INHERITANCE OF RESISTANCE TO CABBAGE YELLOWS CAUSED BY *FUSARIUM OXYSPORUM* F.SP. *CONGLUTINANS* IN MUSTARD CABBAGE (*BRASSICA JUNCEA*)

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ABSTRACT

The inheritance of resistance to cabbage yellows in mustard was studied by using crosses between two resistant varieties, 'Chicken Heart Kaichoy' and 'Wild Type', and three susceptible ones, 'Chinese Round Heading', 'PF-3', and 'Waianae'. Disease evaluations of the parents, F_1, F_2, and backcrosses were made by inoculating 2-week-old seedlings with a suspension of 10^5 spores/ml in a screen house or by transplanting 2-week-old seedlings to outdoor tile beds which were infested with the disease. Disease was graded on a scale of 0 (resistant) to 4 (susceptible) about 15 days after inoculating in the screen house test, and about one month after transplanting in the tile bed test.

All of the parents were either completely resistant or completely susceptible. The F_1s for both resistant x susceptible and resistant x resistant crosses were uniform and intermediate in resistance. The F_2s segregated from complete resistance to complete susceptibility, but there were more resistant plants in the progenies from the resistant x resistant cross than the resistant x susceptible crosses. All backcrosses tested appeared to segregate at a ratio close to 1:1. The data fit neither a simple qualitative ratio nor a normal quantitative distribution. A possible genetic explanation that agrees with all the results follows: each resistant parent differs from the susceptible parents by two pairs of genes, one of which

shows dominance and one of which shows additive gene action and is epistatic to the first. However, the genes for resistance in the two resistant parents are different. Thus, the genotypes of the three levels of resistance observed would be 1) dominant at locus #1 plus homozygous resistant at locus #2; 2) dominant at locus #1 plus heterozygous at locus #2; 3) homozygous susceptible at locus #2. Such a hypothesis would give F_2 ratios of 3:6:7 for the resistant x susceptible crosses and 87:121:48 for the resistant x resistant cross, quite similar to the results observed.

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INTRODUCTION

Brassica juncea Coss., known as mustard, kaichoy, green mustard, leaf mustard, Chinese mustard, Indian mustard, mustard cabbage, etc., is extensively cultivated as a vegetable or salad plant in southern, central, and eastern Asia, eastern Europe and some regions in Africa (Herklots, 1972; Tindall, 1983). In the United States, it is grown to some extent in Texas, California, Florida, Georgia, Louisiana, Mississippi, Tennessee, Arkansas, Alabama, and Hawaii (Peirce, 1987). In the southern states, it is called mustard greens', in Hawaii, it is called kaichoy or green mustard. In Hawaii, the acreage of kaichoy had been increasing, from around 90 acres in the 1970s to a peak of 170 acres in 1983 (Statistics of Hawaiian Agriculture, 1974, 1979, 1984), but then decreased to 140 acres in 1986 and 1987 (Statistics of Hawaiian Agriculture, 1987). In general, Brassica juncea is a relatively minor vegetable, except in parts of Asia.

Brassica juncea is a highly diverse species. Sinskaja (1928) has divided it into four main groups based on leaf shape: B. juncea var. sareptana Sinskaja, B. juncea var. integrifolia (Rupr.) Sinskaja, B. juncea var. japonica Bailey, and B. juncea var. crispifolia Bailey. Brassica juncea var. rugosa (Roxb.) Tsen & Lee, which is cultivated in Hawaii, is included in B. juncea var. integrifolia (Rupr.) Sinskaja and develops a loose leaf head. In recent

years, the production of B. juncea in Hawaii has been seriously limited by cabbage yellows, caused by Fusarium oxysporum f.sp. conglutinans (Wr.) Snyder & Hansen. This organism, which is specialized on crucifers, especially cabbage, can kill an entire field of B. juncea at any stage. In cabbage, the only successful control of this disease has been through the use of resistant varieties, and the nature of resistance to this disease in cabbage has received much study (Walker, 1969). There are no literature reports on resistance in B. juncea yet, however.

The purpose of this study is to confirm what appears to be resistance to cabbage yellows in B. juncea and determine its inheritance.

REVIEW OF LITERATURE

Cytotaxonomic Background and Origin of *Brassica juncea*

The diploid chromosome number of *B. juncea* is 36. It is believed to have originated as a natural amphidiploid hybrid between *B. nigra* (L.) Kock (haploid number of chromosomes = 8) and one or more species with a haploid chromosome number of 10, such as *B. campestris* L., *B. rapa* L., *B. chinensis* L., *B. pekinensis* Rupr., *B. japonica* Sieb. (Morinaga, 1934; U, 1935; Vaughan et al., 1963). Experimental synthesis of pseudo-*juncea* forms very similar to natural *B. juncea* has been carried out by Ramanujam and Srinivaschar (1943), using *B. nigra* and *B. campestris* as the parents .

The center of origin of *B. juncea* is believed to be Central Asia-Himalayas, with migration to three secondary centers in India, China, and the Caucasus (Hemingway, 1976). However, since several of the species which could have been parents of *B. juncea* are of rather local and limited natural distribution in India or China, it is possible that *B. juncea* may have arisen at more than one location, with varied n = 10 parents giving different genetic constitutions to the amphidiploids formed (Vaughan et al., 1963).

Taxonomy of *Brassica juncea*

B. juncea is a highly diverse species with little agreement on its taxonomy. Many varieties have been described by different writers, yet it is still extremely

difficult to fit all the many cultivars into these varieties (Herklots, 1972). Sinskaja (1928) has described four main groups based on leaf shape, and Vaughan et al. (1963) and Herklots (1972) have followed this classification.

Group 1. With lyrately lobed basal leaves: B. juncea var. sareptana Sinskaja.

Group 2. With entire or little lobed basal leaves: B. juncea var. integrifolia (Rupr.) Sinskaja.
B. juncea var. rugosa (Roxb.) Tsen & Lee, B. juncea var. foliosa Bailey, and B. juncea var. subintegrifolia Sinskaja are included in this group.

Group 3. With dissected basal leaves: B. juncea var. japonica Bailey.
B. juncea var. longidens Bailey and B. juncea var. multisecta Bailey are included in this group.

Group 4. With dissected and crisped lower leaves: B. juncea var. crispifolia Bailey.
B. juncea var. subcrispifolia Sinskaja is included in this group.

However, B. juncea var. tumida Tsen & Lee, B. juncea var. strumata Tsen & Lee, and B. juncea var. megarrhiza Tsen & Lee do not fit into any of the four groups (Herklots, 1972).

Botany of *Brassica juncea*

B. juncea is in the *Cruciferae*. Its roots are slender and slightly expanded at the base, except in some varieties which produce large fleshy roots. The plant is usually a rosette with a short stem before bolting, except in the varieties with large expanded stems, and in others which have elongated stems with irregular tumor-like bumps. After bolting, an erect, branched stem grows out, with a height varying from 80 - 180 cm. The basal leaves are elliptic, ovate, obovate, lanceolate, etc. or divided, toothed in shape, and 15 - 30 cm in length. The color of leaves can be green, dark green, light green, or green with red or purple veins, etc. The leaves can be smooth or crinkled, glabrous or hairy. Crinkling and hairiness vary a great deal among varieties or even in different leaves on the same plant. The inflorescence is a corymbose raceme. The flowers first form a short corymbose raceme when the lowest flower opens and then elongate into a long raceme. The flowers are yellow and four-petalled like all *Cruciferae*. *B. juncea* has a very high rate of self-fertilization (Tindall, 1983). Lee (1979) recorded up to 82.6% in one variety in China. Singh (1958) stated self-pollination is the rule. The fruit is a 2-celled silique 3 - 5 cm long. The seeds are brown or dark brown, round, and show marked reticulation over the surface when examined under a microscope. The weight per 1000 seeds is 2 - 3 g (Lee, 1979; Martin, 1984; Singh, 1958; Tindall, 1983).

The Utilization of *Brassica juncea*

B. juncea is extensively cultivated as a vegetable or salad plant in southern, central, and eastern Asia, and less so in eastern Europe, northern America and some regions in Africa (Herklots, 1972; Tindall, 1983). It is widely cultivated as a vegetable in China and Japan, grown for roots, leaves, stems or inflorescences (Khan et al., 1987). It is also a major vegetable for pickling in China (Lee, 1979). In India, the leaves of young *B. juncea* plants have been eaten as a vegetable since the earliest times (Singh, 1958). In the United States, Canada, and Holland, *B. juncea* is grown to a considerable extent for greens (Vaughan and Hemingway, 1959).

B. juncea is also one of the most important oil crops in many parts of the world. The "mustard oil" produced is widely used as a vegetable oil and has also been used as a special lubricant in the place of rape oil (Kester, 1951). In the U.S.S.R., it is the second most important oilseed crop, exceeded only by sunflower (Kirk and Oram, 1978). It is also one of the major edible oils of India (Vaughan and Hemingway, 1959). Goering et al. (1965) analyzed 145 different accessions of this species in the U.S.D.A. collection, and found that the oil content varied from 29 to 44%, with a mean of 35%. In India, the oil content varied from 30 to 42% (Singh, 1958). In the U.S.S.R., varieties with a high oil content have been developed, the cultivar

Zarya averaged 48.8% oil in the period 1959-63 (Pustovoit, 1973).

Another large utilization of B. juncea is as a condiment. The condiment properties of B. juncea arise from the presence within the seed of a class of thioglucosides, known as glucosinolates. When the seed is crushed, the glucosinolates are brought into contact with an enzyme, a thioglucosidase commonly referred to as myrosinase, which in the presence of sufficient moisture hydrolyzes off the glucose, with the concomitant production of an isothiocyanate. The predominant thioglucoside in the seeds of B. juncea is allyl glucosinolate (trivial name: sinigrin); this gives rise to allyl isothiocyanate which is responsible for the pungent character of the mustard paste made from seeds of B. juncea (Kirk and Oram, 1978). The main present-day production of B. juncea for spice use is in North America, in the prairie provinces of Canada and southward into Montana and the Dakotas. Other major production centers are the UK and Denmark (Hemingway, 1976). Mustard condiment is marketed in two forms. In Great Britain, North America, and most of the British Commonwealth, both powder and paste mustard are sold, whereas in France, the rest of Europe, South America, and other parts of the world, the demand is almost exclusively for paste mustard, and very little powder mustard is sold (Vaughan and Hemingway, 1959).

B. juncea is also valuable as green manure or fodder

(Hemingway, 1976). The oil-free meal left after removal of the oil from mustard seed contains up to 42.3% protein with an amino acid composition which is quite suitable for monogastric animals (Kirk and Oram, 1978).

The History and Geographical Distribution of Cabbage Yellows

Cabbage yellows is caused by Fusarium oxysporum f.sp. conglutinans (Wr.) Snyder & Hansen and was first reported by E.F. Smith in the Hudson valley in New York in 1899 (Walker, 1969). Since then it has spread rapidly in the United States and been found in many states such as Ohio, Illinois, Wisconsin, Iowa, New York, etc. (Walker, 1969). Presently, this disease has been reported to occur in all areas where there is production of crucifers, with the exception of China (Bosland and Williams, 1988).

The first record of a wilt disease on B. juncea caused by an unidentified species of Fusarium was from the United States in 1960 (Index of Plant Diseases in the United States, 1960). In 1973, Rai and Singh (1973) identified this Fusarium as Fusarium oxysporum f.sp. conglutinans. In 1988, evidence of a potentially new pathotype from B. juncea was found in Taiwan (Bosland and Williams, 1988). In Hawaii, cabbage yellows in B. juncea was identified by M. Aragaki, Department of Plant Pathology, University of Hawaii in 1982 (M. Aragaki, personal communication).

Infection Cycle of Cabbage Yellows

Cabbage yellows is distributed by means of infested soil on implements, in the wind, or in water. The organism thrives in a variety of soil types, and once it is established, it remains viable indefinitely. While conidia and mycelia are apparently short-lived, the fungus persists as chlamydospores which are stimulated to germinate in the vicinity of host or non-host rootlets, which are penetrated and in which new chlamydospores are formed. Infection takes place through the root-tip region or through wounds created at transplanting. The fungus invades the root cortex with little damage to it, becomes established in the spiral vessels, and progresses upward within the large xylem elements. Occasionally microconidia are produced in the xylem. The organism does not invade other tissue until the plant dies (Walker, 1969).

Variability and Host Range of Cabbage Yellows

Blank (1930) found no pathogenic variation among isolates of the cabbage yellows organism from 11 states within the United States. However, Kendrick and Snyder (1936) and Baker (1948) reported strains of F. oxysporum causing wilt on radish (Raphanus sativus L.) and garden stock (Matthiola incana R.B.), designating them formae speciales raphani and matthiola, respectively. Later, Armstrong and Armstrong (1952) inoculated cabbage, radish, and stock with single spore isolates of Fusarium from

cabbage and radish. Based on the results of this experiment, they proposed that the Fusaria from cabbage, radish, and stock be designated as three physiologic races of F. oxysporum f.sp. conglutinans and not forms of F. oxysporum. Presently, five races of the pathogen have been defined on crucifers (Armstrong and Armstrong, 1952; Armstrong and Armstrong, 1966; Ramirez-Villupadua et al., 1985), and many hosts have been reported (Armstrong and Armstrong, 1966; Armstrong and Armstrong, 1974; Thanassoulopoulos et al., 1978). The hosts relative to different races are listed in Table 1. Race 1 is primarily found from cabbage, race 2 is primarily from radish, and race 3 is primarily from stock; all of above-mentioned races have been found worldwide (Subramanian, 1970). Race 4 is also primarily from stock and reported only in New York (Armstrong and Armstrong, 1966). Recently, in California and the USSR, a new pathotype, which is pathogenic on cabbage cultivars containing the type A monogenic dominant resistance, was found (Bosland and Williams, 1988; Ramirez-Villupadua et al., 1985). This new pathotype was designated Fusarium oxysporum f.sp. conglutinans race 5 (Ramirez-Villupadua et al., 1985).

In 1987, Bosland and Williams (1987) proposed a new nomenclature for the pathotypes of Fusarium oxysporum found on crucifers based on pathogenicity, isozyme polymorphism, vegetative compatibility, and geographic origin. The proposed nomenclature is listed in Table 2. This

nomenclature more clearly depicts the relationship between the races.

Symptomatology of Cabbage Yellows

The disease affects plants at any age. The symptoms on different hosts are somewhat the same. The first sign is a lifeless yellow-green color of the foliage. Sometimes the yellowing is uniform, but more often it is more intense on one side of the leaf or plant, causing a lateral warping or curling of the leaves and stem. The lower leaves become yellow first, and the appearance of symptoms progresses upward. As the yellowed tissue ages, it turns brown and becomes dead and brittle. Affected leaves drop prematurely, and normal growth of the plant is distinctly retarded. The vascular system becomes yellow to dark brown (Walker, 1969). An infected B. juncea plant is shown in Figure 1.

Factors Affecting Cabbage Yellows

Soil temperature is the major factor that influences the development of the pathogen. For all pathotypes, virulence on their respective susceptible hosts is influenced by soil temperature, with disease severity increasing as soil temperatures increased from 10 to 24^{o}C (Bosland et al., 1988). The seasonal curve of disease incidence of radish yellows, caused by Fusarium oxysporum f.sp. conglutinans race 2, closely followed that of soil temperature in north-central Ohio (Wilson, 1962). In B. juncea, the highest

Table 1

Hosts of Physiologic Races 1, 2, 3, and 4 of *Fusarium oxysporum* f.sp. *conglutinans*

Plant species	Common name	Physiologic races 1	2	3	4
Arabis alpina	---	+	+	+	+
Aubrieta	---	+	+	+	+
Brassica carinata	---	-	+	-	-
Brassica napobrassica	rutabaga	+	+	-	-
Brassica oleracea var. *acephale*	kale	+	-	-	-
Brassica oleracea var. *botrytis*	cauliflower	+	-	-	-
Brassica oleracea var. *capitata*	cabbage	+	-	+	+
Brassica oleracea var. *gemmifera*	brussels sprouts	+	+	-	-
Brassica oleracea var. *gongylodes*	kohlrabi	+	-	+	-
Brassica oleracea var. *italica*	broccoli	+	-	-	-
Brassica juncea	mustard	+	+	+	+
Brassica pekinensis	Chinese cabbage	+	-	+	+
Brassica rapa	turnip	+	-	-	+
Capsella bursa-pastoris	shepherd's purse	+			
Cheiranthus	wallflower	+	+	+	+
Crambe abyssinica	Spanish colewort	-	+	-	-
Eruca sativa	rocket	+			
Iberis umbellata	candytuft	+	+	+	
Lepidium sativum	garden cress	+	+	+	+

Table 1. (Continued) Hosts of Physiologic races 1, 2, 3, and 4 of *Fusarium oxysporum* f.sp. *conglutinans*

Plant speciecs	Common name	Physiologic races 1	2	3	4
Lychnis chalcedonica	---	+	-	-	-
Matthiola incana	stock	+	-	+	+
Raphanus sativus	radish	+	+	-	+
Sinapis alba	white mustard	+			

+ = susceptible
- = resistant

Table 2

Present and Proposed New Nomenclature for the Pathotypes of *Fusarium oxysporum* Found on Crucifers (Bosland and Williams, 1987)

Present	Proposed
F. oxysporum f.sp. *conglutinans* race 1	*F. oxysporum* f.sp. *conglutinans* race 1
F. oxysporum f.sp. *conglutinans* race 2	*F. oxysporum* f.sp. *raphani*
F. oxysporum f.sp. *conglutinans* race 3	*F. oxysporum* f.sp. *matthioli* race 1
F. oxysporum f.sp. *conglutinans* race 4	*F. oxysporum* f.sp. *matthioli* race 2
F. oxysporum f.sp. *conglutinans* race 5	*F. oxysporum* f.sp. *conglutinans* race 2

Figure 1. Symptoms of
Fusarium oxysporum f.sp. conglutinans on Brassica juncea

plant mortality caused by the organism in India was observed in late February and early March when the temperature was higher (10.0 - 33.7°C) than in December and January (6.0 - 26.5°C) (Rai and Singh, 1973). When single spore cultures of races 1 and 2 were grown on potato-dextrose agar plates, the optimum temperature for growth was 24 - 28°C (Pound and Fowler, 1953). In soil tests, the severity of the disease in susceptible cabbage varieties increased with soil temperature, reaching a maximum at 26 - 30°C (Tims, 1926; Tisdale, 1923; Walker and Smith, 1930). The disease curve is roughly parallel with the growth curve of the organism, which has been taken to indicate that the effect of soil temperature on disease development is expressed primarily through its effect upon the organism (Walker, 1969). Higher air temperature (up to 28°C) also hastened the disease development (Tims, 1926; Walker and Smith, 1930). Relationships exist between plant nutrition and disease development, but there are differences between types. As salt concentrations increased in 5% to 300% Hoagland's solutions, there was a progressive decline in the rate of disease development in a susceptible cabbage strain (Walker and Hooker, 1945). Ammonium nitrate nitrogen did not have a significant effect on disease severity, but high levels of calcium nitrate nitrogen (300 ug N/ml of irrigation water) reduced the severity of radish Fusarium wilt (Trillas-Gay et al., 1986). In the absence of potassium in the solution, the rate of disease development increased in a susceptible

cabbage strain at 19°C and 25°C, as well as in an intermediately resistant strain at 25°C. The absence of nitrogen or phosphorus decreased the rate of disease development. Type A monogenic dominant resistance was not influenced by plant nutrition, with no sign of disease developed at any salt concentration or at any nutrient levels. The differences in disease development in plants grown in various solutions were due to the effect on the host rather than to a direct effect of the nutrient solutions on the organism before establishment of its parasitic relation to the host (Walker and Hooker, 1945).

Temperature was more important than nutrition in its effect upon disease development. When the temperature was more favorable for disease development, the influence of nutrient concentration upon disease development was less evident. When Hoagland's solutions varying from 5% - 300% concentration were applied to susceptible cabbage plants at 19°C and 25°C, all disease curves at 25°C were higher than the highest curve at 19°C (Walker and Hooker, 1945).

Other microbes also affect the growth of this Fusarium wilt. Heavy growth of some bacterial species, such as Agrobacterium rhizogenes, Serratia marcescens, etc., always inhibited growth and sporulation of the Fusarium (Moore-Landecker and Stotzky, 1974). Reyes and Chadha (1972) found that the severity of cabbage yellows symptoms in Brassica campestris var. chinensis increased when the plants were also infected with turnip mosaic virus.

In addition, severe pruning of the root system during transplanting shortens the incubation period in susceptible varieties, but has no effect on resistant varieties (Walker and Smith, 1930). In the susceptible varieties the appearance of the disease in moderately and severely pruned plants was several days earlier compared with that of slightly pruned plants, but at the end of the period the total infections in all three pruning treatments were not significantly different.

Control of Cabbage Yellows

The only successful control of cabbage yellows is through the use of resistant varieties. There are some resistant varieties available, such as the cabbage varieties Wisconsin Hollander and Wisconsin All Seasons (Anderson, 1933; Blank, 1937), and the radish varieties White Spike and Red Prince (Williams and Pound, 1967). Although there are some other control methods reported, all of them are either impractical or not considered sufficiently effective. Ramirez-Villapudua and Munnecke (1987, 1988) reported a control method by using solar heating and soil amendments of cruciferous residues. After air-dried residues of nine cruciferous plants, broccoli, brussels sprouts, cabbage, cauliflower, collards, kale, mustard, radish, and turnip, were mixed in soil (1% or 2% w/w) and covered with a translucent polyethylene tarp (solar heating) for 4 or 6 weeks, population counts of Fusarium oxysporum f.sp.

conglutinans and cabbage yellows were greatly reduced. Both solar heating alone and plant amendments plus cover under shade were effective but not as effective as the combination of solar heating and plant amendments. Since radish requires only 3 - 4 weeks from seeding to harvest, seed treatment by a fungicide like Arasan might keep the soil surrounding the seedling roots protected long enough to allow the plants to remain healthy until harvest (Pound and Fowler, 1953).

Genetics of Cabbage Yellows Resistance in Cabbage and Radish

Two types of resistance to the disease, type A and type B, have been found in cabbage. Type A resistance, such as in 'Wisconsin Ballhead' (Walker and Blank, 1934), is controlled by a single dominant gene (Walker, 1930). It is expressed as complete resistance or immunity, except at soil temperatures about 26 - 28^{o}C, where atypical symptoms may develop but no invasion of the plant occurs except at the extremities of the root system (Anderson and Walker, 1935). Type B resistance, such as in 'Wisconsin Hollander', is controlled by multiple genes. Typically, the disease symptoms appear in increasing severity with an increase in soil temperature and the resistance tends to be overcome when plants are grown at soil temperatures about 20 - 24^{o}C (Anderson, 1933). Both types of resistance occur in the variety Wisconsin All Seasons (Blank, 1937).

In radish, the resistance is multigenic (Peterson and

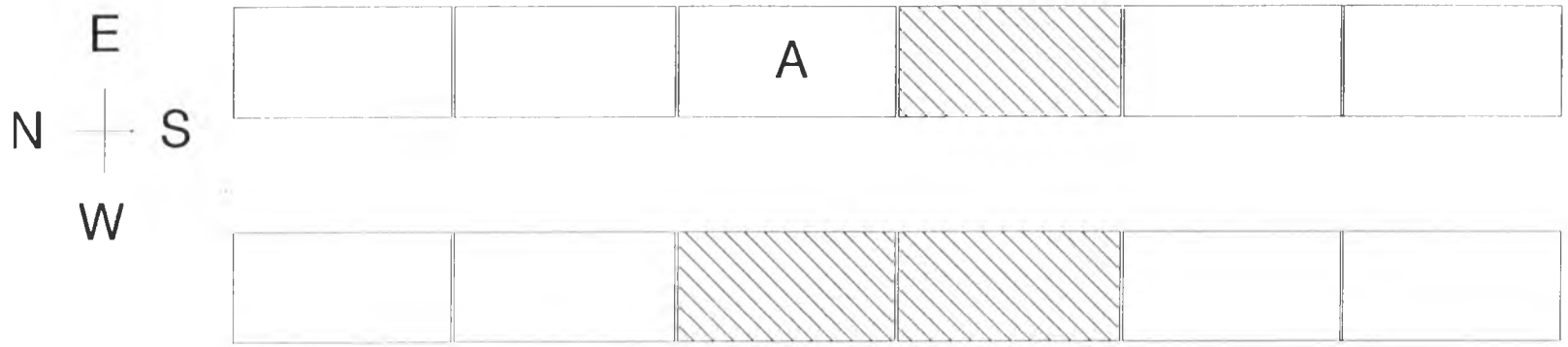
Pound, 1960; Williams and Pound, 1967). Since most of the better Fusarium-resistant varieties are also virus-resistant, some positive correlation between the two has been suspected (Hida and Ashizawa, 1985).

MATERIALS AND METHODS

Pathogen Materials

1. Soil infection

For testing for resistance, four tile beds at the Magoon Greenhouse Facility of the University of Hawaii were inoculated with soil from fields where mustard cabbage had exhibited cabbage yellows symptoms. Each tile bed was constructed from hollow tile blocks set on the ground to a height of 1 foot 3 inches. Each bed was 10 x 4 feet; two of the beds were in one row and the other two were in a different row, each of which has six beds (Figure 2). The beds were filled to the top and were separated in the row only by one row of tile. They were watered by an automatic watering system running along the outer edge of the whole row of beds. Three beds of the four were first inoculated in 1984, but had not been used to grow plants susceptible to cabbage yellows for sometime, so the same three beds were reinoculated on April 29, 1988 with soil from a Waianae farm, where mustard cabbage had been infected with yellows, and again on May 24, 1988, with soil from a Kipapa farm with yellows. On April 29, the infected soil was sprinkled on the surface of the beds. On May 24, it was mixed with the other soil in the beds. From this time until the end of the experiment, the inoculum level was maintained in the tile beds by planting the susceptible variety, Waianae



A Uninoculated bed used in the trials

Inoculated beds used in the trals

Figure 2. Arrangement of tile beds

Strain, whenever the tile beds were not being used for testing the disease.

2. Artificial inoculation

The pathogen was isolated from a diseased mustard plant which had been grown in the infected tile beds and showed typical symptoms of cabbage yellows. M. Aragaki, Department of Plant Pathology, University of Hawaii.

A spore suspension of the pathogen was used for the inoculations. The spore suspension was made by flooding the 7-9 day old culture (No. 2020) grown on a medium of 10% Vegetable Juice Agar plus 0.2% $CaCO_3$ with distilled water, passing it through filter paper to remove mycelial fragments, and diluting it to the desired concentration. The concentration of the spore suspension was determined by counting spore numbers on a Howard Mold Counting Chamber under a microscope.

The optimum spore concentration to be used in inoculation was determined by inoculating a resistant variety, a susceptible variety, and their F_2 with 10^4, 10^5, and 10^6 spores/ml by dipping the roots. Sixteen-day-old seedlings grown in vermiculite in the greenhouse were inoculated on July 17, 1989. Ten days after inoculation, the plants were evaluated on a scale of 0 - 3:

0 = No symptoms on both leaves and roots.

1 = Light yellowing of bottom leaves.

2 = Whole or half portion of some leaves turned obviously yellow or died, and the roots turned black. Plant

growth was hindered.

3 = Plant dead.

Plant Materials

Seven mustard cabbage varieties from China, Hongkong, Taiwan, and Hawaii, plus four head cabbage varieties were evaluated for yellows susceptibility (Table 3). Waianae Strain (WS), a Hawaiian variety which has exhibited cabbage yellows, was used as the susceptible standard. "Wild Type" (WT) was expected to be resistant. This line originated from a plant surviving in a yellows-infected field in Waianae, Hawaii, where a susceptible mustard cabbage variety was growing. The plant surviving had morphological characteristics totally different from the variety (Takeda, personal communication). PF-3 (PF) was a selection from a cross between Waianae Strain and P.I. 174801 (Hartmann, personal communication). Chicken Heart Kaichoy (CHK) is a variety grown in Canton, China. Chinese Round Heading (CRH) and Kaichoy from Hongkong (HK) are commercial varieties from Taiwan and Hongkong. In addition, four head cabbage varieties from American Takii Seed Company, two described as resistant to cabbage yellows and two not resistant to the disease, were also included. These varieties will be referred to by their initials from now on.

Table 3

Mustard Cabbage and Head Cabbage Varieties Tested for Cabbage Yellows Reaction

Variety	Initial	Source
Mustard Cabbage		
Wild Type	WT	K.Y. Takeda U.H. Horticulture
Waianae Strain	WS	U.H. Horticulture
PF-3	PF	R.W. Hartmann U.H. Horticulture
Kaichoy from Hongkong	KH	Hongkong
Chinese Round Heading	CRH	Taiwan
Chicken Heart Kaichoy	CHK	China
Head Cabbage		
C-G (S^Z)	CG	American Takii Seed Company
Emerald Cross (S)	EC	American Takii Seed Company
Green Coronet (R)	GC	American Takii Seed Company
Resist Crown (R)	RC	American Takii Seed Company

[Z] S, R = head cabbage variety susceptible or resistant to *Fusarium oxysporum* f.sp. *conglutinans*

Selection of Parents

Preliminary tests for resistance were conducted in the noninoculated and inoculated tile beds. The first test was planted on March 9, 1988 and included all four head cabbage varieties plus WT and WS mustard cabbage varieties. The second test was planted on May 25, 1988 and included the four head cabbage varieties plus WT and PF mustard cabbage varieties. The third test was planted on July 20, 1988 and included only two head cabbage varieties, EC and GC, plus WT, WS, PF, KH, CRH, and CHK mustard cabbage varieties. They were directly seeded and thinned later. Each entry was replicated one to three times in each of the four beds. Each replication consisted of four plants.

Before being used in crosses, CHK, WT, and PF were selfed. CHK was selfed one generation. WT was selfed three generations. The selfed seeds of PF were obtained by rooting a cutting of a flower stalk from a diseased plant under mist, and growing it in the greenhouse until it flowered and seeded. The seeds of Waianae Strain and Chinese Round Heading were commercial ones.

Crossing Procedures

The mustard cabbage plants to be crossed were grown in a mixture of two parts peat moss, two parts vermiculite, and one part perlite in a screen house at the Magoon Greenhouse Facility. The plants were sown on October 12, October 22, and November 1, 1988 to have overlapping flowering times.

Twelve parental plants CHK-1, CHK-2, CHK-3, CRH-1, CRH-2, PF-1, PF-2, PF-3, WS-1, WS-2, WT-1, and WT-2, were finally involved in crosses.

The crosses were made by bud-pollination. When some flower buds on the lower part of an inflorescence turned yellowish, the younger flower buds on the top were removed, the immature stamens in the yellowish flower buds were removed with tweezers, and the stigmata on the emasculated flowers were pollinated with pollen from the male parent. Pollen was obtained only from flowers which had been bagged before opening. The pollinated inflorescences were bagged and labelled. Three days after pollination, the bags were removed. At the same time as crosses were made, individual plants of both male and female parents were also selfed. The seeds of different combinations and reciprocal crosses were harvested individually.

The F_2 seeds were made by self-pollinating F_1 plants. The F_1 seeds were planted in the screen house on March 24, 1989. Before flowers of the F_1 plants opened, the inflorescences were bagged. When the flowers opened, they were pollinated with pollen from other bagged flowers on the same plant, and the pollinated flowers were covered by bags again. Meanwhile, the F_1 plants were backcrossed to the parents by bud-pollination.

Crosses were made between two resistant and three susceptible parents as well as between the two resistant parents. All backcrosses to both parents were attempted.

Making crosses or backcrosses was continued until there were no more flowers.

Testing for Homozygosity of Parental Lines

Each plant of CHK, CRH, PF, WS, and WT used to make crosses was selfed at the same time and tested for homozygosity in the greenhouse. They were planted in vermiculite on July 17 and inoculated with a 10^5 spores/ml suspension by dipping the roots on August 2. 12 days after inoculation, plants were evaluated on the 0-3 scale previously mentioned.

Testing for Inheritance of Resistance

The F_2's, backcrosses, F_1's, and parents were tested two times to determine the inheritance of resistance. In the first test the seeds were sown in vermiculite in the greenhouse, and in the second they were sown in a cooler screen house. Both times about 15 days later half of the seedlings were inoculated as described next and the other half were transplanted into the infected tile beds the following day.

1. Inoculation tests

In the first test, seedlings sown on August 14, 1989 were lifted on August 29, and the roots washed thoroughly in water, blotted on paper towel, pruned slightly, and dipped in a 10^5 spores/ml inoculum suspension. Control seedlings were dipped in tap water. The inoculated seedlings were

transplanted immediately back into the vermiculite and kept in the greenhouse. After five days, the number of plants surviving was counted. However, no infection occurred, so on September 14, the seedlings were moved from the greenhouse to a cooler screen house, with a plastic shelter over the seedlings to keep the rain off. On September 19, they were reinoculated by pouring more inoculum into furrows made by a knife around the base of the seedlings. After the second inoculation, they were kept in the screen house with the plastic shelter. The plants were evaluated on September 30 on a scale of 0 - 4 (Figure 3) and the number in each class recorded.

Seed for the second test was sown on September 16 in the screen house, inoculated on October 3, kept in the screen house, counted five days after inoculation, and evaluated on the same scale on October 24.

2. Natural infection

The other half of the seedlings inoculated above were transplanted to the tile beds on August 30 and October 4, respectively. Five days later, the number of plants surviving was counted. Evaluation of resistance was done on October 2 and November 10. The plants were dug up. If a plant did not show any top symptoms, its roots were cut off to see whether there were symptoms in the vascular part. Then the plants were sorted on a scale of 0 - 4 (Figure 4) and the number in each class recorded.

0 = No symptoms on both tops and roots.

1 = Light yellowing of one or more bottom leaves.

2 = Whole or half of one or more bottom leaves turned yellow, with crooked abnormal shape.

3 = Whole or half of one or more bottom leaves died and dried up, the roots turned black, the whole plant wilted under the sun, and plant growth hindered.

4 = Plant dead.

Figure 3. Disease grading scale for the inoculation

0 = No symptoms on both tops and roots.

1 = No symptoms on the top; the vascular tissue of roots and bottom parts of stems turned brown.

2 = Whole or half of one or more bottom leaves turned yellow, with crooked abnormal shape.

3 = Whole or half portion of one or more bottom leaves yellow or died, roots turned black, whole plant wilted under the sun, and plant growth hindered.

4 = Plant dead.

Figure 4. Disease grading scale for natural infection

RESULTS AND DISCUSSION

Comparison of Inoculated and Uninoculated Tile Beds

The results of three preliminary trials, in which each variety was replicated one to three times in each of the three inoculated and one uninoculated tile beds, are given in Table 4. The variance of the data from mustard varieties in the June 20 test was analyzed and showed no significant difference between inoculated and uninoculated tile beds (Table 5). Since the uninoculated tile bed is adjacent to an inoculated one, it is likely that infected soil has been moved from one bed to another in the four years since the bed was inoculated, and thus, the uninoculated bed has become infested. Therefore, the uninoculated tile bed data were combined with the data from the three inoculated ones.

Reaction of Cabbage Varieties in the Preliminary Trials

The responses of the cabbbage varieties grown in the yellows-infected beds are shown in Table 6. The two resistant varieties, GC and RC, had more plants surviving than the two susceptible varieties, EC and CG. However, many plants of these two susceptible varieties survived. Aragaki (personal communication) suggested that the Fusarium strain which attacks mustard cabbage in Hawaii is apparently not a strain which infects cabbage.

Table 4

Infection by Cabbage Yellows of
Head Cabbage and Mustard Cabbage Grown
on Inoculated and Uninoculated Tile Beds

	Date Planted					
	3/9		5/25		7/20	
Variety	Bed[Z]	S/P[Y]	Bed	S/P	Bed	S/P
Head Cabbage						
CG(S[X])	INOC	28/32	INOC	28/32		
	NON	10/12	NON	9/12		
EC(S)	INOC	33/36	INOC	27/36	INOC	22/24
	NON	10/12	NON	9/12	NON	8/8
GC (R)	INOC	35/36	INOC	36/36	INOC	22/24
	NON	12/12	NON	11/12	NON	7/8
RC (R)	INOC	32/36	INOC	34/36		
	NON	12/12	NON	11/12		
Mustard Cabbage						
WS	INOC	16/20			INOC	11/24
	NON	3/4			NON	2/8
WT	INOC	13/16	INOC	10/16	INOC	20/24
	NON	7/8	NON	3/4	NON	8/8
PF			INOC	1/16	INOC	6/24
			NON	1/8	NON	1/8
CHK					INOC	23/24
					NON	8/8
CRH					INOC	8/24
					NON	2/8
KH					INOC	15/24
					NON	4/8

[Z] INOC: inoculated beds; NON: uninoculated bed

[Y] Number of plants surviving/number of plants planted

[X] S: susceptible head cabbage variety; R: resistant head cabbage variety

Table 5

Analysis of Variance for Mustard Cabbage
from Inoculated and Uninoculated Tile Beds in 7/20/88 Trial

Source of Variation	df	ss	ms	F
Total	47	5.6875	0.1210	
Inoc. vs. Non. Beds[Z]	1	0.0277	0.0277	0.5947[NS]
Among Varieties	5	3.7500	0.7500	16.1011[**]
Error	41	1.9098	0.0466	

[Z] Inoculated tile beds vs. uninoculated tile beds

[NS] Not significant

[**] Significant at 1% level

Table 6

Survival of Head Cabbage Varieties in the Yellows-inoculated Tile Beds

Variety	Date Planted 3/9	5/25	7/20
CG (S^Z)	38/48[Y]	37/48	
EC (S)	43/48	38/48	30/32
GC (R)	47/48[Z]	47/48	29/32
RC (R)	44/48	45/48	

[Z] S: susceptible variety; R: resistant variety

[Y] Number of plants surviving/Number of plants planted

Evaluation of Resistance of Mustard Cabbage Varieties in the Preliminary Trials

The responses of the mustard cabbage varieties in the yellows-inoculated tile beds are shown in Table 7. In the March 9 trial, only the possibly resistant WT and the susceptible WS were included. There was no difference between the two. In the May 25 trial, after the beds had been reinoculated, there was a large difference between WT and PF, which had been substituted for the WS in the first trial. In the third trial on July 20, in which six varieties were included, not only WT, but also CHK had a very high survival rate. The most susceptible was PF, next were CRH and WS. KH was somewhat intermediate (Table 8). CHK and WT were chosen to be the resistant parents for making crosses, and CRH, PF, and WS were chosen to be the susceptible parents. KH was not used further.

Determination of Optimum Spore Suspension Concentration

A trial to determine an appropriate spore suspension concentration for inoculation of the segregating populations was run. The susceptible CRH, the resistant WT, and their F_2 were tested with three spore concentrations (Table 9). At the concentration of 10^4 spores/ml, only very light yellow symptoms appeared on some leaves of the susceptible CRH, even though two plants died. At the concentrations of 10^5 and 10^6 spores/ml, however, all the plants of CRH either died or were diseased seriously, while eighteen out of

Table 7

Survival of Mustard Cabbage Varieties in
the Yellows-inoculated Tile Beds in 1988 Trials

Variety	Date Planted 3/9	5/25	7/20
WT	20/24[Z]	13/20	28/32
WS	19/24		13/32
PF		2/24	7/32
KH			19/32
CRH			10/32
CHK			31/32

[Z] Number of plants surviving/Number of plants planted

Table 8

Comparison of Survival of Mustard Cabbage Varieties in the Yellows-inoculated Tile Beds in 7/20/88 Trial

Variety	No. of Plants	Mean ± SD[Z]	
CHK	32	96.88% ± 8.27%	a[Y]
WT	32	87.50% ± 12.50%	a
KH	32	59.37% ± 24.80%	b
WS	32	40.63% ± 24.80%	bc
CRH	32	31.25% ± 24.21%	c
PF	32	21.88% ± 19.52%	c

[Z] Mean and standard deviation of percentage of plants surviving

[Y] Duncan's multiple-range test at 5% significant level

Table 9

Influence of Spore Suspension Concentration on Infection of Mustard Cabbage Plants

Suspension con.(spores/ml)	Line	No. of plants at disease grades 0	1	2	3
10^4	CRH-1		8		2
	WT-1	10			
	F_2	6	4		
10^5	CRH-1			5	5
	WT-1	9			1
	F_2	3	3	1	3
10^6	CRH-1			4	6
	WT-1	9			1
	F_2	3	1	3	3

twenty of the resistant WT had no symptoms; and the F_2 ranged from no symptoms all the way to dead plants. It is suspected that the two plants of WT inoculated with 10^5 and 10^6, and the two plants of CRH at 10^4 may have died for other reasons. When analyzed statistically by the Kruskal-Wallis test, the 10^4 concentration was highly significantly different from the 10^5 and 10^6 concentration, but there was no difference between 10^5 and 10^6. Thus, the 10^5 spores/ml concentration was used for later inoculations.

Evaluation of Homozygosity of Parental Lines

The results of the test for homozygosity are given in Table 10. WT was the only variety showing no variation, while the highly susceptible PF was nearly as uniform. Only one plant of the 30 PF plants tested was not class 3. CHK, CRH, and WS each had one plant which seemed to be homozygous, and other plants which seemed to be segregating. Thus, the crosses that had been made with possible heterozygous plants CHK-1, CHK-3, CRH-2 and WS-2 were not used in further studies of inheritance. While it is not too surprising that CHK may not be uniform for resistance, it is surprising to find some seemingly resistant individuals in CRH and WS.

Table 10

Responses of Parental Lines to Cabbage Yellows by Inoculation

Variety	Line	No. of Plants at Disease Grades 0	1	2	3
CHK	1	7	1	2	
	2	10			
	3	9		1	
CRH	1				10
	2	1		2	7
PF	1				10
	2			1	9
	3				10
WS	1				10
	2		1	1	8
WT	1	10			
	2	10			

Inheritance of Resistance

1. Parental Line Disease Resistance

The results of the inoculation tests and tile bed tests of the parental varieties are shown in Table 11. CHK and WT were highly resistant, with only one CHK plant not in Class 0. CRH, PF, and WS were very susceptible. Nearly all plants were in Class 4, with just a few in Class 3. These results agree with those from the July 20, 1988 trial, and the test for homozygosity. It can also be seen that there are no differences between the artificial inoculation trials and natural infection in the tile bed.

2. F_1 Results

Each pair of reciprocal F_1 progeny (Table 12) was tested by the Kruskal-Wallis test which showed no significant differences between the reciprocals. Thus, reciprocal data were combined. Nearly all F_1 plants in all crosses were classified in Class 1, with only a few plants in Class 2 and one plant in Class 0.

3. F_2 Results

The F_2 results are presented in Table 13 and Figure 5. As expected, the F_2 progenies of resistant x susceptible parents segregated from complete resistance to complete susceptibility. Unexpectedly, the F_2 progeny of the two resistant parents also segregated from complete resistance to complete susceptibility. However, the progeny from the two resistant parents had more resistance (means 1.2 - 1.5) than the progeny with one susceptible parent (mean of 2.0 -

Table 11

Reactions of Mustard Cabbage Parental Varieties to Cabbage Yellows

Variety	Test[Z]	No. Plants at Disease Class 0	1	2	3	4	Total	Mean ± SD[Y]
CHK	I-1	9					9	0.0 ± 0.0
	F-1	12					12	0.0 ± 0.0
	I-2	7	1				8	0.1 ± 0.3
	F-2	9					9	0.0 ± 0.0
WT	I-1	10					10	0.0 ± 0.0
	F-1	13					13	0.0 ± 0.0
	I-2	10					10	0.0 ± 0.0
	F-2	10					10	0.0 ± 0.0
CRH	I-1					10	10	4.0 ± 0.0
	F-1				1	12	13	3.9 ± 0.3
	I-2				1	9	10	3.9 ± 0.3
	F-2				4	6	10	3.6 ± 0.5
PF	I-1					10	10	4.0 ± 0.0
	F-1					13	13	4.0 ± 0.0
	I-2					10	10	4.0 ± 0.0
	F-2					10	10	4.0 ± 0.0
WS	I-1					10	10	4.0 ± 0.0
	F-1				1	12	13	3.9 ± 0.3
	I-2					10	10	4.0 ± 0.0
	F-2				2	8	10	3.8 ± 0.4

[Z] I-1: inoculated on 8/29/89; reinoculated on 9/19/89
F-1: transplanted into infected soil on 8/30/89
I-2: inoculated on 10/3/89
F-2: transplanted into infected soil on 10/4/89

[Y] Standard deviation

Table 12

Reactions of F_1 Progeny to Cabbage Yellows

Parents	Test[Z]	No. Plants at Disease Class 0	1	2	3	4	Total	Mean ± SD[Y]
CHK x PF	I-1		10				10	1.0 ± 0.0
PF x CHK	I-1		10				10	1.0 ± 0.0
	F-1		12	1			13	1.1 ± 0.3
	I-2		8	2			10	1.2 ± 0.4
	F-2		9				9	1.0 ± 0.0
CHK x WS	I-1		8				8	1.0 ± 0.0
	F-1		11	1			12	1.1 ± 0.3
WT x PF	I-1		10				10	1.0 ± 0.0
	F-1		12	1			13	1.1 ± 0.3
PF x WT	I-1		10				10	1.0 ± 0.0
	F-1		10				10	1.0 ± 0.0
	I-2		8	2			10	1.2 ± 0.4
WT x WS	I-1		10				10	1.0 ± 0.0
	F-1		12	3			15	1.2 ± 0.4
WS x WT	I-1		10				10	1.0 ± 0.0
	F-1		12	1			13	1.1 ± 0.3
	I-2		7	3			10	1.3 ± 0.5
	F-2		10				10	1.0 ± 0.0
WT x CRH	I-1		9	1			10	1.1 ± 0.3
	F-1		13				13	1.0 ± 0.0
WT x CHK	I-1		14				14	1.0 ± 0.0
	F-1	1	12				13	0.9 ± 0.3
	I-2		7	2			9	1.2 ± 0.4

[Z] I-1: inoculated on 8/29/89; reinoculated on 9/19/89
F-1: transpalnted into infected soil on 8/30/89
I-2: inoculated on 10/3/89
F-2: transplanted into infected soil on 10/4/89

[Y] Standard deviation.

Table 13

Reactions of F_2 Progeny to Cabbage Yellows

Cross	Test[Z]	No. Plants at Disease Class 0	1	2	3	4	Total	Mean ± SD[Y]
CHK x PF	I-1	11	16	3	5	24	59	2.3 ± 1.9
	F-1	15	13	9	5	23	65	2.1 ± 1.6
	I-2	5	7	3	1	14	30	2.4 ± 1.6
	F-2	4	3	1	2	10	20	2.6 ± 1.7
CHK x WS	I-1	6	18	10	1	15	50	2.0 ± 1.4
	F-1	8	3	10	1	17	39	2.4 ± 1.6
	I-2	6	9	9	10	14	48	2.4 ± 1.4
	F-2	5	10	3	7	12	37	2.3 ± 1.5
WT x PF	I-1	14	12	1	8	24	59	2.1 ± 1.8
	F-1	10	8	10	11	14	53	2.2 ± 1.5
	I-2	10	6	12	2	26	56	2.7 ± 1.5
	F-2	8	10	8	6	22	54	2.4 ± 1.5
WT x WS	I-1	11	14	9	3	22	59	2.2 ± 1.6
	F-1	9	7		7	15	38	2.3 ± 1.7
	I-2	3	1	2	3	11	20	2.7 ± 1.6
	F-2	3	6	3	1	7	20	2.2 ± 1.5
WT x CRH	I-1	11	17	14	4	13	59	2.2 ± 1.7
	F-1	9	11	7	1	23	51	2.4 ± 1.6
	I-2	6	9	7	2	16	40	2.4 ± 1.6
	F-2	4	3	5	4	4	20	2.1 ± 1.4
WT x CHK	I-1	21	17	4	9	9	60	1.5 ± 1.5
	F-1	16	20	7	6	3	52	1.2 ± 1.2
	I-2	8	9	7	1	5	30	1.5 ± 1.4
	F-2	10	7	2	2	8	29	1.4 ± 1.7

[Z] I-1: Inoculated on 8/29/89; reinoculated on 9/1989
F-1: Transplanted into infected soil on 8/30/89
I-2: Inoculated on 10/3/89
F-2: Transplanted into infected soil on 10/4/89

[Y] Standard deviation

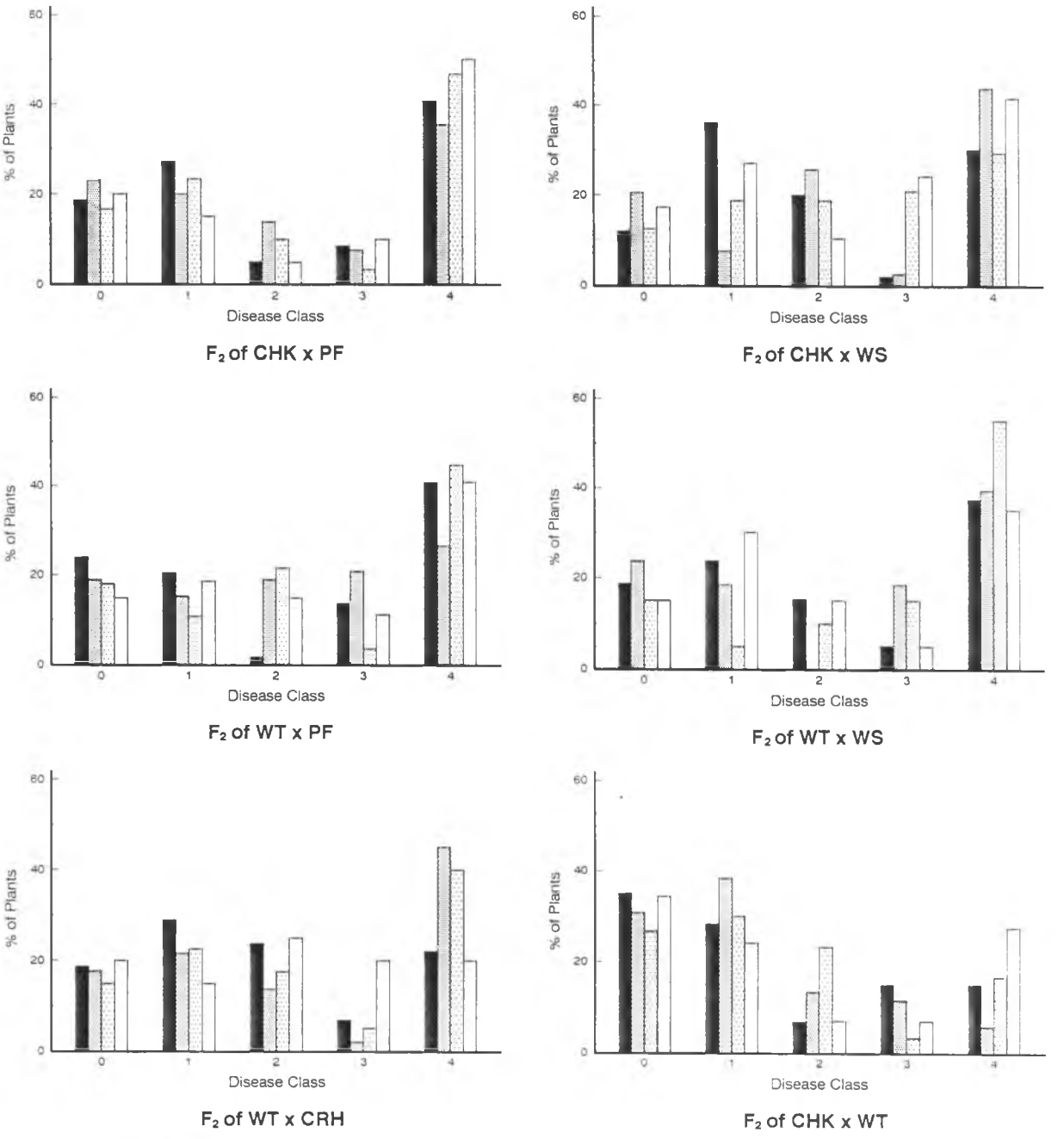


Figure 5. Reaction of F_2 to cabbage yellows

2.7). In Figure 5, it can be seen that in the F_2's of WT x CRH, WT x PF, WT x WS, CHK x PF, and CHK x WS, all of which had one susceptible parent, the most frequent class was Class 4. In WT x CHK, however, in which both parents were resistant, the most resistant classes, 0 and 1, are more frequent than Class 4. In fact, WT x CHK had 30.9% in Class 0 versus 17.5%, 15.1%, 18.8%, 17.0%, and 18.3% for the other crosses. There were no differences in resistance between different resistant x susceptible crosses. There were differences among the number of individuals in specific classes in different tests, but this is not considered important.

4. Backcross Results

Most backcrosses segregated very close to a 1:1 ratio, especially when backcrossed to a resistant parent (Table 14). If the backcross was to a susceptible parent, perhaps less than half were classified in Class 1 like the F_1, and more than half in the more susceptible classes. All backcrosses to resistant parents, however, segregated very closely to half in Class 1 like the F_1, and half in Class 0 like the parent. The F_1 between the two resistant parents also segregated 1:1 in the backcross to one of it parents. The other backcross was not obtained.

Determination of Inheritance

The F_1, F_2, and backcross results presented are quite consistent in all four tests.

Table 14

Reactions of Backcross Progeny to Cabbage Yellows

Parents	Test[Z]	No. Plants at Disease Class 0	1	2	3	4	Total	Mean ± SD[Y]
(CHK x PF) x PF	I-1		16		4	20	40	2.7 ± 1.4
(CHK x PF) x CHK	I-1	22	18				40	0.5 ± 0.5
(WT x PF) x WT	I-1	20	25	5			50	0.0 ± 0.6
	F-1	8	8	2			18	0.7 ± 0.7
(WT x WS) x WS	I-1		16	2	3	27	48	2.9 ± 1.4
	F-1		10		3	17	30	2.9 ± 1.4
	I-2		5	1	4	10	20	3.0 ± 1.2
	F-2		5		1	4	10	2.4 ± 1.4
(WT x WS) x WT	I-1	18	20	2			40	0.6 ± 0.6
(WT x CRH) x WT	I-1	17	20	1	2		40	0.7 ± 0.8
(WT x CHK) x WT	I-1	28	22				50	0.4 ± 0.5
	F-1	20	15				35	0.4 ± 0.5
	I-2	12	7				19	0.4 ± 0.5
	F-2	4	5				9	0.6 ± 0.5

[Z] I-1: Inoculated on 8/29/89; reinoculated on 9/19/89
F-1: Transpanted into infected soil on 8/30/89
I-2: Inoculated on 10/3/89
F-2: Transplanted into infected soil on 10/4/89

[Y] Standard deviation

The F_1's of crosses between a resistant and a susceptible parent fall into a class which is intermediate between the parents; not as susceptible as the susceptible parent, but definitely slightly less resistant than the resistant parent. Thus, complete dominance of either resistance or susceptibility can be ruled out.

The F_2's segregate as expected, into all classes. However, it is not a normal distribution as would be expected if resistance was controlled by quantitative genes. Instead, there is a preponderance of individuals in class 4 with significant numbers in class 0 like the resistant parent and in Class 1 like the F_1. Classes 2 and 3 have fewer individuals with more variation from test to test and progeny to progeny.

All the backcrosses, to either the resistant or the susceptible parent, segregate into mostly two classes in about a 1:1 ratio, suggesting possibly only one pair of qualitative genes is involved.

The results for the progeny of the cross between the two resistant parents, however, add additional complexities. The F_2 segregates, which suggests that the two parents have different resistant genes. In addition, the F_1 is in class 1 just like the other F_1's, and the backcross to one of the parents segregates 1:1, just like the other backcrosses to a resistant parent. This F_2, however, does differ from the other F_2's in having more resistant individuals. About 31% of this F_2 was in Class 0 versus only a little more than 17%

in the F_2's of the other crosses.

Thus, it seems the genetic control is qualitative rather than quantitative, and the genes in both the resistant parents, although they are not the same, do act in the same manner.

Possible Genetic Explanation

After studying various qualitative genetic possibilities, the following was derived which is in agreement with nearly all the results.

Each resistant parent differs from the susceptible parents by two pairs of genes, one of which shows dominance and other of which shows additive gene action and is epistatic to the first. Thus, if the genotype of the resistant parent is AARR, and of the susceptible parent is aarr, the following phenotypes and genotypes occur:

Completely resistant: A-RR

Partially resistant: A-Rr

Susceptible: --rr or aa--

The F_1 would thus be AaRr, which is partially resistant, and the F_2 would segregate into:

3 completely resistant:	1 AARR
	2 AaRR
6 partially resistant:	2 AARr
	4 AaRr
7 susceptible:	1 AArr
	2 Aarr

1 aaRR

2 aaRr

1 aarr

The backcross to the resistant parent would be 1 AARR : 1 AaRR : 1 AARr : 1 AaRr or 1 completely resistant : 1 partially resistant.

The backcross to the susceptible parent would be 1 AaRr : 1 Aarr : 1 aaRr : 1 aarr or 1 partially resistant : 3 susceptible.

If Class 0 is considered as completely resistant, Class 1 plus 2 as partially resistant, and Class 3 plus 4 as susceptible, then the F_2 and backcross ratios for the crosses between a resistant and a susceptible parent all fit the expected ratios except for the backcross of WT x WS to WS, in which there are too many Class 1 and 2 (Table 15). Perhaps some of the Class 2 individuals should have been classified more severely as Class 3.

Assuming that each resistant parent has its own two pairs of genes, one exhibiting dominance and one additive and epistatic to the first, but the genes in one resistant parent are independent of those in the other. Thus, the genotypes and phenotypes would be as follows:

Parents: AAR_1R_1 and BBR_2R_2

F_1: partially resistant: $AaR_1r_1BbR_2r_2$

F_2: 87 completely resistant: $A\text{-}R_1R_1$---- or

$$----B\text{-}R_2R_2$$

121 partially resistant: $A\text{-}R_1r_1B\text{-}R_2r_2$, $A\text{-}R_1r_1B\text{-}r_2r_2$,

Table 15

Segregation Ratios Observed in F_2 and Backcross Progeny of Crosses between One Resistant and One Susceptible Parents

Parents	No. of Plants	No. Plants at Disease Class 0	1+2	3+4	Expected Ratio	X^2 Value	Probability
CHK x PF	174	35	55	84	3:6:7	2.598	0.50-0.10
CHK x WS	174	25	72	77	3:6:7	2.490	0.50-0.10
(CHK x PF) x PF	40		16	24	1:3	4.033	0.05-0.01
(CHK x PF) x CHK	40	22	18		1:1	0.225	0.90-0.50
WT x PF	222	42	67	113	3:6:7	5.770	0.10-0.50
WT x WS	137	26	42	69	3:6:7	3.085	0.50-0.10
WT x CRH	170	30	73	67	3:6:7	2.184	0.90-0.50
(WT x PF) x WT	68	28	40		1:1	1.779	0.90-0.50
(WT x WS) x WS	108		36	72	1:3	7.111	0.01-0.001
(WT x WS) x WT	40	18	22		1:1	0.225	0.90-0.50
(WT x CRH) x WT	40	17	23		1:1	0.500	0.90-0.50

$A\text{-}R_1r_1bb\text{--}$, $A\text{-}r_1r_1B\text{-}R_2r_2$,

$aa\text{--}B\text{-}R_2r_2$

48 susceptible: $A\text{-}r_1r_1B\text{-}r_2r_2$, $A\text{-}r_1r_1bb\text{--}$,

$aa\text{--}B\text{-}r_2r_2$, $aa\text{--}bb\text{--}$

BC: 1 completely resistant: $A\text{-}R_1R_1\text{----}$

1 partially resistant: $A\text{-}R_1r_1\text{----}$

All the data fit the expected ratios with acceptable X^2 probabilities (Table 16).

Table 16

Segregation Ratios Observed in F_2 and Backcross Progeny of Crosses between Two Resistant Parents

Parent	No. of Plants	No. Plants at Disease Class 0	1+2	3+4	Expected Ratio	X^2 Value	Probability
WT x CHK	171	55	73	43	87:121:48	4.655	0.10-0.05
(WT x CHK) x WT	114	64	50		1:1	1.480	0.30-0.20

SUMMARY AND CONCLUSIONS

The inheritance of resistance to cabbage yellows, which has recently become a serious problem for mustard cabbage growers in Hawaii, was investigated. Resistant parents were a line called "Wild Type", which was descended from an off-type plant found in a commercial variety growing in a yellows-infected field, and a variety called "Chicken Heart Kaichoy", which is a variety grown in Canton, China. Crosses were made between the two resistant varieties and three susceptible ones (Waianae Strain, PF-3, and Chinese Round Heading) and between the two resistant varieties. F_1's, F_2's, and backcrosses were made and individual plants evaluated for disease reaction both after inoculation and by natural infection in previously-inoculated soil.

All of the parents used in the study were tested for homozygosity and were either uniformly resistant (Class 0) or uniformly susceptible (Class 4). Most F_1 plants in all crosses were graded in Class 1 and just a few in Class 2. There was no difference between reciprocal crosses. All the F_2's segregated from Class 0 to Class 4, with many plants in Class 0, 1, and 4, and less in Class 2 and 3. However, the two types of crosses had different F_2 distribution. There were more Class 0 plants in the Resistant x Resistant F_2 (26.7% - 34.5%) than in the Resistant x Susceptible F_2's (12.0% - 23.7%). All the backcrosses segregated 1:1 (1 like the F_1 : 1 like the backcross parent) except the backcrosses

to the susceptible parents, which had more than 50% susceptible individuals. However, the numbers tested were not very large and not all backcrosses were obtained.

The data fit neither a normal quantitative distribution, nor any simple qualitative ratio. After many studies, a possible genetic explanation is proposed. The resistant parents each have two pairs of genes for resistance, one of which shows dominance and one of which shows additive gene action and is epistatic to the first. Thus, there are three levels of resistance: 1) when locus 1 is homozygous or heterozygous resistant and locus 2 has two additive resistance genes, the plant is in Class 0; 2) when locus 1 is homozygous or heterozygous resistant and locus 2 has only one additive resistance gene, the plant is in Class 1 or 2; 3) when locus 1 is homozygous recessive or when locus 1 has a dominant resistance gene but none of the locus 2 additive resistance genes are present, the plant is in Class 3 or 4. The F_1 would thus be in Class 1, the F_2 would segregate at a ratio of 3 Class 0 : 6 Class 1 + 2 : 7 Class 3 + 4, and the backcrosses segregate 1 Class 1 + 2 : 3 Class 3 + 4 or 1 Class 0 : 1 Class 1. The data were analyzed by the Chi-square goodness of fit test, and all the progenies except one backcross fit the theoretical ratios.

Although both the resistant parents give the same kind of results when crossed with a susceptible parent, they clearly do not have the same resistance genes, since their F_2 segregates. It is assumed that they each have one locus

that exhibits dominance plus another with additive gene action that is epistatic to the first, as described above. The results obtained fit the hypothesis if it is assumed that the resistance genes from one parent do not interact with the genes from the other parent, even though they appear to be similar in their inheritance and production of resistance. With this assumption, the F_1 would still be Class 1, the F_2 should segregate 87 Class 0 : 121 Class 1 + 2 : 48 Class 3 + 4, and each backcross would give 1 Class 0 : 1 Class 1 + 2. The F_1, F_2, and the one backcross obtained fit this hypothesis very well.

Further studies are necessary to test this hypothesis. For example, F_3's could be made from Class 0 F_2 plants of resistant x susceptible crosses. One third should not segregate, while two third should segregate 3 Class 0 : 1 Class 3 + 4 if the hypothesis is correct. Likewise, Class 1 F_2 plants should segregate either 1 : 2 : 1 or 3 : 6 : 7. Backcrosses could also be tested. The progeny of the resistant x resistant cross can also be tested in the same manner.

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