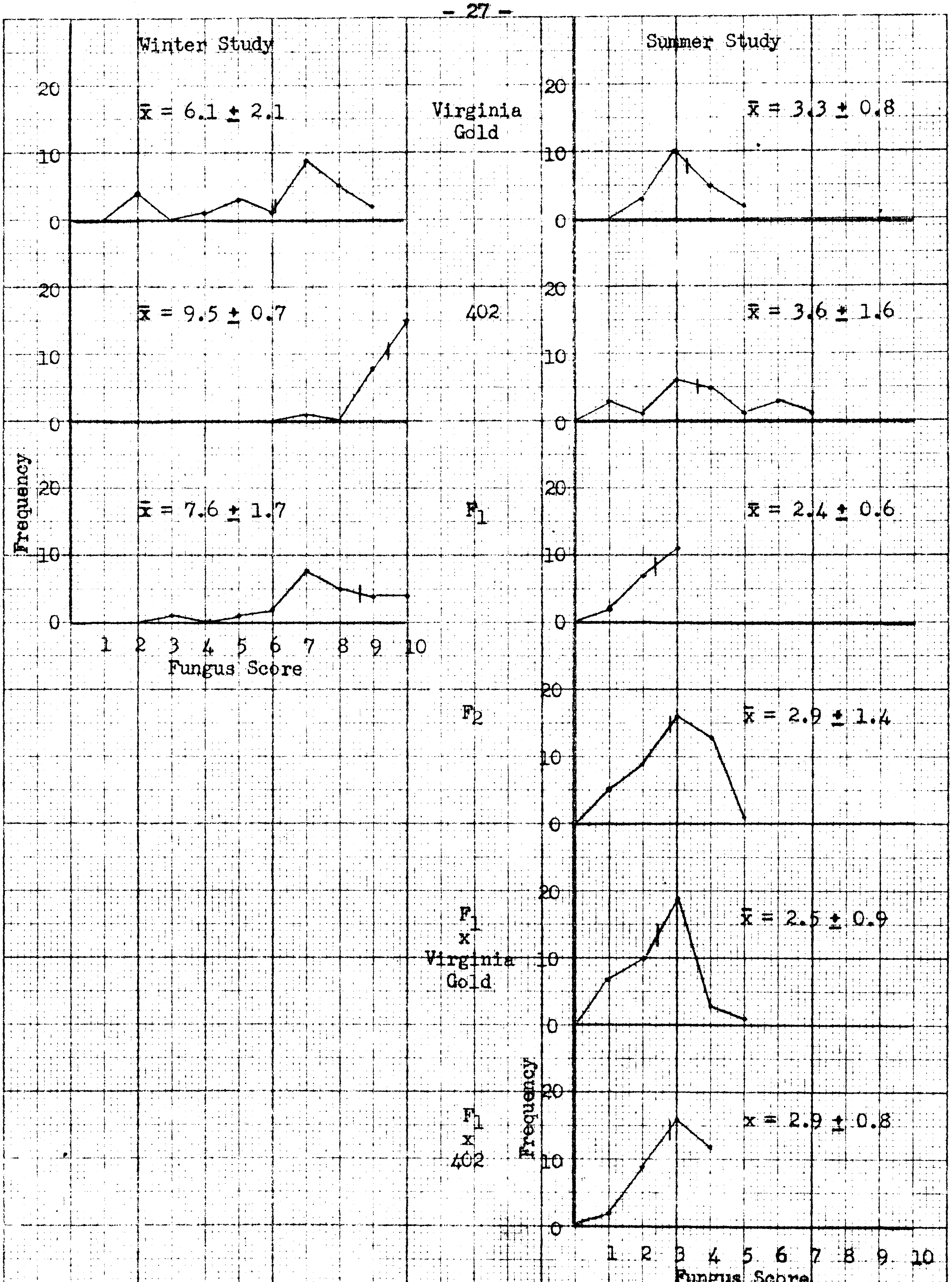

Figure 6. Frequency distribution of plants in various fungus score classes for parental lines, F_1, F_2 and backcross populations of the cross Virginia Gold x 402.

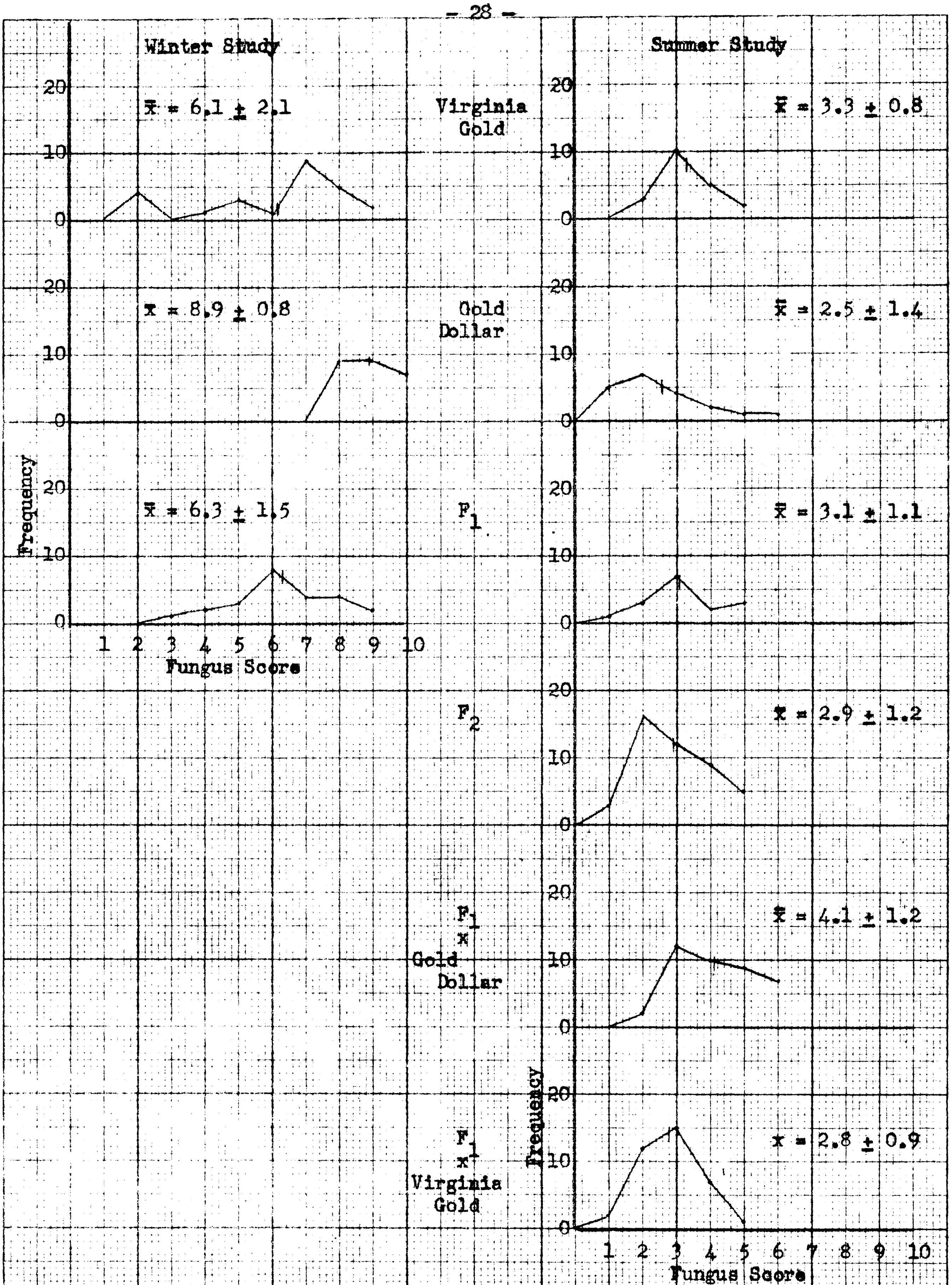

Figure 7. Frequency distribution of plants in various fungus score classes for parental lines, F_1, F_2 and backcross populations of the cross Virginia Gold x Gold Dollar.

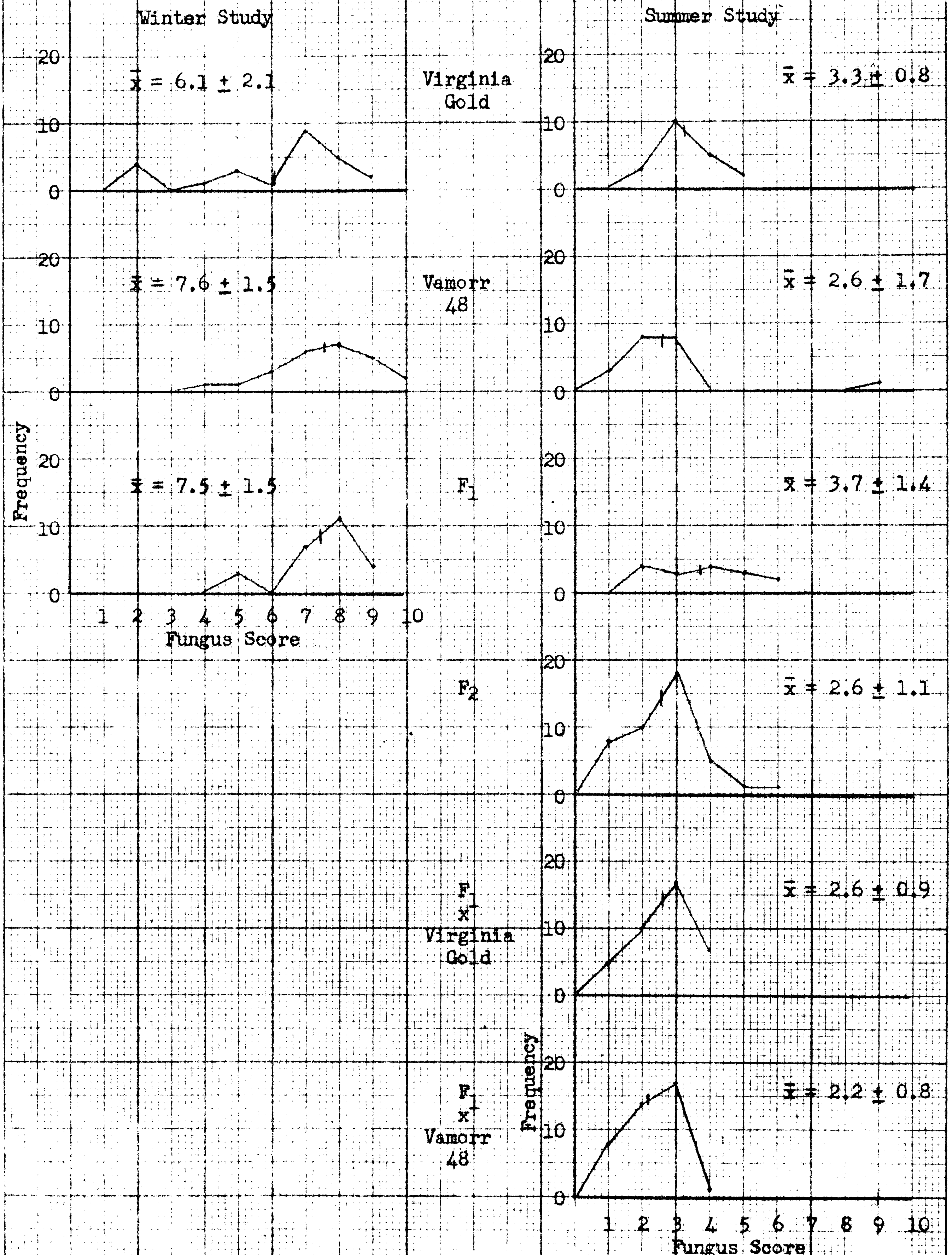

Figure 8. Frequency distribution of plants in various fungus score classes for parental lines, F_1, F_2 and backcross populations of the cross Virginia Gold x Vamorr 48.

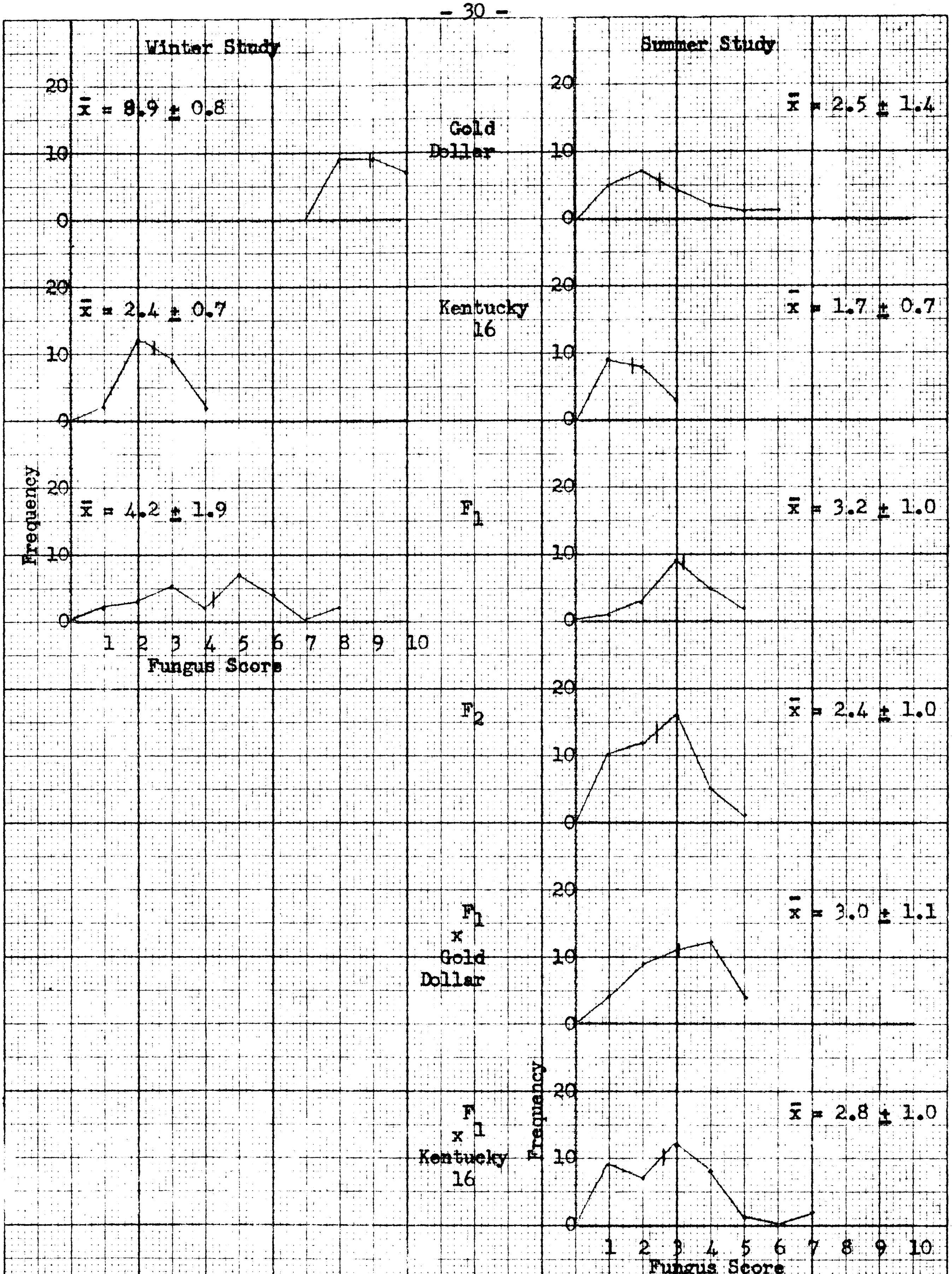

Figure 9. Frequency distribution of plants in various fungus score classes for parental lines, F_1, F_2 and backcross populations of the cross Gold Dollar x Kentucky 16.

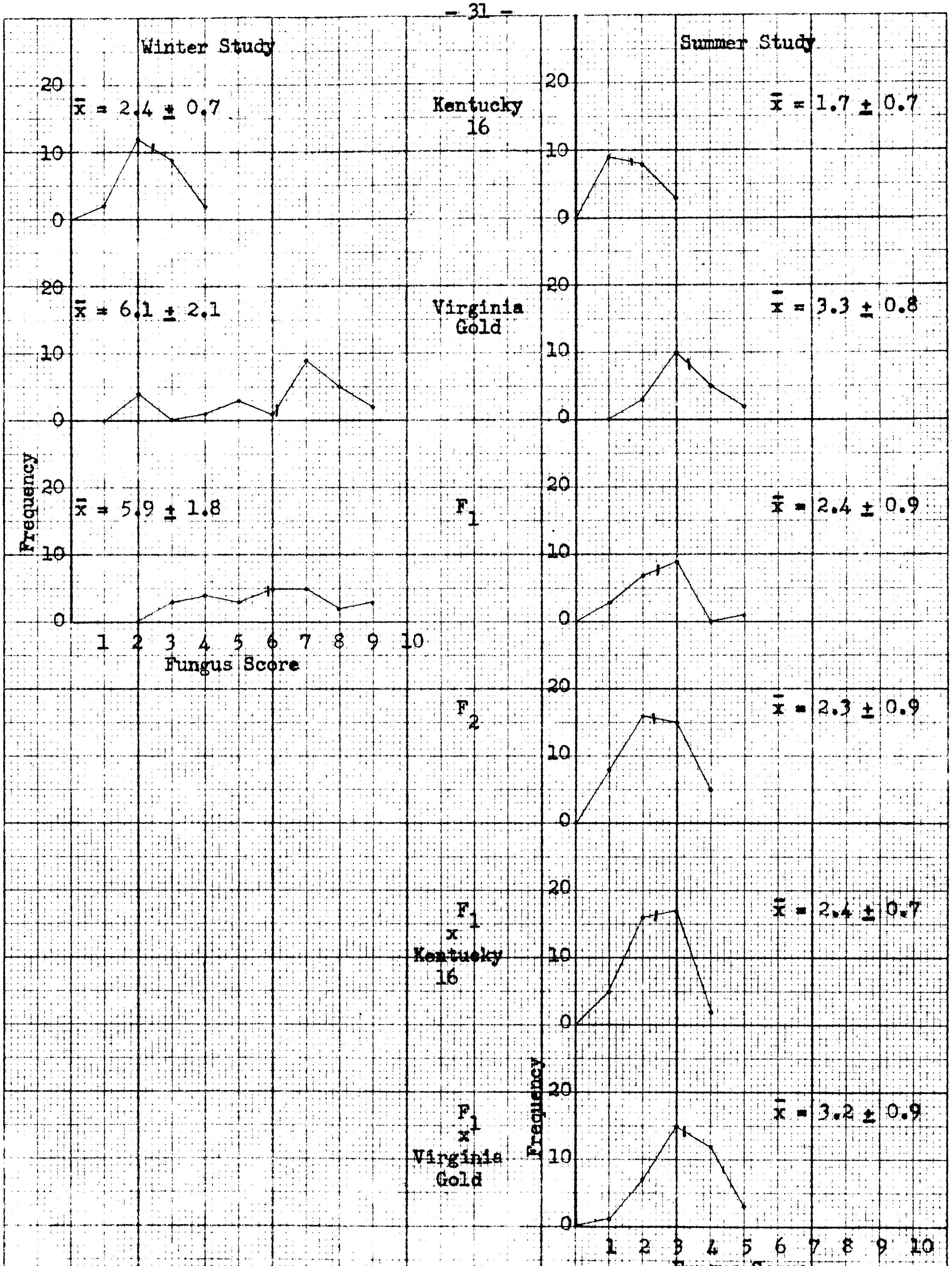

Figure 10. Frequency distribution of plants in various fungus score classes for parental lines, F_1, F_2 and backcross populations of the cross Kentucky 16 x Va. Gold.

some of the parental lines. Varieties 402 and Ky 16 were the extreme types in both the winter and summer studies. In the winter study 402 was found to be the most susceptible variety with a mean root rot score of 9.54 ± 0.75 and a range of 7 to 10, while Ky 16 was the most resistant variety with a mean score of 2.44 ± 0.77 and a range of 1 to 4. Va. Gold, usually classed as a tolerant variety, had a mean score of 6.16 ± 2.12 with a range of 2 to 9. Vamorr 48 was found to be a moderately tolerant variety, with a mean of 7.60 ± 1.50 and a range of 4 to 10. Gold Dollar had a mean of 8.92 ± 0.81 which was slightly less than that of 402.

In the summer study, the average root rot scores of all populations studied were considerably lower than in the winter. The probable cause of this reduction in root rot was the high temperature which prevailed during the period of the summer study. However, the parental varieties maintained, in general, the same relationship in both studies. The only exception was Va. Gold, which was less resistant in summer than Vamorr 48 or Gold Dollar.

The classification of these varieties based on the root observations agrees with the classification based on above-ground symptoms as determined by other workers. The wide range observed within the presumably homozygous parental varieties is attributed to variation between pots as regards environmental factors such as soil temperature, pH and moisture.

<u>Crosses</u>: All the F_1 progeny means were between the mean of their respective parents in the winter study, as shown in table 1, with one

exception: hybrid 402(mean 9.54 ± 0.75) x Gold Dollar(mean 8.92 ± 0.81), a cross between two susceptible varieties, gave an F_1 population with a mean of 9.84 ± 0.12, which is higher than that of either parent. In the five F_2 populations studied in the winter, two had a slightly higher and two slightly lower means than the corresponding F_1 and one was considerably higher than its F_1 mean.

The range of plants obtained in the F_1 generation was intermediate when compared to the two parents. The F_2 populations generally were more variable than the F_1 populations, having plants as resistant as the resistant parent and as susceptible as the susceptible parent. Resistant plants occured less frequently than susceptible ones. The occurance of a wider range in the F_2 than in the F_1 population indicated segregation in the F_2. There was no difinite indication of dominance of resistance or susceptibility.

In the summer, four out of ten F_1 populations had a higher mean root rot score than either parent. The cross 402 x Gold Dollar gave a higher F_1 population mean than either parent in both the summer and winter tests. Most of the F_2 populations had a slightly lower mean and a greater range than their F_1 population with the exception of Va. Gold(mean 3.30 ± 0.83) x 402(mean 3.65 ± 1.60). The F_2 mean of this cross (2.90 ± 1.43) was slightly higher than the F_1 mean(2.45 ± 0.62). While these small differences between the F_1 and F_2 means probably could not be shown to be different statistically, it at least is interesting and probably significant, that the F_2 mean was lower than the F_1 in eight out of ten cases and higher in only one. This may be an indication of partial dominence of susceptibility.

The backcrosses were only studied in the summer and for this reason the observations cannot be considered to be conclusive. The results, however, (table 1) suggested that when the F_1 was backcrossed with the more resistant parent a lower BC_1 mean was obtained than when backcrossed with the more susceptible parent. The exceptions were with the cross Vamorr 48 x Ky 16, in which the mean of the backcross to Vamorr 48 (2.60 ± 1.70) gave a lower mean score of 1.92 ± 0.80 than the backcross to Ky 16 (1.70 ± 0.74) which had a mean of 2.24 ± 0.75; and Va. Gold x Vamorr 48 which when backcrossed with Vamorr 48 (2.60 ± 1.70) gave a BC_1 mean of 2.27 ± 0.81 and with Va. Gold (3.30 ± 0.83) gave a mean of 2.64 ± 0.92. However, the latter exception holds true only if the winter parental mean is considered. As can be seen Va. Gold actually had a higher mean than Vamorr 48 in the summer study.

The data given in table 3, show that the total mean of various F_1, F_2 and backcross populations in which a variety was a common parent, in general, was in proportion to the common parent mean. For instance in the winter study the average of F_1 populations involving 402(9.54 ± 0.75) as one parent was 8.35 ± 1.57, of those involving Gold Dollar(8.92 ± 0.81) was 7.23 ± 1.33, of those involving Vamorr 48 (7.60 ± 1.50) was 7.20 ± 2.17, of those with Va. Gold (6.16 ± 2.12) was 6.86 ± 1.84, and those with Ky 16 (2.44 ± 0.77) was 5.55 ± 2.20. A similar relationship existed in F_2 and backcross populations with the exception that the rank of the

Table - 3 - Mean root rot score of the parents and of all progeny of crosses in which they were involved.

	Winter Study			Summer Study			
	Parental Mean	Mean of all F_1 progeny of indicated parent	Mean of * all F_2 progeny of indicated parent	Parental Mean	Mean of all F_1 progeny of indicated parent	Mean of all F_2 progeny of indicated parent	Mean of all BC_1 progeny of indicated parent
<u>Parents</u>							
402	9.54 ± 0.75	8.35 ± 1.57	8.19 ± 2.22	3.65 ± 1.60	2.94 ± 1.02	2.92 ± 1.17	3.06 ± 1.04
Gold Dollar	8.92 ± 0.81	7.23 ± 1.33	9.10 ± 1.09	2.50 ± 1.40	3.34 ± 1.05	2.98 ± 1.25	3.40 ± 1.03
Vamorr 48	7.60 ± 1.50	7.20 ± 2.17	8.66 ± 2.22	2.60 ± 1.70	2.82 ± 1.29	2.53 ± 1.08	2.15 ± 0.30
Va. Gold	6.16 ± 2.12	6.86 ± 1.84	————	3.30 ± 0.89	2.90 ± 1.16	2.72 ± 1.04	2.85 ± 0.96
Ky 16	2.44 ± 0.77	5.55 ± 2.20	6.87 ± 2.81	1.70 ± 0.74	2.63 ± 0.94	2.32 ± 0.96	2.47 ± 0.97

(*) F_2 means in winter study based on only two crosses.

crosses from Gold Dollar was not always the same as the rank of Gold Dollar among the parental varieties.

DISCUSSION OF RESULTS

One objective of the study was to evaluate the root rot reaction of the five tobacco varieties used in the crosses and to determine which, if any, would be of value as parental varieties in a breeding program for resistance to the disease. The statistical analyses indicated a highly significant difference among the root rot reaction of the varieties in both the winter and summer studies. Ky 16 was the most resistant type in both studies and in no case gave a root rot score higher than 4. Therefore, it could be said that all plants in the variety could be classified as resistant or moderately resistant.

Va. Gold and Vamorr 48 were classified as intermediate or tolerant in their reaction to root rot in the winter study. Va. Gold showed extreme variability, giving plants ranging in root rot score from 2 to 9. As is evident from their means and range, the variety Va. Gold was more tolerant than Vamorr 48. Inasmuch as root rot is caused by an organism which is saprophytic and rather ruthless in its attack on roots, and being highly influenced by environmental conditions, this variability in a moderately resistant variety is not unexpected. In a variety with relatively high resistance, environmental factors may cause a few plants to be given a disease score that is somewhat higher than expected. However, the lower limit for the classification of plants is already close to the population mean, limiting variation in the lower level. Likewise, a variety which is genetically susceptible would have its mean close to the upper end of the classification scale, limiting the variability there. A variety like Va. Gold, having an intermediate reaction and a population mean near the middle of the root rot scale, can vary in both directions. Likewise, varieties intermediate in resistance would probably react more easily to variation in environmental conditions.

Gold Dollar and 402 were classified as susceptible. However, Gold Dollar was less susceptible than 402. Although the difference between the mean root rot score of these two varieties was small, it apparently was a true difference. In F_1 populations from crosses of these two varieties with each of the other three, the mean of crosses with 402 was higher than those with Gold Dollar in all three cases in the winter study. This did not, however, hold true in the summer study. While Gold Dollar itself was less susceptible than 402 in the summer study, the mean root rot scores of F_1 crosses with 402 were slightly lower than those with Gold Dollar. No clear explanation can be given for this reaction.

In the summer study of parental varieties, Va. Gold which had been classified as intermediate or tolerant in the winter study, was more susceptible than Vamorr 48 or Gold Dollar. In summer, the mean of all crosses with Gold Dollar, however, was higher than with Va. Gold, while the mean root rot score of crosses with Vamorr 48 was lower than for those with Va. Gold. It is possible that the relative rank of the varieties would change when grown under different temperatures, and that their ability to contribute resistance, likewise, would change. From the standpoint of a breeding program, it would be desirable to choose a parent which gives resistance under varying environmental conditions.

An examination of the performance of these varieties in cross combinations should give an indication of their value as sources of resistance in a breeding program. While it is obvious that performance under field conditions is the final criterion on which to base such a decision, the greenhouse studies can be used if there is a fairly close correlation between greenhouse performance and performance under field conditions. The classification of the five varieties based on the amount of root rot present on the roots of seedling plants, as made in the present study, does agree fairly well with the classification made by other workers based on above-ground symptoms under field conditions.

Another object of the study was to learn something regarding the inheritance of resistance to root rot. A knowledge of inheritance and number of genes involved in resistance to any disease is valuable in planning a program for breeding for resistance to it. After completing the study it became obvious that the populations grown had not been sufficiently large to draw any definite conclusions regarding mode of inheritance.

A comparison of the parental means and the average mean of their F_1 progeny indicated a rather close correlation between the two in both the winter and summer studies. This indicates a fairly high heritability of reaction to root rot. If this indication is true, then a variety with the highest resistance to root rot generally would be our best source of resistance in a breeding program. It also appears that resistance to the disease was sufficiently simply inherited that plants as resistant as the most resistant parent could be recovered with fairly high frequency in the F_2 populations.

In all cases except one in the winter study and in most cases in the summer study, the mean of the F_1 populations was intermediate to that of the two parents. Generally, there was no definite indication of complete dominance of resistance or susceptibility. In some cases, however, there was evidence of partial dominance. In the winter study of the cross Ky 16 x Vamorr 48, the F_1 mean was closer to that of Ky 16 than to that of Vamorr 48. Likewise, the F_2 mean was significantly higher than that of the F_1, which would indicate partial dominance of resistance. Had there been no dominance, the F_2 mean would be expected to be the same as the F_1 mean. In the cross Ky 16 x 402 the opposite was true. The F_1 mean was nearer to that of the more susceptible parent and the F_2 mean was lower than the F_1, indicating partial dominance of susceptibility.

It is entirely possible that genes for resistance from one variety would be partially dominant in cross with some varieties while they would be partially recessive in another cross. Dominance or susceptibility must be measured in terms of the particular cross being studied, since variation with respect to separate loci and different alleles may be involved.

In the summer study the means of the F_2 populations were slightly higher in practically all cases than the mean of their respective F_1 populations. This could indicate partial dominance of susceptibility, or could result from the fact that all means in the summer study were close to the lower or resistant end of the root rot scale. In the F_2 populations, plants segregating for susceptibility would be more likely to deviate from the population mean than those segregating for resistance.

As in the case of parental varieties, those F_1 populations with the mean root rot scores near the middle of the scale showed more variability than those with means toward either end of the scale. The explanation for this probably would be the same as for the variation in parental varieties.

The F_2 populations in general were considerably more variable than their respective F_1 populations. However, the variability was not sufficiently greater than in parental and F_1 populations to permit drawing definite conclusions regarding segregation. As previously mentioned, the recovery of resistant types in the F_2 from crosses between resistant and susceptible varieties occured with sufficient frequency to indicate that the number of genes conditioning resistance was not large. However, a study of the F_3 progeny from individual F_2 plants would be necessary to show whether homozygous resistant types were being recovered.

In a cross between two susceptible varieties, 402 and Gold Dollar, all F_2 plants recovered were of the susceptible type. However, a few plants more resistant than had been found in either parent were observed. Again, further study from these individual plants would be necessary to determine whether this higher resistance was genetic or environmental.

Unfortunately the F_2 population from the cross of two intermediate or tolerant varieties was not studied in the winter. However, the F_2 population in the summer study of the cross Va. Gold x Vamorr 48 appeared to give a higher frequency of resistant plants than was found in either parent. Likewise, plants were recovered in the F_2 population that were more susceptible than those found in the parental varieties of this cross. While this evidence is not conclusive, it does indicate the possibility of recovering higher resistance by crossing between intermediately resistant varieties.

The backcross populations were studied only in the summer. As previously indicated, the high temperature during the summer study caused most or all plants to be fairly resistant, and the segregation could not easily be observed. In general, however, the backcross to the more resistant parent gave a lower mean root rot score than the backcross to the more susceptible parent, showing recovery of parental genes for root rot reaction. The small populations used and the masking effect of high temperature, prohibits the drawing of definite conclusions from the backcross studies.

Inasmuch as environments play an important role in the development of root rot and mask the genetic variability, it is proposed that in future genetic studies, some consideration be given to studying the F_3 progeny from individual F_2 plants as a means of getting a better estimate of the genotype of the F_2 plants. This would help to reduce the error of mis-classification of F_2 plants and give a better description of the F_2 population.

SUMMARY AND CONCLUSIONS

The inheritance of resistance to black root rot in tobacco, caused by the fungus Thielaviopsis basicola (Bek. and Br.) Ferr., was studied with a view of developing higher resistance by combining genes from various sources. The studies were conducted under greenhouse conditions during the winter and summer seasons of 1951-52. The five parental lines, the F_1 hybrids between them and five of the F_2 populations were included in the winter study when the temperature averaged between 65° and 70° F.. The five parental varieties, all the F_1,F_2, and backcross populations were included in the summer study when the temperature could not be controlled in the greenhouse. The evaluation of resistance was done on the basis of visible damage to the roots of the seedling plants.

The results indicated that:

1. In the winter study only one variety, Ky 16, (mean 2.44 ± 0.77, range 1 to 4), was found to be resistant. Va. Gold (mean 6.16 ± 2.12, range 2 to 9) and Vamorr 48 (mean 7.60 ± 1.50, range 4 to 10) were classed as tolerant types. Gold Dollar (mean 8.92 ± 0.81, range 8 to 10) and 402 (mean 9.54 ± 0.75, range 7 to 10) were classified as susceptible varieties. This classification is in general agreement with the classification based on above ground symptoms as done by other workers under field conditions. This shows that conclusions drawn from the study can be used in a breeding program.

2. While all varieties were resistant to a degree in the summer study, giving, a considerably lower average mean root rot score than that

in the winter study, the relative rank of varieties remained the same as in the winter study.

3. The means of the F_1 populations, in general, were between the means of two parents in both the winter and summer studies, but one cross in winter and four in summer gave higher F_1 population means than that for either parent.

4. The close correlation between the parental means and the average mean of their F_1 hybrids indicated fairly high heritability, showing that the variety with the highest resistance to root rot probably would be the best parental source of resistance.

5. The F_2 populations segregated into plants as resistant and, in one case, higher in resistance, than the more resistant parent and as susceptible as the more susceptible parent.

6. The backcross to the more resistant parent, in general, gave a lower mean root rot score than the backcross to the more susceptible parent, indicating the recovery of parental genes.

7. Generally there was no definite indication of complete dominance of resistance or susceptibility. However, in some cases there was evidence of partial dominance of one of these characters. It was concluded that genes from a variety may be partially dominant in crosses with one variety while they may be partially recessive in crosses with another since variation with respect to separate loci and different alleles may be involved.

8. No definite conclusions regarding the number of genes controlling inheritance could be drawn. This resulted partially from the

small size of the populations studied. However, there was an indication that the number of genes conditioning inheritance was not large, because in the F_2 populations a fairly large number of plants equally as resistant or susceptible as parental types could be recovered.

9. To establish the true genetic identity which is masked by the dominant influence of non-genetic factors, it was proposed that in future studies F_3 progeny from individual F_2 plants be studied to get a better estimate of the genotype of the F_2 population.

BIBLIOGRAPHY

1. Allison, C. C. Physiologic specialization of Thielaviopsis basicola. Phytopath. 28: 1. 1938. (Abstract.)

2. Anderson, P. J. Soil reaction studies on the Connecticut Tobacco crop. Jour. Amer. Soc. Agron. 21: 156-158. 1929.

3. ______________, Osmun, Vincent and Doran, W. L. Soil reaction and black root rot of tobacco. Mass. Agr. Exp. Sta. Bul. 229. 1926.

4. Clayton, E. E. and McMurtrey Jr., J. E. Tobacco diseases and decays. U. S. D. A. F. B. 2023. 1950.

5. Conent, George H. Histological studies of resistance in tobacco to Thielavia basicola. Jour. Agr. Res. 31: 801-825. 1927.

6. Doran, W. L. Effect of soil temperature and reaction on growth of tobacco infected and uninfected with black root rot. Jour. Agr. Res. 39: 825-872. 1929.

7. Gilbert, W. W. The root rot of tobacco caused by Thielavia basicola. U. S. D. A. Bur. Plant Ind. Bul. 158. 1909.

8. Heggested, Howard E. Varietal variation and inheritance studies in natural water-soaking in tobacco. Phytopath. 35: 754-770. 1945.

9. Henderson, R. G. Breeding tobacco for black root rot resistance (Thielaviopsis basicola). Phytopath. 32: 647. 1942. (Abstract.)

10. ______________ Vamorr 48 and 50. Two new flue-cured varieties resistant to root rot. Va. Agr. Exp. Sta. Bul. 427. 1949.

11. Johnson, J. Host plants of Thielavia basicola. Jour. Agr. Res. 7: 289-300. 1921.

12. __________ Breeding tobacco for resistance to Thielavia root rot. U. S. D. A. Tech. Bul. 175. 1930.

13. __________, and Hartman, R. E. Influence of soil environments on the root rot of tobacco. Jour. Agr. Res. 17: 41-86. 1919.

14. Johnson, E. M. and Valleau, W. D. Cultural variation of Thielaviopsis basicola. Phytopath. 25: 1011-1018. 1935.

15. Jones, L. R., Johnson, James and Dickson, James G. Tobacco root rot caused by Thielavia basicola. Wisc. Agr. Exp. Sta. Bul. 71: 40-48. 1926.

16. Kightlinger, C. V. Black-root-rot resistant strains of Havana tobacco. Mass. Agr. Exp. Sta. Bul. 432. 1946.

17. MacRae, N. A. Tobacco growing in Canada. Dom. of Canada Dep. Agr. Bul. 176. 1935.

18. ____________, and Haslam, R. J. Varietal studies of flue-cured, burley and dark tobaccoes. Dom. of Canada Dep. Agr. Bul. 178. 1935.

19. Mathews, M. F. and Henderson, R. G. Yellow Special tobacco, a new flue-cured variety resistant to black root rot. Va. Agr. Exp. Sta. Bul. 346. 1943.

20. McCormick, Florence A. Perithecia of Thielavia basicola Zopf. in culture and stimulation of their production by extracts from other fungi. Conn. Agr. Exp. Sta., Bot. Dep. Bul. 269. 1925.

21. Morgan, M. F. and Anderson, P. J. Relation of the soil reaction to black root rot and good tobacco. Conn. Agr. Exp. Sta. Tob. Bul. 8: 47-49. 1927.

22. Moss, E. G. and Bullock, J. F. Two new varieties of flue-cured tobacco 400 and 401. N. C. Agr. Exp. Sta. Bul. 337. 1942.

23. Nelson, N. T. Tobacco research in Canada. Emp. Jour. Exp. Agr. 9: 265-276. 1941.

24. Ramsey, Glen B., Wiant, James S. and Link, George K. K. Market diseases of fruits and vegetables: Crucifers and cucurbits. U. S. D. A. Misc. Pub. 292: 31. 1938.

25. Selby, A. D. Investigation of plant diseases in forcing house and garden. Ohio Agr. Exp. Sta. Bul. 73. 1896.

26. ____________ Tobacco diseases and tobacco breeding. Ohio Agr. Exp. Sta. Bul. 156. 1904.

27. Stover, R. H. The Black root rot disease of tobacco, 1. Studies on the causal organism Thielaviopsis basicola. Canada Jour. Res. C 28: 445-470. 1950. 11. Physiologic specialization of Thielaviopsis basicola Ibid. C 28: 726-738. 1950.

28. Thaxter, Ronald. Fungus in violet roots. Conn. Agr. Exp. Sta. Anl. Rep. 1891: 166-167. 1891.

29. Valleau, W. D. Breeding tobacco for disease resistance. Eco. Bot. 6: 69-73. 1952.

30. White, F. H. Varietal improvement 1935-1948. Cent. Exp. Farm, Ottowa, Canada. Tob. Div. Prog. Rep. 1950.

31. Wingard, S. A. The nature of disease resistance in plants, 1. Bot. Rev. 7: 59-109. 1941.

The vita has been removed from the scanned document