### ABSTRACT

CALL, ADAM DEAN. Inheritance of Resistance to Downy Mildew in Cucumber (*Cucumis sativus* L.) PI 197088 and Effect of Interaction of Host Plant Resistance, Fungicides, and Environment on Severity of Downy Mildew on Cucumber. (Under the direction of Todd C. Wehner, PhD).

Downy mildew, caused by the oomycete pathogen *Pseudoperonospora cubensis* (Berk. And Curt) Rostov, is a major foliar disease of cucumber (Cucumis sativus L.) Currently, high yield and quality in the presence of downy mildew is achieved with multiple fungicide applications. Most of the currently grown cultivars have some resistance to downy mildew, in that they have lower levels of disease than susceptible check cultivars. Prior to a 2004 outbreak in the United States, host resistance was sufficient to control the disease, and downy mildew was only a minor problem on cucumber. Both host resistance and fungicides contribute to control of downy mildew for growers. There are currently no cultivars that show resistance at a level equal to that seen prior to 2004. However, highly resistant cultigens have been identified. Experiments were conducted to (i) evaluate fungicide programs with different levels of efficacy against downy mildew in combination with cultivars or breeding lines having different levels of resistance for their effect on disease severity and yield; (ii) determine the type of gene action controlling resistance in PI 197088, as well as to estimate genetic variances and inheritance of resistance; (iii) to test a set of cultigens which differed in their resistance to downy mildew to local isolates of P. cubensis in North Carolina each year, to evaluate the effect of environment on disease severity on different cucumber genotypes and to evaluate the stability of resistance in cultigens with good quality traits, to identify genotypes having high or moderate resistance and high stability for resistance, which would be useful in breeding new highly resistant cultivars.

The effects of cultivar resistance and fungicides appear to be additive until a threshold where maximum yield is reached. Highly resistant cultigens, such as PI 197088, required only the least effective fungicides to achieve highest yields, while moderately resistant cultigens required a more effective fungicide to reach a similar level of yield. Susceptible cultigens did not achieve high yield even with the most effective fungicide treatments. It is likely that, even as highly resistant cultivars are released, growers will need to continue a minimal fungicide program to achieve maximum yield.

Resistance in PI 197088 failed to fit the single gene model indicating that resistance is under control of a more complex genetic system. Thus, we suggest that resistance to downy mildew in PI 197088 should be regarded as a quantitative trait for breeding purposes. Genetic effects were greater than environmental effects. Additive variance and broad- and narrow-sense heritability were generally large in our study, with resistance attributed to a few loci. The estimated gain from selection indicated possible improvement of two or more points of resistance (on a nine-point scale) per generation under high selection intensity.

A major breeding objective in cucumber is moving the newly identified high levels of resistance in cultigens such as PI 197088, into adapted varieties with good quality traits. The environment can have a large effect on the severity of plant disease, and it is important to understand the environmental effect on disease tests. 15 cultigens, were tested at Clinton, NC over 7 years to evaluate the effect of genotype, year, and genotype-year interaction. Cultigen had the largest effect (43% of total sums of squares), followed by year (24% of total sums of squares), and cultigen-year interaction (16% of total sums of squares). The cultigens in this study with the lowest mean downy mildew rating, a  $b_i$  close to unity and non-significant  $S^2_d$ , included 'Poinsett 76', a slicing cucumber, and WI 2238, a pickle type

cucumber. These cultigens are recommended for use in breeding with the highly resistant plant introduction accessions.

© Copyright 2012 by Adam Call All Rights Reserved Inheritance of Resistance to Downy Mildew in Cucumber (*Cucumis sativus* L.) PI 197088 and Effect of Interaction of Host Plant Resistance, Fungicides, and Environment on Severity of Downy Mildew on Cucumber

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## BIOGRAPHY

I was born in Kansas City, Kansas on the 25<sup>th</sup> of March, 1983. For as long as I can remember, science has been a primary focus in my education. I realized my interest in plant science while continuing my grandfather's tomato gardening tradition. From that time forward, I began taking classes in horticulture, further expanding my interest and knowledge. During my undergraduate studies at Kansas State University, I worked in a pathology lab which focuses on bacterial leaf blight in rice. My summers were spent managing markets for a vegetable farmer in the area. I also spent one summer as an intern on the Cucurbit Breeding Project at North Carolina State University. Following my undergraduate studies at KSU, the desire to continue my education led me back to NCSU. After completing my M.S. in plant breeding, the desire to continue my research and education let me to continue on to achieve a PhD. I look forward to using my skills and knowledge as a breeder in private industry.

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## **GENERAL INTRODUCTION:**

## A REVIEW OF RESISTANCE TO DOWNY MILDEW ON CUCUMBER

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## Introduction

Cucumber (*Cucumis sativus* L.) is a major vegetable crop used in most countries around the world. In 2008, 61,399 hectares of processing and fresh market cucumbers with a value of \$421 million were grown in the United States. In 2008, in North Carolina, 7,284 hectares were planted with a value of \$25 million (USDA, 2009).

Cucurbit downy mildew, caused by the oomycete pathogen *Pseudoperonospora* cubensis (Berk. And Curt) Rostov, is a major foliar disease of cucumber (Palti and Cohen, 1980). Cucumber cultivars resistant to downy mildew have been developed (Sitterly, 1973; Wehner and Shetty, 1997) over the past 50 yr. In fact, most of the currently-grown cultivars have a moderate level resistance to downy mildew. In many cases, this resistance can be traced back to PI 197087, originally identified as resistant by Barnes and Epps (1954). PI 197087 was used by Carroll Barnes, at Clemson University, to develop resistant cultivars such as 'Polaris', 'Poinsett', 'Pixie', and 'Chipper', which were released from 1961 to 1973 (Wehner and Shetty, 1997). The cultivar 'Polaris', available to growers in 1961, was the first of these to be released. From 1961 to 2003, the dm-1 resistance gene from PI 197087 was sufficient to control downy mildew, and the disease was only a minor problem on cucumber. Between 1982 and 1988, the estimated incidence of downy mildew on cucumbers in North Carolina was 30% (St. Amand and Wehner, 1991). The dollar loss per year averaged 2.9% based on yield and quality reduction. However, cultivars in the United States have been less resistant since 2004. Cucumber yield losses from downy mildew remained minimal compared to other diseases until a resurgence of the pathogen in 2004 when a more virulent

strain of *P. cubensis* caused a 40% yield loss for cucumber growers in the United States (Colucci et al., 2006). The new strains of *P. cubensis* continue to infect cucumber in most production areas in the United States and remain a major threat to cucumber production in warm humid regions around the world. Most cultivars currently grown in the United States have some resistance, but not at the level seen prior to appearance of the new race in 2004. Since then fungicide application programs have been necessary to achieve high yield and quality, resulting in increased cost to growers. Strains of *P. cubensis* resistant to some fungicides have been reported (Reuveni et al., 1980), and new sources of genetic host resistance are in high demand.

#### **Downy Mildew Symptoms**

Symptoms of cucumber downy mildew generally occur only on the foliage. Infection first appears as small, water-soaked lesions on the underside of leaves. Lesions are often angular, being bound by leaf veins, and turning chlorotic to varying degrees. Sporulation occurs on the undersides of the leaves. Chlorotic lesions eventually turn necrotic. Symptoms vary depending on relative susceptibility of the cultigens. The most resistant cultigens exhibit a hypersensitive response (HR) with small necrotic or chlorotic flecks and sparse sporulation, while the most susceptible cultigens are highly chlorotic and necrotic (Personal Observation).

The HR type resistance was first described by Barnes and Epps (1954) in cucumber PI 197087, and controlled by a single resistance gene. The downy mildew lesions on PI 197087 were different from other resistant cultivars. The lesions were described as irregularly shaped, brown, with a slight water-soaked appearance. There was no chlorosis,

and lesions remained as small, brown spots. The leaf tissue died shortly after infection and there was little to no sporulation on the leaf undersides. That accession was previously reported as having some resistance to downy mildew, as well as immunity to anthracnose (Barnes and Epps, 1952). Resistance from PI 197087 was used in the development of new cultivars, and the resistance in many current cultivars traces to PI 197087 (Wehner and Shetty, 1997). That resistance, which was effective for cucumber production in warm humid regions of the United States without fungicides for over 40 years, has since been overcome by a new race of the pathogen.

## **Resistance of Current Cultivars**

Currently, no available cultivars have a high level of resistance to downy mildew. A recent study (Call et al., 2012a) identified the most resistant and high yielding cultivars from a set of 86 cultivars including 23 checks, tested in North Carolina and Michigan. None of the cultigens tested in that study showed a high level of resistance, although differences in resistance were detected. Lines WI 2757 and M 21 and cultivar 'Picklet' were consistently among the top resistant lines in North Carolina and Michigan. The cultivars 'Coolgreen', 'Wis.SMR 18', and 'Straight 8' were identified as moderately to highly susceptible. An unreleased hybrid, 'Nun 5053 F1', and the cultivar 'Cates' were the top yielding lines overall. The highest yield in a single year and location was from the cultivar 'Cates' in Clinton, North Carolina in 2009, with 25.6 Mg/ha. The best cultivars in this study were only moderately resistant, and would likely require fungicide applications to achieve high yield and quality in the presence of downy mildew.

Recent studies (Call et al., 2012b) have focused on identifying higher resistance to the new race of downy mildew in cucumber. Breeders are interested in identifying Plant Introduction (PI) accessions, cultivars, or breeding lines (hereafter collectively referred to as cultigens) having resistance (reduced leaf damage) or tolerance. The combination of different resistance mechanisms may result in higher overall resistance. The development of highly resistant cultivars that are also tolerant should reduce the need for fungicides.

## **Evaluation of Host Resistance**

Several different methods of evaluating resistance to downy mildew have been used by pathologists and breeders interested in developing resistant cultivars. These methods rate the expression of different resistance mechanisms such as chlorosis and necrosis, stunting, sporulation, lesion size, and tolerance (yield with and without disease). Each of these resistance traits could be exploited to develop cultivars highly resistant to downy mildew.

Chlorosis and necrosis are two of the major symptoms of downy mildew. Variation can be seen for each, but the two are highly correlated, and likely controlled by the same gene (Call et al., 2012a; Call et al., 2012b). Nevertheless, the two are often rated separately, and may provide better data than using a combined rating. There are likely genes with small effect that play a role, so there may be additional progress to be made by selection. It would likely be beneficial to select for low overall chlorosis and necrosis and for an increased necrosis to chlorosis ratio because *P. cubensis* is an obligate biotroph and would be unable to survive on necrotic tissue. This may also result in reduced sporulation due to less living leaf tissue available.

Lesion type and size is an important factor in resistance to downy mildew. Bains (1991) described four categories of lesion type: 1. faded green to dull yellow lesions, size restricted, slow necrosis; 2. yellow spots or flecks, non-angular, slow growing, slow necrosis; 3. bright yellow, large, angular, fast growing, susceptible type, high sporulation; 4. necrotic spots or flecks, non-angular, little chlorosis, HR type. The lesion type of most currently-grown cultivars is category 3 (Call, 2012b). Smaller lesions result in less tissue for sporulation. Reduced sporulation is beneficial by limiting local reinfection and reducing inoculum available for long distance aerial dispersal.

Sporangia of *P. cubensis* are wind dispersed and can travel long distances (Ojiambo and Holmes, 2011) in short periods of time. The reduction of the amount of inoculum produced by resistant cultivars should slow overall disease progress and reduce the severity of downy mildew in cucumber production areas. Evaluation of sporulation can be done in the laboratory by examining whole leaves or leaf discs, or using a hemocytometer to estimate spore count. Criswell et al. (2008) reported that rating sporulation in the field using a subjective 0 to 9 scale was as effective as using a hemocytometer to count spores, and was much faster. He also reported was that leaves could be stored in plastic bags for 1 to 2 days without altering results. Leaves from the base of the plant gave a more useful response, because leaves in the middle and tip did not have sufficient time for sporangia to develop.

Vigorous plants can outgrow disease during stages of rapid vegetative growth. Such plants are large and have many leaves. Although large plants can be a problem, such as more surface area for disease spores to infect, there are also benefits. Large plants can give more surface area for photosynthesis, even if some is lost to disease. It may be that yield reduction from downy mildew infection in large plants is compensated for by the increased leaf area relative to smaller plants. Breeders should select for large, vigorous plants with multiple branches. These traits seem to be related to downy mildew tolerance

Chlorosis and necrosis, high vigor, resistance to sporulation, small lesion size, and tolerance are important components of resistance to downy mildew. Breeders should select for improvement in each trait separately in lines that can be intercrossed and later be combined in single cultivar.

### **Evolutionary Potential of** *P. cubensis*

The population genetic structure of a pathogen can be indicative of its potential to overcome resistance or become insensitive to a fungicide (McDonald and Linde, 2002). The mating system of *P. cubensis* is mixed, reproducing asexually and sexually (Cohen and Rubin, 2011), having the highest evolutionary potential. Adding to that are the many spores moving large distances. Effective population sizes are massive with concentrations of sporangia on lesions up to  $4.0 \times 10^3$  /cm<sup>2</sup> (Cohen, 1981). *P. cubensis* is classified as having a high evolutionary potential, along with *Blumeria graminis* (powdery mildew of cereals), and oomycetes *Bremia lactucae* (downy mildew of lettuce) and *Phytopthora infestans* (late blight of potato) (McDonald and Linde, 2002).

Even with such high evolutionary potential, resistance can be effective for long periods of time. From 1961 to 2003, the dm-1 resistance gene from PI 197087 was sufficient to control downy mildew, and the disease was only a minor problem on cucumber in the

United States. Nevertheless, it is important to recognize the potential of *P. cubensis* to overcome host resistance and fungicides.

### **Downy Mildew Control Using Fungicides**

Chemical control of downy mildew is necessary to achieve high yield in the absence of high host plant resistance. Jenkins (1942) mentioned that "various sprays and dusts have been recommended" to control downy mildew and some copper dusts were partially successful. Jenkins (1946) also reported that most commercial growers use dust or spray fungicides, but control was not satisfactory. Barnes and Epps (1950) reported on the use of mancozeb (source Dithane) fungicide in some studies. In these studies, susceptible cultivar 'Marketer' produced roughly twice the yield when treated with mancozeb compared to an unnamed "less effective" fungicide. For the resistant cultivar 'Palmetto', an increase of 7% in fruit number was found when treated with mancozeb compared to the unnamed treatment. Roberts (1955) reported that maneb or zineb sprays and dusts were effective in South Carolina against cucumber downy mildew as well as anthracnose in an annual summary of reports on fungicide tests by 75 pathologists. Mancozeb, maneb, zineb, and nabam are all related dithiocarbamate multi-site inhibitor (protectant) fungicides. Sowell (1958) reported nabam plus zinc sulfate as giving excellent control and high yield. Zineb was equal to nabam+MnSO<sub>4</sub> in disease control but yielded less in one season. Maneb and nabam+MnSO<sub>4</sub> also yielded lower than nabam+ZnSO<sub>4</sub>. Protectant fungicides such as these are now often tank-mixed with more effective systemic fungicides.

The discovery of systemic fungicides was a major advance over protectant fungicides in control of downy mildew. Systemic fungicides, in the absence of resistant pathogen biotypes, can provide effective control, at a cost. Cohen (1979) reported on the effectiveness of two new systemic fungicides, prothiocarb and propamocarb (derivative of prothiocarb) against downy mildew. Both prothiocarb and propamacarb were reported to have very good activity against downy mildew. Briggs et al. (2006) reported fluopicolide as having a novel mode of action controlling a wide range of oomycete pathogens. Typically, a protectant fungicide and a systemic fungicides with different modes of action, in order to reduce selection pressure for resistance in the pathogen. An example of such a program is propamocarb (Previcur Flex) and chlorothalonil (Bravo) alternating with famoxadone + cymoxanil (Tanos) + mancozeb (Manzate).

Resistance to systemic fungicides has been reported by many authors (Pappas, 1982; Cohen and Samoucha, 1984; Baines and Sharma, 1986; Ishii et al., 2001; Zhu et al., 2007). Eshet and Dinur (1970) reported downy mildew on test plots treated with benomyl (source Benlate), suggesting that resistance to benomyl has been observed. At a congression of the Mediterranean Phytopathological Union in 1980, Pappas (1981) reported good control of *P. cubensis* by phosetyl-Al, under conditions favoring disease. Metalaxyl was reported as less effective and *P. cubensis* was shown to have pathotypes that were resistant or insensitive to the fungicide. Cross resistance was reported by Cohen and Samouch (1984) where four systemic fungicides were not effective against strains of *P. cubensis* that were resistant to metalaxyl. Blum et al. (2011) reported resistance by *P. cubensis* to carboxylic acid amide group fungicides such as dimethomorph and mandipropamid is due to a single point mutation in the *CesA3* gene.

The loss of fungicide efficacy through resistance has led to strategies aimed at reducing selection pressure on pathogens. Typically, a protectant fungicide and a systemic fungicide are tank mixed and alternated weekly with a different tank mix consisting of fungicides with different modes of action. An example of such a program is propamocarb (Previcur Flex) and chlorothalonil (Bravo) alternating with famoxadone + cymoxanil (Tanos) + mancozeb (Manzate).

Non-fungicidal secondary effects on yield have been reported for some fungicides, but such effects have been shown only for triazoles and strobilurins, which were not used in this study. Martens et al. (1968) reported no yield effect on oats treated with maneb, but a possible decrease in yield due to treatment with Plantvax (oxathiin;2,3 dihydro-5carboxanilido-6-methyl-1-4-oxathiin-4,4-dioxide). Siefert and Grossman (1996) showed that in wheat, the triazole fungicide epoxiconazole, enhanced green leaf pigmentation, delayed senescence, reduced water consumption and improved stress resistance. Other studies showed no yield effect due to strobilurin fungicides in sugar beet (Beta vulgaris) (Kahn and Carlson, 2009) and soybean (Glycine max (L.) Merr.) (Swoboda and Peterson, 2009) in the absence of disease.

The development of fungicides with different modes of action continues, as the threat of a breakdown in efficacy is unrelenting. Lebeda and Urban (2007) showed that 152

isolates of *P. cubensis* collected over 3 years (2001-2003) exhibited diverse pathogenicity and also shifted to higher pathogenicity and less diversity on a set of 12 cucurbit differentials. They also concluded that *P. cubensis* is a pathogen of high evolutionary potential.

## **Sources of Host Resistance**

There are at least three major genes for resistance to downy mildew in cucumber. One of the earliest sources of resistance came from a Chinese cultivar used in 1933 at the Puerto Rico Agricultural Experiment Station. In 1932, that station and others tested over 150 cultivars and found them to be susceptible to downy mildew. The new Chinese cultivar introduced in 1933 was highly resistant, but had long and curved fruit that were not commercially acceptable. This cultivar was crossed with the best available commercial cultivars in terms of quality to combine the resistance with good horticultural traits. This eventually led to seven highly resistant lines having good agronomic characteristics. Of these, PR 37, PR 39, and PR 40 had high quality fruit and yield superior to commercial checks. These were then used for the development of resistant breeding lines and cultivars (Barnes et al. 1946; Barnes, 1955; Barnes and Epps, 1955).

Barnes (1948) developed the downy mildew resistant cultivar 'Palmetto' from PR 40 crossed with susceptible 'Cubit'. 'Palmetto' was released in 1948 and was highly resistant; it had small lesions and little sporulation, and could be grown without fungicides in the presence of downy mildew (Epps and Barnes, 1952). Limited area was planted in 1948 and 1949. In those years, downy mildew was only found on Palmetto when it was planted adjacent to susceptible cultivars, such as 'Marketer'. Lesion type was as described earlier. In

1950 and 1951, all 'Palmetto' fields inspected showed infection, regardless of proximity to 'Marketer' or other susceptible cultivars. In those years, the lesions were large and sporulated heavily, typical of lesions of susceptible cultivars. Because it is unlikely that the change from 1948 to 1950 was due to a change in resistance, the change likely was in the pathogen population, either through mutation, selection in a mixed population, or migration of a race from a different region.

Wehner and Shetty (1997) summarized downy mildew resistance in the United States Department of Agriculture (USDA) germplasm collection of cucumbers that had been tested in North Carolina in 1988 and 1989 during an unusually severe epidemic of downy mildew. They reported that the most resistant cultigens were of U.S. origin and were primarily elite cultivars and breeding lines. The most resistant cultigens, for which multiple-year data were available, were Gy 4, 'Clinton', PI 234517, 'Poinsett 76', Gy 5, 'Addis', M 21, M 27, and 'Galaxy'. The resistance in those cultigens traced to PI 197087, which was originally identified as resistant by Barnes and Epps (1954). Interestingly, PI 197087 was found to be only intermediate in resistance in 1988 and 1989, indicating a change in the PI accession since its use in breeding in 1952. Because lines tracing resistance to PI 197087 were still highly resistant, it is likely that resistance was lost in PI 197087 either through a mutation or more likely during maintenance increase at the North Central Regional Plant Introduction Station in Ames, IA. PI 401734, which is PR 39 from the Puerto Rico Agricultural Experiment Station, was moderately susceptible, indicating the resistance gene that was overcome in 'Palmetto' was still not effective. This was also reported by Criswell (2008) and

Wehner and Shetty (1997). In both studies, PI 234517 (SC 50) which has resistance from both PI 197087 and PR 40 (through 'Ashley'), was compared to PI 197087, having only one known resistance gene. PI 234517 was equally resistant to PI 197087, indicating that no additional resistance was gained from the presence of the gene tracing to PR 40. The cultivar 'Ashley', which traces its resistance to PR 40, was also no longer resistant. They therefore concluded the gene from PR 40 was no longer effective.

New sources of resistance (reduced leaf damage) and tolerance (good yield under disease presence) to the race(s) of downy mildew in the U.S. since 2004 were identified in a large germplasm screening study and a multiple year re-evaluation of the most resistant and susceptible cultigens conducted at North Carolina State University (Criswell, 2008; Call et al., 2012b). Cultigens were identified that outperformed currently grown cultivars for disease resistance and yield traits. The most resistant cultigens from the germplasm screening test were PI 197088, Ames 2354, PI 267942, Ames 2353, PI 197085, PI 330628, PI 432878, and PI 618931 (Criswell, 2008). Cultigens found to be resistant in the initial screening were also resistant cultigens over all environments in the retest were PI 605996, PI 330628, PI 197088, PI 197086, PI 605924, PI 197085, PI 618893, and PI 432886. PI 618907 and PI 197086 also had tolerance and high yield. See Call et al. (2012b) for the complete results from the screening and retest.

Interestingly, the most resistant cultigens from our recent screening (Criswell, 2008; Call et al., 2012b), including PI 330628, PI 197088, PI 197086, and PI 197085, were only moderately resistant in the screening from 1988 and 1989 (Wehner and Shetty, 1997). The most resistant cultigens from the early study, such as Gy 4, PI 234517, 'Poinsett 76', M 21, were generally intermediate in our recent study. The shift in the pathogen population has changed the resistance ranking of the cultigens, although susceptible cultigens were susceptible in both screening studies. Possible explanations of this include different pathotypes or races in the field, and difference in plant stage at the time of rating. The more recent screening was rated multiple times over a few weeks, while the older screening was rated only on a single occation.

## **Inheritance of Host Resistance**

Several studies have dealt with the inheritance of downy mildew resistance in cucumber. Shimizu et al. (1963) reported that resistance in 'Aojihai' was controlled by three recessive genes (proposed  $s_1$ ,  $s_2$  and  $s_3$ ). Pershin et al. (1988), using cultivar 'Sadao Rischu', determined resistance to be controlled by at least three major genes exhibiting partial dominance that were linked to at least three genes for powdery mildew resistance.

Doruchowski and Lakowska-Ryk (1992) had evidence that downy mildew resistance was controlled by three recessive genes (dm-1, dm-2 and dm-3), where dm-3 and either dm-1 or dm-2 had to be homozygous recessive for maximum resistance. However, there was discrepancy in the F<sub>2</sub> results, which did not agree with their model. They argue that this resulted from testing too narrow a population. Petrov et al. (2000) claimed that the resistance in J-13, which was derived from Wisconsin 2843 (resistance originally from PI 197087 according to Peterson et al., 1985) was not inherited in a clear manner, but suggested it was due to one or two incompletely dominant genes.

El-Hafaz et al. (1990) report that the cultivars 'Palmetto' and 'Yomaki' were resistant in Egypt. They concluded that resistance was the result of an epistatic interaction between a dominant susceptible gene and a recessive resistance gene. Interestingly, 'Palmetto', along with 'Ashley', traces its resistance to P.R. 40 (Barnes, 1948; Barnes, 1955), and was resistant in the U.S. during limited use in 1948 and 1949. By 1950 the resistance was no longer effective.

Resistance in 'Poinsett', which traces to PI 197087 was reported by Van Vliet and Meysing (1974) to be from at least one single recessive gene, dm (dm-1). In addition, they proposed that the downy mildew gene was linked with the genes for powdery mildew resistance and for dull green fruit color (D). This resistance was effective in the United States from 1961 to 2003, suggesting that resistance from dm-1 is a distinctly different from resistance tracing to P.R. 40. In a following study, Van Vliet and Meysing (1977) confirmed that the gene for hypocotyl resistance to powdery mildew was linked with or identical to the gene for resistance to downy mildew. Fanourakis and Simon (1987) reported agreement with Van Vliet and Meysing (1974) confirming that downy mildew resistance is controlled by a single recessive gene. They also reported loose linkage with powdery mildew resistance (pm) and compact plant (cp) genes.

Angelov (1994) reported that PI 197088 resistance was due to two recessive genes. PI 197088 was collected from the same region and at the same time as PI 197087, the source of dm-1. It appears that there are at least three genes for resistance to downy mildew in cucumber: one from the Chinese cultivar used in developing the PR lines, one from PI 197087, and one from PI 197088. It is possible that one of the genes reported by Angelov (1994) in PI 197088 is shared with the dm-1 gene in PI 197088.

Conflicting results regarding the inheritance of downy mildew resistance in cucumber is likely due to multiple factors. First, the pathogen is highly variable and pathogen populations have not been well studied for the factors causing virulence (Lebeda and Urban, 2004). Different genes may be involved in resistance to different races and pathotypes.

Differences in the environment, including temperature, humidity, rainfall and inoculum movement by wind all influence the severity of downy mildew infection (Cohen, 1977). Interactions between pathogen, host and environment are complex and not easily determined. Greenhouse tests are important for reducing environmental variability and should be conducted in addition to field tests. High variability in pathogen-host interactions due to environment can cause simply inherited traits to appear polygenic. This may be misleading and continuous variation with no clear segregation, even in homozygous inbred lines, can also indicate low heritability (Shaner, 1991). Horejsi et al. (2000) measured a low broad-sense heritability for downy mildew resistance, and noted large plant-to-plant variability in their study.

Different mechanisms of resistance have been studied (Angelov and Krasteva, 2000; Baines, 1991; Barnes and Epps, 1950; 1954; Palti and Cohen, 1980; Tarakanov et al., 1988). The previously mentioned inheritance studies used different mechanisms of resistance when

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evaluating plant response. Doruchowski and Lakowska-Ryk (1992) used necrotic lesions; Van Vliet and Meysing (1974; 1977) and El Hafaz et al. (1990) used sporulation intensity; Fanourakis and Simon (1987) used incidence of chlorotic and necrotic lesions on cotyledons; and Petrov et al. (2000) used chlorotic lesions for rating resistance. Other studies did not specify how resistance was measured. Different mechanisms of resistance may have different inheritance patterns.

Original source of resistance varies over downy mildew inheritance studies. Some studies evaluated resistance sources from PI 197087 (India) while other studies evaluated resistance from P.R. 40 (China) and other germplasm sources. Although, P.R. 40 is not available in the germplasm collection, cultivars tracing resistance to P.R. 40 are. Those include 'Ashley' and Ames 4833. The combination of the two different sources should provide either better resistance or more durable resistance. This combination can be found in PI 234517 (SC-50), which does have slightly higher resistance to downy mildew than 'Ashley' or PI 197087. However, the difference between PI 234517 and cultivars having resistance from PI 197087 alone was not significant (Wehner and Shetty, 1997). This is not surprising as this resistance was overcome in 1950 in the cultivar 'Palmetto'.

There are at least three genes for resistance to downy mildew, coming from P.R. 40, PI 197087, and PI 197088. Resistance from P.R. 40 provides only a slight advantage over highly susceptible lines. Cultivars with resistance tracing to PI 197087 are moderately resistant to the current downy mildew in the United States, but still require fungicides to reduce yield loss. PI 197088 is highly resistant, but does not meet fruit type and quality

requirements in the United States. Resistance to downy mildew in PI 197088 does not appear to be inherited as a single gene in our studies. Observations that some of the highly resistant accessions, including PI 197088 are less resistant at the prior to tip-over stage (change from vertical to prostrate growth due to plant physically "tipping over") and more resistant past tip-over stage indicate resistance in PI 197088 may be adult-type resistance (personal observation). New cultivars need to be developed which have a combination of high adult-type resistance with the quality traits and dm-1 resistance of modern cultivars.

## **Pathotypes and Races of Pseudoperonospora cubensis**

Pathotypes and races represent variability in the pathogen population. Pathotypes are defined by their pattern of infection on a standard set of host differentials. Races are defined by differential compatibility on a set of genotypes within a species. Thomas et al. (1987) established a differential set of 26 cultivars spanning 13 species including *Cucumis sativus*, *Citrullus lanatus*, *C. melo* var *reticulatus*, *C. melo* var *conomon*, *C. melo* var *acidulous* and *Cucurbita pepo*. Using this differential, they identified five pathotypes of *P. cubensis* among eight isolates from the United States, Israel, and Japan. An improved differential set was developed by Lebeda and Widrlechner (2003) consisting of 12 genotypes that are well characterized with germplasm available as accessions. Lebeda and Gadasová (2002) reported 13 pathotypes from 22 isolates collected in Czech Republic, Spain, France and the Netherlands. Lebeda and Urban (2007) reported high pathogenic variability along with a shift to high pathogenicity among isolates collected the Czech Republic from 2001 to 2003. In 2001, there were 33 pathotypes among 42 isolates, 16 among 54 isolates in 2002 and 13

among 56 isolates in 2003. There was a decrease in variability from one year to the next, with a higher percentage of isolates reported as highly pathogenic each year.

Pathogenic variation is also high in the United States. Colluci (2008) reported 32 different compatibility patterns on 12 cucurbit hosts from 32 isolates collected in the United States, indicating a high variability in pathogenicity among populations in the United States. All isolates tested showed a high level of compatibility with cucumber. Several races of *P. cubensis* have been reported in differential test studies in many regions of the world (Angelov et al., 2000; Bains and Jhooty, 1976; Lebeda et al., 2006; Palti, 1974; Shetty et al., 2002). Shetty et al. (2002) proposed that at least two races of downy mildew exist, one in Asia, and one in Europe and North America. They also stated that there was no evidence for race differences between the United States and Poland. It is likely that multiple races exist of a pathogen with such high variability. There have been at least 3 races in the United States, corresponding to the 3 resistance sources: resistance from P.R. 40 was effective against *P. cubensis* in 1948 and partially in 1949; dm-*I* conferred resistance in cultivars from 1961 to 2003; PI 197088 (among others) is resistant to the new downy mildew in the United States since 2004.

#### **Disease Resistance and the Cucumber Genome**

In 2009, the genome of cucumber was published (Huang et al., 2009), reporting that of the predicted 26,682 genes of cucumber, 61 were identified as nucleotide-binding site (NBS)-containing resistance (NBS-R) genes. NBS domains, along with lucine-rich repeat (LRR) domains are components of the majority of cytoplasmic resistance proteins (Bent and Mackey, 2007). Huang et al. (2009) also noted that the *LOX* gene family, involved in plant defense and pest resistance, is expanded in cucumber compared to other sequenced genomes, and the that 19 or 23 *LOX* genes are arranged in three clusters, which has only been observed in the grapevine (*Vitus sp.*) genome. In addition, homologues of two genes shown to provide enzymatic resistance to downy mildew when overexpressed in melon (*Cucumis melo* L.) (Benjamin et al. 2009; Taler et al., 2004) were identified in the cucumber genome, *At1* and *At2*. The melon genes *At1* and *At2* are glyoxylate aminotransferase genes, and potential candidate genes for downy mildew resistance in cucumber.

## Conclusion

From 1961 to 2003, the *dm-1* resistance gene from PI 197087 was sufficient to control downy mildew, and the disease was only a minor problem on cucumber. The pathogen reappeared as a major problem in 2004, causing a 40% loss for cucumber growers (Colucci et al., 2006). Since then, downy mildew has continued to be a major disease of cucumber in the eastern United States, where conditions are favorable for disease. Cultivars with a high level of resistance prior to 2004 are only moderately resistant to the new race of *P. cubensis*. Growers in the United States now use these cultivars with moderate resistance in combination with multiple fungicide applications for disease management, which significantly increases the cost of production. We have studied the interaction of cultivars having different levels of restance with fungicides having different efficacys, to determine how host resistance and fungicides interact and the effect on apparent disease and yield.

New sources of resistance have been identified in the USDA germplasm collection. Traits of these accessions include reduced leaf necrosis and chlorosis, high vigor, reduced sporulation, and tolerance. New cultivars need to be developed which have a combination of good agronomic traits and resistance genes. Planning an efficient breeding program requires estimates of heritability, number of effective factors (genes) and gene action of this newly identified resistance. Families have been developed using resistant and susceptible parents to determine the qualitative and quantitative inheritance of this resistance.

Finally, the environment can have a large effect on the severity of plant disease. Differences in the environment, including temperature, humidity, rainfall and inoculum movement by wind all influence the severity of downy mildew infection (Cohen, 1977). Cultigens having high stability for resistance along with good quality traits are ideal for use in breeding with newly identified highly resistant cultigens. A set of cultivars differing in resistance to downy mildew was grown over 7 seasons in North Carolina to evaluate the effect of environment on disease severity on different cucumber genotypes and look for large rank change which may indicate the presence of different races and to evaluate the stability of resistance in cultigens with good quality traits, to identify genotypes having high or moderate resistance and high stability for resistance, which would be useful in breeding new highly resistant cultivars.

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# **CHAPTER ONE:**

# EFFECT OF HOST PLANT RESISTANCE AND FUNGICIDES ON SEVERITY OF CUCUMBER DOWNY MILDEW

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# Effects of Host Plant Resistance and Fungicides on Severity of Cucumber Downy

### Mildew

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Additional index words: Cucumis sativus, Pseudoperonospora cubensis, Disease

#### Abstract

Cucurbit downy mildew caused by the oomycete *Pseudoperonospora cubensis* (Berk. And Curt) Rostov is a major disease of cucumber (Cucumis sativus L.) (Palti and Cohen, 1980) globally. Chemical control of downy mildew is necessary to achieve high yields in the absence of adequate host plant resistance. Most of the currently grown cultivars have some resistance to downy mildew. Prior to the resurgence of the disease in 2004, host plant resistance was sufficient to control the disease without fungicide use, and downy mildew was only a minor problem on cucumber. There are currently no cultivars that show resistance at a level equal to that observed prior to 2004. However, differences in resistance exist among cultivars, ranging from moderately resistant to highly susceptible. In this study, we evaluated the disease severity and yield of four cucumber cultivars that differed in disease resistance and treated with fungicide programs representing a range of efficacy levels. The experiment was a split-plot design with six replications conducted for four years. Disease was evaluated as chlorosis, necrosis, and reduction in plant size on a 0 to 9 scale. Cultigen had a large effect in all four years, while fungicide has a smaller effect on resistance component traits and a larger effect on yield traits. The effects of host resistance and fungicides appear to be additive until a threshold where maximum yield is reached. Highly resistant cultigens, such as PI 197088, required only the least effective fungicides to achieve highest yields, while moderately resistant cultigens required a more effective fungicide to reach a similar level of yield. Susceptible cultigens did not achieve high yields even with the most effective

fungicide treatments. It is likely that, even as highly resistant cultivars are released, growers will need to continue a minimal fungicide program to achieve maximum yield.

#### Introduction

Cucurbit downy mildew caused by the oomycete *Pseudoperonospora cubensis* (Berk. and Curt) Rostov is economically the most important disease of cucumber (*Cucumis sativus* L.) (Palti and Cohen, 1980). Studies on the host range of *P. cubensis* indicate that approximately 20 genera are hosts, including 50 species in the Cucurbitaceae and 19 host species genus Cucumis alone (Palti and Cohen, 1980; Lebeda, 1992; Lebeda and Widrlechner, 2003). In 2010, about 35,840 hectares of cucumbers for processing and fresh market were grown in the United States, with a value of \$378 million (USDA, 2011). Other economically important hosts of *P. cubensis* are melon (*Cucumis melo* L.), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), and squash (*Cucurbita* spp.) (Whitaker and Davis, 1962). The pathogen infects when windblown sporangia are introduced onto cucurbit hosts under favorable environmental conditions.

*P. cubensis* is a biotroph and, with the exception of oospore production, survives only on living host tissue (Bains and Jhooty, 1976). Previously, oospore production was thought to be rare, but Cohen et al. (2011) recently reported on oospore formation in the lab from crosses between different pathotypes, resulting in the production of viable F1 recombinants. In warm production regions, such as southern Florida, overwintering occurs on wild and cultivated cucurbits (Bains and Jhooty, 1976). The pathogen can also overwinter on cucumbers grown in greenhouses. Hausbeck (2007) reported the possibility of cucumbers in greenhouses in Ontario as a local source of *P. cubensis* inoculum for Canada and neighboring states in the Great Lakes region.

Environmental conditions affect overwintering capacity as well as disease development and severity. Leaf moisture is required for germination of sporangia. Rain, dew, or irrigation can easily supply enough moisture for sporangia to germinate. Under optimum temperature, infection can occur with only two hours of leaf wetness (Cohen, 1977). The extent of infection is a result of the combination of time, moisture, temperature, and inoculum concentration (Neufeld and Ojiambo, 2012).

Symptoms of cucumber downy mildew occur almost exclusively on the foliage. Infection first appears as small, water-soaked lesions on the underside of leaves. Symptoms vary by cucurbit species but in cucumber lesions are angular, bounded by leaf veins, and turn chlorotic to varying degrees. Sporulation occurs on the undersides of the leaves. Chlorotic lesions eventually turn necrotic and the entire leaf may be affected by the pathogen as the leaf tissue dies. Symptoms vary depending on relative susceptibility of host plants. The most resistant will exhibit a hypersensitive response (HR) with small necrotic or chlorotic flecks and sparse sporulation, while leaves of the most susceptible will become completely necrotic within two to three weeks.

The HR type resistance was first described by Barnes and Epps (1954) in the accession PI 197087. Resistance from PI 197087 was used to develop resistant cultivars, and most current cultivars are thought to have some resistance derived from PI 197087. This resistance proved highly effective for many years until a resurgence of the disease in 2004. Since then, cultivars having resistance tracing to PI 197087 are only moderately resistant in the United States (Call et al., 2012a). However, differences among cultivars do exist,

ranging from moderately resistant to highly susceptible. New sources of disease resistance have been reported (Call et al., 2012b) but this resistance has not yet been incorporated into cultivars.

Chemical control of downy mildew is necessary to achieve high yields in the absence of high host plant resistance. The discovery of systemic fungicides was a major advance over protectant fungicides in control of downy mildew. Systemic fungicides, in the absence of resistant cultivars, can provide effective control. Cohen (1979) reported on the effectiveness of two systemic fungicides, prothiocarb and propamocarb (derivative of prothiocarb) against downy mildew. Both prothiocarb and propamacarb were reported to have very good activity against downy mildew. Briggs et al. (2006) reported fluopicolide as having a novel mode of action controlling a wide range of oomycete pathogens. Typically, a protectant fungicide and a systemic fungicides with different modes of action. An example of such a program is propamocarb (Previcur Flex) and chlorothalonil (Bravo) alternating with famoxadone + cymoxanil (Tanos) + mancozeb (Manzate).

In this study, we evaluated fungicide programs with different levels of efficacy against downy mildew in combination with cultivars or breeding lines (hereafter collectively referred to as cultigens) having different levels of resistance for their effect on disease severity and yield.

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#### **Materials and Methods**

### I. Field Plot Layout

Field tests were done during the summer from 2008 to 2011 at the Horticultural Crops Research Station in Clinton, NC. All cucumbers were grown using recommended horticultural practices as summarized by Schultheis (1990). Fertilizer was incorporated before planting at a rate of 90-39-74 kg/ha (N-P-K) with an additional 34 kg N/ha applied at the vine-tip-over stage (four to six true leaves). There were six (2008 to 2010) and eight (2011) plot rows surrounded by two border rows. End borders were used at the front and back of test plot rows. End borders were 1.6 m long, separated from test plots by 1.6 m alleys. Plots were hand-seeded on raised, shaped beds with centers 1.5 m apart and plots 1.6 m (2009) or 3.2 m (2008 to 2011) long. Plots were separated at each end by 1.6 m alleys. Two plot lengths were used in 2009 (1.6 m and 3.2 m), each in individual blocks, with the 3.2 m plots planted 11 days after the shorter plots. Two plot lengths were used due to incorrect field layout on the first planting date. The study was planted on 10 July 2008, 18 June 2009 (1.6m), 29 June 2009 (3.2m), 15 July 2010, and 8 July 2011.

Plots and borders were planted when inoculum was present in adjacent cucumber fields (within 50 m). Plots were harvested twice, at two week intervals, by hand and graded into marketable and cull fruit when the largest fruit reached size 4 (>2"), or oversized according to industry standards (6 to 8 weeks after planting). Cull fruit were crooked (curved) or constricted enough to seriously effect fruit appearance, according to industry standards (United States Department of Agriculture, 1958). Number of fruit and total weight were recorded for marketable and cull fruit for each plot. Percent early yield is the percent of yield from harvest one. Yield data for the 1.6 m plots was doubled in the analysis in order to estimate the yield on a 3.2 m plot basis, and to compare with the rest of the study. There was likely a border effect on yield as shown by Wehner (1984) indicating the estimate obtained by doubling will be biased slightly upwards. Because this is equal for all treatments, there will be no rank change, and because these were not yield trials, this bias is acceptable.

## **II. Germplasm and Fungicide Treatments**

Four cucumber cultigens differing in disease resistance were used to evaluate severity of disease, based on previous studies at North Carolina State University (Call et al. 2012a; Call et al. 2012b;Wehner and Shetty, 1997; Shetty et al., 2002).(Table 1.1): M 21 (North Carolina State Univ.) is moderately resistant (MR), 'Sumter'(Clemson Univ.) is slightly resistant (SR), 'Wisconsin SMR 18' (Wisconsin AES) is highly susceptible (HS), and PI 197088 (USDA-ARS) is highly resistant (HR)(2011 only). PI 197088 was added to the study in 2011 to represent a higher level of resistance than the other cultigens in the study. The cultigens used are not elite cultivars that could be marketed directly, but have fruit that can be described as unmarketable if environmental conditions affect development.

Fungicide programs were selected based on results from annual fungicide efficacy tests conducted in North Carolina (Colucci et al., 2008b; Colucci et al. 2008c; Kanetis et al., 2009a; Kanetis et al., 2009b) (Table 1.1). Fungicide treatments were applied using a CO<sub>2</sub>pressurized backpack sprayer equipped with hollow cone nozzles (TXVS-26, TeeJet Inc., Springfield, IL) delivering 61 L/ha at 310 kPa. Application rates were as per label instructions and are presented in Table 1.2. Fungicides were applied weekly for all treatments, beginning at the first true leaf stage, prior to the appearance of disease symptoms on test plots, with 7 applications each year.

# **III. Field Inoculation and Disease Ratings**

No artificial inoculum was used and plots were exposed to natural epidemics during the course of the growing season. Susceptible cultivar 'Coolgreen' (Asgrow) was used for side and end borders to monitor and increase inoculum in the field. Epidemics were encouraged using overhead irrigation at least three days per week.

Disease severity was evaluated weekly as chlorotic and necrotic lesions, and degree of stunting. Chlorosis and necrosis were rated on a 0 to 9 scale based on percentage of symptomatic leaf area (0 = 0%, 1 = 1-3%, 2 = 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99%, 9 = 100%); as described by Jenkins and Wehner (1983). Stunting was rated on the same 0 to 9 scale as reduction in plant size, relative to observations on the same cultigens planted in fungicide-treated, non-inoculated trials planted in adjacent fields. Even without disease, different genotypes have different plant sizes. For instance, M 21 is a dwarf determinate type with a naturally smaller habit than the other cultigens used in this study. Because of this fact, this rating is used to compare the effect of fungicides within a cultigen, but can't be used to compare cultigens. It allows us to identify those fungicide treatments under which plants remain large under a disease epidemic.

## **IV. Design and Data Analysis**

The experiment was a split-plot design with six replications per year. There were three (2008) and four (2009 to 2011) fungicide programs used as the whole plots. Three (2008 to 2010) and four (2011) cultigens were used as the sub plots. Data were analyzed using the General Linear Model, GLIMIX, Means and Correlation procedures of SAS (SAS Institute, Inc., Cary, NC).

## **Results and Discussion**

Data were analyzed using means of all ratings for each trait. There was no plot size effect on disease ratings in 2009 so these data were pooled (data not shown). Two basic datasets were analyzed to account for added treatments:

1. 2008 to 2011 with fungicide programs 1 to 4 (Table 1.1) and cultigens 1 to 3

2. 2011 with fungicide programs 1 to 4 and PI 197088 only.

#### 4 Fungicide Programs, 3 Cultigens (2008 to 2011)

PI 197088 is excluded from this dataset. In 2009 a new fungicide treatment was added to the study to represent a higher efficacy fungicide program (fluopicolide + chlorothalonil alternated with cyazofamid + mancozeb). The analysis of variance (ANOVA) results (Table 1.3) indicate that differences in both cultigen and fungicide treatments contribute significantly (p< 0.001) to differences in disease severity as well as total and percent marketable yield. A larger cultigen effect was observed for chlorosis and necrosis, compared to the fungicide effect on the same traits. For stunting, cultigen had a smaller effect than fungicide. All correlations were calculated using the Pearson product-moment and Spearman's rank methods (data not shown). Yield data was generally highly correlated among environments (p<0.01). Chlorosis and necrosis were significantly highly correlated ( $R^2$ =0.90) for both the Pearson and the Spearman tests, indicating they are likely the same trait. Stunting was less correlated with chlorosis and necrosis (Pearson  $R^2$ =0.26, p<0.001).

Disease and yield data for 2008 to 2011 with all fungicide treatments and cultigens M 21, 'Sumter', and 'Wisconsin SMR-18' are summarized in Table 1.4 as least squares means. The resistant cultigen M 21 on average had higher yields and less disease than the moderately resistant 'Sumter' and susceptible 'Wisconsin SMR-18' in all yield traits and all disease traits with the exception of stunting and fruit size. M 21 is a determinate type and has a naturally smaller habit than indeterminate types. Differences can be seen in both yield and disease traits among cultigen and fungicide treatments. The effects of moving to a cultivar with more resistance appears additive for chlorosis, necrosis, yield, and percent marketable yield. Surprisingly, chlorosis and necrosis severity are reduced only slightly as one moves to higher efficacy fungicide programs. A larger effect is seen on plant stunting. Reduced stunting is equivalent to increased leaf area and more photosynthetic potential. The yield increase observed with more effective fungicide treatments could be partially due to the reduction in stunting.

The highest total and percent marketable yield along with the lowest disease ratings within each cultigen were observed achieved with the high efficacy fungicide treatment fluopicolide + chlorothalonil alt. cyazofamid + mancozeb (Table 1.3). The combination of

the highly susceptible Wisconsin SMR 18 with no fungicide treatment resulted in the highest disease ratings and lowest yield. In general, the disease and yield traits measured improved by moving to a more resistant cultivar or more effective fungicide program.

Disease severity ratings for the new high efficacy fungicide treatment (fluopicolide + chlorothalonil alt. cyazofamid + mancozeb) were not significantly lower than the previous high efficacy (propamocarb + chlorothalonil alternating with famoxadone + cymoxanil + mancozeb) treatment with the exception of necrosis ratings in cultigen M 21, however the means were lower for all traits within each cultivar. Fungicide had a much larger effect on yield traits, with the largest yield difference among fungicide treatments observed in the less resistant cultigens. The mean total yield of 'Sumter' under the fluopicolide + chlorothalonil alternated with cyazofamid + mancozeb treatment was 22.2 Mg/ha, an increase of 20.0 Mg/ha over the non-treated 'Sumter' plots. Similar results were observed for other fungicide treatments. In general, disease ratings for the propamocarb + chlorothalonil alternating with famoxadone + cymoxanil + mancozeb treatment were lower but not significantly different from mancozeb alone for chlorosis, necrosis, and stunting. Although, these plots appeared similar as far as disease appearance, the difference in yield was significant between the treatments.

In a study by Kanetis et al. (2009a) in 2008, conducted in Clayton, NC, propamocarb showed significantly less disease (AUDPC) than both famoxadone + cymoxanil and mancozeb which were not significantly different. In 2009 a study from the same group (Adams and Ojiambo, 2010) at the same location, showed mancozeb and propamocarb (not significantly different) had a significantly lower AUDPC than famoxadone + cymoxanil. In studies conducted in 2009 in Faison, NC, the mancozeb treatment had a significantly lower AUDPC than both propamocarb and famoxadone + cymoxanil (Adams et al., 2010). These results may indicate some loss in effectiveness by famoxadone + cymoxanil and propamocarb, though more studies should be done to evaluate this observation. Yield data was not reported for the above studies, which would have been helpful in determining treatment efficacy.

Our data shows that although apparent disease may not be reduced greatly with improved fungicide programs, the effect on yield was large. In our study, the propamocarb + chlorothalonil alternating with famoxadone + cymoxanil + mancozeb treatment yielded significantly higher (LSD 5%= 1.8) than mancozeb in all cultigens. The newly added program, fluopicolide + chlorothalonil alternating with cyazofamid + mancozeb, generally outperformed all other treatments having less disease and higher yield.

Cultigen resistance seems to be more important for overall disease reduction than fungicides, but each contributed similarly to yield. It is likely that the benefits of fungicide application would be greater than data indicates in terms of severity of disease in a grower field because in our tests, neighboring plots that were not treated with fungicide may have increased spore density in the field, causing more disease. All borders were untreated and planted with a highly susceptible check as well. A field that is treated with complete fungicide coverage should be less affected, assuming neighboring fields are being controlled as well.

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In our study, plots and borders were planted when inoculum was present in adjacent cucumber fields. This means that infection likely occurred prior to fungicides being applied (post infection, prior to disease symptom appearance), which negatively impacts fungicide efficacy (Colucci et al., 2008a). Although fungicides are much more effective if applications are started prior to the arrival of inoculum, a typical grower will begin spraying only when symptoms appear or if disease has been reported in their area, in order to minimize the costs of fungicide application. Growers who initiate a spray program before the appearance of disease symptoms would achieve superior results.

## 4 Fungicide Programs, PI 197088 (2011)

In 2011 the cultigen PI 197088 was added to the test as the representative for high resistance. PI 197088 shows few lesions when challenged with *P. cubensis*, is large, and has high, but late, yield. A summary of disease severity ratings and yield results for PI 197088 is shown in Table 1.5. In this study, PI 197088 was highly resistant, and significantly better for all rated disease and yield traits (except percent early) than other cultigens tested (data not shown). The total yield of PI 197088 was not significantly different for the 4 fungicide programs, but treated plot means were similar and higher than the control. It appears that the highly resistant PI 197088 might receive a small boost to yield from a fungicide treatment. Further testing would likely show a significant difference between fungicide treated and non-treated plots.

#### Conclusions

Both host resistance and fungicide treatment together contribute to plant performance in terms of reducing disease severity and increasing yields. This study examined how fungicide efficacy and host resistance interact to affect these traits. The cultigens used in this study are not isolines differing only in resistance; thus, both resistance and genetic background contribute to treatment differences. The cultigens were chosen to represent different levels of resistance so disease components can be directly compared. Any background genetic effects on cultigen resistance would by definition be part of the overall cultigen resistance. Differences in yield among cultigens are due to both differences in resistance and background genetic potential.

The effects of cultigen resistance and fungicides seem to act additively. In general, a change to a more resistant cultigen or more effective fungicide treatment reduces apparent disease and increases yield. Cultigen treatments affected apparent disease to a far greater extent than fungicide treatments. Fungicides alone are not enough to achieve high yield in susceptible cultigens, but are effective in combination with a moderately resistant cultigen. It is interesting that the overall effect of fungicides on apparent disease severity is low, while the effect on yield and marketable yield is large. More effective fungicide treatments resulted in slightly less apparent disease, higher yield, and a higher percent marketable yield.

In most cases, growers are already using cultivars with resistance comparable to M 21. The most effective fungicide treatment in our study was fluopicolide + chlorothalonil alternating with mancozeb + cyazofamid. For growers using cultivars with moderate resistance, higher yield would likely be achieved with this treatment compared to the previously recommended program (propamocarb + chlorothalonil alternating with famoxadone + cymoxanil + mancozeb).

It is clear that cultivars with resistance coupled with an effective fungicide program are required to achieve high yield at this time. To achieve high yield without fungicide, more resistance is needed. The highly resistant PI 197088 shows little disease, but still appeared to benefit from fungicides, although it seems a low level protectant fungicide (mancozeb) provides enough protection to achieve the highest yields. In other words, with the highest host resistance available to breeders, there was no yield benefit by moving from a low-level protectant fungicide program to a program of alternating systemic and protectant tank-mixes. Nevertheless, even once recently identified high resistance has been incorporated into commercial cultivars, some fungicide applications are likely to be required to achieve the highest yield.

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Table 1.1. Fungicide and cultigen treatments used.

Fungicide	Treatments	Fungicide Efficacy
1.	Control	None
2.	Mancozeb applied weekly	Slightly effective
3.	Famoxadone + cymoxanil + mancozeb alternating weekly with	Moderately effective
	propamocarb-hydrochloride + chlorothalonil	
4.	Cyazofamid + mancozeb alternating weekly with	Highly effective
	fluopicolide + chlorothalonil (added in 2009)	
Cultigens		Cultigen Resistance
curugens		
1.	Wisconsin SMR-18	Susceptible
2.	Sumter	Slightly resistant
3.	M 21	Moderately resistant
4.	PI 197088 (2011 only)	Highly resistant

Table 1.2. Trade names, active ingredients, fungicide group name, and supply company for fungicides used in 2008 and 2009.

Trade	Active	Group	Application	Supply
Name	Ingredient	Name and FRAC Code y	Rate	Company
Manzate <sup>®</sup> Pro-Stick <sup>™</sup>	mancozeb	Multi-site inhibitors	0.37 kg/ha	E.I. Dupont de Nemours and Co.
Bravo Weather-Stick®	chlorothalonil	Multi-site inhibitors	0.39 L/ha	Syngenta Crop Protection, Inc.
Tanos®	famoxadone +	Quinone outside inhibitors (C3)	0.10 L/ha	E.I. Dupont de Nemours and Co.
	cymoxanil	Cyanoacetamide-oximes		
Previcur <sup>®</sup> Flex	propamocarb-	Carbamates (F4)	0.23 L/ha	Bayer CropScience
	hydrochloride			
Presidio <sup>®z</sup>	fluopicolide	Pyridinylmethyl-benzamides (B5)	0.04 L/ha	Valent USA Corporation
Ranman <sup>®z</sup>	cyazofamid	Quinone inside inhibitors (C4)	0.04 L/ha	FMC Agricultural Products

<sup>z</sup> Only used in 2009 <sup>y</sup> Fungicide Resistance Action Committee (http://frac.info)

2008-2011 NO PI 197088		Re	sistance componer	nts <sup>b</sup>	Yield components <sup>c</sup>						
Source	DF	Chlorosis	Necrosis	Stunting	<u>Total yield</u> (Mg/ha)	<u>% mark</u> yield <sup>e</sup>	<u>% early</u> yield <sup>f</sup>	Fruit size (kg/fr)			
Year	3	22.34 ***	3.54 **	23.92 ***	849.85 ***	4084 **	7492 **	0.1666 **			
Rep(Year)	20	0.66 *	0.66	1.55 ***	61.23 **	588 **	1432 ***	0.0256			
Fungicide	3	30.78 ***	33.72 ***	78.38 ***	3161.78 ***	18995 ***	666	0.0653			
Fungicide <sup>*</sup> Year	8	3.05 ***	3.82 ***	5.00 ***	115.82 ***	682	374	0.0352			
Rep(Fungicide*Year)	55	0.31	0.38	0.65	14.72	347	443	0.0418			
Cultigen	2	445.94 ***	224.55 ***	9.08 ***	1926.96 ***	16419 ***	7230 ***	0.0106			
Cultigen*Year	6	3.36 ***	8.50 ***	17.89 ***	115.28 **	726 *	401	0.0642			
Cultigen*Fungicide	6	0.86 *	1.38 *	0.70	99.03 **	2313 ***	450	0.0229			
Year*Cultigen*Fungicide	16	0.75 *	0.62	1.10 *	16.19	294	301	0.0440			
Error	222	0.41	0.63	0.65	31.94	287	437	0.0341			
2011 - PI 197088 Only											
Fungicide	3	0.04	0.24	2.82 **	67.98	40 **	43	0.0002			
Replication	5	0.05	0.15	0.56	89.27	22 *	250 **	0.0052 **			
Error	<u>15</u>	0.03	0.13	0.50	80.44	7	44	0.0012			

Table 1.3. Analysis of variance of downy mildew resistance component and yield trait means for data collected in Clinton, North Carolina in 2008 to 2011, excluding PI 197088 (top) and for data collected in 2011 for only the cultigen PI 197088 (bottom) <sup>a</sup>.

a Data are means of six (2008, 2010, 2011) and 12 (2009) replications.

b Data are means or all ratings.

c Data are from two harvests.

d Percent marketable yield is percent non-cull fruit.

e Percent early yield is yield from harvest 1 of 2. \*,\*\*\*\*\*\*\* Significant at 0.05, 0.01 and 0.001 respectively

Table 1.4. Disease resistance component ratings and yield components for combinations of cultigens and fungicide programs for the control of downy mildew in cucumber. Plots were tested in 2008 to 2011 at Clinton, NC. Data presented is least squares means (EST) and standard error (SEM) of all ratings <sup>a</sup>.

		Re	esistance component	s	Yield components						
		Chlorosis mean Necrosis mean		Stunting mean	<u>Total yield</u> (Mg/ha)	<u>% mark yield d</u>	<u>% early yield<sup>e</sup></u>	Fruit size (kg/fr) <sup>f</sup>			
Cultigen	Fungicide program <sup>b</sup>	$EST \pm SEM$ $EST \pm SEM$		$EST \pm SEM$	$EST \pm SEM$	$EST \pm SEM$	$EST \pm SEM$	$EST \pm SEM$			
M 21	Fluopicolide + chlorothalonil alt. cyazofamid + mancozeb <sup>c</sup>	$2.00\pm0.42~^{\rm A}$	$2.83\pm0.37~^{\rm A}$	$3.40\pm0.53~^{\text{ABC}}$	$20.92\pm2.51\ ^{\rm A}$	$68.80 \pm 8.61 \ ^{\rm A}$	$34.7\pm7.5\ ^{BCD}$	$0.19\pm0.05~^{\rm A}$			
	Famoxadone + cymoxanil + mancozeb alt. propamacarm + chlorothalonil	$2.52\pm0.39~^{\rm AB}$	$3.58\pm0.34~^{\rm B}$	$3.69\pm0.50~^{\text{ABC}}$	$15.99\pm2.35~^{\text{B}}$	$69.47\pm8.26$ $^{\rm A}$	$36.6\pm7.2\ ^{BC}$	$0.18\pm0.05\ ^{\rm A}$			
	Mancozeb	$2.74 \pm 0.39$ AB $3.95 \pm 0.34$ BC		$4.37 \pm 0.50^{\text{CDE}}$ $11.39 \pm 2.35^{\text{CD}}$		$60.90 \pm 8.26$ AB	$41.0 \pm 7.2$ AB	$0.15 \pm 0.05$ <sup>A</sup>			
	None	$3.65 \pm 0.39$ CD	$4.64 \pm 0.34$ <sup>CD</sup>	$5.27 \pm 0.50$ <sup>E</sup>	$08.06 \pm 2.35$ DE	$55.74 \pm 8.26$ <sup>BC</sup>	$48.2 \pm 7.2$ <sup>A</sup>	$0.13 \pm 0.05$ <sup>A</sup>			
Sumter	Fluopicolide + chlorothalonil alt. cyazofamid + mancozeb	$3.07\pm0.42~^{\text{BC}}$	$4.07\pm0.37~^{\text{BC}}$	$2.27\pm0.53$ $^{\rm A}$	$22.22\pm2.51~^{\rm A}$	$66.19\pm8.63~^{\text{AB}}$	$23.5\pm7.5~^{\text{CDE}}$	$0.23\pm0.05$ $^{\rm A}$			
	Famoxadone + cymoxanil + mancozeb alt. propamacarm + chlorothalonil	$3.86\pm0.39~^{\rm C}$	$4.94\pm0.34~^{\text{CD}}$	$3.15\pm0.50~^{\text{ABC}}$	$10.90\pm2.35~^{\text{CD}}$	$42.67\pm8.28\ ^{\rm C}$	$26.6\pm7.2~^{\rm CD}$	$0.17\pm0.05~^{\rm A}$			
	Mancozeb	$4.29\pm0.39~^{\text{DE}}$	$5.28\pm0.34~^{\text{DE}}$	$4.00\pm0.50~^{\text{BCDE}}$	$05.91 \pm 2.35$ EF	$28.17 \pm 8.26$ <sup>D</sup>	$21.6\pm7.2~^{\rm DE}$	$0.15\pm0.05$ $^{\rm A}$			
	None	$4.75 \pm 0.39^{\text{E}}$ $6.06 \pm 0.34^{\text{FG}}$		$4.92 \pm 0.50$ DE	$02.19 \pm 2.35$ FG	$11.68 \pm 8.58$ <sup>E</sup>	$27.9 \pm 7.5$ <sup>BCD</sup>	$0.14 \pm 0.06$ <sup>A</sup>			
Wis. SMR18	Fluopicolide + chlorothalonil alt. cyazofamid + mancozeb	$6.14\pm0.42~^{\rm F}$	$6.05\pm0.37~^{\text{EF}}$	$2.86\pm0.53~^{\rm AB}$	$14.60\pm2.51~^{\text{BC}}$	$64.23\pm8.63~^{\rm AB}$	$23.4\pm7.5~^{\text{CDE}}$	$0.23\pm0.05$ $^{\rm A}$			
	Famoxadone + cymoxanil + mancozeb alt. propamacarm + chlorothalonil	$6.72\pm0.39~^{\text{FG}}$	$6.80\pm0.34~^{\rm GH}$	$3.58\pm0.50~^{\text{ABCD}}$	$04.69\pm2.35~^{\text{EFG}}$	$44.79\pm8.28\ ^{\rm C}$	$30.0\pm7.2~^{\text{BCD}}$	$0.27\pm0.05$ $^{\rm A}$			
	Mancozeb	$7.10\pm0.39~^{\rm GH}$	$6.89\pm0.34~^{\rm GH}$	$4.30\pm0.50~^{\text{CDE}}$	$01.96 \pm 2.35$ FG	$26.01 \pm 8.36$ DE	$13.5 \pm 7.3$ <sup>E</sup>	$0.16\pm0.06\ ^{\rm A}$			
	None	$7.40\pm0.39$ $^{\rm H}$	$7.17\pm0.34$ $^{\rm H}$	$5.36\pm0.50~^{\rm E}$	$00.51\pm2.35~^{G}$	$\text{-}2.50\pm8.79~\text{F}$	$20.8\pm7.9~^{\rm DE}$	$0.16\pm0.14$ $^{\rm A}$			
M 21		$2.73 \pm 0.34$ <sup>A</sup>	$3.75\pm0.28~^{\rm A}$	$4.19 \pm 0.43$ <sup>A</sup>	$14.09 \pm 2.02$ <sup>A</sup>	$63.73 \pm 7.56$ <sup>A</sup>	$40.1\pm6.4$ <sup>A</sup>	$0.16\pm0.04~^{\rm A}$			
Sumter		$3.99 \pm 0.34$ <sup>B</sup>	$5.09 \pm 0.28$ <sup>B</sup>	$3.58 \pm 0.43$ <sup>A</sup>	$10.31 \pm 2.02$ <sup>B</sup>	$37.18 \pm 7.60$ <sup>B</sup>	$24.9 \pm 6.5$ <sup>B</sup>	$0.17 \pm 0.04$ <sup>A</sup>			
Wis. SMR18		$6.84\pm0.34~^{\rm C}$	$6.72\pm0.28~^{\rm C}$	$4.02\pm0.43~^{\rm A}$	$05.44 \pm 2.02$ <sup>C</sup>	$33.13\pm7.62\ ^{\text{B}}$	$21.9\pm6.5\ ^{\text{B}}$	$0.20\pm0.05$ $^{\rm A}$			
	Fluopicolide + chlorothalonil alt. cyazofamid + mancozeb	$3.74\pm0.36~^{\rm A}$	$4.32\pm0.27~^{\rm A}$	$2.84\pm0.39\ ^{\rm A}$	$19.25\pm2.20~^{\rm A}$	$66.40\pm7.90\ ^{\rm A}$	$27.2\pm6.5~^{\rm A}$	$0.22\pm0.04~^{\rm A}$			
	Famoxadone + cymoxanil + mancozeb alt. propamacarm + chlorothalonil	$4.37\pm0.34~^{\rm AB}$	$5.11\pm0.24~^{\text{B}}$	$3.47\pm0.36~^{\rm AB}$	$10.53\pm2.06~^{\text{B}}$	$52.31\pm7.65\ ^{\text{B}}$	$31.1\pm6.3~^{\rm A}$	$0.21\pm0.04$ $^{\rm A}$			
	Mancozeb	$4.71\pm0.34~^{\text{BC}}$	$5.38\pm0.24~^{BC}$	$4.22\pm0.36\ ^{\rm B}$	$06.42 \pm 2.06$ <sup>C</sup>	$38.36 \pm 7.66$ <sup>C</sup>	$25.4\pm6.3$ $^{\rm A}$	$0.15\pm0.04$ $^{\rm A}$			
	None	$5.27 \pm 0.34$ <sup>C</sup>	$5.96 \pm 0.24$ <sup>C</sup>	$5.18 \pm 0.36$ <sup>C</sup>	$03.59 \pm 2.06$ <sup>C</sup>	$21.64 \pm 7.78$ <sup>D</sup>	$32.3 \pm 6.5$ <sup>A</sup>	$0.14\pm0.06$ $^{\rm A}$			

a Data are means of 12 (2009) and six (2008,2010, 2011) replications and 2 harvests. Ratings were 0 to 9 (0 = 0%, 1 = 1-3%, 2 = 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99%, 9 = 100%) for chlorosis, necrosis, and stunting.

b Fungicide treatments were applied weekly.

c Treatment not tested in 2008.

d Percent marketable yield is total yield that is non-culled fruit.

Table 1.4. continued

e % early yield was the percentage of total yield obtained in harvest 1 (out of 2). f Fruit size was calculated from marketable fruit. A,B,...,H Results with the same letter in the same column are not significantly different (P < 0.05)

Table 1.5. Disease resistance component ratings and yield components for the combinations of highly resistant PI 197088 and fungicide programs for the control of downy mildew in cucumber. Plots were tested in 2011 at Clinton, NC <sup>a</sup>.

		Resistance components						Yield components								
		Chlor	Chlorosis <u>Necrosis</u>		Stunting		Total yield (Mg/ha)		<u>% mark yield <sup>c</sup></u>		% early yield d		<u>Fruit size (kg/fr)<sup>e</sup></u>			
Cultigen	Fungicide program <sup>b</sup>	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	
PI 197088	Fluopicolide + chlorothalonil alt. cyazofamid + mancozeb	1.0 <sup>A</sup>	0	$1.8^{AB}$	0.46	1.6 <sup>A</sup>	0.80	29.2 <sup>A</sup>	9.30	90 <sup>A</sup>	3.89	4 <sup>A</sup>	6.19	0.32 <sup>A</sup>	0.04	
	Famoxadone + cymoxanil + mancozeb alt. propamacarm + chlorothalonil	1.1 <sup>A</sup>	0.17	1.6 <sup>B</sup>	0.27	1.6 <sup>A</sup>	0.74	28.8 <sup>A</sup>	9.15	93 <sup>AB</sup>	3.65	4 <sup>A</sup>	6.92	0.31 <sup>A</sup>	0.04	
	Mancozeb	1.2 <sup>A</sup>	0.18	1.9 <sup>AB</sup>	0.27	1.8 <sup>A</sup>	0.69	31.1 <sup>A</sup>	6.88	95 <sup>в</sup>	2.06	8 <sup>A</sup>	17.08	0.31 <sup>A</sup>	0.05	
	None	1.2 <sup>A</sup>	0.28	2.0 <sup>A</sup>	0.42	3.0 <sup>B</sup>	0.63	23.3 <sup>A</sup>	10.63	95 <sup>B</sup>	3.09	1 <sup>A</sup>	1.81	0.32 <sup>A</sup>	0.05	
PI 197088		1.1	0.19	1.8	0.38	2.0	0.9	28.1	8.99	93	3.8	4	9.41	0.32	0.04	

a Data are means of six replications and two harvests. Ratings were 0 to 9 (0 = 0%, 1 = 1-3%, 2 = 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99\%, 9 = 100%) b Fungicide treatments were applied weekly.
c Percent marketable yield is total yield that is non-culled fruit.
d % early yield was the percentage of total yield obtained in harvest 1 (out of 2).

e Fruit size was calculated from marketable fruit.

A,B Results with the same letter in the same column are not significantly different (P < 0.05)

# **CHAPTER TWO:**

# HERITABILITY AND GENETIC VARIANCE ESTIMATES FOR HIGH RESISTANCE TO DOWNY MILDEW IN CUCUMBER PI 197088

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# Heritability and Genetic Variance Estimates For High Resistance to Downy Mildew in

# Cucumber PI 197088

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#### Abstract

Downy mildew (DM) of cucumber, caused by *Pseudoperonospora cubensis*, is a devastating disease of cucurbits. Resistance is available, but is not sufficient to eliminate the need for fungicides to control the disease. Previously, the USDA Plant Introduction (PI) collection of cucumber germplasm was screened and PI 197088 was identified as highly resistant. The objective of this study was to determine the type of gene action controlling resistance in PI 197088. Four families were developed using cultigens ranging from susceptible to moderately resistant in crosses with PI 197088 to make 7 generations for the study: P1, P2, F1, F1 reciprocal, F2, BC1P1 and BC1P2. Families were tested at two locations in North Carolina for one or two years. Failure of the data to fit the single gene model indicated that resistance is under control of a more complex genetic system. Thus, we suggested that resistance to downy mildew in PI 197088 should be regarded as a quantitative trait for breeding purposes. Generation variances were measured and genetic parameters estimated, including genetic variances, heritability, number of effective factors, and gain from selection. Genetic effects were greater than environmental effects. Additive variance and broad- and narrow-sense heritability were generally large in our study, with resistance attributed to a few loci. The estimated gain from selection indicated possible improvement of two or more points of resistance (on a nine-point scale) per generation under high selection intensity.

#### Introduction

Cucurbit downy mildew, caused by the oomycete pathogen *Pseudoperonospora cubensis* (Berk. And Curt) Rostov, is a major foliar disease of cucumber (*Cucumis sativus* L.) (Palti and Cohen, 1980). The disease was first described in Cuba in 1868 (Berkeley and Curtis, 1868). It was then reported in Russia in 1903 (Rostowzew, 1903) and Japan in 1927 (Kurosawa, 1927). Epidemics of downy mildew on the genus *Cucumis* have now been observed in over 70 countries worldwide (Palti, 1974; Cohen, 1981). A resurgence of the disease in 2004 was unexpected in the United States and resulted in an estimated yield loss of 40% (Colucci et al., 2006). The new strains of *P. cubensis* continue to infect cucumber in most production areas in the United States and remain a major threat to cucumber production in warm humid regions around the world. Most cultivars currently grown in the United States have some resistance, but not at the level seen prior to appearance of the new race in 2004. A recent study of resistance of available cultivars concluded that although there are differences in cultivar resistance among the cultigens tested, none of the cultigens tested were highly resistant (Call et al., 2012a).

The hypersensitive response (HR) type resistance was first described by Barnes and Epps (1954) in cucumber PI 197087, being controlled by a single resistance gene. Downy mildew lesions on PI 197087 were different from other resistant cultivars. The lesions were described as irregularly shaped, brown, with a slight water-soaked appearance. There was no chlorosis, and lesions remained as small, brown spots. The leaf tissue died shortly after infection and there was little to no sporulation on the leaf undersides. Resistance from PI

197087 was used in the development of new cultivars, and the resistance in many current cultivars traces to PI 197087 (Wehner and Shetty, 1997). That resistance, which was sufficiently effective for growers to produce cucumbers in warm humid regions of the United States without fungicides for over 40 years, has since been overcome by a new race of the pathogen.

Without high resistance, fungicide programs have been necessary to achieve high yield and quality, resulting in increased cost to growers. *P. cubensis* is a pathogen with high evolutionary potential (McDonald and Linde, 2002). The pathogen reproduces both asexually and sexually and produces a large quantity of asexual sporangia that can be transported aerially over a long distance (Cohen and Rubin, 2011). Resistance to systemic fungicides has been reported in several studies (Pappas, 1982; Cohen and Samoucha, 1984; Baines and Sharma, 1986; Ishii et al., 2001; Zhu et al., 2007). Eshet and Dinur (1970) reported downy mildew in test plots treated with benomyl (source Benlate), suggesting that resistance to benomyl has been observed. At the Fifth Congress of the Mediterranean Phytopathological Union in 1980, Pappas (1981) reported good control of *P. cubensis* by phosetyl-Al, under conditions favoring disease. Metalaxyl was reported as less effective and also was shown to have resistant or insensitive biotypes. Cross resistance has also been reported (Cohen and Samouch, 1984) where four systemic fungicides were not effective against strains of P. *cubensis* that were resistant to metalaxyl. As the threat of fungicide efficacy breakdown continues, new sources of high resistance have been identified.

New sources of resistance (reduced leaf damage) and tolerance (good yield under disease presence) to the race(s) of downy mildew in the United States since 2004 were identified in a large germplasm screening study and a multiple year re-evaluation of the most resistant and susceptible cultigens conducted at North Carolina State University (Call et al., 2012b). Cultigens were identified that outperformed currently grown cultivars for disease resistance and yield traits, including PI 197088. PI 197088 was collected at the in the same location and collection trip as PI 197087, the source of resistance that was overcome in 2004. Also among that collection are PI 197086 and PI 197085, both testing as highly resistant to the new race of *P. cubensis* in the Southeast United States. Angelov (1994) reported that PI 197088 resistance to the European race(s) was due to two recessive genes. Resistance in 'Poinsett', which traces to PI 197087 was reported by Van Vliet and Meysing (1977) to be from at least one single recessive gene, dm (dm-1).

Interestingly, the most resistant cultigens from the recent screening (Call et al., 2012), were only moderately resistant in the screening done in 1988 and 1989 (Wehner and Shetty, 1997). The most resistant cultigens from the early study, such as Gy 4, PI 234517, 'Poinsett 76', M 21, were generally intermediate in the recent study. It is possible the shift in the pathogen population has changed the resistance ranking of the cultigens. It has been observed (personal observation) that resistance in PI 197088 may be adult stage resistance, as young plants are somewhat susceptible, but become resistant as tip-over stage is reached. It is possible that in the screening in 1988 and 1989, this resistance was not yet expressing,

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resulting in them being ranked as moderately resistant. Another possibility is the presence of different races in different locations, years, or even at different times of the year.

The objective of this experiment was to study the inheritance of resistance to downy mildew in cucumber PI 197088. In addition, under the hypothesis that resistance to downy mildew in cucumber could be a quantitative trait, we estimated genetic variances and heritability of resistance.

# **Materials and Methods**

In this experiment, we used four families developed from the four crosses PI 197088 × 'Coolgreen', PI 197088 × 'Ashley', PI 197088 × 'Poinsett 76', and PI 197088 × 'Polaris'. For each family, we developed six generations (Pa, Pb, F1, F2, BC1Pa, BC1Pb) in the greenhouses at North Carolina State University in Raleigh, North Carolina. PI 197088 is a highly resistant plant accession. 'Coolgreen' is a highly susceptible cultivar. 'Ashley' is a moderately susceptible cultivar, having slightly effective resistance tracing to a long fruited Chinese cultivar with resistance identified at the Puerto Rico Agricultural Experiment Station. The same resistance was used in the cultivar 'Palmetto' which was highly resistant when released in 1948, but within two years was no longer effective (Epps and Barnes, 1952). 'Poinsett 76' is a cultivar developed by Henry Munger at Cornell, which traces its resistance to PI 197087. Cultivars with this resistance are moderately resistant. 'Polaris' is a moderately resistant cultivar from the Clemson breeding program developed from crosses of PI 197087 with breeding lines.

All tests were conducted in the field at the Horticultural Crops Research Stations at Clinton, and Castle Hayne, North Carolina. The family developed from the cross of 197088 x 'Coolgreen' was tested in 2010 and 2011, while the other families were tested in 2011 only. The experiment had two sets, divided equally among the two locations, each set including all six generations (Parent A, Parent B, F<sub>1</sub>, Backcross to Parent A, Backcross to Parent B, and  $F_2$ ). Single plant hills were grown on plastic mulch, 1.2 m apart within the row, with 50 hills per row. Plots were hand seeded and thinned to a single plant at true leaf stage. Generations were planted in the following order and number (no serpentine): ( $P_a(10)$ ,  $P_b(10)$ ,  $BC_1P_a(30)$ ,  $F_1(20)$ , BC<sub>1</sub>P<sub>b</sub>(30),  $F_2(100)$ ). Rows were spaced with centers 1.6 m apart. A spreader row planted with susceptible cultivar 'Coolgreen' was planted every 9<sup>th</sup> row to monitor and increase inoculum in the field. 'Coolgreen' was also used for side and end borders. In the field, no artificial inoculum was used. Plots were exposed to natural epidemics in the course of the growing season. Epidemics were encouraged using overhead irrigation at least 3 days a week. The study was planted at Castle Hayne, NC on 15 July 2010, and 8 July 2011, and at Clinton, NC on 15 July 2010, and 8 July 2011

Disease was evaluated weekly starting when symptoms appeared for a total of three ratings. Chlorosis and necrosis were rated on a 0 to 9 scale based on percentage of symptomatic leaf area (0 = 0%, 1 = 1-3%, 2 = 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99%, 9 = 100%); as described by Jenkins and Wehner (1983). Stunting was rated on the final rating day on the same 0 to 9 scale as reduction in plant size, relative to observations of the largest parent (PI 197088) planted in fungicide-treated, non-

inoculated trials. Due to uneven emergence in 2011, stunting was rated in 2010 only. Our analysis will highlight data from the third rating along with means of all ratings for comparison. We believe the nature of this rating results in minimized chance for error for the following reasons:

- 1. Possible adult plant resistance not expressing at earlier ratings.
- 2. Rating 3 had the most amount of disease present.
- 3. Less chance of having a miss or non-inoculated plants in the field.

We performed segregation analysis and goodness-of-fit tests with the SAS-STAT statistical package (SAS Institute, Cary, NC) and the SASGene 1.2 program (Liu et al. 1997). All  $\chi^2$  tests were performed at the 95% confidence level. This experiment was designed to determine qualitative inheritance of resistance to downy mildew. Since there was strong evidence against the single gene hypothesis, we verified the distribution of the F<sub>2</sub> data for each family using the UNIVARIATE procedure of SAS-STAT and by plotting the disease ratings against their frequency, prior to analyzing resistance to downy mildew as a quantitative trait. Due to the experimental design, variance estimates for generations are obtained from different sample sizes.

We tested the  $F_2$  data for homogeneity of variances using the Bartlett's method (Ostle and Malone 1988; Steel et al. 1997). When variances were homogeneous among tests within a family, we pooled the data for that family. We also analyzed the data for each family and test, to highlight possible differences among tests. Phenotypic (P), environmental (E), genotypic (G), and additive (A) variances were estimated from generation variances as follows (Warner 1952; Wright 1968):

$$\sigma^{2}(P) = \sigma^{2}(F_{2}) \qquad \sigma^{2}(E) = \frac{\sigma^{2}(P_{a}) + \sigma^{2}(P_{b}) + [2 \times \sigma^{2}(F_{1})]}{4}$$
$$\sigma^{2}(G) = \sigma^{2}(P) - \sigma^{2}(E) \qquad \sigma^{2}(A) = [2 \times \sigma^{2}(F_{2})] - [\sigma^{2}(BC_{1}P_{a}) + \sigma^{2}(BC_{1}P_{b})]$$

Phenotypic variance is estimated as the variance of the  $F_2$  progeny. The means of the variances in non-segregating generations ( $P_a$ ,  $P_b$ , and  $F_1$ ) provides an estimate of environmental variance. Genotypic variance is the difference in the phenotypic variance and environmental variance. Additive variance is derived from the single-locus model (Warner, 1952) and estimated by subtracting the backcross (BC<sub>1</sub>P<sub>a</sub>, BC<sub>1</sub>P<sub>b</sub>) variances from twice the variance of the F<sub>2</sub> progeny.

Heritability was estimated using the ratio of genotypic or additive variances to phenotypic variance. In all quantitative genetic studies, there is a large variation associated with variance component estimates. Negative estimates for genetic variances and heritability estimates outside the expected range of 0 to 1 are possible with the experiment design adopted. Negative estimates should be considered equal to zero (Robinson et al. 1955), but should be reported "in order to contribute to the accumulation of knowledge, which may, in the future, be properly interpreted" (Dudley and Moll 1969). We considered negative estimates equal to zero for the calculation of the mean estimates over families or locations. When a negative estimate was derived from another negative value (for example, narrowsense heritability and gain from selection, calculated from additive variance), it was considered to be zero and omitted.

The number of effective factors, an estimate of the genetic factors determining a quantitative trait (Mendelian genes or Quantitative Trait Loci), was estimated using the following methods (Lande 1981; Mather and Jinks 1982; Wright 1968):

Lande's method I:  

$$\frac{\left[\mu(P_b) - \mu(P_a)\right]^2}{8 \times \left\{\sigma^2(F_2) - \frac{\sigma^2(P_a) + \sigma^2(P_b) + \left[2 \times \sigma^2(F_1)\right]\right\}}{4}\right\}}$$

Lande's method II:

$$\frac{\left[\mu(P_{b})-\mu(P_{a})\right]^{2}}{8\times\left\{\left[2\times\sigma^{2}(F_{2})\right]-\left[\sigma^{2}(BC_{1}P_{a})+\sigma^{2}(BC_{1}P_{b})\right]\right\}}$$

Lande's method III:

$$\frac{\left[\mu(P_b) - \mu(P_a)\right]^2}{\left\{8 \times \left[\sigma^2(BC_1P_a) + \sigma^2(BC_1P_b) - \sigma^2(F_1)\right]\right\} - \frac{\left[\sigma^2(P_a) + \sigma^2(P_b)\right]}{2}$$

Mather's method: 
$$\frac{\left[\mu(P_b) - \mu(P_a)\right]^2}{\left[2 \times \sigma^2(F_2)\right] - \left[\sigma^2(BC_1P_a) + \sigma^2(BC_1P_b)\right]}$$

Wright's method:

$$\frac{\left[\mu(P_{b})-\mu(P_{a})\right]^{2}\times\left\{1.5-\left[2\times\frac{\mu(F_{1})-\mu(P_{a})}{\mu(P_{b})-\mu(P_{a})}\times\left(1-\frac{\mu(F_{1})-\mu(P_{a})}{\mu(P_{b})-\mu(P_{a})}\right)\right]\right\}}{8\times\left\{\sigma^{2}(F_{2})-\frac{\sigma^{2}(P_{a})+\sigma^{2}(P_{b})+\left[2\times\sigma^{2}(F_{1})\right]\right\}}{4}\right\}}$$

The assumptions for the estimates of number of effective factors were as follows: 1) with respect to all relevant loci, one parent is fixed with the alleles increasing the trait of interest and the other parent is fixed with alleles decreasing the trait of interest; 2) additive gene effects; 3) unlinked loci; and 4) equal allelic effects at all loci.

The possible gain from selection per cycle was predicted as  $h_n^2 \times \sqrt{\sigma^2(P)}$  multiplied by the selection differential in standard deviation units k for selection intensities of 5%, 10%, or 20% (Hallauer and Miranda 1988). The statistical analysis was performed using the SAS-STAT statistical package (SAS Institute, Cary, North Carolina). Correlations for disease trait ratings were calculated using the Pearson product-moment and Spearman rank methods.

# **Results and Discussion**

The mean of all F2 ratings for chlorosis, necrosis, and stunting are shown in Table 2.1. Overall, the highest disease severity for chlorosis and necrosis was on the final rating date (rating 3). The mean disease severity for both traits rated increased over time (ratings) for all environments (years x locations) with the exception of Castle Hayne in 2010. In this environment, the second rating date had a higher mean  $F_2$  rating than the final rating date (6.65 vs 5.85). It is possible that the environmental conditions had become less favorable for the disease, and plants had begun to recover.

Environmental variance  $[\sigma^2(E)]$  can have a large effect on inheritance studies as it is used to estimate genetic variance  $[\sigma^2(G) = \sigma^2(P) - \sigma^2(E)]$ . Broad-sense heritability (H<sup>2</sup>) is an estimate of the relative contribution of variance due to genotype to the total estimated variance  $[\sigma^2(G)/\sigma^2(P)]$ . Therefore, for our analysis, the mean of all ratings for each trait was chosen over individual ratings as the mean showed the least amount of environmental variance and highest genotypic contribution to overall variance (Table 2.2). The single rating for both chlorosis and necrosis having the least environmental variance and highest broadsense heritability over all families was rating two. Call et al. (2012a) showed that stunting ratings were progressively more informative as plants grew larger, and differences among plants were most apparent. Therefore, stunting was rated only on the final rating date. In 2011, stunting was not rated due to large differences among plants in time to emergence. There was no obvious difference between the parents, so we believe that the differences seen were due to weak seeds.

Call et al. (2012b) reported PI 197088 to be highly resistant to the current race of *P*. *cubensis* in the southeast United States. Seeds of PI 197088 were originally obtained from the United States Department of Agriculture's National Plant Germplasm System (USDA NPGS). Due to time limitations, only a single generation of selfing prior to using PI 197088 as the parent in each cross. It is therefore possible, and likely, that the parent used in the cross was not fully homozygous at all loci.

No segregation for high resistance has been observed in other studies conducted at NCSU using multiple PI 197088 sources (NPGS, greenhouse  $S_1$ , isolation block bulk increase) (data not shown). Nevertheless, it is possible that the parent was not homozygous at some loci affecting overall resistance to downy mildew. A high variance in the  $F_1$  generation relative to the parents in some crosses indicates this is likely. To account for this, we have redone the analysis using only the parents to estimate environmental variance

 $[\sigma^2(E)=(\sigma^2 P_a+\sigma^2 P_b)/2]$ . Estimates of environmental variance and broad-sense heritability using the modified formula are in Table 2.3. For reader information, this will be included in all relevant tables. But because the adjusted analysis may be inflating heritability estimates, discussion will be based on the original analysis which uses the parents and F<sub>1</sub> to estimate environmental variance  $[\sigma^2(E)]$ . In choosing the best rating for the analysis, it was determined under both methods that the mean of all ratings for each trait should be used as it showed the least amount of environmental variance and largest genotypic contribution to overall variance (Table 2.2). We also considered using the maximum single rating over reps for the analysis (Table 2.4).

All correlations were significant at p<0.001(Table 2.5). The chlorosis and necrosis mean ratings were highly correlated in both the Pearson and Spearman tests ( $R^2 = 0.90$  and 0.89, respectively), indicating they are likely the same trait. These traits were also highly correlated in other downy mildew studies (Call et al., 2012a; Call et al., 2012b). Therefore we will use only chlorosis in our analysis. Both chlorosis and necrosis were also significantly correlated with stunting, but to a lesser extent at  $R^2$ =0.73 and 0.75 for chlorosis and  $R^2$ =0.54 and 0.58 for necrosis, respectively. This indicates stunting ratings are related to tissue lesions, but other factors influence stunting as well, namely differences in genotypes. Correlations of chlorosis and necrosis with stunting were higher in this study compared to those reported by Call et al. (2012a; 2012b). This is likely due to the limited number of genotypes and the fact that the resistant parent (PI 197088) tended to be large and vigorous, with few lesions. We tested the F<sub>2</sub> data for homogeneity of variances using the Bartlett's method (Ostle and Malone 1988; Steel et al. 1997) (Table 2.6). Since variances were homogeneous among locations (Clinton, NC vs. Castle Hayne, NC) within family for PI 197088 x 'Ashley, PI 197088 x 'Poinsett 76', and PI 197088 x 'Polaris', we pooled the data for each family. The variances of family PI 197088 x 'Coolgreen' were homogeneous among locations for 2010, but not for 2011, or over both locations and years. We investigated the homogeneity of variances for that family over years within location and found that the data from Castle Hayne, NC was poolable, but data from Clinton, NC was not. To take it one step further, we looked at pooling 3 of the 4 environments (years x locations) and found that the combination of data from both locations in 2010, with data from Castle Hayne, NC in 2011 were poolable. We also analyzed the data for each family and test, to highlight possible differences among tests.

### **Qualitative Inheritance**

In our study, both resistance to downy mildew and stunting due to downy mildew in cucumber was not inherited as a single gene, (Tables 2.7 to 2.11). Plants having a mean rating less than 3.5 were classified as resistant, and those greater than 3.5 were classified as susceptible. This separation point was chosen to be between both parents, with emphasis on the high level of resistance from PI 197088. The expected segregation ratios for the inheritance of a single gene were not observed in the  $F_2$  and backcross generations (Tables 2.7 to 2.11). Interestingly, there were two combinations of family and environment in which segregation patterns resembled a single gene: PI 197088 x 'Coolgreen' at Clinton, NC in

2011, and PI 197088 x 'Ashley' at Castle Hayne, NC in 2011 (Tables 2.7 and 2.8). This appears to be a product of environment and chance, as well as the chosen cutoff for classifying plants as resistant or susceptible. The distribution of  $F_2$  plants for each of these cases also indicates that more than a single gene is involved (Table 2.12).

Stunting resistance in our study is a combination of the ability of the plant to grow vigorously with or without the pathogen present, and is therefore affected by resistance. PI 197088 is a large and vigorous cultigen, and would rate as more "stunting resistant" than 'Coolgreen', even in the absence of disease. To determine if this large vigorous habit was a single gene, plants were classified as resistant only if they rated at a 1 or 2 for stunting, as all of the PI 197088 plants did. The goodness of fit test (Table 2.11) and the distribution of  $F_2$  plants both indicate the trait is not under control of a single gene.

The lack of fit to the single gene hypothesis suggests that resistance could be inherited quantitatively, with multiple loci regulating the level of expression. For both chlorosis and stunting, the F1's were generally intermediate between the parents in all families, indicating at least 1 dominant gene is involved in both traits (Tables 2.12 to 2.14). In general, the test at Castle Hayne, NC tended to rate higher (more disease, smaller plants), pushing the means towards that of the susceptible parent. It is possible this is due to higher humidity near the coast and indicates that Castle Hayne, NC may be a better selection environment to identify high resistance. It is also possible that there are different races of *P. cubensis* at each location, resulting in differences among tests.

The distributions of the  $F_2$  data (Tables 2.12 to 2.14 and Figure 2.1) are close to the expected bell-shaped distribution of a quantitative trait, and ranging between both parents, though somewhat skewed towards susceptibility for most families and environments. Exceptions were crosses involving moderately resistant cultivars 'Poinsett 76' and 'Polaris'. In these crosses, the  $F_2$  distribution was much narrower with more plants rating as more resistant, especially at Clinton, NC, which had less disease pressure than Castle Hayne. For stunting, none of the  $F_2$ 's rated as high as the resistant parent within the respective locations (Table 2.14), but we did observe plants with stunting equal to or greater than the susceptible 'Coolgreen'. Since there was strong evidence against the single gene hypothesis, analyzed resistance to downy mildew as a quantitative trait.

#### **Quantitative Inheritance**

In our analysis, the variances of the six generations tested were mostly consistent across locations (Tables 2.15 and 2.16). One exception is the  $F_1$  generation of PI 197088 x 'Coolgreen' at Castle Hayne, NC in 2011, which had a much higher variance compared to other homogenious generations. It can also be observed in the distribution (Table 2.13) that half of the plants in that cross were resistant and half were susceptible. This indicates that there may have been a dominant resistance gene in PI 197088 present in its heterozygous state. It is interesting that this phenomenon is only observed in this family in 2011, and only in the  $F_1$  (not observed in reciprocal  $F_1$ ). Field tests of selfed PI 197088 show no segregation for high resistance (Call, personal observation). Segregation of a gene with such a large effect should be obvious within a plot unless it is masked by other resistance genes. At Castle Hayne, NC, generation variances involving the stunting resistant parent (Pa, F1, BC1Pa) tended to be higher compared to the same generations at Clinton, NC (Table 2.16). The environment at Castle Hayne, NC may have been less favorable for large plants. Mean ratings for both chlorosis and stunting were higher at Castle Hayne, NC compared to Clinton, NC, suggesting disease pressure was most likely greater at Castle Hayne, NC, and indicating that even vigorous, highly resistant plants are negatively affected by downy mildew.

Genetic variance for downy mildew resistance (Table 2.17) was larger than environmental variance for all families and locations except PI 197088 x 'Coolgreen' at Castle Hayne, NC in 2011. This was due the large environmental variance estimate originating with high variance in the F<sub>1</sub>. With the modified estimate of environmental variance (Table 2.18) it was observed that this is not the case. In general, the genetic variance was at least twice the environmental variance, further supporting the hypothesis that downy mildew resistance in PI 197088 is a quantitative trait. Additive genetic effects were estimated, but a comparison with dominance effects was not possible due to the experiment design. Dominance variance could be indirectly estimated by subtraction of additive variance from the genetic variance, but this estimate would not be precise. Negative estimates for additive variance were found for 3 families at Castle Hayne, NC: 'Coolgreen' 2010, 'Coolgreen' 2011, and 'Ashley' 2011, resulting from high variance in the backcross to PI 197088. This indicates the presence of either a major recessive or additive resistance gene in PI 197088.

For stunting, the genetic variance was larger than environmental variance at Clinton, NC but smaller than environmental variance at Castle Hayne, NC (Table 2.19). The large environmental variance at Castle Hayne, NC is due to the large variance in the F1 plants tested. It is likely this is partially due to missing plots (11 of 20 missing) as well as environmental effects in the field. Interestingly, both of the parent generations at Castle Hayne, NC had a very low variance. Downy mildew progression is highly influenced by environment, and it is easy to imagine that the environmental influence would be greater on moderately resistant plants compared to highly resistant or highly susceptible plants. That is, the range of observed phenotypes is greater on moderately resistant genotypes versus those at the extremes. Nevertheless, a large genetic component was found for both locations, corroborating the hypothesis of stunting resistance as a quantitative trait. To reduce overall environmental variance, greenhouse tests could be used, which may result in even higher additive variance and narrow-sense heritability. Additive effects in our experiment were large at both locations (1.91 and 1.28 at Clinton, NC and Castle Hayne, NC, respectively), contributing to a high narrow-sense heritability.

The heritability of resistance to downy mildew and stunting in PI 197088 were high (Tables 2.18 and 2.19), with all families at Clinton, NC approaching or above 1. Estimates at Castle Hayne, NC were lower (0.23) for the 'Poinsett 76' cross and high (1.15) for the 'Polaris' cross. This is interesting because both of these cultigens are moderately resistant and share the dm-1 gene (Wehner and Shetty, 1997), and have tested similarly in other

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studies (Call et al., 2012). The observed difference at Castle Hayne, NC could be due to the environment with higher disease pressure.

For stunting, the narrow-sense heritability was much larger at Clinton, NC than Castle Hayne, NC (1.26 vs. 0.66, respectively) (Table 2.19). The broad-sense heritability was also high at Clinton, NC (0.61) and moderate at Castle Hayne, NC (0.44). The high broad-sense heritability indicates genetic variability was important at both locations. The large value of narrow-sense heritability indicates that additive effects are likely more important than dominance effects in improving stunting resistance, but this could be further studied using an experimental design that can estimate dominance variance directly, such as NC Design I.

Dominance and epistatic effects could not be estimated in our analysis. Thus, the estimates of the minimum number of effective factors (genes) for resistance may be biased. For downy mildew resistance, the number of effective factors ranged from 1 to 17, with all but two estimates ranging from one to seven (Table 2.20). Mean estimates of families within environments ranged from two to five. For stunting, five estimates were used but only four methods that were most consistent among locations are presented (Table 2.21). Estimates range from two to seven effective factors with a mean of 3.55 for the pooled data. These estimates suggest that stunting resistance to downy mildew in cucumber is a quantitative trait controlled by few genetic factors.

Our analysis showed that good progress can be made using field selection for both downy mildew and stunting resistance, potentially leading to a gain of two to three points (on a 0 to 9 scale) in a single generation under high selection intensity (k = 5%) (Table 2.20).

Even under the lower selection intensities (i.e., k = 20%) typically used in recurrent selection programs, a gain of one to two points can be expected in a single generation of selection.

Significant progress for stunting resistance or large vigorous plants would likely be made relatively quickly using a susceptible line for population development such as 'Coolgreen', used in this study. It is more likely, however, that breeders would use moderately resistant lines with good fruit quality to cross with vigorous and stunting resistant lines. In such a population, heritability estimates may be smaller than found in our study, and progress may be slower than estimated. Nevertheless, the potential for progress and the opportunity to capitalize on additive variance remain.

# Conclusion

Our study indicates that downy mildew and stunting resistance in cucumber should be regarded as a quantitative traits for breeding purposes, although it appears that both traits are under the control of relatively few genes. Resistant by moderately resistant crosses seem to be the most useful for breeding. Breeding techniques taking advantage of the high additive variance, such as recurrent selection, should be used for population improvement. Field resistance is easily tested using natural sources of inoculum in areas where downy mildew is an endemic disease. Greenhouse testing could be used to reduce environmental variance as the combination of quantitative inheritance and high environmental variability could limit the gain from selection. Nevertheless, the high narrow-sense heritability indicates single plants could be selected for inbred line development. In addition, PI 197088 is far from marketable, and linkage drag will likely bring along undesirable traits. Pedigree selection or backcross

breeding will likely be useful in moving qualitative traits, such as white spine, non-netted, mottled, and bitterfree, into breeding lines with vigor and high resistance.

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	Chloros	sis Rating			Necrosi		Stunting		
Year, Loc	Rt. 1	Rt. 2	Rt. 3	Mean	Rt. 1	Rt. 2	Rt. 3	Mean	
2010	4.94	5.84	5.99	5.55	4.83	5.55	6.21	5.43	4.87
Clinton	3.96	5.08	6.12	5.01	4.96	5.08	6.64	5.53	4.19
C. Hayne	5.96	6.65	5.85	6.11	4.70	6.06	5.72	5.32	5.59
2011	3.44	3.78	4.89	3.97	3.39	3.50	4.53	3.75	-
Clinton	2.22	2.89	4.12	2.99	2.29	2.55	3.67	2.77	-
C. Hayne	4.62	4.57	5.53	4.92	4.47	4.34	5.26	4.70	-
Mean	4.19	4.80	5.42	4.76	4.11	4.52	5.34	4.58	4.88

Table 2.1. Mean of F2 plants by year and location within year for all ratings <sup>a</sup>.

a Data are ratings from four *Cucumis sativus* families developed from the cross of resistant PI 197088 by susceptible cultivars 'Coolgreen', 'Ashley', 'Poinsett 76', and 'Polaris'. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: were 0 to 9 (0 = 0%, 1 = 1-3%, 2 = 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99%, 9 = 100%)

Environmental Variance ( $\sigma^2(E)$ ) <sup>b</sup>												
Family			Chlorosi	is Rating	3		Necrosi	is Rating	ç	Stunting		
197088 x	Year x Loc	<b>Rt.</b> 1	Rt. 2	<b>Rt. 3</b>	Mean	Rt. 1	<b>Rt. 2</b>	<b>Rt. 3</b>	Mean			
Coolgreen	10 Clinton	0.89	0.61	0.55	0.25	0.93	0.87	0.64	0.30	0.59		
	10 C. Hayne	1.19	0.32	1.04	0.33	1.43	0.61	0.95	0.33	1.08		
	11 Clinton	0.99	1.29	1.39	0.89	2.84	2.62	2.62	1.99	-		
	11 C. Hayne	3.25	1.74	3.23	2.55	3.23	1.62	2.69	2.08	-		
Ashley	11 Clinton	1.34	2.10	1.08	1.07	2.38	3.36	2.37	1.64	-		
	11 C. Hayne	0.82	1.22	0.78	0.48	1.80	1.14	1.34	0.74	-		
Poinsett 76	11 Clinton	0.40	0.48	1.20	0.23	2.38	1.95	3.38	0.98	-		
	11 C. Hayne	0.87	1.03	0.82	0.42	1.04	1.60	1.09	0.65	-		
Polaris	11 Clinton	0.87	0.32	0.64	0.26	2.52	2.25	1.64	0.79	-		
	11 C. Hayne	C. Hayne 0.61 0.57 0.47 0.23					1.01	1.06	0.37	-		
	Mean	1.12	0.97	1.12	0.67	1.95	1.70	1.78	0.99	0.84		

Table 2.2. Environmental variance estimates and broad-sense heritability for downy mildew disease trait ratings and their means by family and environment (Year x Location)<sup>a</sup>.

Broad-sense Heritability – $(\sigma^2(G)/\sigma^2(P))$													
Family			Chloros	is Rating	3		Necros	is Rating	5	Stunting			
197088 x	Year x Loc	Rt. 1	Rt. 2	Rt. 3	Mean	Rt. 1	<b>Rt. 2</b>	Rt. 3	Mean				
Coolgreen	10 Clinton	0.34	0.82	0.73	0.84	-1.10	0.77	0.70	0.75	0.61			
	10 C. Hayne	0.23	0.86	0.58	0.78	0.31	0.84	0.64	0.83	0.44			
	11 Clinton	0.82	0.77	0.62	0.76	0.44	0.55	0.54	0.47	-			
	11 C. Hayne	-0.60	-0.15	-0.99	-0.94	-0.05	-0.02	-0.67	-0.53	-			
Ashley	11 Clinton	0.12	0.39	0.80	0.53	-0.82	0.02	0.48	0.26	-			
	11 C. Hayne	0.73	0.42	0.65	0.74	0.45	0.41	0.42	0.56	-			
Poinsett 76	11 Clinton	0.77	0.76	0.44	0.85	-0.39	-0.37	-0.57	0.26	-			
	11 C. Hayne	0.61	0.51	0.71	0.73	0.68	0.13	0.61	0.62	-			
Polaris	11 Clinton	0.02	0.84	0.81	0.78	-0.97	0.11	0.54	0.46	-			
	11 C. Hayne	0.84	0.79	0.76	0.87	0.77	0.27	0.51	0.75	-			
	Mean <sup>c</sup>	0.45	0.62	0.61	0.69	0.17	0.31	0.44	0.50	0.53			

a Data are ratings from four *Cucumis sativus* families developed from the cross of resistant PI 197088 by susceptible cultivars 'Coolgreen', 'Ashley', 'Poinsett 76', and 'Polaris'. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: 0 to 9 (0 = 0%, 1 = 1-3%, 2= 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99%, 9 = 100%)

$$=\frac{\sigma^{2}(P_{a})+\sigma^{2}(P_{b})+\left[2\times\sigma^{2}(F_{1})\right]}{4}$$

b 
$$\sigma^2(E)$$
 = environmental variance = -

c Broad-sense heritability means calculated with negative estimates as zero.

Environmental Variance (σ <sup>2</sup> (E)) <sup>b</sup>													
Family			Chlorosi	is Rating	5		Necrosi	s Rating		Stunting			
197088 x	Year x Loc	Rt. 1	<b>Rt. 2</b>	Rt. 3	Mean	Rt. 1	<b>Rt. 2</b>	Rt. 3	Mean				
Coolgreen	10 Clinton	1.13	0.11	0.26	0.13	1.29	0.48	0.55	0.15	0.38			
	10 C. Hayne	0.18	0.14	0.14	0.06	0.50	0.21	0.38	0.59	0.13			
11 Clinton 0.09 0.09 0.33 0.07 0.75 0.40 1.03 0.25 -													
	11 C. Hayne	0.40	0.29	0.34	0.20	0.95	0.63	0.65	0.35	-			
Ashley	11 Clinton	0.47	0.22	0.58	0.21	1.30	0.84	1.76	0.47	-			
	11 C. Hayne	0.49	0.89	0.62	0.41	1.36	1.43	1.24	0.74	-			
Poinsett 76	11 Clinton	0.21	0.16	0.37	0.15	2.73	0.53	1.17	0.41	-			
	11 C. Hayne	0.74	0.56	1.01	0.55	0.87	2.06	1.14	0.76	-			
Polaris	11 Clinton	0.75	0.21	0.32	0.27	1.33	1.82	1.42	0.54	-			
	11 C. Hayne	0.40	0.70	0.50	0.34	0.75	1.66	1.05	0.50	-			
	Mean	0.49	0.34	0.45	0.24	1.18	1.01	1.04	0.48	0.26			
Broad-sense H	- (σ	$^{2}(G)/\sigma^{2}(G)$	<b>P</b> ))			•				•			

Table 2.3. Modified environmental variance estimates and broad-sense heritability for downy mildew disease trait ratings and their means by family and environment (Year x Location)<sup>a</sup>.

Family			Chlorosi	is Rating	Ş			Stunting		
197088 x	Year x Loc	Rt. 1	Rt. 2	Rt. 3	Mean	Rt. 1	<b>Rt. 2</b>	Rt. 3	Mean	
Coolgreen	10 Clinton	0.16	0.97	0.87	0.92	-1.93	0.87	0.74	0.87	0.75
	10 C. Hayne	0.88	0.94	0.94	0.96	0.80	0.98	0.83	0.78	0.93
	11 Clinton	0.98	0.98	0.90	0.98	0.85	0.93	0.81	0.93	-
	11 C. Hayne	0.80	0.81	0.78	0.84	0.69	0.60	0.56	0.73	-
Ashley	11 Clinton	0.67	0.92	0.88	0.89	-0.04	0.69	0.55	0.71	-
	11 C. Hayne	0.84	0.58	0.72	0.77	0.59	0.26	0.47	0.56	-
Poinsett 76	11 Clinton	0.87	0.92	0.83	0.89	-0.67	0.62	0.47	0.69	-
	11 C. Hayne	0.67	0.73	0.64	0.64	0.73	-0.12	0.59	0.56	-
Polaris	11 Clinton	0.17	0.89	0.91	0.78	-0.05	0.28	0.62	0.63	-
	11 C. Hayne	0.90	0.74	0.75	0.81	0.81	-0.20	0.52	0.66	-
	Mean	0.69	0.85	0.82	0.85	0.45	0.52	0.62	0.71	0.84

a Data are ratings from four *Cucumis sativus* families developed from the cross of resistant PI 197088 by susceptible cultivars 'Coolgreen', 'Ashley', 'Poinsett 76', and 'Polaris'. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: 0 to 9 (0 = 0%, 1 = 1-3%, 2= 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99%, 9 = 100%)

b Modified  $\sigma^2(E)$  = environmental variance =  $(\sigma^2(Pa) + \sigma^2(Pb))/2$ 

Family		Environme	ntal Varianc	$e(\sigma^2(E))^b$	Broad-sense Heritability – $(\sigma^2(G)/\sigma^2(P))$				
197088 x	Year x Loc	Chlorosis	Necrosis	Mean	Chlorosis	Necrosis	Mean		
Coolgreen	10 Clinton	0.79	0.94	0.56	0.60	0.41	0.67		
	10 C. Hayne	0.67	2.39	1.09	0.52	0.37	0.49		
	11 Clinton	0.72	1.67	0.90	0.78	0.63	0.7		
	11 C. Hayne	1.99	1.82	1.76	-1.00	-0.73	-1.23		
Ashley	11 Clinton	0.81	1.92	1.10	0.81	0.50	0.69		
	11 C. Hayne	0.52	1.14	0.48	0.77	0.50	0.78		
Poinsett 76	11 Clinton	0.62	2.00	0.83	0.73	0.17	0.62		
	11 C. Hayne	0.66	0.81	0.64	0.69	0.68	0.7		
Polaris	11 Clinton	0.58	2.20	1.04	0.84	0.4	0.68		
	11 C. Hayne	0.47	0.97	0.51	0.76	0.58	0.71		
	Mean	0.78	1.59	0.89	0.65	0.42	0.60		

Table 2.4. Environmental variance and broad-sense heritability for downy mildew disease trait maximum ratings and their means by family and environment (Year x Location)<sup>a</sup>.

a Data are ratings from four *Cucumis sativus* families developed from the cross of resistant PI 197088 by susceptible cultivars 'Coolgreen', 'Ashley', 'Poinsett 76', and 'Polaris'. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: 0 to 9 (0 = 0%, 1 = 1-3%, 2= 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75\%, 7 = 75-87\%, 8 = 87-99\%, 9 = 100\%)

$$\sigma^{2}(P_{a}) + \sigma^{2}(P_{b}) + \left[2 \times \sigma^{2}(F_{1})\right]$$

b  $\sigma^2(E)$  = environmental variance = -

	Chlorosis 1	Rating			Necrosis R		Stunting		
Rating	1	2	3	Mean	1	2	3	Mean	
Chlorosis	Rating								
1		$0.79^{***}$	$0.70^{***}$	0.91***	$0.81^{***}$	$0.76^{***}$	$0.68^{***}$	$0.84^{***}$	$0.57^{***}$
2	0.78***		$0.81^{***}$	0.94***	0.64***	0.83***	$0.79^{***}$	$0.86^{***}$	$0.78^{***}$
3	$0.70^{***}$	0.81***		0.91***	$0.60^{***}$	$0.68^{***}$	$0.84^{***}$	$0.80^{***}$	$0.68^{***}$
Mean	$0.90^{***}$	0.94***	0.92***		$0.74^{***}$	$0.81^{***}$	$0.84^{***}$	$0.90^{***}$	0.73***
Necrosis I	Rating								
1	$0.80^{***}$	0.65***	$0.60^{***}$	0.75***		$0.66^{***}$	0.61***	$0.86^{***}$	0.30***
2	$0.75^{***}$	$0.82^{***}$	$0.67^{***}$	$0.82^{***}$	0.66***		0.73***	0.91***	$0.65^{***}$
3	$0.68^{***}$	$0.79^{***}$	$0.84^{***}$	$0.84^{***}$	0.61***	0.73***		$0.89^{***}$	$0.52^{***}$
Mean	0.83***	$0.85^{***}$	$0.81^{***}$	$0.89^{***}$	$0.84^{***}$	$0.90^{***}$	0.90***		$0.54^{***}$
Stunting 1	Rating								
	$0.58^{***}$	$0.75^{***}$	0.63***	0.75***	$0.27^{***}$	$0.62^{***}$	$0.44^{***}$	$0.58^{***}$	

Table 2.5. Pearson product-moment correlation coefficients (above diagonal) and Spearman's rank correlation coefficients (below diagonal) for downy mildew chlorosis, necrosis and stunting ratings and means in Clinton and Castle Hayne, North Carolina from 2010 to 2011 <sup>a</sup>.

a Data are ratings from four *Cucumis sativus* families developed from the cross of resistant PI 197088 by susceptible cultivars 'Coolgreen', 'Ashley', 'Poinsett 76', and 'Polaris'. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: 0 to 9 (0 = 0%, 1 = 1-3%, 2= 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99%, 9 = 100%)

\*, \*\*, \*\*\* Significant at 0.05, 0.01, and 0.001, respectively.

	Chlorosis Mean	Rating	<u>Stunting</u>	
	$X^2$	P-value	$\mathbf{X}^2$	P-value
Poolability by family over locations				
<u>2010</u>				
PI 197088 x Coolgreen	0.83	0.36	1.06	0.30
<u>2011</u>				
PI 197088 x Coolgreen	13.91*	0.00	-	-
PI 197088 x Ashley	0.02	0.89	-	-
PI 197088 x Poinsett 76	0.20	0.66	-	-
PI 197088 x Polaris	2.64	0.10	-	-
Poolability by location over years (2010 to 2011)				
<u>Clinton, NC</u>				
PI 197088 x Coolgreen	9.36*	0.00	-	-
<u>Castle Hayne, NC</u>				
PI 197088 x Coolgreen	0.01	0.92	-	-
Poolability by location and years (2010 to 2011)				
PI 197088 x Coolgreen	19.75*	0.00	-	-
Poolability of 2010 and Castle Hayne, NC 2011				
PI 197088 x Coolgreen	1.24	0.54	-	-

Table 2.6. Chi-squares and P-values from Bartlett's Homogeneity of Variances test for validity in pooling data.

\*P-value<.05 not poolable.

Trait: Chlorosis Mean												
Family: 1	97088 x C	oolgreei	1									
Clinton, N	NC 2010						Castle	Hayne,	NC 201	0		
	Dom. <sup>b</sup>	Rec. <sup>c</sup>	Exp. <sup>d</sup>	$X^2$	df	Р	Dom. <sup>b</sup>	Rec. <sup>c</sup>	Exp. <sup>d</sup>	$X^2$	d	Р
$P_a^e(R)$	0	4					0	7				
$P_b^{f}(S)$	10	0					9	0				
F1	19	0					9	0				
F2	66	7	3:1	9.25	1	0.002*	67	1	3:1	20.08	1	0.000*
$BC_1P_a$	4	21	1:1	11.56	1	0.000*	15	11	1:1	0.62	1	0.43
$BC_1P_b$	27	0	1:0	0	1	1.00	20	0	1:0	0.05	1	1.00
Clinton, N	NC 2011						Castle	Hayne,	NC 201	1		
	Dom. <sup>b</sup>	Rec. <sup>c</sup>	Exp. <sup>d</sup>	$X^2$	df	Р	Dom. <sup>b</sup>	Rec. <sup>c</sup>	Exp. <sup>d</sup>	$X^2$	d	Р
$P_a^e(R)$	0	24					0	33				
$P_b^{f}(S)$	6	0					6	0				
F1	12	1					14	4				
F2	25	12	3:1	1.09	1	0.29	61	1	3:1	18.09	1	0.000*
$BC_1P_a$	7	13	1:1	1.80	1	0.17	19	5	1:1	8.17	1	0.004*
$BC_1P_b$	26	0	1:0	0	1	1	30	0	1:0	0	1	1.00
Clinton,	C. Hayne	2010 + 0	C. Hayn	e 2011								
$P_a^e(R)$	0	44										
$P_b^{f}(S)$	25	0										
F1	42	4										
F2	194	9	3:1	45.21	1	0.000*						
$BC_1P_a$	38	37	1:1	0.01	1	0.90						
$BC_1P_b$	77	0	1:0	0.01	1	1.00						

Table 2.7. Single locus goodness-of-fit-test of mean chlorosis ratings for resistance to downy mildew at Clinton and Castle Hayne, NC in 2010 and 2011 for the cross PI 197088 x 'Coolgreen' <sup>a</sup>.

a Data are ratings from families developed from the cross of resistant PI 197088 by susceptible cultivar 'Coolgreen'. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: 0 to 9 (0 = 0%, 1 = 1-3%, 2= 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99%, 9 = 100%)

b Plants classified as hypothetic carrier of dominant gene

c Plants classified as hypothetic carrier of susceptible gene

d Expected was the hypothesized segregation ratio for single gene inheritance for each segregating generation

e Resistant plants had a mean disease rating < 3.5

Trait: Chlorosis Mean													
Family: 197088 x Ashley													
Clinton, N	NC 2011						Castle Hayne, NC 2011						
	Dom. <sup>b</sup>	Rec. <sup>c</sup>	Exp. <sup>d</sup>	$X^2$	df	Р	Dom. <sup>b</sup>	Rec. <sup>c</sup>	Exp. <sup>d</sup>	$X^2$	df	Р	
$P_a^{e}(R)$	0	24					0	28					
$P_b{}^{f}(S)$	9	0					9	0					
F1	8	7					16	2					
F2	15	49	3:1	90.75	1	0.000*	49	12	3:1	0.92	1	0.33	
$BC_1P_a$	3	22	1:1	14.44	1	0.000*	13	11	1:1	0.17	1	0.68	
$BC_1P_b$	13	15	1:0	8.04	1	0.004*	21	0	1:0	0	1	1	
Clinton, C	C. Hayne 2	2011 Po	oled										
$P_a^{e}(R)$	0	52											
$P_b{}^{f}(S)$	18	0											
F1	24	9											
F2	64	61	3:1	37.76	1	0.000*							
$BC_1P_a$	16	33	1:1	5.90	1	0.015*							
$BC_1P_b$	34	15	1:0	4.59	1	0.032*							
a Data	are ratings	from for	milios do	valopad fr	om t	ha cross of r	originat D	107089	by succe	ntible c		or	

Table 2.8. Single locus goodness-of-fit-test of mean chlorosis ratings for resistance to downy mildew at Clinton and Castle Hayne, NC in 2010 and 2011 for the cross PI 197088 x 'Ashley' <sup>a</sup>.

a Data are ratings from families developed from the cross of resistant PI 197088 by susceptible cultivar 'Ashley'. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: 0 to 9 (0 = 0%, 1 = 1-3%, 2 = 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99%, 9 = 100%)

b Plants classified as hypothetic carrier of dominant gene

c Plants classified as hypothetic carrier of susceptible gene

d Expected was the hypothesized segregation ratio for single gene inheritance for each segregating generation

e Resistant plants had a mean disease rating < 3.5

Trait: Chlorosis Mean													
Family: 197088 x Poinsett 76													
Clinton,	NC 2011						Castle Hayne, NC 2011						
	Dom. <sup>b</sup>	Rec. <sup>c</sup>	Exp. <sup>d</sup>	$X^2$	df	Р	Dom. <sup>b</sup>	Rec. <sup>c</sup>	Exp. <sup>d</sup>	$X^2$	df	Р	
$P_a^e(R)$	24	0					0	28					
$P_b^{f}(S)$	0	7					5	0					
F1	15	1					20	0					
F2	47	4	3:1	8.01	1	0.001*	31	42	3:1	41.2	1	0.000*	
$BC_1P_a$	23	4	1:1	0.59	1	0.58	15	15	1:1	0	1	1	
$BC_1P_b$	23	3	1:0	15.38	1	0.000*	17	6	1:0	1.57	1	0.21	
Clinton,	C. Hayne	2011 Po	oled										
$P_a^e(R)$	0	52											
$P_b^{f}(S)$	12	0											
F1	21	15											
F2	36	100	3:1	170.82	1	0.000*							
$BC_1P_a$	18	42	1:1	9.60	1	0.001*							
$BC_1P_b$	20	29	1:0	17.16	1	0.000*							
o Doto	oro rotingo	from fo	milia da	walamad f	nom t	he erece of	magistant D	1 10700	0 hrs and a	antihla	a		

Table 2.9. Single locus goodness-of-fit-test of mean chlorosis ratings for resistance to downy mildew at Clinton and Castle Hayne, NC in 2010 and 2011 for the cross PI 197088 x 'Poinsett 76' <sup>a</sup>.

a Data are ratings from families developed from the cross of resistant PI 197088 by susceptible cultivar Poinsett 76'. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: 0 to 9 (0 = 0%, 1 = 1-3%, 2= 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99%, 9 = 100%)

b Plants classified as hypothetic carrier of dominant gene

c Plants classified as hypothetic carrier of susceptible gene

d Expected was the hypothesized segregation ratio for single gene inheritance for each segregating generation

e Resistant plants had a mean disease rating < 3.5

Trait: Chlorosis Mean												
Family: 19	97088 x P	olaris										
Clinton, N	C 2011						Castle l	Hayne, I	NC 2011			
	Dom. <sup>b</sup>	Rec. <sup>c</sup>	Exp. <sup>d</sup>	$X^2$	df	Р	Dom. <sup>b</sup>	Rec. <sup>c</sup>	Exp. <sup>d</sup>	$X^2$	df	Р
<u>Clinton</u>												
$P_a^{e}(R)$	24	0					0	28				
$P_b^{f}(S)$	0	7					10	0				
F1	14	0					17	2				
F2	59	5	3:1	10.08	1	0.001*	62	11	3:1	3.84	1	0.05
$BC_1P_a$	26	0	1:1	0	1	1	2	18	1:1	12.8	1	0.000*
$BC_1P_b$	23	1	1:0	20.17	1	0.000*	26	0	1:0	0	1	1
Clinton, C	. Hayne 2	2011 Po	oled									
$P_a^e(R)$	0	52										
$P_b^{f}(S)$	17	0										
F1	17	16										
F2	67	70	3:1	49.75	1	0.000*						
$BC_1P_a$	2	44	1:1	38.35	1	0.000*						
$BC_1P_b$	28	22	1:0	9.68	1	0.001*						

Table 2.10. Single locus goodness-of-fit-test of mean chlorosis ratings for resistance to downy mildew at Clinton and Castle Hayne, NC in 2010 and 2011 for the cross PI 197088 x 'Polaris' <sup>a</sup>.

a Data are ratings from families developed from the cross of resistant PI 197088 by susceptible cultivar 'Polaris'. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: 0 to 9 (0 = 0%, 1 = 1-3%, 2 = 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99%, 9 = 100%)

b Plants classified as hypothetic carrier of dominant gene

c Plants classified as hypothetic carrier of susceptible gene

d Expected was the hypothesized segregation ratio for single gene inheritance for each segregating generation

e Resistant plants had a mean disease rating < 3.5

Family: PI 197088 x Coolgreen												
Trait: Stunting												
Clinton, NC 2010							Castle Hayne, NC 2010					
2010	Dom. <sup>d</sup>	Rec. <sup>e</sup>	Exp. <sup>f</sup>	$X^2$	df	Р	Dom. <sup>d</sup>	Rec. <sup>e</sup>	Exp. <sup>f</sup>	$X^2$	df	Р
Clinton												
$P_a{}^g(R)$	0	4					0	7				
$P_b{}^h(S)$	10	0					8	0				
F1	16	3					9	0				
F2	66	7	3:1	9.25	1	0.002*	68	0	3:1	22.67	1	0.000*
$BC_1P_a$	2	23	1:1	17.64	1	0.000*	9	17	1:1	2.46	1	0.110
$BC_1P_b$	27	0	1:0	0	1	1.000	6	0	1:0	0	1	1.000
Pooled:	Clinton a	and Cas	tle Hayı	ne, NC 2	2010							
$P_a{}^g(R)$	0	11										
$P_b{}^h(S)$	18	0										
F1	25	3										
F2	134	7	3:1	30.19	1	0.000*						
$BC_1P_a$	11	40	1:1	16.49	1	0.000*						
$BC_1P_b$	33	0	1:0	0	1	1.000						

Table 2.11. Single locus goodness-of-fit-test for stunting resistance to downy mildew in the cross PI 197088 (R) x 'Coolgreen' (S) tested at Clinton and Castle Hayne, NC in 2010 <sup>a</sup>.

a Data are ratings from a *Cucumis sativus* family developed from the cross of resistant PI 197088 by susceptible cultivar 'Coolgreen'.

b Susceptible plants had a disease rating > 2.5; Resistant plants had a disease rating < 2.5 on a 0 to 9 scale.

d Plants classified as having the predicted dominant phenotype.

e Plants classified as having the predicted recessive phenotype.

f Expected was the hypothesized segregation ratio for single gene inheritance for each segregating generation

g P<sub>a</sub> was the resistant parent, PI 197088

h P<sub>b</sub> was the susceptible parent, 'Coolgreen'.

Comonstinue	No. of	No. of plants in classes <sup>b</sup>										
Generation	plants	0 1	2	3	4	5	6	7	8	9	DSI	
197088 x Coolg	reen – Clin	ton and Cas	stle Hayı	ne, NC 2	010 + Ca	astle Hay	ne, NC	2011				
P1	44	25	15	4							1.5	
P2	25							4	11	1	8.2	
F1	23	1	3		2	5	3	8	1		5.3	
RF1	23					5	7	11			6.3	
F2	28	1	2	7	26	31	67	6	14		5.9	
Bc1P1	75	3	16	18	11	8	1	9			3.9	
Bc1P2	77						3	43	3	1	7.4	
197088 x Ashley – Clinton and Castle Hayne, NC 2011												
P1	52	33	16	3							1.4	
P2	18				3	6	1	4	4		6.0	
F1	14			1	4	7	1		1		4.9	
RF1	19			8	9	2					3.7	
F2	125	1	27	24	22	22	12	6	2		3.7	
Bc1P1	49	7	15	11	9	3	2	2			3.0	
Bc1P2	49		4	11	12	4	8	6	4		4.7	
197088 x Poinse	ett 76 – Cli	nton and Ca	stle Hay	ne, NC 2	2011						_	
P1	52	33	16	3							1.4	
P2	12				3	7	2				4.9	
F1	18		4	4	2	7	1				3.8	
RF1	18		4	3	6	4	1				3.7	
F2	124	3	28	31	17	1	6	2			2.8	
Bc1P1	57	7	23	8	14	5					2.8	
Bc1P2	49	7	9	13	11	2	6	1			3.3	
197088 x Polari	s – Clinton	and Castle	Hayne, I	NC 2011								
P1	52	33	16	3							1.4	
P2	17				2	5	3	7			5.9	
F1	20	5	5	2	7	1					2.7	
RF1	13		3	1	9						3.5	
F2	137	19	25	26	21	17	18	11			3.7	
Bc1P1	46	19	18	7		2					1.9	
Bc1P2	50	2	10	10	1	4	12	11			4.5	

Table 2.12. Frequency distribution and disease severity index (DSI) of downy mildew mean chlorosis ratings for pooled data <sup>a</sup>.

a Data are ratings from families developed from the cross of resistant PI 197088 by susceptible cultivar 'Poinsett 76'. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: 0 to 9 (0 = 0%, 1 = 1-3%, 2= 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99%, 9 = 100%)

b Data were means of three ratings, rounded to nearest class.

C	No. of	No. of pla	No. of plants in classes <sup>b</sup>										
Generation	plants	0 1	2	3	4	5	6	7	8	9	DSI		
PI 197088 x Coolg	reen – Cl	inton 2010											
P1	4	3	1								1.3		
P2	10							4	6		7.6		
F1	9				2	5	2				5.0		
RF1	10					4	6				5.6		
F2	76		2	6	18	2	21	8	1		5.1		
Bc1P1	25	1	13	7	3		1				2.6		
Bc1P2	27							17	1		7.4		
PI 197088 x Coolgreen – Castle Hayne 2010													
P1	7	6	1	÷		-	-	÷	÷		1.1		
P2	9								5	4	8.4		
F1	6						1	4	1		7.0		
RF1	3					1	1	1			6.0		
F2	68	1			5	7	24	29	2		6.2		
Bc1P1	26	2	1	8	3	5	3	4			4.3		
Bc1P2	20						2	4	13	1	7.7		
PI 197088 x Coolgreen – Clinton 2011													
P1	24	19	5	Ċ.	-	-	-	Ċ.		_	1.2		
P2	6									6	9.0		
F1	4			1				1	2		6.5		
RF1	9						3	6			6.7		
F2	37	1	3	8	7	7	4	3	2	2	4.7		
Bc1P1	20	5	5	3	3	2	2				2.9		
Bc1P2	26				1	11	6	8			5.8		
PI 197088 x Coolg	reen – Ca	stle Hayne	2011										
P1	33	16	13	4							1.6		
P2	6									6	9.0		
F1	8	1	3					4			4.4		
RF1	1							1			7.0		
F2	62			1	4	4	19	22	12		6.5		
Bc1P1	24		2	3	5	3	6	5			5.0		
Bc1P2	30						1	22	7		7.2		
PI 197088 x Ashle	y – Clinto	on 2011											
P1	24	19	5				-				1.2		
P2	9				3	6					4.7		
F1	5			1	2	1			1		4.8		
RF1	10			6	4						3.4		
F2	64	1	21	18	8	3	2		2		2.8		
Bc1P1	25	5	11	6	3						2.3		
Bc1P2	28		4	11	12	1					3.4		

Table 2.13. Frequency distribution and disease severity index (DSI) of downy mildew mean chlorosis ratings for families by year and location.<sup>a</sup>

PI 197088 x Ashley – Castle Hayne 2011

Table 2.13 continu	ed									
P1	28	14	11	3						1.6
P2	9						1	4	4	7.3
F1	9				2	6	1			4.9
RF1	9			2	5	2				4.0
F2	61		6	6	14	19	10	6		4.6
Bc1P1	24	2	4	5	6	3	2	2		3.8
Bc1P2	21					3	8	6	4	6.5
PI 197088 x Poinse	ett 76 – Clinto	on 2011								
P1	24	19	5							1.2
P2	7				2	5				4.7
F1	8		4	4						2.5
RF1	8		4	3	1					2.6
F2	51	29	13	5	2			2		1.8
Bc1P1	27	7	14	2	4					2.1
Bc1P2	26	7	9	7	3					2.2
PI 197088 x Poinse	ett 76 – Castle	Hayne	2011							
P1	28	14	11	3						1.6
P2	5				1	2	2			5.2
F1	10				2	7	1			4.9
RF1	10				5	4	1			4.6
F2	73	1	15	26	15	10	6			3.5
Bc1P1	30		9	6	10	5				3.4
Bc1P2	23			6	8	2	6	1		4.5
PI 197088 x Polari	s – Clinton 20	)11								
P1	24	19	5							1.2
P2	7				2	4	1			4.9
F1	10	5	5							1.5
RF1	4		3	1						2.3
F2	64	19	22	18	3		1	1		2.2
Bc1P1	26	15	9	2						1.5
Bc1P2	24	2	10	10	1	1				2.5
PI 197088 x Polari	s – Castle Ha	yne 201	1							
P1	28	14	11	3			-			1.6
P2	10					1	2	7		6.6
F1	10			2	7	1				3.9
RF1	9				9					4.0
F2	73		3	8	18	17	17	10		4.9
Bc1P1	20	4	9	5		2				2.4
Bc1P2	26					3	12	11		6.3

a Data are ratings from families developed from the cross of resistant PI 197088 by susceptible cultivar 'Poinsett 76'. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: 0 to 9 (0 = 0%, 1 = 1-3%, 2= 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99%, 9 = 100%)

b Data were means of three ratings, rounded to nearest class.

<b>C</b>	No. of	No. o	f plan	ts in c	lasses							DCI
Generation	plants	0	1	2	3	4	5	6	7	8	9	DSI
197088 x Coolgreen – Clinton 2011												
P1	4		4									1.0
P2	10						4	3	3			5.9
F1	9			2	6	1						2.9
RF1	10			1	2	5	2					3.8
F2	73			7	12	27	17	7	3			4.2
Bc1P1	25		22	1	2							1.2
Bc1P2	27						1	12	3	2		5.9
197088 x Coolgreen – Castle Hayne, NC 2010												
P1	7		1	6								1.9
P2	8							7	1			6.1
F1	6					2	2		1	1		5.5
RF1	3					1		2				5.3
F2	68				2	16	16	16	11	6	1	5.6
Bc1P1	26		12	5	6	1	1		1			2.2
Bc1P2	6								1	4	1	8.0
197088 x Coolgreen -	Pooled: Clin	ton and	d Castl	e Hay	ne, NC	2011						
P1	11		5	6								1.5
P2	18						4	1	4			6.0
F1	15			2	6	3	2		1	1		3.9
RF1	13			1	2	6	2	2				4.2
F2	141			7	14	43	33	23	14	6	1	4.9
Bc1P1	51		34	6	8	1	1		1			1.7
Bc1P2	33						1	12	4	6	1	6.3

Table 2.14. Frequency distribution and disease severity index (DSI) of downy mildew mean stunting ratings for the cross PI 197088 x 'Coolgreen' in 2011 <sup>a</sup>.

a Data are ratings from a *Cucumis sativus* family developed from the cross of resistant PI 197088 by susceptible cultivar 'Coolgreen'.



Mean Stunting rating

Figure 2.1. Distribution of F2 mean stunting ratings for the cross PI 197088 x 'Coolgreen' in 2011 at Clinton, NC and Castle Hayne, NC in 2010 <sup>a</sup>.

a Data are ratings from a *Cucumis sativus* family developed from the cross of resistant PI 197088 by susceptible cultivar 'Coolgreen'.

Pedigree	$\sigma^2(P_a)$	$\sigma^2(P_b)$	$\sigma^2(\mathbf{F}_1)$	$\sigma^2(\mathbf{F}_2)$	$\sigma^2(BC_1P_a)$	$\sigma^2(\mathbf{B}\mathbf{C}_1\mathbf{P}_b)$
Clinton, NC						
2010-PI 197088 × 'Coolgreen'	0.22	0.03	0.37	1.60	1.14	0.17
2011-PI 197088 × 'Coolgreen'	0.12	0.02	1.71	3.77	2.63	0.82
2011-PI 197088 × 'Ashley'	0.12	0.31	1.93	2.30	0.79	0.77
2011-PI 197088 × 'Poinsett 76'	0.12	0.10	0.35	1.51	0.75	0.92
2011-PI 197088 × 'Polaris'	0.12	0.33	0.31	1.23	0.25	0.84
Castle Hayne, NC						
2010-PI 197088 × 'Coolgreen'	0.05	0.06	0.50	1.25	3.21	0.50
2011-PI 197088 × 'Coolgreen'	0.36	0.03	4.91	1.31	2.57	0.14
2011-PI 197088 × 'Ashley'	0.35	0.43	0.57	1.80	2.80	1.04
2011-PI 197088 × 'Poinsett 76'	0.35	0.75	0.29	1.55	1.13	1.61
2011-PI 197088 × 'Polaris'	0.35	0.28	0.15	1.83	1.12	0.43
Pooled over location						
2011-PI 197088 × 'Ashley' $^{\rm b}$	0.23	0.37	1.25	2.05	1.79	0.90
2011-PI 197088 × 'Poinsett 76' °	0.23	0.43	0.32	1.53	0.94	1.26
2011-PI 197088 × 'Polaris' <sup>d</sup>	0.23	0.30	0.23	1.53	0.68	0.63
Pooled over locations and years						
2010 + Castle Hayne, 2011 PI 197088 × 'Coolgreen' <sup>e</sup>	0.21	0.04	1.93	1.39	2.30	0.27

Table 2.15. Phenotypic variances by generation for the four cucumber families screened for resistance to downy mildew at Clinton and Castle Hayne, North Carolina (2010-20113) a.

а Data are the mean of 3 chlorosis ratings from four Cucumis sativus families developed from the cross of resistant PI 197088 by susceptible cultivars 'Coolgreen', 'Ashley', 'Poinsett 76', and 'Polaris'. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: 0 to 9 (0 = 0%, 1 = 1-3%, 2= 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99%, 9 = 100%)

 $\begin{array}{l} F_2 \text{ Bartlett's } \chi^2 = 0.02; \text{ P-value} = 0.00\\ F_2 \text{ Bartlett's } \chi^2 = 0.20; \text{ P-value} = 0.66 \end{array}$ b

с

F<sub>2</sub> Bartlett's  $\chi^2 = 2.64$ ; P-value = 0.10 F<sub>2</sub> Bartlett's  $\chi^2 = 1.24$ ; P-value = 0.54 d

e

Pedigree	$\sigma^2(P_a)$	$\sigma^2(P_b)$	$\sigma^2(F_1)$	$\sigma^2(\mathbf{F}_2)$	$\sigma^2(BC_1P_a)$	$\sigma^2(BC_1P_b)$
Clinton, NC						
2010-PI 197088 × 'Coolgreen'	0.00	0.77	0.80	1.52	0.33	0.79
Castle Hayne, NC						
2010-PI 197088 × 'Coolgreen'	0.14	0.13	2.03	1.95	2.22	0.40
Pooled						
2010-PI 197088 × 'Coolgreen' $^{\rm b}$	0.07	0.45	1.41	1.73	1.27	0.60

Table 2.16. Phenotypic variances of stunting by generation for the family from cross PI 197088 x 'Coolgreen' screened for resistance to downy mildew at Clinton and Castle Hayne, North Carolina (2010) <sup>a</sup>.

a Data are ratings from a *Cucumis sativus* family developed from the cross of resistant PI 197088 by susceptible cultivar 'Coolgreen'. Disease assessment scale adopted for evaluating stunting based on percent reduction in plant size relative to fungicide-treated, non-inoculated trials planted in adjacent fields. 0 = 0%, 1 = 1-3%, 2= 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99%, 9 = 100%.

b  $F_2$  Bartlett's  $\chi^2 = 1.06$ ; P-value = 0.30

Pedigree	$\sigma^2(\mathbf{P})^{b}$	$\sigma^2(E)^c$	$\sigma^2(G)^d$	σ <sup>2</sup> (A) <sup>e</sup>	$\mathrm{H}^{2\mathrm{f}}$	$\mathbf{h}^{2\mathrm{g}}$
Clinton, NC						
2010-PI 197088 × 'Coolgreen'	1.60	0.25	1.35	1.88	0.84	1.18
2011-PI 197088 × 'Coolgreen'	3.77	0.89	2.88	4.08	0.76	1.08
2011-PI 197088 × 'Ashley'	2.30	1.07	1.22	3.03	0.53	1.32
2011-PI 197088 × 'Poinsett 76'	1.51	0.23	1.28	1.35	0.85	0.90
2011-PI 197088 × 'Polaris'	1.23	0.26	0.96	1.36	0.78	1.11
Mean	2.08	0.54	1.54	2.34	0.75	1.12
Castle Hayne, NC						
2010-PI 197088 × 'Coolgreen'	1.25	0.28	0.97	-1.20	0.78	<sup>h</sup>
2011-PI 197088 × 'Coolgreen'	1.31	2.55	-1.24	-0.07	<sup>h</sup>	<sup>h</sup>
2011-PI 197088 × 'Ashley'	1.80	0.48	1.33	-0.23	0.74	<sup>h</sup>
2011-PI 197088 × 'Poinsett 76'	1.55	0.42	1.13	0.35	0.73	0.23
2011-PI 197088 × 'Polaris'	1.83	0.23	1.59	2.11	0.87	1.15
Mean	1.55	0.79	1.00	0.19	0.78	0.69
Pooled						
2011-PI 197088 × 'Ashley' $^{\rm i}$	2.05	0.77	1.28	1.40	0.62	0.68
2011-PI 197088 × 'Poinsett 76' $^{j}$	1.53	0.32	1.21	0.85	0.79	0.56
2011-PI 197088 × 'Polaris' $^{\rm k}$	1.53	0.25	1.28	1.73	0.84	1.14
Mean	1.70	0.45	1.26	1.33	0.75	0.79
Pooled over locations and years						
2010 + Castle Hayne, 2011 PI 197088 × 'Coolgreen' <sup>1</sup>	1.39	1.03	0.36	0.20	0.26	0.15

Table 2.17. Variance and heritability estimates for the four cucumber families screened for resistance to downy mildew at Clinton and Castle Hayne, North Carolina (2010-2011) a.

Data are mean of three chlorosis ratings from four Cucumis sativus families developed from the cross of а resistant PI 197088 by susceptible cultivars 'Coolgreen', 'Ashley', 'Poinsett 76', and 'Polaris'. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: 0 to 9 (0 = 0%, 1 = 1 - 1)3%, 2=3-6%, 3=6-12%, 4=12-25%, 5=25-50%, 6=50-75%, 7=75-87%, 8=87-99%, 9=100%)

b 
$$\sigma^2(P)$$
 = phenotypic variance =  $\sigma^2(F_2)$ 

$$\sigma^{2}(E) = \text{environmental variance} = \frac{\sigma^{2}(P_{a}) + \sigma^{2}(P_{b}) + \left[2 \times \sigma^{2}(F_{1})\right]}{4}$$

 $\sigma^{2}(G) = \text{genetic variance} = \sigma^{2}(P) - \sigma^{2}(E)$ d

e 
$$\sigma^2(A) = \text{additive variance} = \sigma^2(A) = [2 \times \sigma^2(F_2)] - [\sigma^2(BC_1P_a) + \sigma^2(BC_1P_b)]$$

 $H^2$  = broad-sense heritability f

с

- $h^2 =$  narrow-sense heritability g
- Negative estimate from a negative estimate of additive variance h
- $\begin{array}{l} F_2 \text{ Bartlett's } \chi^2 = 0.02; \text{ P-value} = 0.00 \\ F_2 \text{ Bartlett's } \chi^2 = 0.20; \text{ P-value} = 0.66 \end{array}$ i
- j
- $F_2$  Bartlett's  $\chi^2 = 2.64$ ; P-value = 0.10 k
- 1 F<sub>2</sub> Bartlett's  $\chi^2 = 1.24$ ; P-value = 0.54
| Pedigree  | $\sigma^2(P)^{b}$ | $\sigma^2(E)^c$ | $\sigma^2(G)^d$ | $\sigma^2(A)^e$ | $\mathbf{H}^{2\mathrm{f}}$ | $\mathbf{h}^{2\mathrm{g}}$ |
|---|-------------------|-----------------|-----------------|-----------------|----------------------------|----------------------------|
| Clinton, NC   |                   |                 |                 |                 |                            |                            |
| 2010-PI 197088 × 'Coolgreen'                                      | 1.60              | 0.13            | 1.47            | 1.88            | 0.92                       | 1.18                       |
| 2011-PI 197088 × 'Coolgreen'                                      | 3.77              | 0.07            | 3.70            | 4.08            | 0.98                       | 1.08                       |
| 2011-PI 197088 × 'Ashley'   | 2.30              | 0.21            | 2.08            | 3.03            | 0.91                       | 1.32                       |
| 2011-PI 197088 × 'Poinsett 76'                                    | 1.51              | 0.11            | 1.40            | 1.35            | 0.93                       | 0.90                       |
| 2011-PI 197088 × 'Polaris'  | 1.23              | 0.22            | 1.00            | 1.36            | 0.82                       | 1.11                       |
| Mean  | 2.08              | 0.15            | 1.93            | 2.34            | 0.91                       | 1.12                       |
| Castle Hayne, NC  |                   |                 |                 |                 |                            |                            |
| 2010-PI 197088 × 'Coolgreen'                                      | 1.25              | 0.05            | 1.20            | -1.20           | 0.96                       | h                          |
| 2011-PI 197088 × 'Coolgreen'                                      | 1.31              | 0.20            | 1.12            | -0.07           | 0.85                       | h                          |
| 2011-PI 197088 × 'Ashley'   | 1.80              | 0.39            | 1.42            | -0.23           | 0.79                       | h                          |
| 2011-PI 197088 × 'Poinsett 76'                                    | 1.55              | 0.55            | 1.00            | 0.35            | 0.64                       | 0.23                       |
| 2011-PI 197088 × 'Polaris'  | 1.83              | 0.31            | 1.51            | 2.11            | 0.83                       | 1.15                       |
| Mean  | 1.55              | 0.30            | 1.25            | 0.49            | 0.81                       | 0.69                       |
| Pooled  |                   |                 |                 |                 |                            |                            |
| 2011-PI 197088 × 'Ashley' $^{i}$                                  | 2.05              | 0.30            | 1.75            | 1.40            | 0.85                       | 0.68                       |
| 2011-PI 197088 × 'Poinsett 76' <sup>j</sup>                       | 1.53              | 0.33            | 1.20            | 0.85            | 0.78                       | 0.56                       |
| 2011-PI 197088 × 'Polaris' <sup>k</sup>                           | 1.53              | 0.27            | 1.26            | 1.73            | 0.82                       | 1.14                       |
| Mean  | 1.66              | 0.30            | 1.37            | 1.04            | 0.82                       | 0.79                       |
| Pooled over locations and years                                   |                   |                 |                 |                 |                            |                            |
| 2010 + Castle Hayne, 2011 PI<br>197088 × 'Coolgreen' <sup>1</sup> | 1.39              | 0.13            | 1.26            | 0.20            | 0.91                       | 0.15                       |

Table 2.18. Modified variance and heritability estimates for the four cucumber families screened for resistance to downy mildew at Clinton and Castle Hayne, North Carolina (2010-2011)<sup>a</sup>.

a Data are mean of three chlorosis ratings from four *Cucumis sativus* families developed from the cross of resistant PI 197088 by susceptible cultivars 'Coolgreen', 'Ashley', 'Poinsett 76', and 'Polaris'. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: 0 to 9 (0 = 0%, 1 = 1-3%, 2= 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99%, 9 = 100%)

b  $\sigma^2(\mathbf{P}) = \text{phenotypic variance} = \sigma^2(F_2)$ 

c Modified  $\sigma^2(E)$  = environmental variance =  $(\sigma^2(Pa) + \sigma^2(Pb))/2$ 

d  $\sigma^2(G) = \text{genetic variance} = \sigma^2(P) - \sigma^2(E)$ 

e  $\sigma^2(A) = \text{additive variance} = \sigma^2(A) = [2 \times \sigma^2(F_2)] - [\sigma^2(BC_1P_a) + \sigma^2(BC_1P_b)]$ 

- f  $H^2$  = broad-sense heritability
- g  $h^2 = narrow-sense heritability$
- h Negative estimate from a negative estimate of additive variance
- i  $F_2$  Bartlett's  $\chi^2 = 0.02$ ; P-value = 0.00
- j  $F_2$  Bartlett's  $\chi^2 = 0.20$ ; P-value = 0.66
- k  $F_2$  Bartlett's  $\chi^2 = 2.64$ ; P-value = 0.10
- 1 F<sub>2</sub> Bartlett's  $\chi^2 = 1.24$ ; P-value = 0.54

Table 2.19. Variance and heritability estimates of stunting by generation for the family from cross PI 197088 x 'Coolgreen' screened for resistance to downy mildew at Clinton and Castle Hayne, North Carolina (2010) <sup>a</sup>.

Pedigree	$\sigma^2(\mathbf{P})^{b}$	$\sigma^2(E)^{c}$	$\sigma^2(G)^d$	σ <sup>2</sup> (A) <sup>e</sup>	$\mathrm{H}^{2\mathrm{f}}$	$h^{2 g}$			
F1 included in estimating environmental variance ( $\sigma^2(E)$ )									
Clinton, NC									
2010-PI 197088 × 'Coolgreen'	1.52	0.59	0.93	1.91	0.61	1.26			
Castle Hayne, NC									
2010-PI 197088 × 'Coolgreen'	1.95	1.08	0.87	1.28	0.44	0.66			
Pooled									
2010-PI 197088 × 'Coolgreen' $^{\rm h}$	1.73	0.84	0.90	1.59	0.52	0.92			
No F1 included in estimating environ	mental varia	nce $(\sigma^2(E))$							
Clinton, NC									
2010-PI 197088 × 'Coolgreen'	1.52	0.38	1.13	1.91	0.75	1.26			
Castle Hayne, NC									
2010-PI 197088 × 'Coolgreen'	1.95	0.13	1.81	1.28	0.93	0.66			
Pooled									
2010-PI 197088 × 'Coolgreen' $^{\rm h}$	1.73	0.26	1.47	1.59	0.85	0.92			

a Data are ratings from a *Cucumis sativus* family developed from the cross of resistant PI 197088 by susceptible cultivar 'Coolgreen'. Disease assessment scale adopted for evaluating stunting based on percent reduction in plant size relative to fungicide-treated, non-inoculated trials planted in adjacent fields. 0 = 0%, 1 = 1-3%, 2= 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99%, 9 = 100%.

b 
$$\sigma^2(\mathbf{P}) = \text{phenotypic variance} = \sigma^2(F_2)$$

$$\sigma^{2}(E) = \text{environmental variance} = \frac{\sigma^{2}(P_{a}) + \sigma^{2}(P_{b}) + \left[2 \times \sigma^{2}(F_{1})\right]}{4}$$

d 
$$\sigma^2(G)$$
 = genetic variance =  $\sigma^2(P) - \sigma^2(E)$ 

e  $\sigma^2(\mathbf{A}) = \text{additive variance} = \sigma^2(\mathbf{A}) = [2 \times \sigma^2(F_2)] - [\sigma^2(BC_1P_a) + \sigma^2(BC_1P_b)]$ 

- f  $H^2$  = broad-sense heritability
- g  $h^2 = narrow-sense heritability$

с

h  $F_2$  Bartlett's  $\chi^2 = 1.06$ ; P-value = 0.30

	Effecti	ve Factor	s				Gain <sup>1</sup>	)	
Pedigree	W <sup>c</sup>	$\mathbf{M}^{\mathbf{d}}$	L1 e	L2 <sup>f</sup>	L3 <sup>g</sup>	Mean	5%	10%	20%
Clinton, NC									
2010-PI 197088 × 'Coolgreen'	3.7	10.2	3.6	2.6	5.9	5.2	3.1	2.6	2.1
2011-PI 197088 × 'Coolgreen'	1.7	3.3	1.7	0.8	<sup>h</sup>	1.9	8.0	6.8	5.4
2011-PI 197088 × 'Ashley'	2.7	7.2	2.5	1.8	4.4	3.7	4.3	3.7	2.9
2011-PI 197088 × 'Poinsett 76'	1.2	4.4	1.2	1.1	1.2	1.8	2.3	1.9	1.5
2011-PI 197088 × 'Polaris'	2.1	4.6	1.6	1.1	2.7	2.4	2.5	2.2	1.7
Mean	2.3	5.9	2.1	1.5	3.6	3.1	4.1	3.5	2.8
Castle Hayne, NC									
2010-PI 197088 × 'Coolgreen'	7.4	<sup>h</sup>	6.6	<sup>h</sup>	2.0	5.3	<sup>h</sup>	<sup>h</sup>	<sup>h</sup>
2011-PI 197088 × 'Coolgreen'	<sup>h</sup>	<sup>h</sup>	<sup>h</sup>	<sup>h</sup>	<sup>h</sup>	<sup>h</sup>	<sup>h</sup>	<sup>h</sup>	<sup>h</sup>
2011-PI 197088 × 'Ashley'	3.0	<sup>h</sup>	3.0	<sup>h</sup>	1.4	2.5	<sup>h</sup>	<sup>h</sup>	<sup>h</sup>
2011-PI 197088 × 'Poinsett 76'	1.8	17.9	1.4	4.5	0.8	5.3	0.6	0.5	0.4
2011-PI 197088 × 'Polaris'	1.9	5.7	1.9	1.4	2.8	2.7	3.2	2.7	2.2
Mean	3.5	11.8	3.2	3.0	1.8	4.7	4.1	3.5	2.8
Pooled									
2011-PI 197088 × 'Ashley' $^{\rm i}$	2.1	7.5	2.1	1.9	2.3	3.2	2.0	1.7	1.4
2011-PI 197088 × 'Poinsett 76' $^{\rm j}$	1.3	7.2	1.3	1.8	1.0	2.5	1.4	1.2	1.0
2011-PI 197088 × 'Polaris' $^{\rm k}$	1.8	5.1	1.7	1.3	2.7	2.5	2.9	2.5	2.0
Mean	2.2	7.9	2.1	2.0	1.9	3.2	3.3	2.9	2.3
Pooled over locations and years									
2010 + Castle Hayne, 2011 PI 197088 × 'Coolgreen' <sup>1</sup>	17.0	115.6	16.1	28.9	11.2	37.8	0.4	0.3	0.2

Table 2.20. Estimates of number of effective factors and predicted gain from selection under different selection intensities of the mean of three chlorosis ratings by generation for the four cucumber families screened for resistance to downy mildew at Clinton and Castle Hayne, North Carolina (2010-2011) <sup>a</sup>.

a Data are ratings from four *Cucumis sativus* families developed from the cross of resistant PI 197088 by susceptible cultivars 'Coolgreen', 'Ashley', 'Poinsett 76', and 'Polaris'. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: 0 to 9 (0 = 0%, 1 = 1-3%, 2= 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75\%, 7 = 75-87\%, 8 = 87-99\%, 9 = 100\%)

b Gain from selection =  $k \times h_n^2 \times \sqrt{\sigma^2(P)}$ 

#### Table 2.20 continued

c Lande's method I:  

$$\frac{\left[\mu(P_{\delta}) - \mu(P_{a})\right]^{2}}{8 \times \left\{\sigma^{2}(F_{1}) - \frac{\sigma^{2}(P_{a}) + \sigma^{2}(P_{a}) + \left[2 \times \sigma^{2}(F_{1})\right]\right\}}{4}}$$
d Lande's method II:  

$$\frac{\left[\mu(P_{\delta}) - \mu(P_{a})\right]^{2}}{8 \times \left\{\left[2 \times \sigma^{2}(F_{2})\right] - \left[\sigma^{2}(BC_{1}P_{a}) + \sigma^{2}(BC_{1}P_{\delta})\right]\right\}}$$
e Lande's method III:  

$$\frac{\left[\mu(P_{\delta}) - \mu(P_{a})\right]^{2}}{\left\{8 \times \left[\sigma^{2}(BC_{1}P_{a}) + \sigma^{2}(BC_{1}P_{\delta}) - \sigma^{2}(F_{1})\right]\right\} - \frac{\left[\sigma^{2}(P_{a}) + \sigma^{2}(P_{\delta})\right]}{2}}{\left[2 \times \sigma^{2}(F_{2})\right] - \left[\sigma^{2}(BC_{1}P_{a}) + \sigma^{2}(BC_{1}P_{\delta})\right]}$$
f Mather's method:  

$$\frac{\left[\mu(P_{\delta}) - \mu(P_{a})\right]^{2}}{\left[2 \times \sigma^{2}(F_{2})\right] - \left[\sigma^{2}(BC_{1}P_{a}) + \sigma^{2}(BC_{1}P_{\delta})\right]}$$
g Wright's method:  

$$\frac{\left[\mu(P_{\delta}) - \mu(P_{a})\right]^{2} \times \left\{1.5 - \left[2 \times \frac{\mu(F_{1}) - \mu(P_{a})}{\mu(P_{\delta}) - \mu(P_{a})} \times \left(1 - \frac{\mu(F_{\epsilon}) - \mu(P_{a})}{\mu(P_{\delta}) - \mu(P_{a})}\right)\right]\right\}}{8 \times \left\{\sigma^{2}(F_{2}) - \frac{\sigma^{2}(P_{a}) + \sigma^{2}(P_{\delta}) + \left[2 \times \sigma^{2}(F_{1})\right]}{4}\right\}}$$

Table 2.21. Estimates of number of effective factors and predicted gain from selection under different selection intensities for stunting by generation for the family from cross PI 197088 x 'Coolgreen' screened for resistance to downy mildew at Clinton and Castle Hayne, North Carolina (2010) <sup>a</sup>.

	Effective Factors						Gain <sup>b</sup>		
Pedigree	W <sup>c</sup>	$\mathbf{M}^{d}$	L1 <sup>e</sup>	$L2^{f}$	Mean	5%	10%	20%	
Clinton, NC									
2010-PI 197088 × 'Coolgreen'	3.2	6.3	3.2	1.6	3.58	3.2	2.7	2.2	
Castle Hayne, NC									
2010-PI 197088 × 'Coolgreen'	3.2	7.1	2.6	1.8	3.68	1.9	1.6	1.3	
Pooled									
2010-PI 197088 × 'Coolgreen'	3.1	6.6	2.9	1.6	3.55	2.5	2.1	1.7	

a Data are ratings from a *Cucumis sativus* family developed from the cross of resistant PI 197088 by susceptible cultivar 'Coolgreen'. Disease assessment scale adopted for evaluating stunting based on percent reduction in plant size relative to fungicide-treated, non-inoculated trials planted in adjacent fields. 0 = 0%, 1 = 1-3%, 2 = 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99%, 9 = 100%.

b Gain from selection =  $k \times h_n^2 \times \sqrt{\sigma^2(P)}$ 

$$\frac{\left[\mu(P_{b})-\mu(P_{a})\right]^{2}\times\left\{1.5-\left[2\times\frac{\mu(F_{1})-\mu(P_{a})}{\mu(P_{b})-\mu(P_{a})}\times\left(1-\frac{\mu(F_{1})-\mu(P_{a})}{\mu(P_{b})-\mu(P_{a})}\right)\right]\right\}}{8\times\left\{\sigma^{2}(F_{2})-\frac{\sigma^{2}(P_{a})+\sigma^{2}(P_{b})+\left[2\times\sigma^{2}(F_{1})\right]}{4}\right\}}$$

$$\frac{\left[\mu(P_b) - \mu(P_a)\right]^2}{2}$$

$$\frac{2}{\left[2 \times \sigma^2(F_2)\right] - \left[\sigma^2(BC_1P_a) + \sigma^2(BC_1P_b)\right]}$$

d Mather 5 method.

Lande's method I:

Wright's method:

с

e

$$\frac{\left[\mu(P_b) - \mu(P_a)\right]^2}{8 \times \left\{\sigma^2(F_2) - \frac{\sigma^2(P_a) + \sigma^2(P_b) + \left[2 \times \sigma^2(F_1)\right]\right\}}{4}\right\}}$$

1 .

$$\frac{\left[\mu(P_b) - \mu(P_a)\right]^{\epsilon}}{8 \times \left\{\left[2 \times \sigma^2(F_2)\right] - \left[\sigma^2(BC_1P_a) + \sigma^2(BC_1P_b)\right]\right\}}$$

1 2-0

g  $F_2$  Bartlett's  $\chi^2 = 1.06$ ; P-value = 0.30

## **CHAPTER THREE:**

# GENOTYPE X ENVIRONMENT INTERACTION AND STABILITY ANALYSIS OF DOWNY MILDEW RESISTANCE IN CUCUMBER TESTED IN THE SOUTHEASTERN UNITED STATES

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### Genotype x Environment Interaction and Stability Analysis of Downy Mildew Resistance in Cucumber Tested in the Southeastern United States

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Additional index words: Cucumis sativus, Pseudoperonospora cubensis, Disease

#### Abstract

Downy mildew (Pseudoperonospora cubensis (Berk. & Curt.) Rostov) is an important disease in most cucumber (*Cucumis sativus* L.) production areas worldwide. A major breeding objective in cucumber is moving the newly identified high levels of resistance in cultigens such as PI 197088, into adapted varieties with good quality traits. The environment can have a large effect on the severity of plant disease, and it is important to understand the environmental effect on disease expression. A set of 15 cucumber cultivars and breeding lines ranging from moderately resistant to susceptible to downy mildew in the were tested at Clinton, NC from 2005 to 2011. These cultigens were evaluated against local isolates of *P. cubensis* under field conditions to analyze the effect of genotype, year, and genotype-year interaction, as well as stability of resistance do downy mildew. We were also interested in looking for evidence that different races were present in different years. Cultigen had the largest effect (43% of total sums of squares), followed by year (24% of total sums of squares), and cultigen-year interaction (16% of total sums of squares). The cultigens in this study with the lowest mean downy mildew rating, a slope close to unity and nonsignificant deviation from regression included 'Poinsett 76', a slicing cucumber, and WI 2238, a pickle type cucumber, and are considered the most stable across environments.

#### Introduction

Downy mildew, caused by the oomycete pathogen *Pseudoperonospora cubensis* (Berk. & Curt) Rostov, is a major foliar disease of cucumber (*Cucumis sativus* L.) (Palti and Cohen, 1980). Other economically important hosts of *P. cubensis* are melon (*Cucumis melo*  L.), watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai), and squash (*Cucurbita* spp.) (Whitaker and Davis, 1962). Host range studies with *P. cubensis* were summarized on cucurbits by Palti (1974). The host range of *P. cubensis* is reported to include 20 genera, including 50 species in the Cucurbitaceae, as hosts, of which 19 species are in *Cucumis* (Palti and Cohen, 1980; Lebeda, 1992; Lebeda and Widrlechner, 2003). Downy mildew has been reported on species of the genus *Cucumis* from 70 countries worldwide (Cohen, 1981; Palti, 1974; Thomas, 1986). The pathogen can overwinter in areas with mild winter temperatures, such as the extreme southern United States in the form of active mycelium in either cultivated or wild species of cucurbits (Bains and Jhooty, 1976). The primary source of inoculum in the field is through airborne sporangia, which also serve as the secondary source of high humidity in combination with high day and moderate night temperatures. Dew is also a major factor in the initiation, development, and spread of the disease (Thomas, 1996).

The population genetic structure of a pathogen can be indicative of its potential to overcome resistance or become insensitive to a fungicide (McDonald and Linde, 2002). The mating system of *P. cubensis* is mixed, reproducing asexually and sexually (Cohen and Rubin, 2011), resulting in being classified as having the highest evolutionary potential. Adding to that are the many spores moving large distances. Effective population sizes are massive with concentrations of sporangia on lesions up to  $4.0 \times 10^3$  /cm<sup>2</sup> (Cohen, 1981). *P. cubensis* is classified as having a high evolutionary potential, among *Blumeria graminis* 

(powdery mildew of cereals), and oomycetes *Bremia lactucae* (downy mildew of lettuce) and *Phytopthora infestans* (late blight of potato).

Different pathotypes and races of *P. cubensis* have been reported. Using a differential set of 26 cultivars spanning 13 species of cucurbits, Thomas et al. (1987) identified five pathotypes of *P. cubensis* among eight isolates from the United States, Israel, and Japan. Lebeda and Gadasová (2002) reported 13 pathotypes from 22 isolates collected in Czech Republic (19 isolates), Spain, France and the Netherlands. Lebeda and Urban (2007) reported high pathogenic variability along with a shift to high pathogenicity among isolates collected the Czech Republic from 2001 to 2003. In 2001, there were 33 pathotypes among 42 isolates, 16 among 54 isolates in 2002 and 13 among 56 isolates in 2003. There was a decrease in variability from one year to the next, with a higher percentage of isolates reported as highly pathogenic each year.

High variability of *P. cubensis* has been reported in the United States as well. Colluci (2008) reported 32 different compatibility patterns on 12 cucurbit hosts from 32 isolates collected in the United States, indicating a high variability in pathogenicity among populations in the United States. All isolates tested showed a high level of compatibility with cucumber. Several races of *P. cubensis* have been reported in differential test studies in many regions of the world (Angelov et al., 2000; Bains and Jhooty, 1976; Lebeda et al., 2006; Palti, 1974; Shetty et al., 2002). Shetty et al. (2002) proposed that at least two races of downy mildew exist, one in Asia, and one in Europe and North America. They also stated that there was no evidence for race differences between the United States and Poland.

It is likely that multiple races of *P. cubensis* exist, given it is pathogen of high variability and evolutionary potential. Even with such high evolutionary potential, resistance can be effective for long periods of time. Nevertheless, it is important to recognize the potential to overcome host resistance and fungicides. In the United States there have been at least 3 races of *P. cubensis*, corresponding to the 3 resistance sources: resistance from P.R. 40 was effective against *P. cubensis* in 1948 and partially in 1949; dm-*I* conferred resistance in cultivars from 1961 to 2003; PI 197088 (among others) is resistant to the new downy mildew in the United States since 2004. Therefore, the number of possible races of downy mildew on cucumber that have been in the United States could range up to eight if there were a gene-for-gene system for the disease.

Currently, no available cultivars have high levels of resistance to the downy mildew in the United States (Call et al., 2012a). Most cultivars currently grown in the United States have some resistance, but not at the level seen prior to appearance of the new race in 2004. New sources of resistance (reduced leaf damage) and tolerance (good yield under disease presence) to the race(s) of downy mildew in the United States since 2004 were identified in a large germplasm screening study and a multiple year re-evaluation of the most resistant and susceptible cultigens conducted at North Carolina State University (Criswell, 2008; Call et al., 2012b). Cultigens were identified that outperformed currently grown cultivars for disease resistance and yield traits. Unfortunately, these cultigens lack the quality traits required for commercial use. The environment can have a large effect on the severity of plant disease. Differences in the environment, including temperature, humidity, rainfall, and inoculum movement by wind all influence the severity of downy mildew infection (Cohen, 1977). Cultigens having high stability for resistance along with good quality traits are ideal for use in breeding with newly identified highly resistant cultigens. The objective of this study was (i) to test a set of cultigens which differed in their resistance to downy mildew to local isolates of *P. cubensis* in North Carolina each year, to evaluate the effect of environment on disease severity on different cucumber genotypes and look for large rank change (change in the relative resistance to other tested cultigens) which may indicate the presence of different races and (ii) to evaluate the stability of resistance in cultigens with good quality traits, to identify genotypes having high or moderate resistance and high stability for resistance, which would be useful in breeding new highly resistant cultivars.

#### **Materials and Methods**

Experiments were conducted at the Horticultural Crops Research Station in Clinton, North Carolina during the summers of 2005 to 2011. The cucumber cultigens used in this study were chosen because they differed in their resistance to downy mildew (Call et al., 2012a; Call et al., 2012b; Wehner and Shetty, 1997). It is difficult to separate cultigens into resistant and susceptible classes since there were no obvious gaps in their distribution over a 0 to 9 scale. However, plant breeders often use those terms for quantitative traits. In keeping with that practice, and to remain consistent with previous studies, cultigens having ratings less than 3.0 were classified highly resistant, from 3.1 to 4.0 moderately resistant, from 4.1 to 6.0 intermediate, from 6.1 to 7.0 moderately susceptible, and from 7.1 to 9.0 highly susceptible. The cultigens tested in this study ranged from moderately resistant to highly susceptible. Although highly resistant plant introduction accessions have been identified (Call et al., 2012b), none of the cultigens tested in this study were reported as highly resistant to the downy mildew in the United States since 2004. Cultigens tested were 'Calypso', 'Coolgreen', 'Dasher II', Gy 4, 'Heidan #1', 'Homegreen #2', M 21, 'Marketmore 76', 'NongChen #4', 'Poinsett 76', 'Slice', 'Sumter', WI 2238, WI 2757, and Wisconsin SMR 18.

Cucumbers were grown using recommended horticultural practices as summarized by Schultheis (1990). Fertilizer was incorporated before planting at a rate of 90-39-74 kg/ha (N-P-K) with an additional 34 kg N/ha applied at the vine-tip-over stage (four to six true leaves). Plots were planted after downy mildew was reported in the county of the test. The field was surrounded by border rows, and spreader rows were spaced every ninth row, planted with susceptible 'Coolgreen', a highly susceptible having leaves that sporulate heavily and so are ideal for increasing field inoculum. Border and spreader rows were planted on the same day as the test plots, with seeds 4" to 6" apart in the row. Test plots 1.5 m long were hand-seeded on raised, shaped beds with centers 1.5 m apart and thinned to 15 plants prior to vine-tipover-stage (4 to 6 true leaves), equaling a density of 66,666 plants/ha. No artificial inoculation was used in the field tests in North Carolina. Plots were exposed to natural epidemics during the growing season. Epidemics were encouraged using overhead irrigation.

The experiment was a randomized complete block design with four replications of 15 cultigens. Disease severity was evaluated weekly based on percentage of symptomatic leaf

area (0 = 0%, 1 = 1-3%, 2= 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99%, 9 = 100%); as described by Jenkins and Wehner (1983).

Prior to analysis, data were checked for normality and error variance homogeneity. Residual plots had a random distribution, indicating that the statistical model was valid and its assumptions were met (Fernandez, 1992). Rating scale data were not transformed because assumptions for the analysis of variance were met (Little, 1985). For stability parameters, tests for significance were derived using a t-test for each  $b_i$  and an *F* test for each  $S^2_d$  for statistical differences from unity and zero, respectively. Data presented is the mean of all downy mildew ratings, as it had the highest *F* value and lowest coefficient of variation. Data were analyzed using the GLM, CORR, and REG procedures of SAS 9.3 and JMP 10 (SAS Institute, Inc., Cary, NC).

#### **Results and Discussion**

The analysis of variance (Table 3.1) showed significant differences among cultigens and environments (years) at P < 0.001 for the mean of all downy mildew ratings. This indicates the presence of variability in genotypes as well as variation in growing conditions each year. The significant cultigen-year interaction (P < 0.001) indicates differential response of the genotypes in different years. Cultivar had the largest effect (43% of total sums of squares), followed by year (24% of total sums of squares), and cultigen-year interaction (16% of total sums of squares). The large GXE interaction reveals the importance of testing for downy mildew in multiple environments. Significant differences among cultigens (P < 0.0001) were observed each year (Table 3.2). This was expected as cultigens with varying levels of resistance were purposely chosen for this study.

Tests in this study were conducted at only one location, but testing at multiple locations is recommended as it provides more data in less time. In another study on downy mildew on cucumbers tested at two locations in North Carolina, Call et al. (2012b) reported a higher correlation of the locations within a year, compared to a single location over two years. This effect was likely at least partially due to the proximity of the two testing locations, which are approximately 60 miles apart. Breeders should choose the most diverse testing environments available with conditions favorable for disease. This is especially critical in crops grown over a wide area, with a pathogen shown to be highly variable.

The genotype-environment interaction of the 15 cultigens is depicted in Figure 3.1. This figure depicts genotype means over the environmental index. The environmental index is equal to the difference in the average performance of genotypes in each environment and the average performance of genotypes in all environments. This figure illustrates the importance of multi-year testing, as genotype rankings changed often. But, in general, the most susceptible cultigens overall were ranked lowest in individual environments, and the most resistant cultigens overall tended to rank in the upper third.

Correlations for mean downy mildew ratings for the different years were calculated (Table 3.3). Both Pearson and Spearman correlations are presented. Based on Pearson correlations, which tend to be a better measure for linear relationships and interval data, all years were correlated at least P = 0.05. Rankings of cultigens were less correlated than were

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actual ratings, indicating that changes in rank often occurred. Most of this rank change is likely due to environment and error involving cultigens with moderate resistance, as they tend to be more variable than highly resistant or susceptible cultigens. The significant correlations of years for downy mildew ratings indicates that the downy mildew race(s) present each are likely the same or at have similar pathogenicity.

Highly significant differences were observed among cultigens for resistance to downy mildew each year (Table 3.4). The mean rating was computed based on the mean of all ratings each year for the particular cultigen. The means for downy mildew ratings in the different years ranged from 3.8 to 5.8. Mean ratings in 2005 to 2007 were lower (3.8 to 4.2) than mean ratings from 2008 to 2011 (5.0 to 5.8). The ratings were standardized to a mean of 4.5 and a standard deviation of 1.5 for comparison of resistance across locations (Table 3.5). Standardization permitted comparison of cultigens across years uniformly. The value of 4.5 was chosen as the midpoint of the 0 to 9 rating scale, and 1.5 was chosen as a standard deviation because a range of  $\pm$  3 standard deviations would include 99% of the population and range from 0 to 9, corresponding to the original, unstandardized rating. Cultigens differed only slightly in their resistance level in different years, and no pattern emerged to indicate different races were present.

#### Stability analysis

Genotype means for mean of all downy mildew resistance ratings ranged from 3.7 to 7.1 (Table 3.6). Based on the mean response ratings, the most resistant cultigens across all years were 'Poinsett 76' and WI 2238; 'Coolgreen' and 'Wisconsin SMR 18' were the most

susceptible. Similar results were reported by Call et al. (2012b). The regression (mean downy mildew rating on to environmental index) coefficient or slope (*bi*) of most of the cultigens was not significantly different (P>0.05) from unity, except for 'Calypso', and 'Dasher II'. A slope significantly different from unity indicates large differences in genotype response to different environments (Eberhart and Russell, 1966). A  $b_i$  greater than unity indicates the genotype response was higher than the mean response of all genotypes to different environments, while a lower  $b_i$  indicates the genotype response was lower. Cultigens with a  $b_i$  close to unity included 'Homegreen #2', M 21, 'Slice', and 'Sumter'.

Regression of cultigen mean downy mildew ratings on the environmental index along with 95% confidence of fit are shown in Figure 3.2. All regressions are positive, that is no cultigens performed better in higher disease environments than in low disease environments, on average. Figure 3.3 shows the individual replication downy mildew means of cultigens on the environmental index, with a smooth loess (for "local regression") fit curve, a non-parametric method to summarize central tendency of the distribution of the mean downy mildew rating. A flat line indicates no relationship between the variables, while any deviation from a horizontal line indicates some relationship between the variables (Jacoby, 2000). The figure is further indication of the effect of environment on each cultigen.

A  $b_i$  close to unity combined with a non-significant deviation from regression ( $S^2_d$ ) indicate average stability (Eberhart and Russell, 1966). Therefore, the ideal genotype for downy mildew resistance would have these traits, along with a low overall mean. Three cultigens, 'Heidan#1', M 21, and WI 2757, had a  $S^2_d$  significantly different from zero, indicating their means tended to be farther from the fit regression line, and that they may not be ideal for use in breeding for resistance to downy mildew. Cultigen M 21 is a dwarf determinate pickle, and had regression slope close to unity, and therefore may be useful in the introducing that type into elite lines, with the understanding that the environmental effect on the apparent disease severity can be large. The cultigens with the lowest mean downy mildew rating in this study included 'Poinsett 76', a slicing cucumber, and WI 2238, a trellis type cucumber. Both of these cultigens had a  $b_i$  close to unity and non-significant  $S^2_d$ , indicating the resistance of these cultigens is stable.

#### Conclusions

Significant variation existed for genotype, year, and genotype-year interaction. Several cultigens had showed a strong response to environment for downy mildew resistance. The high environmental effect along with genotype-environment interaction demonstrates the importance of testing for downy mildew resistance in multiple environments. Breeders must balance the cost of testing in locations and seasons to get the best data with meeting budget and time goals, by maximizing efficiency. Downy mildew is a highly variable pathogen, influenced greatly by environmental conditions. Therefore multiple locations over a wide area for testing over multiple years is recommended.

Cultigens that are highly resistant to the downy mildew currently in the United States have been reported (Call et al., 2012b). The most resistant cultigens in this study were only moderately resistant. The cultigens in this study with the lowest mean downy mildew rating, a  $b_i$  close to unity and non-significant  $S^2_d$ , included 'Poinsett 76', a slicing cucumber, and WI 2238, a trellis type cucumber. These cultigens are considered the most stable across the environments tested.

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Source of variation	df	Sum of Squares	Mean Square	F	Р
Year	6	225.24	37.54	80.37	<.0001
Replication(Year)	21	19.59	0.93	2.00	0.0067
Cultigen	14	407.35	29.10	14.81	<.0001
Cultigen*Year	80	157.20	1.97	4.21	<.0001
Pooled Error	282	131.72	0.47		

Table 3.1. Analysis of variance of the mean of all downy mildew disease ratings for data collected in Clinton, North Carolina from 2005-2011 <sup>a</sup>.

a Data are from four replications with four weekly ratings.

Source of variation	df	Mean	F	Р
2005		Square		
2005				
Replication	3	0.15	0.63	0.5969
Cultigen	14	8.34	35.62	<.0001
<u>2006</u>				
Replication	3	1.23	2.25	0.0964
Cultigen	14	7.07	12.95	<.0001
<u>2007</u>				
Replication	3	1.20	5.25	0.0036
Cultigen	14	2.55	11.20	<.0001
2008				
Replication	3	1.85	3.80	0.0182
Cultigen	12	4.07	8.35	<.0001
2009				
Replication	3	1.41	2.95	0.0433
Cultigen	14	7.58	15.83	<.0001
<u>2010</u>				
Replication	3	0.31	0.45	0.7162
Cultigen	12	6.04	8.74	<.0001
2011				
Replication	3	0.40	0.63	0.6012
Cultigen	14	5.77	9.02	<.0001

Table 3.2. Analysis of variance of the mean of all downy mildew disease ratings by year for data collected in Clinton, North Carolina from 2005-2011 <sup>a</sup>.

a Data are from four replications with four weekly ratings.



Figure 3.1. Genotype by environment interaction of 15 cucumber genotypes in 7 environments for mean downy mildew ratings <sup>a</sup>.

a Data are from four replications with four weekly ratings. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: 0 to 9 (0 = 0%, 1 = 1-3%, 2= 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75\%, 7 = 75-87\%, 8 = 87-99\%, 9 = 100\%). Environmental index = mean of environment – mean of all environments.

	2005	2006	2007	2008	2009	2010	2011
2005		0.90***	0.64**	0.57*	0.66**	0.83***	0.64*
2006	0.79***		0.57**	0.62*	0.70**	0.80***	0.65*
2007	0.23	0.20		0.61**	0.72**	0.56*	0.57*
2008	0.10	0.35	0.32		0.58*	0.61*	0.79***
2009	0.44	0.50*	0.59*	0.42		0.69**	0.65**
2010	0.64**	0.64**	0.17	0.32	0.55*		0.76**
2011	0.45	0.51*	0.31	0.77**	0.60*	0.75**	

Table 3.3. Pearson (above diagonal) and Spearman (below diagonal) correlations of mean downy mildew resistance ratings for years <sup>a</sup>.

a Data are from four replications with four weekly ratings.

\*, \*\*, \*\* Significant at 0.05, 0.01 and 0.001, respectively.

Cultigen	Source	2005	2006	2007	2008	2009	2010	2011
Calypso	NCStateUniv	2.8	2.5	3.5	5.8	4.5	5.1	6.1
Coolgreen	AsgrowSeed	7.0	6.6	6.1	7.6	7.3	7.9	7.5
Dasher II	PetoSeed	2.4	2.6	4.2	5.9	5.8	5.3	6.7
Gy 4	NCStateUniv	4.4	4.4	3.6	4.5	4.5	6.0	5.4
Heidan#1	PRChina	3.6	3.6	4.3	4.3	6.1	4.1	4.4
Homegreen #2	USDA-Wis	3.3	3.3	4.9	4.1	5.1	5.4	5.3
M 21	NCStateUniv	3.4	4.5	3.0	5.4	3.4	5.1	5.3
Marketmore 76	CornellUniv	2.9	4.2	4.9	6.3	5.6	-	6.7
NongChn#4	PRChina	4.1	4.2	3.7	5.0	5.8	5.4	5.4
Poinsett 76	CornellUniv	2.9	2.9	3.9	5.0	3.6	3.6	3.9
Slice	ClemsonUniv	3.4	2.6	4.1	4.9	4.4	-	4.7
Sumter	ClemsonUniv	4.7	4.3	3.9	5.3	5.2	5.8	7.3
WI 2238	USDA-Wis	2.4	2.4	4.0	-	3.3	4.9	5.2
WI 2757	USDA-Wis	3.8	2.6	4.1	-	2.8	3.8	5.3
Wis.SMR 18	Univ.Wis.	7.1	6.3	5.5	6.9	7.3	7.6	8.1
Mean		3.9	3.8	4.2	5.5	5.0	5.4	5.8
CV (%)		12.5	19.5	11.3	12.8	13.9	15.5	13.7
LSD (5%)		0.7	1.1	0.7	1.0	1.0	1.2	1.1
Range/LSD		6.7	3.8	4.4	3.5	4.5	3.6	3.8
Range		4.7	4.2	3.1	3.5	4.5	4.3	4.2

Table 3.4. Mean ratings for resistance of cucumber cultivars by year (2005-2011) at Clinton, North Carolina<sup>a</sup>.

a Data are from four replications with four weekly ratings. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: 0 to 9 (0 = 0%, 1 = 1-3%, 2 = 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99%, 9 = 100%)

Cultigen	Source	2005	2006	2007	2008	2009	2010	2011
Calypso	NCStateUniv	Ι	Ι	Ι	Ι	Ι	Ι	Ι
Coolgreen	AsgrowSeed	S	S	S	S	S	S	S
Dasher II	PetoSeed	Ι	Ι	Ι	Ι	Ι	Ι	Ι
Gy 4	NCStateUniv	Ι	Ι	Ι	Ι	Ι	Ι	Ι
Heidan#1	PRChina	Ι	Ι	Ι	Ι	Ι	Ι	R
Homegreen #2	USDA-Wis	Ι	Ι	Ι	Ι	Ι	Ι	Ι
M 21	NCStateUniv	Ι	Ι	Ι	Ι	Ι	Ι	Ι
Marketmore 76	CornellUniv	Ι	Ι	Ι	Ι	Ι	-	Ι
NongChn#4	PRChina	Ι	Ι	Ι	Ι	Ι	Ι	Ι
Poinsett 76	CornellUniv	Ι	Ι	Ι	Ι	Ι	R	R
Slice	ClemsonUniv	Ι	Ι	Ι	Ι	Ι	-	Ι
Sumter	ClemsonUniv	Ι	Ι	Ι	Ι	Ι	Ι	S
WI 2238	USDA-Wis	R	R	Ι	-	R	Ι	Ι
WI 2757	USDA-Wis	Ι	R	Ι	-	R	R	Ι
Wis.SMR 18	Univ.Wis.	S	S	S	S	S	S	S

Table 3.5. Ratings for resistance of cucumber cultivars to local race(s) of *P. cubensis* in different years at Clinton, NC <sup>a</sup>.

a Data are from four replications with four weekly ratings, and standardized to a mean of 4.5 and standard deviation of 1.5. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: 0 to 9 (0 = 0%, 1 = 1-3%, 2= 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99\%, 9 = 100\%)

		Downy mildew rating					
Cultivar	Source	Mean	$b_i$	$S^2_d$			
Calypso	NCStateUniv	4.3	1.72***	0.037			
Coolgreen	AsgrowSeed	7.1	0.62	0.185			
Dasher II	PetoSeed	4.7	1.95**	0.315			
Gy 4	NCStateUniv	4.7	0.63	0.412			
Heidan#1	PRChina	4.3	0.42	0.667*			
Homegreen #2	USDA-Wis	4.5	0.81	0.480			
M 21	NCStateUniv	4.3	0.87	0.670*			
Marketmore 76	CornellUniv	5.1	1.51	0.367			
NongChn#4	PRChina	4.8	0.78	0.253			
Poinsett 76	CornellUniv	3.7	0.63	0.310			
Slice	ClemsonUniv	4.0	0.92	0.207			
Sumter	ClemsonUniv	5.2	1.15	0.446			
WI 2238	USDA-Wis	3.7	1.27	0.446			
WI 2757	USDA-Wis	3.7	0.66	0.834*			
Wis.SMR 18	Univ.Wis.	7.0	0.73	0.444			
LSD (5%)		0.4					

Table 3.6. Means and stability parameters (regression coefficient  $[b_i]$  and deviation from regression  $[S^2_d]$ ) for mean downy mildew rating of 15 cucumber cultigens tested in 7 years at Clinton, NC.

a Data are from four replications with four weekly ratings. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: 0 to 9 (0 = 0%, 1 = 1-3%, 2 = 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99%, 9 = 100%)

\*, \*\*, \*\*\* indicate significantly different from unity for the regression coefficients or slope  $(b_i)$  and from zero for the deviation from regression  $(S^2_d)$  at 0.05, 0.01 and 0.001 levels of probability, respectively



Figure 3.2. Regression (dark line) and 95% confidence of fit (light blue) of mean downy mildew rating on environmental index for 15 cucumber cultigens <sup>a</sup>.

a Data are from four replications with four weekly ratings. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: 0 to 9 (0 = 0%, 1 = 1-3%, 2= 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99%, 9 = 100%). Environmental index = mean of environment – mean of all environments.



Figure 3.3. Mean resistance by environmental index for cultigen downy mildew rating means over ratings and reps <sup>a</sup>.

a Data are from four replications with four weekly ratings. Disease assessment scale adopted for evaluating cucumber for resistance to downy mildew: 0 to 9 (0 = 0%, 1 = 1-3%, 2= 3-6%, 3 = 6-12%, 4 = 12-25%, 5 = 25-50%, 6 = 50-75%, 7 = 75-87%, 8 = 87-99%, 9 = 100%) Environmental index = mean of environment – mean of all environments.