77

BARD

FINAL REPORT
PROJECT NO. US-752-84

**Utilization, Nature, and Genetics of Mildew
Resistances in Cantaloup**

C.E. Thomas, Y. Cohen, J.K. Peterson

1987

Date: January 26, 1988

BARD
P. O. Box 6
Bet Dagan, ISRAEL

BARD Project No. US-752-84C

Title of Research Project:

Utilization, Nature, and Genetics of Mildew Resistances in Cantaloup

Names of Investigators:

Principal Investigator: C. E. Thomas

Cooperating Investigator(s): Y. Cohen and J. K. Peterson

Name and Address of Affiliated Institutions:

Principal Institution: U. S. Vegetable Laboratory, Agricultural Research
Service, USDA, South Atlantic Area, Charleston, SC,
USA

Cooperating Institution: Dept. of Life Sciences, Bar-Ilan University, Ramat
Gan, Israel

Project's starting date: October 1, 1985

Type of Report: 1st Annual _____ 2nd Annual _____ Final x

Signatures:

Principal Investigator

Charles E. Thomas
Research Plant Pathologist

Institution's Authorizing Official

George R. Burns
acting Area Director
USDA, ARS, South Atlantic Area
Richard B. Russell Ag. Research Center
P. O. Box 5677
Athens, GA 30613

USDA, ARS, South Atlantic Area
Richard B. Russell Ag. Research Center
P.O. Box 5677, 950 College Station Road
Athens, GA. 30613

Table of Contents

Abstract	1
Objectives of original research proposal	2
Body of report:	
Objective 1 - Exploit the superior levels of downy and powdery mildew resistance identified and stabilized from PI 124111 by ensuring their incorporation into high quality cantaloup germ plasm for production in Israel	3
Objective 2 - Study the mechanisms and durability of resistance of PI 124111 to both mildew pathogens	7
Objective 3 - Further elucidate the modes of inheritance of these resistances to mildew	45
Unstated objectives	52
Literature Cited	71
Description of cooperation	72
Evaluation of research achievements with respect to the original research proposal	72
Benefits to agriculture	73
List of publications	74

Abstract

Downy and powdery mildew resistant parental lines of cantaloup with high quality were produced and tested in crosses with commercial cultivars to generate some promising hybrid combinations for production in Israel. The potential for high durability of downy mildew resistance from Cucumis melo PI 124111 was demonstrated in field and growth chamber studies. Isoelectric focusing studies located common peroxidase and esterase bands with activity levels which corresponded to the levels of resistance expressed by leaves of susceptible, moderately resistant, and resistant plants. In downy mildew resistant PI 124111 host cell and haustorial containment by callose- and lignin-like materials which disconnect the flow of nutrients from and into invaded host cells were implicated in the resistance mechanism. Inhibition of the production of the second and third germ tubes, hyphal growth, and sporulation were found to be expressions of the resistance mechanism of PI 124111 against powdery mildew. Resistance to downy mildew from PI 124111 is conferred by two incompletely dominant genes designated Pc1 and Pc2. Resistance to race 2 of powdery mildew from this PI is conferred by an incompletely dominant gene. PI 124111 was found to also be resistant to races 0, 1, and 2 of Fusarium wilt.

D. Objectives of original research proposal.

The three objectives as stated in the original research proposal were:

1. Exploit the superior levels of downy and powdery mildew resistances identified and stabilized from PI 124111 by ensuring their incorporation into high quality cantaloup germ plasm for production in Israel.
2. Study the mechanisms and durability of resistance of PI 124111 to both mildew pathogens.
3. Further elucidate the modes of inheritance of these resistances to mildews.

In the following body of this report, each objective and the research pursuant to its attainment will be discussed in individual sections in the order listed above. Wherever possible, publications or manuscripts that have been submitted for publication are used as part of this report. Tables and figures are numbered by section and 'Literature Cited' is inclusive for all sections.

- E. Objective 1. Exploit the superior levels of downy mildew and powdery mildew resistance identified and stabilized from PI 124111 by ensuring their incorporation into high quality cantaloupe germ plasm for production in Israel.

Introduction

In breeding work performed under BARD Research Project US-287-81 (3), F₅ through F₆ generations of a disease resistant parental line were developed using an inbred line of Cucumis melo PI 124111 as a source of resistance to downy mildew (Pseudoperonospora cubensis) and powdery (Sphaerotheca fuliginea races 1, 2, and 3) mildews. These disease resistant lines were crossed to a group of representative commercial cultivars and hybrids resulting from these crosses were evaluated for downy and powdery mildew resistance and horticultural acceptability. This work was continued under the present BARD Research Project.

Materials and Methods

Parental lines were produced in the greenhouse using standard techniques for muskmelon (8). Previously developed inbred lines (F₅ - F₆) with PI 124111 as the source of resistance to mildews were used to generate additional disease resistant inbred generations (F₇ and F₈) for crossing to commercial cultivars or were themselves crossed directly to commercial cultivars. Resultant hybrids were evaluated in field plots at Ramat Gan for disease resistance and horticultural acceptability in 1986 and 1987 under both natural and artificial inoculations.

Results and Discussion

Results of these studies are summarized in Tables 1-1 for the parental lines and 1-2 for the hybrids.

These results indicate continued progress in the development of horticulturally acceptable muskmelon hybrids with high levels of resistance to downy and powdery mildews for production in Israel. However, additional work is necessary to fully attain this objective and this work is continuing in Israel.

Table 1-1. Progeny, resistance, and fruit characteristics of advanced breeding lines of Cucumis melo.

Breeding line	Progeny	Mildew resistance downy ^a	powdery ^b	Fruit characteristics			
				flesh color	fruit shape	net	% soluble solids
16-4	PI 124111 x Hemed, F ₉	R(44)	R	orange	round	-	11
36	16-4 x Perlita, F ₅	R(34)	R	orange	oval	+	12
29	Hemed x PI 124111 x unknown, F ₉	MR(24)	R	orange	round	-	12
31	Hemed x PI 124111, F ₉	R(44)	R	green	round	-	13

aR = resistant; numbers in parentheses represent reaction type: 34 & 44 = highly resistant, 24 = moderately resistant.

bR = resistant to powdery mildew incited by races 1 and 2 of Sphaerotheca fuliginea.

Table 1-2. Resistance to downy mildew, powdery mildew race 2 and fruit characteristics of F₁ muskmelon hybrids under field conditions^z.

Hybrid	% defoliation ^y		Fruit characteristics		
	Spring	Summer	Shape	Net ^x	% soluble solids
16-4 F ₅ x Charantais	70	20	round	-	11
16-4 F ₆ x Cinco	65	70	oval	+	13
16-4 F ₆ x Topmark	55	--	round	+	12
16-4 F ₆ x PMR 45	75	45	round	+	10
16-4 F ₆ x TAM-Uvalde	50	--	oval	+	13
16-4 F ₆ x Persian	80	60	round	+	12
16-4 F ₆ x Charantais	70	30	round	-	11
16-4 F ₆ x Edisto 47	45	35	oval	+	13
16-4 F ₇ x Mainstream	55	30	oval	+	10
16-4 F ₈ x Gulfstream	65	45	oval	+	10
16-4 F ₈ x Goldenperfect	70	15	oval	+	8
16-4 F ₈ x PMR 45	70	45	round	+	10
16-4 F ₈ x Cum Laude	50	--	segregate	+	11
16-4 F ₈ x Topmark	70	20	oval	+	10
Ananas Yokneam	95	90			
Hemed	60	--			
Cinco	75	60			
TAM Uvalde	90	--			
Ga. 47	35	35			
Topmark	100	--			
PMR 45	95	85			
Mainstream	65	55			
Persian	90	85			
Cum Laude	95	--			
Golden Perfection	65	45			
Gulfcoast	100	--			

^zPlants transplanted at the two-leaf stage and inoculated with a sporangial suspension (500/ml) of *P. cubensis* one month later. Powdery mildew developed from natural inoculum.

^yDefoliation estimated visually and due mainly to downy mildew.

^x"+" = netted, "-" = not netted.

^wAs % sucrose.

E. Objective 2. Study the mechanisms and durability of resistance of PI 124111 to both mildew pathogens.

Some of the work under this objective has either been accepted for publication or submitted for publication and is therefore included at the end of this section in the form of the respective manuscripts.

In the section directly below, durability and isozyme studies are presented in separate write-ups.

Durability studies.

Introduction

Many downy mildew "resistant" cantaloup cultivars have been released, but these resistances have not held up due to either the various pathotypes (6) of the downy mildew pathogen (Pseudoperonospora cubensis) or because of adaptability of the pathogen for virulence towards the resistant genotype. In order to study the durability of downy mildew resistance from Cucumis melo PI 124111, research was conducted at Charleston, S. C. under field, growth chamber, and greenhouse conditions.

Materials and Methods

The released inbred muskmelon breeding line MR-1 (4) which was developed from PI 124111 was used as the downy mildew resistant genotype in these studies.

Field studies. Field plots of downy mildew resistant line MR-1 and the susceptible cultivar Perlita were planted and maintained from April through December 1985. When each set of plants reached mid-maturity, a new row of each genotype was planted within the same plot so that candidate susceptible and resistant material in the early, medium, and late stages of development

was always available within the plot area. In this way six sets of both the susceptible and resistant genotypes were tested in this study. Initially these plots were artificially inoculated with a sporangial suspension (2.0×10^4 per ml) of P. cubensis when the first set of plants reached the 10-leaf stage of development. This original artificial inoculation coupled with the continuous availability of candidate susceptible material of the cultivar Perlita was sufficient to establish an epiphytotic which was maintained by natural conditions.

Plants in the plots were examined at 7-day intervals for downy mildew reaction type for the duration of the study.

Growth chamber studies. Cotyledons were used as the test substrate in this work because preliminary studies showed that insufficient sporangia were produced on true leaves of line MR-1 to provide sufficient inoculum for the next generation of that subpopulation. Cotyledons of downy mildew resistant line MR-1 and the susceptible cultivar Ananas Yokneam were inoculated with a 10^4 suspension of sporangia of P. cubensis. This suspension was a 50-50 mix from pathotypes 4 and 5. Four replications of twelve plants each were inoculated for each genotype. Following inoculation plants were placed in a dark dew chamber at 20C for 18 hr. Plants were removed from the dew chamber and placed in a 20C growth chamber with a 12/24 hr photoperiod for five days. For the sixth night after inoculation plants were returned to the 20C dew chamber for 18 hr to enhance sporulation. Plants were removed from the dew chamber seven days after inoculation and cotyledons were removed. Sporangia were washed from cotyledons, concentrated by centrifugation, resuspended in a known volume of water, and counted with a hemacytometer. The area of the cotyledons was determined with a Li-Cor area meter and the number of sporangia produced per cm^2 of

cotyledon was calculated for each genotype. Sporangia recovered from cotyledons of each genotype were used to inoculate a second generation of cotyledons of that respective genotype. In this way two subpopulations were established, one maintained on the resistant line MR-1 and designated D_{MR-1} and the other maintained on the susceptible cultivar Ananas Yokneam and designated D_{AY} . These subpopulations were maintained for a total of 36 generations.

Sporulation efficiency as a measure of pathogen adaptability to indicate a loss of the durability of the resistant genotype was calculated for each generation as number of sporangia produced/number of sporangia applied. Linear regressions, correlation coefficients, and coefficients of determination were calculated from this data.

As an additional check for pathogen adaptability, cross inoculations were conducted with each subpopulation at generations 20, 24, 25, and 26. In these inoculations D_{MR-1} and D_{AY} were inoculated to both MR-1 and Ananas Yokneam. These inoculations and determinations of sporulation efficiency were performed as described above.

Results and Discussion

Field studies. Throughout this study all plants of line MR-1 exhibited the highly resistant RT 44 (5) and all plants of the susceptible cultivar Perlita exhibited the susceptible RTs 11 and 12. There was no indication of any adaptability of the pathogen to overcome the resistance of MR-1.

While this study indicated a high potential for the durability of the resistance to downy mildew from PI 124111, only the deployment of the genes which condition resistance from this source in varied and widespread

geographic areas in large acreages for a number of years can absolutely attest to their durability.

Growth chamber studies. The statistical analyses (Table 2-1) did not indicate any tendency toward adaptability of P. cubensis toward either the highly susceptible Ananas Yokneam or the highly resistant MR-1 over the time period of the 36 generations of this study. This again indicates a high potential for durability of resistance against downy mildew derived from PI 124111. These data also indicate that the two genotypes used in this study do represent the fully susceptible and fully resistant host situation for this pathosystem

The results of the cross inoculations that were performed with each subpopulation of P. cubensis (Table 2-2) did not indicate any tendency towards adaptation to overcome the resistance of MR-1 by either subpopulation. Sporulation per cm² was always greater when either subpopulation was inoculated to Ananas Yokneam than when that same subpopulation was inoculated to MR-1.

Isozyme studies.

Introduction

Some studies have implicated peroxidase activity in leaves as being involved in the mechanism of resistance of muskmelon foliage to downy and powdery mildews (1). In our work peroxidase and other isozymes in susceptible and resistant muskmelon leaves were studied.

Materials and Methods

Muskmelon plants at the two leaf stage were inoculated with Pseudoperonospora cubensis and evaluated for downy mildew reaction type (RT)

(5). These plants were produced from seed of the susceptible cultivar Ananas Yokneam (AY), the resistant breeding line MR-1, and the F₂ generation of AY x MR-1. Plants of RTs which were representative of susceptible, resistant, and moderately resistant reactions to downy mildew were selected and transplanted to the greenhouse bench and grown to the 15 leaf stage. Leaves were then picked from positions 3, 4, and 5 and prepared for isozyme analysis.

Leaf samples were weighed, rinsed, and placed in a chilled mortar and pestle. For every g of leaf tissue 1.5 ml of extraction buffer (B. Rhodes, personal communication) was added. Leaf tissue was then macerated with the mortar and pestle. The resultant slurry was centrifuged at 40,000xg for 1 hr at 4C. The supernatants were poured off and frozen until used within seven days of preparation.

Five percent T, three percent C, polyacrylamide slabs, 240 x 125 x 0.5 mm, were prepared using two percent (w:v) Ampholine, pH 3.5-9.5. Anolyte and catholyte were 0.1 M HCl and 0.5 M NaOH, respectively. Samples were loaded on 5 x 10 mm Whatman filter paper wicks and run on a LKB Multiphor II electrophoresis unit for 1.25 hr at 2.5 w. Wicks were then removed and samples were run an additional 2.25 hr at 2.5 w, followed by 5.0 w for 0.5 hr.

Slabs were stained for three enzymes: esterase, acid phosphatase, and peroxidase (2, 7) and examined with a LKB Ultrosan laser densitometer for common bands.

Results and Discussion

For peroxidase, a common band was located which corresponded to the resistance levels characteristic of the respective susceptible, resistant,

Table 2-1. Analysis of tendencies in sporulation efficiency of Pseudoperonospora cubensis subpopulation maintained on the downy mildew susceptible cultivar Ananas Yokneam and the resistant line MR-1 for 36 generations.

	Subpopulation	
	D_{MR-1}	D_{AY}
y-intercept	2.18	296.50
slope	0.13	-2.06
r	0.35	-0.10
r^2	0.13	0.01

Table 2-2. Sporulation efficiency of Pseudoperonospora cubensis subpopulations on downy mildew resistant line MR-1 and the susceptible cultivar Ananas Yokneam at selected subpopulation generations.

Subpopulation	Host	Spore Production/Unit Cotyledon Area (1000s/cm ²)			
		Generation			
		20	24	25	26
D _{AY}	AY	47.00	51.52	37.46	23.90
D _{AY}	MR-1	4.00	1.15	0.91	1.11
D _{MR-1}	AY	6.85	39.51	38.61	24.70
D _{MR-1}	MR-1	0.97	0.47	0.59	0.80

Ultrastructure of Pseudoperonospora cubensis in muskmelon
genotypes susceptible and resistant to downy mildew

Y. COHEN, H. EYAL, J. HANANIA and Z. MALIK

Department of Life Sciences, Bar-Ilan University
Ramat-Gan, Israel 52100

The downy mildew causal agent Pseudoperonospora cubensis (Berk. et Curt.) Rost. produced 10-15 mm lesions in the susceptible Cucumis melo L. cv. Ananas-Yokneam (AY) but 1-3 mm, water-soaked lesions in the resistant C. melo PI 124111F(PI). Sporulation in PI was extremely limited compared to the conspicuous sporulation in AY. In both genotypes the fungus produced intercellular hyphae, rich in B-1,3-glucans, from which digitate (cauliflower-like) haustoria grew into the host cells. The haustoria induced minor histochemical and ultrastructural changes in the cytoplasm or walls of AY at 144h after inoculation. In contrast, remarkable changes were observed in the infected PI cells as early as 20 h post inoculation. Changes induced in PI included a heavy deposition of paramural, layered, callose-like materials along the inner surface of the host cells; enrichment of host cell walls with lignin-like materials; and encasement of the haustoria with heavy deposits of callose-like materials. In addition, the intercellular fungus in PI, but not in AY, could produce haustoria (and necrosis) in epidermal cells, which gave rise to the early appearance (72h) of water-soaked lesions. We suppose that host cell - and haustorial - containment by callose- and lignin-like materials may disconnect the flow of nutrients from and into the invaded host cells. This containment could lead to an enhanced necrosis and hence to the limitation of lesion expansion and sporulation.

INTRODUCTION

Downy mildew, incited by Pseudoperonospora cubensis (Berk. et

(Cucumis sativus L.) and muskmelon (Cucumis melo L.) in temperate growing areas of the world [5,25]. Muskmelon (C. melo var. reticulatus) is susceptible to all five pathotypes of the fungus recently delineated by Thomas, Inaba and Cohen [38], while oriental pickling melon (C. melo var. conomon) and oriental melon (C. melo var. acidulus) are resistant to pathotypes 1 and 2, respectively [38].

In 1981 we reported on a high level of resistance against pathotype 3 of P. cubensis in C. melo var. reticulatus Plant Introduction (US) 124111 [5] and Thomas [37] reported in 1982 on resistance in this line against pathotypes 4 and 5. Tests conducted in Japan by T. Inaba (personal communication) showed that PI124111 is resistant to pathotypes 1 and 2 of the pathogen. Other studies showed that PI124111F derived from PI124111 by selfing and selection for 7 generations, is resistant to powdery mildew and Fusarium wilt [7,8].

The aim of this investigation was to follow the course of development of P. cubensis in PI124111 and the reactions of the invaded plant using TEM and histochemical staining, with a view to elucidating the mechanisms involved in its resistance to downy mildew.

MATERIALS AND METHODS

Plant genotypes

Tests were conducted with C. melo var. reticulatus cultivars Ananas-Yokneam (=AY, susceptible) and PI124111F(=PI, resistant). Seeds of AY were purchased from Hazera Seed Corp. (Haifa, Israel). Seeds of

of AY were purchased from Hazera Seed Corp. (Haifa, Israel). Seeds of PI were obtained in 1979 as a gift from G. Sowell, Jr., USDA, Experiment, GA (USA). This line was brought to homosigosity for resistance to downy mildew during 7 generations of selfing using the methods described earlier [7].

Plants were grown in the greenhouse (19-34 C) in 0.5 l pots filled with sandy loam and taken for inoculation at 3 wks after sowing, when they had 2 fully expanded true leaves.

Fungus and inoculation

Studies were made using pathotype 3 of Pseudoperonospora cubensis from Shuval (Northern Negev). The fungus was maintained on AY plants in growth chambers at 20 C. Sporangia were produced on AY, collected in cold (4 C) water and used immediately. Leaf No. 2 (from stem base) was detached from test plants (AY and PI), placed on a wet filter paper in 9 cm petri dishes (1 leaf/dish), abaxial surface uppermost and inoculated with 10 inoculum droplets (10 ul each containing about 400 sporangia). Dishes were incubated in a 20 C growth chamber with a 12h photoperiod with light intensity (Cool White fluorescent supplemented by incandescent light) of 180 uEinstins.m s.

Sampling and processing

For bright-field and fluorescence microscopy, leaves were sampled 1-7 days after inoculation at 1 day interval. For transmission electron microscopy (TEM), leaves were sampled at 1, 4, and 7 days after inoculation. For TEM, leaf discs, consisting of infected tissue and green tissue from the margins of lesions, were cut using a 3 mm diameter cork borer and immediately fixed under vacuum in 2.5% (v/v)

glutaraldehyde and 2% (v/v) formaldehyde in 0.1M phosphate buffer pH 7.4 for 2h. Tissue was then rinsed in phosphate buffer and post-fixed for 1h at 4C in 1% (v/v) osmium tetroxide in phosphate buffer.

After

several washings with phosphate buffer and then with Veronal acetate buffer (0.1M, pH 7.4) samples were prestained with 0.5% (v/v) uranyl acetate for 30 min at 4 C. After rinsing with Veronal acetate buffer samples were dehydrated in a graded ethanol series, and embedded in Spurr's resin [35]. Thin sections were cut with an LKB Ultratome III, stained for 5 min at 60 C in saturated uranyl acetate in 50% ethanol and poststained for 1 min with lead citrate [30]. Specimens were examined using a J.E.O.L. 1200 EX electron microscope operated at 80 kV.

To observe mycelium and haustoria of P. cubensis within muskmelon leaves, the whole-leaf clearing (alcoholic lactophenol) and staining (aniline blue) technique of Herzog & Schuepp [18] was used.

Samples for histochemical studies were fixed and decolorized in boiling ethanol for 10 min and then stained as follows:

For callose

The aqueous basic aniline blue fluorescence method was applied according to Eschrich & Currier [13] and Heath [17] with a Zeiss standard Epifluorescence Microscope (see below). Resorcinol blue was applied according to Eschrich & Currier [13] and Smart et al. [34]. Lacmoid was applied according to Smart et al. [34].

For lignin

The phloroglucinol method of Johansen [22] was applied. Chlorine sulfite was used according to Cadena-Gomez and Nicholson [4], and

Suberin was tested with gentian violet according to Bowen & Heale [3]; Pectin was tested with ruthenium red according to Smart et al. [34]. Lipids were tested with Sudan IV according to Smart et al. [34].

Autofluorescence

Discs were cut from test leaves and mounted fresh on glass slides, or fixed in boiling ethanol for 10 minutes then kept in 0.07M K_2HPO_4 (pH 8.9) overnight, and examined with the aid of a Zeiss Standard Epifluorescence Microscope. The microscope was equipped with an Halogen lamp (HBO, 50W), epifluorescence condensor IVFI and a filter set No. 18. This set included excitation filter BP 395-425, a dichromatic beam splitter FT 425 and a barrier filter LP 450. Neofluor objectives were used.

Photographs were taken using Agfa Pan 100 ASA black and white film.

RESULTS

Symptoms and Sporulation

Pale-yellow lesions one to 3mm in diameter were developed on PI at about 72 h after inoculation. No lesions were visible on AY after this time interval. Lesions in PI were water-soaked in appearance when observed from the abaxial surface. By 120 h first symptoms were seen in AY as greenish lesions. At 168 h (7 days) lesions in PI were chlorotic, 1-3 mm in size, whereas those in AY were 10-15 mm in size, chlorotic with some necrosis in the center.

Abundant sporangia (200-500 per mm^2) were produced on AY whereas very few sporangia (maximum of 1-2 per mm^2) were formed in PI.

Symptoms developed in AY and PI under heavy downy mildew epiphytotics in the field are shown in Fig. 1. Sporangial production in the field was 370-790 sporangia/mm² in AY compared to none in PI.

Mycelium and haustoria

Encysted zoospores settled mostly on leaf stomatal openings. Alcoholic aniline blue staining [18] revealed no significant difference in receptivity (proportion of stomatal openings occupied by cystospores) between AY and PI. Prolific hyphal development was observed in whole-leaf mounts of AY lesions starting from 96 h post inoculation (Fig. 2a). In contrast, no hyphae were detectable with this method in PI due to an overstaining of host cells in the lesions (Fig. 2b).

The haustorium, as observed in AY, had a "cauliflower" shape. It consisted of a thick basal portion from which thinner digitate extensions had grown (Fig. 3a). Usually, one haustorium was observed in a host cell. Haustoria colonized the mesophyll cells, but, in PI occasional haustorial development was also seen in abaxial epidermal cells. Such epidermal cells had collapsed and gave a water-soaked appearance to the lesion when viewed from the abaxial surface (Fig. 3b).

Histochemistry

Autofluorescence and histochemical data concerning callose-like materials formation are listed in Table 1. Autofluorescence was not detected in the susceptible AY until 120 h post inoculation. AT 120-168h a weak, yellow-green autofluorescence was emitted by hyphae and host cell wall corners. A different reaction was observed in the resistant PI: host cell walls autofluoresced yellow from 72 h onwards

and host cell wall corners. A different reaction was observed in the resistant PI: host cell walls autofluoresced yellow from 72 h onwards with a weak to moderate intensity.

Specimens treated with aniline blue emitted a much stronger yellow-green fluorescence. In AY, hyphae and haustorial bases fluoresced (Fig. 4a, 5a) as compared to host cells (walls and content) in PI (Fig. 4b, 5b). Resorcinol blue and Lacmoid stainings confirmed the presence of callose-like deposits in haustorial bases and at a later stage in haustoria and host cells in PI (Table 1).

Staining with phloroglucinol for lignin was negative in AY lesions (Fig. 6a) all along the colonisation period (168 h). Nevertheless, some lignification was seen in colonized parenchyma cells adjacent to major veins. A positive staining for lignin was observed in mesophyll cell walls of PI as early as 72 h post inoculation (Fig. 6b). Staining was intensified at 96 h and could be also seen in epidermal cells. At 120 h, phloroglucinol slightly stained the hyphae and haustoria in PI. Toluidine blue O stained hyphae and host cell-walls in PI. Chlorine sulfite stained host cell-walls in PI only, including epidermal cells. The only positive staining for suberin (gential violet) was observed in epidermal cells of PI. Staining for pectin was negative in AY and very minor in cell walls of PI at 144h.

Ultrastructure

The single germtube produced by the cystospore penetrated the mesophyll, produced a vesicle in the substomatal chamber and developed intercellular infective hyphae. First haustoria were seen in either hosts at 20 h (earliest observation) after inoculation. Intercellular hyphae contained spherical nuclei, mitochondria with tubular cristae,

appressed to host cell walls. In AY cells there was no discernable change in the cell wall (Fig. 8) except at the penetration site of a haustorium (Fig. 9). In PI cells a considerable thickening of the walls was observed (Fig. 10). Haustoria were initiated as lateral extensions of intercellular hyphae (Fig. 11). A collar of callose-like material surrounded the haustorial neck (Fig. 12 and 13). Callose-like materials were layered in massive amounts in penetrated cells of PI (Fig. 14 and 15) but not in AY (Figs. 11-13). In the resistant host (PI), wall thickening of the intercellular hyphal was observed (Fig. 16). Callose containing deposits were characteristically marbled or layered in appearance under TEM. Haustoria were usually constricted at the penetration site (Fig. 11) and then expanded inside the host cell (Fig. 13) digitating as a cauliflower (Fig. 3a). In trasverse sections, through a colonised host cell of AY, the basal (proximal) portion (Fig. 17) and the distal portion of a houstorium (Fig. 18) could be seen. The distal portion was made of many lobes separated to a distance of about 15 μ m (Fig. 18). Transverse sections through haustoria could also be seen in longitudinal sections through the host cell (Fig. 19). Haustoria were very rich in mitochondria, but lacked any nuclei. Vacuolisation in haustoria and hyphae appeared earlier and was more common in PI- than in AY-infected tissue.

An electron dense extrahaustorial matrix (E) completely surrounded the haustorium (Fig. 17). It was thinner in the base compared to the distal parts (lobes) of the haustorium (Fig. 13). Occasionally, the extrahaustorial matrix was continuous between close-enough lobes (Figs. 18, 19, 20). At 144-168 h after inoculation, callose-like deposits encased the haustoria in the susceptible AY (Figs. 21, 22, 23)

leaving the host cytoplasm apparently alive. Encasement of the haustoria with callose-like materials occurred much earlier during the colonisation process and was much heavier in the resistant PI. In PI it started at 20 h after inoculation and was very intensive at 96 h (Fig. 24). Haustorial encasement was accompanied by necrosis of the host cytoplasm and wall thickening of the intercellular hypha (Fig. 25). Collapse of epidermal cells including stomatal guard cell was often observed in PI, but never in AY.

DISCUSSION

The results extend our previous observations [7] and confirm that there is a striking difference in reaction of the susceptible Cucumis melo Cv. Ananas-Yokneam and the resistant breeding line PI 124111F as a result of infection with the downy mildew pathogen Pseudoperonospora cubensis. The major differences between these two genotypes were in lesion size and sporulation capacity. PI produced minute lesions with a negligible sporulation, while AY produced large lesions with conspicuous sporulation. These differences were attributed by Cohen et al. [6] to the incompletely dominant genes Pc-1 and Pc-2 present in PI [29].

The restriction of hyphal development in PI is probably related to the extensive defense reactions of the host cells. These defense reactions were of three types: a heavy deposition of paramural callose-like materials along the inner surface of host cell walls; encasement of the haustoria with callose-like (and probably lignin-like) deposits; a massive lignification of host cell walls.

Such reactions were clearly distinguished in the resistant PI as early as 72 h post inoculation. Using TEM, callose accumulation was observed at 20 h post inoculation.

The early encasement by callose- and lignin-like materials of the host cells and the haustoria in PI probably contained both the fungus and the infected cells, thus preventing intercellular hyphal growth, suppressing sporulation and inducing host cells death by disconnecting the nutrient flow into and from either haustoria or host cells [11,15,16,17,31,40].

Aniline blue fluorescence, resorcinol blue and Lacmoid stainings confirmed the presence of callose-like materials around the haustorial bases (collar) of P. cubensis colonising the susceptible AY, similar to that observed in other Peronosporales [12,14,28]. The strong aniline blue fluorescence of hyphae of P. cubensis confirmed earlier studies by Bartnicki-Garcia & Hemmes [2] and Hicky & Coffey [20] that β -1,3-glucans predominate in the construction of cell walls of Peronosporales. While callose deposition and lignification were abundant in host cells of PI, they were never observed in cell walls of affected AY cells. Nevertheless, encasement of haustoria by callose did appear in AY as late as 7 days after inoculation. This late encasement may explain the declining sporulating capacity of P. cubensis in mature lesions [9].

Encasement of haustoria by callose-like deposits was observed in a susceptible Nicotiana tabacum infected with Peronospora tabacina by Trigiano et al. [39], but not by Svircev and McKeen [36].

Hauستoria of P. cubensis in muskmelon resemble those of

Pseudoperonospora humuli in the hop [14,32]. They contain a large number of mitochondria, but have no nuclei as was reported for P. humuli [26,27] and for P. cubensis in cucumber [23]. Unlike other downy mildews which produce simple, cylindrical haustoria [10,12,14,19,28,36,39], Pseudoperonospora produces digitate "cauliflower-type" haustoria [14,32, and this study]. A unique feature of P. cubensis was the capacity of the intercellular hyphae growing in the mesophyll to penetrate the epidermal (including stomatal) cells of PI, but not those of AY, from beneath. Such cells collapsed after penetration giving the lesion a water-soaked appearance.

To the best of our knowledge, this is the first comparative study on the ultrastructure of P. cubensis in downy mildew-susceptible and-resistant genotypes of cucurbits. It is clear that callose- and lignin-like materials play a key role in restricting the growth of the fungus. This defense mechanism was described in detail elsewhere [1,31,40]. According to Aist [1], when cell damage occurs during infection by pathogens, localized elevation of Ca^{2+} levels results in localized deposition of callose, thus blocking sites of pathogen invasion. Delmer [11], in her recent review on the regulation of cellulose and callose biosynthesis, suggested that there is one plasma membrane-associated glucan synthase that is capable of catalyzing the synthesis of either cellulose or callose. Callose is synthesized when Ca^{2+} levels rise and the negative electrical potential across the plasma membrane dissipates. We are currently looking into the changes in Ca^{2+} in AY and PI in order to achieve a better understanding of the extremely extensive deposition of callose in the resistant host.

Current studies are also aimed at elucidation as to whether the extreme capability of PI to produce callose under stress is also related to its resistances against powdery mildew and Fusarium wilt.

This research was supported by BARD grant no. US-752-84C.

REFERENCES

1. AIST, J.R. (1983). Structural responses as resistance mechanisms. In The Dynamics of Host Defence , Ed. by J.A.Bailey & B.J. Deverall, pp. 33-70. Academic Press, Australia, Sydney.
2. BARTNICKI-GARCIA, S. & HEMMES, D.E. (1976). Some aspects of the form and function of Oomycetes spores. In The Fungal Spore: Form and Function , Ed. by D.J. Weber & W.M. Hess, pp. 593-641, J. Wiley & Sons, Inc., New York.
3. BOWEN, R.M. & HEALE, J.B. (1987). Endogenous elicitor activity from damaged carrot root tissue and induced resistance to Botrytis cinerea. Annals of Botany 59, 351-358.
4. CADENA-GOMEZ, G. & NICHOLSON, R.L. (1987). Papilla formation and associated peroxidase activity: A non-specific response to attempted fungal penetration of maize. Physiological and Molecular Plant Pathology 31, 51-67.
5. COHEN, Y. (1981). Downy mildew of cucurbits. In The Downy Mildew, Ed. by D.M. Spencer, pp. 341-354, Academic Press
6. COHEN, Y., COHEN, S. & EYAL, H. (1985). Inheritance of resistance to downy mildew in Cucumis melo PI124111. Cucurbit Genetics Cooperative 8, 36-38.
7. COHEN, Y. & EYAL, H. (1987). Downy mildew-, powdery mildew-, and Fusarium wilt-resistant muskmelon breeding line PI-124111F. Phytoparasitica 15, 187-195.
8. COHEN, Y., EYAL, H. & THOMAS, C.E. (1984). Stabilizing resistance in Cucumis melo against downy mildew and powdery mildew in Israel and the USA. Phytopathology 74, 829.
9. COHEN, Y. & ROTEM, J. (1971). Rate of lesion development in relation to

- sporulating potential of Pseudoperonospora cubensis in cucumber. Phytopathology 61, 265-268.
10. CHOU, C.K. (1970). An electron-microscope study of host penetration and early stages of houstorium formation of Peronospora parasitica (Fr.) Tul. on cabbage cotyledons. Annals of Botany 34, 189-204.
 11. DELMER, D.P. (1987). Cellulose biosynthesis. Annual Review of Plant Physiology 38, 259-290.
 12. EHRLICH, M.A. & EHRLICH, H.G. (1966). Ultrastructure of the hyphae and haustoria of Phytophthora infestans and hyphae of P. parasitica. Canadian Journal of Botany 44, 1495-1507.
 13. ESCHRICH, W. & CURRIER, H.B. (1964). Identification of callose by its diachrome and fluorochrome reactions. Stain Technology 39, 303-307.
 14. FRAYMOUTH, J. (1956). Haustoria of the Peronosporales. Transactions of the British Mycological Society. 39, 79-107.
 15. GODWIN, J.R., MANSFIELD, J.W. & DARBY, P. (1987). Microscopical studies of resistance to powdery mildew disease in hop cultivar Wye Target. Plant Pathology 36, 21-32.
 16. HAMMOND, K.E. & LEWIS, B.G. (1986). Ultrastructural studies of the limitation of lesions caused by Leptosphaeria maculans in stems of Brassica napus var. oleifera. Physiological and Molecular Plant Pathology. 28, 251-265.
 17. HEATH, M.C. (1974). Light and electron microscope studies of the interactions of host and non-host plants with cowpea rust - Uromyces phaseoli var. vignae. Physiological Plant Pathology 4, 403-414.

18. HERZOG, J. & SCHUEPP, H. (1985). Haustorial development test to characterize metalaxyl resistance and genetic variability in Plasmopara viticola. Bulletin OEPP/EPPO Bulletin 15, 431-435.
19. HICKEY, L.I. & COFFEY, M.D. (1977). A fine-structural study of the pea downy mildew fungus Peronospora pisi in its host Pisum sativum. Canadian Journal of Botany 55, 2845-2858.
20. HICKEY, E.L. & COFFEY, M.D. (1978). A cytochemical investigation of the host-parasite interface in Pisum sativum infected by the downy mildew fungus Peronospora pisi. Protoplasma 97, 201-220.
21. HINCH, J.M. & CLARKE, A.E. (1982). Callose formation in Zea mays as a response to infection with Phytophthora cinnamomi. Physiological Plant Pathology 21, 113-124.
22. JOHANSEN, D.A. (1940). Plant Microtechnique. McGraw-Hill Comp., Inc., 523 pp.
23. KAJIWARA, T. (1969). Ultrastructure of the host - parasite relationships in cucumber downy mildew caused by Pseudoperonospora cubensis (Berk. et Curt.) Rostow. Bulletin of the National Institute of Agricultural Sciences. (Japan) Series C. No. 23, 63-91.
24. KAUSS, H. (1987). Some aspects of calcium dependent regulation in plant metabolism. Annual Review of Plant Physiology 38, 47-72.
25. PALTÍ, J. & Cohen, Y. (1980). Downy mildew of cucurbits (Pseudoperonospora cubensis): the fungus and its hosts, distribution, epidemiology and control. Phytoparasitica 8, 109-147.
26. PARES, R.D. & GREENWOOD, A.D. (1977). Ultrastructure of the host - parasite relationships of Pseudoperonospora humuli on hops.

Australian Journal of Botany 25, 585-598.

27. PARES, R.D. & GREENWOOD, A.D. (1981). Ultrastructure of Pseudoperonospora humuli mycelium. The new Phytologist 89, 289-294.
28. PEYTON, G.A. & BOWER, C.C. (1963). The host - parasite interface of Peronospora manshurica on Glycine max. American Journal of Botany 50, 787-797.
29. PITRAT, M. (1986). Gene list of muskmelon (Cucumis melo L.). Cucurbit Genetics Cooperative 9, 111-120.
30. REYNOLDS, E.S. (1963). The use of lead citrate at high pH as an electron opaque stain in electron microscopy. Journal of Cell Biology 17, 208-214.
31. RIDE, J.P. (1983). Cell walls and other structural barriers in defence. In Biochemical Plant Pathology, Ed. by J.A. Callow, pp. 215-236. John Wiley & Sons, Chichester.
32. ROYLE, D. J., & KREMHELLER, T. TH. (1981). Downy mildew of the hop. In The Downy Mildews, Ed. by D.M. Spencer, pp. 395-422, Academic Press.
33. SKOU, J.P., JORGENSEN, J.H. & LILHOLT, U. (1984). Comparative studies on callose formation in powdery mildew compatible and incompatible barley. Phytopathologische Zeitschrift 109, 147-168.
34. SMART, M.G., AIST, J.R. & ISRAEL, H.W. (1986). Structure and function of wall appositions. 1. General histochemistry of papillae in barley coleoptiles attacked by Erysiphe Graminis f. sp. hordei . Canadian Journal of Botany 64, 793-801.
35. SPURR, A.R. (1969). A low viscosity epoxy resin embedding medium for electron microscopy. Journal of Ultrastructural Research 26, 31-34.
36. SVIRCEV, A.M. & MCKEEN, W.E. (1982). The haustorium of Peronospora

hyoscyami f. sp. tabacina in the susceptible tobacco leaf cell.

Canadian Journal of Plant Pathology 4, 119-128.

37. THOMAS, C.E. (1982). Resistance to downy mildew in Cucumis melo plant introductions and American cultivars. Plant Disease 66, 500-502.
38. THOMAS, C.E., INABA, T. & COHEN, Y. (1987). Physiological specialization in Pseudoperonospora cubensis. Phytopathology 77, (in press).
39. TRIGIANO, R.N., VAN DYKE, C.G. & SPURR, H.W., JR. (1983). Haustorial development of Peronospora tabacina infecting Nicotiana tabacum. Canadian Journal of Botany 61, 3444-3453.
40. VANCE, C.P., KIRK, T.K., & SHERWOOD, R.T. (1980). Lignification as a mechanism of disease resistance. Annual Review of Phytopathology 18, 259-288.

LEGEND TO FIGURES

- Fig. 1 Symptoms of downy mildew, incited by Pseudoperonospora cubensis in muskmelons. AY, in cv. Ananas-Yokneam (susceptible). PI, in PI 124111F (resistant).
- Fig. 2 Hyphae of Pseudoperonospora cubensis in AY (a) and a lesion of downy mildew in PI (b) as seen after clearing with alcoholic lectophenol and staining with aniline blue [18]. x 80. Bright field microscopy. 96h.
- Fig. 3a Hyphae and a hanstorium (middle of photograph) of Pseudoperonospora cubensis in AY. Note the digitate (cauliflower-like) shape of the hanstorium. X500. 96h.
- Fig. 3b Collapsed epidermal cells in PI after infection with Pseudoperonospora cubensis. X500. 96h.
- Fig. 4 Hyphae of Pseudoperonospora cubensis in AY (a) and lesions of downy mildew in PI (b) after clearing with boiling ethanol and staining with aniline blue. X30. Epifluorescence microscopy. Note the trichomes in (b). 96h.
- Fig. 5 As in Fig. 4 but at a magnification of X200. (a) AY. Note intensive fluorescence of hanstorial bases. (b) PI. Note intensive fluorescence of host cell. Mycelium could hardly be seen.
- Fig. 6 Whole leaf mounts in phloroglucinol - HCl[22] for lignin staining. (a) AY. X 30. Note sporulation. (b) PI. X 30. Note a positive staining of host cells. Sporangia of the original inoculum are seen. Bright field microscopy. 96h.
- Fig. 7 An electron micrograph of a transverse section through an

intercellular hypha of Pseudoperonospora cubensis in AY. X 12,000.

Fig. 8 The interface of an intercellular hyphae of Pseudoperonospora cubensis and a mesophyll cell of AY. X21,000. 20h.

Fig. 9 The initial response of an AY mesophyll cell to a potential penetration of Pseudoperonospora cubensis. X28,000. 20h.

Fig. 10 The interface of an intercellular hypha of Pseudoperonospora cubensis and a mesophyll cell of PI. Note lomasomes (L) in hypha and the thickening of host cell wall. X18,000. 20h.

Fig. 11 Penetration of Pseudoperonospora cubensis into a mesophyll cell of AY. X 10,000. 20h.

Fig. 12 A median section through the haustorial neck of Pseudoperonospora cubensis invading a mesophyll cell of AY. Note the collar surrounding the haustorial neck. X15,000. 20h.

Fig. 13 A haustorium of Pseudoperonospora cubensis in a mesophyll cell of AY. X9,000. 20h.

Fig. 14 Attempted penetration of Pseudoperonospora cubensis into a mesophyll cell of PI. Note paramural deposits of callose-like material along host cells. X 11,000. 96h.

Fig. 15 A successful penetration of Pseudoperonospora cubensis into a mesophyll cell of PI. Note the heavy deposits of callose-like materials along host cell walls and around the haustorium. X11,000. 96h.

Fig. 16 A heavy thickening, due to callose-like materials, of PI mesophyll cells infected with Pseudoperonospora cubensis. The intercellular hyphal-cell wall is also thickened but with no membranal blebs. X27,000. 96h.

- Fig. 17 The proximal part of an haustorian of Pseudoperonospora cubensis in a mesophyll cell of AY. Note the extrahaustorial matrix (E) surrounding the fungus. X11,000. 20h.
- Fig. 18 Haustorial lobes of Pseudoperonospora cubensis surrounded by extrahaustorial matrix in a transverse section through a mesophyll cell of AY. X6,000. 20h.
- Fig. 19 Transverse section through the intermediate part of an haustorium of Pseudoperonospora cubensis in a mesophyll cell of AY. Note no discernable damage to the host cell. X3,900. 20h.
- Fig. 20 As in Fig. 19. Note continuity of extrahaustorial matrix between some of the lobes and the distortion of chloroplasts. X8,200.
- Fig. 21 The encasement by callose-like materials of an haustorium of Pseudoperonospora cubensis in AY in a median section as observed at 7 days after inoculation. X5,500.
- Fig. 22 As in Fig. 21 but in a transverse section. Note the lack of necrosis in the host cell cytoplasm. X5,500.
- Fig. 23 An encased haustorium of Pseudoperonospora cubensis in AY mesophyll cell at days after inoculation. Note the extensive vacuolization in the wider part of the haustorium. X14,000. 7 days.
- Fig. 24 Median section of an intercellular hypha of Pseudoperonospora cubensis in a PI mesophyll cell at 4 days after inoculation. Note the difference in the extensive thickenings of fungal vs. host cell walls. That of the fungus lacks membranal blebs. X9,200. 96h.
- Fig. 25 An haustorium of Pseudoperonospora cubensis in a PI mesophyll cell heavily encased with callose-like materials at 4 days after inoculation. Host cytoplasm is necrotic. X6,500. 96h.

Table 1
Autofluorescence and callose formation in the Cucumis melo - Pseudoperonospora cubensis pathosystem

Reagent	genotype	24	48	72	96	120	144	168
Magnitude and site of reaction at various hours after inoculation								
Autofluorescence (yellow-green)	AY	-	-	-	-	+w,m	+w,m	+w,m
	PI	-	-	+w	+w	+w	+w	nt
Aniline-blue fluorescence (yellow-green)	AY	-	+m	+m,hb	+m,hb	+++m,hb	+++m,hb	+++m,hb
	PI	-	-	+++w,c,(m)	+++w,c(m)	+++w,c(m)	+++w,c(m)	+++w,c(m)
Resorcinol blue (dark blue)	AY	-	-	-	+hb	nt	+hb	+h
	PI	-	-	+h,w	+h,w	nt	+w	+w
Lecmoid (blue)	AY	-	-	+hb	+hb	nt	+hb	+hb,m
	PI	-	-	+h	+++h,w	nt	+++w,c	+++w,c

- no response; + trace of response; + → +++ increasing magnitude of response; c = cell content; h = haustoria;

hb = haustorial bases;

m = mycelium; w = host cell-walls; () seen when not mused; AY = Ananas-Yokneam; PI = PI124111 F; nt = not tested

AY

PI

4a

4b

5a

5b

6a

6b

7	8
9	10

11

12

13

14

15

16

17	18
19	20

21

22

23

24

25

Epifluorescence Microscopy of Sphaerotheca fuliginea Race 2 on
Susceptible and Resistant Genotypes of Cucumis melo

Yigal Cohen and Helena Eyal
Professor and Senior Technician, respectively
Department of Life Sciences, Bar-Ilan University, Ramat-Gan, Israel.

The useful technical assistance of A. Cohen and Z. Gov is acknowledged.
Research supported by BARD Grant US 752-84C granted to C.E. Thomas and
Y. Cohen.

ABSTRACT

Cohen, Y. and Eyal, H. 1987. Epifluorescence microscopy of
Sphaerotheca fuliginea race 2 on susceptible and resistant genotypes
of Cucumis melo. Phytopathology 77: 000-000.

Fungal growth and host response were investigated in leaves of
susceptible (Ananas-Yokneam, PMR-45), moderately resistant (Edisto 47,
Ananas-Yokneam x PI 124111 F1, Seminole) and resistant (PI 124111, PI
124112, PMR-5, PMR-6, Male Sterile-1, Dulce) genotypes of muskmelon with
the aid of the fluorescent stain, calcofluor, using UV epifluorescence
microscopy. Fungal germination at 24 hr with a single germtube proceeded
equally well on all genotypes, but the production of the second and
third germtubes at 48 hr, hyphal growth, and sporulation were inhibited
in resistant genotypes, probably due to the hypersensitive reaction of
epidermal cells that was associated with germtubes and hyphae. The
possibility is raised that the resistant genotypes studied share a
common gene for resistance to race 2 of Sphaerotheca fuliginea with
PI 124111.

Additional keywords: powdery mildew, genetics.

- 2 -

Powdery mildew is a major disease of muskmelon all over the world. Three races of the fungal causal agent, Sphaerotheca fuliginea (Schlech. ex. Fr.) Poll, were reported in the U.S.A. (9) and two, races 1 and 2, in Israel (4).

The muskmelon (Cucumis melo L.) line PI 124111 was reported to be resistant to races 1 and 2 of S. fuliginea in Israel and to races 1, 2, and 3 in the U.S. (4). The breeding line MR-1, derived from PI 124111, was reported to be resistant to all three races of the pathogen (12).

PI 124111 has been grown in our field plots on campus since 1978 along with the susceptible domestic cultivar Ananas-Yokneam. While Ananas-Yokneam suffered heavy epiphytotics of powdery mildew each year, PI 124111 remained disease free. Cohen and Cohen (3) studied the genetics and nature of resistance to powdery mildew race 2 in PI 124111. In crosses they made between PI 124111 and Ananas-Yokneam they found that resistance is conferred by a partially dominant gene. Resistance in PI 124111 was attributed to reduced conidial germination of S. fuliginea on the leaf surface, reduced colony formation, and reduced sporulation in comparison to the susceptible Ananas-Yorkneam (3).

The aims of this study were: (a) further explore the interaction between S. fuliginea and C. melo PI 124111 with the aid of fluorescence microscopy; and (b) compare fungal growth on PI 124111 with that on other powdery mildew-resistant genotypes of C. melo.

Materials and Methods

Plants. The various genotypes of Cucumis melo were grown in the greenhouse (20-34 C) in 0.5 l pots containing sandy loam, 1 plant per pot. Once a week plants were fertilized with a 1% solution of N:P:K (20:20:20). Three weeks after sowing, at the 2-leaf stage, plants were transferred to growth chambers at 23 C for inoculation.

Pathogen. A culture of Sphaerotheca fuliginea race 2 collected from C. melo cv PMR-45 (resistant to race 1 and susceptible to race 2) was used. This culture was maintained in a growth chamber at 23 C on PMR-45 plants.

Inoculation. One week after having been inoculated, PMR-45 plants were thoroughly shaken to dislodge conidia over the test plants. Conidial density was determined in leaf discs (2 cm diameter) taken from the inoculated plants immediately after inoculation. Leaf discs were stained with calcofluor and conidia counted with the aid of a fluorescence microscope (see below). Inoculated test plants were thereafter kept in a growth chamber at 23 C (60-70% RH) illuminated 12 hr a day with CW fluorescent light at an intensity of about 100 micro Einsteins per m² per second.

Microscopical examinations. The calcofluor staining technique reported earlier (5) for the Peronospora tabacina - tobacco pathosystem was used. One cm leaf disks were removed from leaf 2 of the inoculated plants at 1, 2 and 7 days after inoculation and mounted on microscope slides with the adaxial surface uppermost. Fifty μ l of aqueous 0.01% calcofluor (Calcofluor White M2R New, disodium salt of 4,4'-bis

(4-anilino-6-diethyl-amino- s -triazin-2-ylamino) -2,2' -stilbene disulfonic acid; Polyscience, Warrington, PA) solution were applied to the surface of each disk and then covered with a glass coverslip. Conidial germination, fungal growth and plant response were examined with the aid of a Zeiss Standard Epifluorescence Microscope equipped with an Osram halogen lamp (HBO, 50 W), epifluorescence condenser IVFI and filter set No. 18. This set includes excitation filter BP395-425, a dichromatic beam splitter FT 425 and a barrier filter LP 450. Neofluor objectives 6.3/0.20, 16/0.40, 40/0.75 and 100/1.30 were used with CPL W10x eyepieces. With this setup, fungal hyphae and conidia fluoresced blue, and plant cells remained dark or emitted a weak, red autofluorescence. Plant trichomes and physically injured cells also autofluoresced blue. Affected epidermal cells in resistant genotypes autofluoresced yellow.

To observe fungal penetration into plant epidermal cells infected leaf disks, at 1, 2 and 7 days after inoculation, were cleared by boiling in alcoholic lactophenol (phenol 10g, lactic acid 10 ml, glycerine 10 ml, 96% ethanol 20 ml) for 15 min and then washed twice with 96% ethanol and twice with deionized water. Leaf disks were then mounted on microscope slides with the adaxial surface uppermost and treated with calcofluor as described above. When examined with fluorescence microscopy, fungal hyphae and conidia fluoresced blue, and plant cells remained dark-brown. Affected epidermal cells in resistant genotypes retained their yellow autofluorescence. Points of penetration of fungal hyphae into epidermal cells fluoresced blue, especially in leaf disks sampled at 7 days postinoculation. Bright field microscopy

revealed the presence of circular holes at points of penetration into the epidermis.

To observe haustorial sacs inside epidermal cell, adhesive Scotch tape was attached to the adaxial surface of inoculated leaves, pressed lightly and peeled off. Some hyphae and conidia were thus removed from leaf surface. Peeled leaf pieces were cleared with lactophenol, mounted on microscope slides, and examined with bright field microscopy. Points of hyphal penetration and haustorial sacs were clearly visible.

Fungal sporulation. 23 mm diameter leaf disks were taken from leaf 2 of infected plants 10 days after inoculation and placed in 5 ml of FAA (formalin : acetic acid : ethanol, 5:5:90). Conidia were brushed off the leaf disks with the aid of a small camel hair brush and the number of conidia was determined with the aid of a cytometer (5 counts per disk).

Field observations. Various genotypes of C. melo were planted in microplots (5 x 10 m) on campus on May 1 and June 1, 1986. A source of PMR-45 infected plants was positioned in the middle of each plot one week after planting. Two mo after planting, disease severity was visually assessed using a 0-4 scale (0 - no disease symptoms apparent, 1, 2, 3 and 4 about 25, 50, 75 and 100% respectively, of the foliage affected with powdery mildew).

Results

Disease development in the field. Occurrence of powdery mildew was assessed 2 mo after planting. Ananas-Yokneam (AY), Topmark, Delicious 51, Planter's Jumbo and Hale's Best Jumbo were all severely affected (rated 4 on a visual scale 0-4). PMR-45, Edisto-47 and Rio Gold were moderately diseased (rated 2). No disease was recorded on Perlita, Male Sterile-1, Seminole, Dulce, PI 124111, PI 124112, PMR-5 and PMR-6. Seminole was slightly diseased (rated 1) at 3 mo after planting.

Fungal sporulation in controlled environment. Sporulation on plants artificially inoculated with 70-160 conidia per cm^2 at 10 days after inoculation in growth chambers is given in Table 1. The highest conidial yields were obtained in AY and PMR-45, a moderate yield in Edisto-47, low yields in AY x PI 124111 F1 hybrid and Seminole, and very low yields in PI 124112, PMR 5, PI 124111, Male Sterile-1, Dulce and PMR-6. In plants inoculated with 10-40 conidia per cm^2 no sporulation was detected in the resistant genotypes; conidial yields in AY and PMR-45 were 41.7 ± 21.4 and 19.7 ± 11.6 thousands conidia/ cm^2 , respectively.

Fungal growth on susceptible and resistant genotypes. Repeated epifluorescence microscopical examinations showed that conidia produced their first germtube (unbranched or bifurcate) in about 16-24 hr regardless of whether they were placed on leaves of resistant or susceptible genotypes. Percentage of conidia germinating with a single germtube at 24 hr postinoculation ranged between 40 and 70 in different experiments and no significant differences were noted between host genotypes. The production of the second and third germtubes and the

extension of the first one occurred within 48 hr with significant differences emerging between host genotypes. A representative experiment given in Table 2 shows that 42 and 47% of the conidia in a population developed 2-3 germtubes on the susceptible genotypes but only 6-18% did so on the resistant genotypes. At 72 hr after inoculation, an additional 2-3 germtubes and extension and ramification of the two first germtubes were seen on susceptible genotypes. Such a development was rarely seen in resistant genotypes. By 96 hr discrete colonies and conidiophores bearing conidial chains (7-9 conidia per conidiophore) were observed in susceptible genotypes. No conidiophores were seen in resistant genotypes at this stage. Profuse sporulation was observed on the susceptible genotypes a week after inoculation (Fig. 1 F), but only very few conidiophores bearing conidia (3-7 conidia per conidiophore) were seen in resistant ones (Fig. 1 C) except in Dulce and PMR-6 on which no sporulation was seen (compare with Table 1).

Host response. A strong yellow autofluorescence of epidermal cells was observed in all resistant genotypes as early as 16-24 hr after inoculation with S. fuliginea (Fig. 1 A). This fluorescence was not dependent on calcofluor staining and did not disappear after clearing with lactophenol. Autofluorescing epidermal cells were always associated with germtubes or hyphae of the pathogen and did not show up in plants inoculated with non-viable conidia (killed by chloroform vapour) nor in non-inoculated plants. Trichomes did show blue autofluorescence in all genotypes whether inoculated or not. The subjacent location of the yellow-fluorescing epidermal cells relative to germtubes and hyphae (Fig. 1 A-C) suggested that penetration of the

fungus into these host cells had occurred. Penetrations into normal and yellow-fluorescing epidermal cells was difficult to detect at 1 day after inoculation. In contrast, penetrations into fluorescing cells were distinguished at 7 days in calcofluor-stained tissue after clearing with lactophenol (Fig. 2 C, F). No yellow autofluorescing epidermal cells were seen in the susceptible AY and PMR-45 even on the 7th day after inoculation (Fig. 1 D-F) although numerous penetrations and haustorial sacs were clearly seen in epidermal cells of these genotypes (Fig. 2 A,B,D,E). Some epidermal cells, especially in the center of the colony, did autofluoresce yellow in Edisto 47, AYxPI hybrid, and Seminole a week after inoculation.

Fungal growth and host cell response are shown in color micrographs taken 1, 2 and 7 days after inoculation of the resistant PI 124111 (Fig. 1, A-C) and the susceptible AY (Fig. 1, D-F).

Discussion

Despite extensive studies on the occurrence and inheritance of genes for resistance to powdery mildew in muskmelon (1,2,6-12), very little is known about their nature. Cohen and Cohen (3) showed that a partially dominant gene is responsible for the resistance of PI 124111 to race 2 of Sphaerotheca fuliginea. In this line, advanced stages of conidial germination, hyphal growth and sporulation of S. fuliginea are suppressed (3).

The results presented in this paper show that the reduced fungal growth in PI 124111 is associated with a hypersensitive reaction of the host subsequent to a challenge inoculation with S. fuliginea. Leaf epidermal cells associated with germ tubes and hyphae showed extensive yellow autofluorescence. We postulate that epidermal cells produce upon fungal penetration yellow-fluorescing (probably phenolic) substances. The initial appearance of such autofluorescing cells was noticed as early as 16-20 hr after inoculation. Their number increased as the fungus continued to grow on the surface. This hypersensitive response of the host did not completely stop the growth of the fungus, which was able to produce a few conidia.

Other microscopical examinations showed that resistance to race 2 in PI 124112, PMR-5, PMR-6, Dulce and Male Sterile-1 is also associated with an hypersensitive response similar to that described in PI 124111. The difference observed in the capacity of the fungus to sporulate on the various resistant genotypes indicates that hypersensitivity is a major, but not the sole, mechanism responsible for resistance to race 2 of the fungus. All genotypes showing a strong hypersensitive reaction

supported a reduced hyphal growth and severely inhibited sporulation. Ananas-Yokneam and PMR-45, which showed no fluorescing epidermal cells throughout the first week after inoculation supported profuse sporulation. Interestingly, Edisto-47, AY x PI 124111 (F1) and Seminole, which supported a moderate-to-weak sporulation, showed some fluorescing epidermal cells in the center of the fungal colonies.

According to Bohn and Whitaker (2) resistance in PMR-5 and PMR-6 is governed by the partially dominant gene Pm-2. These lines have PI 124111 in their pedigree together with additional sources of resistance to race 2 such as PI 79376. No information is yet available on the race 2 resistance genes in PI 124112, Male Sterile-1 and Dulce. It is possible that they share a common gene with PI 124111 against race 2 of S. fuliginea. This possibility is being investigated.

The calcofluor epifluorescence technique reported here was found to be useful for rapid characterization of resistance to powdery mildew in muskmelon genotypes. It may be adapted easily for similar purposes in other fungal-plant pathosystems.

References

1. Bohn, G.W. 1961. Inheritance and origin of nectarless muskmelon. J. Hered. 52: 233-237.
2. Bohn, G.W. and Whitaker, T.W. 1964. Genetics of resistance to powdery mildew race 2 in muskmelon. Phytopathology 54: 587-591.
3. Cohen, S. and Cohen, Y. 1986. Genetics and nature of resistance to race 2 of Sphaerotheca fuliginea in Cucumis melo PI 124111. Phytopathology 76: 1165-1167.
4. Cohen, Y., Eyal, H. and Thomas, C.E. 1984. Stabilizing resistance in Cucumis melo against downy and powdery mildew in Israel and the USA. Phytopathology 74: 829.
5. Cohen, Y., Peer, S., Ballas, O. and Coffey, M.D. 1987. A fluorescence technique for studying growth of Peronospora tabacina on leaf surfaces. Phytopathology 77: 201-204.
6. Harwood, R.R. and Markarian, D. 1968. The inheritance of resistance to powdery mildew in cantaloupe variety Seminole. J. Hered. 59: 126-130.
7. Harwood, R.R. and Markarian, D. 1968. A genetic survey of resistance to powdery mildew in muskmelon. J. Hered. 59: 213-217.
8. Jagger, I.C. and Scott, G.W. 1937. Development of powdery mildew resistant cantaloupe No. 45. U.S. Dept. Agr. Circ. 441: 1-5.

9. McCreight, J.D., Pitrat, M., Thomas, C.E., Kishaba, A.N. and Bohn, G.W. 1986. Powdery mildew resistance genes in muskmelon. J. Amer. Soc. Hort. Sci. (in press).
10. Robinson, R.W., Munger, H.M., Whitaker, T.W. and Bohn, G.W. 1976. Genes of the Cucurbitaceae. HortScience 11: 554-568.
11. Sitterly, W.R. 1979. Powdery mildew of cucurbits. Pages 359-379 in: The Powdery Mildews. D.M. Spencer, ed. Academic Press, 565 pp.
12. Thomas, C.E. 1986. Downy and powdery mildew resistant muskmelon breeding line MR-1. HortScience 21: 329.

Table 1. Sporulation of Sphaerotheca fuliginea race 2 on susceptible and resistant genotypes of Cucumis melo at 23 C.

<u>Genotype</u>	<u>Class</u> ¹	<u>Conidia/cm², thousands</u> ²
Ananas-Yokneam (=AY)	S	71.1 $\pm$ 16.6 a ³
PMR-45	S	70.6 $\pm$ 19.3 a
Edisto 47	S	43.9 $\pm$ 20.0 b
AY x PI (F1)	MR	22.3 $\pm$ 11.8 c
Seminole	MR	16.0 $\pm$ 8.4 c
PI 124112	R	3.9 $\pm$ 4.6 d
PMR-5	R	2.1 $\pm$ 3.1 d
PI 124111 (=PI)	R	1.4 $\pm$ 2.4 d
Male Sterile-1	R	0.4 $\pm$ 0.8 d
Dulce	R	0.1 $\pm$ 0.2 d ⁴
PMR-6	R	0.1 $\pm$ 0.2 d ⁴

¹S = susceptible, MR = Moderately resistant and R = resistant to powdery mildew.

²Inoculum density was 70-160 conidia per cm² of leaf surface. Conidial production was measured at 10 days after inoculation with the aid of a cytometer in a 23 mm diameter leaf disc taken from leaf 2 of 4-leaf plants. Six plants were examined per entry.

³Different letters indicate significant differences at the 5% level according to Duncan's multiple range test.

⁴Since no sporulation was observed with epifluorescence microscopy these conidia may represent the original inoculum.

Table 2. Germination of Sphaerotheca fuliginea race 2 on the adaxial leaf surface of susceptible and resistant Cucumis melo genotypes at 23 C¹

<u>Genotype</u>	<u>Class</u> ²	<u>Percentage (+SD) conidia developing 2-3 germ tubes at 48 hr</u>
Ananas-Yokneam	S	47 ± 13 a ³
PMR-45	S	42 ± 6 a
PI 124111	R	19 ± 12 b
PI 124112	R	10 ± 11 bc
Male Sterile-1	R	11 ± 2 bc
PMR-5	R	18 ± 16 bc
PMR-6	R	6 ± 4 c

¹ The calcofluor assay was conducted in leaf 2 of 2-leaf plants, five plants per genotype, and one hundred conidia were counted per plant at 48 hr after inoculation.

² S = susceptible and R = resistant to powdery mildew.

³ Different letters indicate significant differences at the 5% level according to Duncan's multiple range test.

Legend to Figures

Fig. 1 Development of Sphaeroteca fuliginea race 2 on leaf surface of PI 124111 (resistant, A-C) and Ananas-Yokneam (susceptible, D-F), at 1 (A, D, x450), 2 (B, E, x450), and 7 (C, F, x70) days after inoculation. Photos were taken with the aid of a UV epifluorescence microscope after calcofluor staining, with Agfa xR 100 i color film.

Tissue photographed in A was first clear with boiling lactophenol. Note the association of fluorescing epidermal cells with germ tubes in A and with hyphae in B, C.

Fig. 2 Penetration of Sphaeroteca fuliginea race 2 into leaf epidermal cells of Ananas-Yokneam (susceptible, A, B, D, E, x450) and PI 124111 (resistant, C x 1125, F x 450) at 7 days after inoculation. A and D are matching photos taken under UV (A) and bright field (D) microscopy, with calcofluor staining. Note that fluorescing points of penetration in A correspond with holes in epidermal cells in D. B and E are matching photos taken similarly after peeling with adhesive tape. Note that fluorescing points of penetration in B correspond with holes and haustorial sacs in epidermal cells in D. C and F show penetration into a fluorescing cell of PI 124111, under mixed UV and incandescent light.

- E. Objective 3. Further elucidate the modes of inheritance of these resistances to mildews.

The work under this objective has been published. Therefore, copies of these publications are included here.

F. Description of Cooperation:

Cooperation between USA and Israeli scientists in the conduct of this research project has taken several forms including germ plasm exchange, information exchange, joint planning of key phases, and joint publication of results.

USA scientists provided germ plasm stocks to the Israeli scientist as needed. Many joint discussions of research problems were conducted through either written correspondence or in person. Dr. Cohen visited the U. S. Vegetable Laboratory on two occasions for this purpose and other in depth discussions were held at meetings of the American Phytopathological Society.

G. Evaluation of the research achievements with respect to the original research proposal:

The achievements of this research have either accomplished or substantially advanced towards the accomplishment of the originally proposed research objectives.

Superior levels of downy and powdery mildew resistance have been incorporated into high quality parental lines of cantaloup for hybrid production in Israel.

The physical mechanisms involved in the resistance of PI 124111 to both downy and powdery mildews have been described and studies of the chemical nature of the mildew resistances from this PI have been initiated and are in progress. Experimental evidence has been developed which indicates that downy mildew resistance from this PI will be durable.

The inheritance of resistance to downy mildew from PI 124111 has been further clarified and the inheritance of resistance to race 2 of powdery mildew from this PI source has been determined.

H. Benefit to agriculture:

The development of advanced parental lines with superior levels of downy and powdery mildew resistances and their hybrid combinations for cantaloup production in Israel will enhance production efficiency and reliability and will also provide revenues from sales of seed.

Elucidation of the nature of the resistant reactions against both downy and powdery mildews in cantaloup advanced knowledge of resistance for these and other pathosystems. The demonstrated potential durability of downy mildew resistance from PI 124111 enhances the value of this resistance to plant breeders.

Knowledge on the modes of inheritance of these superior levels of mildew resistances from PI 124111 will contribute to their utilization and further exploitation by plant breeders in the development of disease resistant cantaloups.

I. List of Publications

Cohen, S. and Cohen, Y. 1986. Genetics and nature of resistance to race 2 of Sphaerotheca fuliginea in Cucumis melo PI 124111. Phytopathology 76:1165-1167.

Cohen, Y. and Eyal, H. 1986. Sphaerotheca fuliginea sexual reproduction on cucurbits in Israel. Phytopathology 76:1082.

Cohen, Y. and Eyal, H. 1987. Downy mildew-, powdery mildew-, and Fusarium wilt-resistant muskmelon breeding line PI 124111F. Phytoparasitica 15:187-195.

Cohen, Y. and Eyal, H. 1988. Epifluorescence microscopy of Sphaerotheca fuliginea race 2 on susceptible and resistant genotypes of Cucumis melo. (Accepted by Phytopathology).

Thomas, C. E., Cohen, Y., McCreight, J. D., Jourdain, E. L., Cohen, S. 1988. Inheritance of resistance to downy mildew in Cucumis melo. Plant Disease 72:33-35.

100-100-100