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INHERITANCE OF RESISTANCE TO
DROP (SCLEROTINIA SCLEROTIORUM) IN LETTUCE

presented by

Abdul Madjid

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INHERITANCE OF RESISTANCE TO DROP (*SCLEROTINIA SCLEROTIORUM*) IN LETTUCE

By

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ABSTRACT

INHERITANCE OF RESISTANCE TO
DROP (*SCLEROTINIA SCLEROTIORUM*) IN LETTUCE

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Using a large number of seedlings, it was possible to screen for resistance to lettuce drop caused by (*Sclerotinia sclerotiorum* under greenhouse conditions. Reliable results were obtained by placing ½ cm^2 of colonized agar block 1 cm from the base of 18 to 24 day old seedlings.

Since the degree of resistance among cultivars varied with the time of observation, resistance was measured by the number of days required to reach 50% mortality and by the number of plants that survived in each cultivar. Cultivars and accessions that were found significantly higher in level of resistance than Grand Rapids at 9, 12 and 15 days following innoculation were PI 250427, Taiwan, PI 251790, PI 255568, MSU 73-44 and Bibb.

A correlation coefficient of r =.77 significant at P = .001 was found between the plants grown in the greenhouse ground bed and those grown in flats, suggesting that similar results were obtained by both growing methods.

A study of inheritance of resistance to lettuce drop was made by using a large number of seedlings from various

generations involving nine crosses using the following parents: Grand Rapids, PI 250427, PI 278110, and 4 Taiwan strains. Variation in the time required to reach 50% mortality was observed to be primarily due to environmental effects, although low genetic variation for resistance was noted based on (1) variation in survival percentage as observed in the parents, and (2) genetic gain that was evident in the F_3 generation when a large number of lines were sampled; and no gain was observed when a low number of lines were sampled in the F_4 generation. These results suggest that resistance to lettuce drop in the cultivars studied was polygenically controlled with low level of resistance.

The possibility of obtaining a greenhouse cultivar resistant to lettuce drop is discussed.

DEDICATION

To my wife, Lilis Isminatun, for her understanding and encouragement, and

To my children Winarto, Yulia, Dedi, Suzy and Dewi, and

To my niece, Yati,
for their understanding

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INTRODUCTION

Lettuce drop is a disease of greenhouse and field lettuce caused by Sclerotinia sclerotiorum (Libert) de Bary. The fungus attacks various other species of crops and weeds in the field, and also causes decay in transported and stored lettuce.

The fungus attacks the lettuce at the base of the plant, girdling the stem and causing the plant to collapse or drop. The fungus survives in a dormant state as sclerotia, which are able to withstand extreme variation in environmental conditions. Sclerotia under cool and moist conditions may germinate, either forming mycelia or apothecia which release ascospores. Ascospores may be the source of primary infection in the spring. The wide host range of Sclerotinia, its ability to grow saprophytically, and the ability of the sclerotia to survive long periods of time under extreme environmental conditions, help make lettuce drop an important disease of lettuce.

Several control measures have been attempted (flooding the field, crop rotation, chemical control, etc.) with varying degrees of success. Field resistance was reported on several accessions of lettuce (Newton and Sequeira, 1972b). Under greenhouse conditions, however, these accessions were found

to be susceptible. Knowledge on the inheritance of lettuce drop resistance would be valuable for breeding for disease resistance in greenhouse lettuce.

The purpose of this study was to determine a simple and reliable screening technique for lettuce drop and to study the inheritance of resistance to lettuce drop.

REVIEW OF LITERATURE

The Pathogen

The genus Sclerotinia was originally established by Fuckel in 1870 covering 5 species, 4 of which are known to be important as a plant pathogen (Korf and Dumont, 1972). A new generic name Whetzelinia sclerotiorum (Libert) Korf and Dumont (Korf and Dumont, 1972) was recently proposed for Sclerotinia sclerotiorum (Libert) de Bary. A proposal to preserve the previous generic name, however, has been accepted by the International Association of Plant Taxonomists (Kohn, 1979), and therefore the generic name Sclerotinia is retained.

According to Adams et al., (1974) the fungus attacks 190 crop plants and weed species in 130 genera and 45 families of plants. Schwartz (Schwartz et al.,1978) mentioned a host range covering 374 species of 237 genera in 62 plant families. In vegetable crops, Chupp and Sherf (1960) listed 53 crops which are attacked by the disease. They also listed the different common names of the pathogen when it infests various hosts. The common name for the disease on lettuce is lettuce drop; it is less commonly known as collar rot.

The rot begins at the stem near the soil surface and spreads downward towards the roots. The peticles decay,

starting from the point of attachment to the stem upward, causing the leaves to drop. The fungus then spreads over all collapsed leaves. Cottony mycelia covers the decayed plant parts. The sclerotial initials were white, the sclerotia then become brown and finally black.

The fungus is known to be adaptable to a wide range of environmental conditions. The most favorable temperature for infection in lettuce is about 10-25 C (Abawi and Grogan, 1979), but the optimal growth *in vitro* ranges from 20 C (Tanrikut and Vaughan, 1951) to 25 C (Bedi, 1962).

The fungus grows best under abundant moisture from rain, fog, or sprinkler irrigation (Chupp and Sherf, 1960). Prolonged soil flooding, however, kills the sclerotia (Moore, 1949).

Primary infection by *S. sclerotiorum* results from infection by ascospores produced by apothecia (Natty, 1971; Newton and Sequeira, 1972a) and mycelia from sclerotia. Under natural conditions at temperatures of 9-17 C for 14 days, apothecia often arise from sclerotia (Letham *et al.*, 1976). Under natural conditions, plant canopy characteristics were found to have direct effects on the types of inoculum formed. In cauliflower or lettuce, the canopy was dense which prevented the drying of the soil surface. This condition resulted in the inhibition of germination of sclerotia to produce mycelium, but maintained suitable conditions for apothecia formation. On the other hand, tomatoes trained on trellies left the soil exposed and dry, which stimulated the sclerotia to produce mycelium.

The apothecia remain functional (unshriveled) for about 7 days after germination (Schwartz and Steadman, 1978). A sclerotium under natural conditions produces an average of 2 apothecia. Ascospore spreading is by wind or in the case of rape seed (Stelfox et al., 1978) was reported to be spread by bees.

When ascospores landed on susceptible plant parts, infection occurred within two days and symptoms appeared in four days (Chupp and Sherf, 1960).

Secondary infection of S. sclerotiorum may occur from mycelia grown from sclerotia and from mycelium fragmentation. Adams and Tate (1976) showed that eight weeks after placing the sclerotia on the soil, more than 22% of sclerotia germinated. Only two days after germination, the mycelium infected the hosts. In the case of S. sclerotiorum minor, the authors found the mycelium directly attacked the lettuce plants without first colonizing organic matter.

The formation of sclerotia by the fungus was apparently affected by temperature as demonstrated by Bedi (1962). Potato Dextrose Agar (PDA) cultures of the fungus produce sclerotia slower and there are fewer sclerotia formed at low temperatures. At a temperature of 25 C sclerotia are formed in four days. Studies by Smith (1972) showed that the sclerotia could be induced to produce mycelia by alternate drying and rewetting of the sclerotia.

The sclerotia have been known as a potential source of inoculum in soil. The survivability depends on:

(a) Weather conditions in summer; dry summers being favorable for survival.

(b) Cropping practices; cropping with susceptible crops increases the number of the potential inocula.

(c) Depth of sclerotia in the soil. Adams (1975) showed that sclerotia of S. sclerotiorum buried in the field at 1, 6 and 12 inch depths survived well over a 15 month period, but it survived poorly at a depth of 24 inches.

Plant debris in the field under favorable conditions (cool and moist) can be covered by lettuce drop mycelia within 5 days. Tanrikut and Vaughan (1951), supported by Purdy and Grogan (1954), postulate that since S. sclerotiorum is able to grow in limited substrates, it can live for a long period as a saprophyte. This extreme adaptability and habit of producing tough, resistant sclerotia indicate that S. sclerotiorum is a disease that is difficult to control by growing nonsusceptible crops in a rotation.

Screening Techniques

1. Age of Plants

The effect of age on the susceptibility of lettuce plants to drop has not been carefully studied. Different authors tested various ages of seedlings with several inocula. Adams and Tate (1976) infected 2 week old transplanted seedlings of lettuce of sclerotia of S. sclerotiorum minor. Six

percent of the sclerotia were found to germinate and cause infection. Using mycelia in agar blocks, Newton and Sequeira (1972b) found that inoculating 28 day old plants in the greenhouse killed all of the seedlings.

The effect of stage of growth on infection by ascospores in bean is remarkable. Ascospore inoculation has been successful when applied to blossoms, but not to leaves (Abawi, et al., 1975).

2. Type of Inocula

Inocula used to study pathogenicity of S. sclerotiorum have been either sclerotia, mycelia or ascospores. The use of sclerotia on lettuce by Adams and Tate (1976) has been referred to earlier. These authors found that germination of sclerotia were observed only on the small sclerotial isolates, but not on the large isolates. Adams (1975) reported that using sclerotia produced an oat seed media gave 90% infestation on Romaine and head lettuce (Mesa 659), but did not infest escarole (Cichorium endivia). Abawi and Grogan (1975) demonstrated that in bean, mycelium from sclerotia infected bean readily when an available source of energy such as dead or senescent tissues were found in direct contact between sclerotia and the bean stem. The need of the presence of non-living organic matter to initiate infection by mycelia from sclerotia was confirmed in lettuce study (Purdy and Grogan, 1952). No infection was observed when dead lower leaves were removed, but infection occurred when

sclerotia were placed at 6 mm from the stem and covered with dead leaves which touched the stem. These findings suggest that sclerotia do not provide sufficient nutrient for mycelial development in plant.

Using a 1.0 cm diameter agar blocks of PDA culture or infected oat kernel placed near the base of the plants, Newton and Sequeira (1972b) found that 2 to 3 seedlings of each field resistant line of lettuce died within 2 to 4 days when inoculated with colonized oat seeds or agar blocks in the greenhouse. However, field inoculation with residue of the infected plants resulted no infection in the field for the lines PI 184787, PI 187239, PI 250427, PI 251790 and PI 255568. When mycelial inoculation was done in the field, all lines died except PI 184787, PI 165063, PI 250427, PI 250429 and PI 255568. The difference in the results was probably due to succulence, low fiber content and undetermined factors found in plants grown in the greenhouse.

Kreitlow (1951) working with Ladino clover did not find satisfactory results by scattering pieces of agar culture on the soil among the plants grown in flats. The infection was not uniform and the agar was quickly overgrown by contaminants.

The use of grain inoculum in studying pathogenicity of S. sclerotiorum on several species of plants was reported by Price and Colhoun (1975b). Using S. trifoliorum, Kreitlow

(1951) obtained good results in the field and greenhouse tests by applying moist infected grain to clover. Susceptible plants in the greenhouse were killed in 5 to 15 days following the inoculation. The disadvantage of applying moist infected grain was the wet grain tended to lump and was difficult to apply evenly to individual seelings.

Mycelia of S. trifoliorum inoculated by spraying was studied by Frandsen (1946) on pasture legumes. The suspension was prepared by mixing the culture with clover decoction. One dosage consisted of 5 plates of mycelia 5-6 cm diameter suspended in 1 liter of clover decoction for 8 flats of 44 x 40x 15 cm. The number of dead plants found varied with the different concentration of inocula, viz. 83%, 79.5% and 68.5% with the concentration at 1½, 1 and ½ dosage, respectively.

S. sclerotiorum does not form conidia, it produces ascospores liberated from apothecia. According to Kreitlow (1951), it is difficult to secure adequate numbers of ascospores for large scale plant inoculations. The simplest technique in producing apothecia was reported by Bedi (1956). It consisted of floating the sclerotia in water for approximately 6 weeks at 15-20 C. But this technique, and several other techniques developed by different authors, when tested by Price and Colhoun (1975a) did not result in mature apothecia formation, although stipes were formed. In addition, since the viability of ascospores is limited to 4 days

(Newton and Sequeira, 1972a), a continuous supply of mature apothecia would be needed during the inoculation studies.

3. Optimum Time for Cultivar Evaluation

Infection of lettuce plants inoculated with sclerotia of S. sclerotiorum begins 2 days after inoculation (Adams and Tate, 1976). The percentage of plants infected increased and reached its maximum 14 days following inoculation, and remained the same when observation was ceased 22 days following inoculation.

In several generations of tomato crosses which were assessed for resistance to tobacco mosaic virus, the degree of resistance changed with the age of the plants (Phillip et al., 1965). Similar results were found in evaluating host resistance to Stewart's disease (Erwinia stewartii) in corn (Blanco et al., 1979). From data collected at 3 different times of observation, the authors were able to differentiate lines contributing genes for suppressed disease development.

Disease Resistance

Literature on the resistance to lettuce drop is limited. A lettuce cultivar grown in Europe was mentioned by Chupp and Sherf (1960) as almost completely immune to drop. Fifteen cultivars with a high level of field resistance were observed among 125 cultivars tested during 2 summer seasons by Elia and Piglionica (Newton and Sequeira, 1972b). The

resistance was associated with red pigmentation and leaf type of the cultivars.

In field evaluation of 178 lettuce accessions, Newton and Sequeira (1972b) found 21 lines with varying degrees of field resistance. Five accessions (PI 184787, PI 165063, PI 250427, PI 250429 and PI 255568) were found to be most resistant. The nature of resistance was associated with the structure of the plant. The erect type of plant with its open foliage prevented the build up of high relative humidity favorable to disease infestation, while in butterhead and leaf types the resistance was postulated as caused by less wounding by wind blown particles. Wounding has been considered to predispose lettuce to ascospore infection. The resistance,however, was less when the plants were tested under greenhouse conditions, probably due to more succulence, less fiber content, etc. Based on the breeding tests, the authors believed that the field resistance was genetically inherited; however, no genetic ratio of resistance: susceptibility was reported.

Resistance to S. sclerotiorum associated with plant structure was also found on bean (Anderson et al. 1960; Coyne et al., 1977; and Schwartz et al., 1978). A low level of disease incidence was found in cultivars with an open upright plant habit. Plants with indeterminate habit were likely to be susceptible owing to the heavy canopy. Among the determinate plants, cultivars with a dense leaf canopy

were susceptible. Coyne et al. (1977) found a high correlation between high yielding ability of GN Nebraska and susceptibility to white mold. This high yielding cultivar had a heavy canopy, causing lack of air circulation and light penetration.

Dow and Lumsden (1975) found differences in the nature of infection between resistant Scarlet Runner bean lines (P.coccineus) and susceptible P. vulgaris. The resistant tissues acted as a physical barrier for the rapid penetration of the pathogen. Such a barrier was not found in the susceptible tissues of P. vulgaris. Inheritance studies of the resistance showed that the resistance was governed by a single dominant gene (Abawi et al., 1978), assigned the gene symbol Ws.

Penetration

Interpreting genetic data may be complicated by incomplete penetrance of a character. As defined by Allard (1960), penetrance is the ability of a gene to be repressed in individuals carrying it. The phenomenon was demonstrated in deficient chlorophyll in the tip and leaf margins of unifoliate leaved of lima bean cv. Ventura. Deficiency of chlorophyll is a dominant character, but even in the homozygous condition it is rarely found in more than at 10% frequency. The penetrance is approximately 10%. It was reported that under certain environmental conditions the penetrance is complete, while in another condition is 0.

In mosquito (Aedes aegypti L.), inheritance studies with the gene Gold affecting mesonotal scale color were inconclusive until Klassen (1964) showed the existence of varying degrees of penetrance for the gene in male and female insects.

Incomplete penetrance was suggested in the resistance to Fusarium wilt race I in tomate (Retig et al., 1967). In a backcross with the susceptible parent, 150 healthy: 170 diseased plants were found, suggested 90.6% penetrance in the F_1.

In the study of resistance to internal browning in tomato, Phillip (1964) found incomplete penetrance in the cultivar Fireball. Resistance was suggested as caused by the interaction of 2 genes, and the level of penetrance was dependent upon the genotype of the plant.

PART I: SCREENING TECHNIQUES

MATERIALS AND METHODS

Plant Materials

Ten accessions were utilized in evaluating variable response due to seedling age while 16 accessions were used in evaluating the various inoculation techniques. Under field conditions, PI 165063, PI 184787, PI 187239, PI 250427, PI 250429 and PI 255568 were described as resistant (Table 1), while PI 206965 and PI 251790 were described as tolerant to lettuce drop by Newton and Sequeira (1972b). All Plant Introduction accessions were obtained from the Western Regional Plant Introduction Station, Pullman, Washington. The six cultivars tested represent the four types of lettuce, Lactuca sativa, (leaf, Romaine, butterhead and head types). The seeds of these cultivars were obtained from Joseph Harris Company, Inc., Rochester, New York.

To obtain uniform germination, the seeds were sown in the evening in ½ cm deep furrows of moist vermiculite in clay pots. Next morning, the seeds were thinly covered with moist vermiculite. The seedlings were transplanted at the cotyledon leaf stage 7 to 10 days after sowing into sterilized soil in flats. The planting distance was 5 x 5 cm,

TABLE 1: LETTUCE CULTIVARS AND ACCESSIONS USED IN THE STUDY OF SCREENING TECHNIQUES

Cultivars	Origin	Resistance to Drop*	Type	Leaf Color	Source
1. PI 165063	Turkey	R(27)	Romaine	Green	W-6 Regional
2. PI 184787	Netherlands	R-27)	Bibb	Yellow	P.I. Pullman
3. PI 187239	Belgium	R-27)	Bibb	Yellow	"
4. PI 206965	Turkey	T(27)	Leaf	Lt.Green	"
5. PI 250427	Czeckoslo-vakia	R(27)	Leaf	Green	"
6. PI 250429	Czeckoslo-vakia	R(27)	Bibb	Yellow	"
7. PI 251790	Yugoslavia	T(27)	Leaf	Lt. Green	"
8. PI 255568	Yugoslavia	R(27)	Romaine	Green	"
9. Taiwan	Netherlands	N	Leaf	Green	Dept. Hort, MSU
10. MSU 73-44	MSU	N	Head	Green	"
11. Grand Rapids	(Cultivar)	S	Leaf	Yellow	Joseph Harris,NY
12. Ithaca	(cultivar)	S	Head	Lt. Green	"
13. Valmaine	(Cultivar)	S	Romaine	Green	"
14. Bibb	(Cultivar)	S	Bibb	Yellow	"
15. Parris Island	(Cultivar)	S(5)	Bibb	Yellow	"
16. Mesa 659	(Cultivar)	S(5)	Head	Green	"

*) R= resistant; T = tolerant; S= susceptible; N = not available.

numbers in parentheses show source of reference, correspond to numbers in the "References Cited".

70 seedlings per flat. Plants were fertilized 7 and 21 days after transplanting with a solution of 2 g of 20-20-20 (N-P-K) soluble fertilizer per liter of water.

Age of Plants

In preliminary studies, 140 seedlings from each of 4 cultivars were inoculated as 30 day old plants, died in 7 days following inoculation. When the same cultivars were inoculated at 3 weeks of age a variation in the age at which the plants succumbed to disease was observed. To learn the optimum age for inoculation, plants were inoculated at 18 and 24 days after transplanting.

Types of Inocula

The culture of S. sclerotiorum used in this study was isolated from lettuce and obtained from Mr. David Willis of the Department of Botany and Plant Pathology, Michigan State University. The inoculum was cultured on potato dextrose agar (PDA) medium and incubated at 20-25 C for 5 days prior to its use.

The types of inocula used were: (1) agar block colonized with mycelia, hereafter referred to as agar block, (2) mycelial suspension applied as a spray (3) sclerotia.

The agar block inoculation technique was adapted from that described by Newton and Sequeira (1972b), by placing either 1 cm^2 or ½ cm^2 blocks of agar, 1 cm from the base of

the plant. The mycelial suspensions were prepared by blending 200 ml of water with the equivalent 1 cm^2 or ½ cm^2 of agar block per plant with a blender. One and a half petri dishes of PDA culture were used for 70 plants which approximated 1 cm^2 of agar block per plant. Mycelial suspensions using cultures equivalent to 1 cm^2 of agar block per plant will be referred to as full dosage concentration, while that equal to using ½ cm^2 of agar block per plant will be referred to as a half dosage concentration.

Sclerotia used were obtained from 10 day old agar cultures. The size of sclerotia averaged 3 mm in diameter. The sclerotia were mixed into the soil, 20 sclerotia per flat.

The experiments were conducted in the greenhouse in a completely randomized design with 2-10 replicates, and 4-7 seedlings per plot. The total number of seedlings included in the inoculation studies were 1960 plants.

Time of Evaluation

To study the optimum time for cultivar evaluation, plant stand was recorded every day starting from the sixth day following inoculation and was terminated on the 18th day. A plant was considered as dead when it collapsed or the stem was girdled (Figure 1). A total of 512 seedlings from 16 lines were observed in this test.

Figure 1: Resistance and collapsed susceptible plants, 15 days after inoculation.

Relationship Between Bed and Flat Culture

To study the relationship between data obtained from experiments conducted in the greenhouse ground bed and those made in the flats, F_2 populations from 5 families were used. These families were derived from crosses between Grand Rapids x PI 250427 and Grand Rapids x 4 Taiwan lines. The F_2 seedlings grown in the greenhouse bed were part of an experiment conducted to study inheritance of resistance to lettuce drop. Materials grown in the flats were grown in the same houses as the greenhouse bed.

Seeds for both experiments were germinated in moist vermiculite in the greenhouse. Ten-day-old seedlings were transplanted to 5 x 5 cm peat pots containing artificial medium. The pots were placed in flats, 70 pots per flat. Fertilizer was given 3 and 17 days after transplanting by applying a solution of 2 g of 20-20-20 (N-P-K) soluble fertilizer per liter of water. The plot in the flats consisted of 28 seedlings per line, while those in the greenhouse ground bed contained 50 seedlings. Each plot in both planting methods was replicated 3 times in a completely randomized block design. A total of 420 and 750 seedlings respectively were planted from the experiments in the flat and in the bed. Data on the percentage of dead plants were taken 10 days after inoculation.

Statistical Interpretation

An analysis of variance was carried out for data on age of plants, techniques of inoculation and the optimum time for cultivar evaluation. Regression analysis was conducted for cumulative mortality recorded at five observation dates following the inoculation of 16 cultivars. Homogeneity test for regression coefficient was made following the procedure described by Steel and Torrie (1960) (Table 2). The number of days following inoculation when 50% of plants succumbed was estimated using the following linear regression formula:

$$y = a + bx, \text{ hence } x = (y - a)/b$$

where x = the number of days when 50% of the plants died,

y = 50% of the total number of the plants that died at the final count,

a = intercept of the linear regression,

b = slope of the linear regression.

Differences in the percent survivors of cultivars tested were analyzed using Tukey's test for significant difference.

TABLE 2: STATISTICS UTILIZED TO CALCULATE HOMOGENEITY TEST OF REGRESSION COEFFICIENT (ADAPTED FROM STEEL AND TORRIE, 1960).

Cultivars	n	b	a	SSX	SSY	SSXY (=b x SSX)	Reduced SS (=SSY - $(SSXY)^2$ /SSX)
PI 165063	.	.	.	.	.	.	.
. .	.	.	.	.	.	.	.
Mesa 659	.	.	.	.	.	.	.
Total(T)	TN			TSSX	TSSY	TSSXY	T Reduced SS (=A)

$B = TSSY - (TSSXY)^2/TSSX$

Test for homogeneity $F = ((B-A)/(cv-1))/((A/(Tn-2cv))$, with df $=(cv-1)$ and $(Tn-2cv)$

RESULTS AND DISCUSSION

1. Effect of Age of Seedlings on Disease Incidence

In an earlier study utilizing 140 seedlings from each of 4 cultivars, 30 day old plants grown in flats succumbed a week after inoculation. Further observations with the same materials showed that when 3-week-old plants were inoculated, it took a longer period before the plants died. The difference in the length of time for the plants to die was probably due to the dense canopy found in the 30-day-old plants which created a condition more favorable for the rapid development of the fungus.

The optimum seedling age for inoculation was found to be at 18 to 24 days (Table 2). The plants inoculated at 18 days of age showed 69% mortality, while those inoculated at the age of 24 days had 66% mortality.

2. Types of Inocula

a. Sclerotia

When sclerotia was used as inocula, none of the plants died (Table 3), although more than 60% of plants inoculated with mycelium died. The results were observed 10 days after inoculation, which probably was too early, Rudorf (1937) found that under favorable conditions, infestation through

TABLE 3: THE EFFECTS OF SEEDLING AGE AND INOCULATION TECHNIQUE ON PERCENT SEEDLING MORTALITY AT 10 DAYS AFTER INOCULATION

Inoculation Technique	Seedling Age (Days)	
	18	24
	Percent Seedling Mortality	
Control	0	0
Sclerotia	0	0
1 cm^2 agar block	65	65
full dosage spray[1]	69	73
half dosage spray	75	61

no significant difference between ages of plants, and between agar block and spraying technique.

[1]equal to 1 cm^2 agar block culture per plant

sclerotial inoculation was obtained in three weeks. Kreitlow (1951) did not obtain infection with this method although the environmental conditions were favorable for the growth of the mycelium. According to Adams and Tate (1976), dormancy sclerotia of each isolate varied. Therefore, it was assumed that the differences in Rudorf and Kreitlow results may be due to differences in length of dormancy period among the isolates used in the studies. Since no attempt was made to use sclerotia from other isolates, the effect of dormancy period could not be confirmed.

Results of this study suggested that the use of sclerotia was not satisfactory for evaluating disease resistance.

b. Mycelial suspensions and agar blocks

No significant differences were found between the use of agar block and the mycelial suspension spray, and between the size of agar block. Applying full dosage spray was not statistically different with that of half dosage (Table 3). However, in the second experiment (Table 4), these dosages spray were found significantly different at the 5% level (Table 5). The inconsistency of the results of spraying may have been due to the lowering of the inoculum potential.

Although placing the blocks individually next to the plants required more time, the agar block technique was considered as more reliable for screening lettuce cultivars than the spray technique, because the technique insured the same amount of inoculum given.

3. The Optimum Time for Cultivar Evaluation

Tests for homogeneity of the regression coefficients for cumulative mortality at 5 different dates following inoculation for the 16 cultivars did not show significant differences (P 10%). The slopes of regression lines for all the cultivars were similar (Table 6). Cultivar differences were found in the intercept (a) values, suggesting variation in resistance can be measured by differences when the plants from each cultivar died. The number of days when 50% of the plants died for the 16 cultivars ranged from 8.4 to 12.7 days (Table 7). In most of the cultivars this value was between 9 and 12 days and in PI 250427 and Taiwan it exceeded 12 days.

TABLE 4: THE EFFECT OF THE INOCULATION TECHNIQUE ON PERCENT OF MORTALITY

Inoculation Technique	Percent Mortality
1 cm^2 agar block per plant	50
0.5 cm^2 agar block per plant	49
full dosage spray[1]	53
half dosage spray	43[2]

[1] equal to 1 cm^2 agar block culture per plant

[2] significantly different at the 5% level from the full dosage spray (LSD .05 = 9%).

TABLE 5: VARIANCE ANALYSIS FOR THE EFFECTS OF INOCULATION TECHNIQUES

Source of Variance	df	ms	F
Total	19		
Time	1	47.74	1.96
Blocks	3	114.50	4.69*
Treatments	3	103.80	4.25*
Error	12	24.41	

TABLE 6: SLOPES (b), INTERCEPTS (a) AND CORRELATION COEFFICIENTS (r) FOR CUMULATIVE NUMBER OF MORTALITY OF LETTUCE PLANTS AT 5 OBSERVATIONS AFTER INOCULATION

Cultivars	Slopes(b)	Intercepts (a)	Correlation Coefficient(r)
PI Accessions:			
PI 165063	1.63	-5	.97
PI 184787	1.93	-1.8	.92
PI 187239	1.73	- .6	.95
PI 206965	1.73	-1.4	.98
PI 250427	1.97	-11.8	.99
PI 250427	1.93	-4.8	.96
PI 251790	1.4	-5	.99
PI 255568	1.7	-7.4	.97
MSU Breeding Lines:			
Taiwan	1.7	-11.6	.99
MSU 73-44	1.83	-9	.98
Cultivars:			
Grand Rapids	2.37	-11	.95
Ithaca	1.9	-7	.97
Valmaine Cos	1.8	-3.2	.94
Bibb	1.67	-7.8	.98
Parris Island	1.97	-7.4	.98
Mesa 659	2	-4.4	.94

TABLE 7: NUMBER OF DAYS WHEN 50% OF PLANTS DIED AND SURVIVAL PERCENTAGE AT 15 DAYS AFTER INOCULATION FOR 16 CULTIVARS

Cultivars	Number of Days for 50% mortality	Survivors*) (%)
PI accessions:		
PI 165063	10.4	44c
PI 184787	8.7	16a
PI 187239	8.4	19ab
PI 206965	9.2	25ab
PI 250427	12.1	50e
PI 250429	9.7	25ab
PI 251790	10.7	50e
PI 255568	11.1	47de
MSU Breeding Lines:		
Taiwan	12.7	60e
MSU 73-44	11.5	44c
Cultivars:		
Grand Rapids	10.5	19ab
Ithaca	10.3	32bcd
Valmaine Cos	9.0	22ab
Bibb	11.3	50e
Parris Island	10.4	28abc
Mesa 659	9.5	21ab

*) Percentage within column followed by the same letter not significantly different at the 5% level (Tukey's test).

If the degree of cultivar resistance was measured from the data obtained at the 12th day, one cannot measure the degree of resistance of the cultivars that survived beyond the 12th day. This is evident since a small number of plants died at the 15th and 18th day (Appendix Table A-1). Since resistance is measured from inoculation to death, evaluating resistance should be based on the greatest number of days when 50% of plants succumbed between cultivars. The greatest number of days for 50% mortality was 12.7 (Table 6); therefore evaluation of susceptibility was based on the cumulative number of survivors up to the 15th day following inoculation. Cultivars showing significant differences ($P = 0.05$) compared to Grand Rapids at 15 days following inoculation were PI 250427, PI 251790, PI 255568, Taiwan, MSU 73-44 and Bibb (Table 7). Cultivars showing significant differences from Grand Rapids from the second observation to the fourth observation were PI 250427 and Taiwan (Appendix Table A-1).

Experiment in the Greenhouse Ground Bed vs. Using Flats

Results of the study on the relationship between greenhouse ground bed vs. flats showed a correlation coefficient of $r = .77$ (Table 8), suggests that the results from growing in the flats were similar to those in the ground beds.

TABLE 8: CORRELATION BETWEEN PERCENTAGES OF SURVIVING PLANTS GROWN IN THE GREENHOUSE GROUND BED AND THOSE GROWN IN FLATS IN THE SAME GREENHOUSE

F_2 Families	Greenhouse Bed Replicates 1	2	3	Flats Replicates 1	2	3
Grand Rapids x PI 250427	52	38	50	57	61	57
Grand Rapids x Taiwan-1	53	70	46	89	96	54
Grand Rapids x Taiwan-2	64	60	48	100	64	75
Grand Rapids x Taiwan-3	68	80	66	96	100	82
Grand Rapids x Taiwan-4	56	64	60	86	89	89

r= .77***, P = .001.

CONCLUSIONS

Age of Seedlings

In a study carried out by Newton and Sequeira (1972b) with PI lines, resistant to Sclerotinia in the field, 2 to 3 plants of each line grown in 15 cm clay pots died within 2 to 4 days when inoculated in the greenhouse at the age of 28 days.

Preliminary studies were made in the greenhouse in winter 1978 using 140 30 day-old seedlings of each of the 4 cultivars. Seedlings were grown at 5 x 5 cm in flats. All plants died one week following inoculation using 1 cm^2 agar block. When the test was repeated using 3 week old seedlings, there was a variation in time when each of the plants died. The difference between the results obtained between these two plant ages was probably due to the dense canopy noted in the 30 day old seedlings, which provided a more favorable environment at the base of the plants for mycelial development. Such a condition could also have happened with the materials studied by Newton and Sequeira. These authors suggested that under greenhouse conditions, the PI lines lost their tolerance to lettuce drop. No test at different ages was made by these authors.

In preliminary studies using a large number of seedlings inoculated at the 21st and 30th day after transplanting

suggest that screening for susceptibility to lettuce drop in the greenhouse is possible by inoculating plants less than 30 days old.

When plants were inoculated made using 350 seedlings of 18 and 24 day old plants, no significant difference at the 5% level was noted in mortality for the two age groups. Although statistically not significant, plants incoulated at the 18th day after transplanting showed more dead plants. Mellinger (1968) reported similar observations working with S. sclerotiorum on potato.

It appears that the best age for screening for lettuce drop in the greenhouse is 18 to 24 day old plants.

Type of Incoula

Results using sclerotia as an inoculum were not satisfactory since it required a longer period (3 to 6 weeks) to obtain infection (Rudorf, 1937), and infection was dependent upon the presence of non-living organic matter which was necessary for the mycelia from sclerotia to initiate infection (Purdy and Grogan, 1952). Although 268 seedings were used in this study, no infection was observed 10 days following inoculation on plants grown in sandy soil low in organic matter.

The use of mycelium on agar plugs was reported effective in inoculating lettuce grown in the greenhouse (Newton and Sequeira, 1972b). One centimeter diameter agar

plug was placed at the base of each plant. In this study, 1/2 cm^2 or 1 cm^3 of agar block was placed 1 cm from the base of each plant. Both of the blocks were effective. It is suggested 1/2 cm^2 of agar block can be used for screening lettuce for disease resistance.

Mycelium as a suspension spray to a concentration equal to 1/2cm^2 of agar block per plant gave inconsistent results. This is probably due to the lowering of the inoculum potential.

Although the placing of agar blocks is time consuming, it was found to be the best method for screening resistance to drop.

Optimum Time to Evaluate Cultivars

Since the degree of susceptibility among cultivars to lettuce drop varied with time, it was necessary to establish the optimum time to evaluate cultivars. Resistance was measured by the cultivar's delay in succumbing to the disease. This is shown by number of days required for the cultivars to reach 50% mortality.

When this criterion was applied to the 16 PI accessions and cultivars (Table 7), it was noted that the highest number of days for 50% mortality was 12.7. Therefore, differences in susceptibility among cultivars were compared using cumulative mortality data up to the 15th day following inoculation. Statistical analysis showed that Grand Rapids, a cultivar grown in Michigan greenhouses, was one of the most susceptible. Cultivars found significantly different at the

5% level compared with Grand Rapids were PI 250427, Taiwan, PI 251790, PI 165063, PI 255568, Bibb and MSU 73-44.

Greenhouse Ground Bed vs. Flats Culture

A correlation coefficient of r = .77 was found between the results of planting in the greenhouse bed and those planted in flats. This r value was significant at P = .001 suggesting that the results obtained by growing in the soil bed and flats were closely related.

PART II. RESISTANCE TO LETTUCE DROP

MATERIALS AND METHODS

1. Parental materials

Since it is difficult to obtain 100 percent hybridization in lettuce due to the nature of the reproductive mechanism, variable amounts of selfed progenies occur in the female parents when hybridized. Since the likelihood of self progenies occurring in the population is high, leaf color was used as a genetic marker. The green leaf trait, which is monogenic dominant to yellow-green leaf, was used as the male parents. No reciprocal crosses was made, since it would be difficult to distinguish the hybrids from the selfs if the green leaf color parent was used as the seed parent.

The resistant parents selected for the genetic studies were derived from cultivars studied in the previous section (Figure 2), viz.:

-PI 250427, and 4 selections from the cultivar Taiwan, designated as Taiwan-1, Taiwan-2, Taiwan-3, and Taiwan-4.

All the parental material was allowed to self pollinate.

PI 250427 had a dense green foliage. The leaves were oval, with a length to wide ratio of 3:1. The leaf margins

were serrate, and the leaf blades were slightly crinkled.

The 4 selections derived from the cultivar Taiwan were morphologically similar. The leaf form was oval, with a length to width ratio of 3:1 and leaf color was dark green.

The susceptible parents for these studies were the cultivars Grand Rapids and PI 278110 (Figure 2).

The cultivar Grand Rapids is a leaf lettuce grown commercially in greenhouses in Michigan. The length-to-width ratio of leaves is 2:1, shorter than the other cultivars. The leaves are yellow-green with serrate margins. The cultivar Grand Rapids is late flowering and therefore bolting was induced by spraying 20 ppm gibberellic acid when the plants were at the 5 leaf stage.

PI 278110 is a butterhead lettuce which has yellow-green leaves with a smooth leaf margin.

2. Pollination

Pollination was done in the greenhouse, using a technique developed by Oliver (1910) and described by Newton and Sequeira (1972b). The petals of unopen flowers were cut with scissors approximately 2 cm from the tip. When the pollen laden stigma protruded a few hours later, it was washed by a jet stream of water. After the flower dried, they were pollinated. One male flower was used to pollinate 1-3 female flowers. Seeds matured approximately 12 days after pollination. Hybridization was made in April, while backcrossing and selfing were in August and early September, 1979.

Figure 2: Parental materials: Top left: PI 278110 (susceptible); Top Right: PI 250427 (resistant); Bottom left: Grand Rapids (susceptible); Bottom Right: Taiwan (resistant)

One plant of PI 250427 and one plant from each of the 4 Taiwan cultivars were allowed to self to obtain parental seeds. F_2 seeds were obtained by allowing the F_1 plants to self.

A random sample of alleged resistant plants showing Grand Rapids phenotype from the F_2 population were selfed for the F_3 generation (Table 9).

The F_2 population was obtained by selfing randomly selected alleged resistant plants from the F_3 population.

3. Screening for Disease Resistance

Seeds were sown in moist vermiculite in flats in the greenhouse. Seedlings were transplanted 7-10 days after sowing into 5 x 5 cm peat pots containing artificial mixture medium. Seventy peat pots were placed per flat. Liquid fertilizer was applied 3 and 17 days following transplanting with 2 g of 20-20-20 (N-P-K) soluble fertilizer per liter of water.

The greenhouse soil bed was sterilized prior to growing a standard lettuce crop. Following harvest the bed was used to grow the experimental materials. The soil was tilled 20 cm deep prior to planting. Tilling was repeated after the third planting. The soil was leveled using a hand rake before planting.

S. sclerotiorum was grown at 20 C for 5 days on potato dextrose agar (PDA) medium prior to inoculation. Plants were

TABLE 9: NUMBER OF THE F_3 LINES SCREENED FOR RESISTANCE TO LETTUCE DROP

Parentage	Number of Lines
Grand Rapids x PI 250427	10
Grand Rapids x Taiwan-1	20
Grand Rapids x Taiwan-2	27
Grand Rapids x Taiwan-3	24
Grand Rapids x Taiwan-4	19
PI 278110 x PI 250427	8
PI 278110 x Taiwan-1	9
PI 278110 x Taiwan-2	11
PI 278110 x Taiwan-3	14

inoculated by placing a 1/2 cm^2 agar block culture 1 cm from the base of the 3-week-old seedlings one day prior to transplanting into the greenhouse ground bed. The planting distance in the bed was 15 x 15 cm.

The experiments were in a completely randomized block design and replicated 3 times. The randomization was done within and between families for each of the plantings. Numbers of plants used in the experiments for each replicate were 10 plants for P_1, P_2 and F_1, 30 plants for the reciprocal backcrosses, and 50 plants for the F_2 population.

Total number of plants per family in each experiment were 30 plants for P_1, P_2 and F_1, 90 plants for reciprocal backcrosses, and 150 plants for the F_2 generation. The cultivars Grand Rapids and PI 278110 were used as guard rows. Crosses involving Grand Rapids were tested in 5 different plantings during the fall of 1979 and winter 1980, while that of PI 278110 was done once in March 1980. The air temperature and relative humidity were recorded using a hygrothermograph placed 20 cm above soil level. Taylor Weather-Hawk recording thermometer number 2354 (Taylor Instrument Companies, Rochester, N.Y) was used to record the soil temperature (Table 10). The temperature sensing bulb was inserted 7 cm in the ground bed. The relative humidity of the greenhouse was maintained at 80% ± 10%. The numbers of dead plants were recorded every 2 days starting from the 6th day and ending on the 18th day after transplanting.

4. Screening of the F_3 Population

Seedlings were grown in the greenhouse as described earlier for 19 days after transplanting, then transferred to the cold chamber at the Horticultural Research Farm. The temperature in the cold chamber was 14 C and the relative humidity was maintained at 80%±5%. Fluorescent lamps were used as the source of light.

Plants were inoculated when the seedlings were 21 days old using the similar technique used for the F_2 population.

TABLE 10: THE SOIL AND AIR TEMPERATURES IN THE GREENHOUSE DURING EACH OF THE SIX PLANTINGS

	1 Nov.1- Nov.20	2 Nov.24- Dec.17	3 Dec.22- Jan.11	4 Jan.19- Jan.29	5 Febr. 3- Febr.26	6 March 1- March 21
Soil Temperature (°C):						
Mean	14	12	13	16	14	16
Range	10-17	9-16	11-16	13-19	11-19	11-24
Air Temperature (°C):						
Mean	15	16	14	14	12	13
Range	11-28	11-28	11-26	10-24	9-24	11-23

The plot consisted of 10 seedlings for P_1 and P_2, and 20 seedlings for each line of F_2 and F_3, replicated 3 times in a completely randomized block design. A total of 3 plantings were made. Two plantings were materials hybridized with Grand Rapids, while the third was those hybridized with PI 278110. Numbers of dead plants were recorded every 2 days beginning after the 6th day. Data were reported as percent survivors.

5. Screening of the F_4 Population

F_4 seedlings were grown in the greenhouse similar to that described earlier. Twenty-one day old seeldings were inoculated using the method described for the F_2 and F_3 populations. The plot consisted of 7 seedlings for each P_1 and P_2, 14 seedlings for each F_2 and F_4, replicated 5 times in

a completely randomized design. Data were recorded every day following the 3rd day after inoculation.

6. Weighting of the Backcross Populations

Difficulty in obtaining complete control in hybridization of lettuce due to the nature of the reproductive mechanism made it necessary to formulate a weighting factor. Leaf color was used as a genetic market to identify the hybrids from the selfs. The magnitude of self pollination was found to be 30%. Prior to transplanting into the greenhouse bed, selfed seedlings were discarded from the F_1 populations. Since selfed seedlings were not discernable from the BC to P_1 (BC_1) to BC to P_2 (BC_2) seedlings, a weighting factor was formulated to remove the selfs from the crossed seedlings. The presence of incomplete penetrance in the resistance to lettuce drop was included in the weighting. The magnitude of this factor in the BC_1 is estimated from that of the P_1 population. The weighting formula for the BC_1 (Grand Rapids or PI 278110 x F_1) is as follows:

$R_w = R_o$ - number of seedlings grown in BC_1 population
x percentage of self-pollination
x percentage of penetrance in P_1

where R_w = weighted number of the resistant plants

R_o = observed number of the resistant plants

The weighting formula for BC_2 (F_1 X P_2) was formulated as follows:

Since the F_1 was used as the female parent, the penetration factor was based on the F_2 segregating generation.

$R_w = R_o$ - number of seedlings grown in BC_2 population
x percentage of self-pollination
x percentage of penetrance in F_2

7. Genetic Interpretation

Resistance to lettuce drop was manifested by differences in the length of time required for the plants to die and by differences in number of plants that survived on a given date for each cultivar. The resistant cultivars had a larger number of survivors at the greatest time to reach 50% mortality among cultivars. To establish the degree of resistance for the cultivars, the optimum time to evaluate the cultivars was based on the survivors from several observations.

The differences in the time to reach 50% mortality between the various generations were measured by using regression analysis. LSD test was applied to measure the significant differences in the number of survivors found between generations. The Chi-Square analysis for a 2 x 2 contingency table as described by Briggs and Knowles (1967) and Skory (1952) was applied to the parent materials in F_2 tests.

RESULTS AND DISCUSSION

Parental Materials

Grand Rapids seedlings were found to be the lowest in number of days following inoculation to reach 50% mortality among the parent lines tested (Table 11). The percentage survivors of Grand Rapids and PI 278110 were significantly lower ($P = 0.05$) than PI 250427 and the four Taiwan strains. This observation was similar to that reported in the previous section where 16 cultivars were tested. The results suggest that the four Taiwan strains possessed greater resistance to lettuce drop than Grand Rapids. In comparison to PI 250427 and the four Taiwan strains, PI 278110 showed a similarity in number when 50% of plants succumbed.

Six days after inoculation, 10% of the PI 278110 seedlings survived as compared to more than 20% for PI 250427 and the four Taiwan strains. At the final observation, only 5% of PI 278110 seedlings survived, while those of PI 250427 and the four Taiwan strains showed more than 20% survivors (Appendix Table A-2).

Pooling of the Data

Bartlett's test for homogeneity of the five plantings of Grand Rapids x PI 250427 and Grand Rapids x four Taiwan

TABLE 11: NUMBER OF DAYS AFTER INOCULATION TO REACH 50% MORTALITY AND PERCENT SURVIVORS ON THE 6TH DAY FOLLOWING INOCULATION OF THE PARENT LINES

Lines	Number of days to 50% mortality	Survivors 1) (%)
Grand Rapids	4.6	0a
PI 278110	5.3	10a
PI 250427	5.7	29b
Taiwan-1	5.8	21b
Taiwan-2	5.5	29b
Taiwan-3	5.0	21b
Taiwan-4	5.2	50b

1) Percentage within column followed by the same letter are not significantly different at the 5% level.

strains crosses did not show significant differences (P = 0.25 - 0.5), and therefore the data were pooled. The pooling of the data was based on the time of observation rather than the number of days after inoculation. The first observation was made, when approximately 10% of the seedlings of each planting died. Since the rate of mortality of each of the families was not similar, some of the families reached 50% mortality prior to the first observation. The number of days when 10% of the seedlings died varied from 5 to 8 days. In the first and second plantings it took 8 days, while in the fourth planting, 6 days, and in the third and fifth plantings, 5 days. Frequency distribution on the number of dead plants at each observation (Appendix Table A-3) showed that the four Taiwan strains in crosses with Grand Rapids and the three Taiwan strains in crosses with PI 278110 were not homogeneous.

Inheritance of Resistance

Grand Rapids Crosses

The time of observation for 50% mortality for the Grand Rapids cultivar showed negative values in four out of five cases, while the other parental cultivars two of the parents showed negative values (Table 12). The data suggest that Grand Rapids succumbed earlier than the other cultivars.

The negative values for the time of observation indiated that more than 50% mortality occurred prior to the first observation. The occurrence of negative values was

due to the fact that the observation was made when 10% of the plants in the entire experimental plots succumbed, rather than on the mortality of the susceptible parent in each of the families.

In the F_1 generations of Grand Rapids crosses, the time of observation for 50% mortality was less than one, suggesting that the 50% mortality occurred prior to the second observation. In three of the crosses, the time of observation for 50% mortality for the F_1 generation was greater than the parent mean.

The time for 50% mortality in the F_2 generations was found to be negative in four of the crosses, suggesting mortality in the F_2 generation was observed to be sooner than in the F_1 population.

In four of the crosses, the backcrosses to the supposedly resistant parents resulted in larger values for time for 50% mortality than those values for backcrosses to the supposedly susceptible parent.

The differences among the cultivars in survival percentage were small (Table 13). Grand Rapids, the supposedly susceptible cultivar at the final observation showed total survival percentage of 48 to 62 percent. The supposedly resistant cultivars had survival percentages ranging from 57 to 63 percent.

Survival percentages in the F_1 populations were intermediate in three crosses, and lower than either parent in two of the crosses. The survival percentages in the F_2 population

TABLE 12: TIME OF OBSERVATION TO REACH 50% MORTALITY FOR GRAND RAPIDS CROSSES

Generation	Other Parents				
	PI 250427	Taiwan-1	Taiwan-2	Taiwan-3	Taiwan-4
Grand Rapids	-.2	-.6	-1.7	.3	-1.8
Other Parents	.8	.1	- .8	.3	-4.0*)
F_1	.2	.2	.5	.3	.5
F_2	.6	-.7	-.7	-.7	-1.0
BC_1	1.6	-.1	.0	.4	-1.5
BC_2	.2	-2.7*)	-.1	-.7	-1.0

*) These figures did not measure the time of mortality for these generations, due perhaps to regression model used for the above data or random error.

TABLE 13: SURVIVAL PERCENTAGE AT THIRD AND LAST OBSERVATION FOR GRAND RAPIDS CROSSES*)

Generation	Other Parents									
	PI 250427		Taiwan-1		Taiwan-2		Taiwan-3		Taiwan-4	
	3rd	last	3rd	last	3rd	last	3rd	last	3rd	last
Grand Rapids	57	54	57	48	66	62	59	52	60	56
Other Parents	65	59	67	62	63	60	62	58	61	57
F_1	63	56	62	54	62	58	59	53	62	55
F_2	65	58	56	52	62	58	58	55	56	50
BC_1	64	59	53	47	67	62	53	48	56	52
BC_2	51	44	55	50	57	55	58	54	53	49
Parental Difference (P = .05)	No	No	Yes	Yes	No	No	No	No	No	No

*) There were approximately 150 seedlings for each parent and F_1 generation for each cross; 750 seedlings for F_2; 320 for each backcross

were also intermediate in three of the crosses, and lower than either parent in two of the crosses.

In three of the crosses the percentage of the survivors was higher in the backcross to the supposedly susceptible Grand Rapids cultivar than in the backcrosses to the supposedly resistant other parents.

PI 278110 crosses

The time to reach 50% mortality in PI 278110 was less than that of the three Taiwan strains suggesting that PI 278110 was more susceptible than Taiwan strains (Table 14). In comparison with PI 250427, the time for 50% mortality was greater for PI 278110.

The F_1 generation showed a higher value for the time to reach 50% mortality than both of the parents in two of the four crosses (PI 278110 x Taiwan 1 and PI 250427) and a lower value than either parents (PI 278110 and Taiwan-2) and a similar value in the cross PI 278110 x Taiwan-3. The F_2 generation showed larger value in three of the crosses and lower than either parent in one of the crosses. The backcrosses to the supposedly resistent parent resulted in a lower value than that obtained from backcrosses to the supposedly susceptible parents.

The survival percentage PI 278110 was significantly greater than that of supposedly resistant parents PI 250427 and Taiwan-1 (Table 15). In the final observation PI 278110 showed higher survival percentage than Taiwan-2 by 13%, but

TABLE 14: TIME OF OBSERVATION FOR 50% MORTALITY FOR PI 278110 CROSSES

Generation	Other Parents			
	PI 250427	Taiwan-1	Taiwan-2	Taiwan-3
PI 278110	1.7	2.5	3.1	3.3
Other Parents	1.1	2.6	3.6	3.4
F_1	2.5	4.5	2.6	3.2
F_2	4.0	3.1	3.3	3.7
BC_1	3.5	1.0	2.0	3.7
BC_2	2.6	.0	1.9	3.1

was not significant (P = 0.05). When comparison was made between survival percentage of PI 278110 Taiwan-3, the latter cultivar showed 13% greater survival percentage, but not significant at the 5% level.

F_3 and F_4 generations

The observed mean survival percentages for the F_3 and F_4 generations in comparison to the parental means showed that a large percentage of survivors occurred in the F_3 generation in all of Grand Rapids crosses observed in these two plantings (Table 16). These differences, however, were not significantly different from the parents. In the F_4 generation, two out of those 5 crosses mentioned in the F_3 showed greater survival percentage over the parent means, but not significantly (P = 0.05).

TABLE 15: SURVIVAL PERCENTAGE AT THIRD AND LAST OBSERVATION FOR PI 278110 *)

	Other Parents							
	PI 250427		Taiwan-1		Taiwan-2		Taiwan-3	
	Observations							
	3rd	Last	3rd	Last	3rd	Last	3rd	Last
PI 278110	93	93	97	97	87	83	87	73
Other Parents	75	68	75	75	90	70	89	86
F_1	93	90	97	87	83	80	93	87
F_2	91	89	84	79	93	89	91	83
BC_1	76	75	70	67	87	86	71	62
BC_2	87	86	95	94	76	71	86	76
Parental Difference (P =.05)	No	Yes	Yes	Yes	No	No	No	No

*) Seedlings raised for the test of each cross were approximately 30 for each parent and the F_1 generation, 150 for the F_2 and 63 for each backcross.

TABLE 16: MEANS SURVIVAL PERCENTAGES OF F_3 AND F_4 LINES COMPARED TO THE PARENTAL MEANS AND F_2, FOR GRAND RAPIDS ^{3}CROSSES. PARENTAL MEANS = 100%

Generation	Other Parents				
	PI 250427	Taiwan-1	Taiwan-2	Taiwan-3	Taiwan-4
F_3 generation tests:					
First planting:					
Parental means	100	100	100	100	100
F_2	141	191	84	114	135
F_3^2	128(10)*	159(10)	92(10)	121(10)	157(10)
Second Planting:					
Parental means	---	100	100	100	100
F_2	---	119	122	91	104
F_3^2	---	95(10)	104(17)	100(14)	98(9)
F_4generation tests:					
Parental means	---	---	100	100	100
F_2	---	---	124	125	115
F_4^2	---	---	109(2)	125(3)	115(3)

*Total number of lines tested in F_3 and F_4 generations are shown in parentheses.

The F_3 generations of PI 278110 crosses showed a larger mean for survival percentage than the parental means in 2 of the 4 crosses, but the differences was not significant (Table 17). No change in survival percentage was observed in the F_4 generation of these crosses.

When survival percentage for individual lines was considered, several F_3 lines were significantly greater ($P = .05$) than parental means (Table 18). In crosses involving Grand Rapids, nine out of 100 F_3 lines tested showed significantly greater survival percentage, while crosses involving PI 278110 only one out of 42 lines tested was significant.

In the F_4 generations, none of the eight lines of the Grand Rapids crosses and 11 lines of PI 278110 crosses showed significantly greater in survival percentage than that of the parents.

Interpretation of the Data

It is difficult to explain the results genetically. The most likely explanation is that the time for 50% mortality and survival percentage in all populations were primarily under the control of environment, while the genetic contribution to the variation for resistance was low. The variation is discernable especially between the parents, since they were included in each planting.

Low genetic variation for resistance was observed based on the following:

TABLE 17: MEANS SURVIVAL PERCENTAGES OF F_3 AND F_4 LINES COMPARED TO THE PARENTAL MEANS AND F_2, FOR PI 278110 CROSSES. PARENTAL MEANS = 100%

Generation	PI 250427	Other parents Taiwan-1	Taiwan-2	Taiwan-3
F_3 Generation Test:				
Parental Means	100	100	100	100
F_2	91	89	102	103
F_3	89(8)*	104(9)	113(11)	95(14)
F_4 Generation Test:				
Parental Means	---	100	100	---
F_2	---	98	76	---
F_4	---	95(5)	80(6)	---

* Total number of lines tested in F_3 and F_4 generations are shown in parentheses.

TABLE 18: NUMBER OF LINES THE F_3 AND F_4 GENERATIONS HAVING SIGNIFICANTLY GREATER SURVIVAL PERCENTAGE THAN THAT OF THE PARENT MEANS. (TOTAL NUMBER OF LINES TESTED IN EACH GENERATION SHOWN IN PARENTHESES).

Generation	PI 250427	Other Parents Taiwan -1	Taiwan -2	Taiwan -3	Taiwan -4
Grand Rapids Crosses					
F_3	2 (10)	2 (20)	0(27)	1(24)	3 (19)
F_4	--	--	0(2)	0(3)	0 (3)
PI 278110 Crosses					
F_3	0 (8)	1 (9)	0(11)	0(14)	--
F_4	--	0 (5)	0(6)	--	--

(1) Variation in survival percentage was observed for the parents.

(2) Genetic gains were evident in the F_3 generation when a large number of lines were sampled, while only an insignificant gain was observed when fewer of lines were sampled in the F_4 generation.

This suggested that the resistance to lettuce drop in the materials under study was polygenically controlled with low level of penetrance.

CONCLUSIONS

Data on inheritance of resistance to lettuce drop reported here were based on 14,352 seedlings which included parents, F_1, F_2 and backcross generations grown in the greenhouse ground bed. In addition, results obtained were supported by data from performance of 10,140 F_3 plants and 2030 F_4 plants grown in flats. The level of penetrance for resistance to lettuce drop varied with time of observation. Resistance was manifested by the delay in time of death from the pathogen and by the greater number of survivors. The level of penetrance was modified by environmental conditions, such as fluctuation of the soil and air temperatures observed. Penetrance level was also modified by the inoculum load since the soil was not sterilized between planting the first and the third planting, and the fourth and the sixth planting.

The variation in penetrance level was discernable especially with the parents since they were included in each of the plantings. Genetic gain was observed when a large number of F_3 lines were tested. No genetic gain was observed in the F_4 since fewer number of lines were tested. These suggested that the resistance to lettuce drop in the materials under study was polygenically controlled with low level of penetrance.

It was observed that in some of the plantings the difference in the level of penetrance between two parents was not significant. This suggests that there is a possibility of selecting for resistance strains from the Grand Rapids cultivar. Robinson (1980) suggested since transferring polygenically inherited characters is difficult, selection can start from susceptible parents simultaneously with other desirable characters. A selection that began from low level of resistance has been demonstrated successful in breeding red clover resistant to Sclerotinia trifoliorum by Frandsen (1946). An increase from 4.6% to 67.3% of level of resistance was achieved in 6 years of selection. In the Grand Rapids cultivar the lowest level of penetrance for resistance at the final observation was 3%.

APPENDIX

TABLE A-1. SURVIVAL PERCENTAGE FOR 16 CULTIVARS OBSERVED AT SEVERAL NUMBER OF DAYS FOLLOWING INOCULATION *)

Cultivars	Days Following Inoculation 6	9	12	15	18
			% of Survival		
PI accessions:					
PI 165063	91abc	66abc	47cde	44c	25abc
PI 184787	81abc	47a	16a	16a	6a
PI 187239	78a	50a	25ab	19ab	12ab
PI 206965	78a	50a	34bcde	25ab	9ab
PI 250427	100c	84cd	56ef	50e	25abc
PI 250429	87abc	56ab	31abcd	25ab	12ab
PI 251790	87abc	81cd	59f	50e	37c
PI 255568	91abc	81cd	50cdef	47de	28abc
MSU Breeding lines:					
Taiwan	100c	94d	72f	59e	37c
MSU 73-44	91abc	84cd	53def	44c	25abc
Cultivars:					
Grand Rapids	97c	72abc	28ab	19ab	12ab
Ithaca	94bc	66abc	41cde	31bcd	27abc
Valmanine Cos	87abc	53a	30abc	22ab	19abc
Bibb	94bc	81cd	53def	50e	31bc
Parris Island	91abc	69abc	41bcde	28abc	19abc
Mesa 659	87abc	53a	22ab	22ab	9ab

*) Survival percentages within a column followed by the same letter are not significantly different at the 5% level (Tukey's test).

TABLE A-2 SURVIVAL PERCENTAGES OF THE PARENT MATERIALS OBSERVED AT DIFFERENT NUMBER OF DAYS FOLLOWING INOCULATION

Cultivars	Number of days following inoculation						
	3	4	5	6	7	8	10
	% of Survival						
Grand Rapids	86	57	32	25	18	0	0
PI 278110	95	76	43	29	29	10	5
PI 250427	96	82	71	61	54	29	29
Taiwan-1	96	86	82	54	39	21	21
Taiwan-2	100	79	71	50	29	29	29
Taiwan-3	93	71	57	50	21	21	21
Taiwan-4	100	86	71	57	57	50	50

TABLE A-3 FREQUENCY DISTRIBUTION OF THE NUMBER OF DEAD PLANTS AT DIFFERENT TIMES OF OBSERVATION FROM 5 CROSSES INVOLVING GRAND RAPIDS.

Generation	Total Number of Plants	Total Number of dead Plants	Time of Observation							Mean	SD
			1	2	3	4	5	6	7		
	Grand Rapids x PI 250427		Number of Dead Plants								
Grand Rapids (P_1)	149	69	37	19	8	3	2	0	0	1.75	1.02
PI 250427 (P_2)	151	62	25	24	4	4	5	0	0	2.03	1.21
F_1	145	64	24	25	5	4	4	1	1	2.16	1.38
F_2	739	309	121	79	60	20	19	8	2	2.25	1.38
BC to P_1	316	130	27	56	30	6	7	4	0	2.40	1.20
BC to P_2	307	171	71	63	17	8	10	2	0	2.00	1.19
	Grand Rapids X Taiwan-1										
Grand Rapids (P_1)	151	79	43	19	3	11	2	1	0	1.90	1.26
Taiwan-1 (P_2)	152	57	24	22	4	3	4	0	0	1.96	1.16
F_1	143	66	31	19	5	7	4	0	0	2.00	1.24
F_2	738	353	170	127	30	13	12	1	0	1.79	1.01
BC to P_1	313	166	77	53	17	6	6	7	0	1.99	1.31
BC to P_2	319	158	104	27	14	7	3	3	0	1.65	1.13
	Grand Rapids x Taiwan-2										
Grand Rapids (P_1	154	58	39	8	5	2	4	0	0	1.69	1.20
Taiwan-2 (P_2)	148	59	33	20	2	4	0	0	0	1.61	0.85
F_1	147	62	28	21	7	5	1	0	0	1.87	1.02
F_2	723	304	139	96	38	13	13	3	2	1.95	1.21
BC to P_1	318	121	46	48	11	5	4	5	2	2.14	1.42
BC to P_2	319	142	80	49	7	6	0	0	0	1.57	0.78
	Grand Rapids x Taiwan-3										
Grand Rapids (P_1)	149	71	29	18	14	5	1	3	1	2.21	1.42
Taiwan-3 (P_2)	147	62	24	26	6	3	2	1	0	1.96	1.12
F_1	140	66	24	23	11	4	1	2	1	2.17	1.33
F_2	752	342	152	108	54	13	9	5	1	1.94	1.14
BC to P_1	316	164	66	57	26	7	3	5	0	2.02	1.18
BC to P_2	319	147	67	43	24	10	1	0	2	1.93	1.14
	Grand Rapids x Taiwan-4										
Grand Rapids (P_1)	151	66	42	17	1	4	2	0	0	1.59	1.01
Taiwan-4 (P_2)	150	64	41	14	3	2	2	1	1	1.70	1.29
F_1	143	64	26	24	4	1	7	2	0	2.14	1.42
F_2	739	373	186	106	32	19	20	8	2	1.96	1.32
BC to P_1	315	152	72	54	14	2	3	4	0	1.77	1.12
BC to P_2	317	161	80	60	8	8	3	2	0	1.76	1.03

Generation	Total Number of Plants	Total Number of Dead Plants	Time of Observation							Mean	SD
			1	2	3	4	5	6	7		
	PI 278110 x PI 250427		Number of Dead Plants								
PI 278110 (P_1)	29	2	0	2	0	0	0	0	0	2.0	0.0
PI 250427 (P_2)	38	9	0	4	0	3	0	2	0	3.56	1.67
F_1	30	3	0	2	0	0	0	1	0	3.33	2.31
F_2	150	16	0	2	3	2	6	0	3	4.50	1.63
BC to P_1	63	16	0	3	4	0	8	1	0	4.00	1.37
BC to P_2	63	9	0	4	3	1	0	0	1	3.11	1.62
	PI 278110 x Taiwan-1										
PI 278110 (P_1)	29	1	0	0	1	0	0	0	0	3.0	0.0
Taiwan-1 (P_2)	28	7	1	2	1	1	2	0	0	3.14	1.57
F_1	30	4	0	1	0	0	0	3	0	5.00	2.00
F_2	150	32	0	14	6	1	3	7	1	3.56	1.76
BC to P_1	63	21	7	7	4	1	0	2	0	2.33	1.49
BC to P_2	62	4	3	1	0	0	0	0	0	1.25	0.50
	PI 278110 x Taiwan-2										
PI 278110 (P_1)	30	5	0	0	3	1	1	0	0	3.6	0.89
Taiwan-2 (P_2)	20	6	0	1	0	2	2	1	0	4.3	1.37
F_1	30	6	0	3	2	0	0	1	0	3.0	1.55
F_2	149	17	2	2	5	1	3	4	0	3.76	1.75
BC to P_1	63	9	3	2	0	3	1	0	0	2.67	1.58
BC to P_2	62	18	2	11	2	0	1	2	0	2.61	1.50
	PI 278110 x Taiwan-3										
PI 278110 (P_1)	30	8	0	3	1	0	3	1	0	3.75	1.67
Taiwan-3 (P_2)	28	4	0	0	2	1	0	1	0	4.00	1.41
F_1	30	4	1	0	1	0	1	1	0	3.75	2.22
F_2	150	25	0	3	6	4	7	5	0	4.20	1.35
BC to P_1	63	24	3	2	4	3	6	4	2	4.13	1.85
BC to P_2	63	15	3	1	5	0	4	1	1	3.53	1.88

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